Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2427741 A1 2002/08/22

(21) 2 427 741

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2001/11/01
(87) Date publication PCT/PCT Publication Date: 2002/08/22
(85) Entrée phase nationale/National Entry: 2003/05/01
(86) N° demande PCT/PCT Application No.: US 2001/045385
(87) N° publication PCT/PCT Publication No.: 2002/064732
(30) Priorités/Priorities: 2000/11/02 (60/245,383) US; 2000/12/21 (60/257,780) US; 2001/02/20 (60/269,854) US

(51) Cl.Int.^{7}/Int.Cl.^{7} C12N 15/12, A61K 31/7088, A61K 48/00, A61K 38/17, C12N 1/21, C12N 5/10, C12Q 1/02, C12N 15/63, C12P 19/34, C07K 16/28, C12P 21/02, C07K 14/705, C12Q 1/68, G01N 33/68, G01N 33/53

(71) Demandeur/Applicant:
BRISTOL MYERS SQUIBB COMPANY, US

(72) Inventeurs/Inventors:
FEDER, JOHN NATHAN, US;
LEE, LIANA M., US;
CHEN, JIAN, US;
JACKSON, DONALD, US;
...

(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : POLYNUCLEOTIDE CODANT POUR UNE NOUVELLE SOUS-UNITE ALPHA DE CANAL POTASSIQUE HUMAIN, K+alphaM1, ET VARIANTS DE CELUI-CI

(54) Title: POLYNUCLEOTIDE ENCODING A NOVEL HUMAN POTASSIUM CHANNEL ALPHA-SUBUNIT, K+alphaM1, AND VARIANTS THEREOF

(57) Abrégé/Abstract:
The present invention provides novel polynucleotides encoding K+alphaM1 polypeptides, fragments and homologues thereof. The invention also provides novel polynucleotides encoding the K+alphaM1 variant polypeptides, K+alphaM1.v1 and K+alphaM1.v2, in addition to fragments and homologues thereof. Also provided are vectors, host cells, antibodies, and recombinant and synthetic methods for producing said polypeptides. The invention further relates to diagnostic and therapeutic methods for applying these novel K+alphaM1, K+alphaM1.v1, and K+alphaM1.v2 polypeptides to the diagnosis, treatment, and/or prevention of various diseases and/or disorders related to these polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of the polynucleotides and polypeptides of the present invention.

CA 2427741 A1 2002/08/22

(21) 2 427 741

(13) A1

(72) **Inventeurs(suite)/Inventors(continued)**: RAMANATHAN, CHANDRA, US; SIEMERS, NATHAN, US; CHANG, HAN, US

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 August 2002 (22.08.2002)

PCT

(10) International Publication Number
WO 02/064732 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US01/45385

(22) International Filing Date:
1 November 2001 (01.11.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/245,383	2 November 2000 (02.11.2000)	US
60/257,780	21 December 2000 (21.12.2000)	US
60/269,854	20 February 2001 (20.02.2001)	US

(71) Applicant *(for all designated States except US)*: **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; P.O. Box 4000, Lawrenceville-Princeton Road, Princeton, NJ 08543-4000 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FEDER, John, Nathan** [US/US]; 277 Dutchtown Zion Rd., Belle Mead, NJ 08502 (US). **LEE, Liana, M.** [US/US]; 8 Petunia Drive, Apt. 1J, North Brunswick, NJ 08902 (US). **CHEN, Jian** [US/US]; 121 York Drive, Princeton, NJ 08540 (US). **JACKSON, Donald** [US/US]; 2641 Main St., Apt. 1, Lawrenceville, NJ 08648 (US). **RAMANATHAN, Chandra** [IN/US]; 41 Alison Avenue, Wallingford, CT 06492 (US). **SIEMERS, Nathan** [US/US]; 171 East Delaware Ave., Pennington, NJ 08534 (US). **CHANG, Han** [US/US]; 2 Ann's Court, Princeton Junction, NJ 08550 (US).

(74) Agents: **D'AMICO, Stephen, C.** et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Lawrenceville-Princeton Road, Princeton, NJ 08543-4000 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/064732 A2

(54) Title: POLYNUCLEOTIDE ENCODING A NOVEL HUMAN POTASSIUM CHANNEL ALPHA-SUBUNIT, K+alphaM1, AND VARIANTS THEREOF

(57) Abstract: The present invention provides novel polynucleotides encoding K+alphaM1 polypeptides, fragments and homologues thereof. The invention also provides novel polynucleotides encoding the K+alphaM1 variant polypeptides, K+alphaM1.v1 and K+alphaM1.v2, in addition to fragments and homologues thereof. Also provided are vectors, host cells, antibodies, and recombinant and synthetic methods for producing said polypeptides. The invention further relates to diagnostic and therapeutic methods for applying these novel K+alphaM1, K+alphaM1.v1, and K+alphaM1.v2 polypeptides to the diagnosis, treatment, and/or prevention of various diseases and/or disorders related to these polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of the polynucleotides and polypeptides of the present invention.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 275

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 275

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

POLYNUCLEOTIDE ENCODING A NOVEL HUMAN POTASSIUM CHANNEL ALPHA-SUBUNIT, K+alphaM1, AND VARIANTS THEREOF

This application claims benefit to provisional application U.S. Serial No. 60/245,383, filed November 2, 2000; to provisional application U.S. Serial No. 60/257,780, filed December 21, 2000; and to provisional application U.S. Serial No. 60/269,854, filed February 20, 2001.

FIELD OF THE INVENTION

The present invention provides novel polynucleotides encoding K+alphaM1 polypeptides, fragments and homologues thereof. The invention also provides novel polynucleotides encoding the K+alphaM1 variant polypeptides, K+alphaM1.v1 and K+alphaM1.v2, in addition to fragments and homologues thereof. Also provided are vectors, host cells, antibodies, and recombinant and synthetic methods for producing said polypeptides. The invention further relates to diagnostic and therapeutic methods for applying these novel K+alphaM1, K+alphaM1.v1, and K+alphaM1.v2 polypeptides to the diagnosis, treatment, and/or prevention of various diseases and/or disorders related to these polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of the polynucleotides and polypeptides of the present invention.

BACKGROUND OF THE INVENTION

Voltage-gated potassium channels are a large and diverse family of proteins critical for the regulation of resting membrane potential in nearly all cell types. The importance of these proteins in the maintenance of cellular homeostasis is highlighted by the fact that defective potassium channels have been implicated in several human diseases, myokymia, long QT syndrome, epilepsy, and Bartter's syndrome (Ackerman and Clapham, 1997). Potassium channels are classified in various functional categories by the number of transmembrane domains (Jan and Jan, 1997). A large class of channels, the outward recitifiers, contain 6 transmembrane domains. Within this family are

6 subfamilies of functional alpha chains, Shaker (Kv1), Shab (Kv2), Shaw (Kv3), Shal (Kv4), KvLQT, and EAG.

In addition, potassium channels can undergo hetero-multimerization with a class of alpha subunits, which by themselves, do not form functional channels (Salians et al., 1997; Shepard and Rae, 1999). These proteins, referred to as electrically silent channels or alpha chains, inhibit functional channels when expressed at high levels and when expressed at lower levels, shift the voltage dependence of inactivation. Within this group are several additional subfamilies, Kv5, Kv6, Kv8 and Kv9.

Heteromultimerization of alpha subunits to potassium channels appears to contribute significantly to the diversity of potassium channel function. Such diversity is also affected by alternative splicing of alpha subunits (Luneau, C.J., et al., P.N.A.S USA, 88:3932-3936 (1991); and Attali, B., et al., J. Biol. Chem.., 268:24283-24289 (1993)), in addition to, the interplay of potassium channel beta subunits with their cognate alpha subunits (Rehm, H., P.N.A.S USA, 85:4919-4923 (1988); Pongs, O., Semin. Neurosci., 7:137-146 (1995); and Fink, M. et al., J. Biol. Chem.., 271:26341-26348 (1996)).

The central role of electrically silent potassium channel alpha subunits in regulating the biological activity of various potassium channels, which, in turn, regulate numerous physiological functions, makes them particularly important targets for specific therapeutic development. Thus, there is a clear need for the identification and characterization of such subunits, in addition to, their association to disease states and/or processes. In particular, there is a need to isolate and characterize additional novel potassium channel alpha subunits.

BRIEF SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the K+alphaM1 protein having the amino acid sequence shown in Figures 1A-C (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA clone K+alphaM1 (also referred to as BAC15, clone

E1, and/or clone Bb1-E3) deposited as ATCC Deposit Number PTA-2766 on December 8, 2000.

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the K+alphaM1.v1 protein having the amino acid sequence shown in Figures 6A-C (SEQ ID NO:34) or the amino acid sequence encoded by the cDNA clone K+alphaM1.v1 (also referred to as BAC15-FL2A) deposited as ATCC Deposit Number PTA-2966 on January 24, 2001.

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the K+alphaM1.v2 protein having the amino acid sequence shown in Figures 7A-C (SEQ ID NO:36) or the amino acid sequence encoded by the cDNA clone K+alphaM1.v2 (also referred to as BAC15-FL2B).

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells, in addition to their use in the production of K+alphaM1, K+aplhaM1.v2, K+alphaM1.v2 polypeptides or peptides using recombinant techniques. Synthetic methods for producing the polypeptides and polynucleotides of the present invention are provided. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the K+alphaM1, K+aplhaM1.v1, or K+alphaM1.v2 polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

The invention further relates to a method of identifying a compound that modulates the biological activity of K+alphaM1, comprising the steps of, (a) combining a candidate modulator compound with K+alphaM1 having the sequence set forth in one or more of SEQ ID NO:2, 34, and/or 36; and measuring an effect of the candidate modulator compound on the activity of K+alphaM1.

The invention further relates to a method of identifying a compound that modulates the biological activity of a potassium channel alpha subunit, comprising the steps of, (a) combining a candidate modulator compound with a host cell expressing K+alphaM1 having the sequence as set forth in SEQ ID NO:2, 34, and/or 36; and , (b)

measuring an effect of the candidate modulator compound on the activity of the expressed K+alphaM1.

The invention further relates to a method of identifying a compound that modulates the biological activity of K+alphaM1, comprising the steps of, (a) combining a candidate modulator compound with a host cell containing a vector described herein, wherein K+alphaM1 is expressed by the cell; and, (b) measuring an effect of the candidate modulator compound on the activity of the expressed K+alphaM1.

The invention further relates to a method of screening for a compound that is capable of modulating the biological activity of K+alphaM1, comprising the steps of: (a) providing a host cell described herein; (b) determining the biological activity of K+alphaM1 in the absence of a modulator compound; (c) contacting the cell with the modulator compound; and (d)determining the biological activity of K+alphaM1 in the presence of the modulator compound; wherein a difference between the activity of K+alphaM1 in the presence of the modulator compound and in the absence of the modulator compound indicates a modulating effect of the compound.

The invention further provides an isolated K+alphaM1 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The invention further provides an isolated K+alphaM1.v1 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The invention further provides an isolated K+alphaM1.v2 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

BRIEF DESCRIPTION OF THE FIGURES/DRAWINGS

Figures 1A-C show the polynucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of the novel potassium channel alpha-subunit, K+alphaM1, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence contains a sequence of 2850 nucleotides (SEQ ID NO:1), encoding a polypeptide of 545 amino acids (SEQ ID NO:2). An analysis of the K+alphaM1 polypeptide determined that it comprised the following features: six transmembrane

domains (TM1 to TM6) located from about amino acid 155 to about amino acid 180 (TM1), from about amino acid 254 to about amino acid 282 (TM2), from about 301 to about amino acid 322 (TM3), from about 333 to about amino acid 356 (TM4), from about 406 to about amino acid 432 (TM5), and/or from about 469 to about amino acid 492 (TM6) of SEQ ID NO:2 represented by double underlining. A comparison of two independent cDNA sequences used in the determination of the consensus sequence (SEQ ID NO:1), revealed 3 single base pair polymorphisms labeled on the sequence above as 'S', in bold letters. Either a 'C' or a 'G' can be found at nucleotide position 841, 1065, 1677 of SEQ ID NO:1. The last two polymorphisms occur in the coding region but are silent with respect to the amino acid code. Additional K+alphaM1 polymorphisms have been identified by comparing the K+alphaM1 polynucleotide to the K+alphaM1.v1 and K+alphaM1.v2 polynucleotides (see Figures 10A-E) located at nucleotide position 894, 1937, and 2197 of SEQ ID NO:1 and are represented in bold. The present invention encompasses the presence of either a "G" or a "T" at nucleotide position 894; the presence of either a "T" or a "C" at nucleotide position 1937; and/or the presence of either an "A" or a "G" at nucleotide position 2197 of SEQ ID NO:1. These polymorphisms are useful as genetic markers for any study that attempts to look for linkage between K+alphaM1 and a disease or disease state related to this polypeptide. Moreover, the K+alphaM1 polypeptide contains six amino acid residue alternations that are characteristic of the class of potassium channel alpha subunits that do not conduct potassium ions. These six amino acid residues are represented by shadowing.

Figure 2 shows the regions of identity and similarity between K+alphaM1 and other electrically silent alpha subunits, specifically, the Shab-related (Genbank Accession No. gi|2815899; SEQ ID NO:3), Kv9.3 (Genbank Accession No. gi|7514119; SEQ ID NO:4), and Kv8.1 (Genbank Accession No. gi|6604550; SEQ ID NO:5) proteins. The six residues found to be altered in electrically silent alpha subunits in the S6 domain are denoted in bold and in larger font. The alignment was perfomed using the CLUSTALW algorithm described elsewhere herein. Lines between residues indicate gapped regions of non-identity for the aligned polypeptides, asterisks below the aligned polypeptides indicate identical amino acids, double dots indicate conservative

amino acid differences, and single dots indicate non-conservative amino acid differences.

Figure 3 shows a hydrophobicity plot of K+alphaM1 (top panel) compared to that of the electrically silent Shab-related channel (bottom panel) according to the BioPlot Hydrophobicity algorithm of Vector NTI (version 5.5).

Figure 4 shows an expression profile of the novel human potassium channel modulatory alpha subunit, K+alphaM1. As shown, transcripts corresponding to K+alphaM1 expressed highly in the lung, pancreas, prostate and small intestine. Expression data was obtained by measuring the steady state K+alphaM1 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NO:7 and 8 as described herein.

Figure 5 shows a table illustrating the percent identity and percent similarity between the K+alphaM1, K+alphaM1v1, and K+alphaM1v2 polypeptides of the present invention with the Shab-related (SEQ ID NO:3), Kv9.3 (SEQ ID NO:4), and Kv8.1 (SEQ ID NO:5) proteins. The percent identity and percent similarity values were determined using the GAP algorithm (Genetics Computer Group suite of programs; and Henikoff, S. and Henikoff, J. G., Proc. Natl. Acad. Sci. USA 89: 10915-10919(1992)) using default parameters (Scoring Matrix: Blosum62; Gap Creation Penalty: 8; and Gap Extension Penalty:2; No penalty for gaps at end of aligment).

Figures 6A-C show the polynucleotide sequence (SEQ ID NO: 33) and deduced amino acid sequence (SEQ ID NO:34) of the novel potassium channel alpha-subunit, K+alphaM1.v1, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence contains a sequence of 1871 nucleotides (SEQ ID NO:33), encoding a polypeptide of 545 amino acids (SEQ ID NO:34). An analysis of the K+alphaM1.v1 polypeptide determined that it comprised the following features: six transmembrane domains (TM1 to TM6) located from about amino acid 156 to about amino acid 178 (TM1), from about amino acid 261 to about amino acid 282 (TM2), from about 333 to

about amino acid 355 (TM3), from about 411 to about amino acid 429 (TM4), from about 441 to about amino acid 461 (TM5), and/or from about 472 to about amino acid 492 (TM6) of SEQ ID NO:34 represented by double underlining; and six amino acid residue alternations that are characteristic of the class of potassium channel alpha subunits that do not conduct potassium ions. These six amino acid residues are represented by shadowing.

Figures 7A-C show the polynucleotide sequence (SEQ ID NO: 35) and deduced amino acid sequence (SEQ ID NO:36) of the novel potassium channel alpha-subunit, K+alphaM1.v2, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence contains a sequence of 1871 nucleotides (SEQ ID NO:35), encoding a polypeptide of 545 amino acids (SEQ ID NO:36). An analysis of the K+alphaM1.v1 polypeptide determined that it comprised the following features: six transmembrane domains (TM1 to TM6) located from about amino acid 156 to about amino acid 178 (TM1), from about amino acid 261 to about amino acid 279 (TM2), from about 333 to about amino acid 352 (TM3), from about 410 to about amino acid 430 (TM4), from about 443 to about amino acid 461 (TM5), and/or from about 472 to about amino acid 491 (TM6) of SEQ ID NO:36 represented by double underlining; and six amino acid residue alternations that are characteristic of the class of potassium channel alpha subunits that do not conduct potassium ions. These six amino acid residues are represented by shadowing.

Figure 8 shows the regions of identity and similarity between K+alphaM1 (SEQ ID NO:2) and the variants K+alphaM1.v1 (SEQ ID NO:34) and K+alphaM1.v2 (SEQ ID NO:36) of the present invention. The six residues found to be altered in electrically silent alpha subunits in the S6 domain are conserved amonst the variants as shown. The alignment was perfomed using the CLUSTALW algorithm using default parameters as described elsewhere herein (CLUSTALW parameters: gap opening penalty: 10; gap extension penalty: 0.5; gap separation penalty range: 8; percent identity for alignment delay: 40%; and transition weighting: 0). The darkly shaded amino acids represent regions of matching identity. The lightly shaded amino acids

represent regions of matching similarity. Lines between residues indicate gapped regions for the aligned polypeptides.

Figures 9A-B show the regions of identity and similarity between K+alphaM1 (SEQ ID NO:2), the variants K+alphaM1.v1 (SEQ ID NO:34) and K+alphaM1.v2 (SEQ ID NO:36), and the other electrically silent alpha subunits, specifically, the Shab-related (SEQ ID NO:3), Kv9.3 (SEQ ID NO:4), and Kv8.1 (SEQ ID NO:5) proteins. The six residues found to be altered in electrically silent alpha subunits in the S6 domain are conserved amonst the variants as shown. The alignment was perfomed using the CLUSTALW algorithm using default parameters as described elsewhere herein (CLUSTALW parameters: gap opening penalty: 10; gap extension penalty: 0.5; gap separation penalty range: 8; percent identity for alignment delay: 40%; and transition weighting: 0). The darkly shaded amino acids represent regions of matching identity. The lightly shaded amino acids represent regions of matching similarity. Lines between residues indicate gapped regions for the aligned polypeptides.

Figures 10A-E show the regions of identity and similarity between the K+alphaM1 polynucleotide (SEQ ID NO:1), and the variants K+alphaM1.v1 (SEQ ID NO:33) and K+alphaM1.v2 (SEQ ID NO:35). The alignment was perfomed using the CLUSTALW algorithm using default parameters as described elsewhere herein (CLUSTALW parameters: gap opening penalty: 10; gap extension penalty: 0.5; gap separation penalty range: 8; percent identity for alignment delay: 40%; and transition weighting: 0). The darkly shaded nucleic acid residues represent regions of matching identity. The lightly shaded nucleic acid residues represent regions of matching similarity. Lines between residues indicate gapped regions for the aligned polynucleotides.

Figures 11A-C show the polynucleotide sequence (SEQ ID NO:) and deduced amino acid sequence (SEQ ID NO:290) of the human K+alphaM1 potassium channel alpha subunit protein comprising, or alternatively consisting of, one or more of the predicted polynucleotide polymorphic loci, in addition to, the encoded polypeptide polymorphic loci of the present invention for this particular protein, which include but are not

limited to the following polynucleotide polymorphisms: K+alphaM1-C841G, K+alphaM1-C1065G, K+alphaM1-C1677G, K+alphaM1-G894T, K+alphaM1-T1937C, and/or K+alphaM1-A2197G of SEQ ID NO:1; and polypeptide polymorphisms - K+alphaM1-L352P, and/or K+alphaM1-T439A of SEQ ID NO:2. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence contains a sequence of 2850 nucleotides (SEQ ID NO:115), encoding a polypeptide of 545 amino acids (SEQ ID NO:116). The polynucleotide polymorphic sites are represented by an "N", in bold. The polypeptide polymorphic sites are represented by an "X", in bold. The present invention encompasses the polynucleotide at nucleotide position 841 as being either a "C" or a "G", the polynucleotide at nucleotide position 1065 as being either a "C" or a "G", the polynucleotide at nucleotide position 1677 as being either a "C" or a "G", the polynucleotide at nucleotide position 894 as being either a "G" or a "T", the polynucleotide at nucleotide position 1937 as being either a "T" or a "C", and the polynucleotide at nucleotide position 2197 as being either an "A" or a "G" of Figures 11A-C (SEQ ID NO:115), in addition to any combination thereof. The present invention also encompasses the polypeptide at amino acid position 352 as being either a "Leu" or a "Pro", and the polypeptide at amino acid position 439 as being either a "Thr" or an "Ala" of Figures 11A-C (SEQ ID NO:116).

Figures 12A-C show the polynucleotide sequence (SEQ ID NO:117) and deduced amino acid sequence (SEQ ID NO:118) of the human K+alphaM1.v1 potassium channel alpha subunit variant protein comprising, or alternatively consisting of, one or more of the predicted polynucleotide polymorphic loci, in addition to, the encoded polypeptide polymorphic loci of the present invention for this particular protein, which include but are not limited to the following polynucleotide polymorphisms: K+alphaM1.v1-C37G, K+alphaM1.v1-C261G, K+alphaM1.v1-C873G, K+alphaM1.v1-G90T, K+alphaM1.v1-T1133C, and/or K+alphaM1.v1-A1393G of SEQ ID NO:33; and polypeptide polymorphisms - K+alphaM1.v1-P352L, and/or K+alphaM1.v1-T439A of SEQ ID NO:34. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence contains a sequence of 1871 nucleotides (SEQ ID NO:117), encoding a

polypeptide of 545 amino acids (SEQ ID NO:118). The polynucleotide polymorphic sites are represented by an "N", in bold. The polypeptide polymorphic sites are represented by an "X", in bold. The present invention encompasses the polynucleotide at nucleotide position 37 as being either a "C" or a "G", the polynucleotide at nucleotide position 261 as being either a "C" or a "G", the polynucleotide at nucleotide position 873 as being either a "C" or a "G", the polynucleotide at nucleotide position 90 as being either a "G" or a "T", the polynucleotide at nucleotide position 1133 as being either a "T" or a "C", and the polynucleotide at nucleotide position 1393 as being either an "A" or a "G" of Figures 12A-C (SEQ ID NO:117), in addition to any combination thereof. The present invention also encompasses the polypeptide at amino acid position 352 as being either a "Leu" or a "Pro", and the polypeptide at amino acid position 439 as being either a "Thr" or an "Ala" of Figures 12A-C (SEQ ID NO:118).

Figures 13A-C show the polynucleotide sequence (SEQ ID NO:119) and deduced amino acid sequence (SEQ ID NO:120) of the human K+alphaM1.v2 potassium channel alpha subunit variant protein comprising, or alternatively consisting of, one or more of the predicted polynucleotide polymorphic loci, in addition to, the encoded polypeptide polymorphic loci of the present invention for this particular protein, which include but are not limited to the following polynucleotide polymorphisms: K+alphaM1.v2-C37G, K+alphaM1.v2-C261G, K+alphaM1.v2-C873G, K+alphaM1.v2-G90T, K+alphaM1.v2-T1133C, and/or K+alphaM1.v2-A1393G of SEQ ID NO:35; and polypeptide polymorphisms - K+alphaM1.v2-P352L, and/or K+alphaM1.v2-T439A of SEQ ID NO:36. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence contains a sequence of 1871 nucleotides (SEQ ID NO:119), encoding a polypeptide of 545 amino acids (SEQ ID NO:120). The polynucleotide polymorphic sites are represented by an "N", in bold. The polypeptide polymorphic sites are represented by an "X", in bold. The present invention encompasses the polynucleotide at nucleotide position 37 as being either a "C" or a "G", the polynucleotide at nucleotide position 261 as being either a "C" or a "G", the polynucleotide at nucleotide position 873 as being either a "C" or a "G", the polynucleotide at

nucleotide position 90 as being either a "G" or a "T", the polynucleotide at nucleotide position 1133 as being either a "T" or a "C", and the polynucleotide at nucleotide position 1393 as being either an "A" or a "G" of Figures 12A-C (SEQ ID NO:119), in addition to any combination thereof. The present invention also encompasses the polypeptide at amino acid position 352 as being either a "Leu" or a "Pro", and the polypeptide at amino acid position 439 as being either a "Thr" or an "Ala" of Figures 12A-C (SEQ ID NO:120).

DETAILED DESCRIPTION OF THE INVENTION

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the Examples included herein. All references to "K+alphaM1" shall be construed to apply to K+alphaM1, K+alphaM1.v1, and/or K+alphaM1.v2 unless otherwise specified herein.

The invention provides a novel human sequence that encodes a potassium channel alpha subunit with substantial homology to the class of electrically silent potassium channels. The protein encoded by the novel sequence possesses 6 transmembrane domains with a truncated cytoplasmic tail. Alignment of the novel protein with those in the public domain shows that the novel protein contains a collection of 6 amino acid alterations in a specific portion of the protein that are characteristic of the class of alpha chains that do not conduct potassium ions and are referred to as electrically silent alpha modulatory subunits (Salians et al., 1997; Shepard and Rae, 1999). Based on this we have provisionally named the gene and protein K+alphaM1. Transcripts for K+alphaM1 are found in the testis and the brain, suggesting that the invention modulates potassium channel functions in these tissues. All information relevant to K+alphaM1 is also applicable to K+alphaM1.v1 and K+alphaM1.v2 unless stated otherwise herein.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be

contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1, SEQ ID NO:33, SEQ ID NO:35, or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without a signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:1, SEQ ID NO:33, and/or SEQ ID NO:35, was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:1 was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is

identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure. The deposited clone is inserted in the pSport1 plasmid (Life Technologies) using the NotI and SalI restriction endonuclease cleavage sites.

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequnencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined above. Therefore, as is known in the art for any DNA seuqnece detemrined by this automated approach, any nucleotide seqence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide seqnece of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a detemrined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded bt the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucletide sequence in Figures 1A-C (SEQ ID NO:1), a nucleic acid molecule of the present invention encoding the K+alphaM1 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figures 1A-C (SEQ ID NO:1) was discovered in a cDNA library derived from human brain.

The determined nucleotide sequence of the K+alphaM1 cDNA in Figures 1A-C (SEQ ID NO:1) contains an open reading frame encoding a protein of about 545 amino acid residues, with a deduced molecular weight of about 62.5kDa. The amino

acid sequence of the predicted K+alphaM1 polypeptide is shown in Figures 1A-C (SEQ ID NO:2). The K+alphaM1 protein shown in Figures 1A-C is about 41% identical and about 61% similar to the human Shab-related delayed-rectifier K+ channel alpha subunit (Figure 5).

The determined nucleotide sequence of the K+alphaM1.v1 cDNA in Figures 6A-C (SEQ ID NO:33) contains an open reading frame encoding a protein of about 545 amino acid residues, with a deduced molecular weight of about 62.24kDa. The amino acid sequence of the predicted K+alphaM1.v1 polypeptide is shown in Figures 6A-C (SEQ ID NO:34).

The determined nucleotide sequence of the K+alphaM1.v2 cDNA in Figures 7A-C (SEQ ID NO:35) contains an open reading frame encoding a protein of about 545 amino acid residues, with a deduced molecular weight of about 62.43kDa. The amino acid sequence of the predicted K+alphaM1.v2 polypeptide is shown in Figures 7A-C (SEQ ID NO:36).

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:1, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH2PO4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed

following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids.

The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:1", "SEQ ID NO:33", and "SEQ ID NO:35" refers to a polynucleotide sequence while "SEQ ID NO:2", "SEQ ID NO:34", and "SEQ ID NO:36" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the

present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

The term "organism" as referred to herein is meant to encompass any organism referenced herein, though preferably to eukaryotic organsisms, more preferably to mammals, and most preferably to humans.

The present invention encompasses the identification of proteins, nucleic acids, or other molecules, that bind to polypeptides and polynucleotides of the present invention (for example, in a receptor-ligand interaction). The polynucleotides of the present invention can also be used in interaction trap assays (such as, for example, that discribed by Ozenberger and Young (Mol Endocrinol., 9(10):1321-9, (1995); and Ann. N. Y. Acad. Sci., 7;766:279-81, (1995)).

The polynucleotide and polypeptides of the present invention are useful as probes for the identification and isolation of full-length cDNAs and/or genomic DNA which correspond to the polynucleotides of the present invention, as probes to hybridize and discover novel, related DNA sequences, as probes for positional cloning of this or a related sequence, as probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides, as probes to quantify gene expression, and as probes for microarays.

In addition, polynucleotides and polypeptides of the present invention may comprise one, two, three, four, five, six, seven, eight, or more membrane domains.

Also, in preferred embodiments the present invention provides methods for further refining the biological fuction of the polynucleotides and/or polypeptides of the present invention.

Specifically, the invention provides methods for using the polynucleotides and polypeptides of the invention to identify orthologs, homologs, paralogs, variants, and/or allelic variants of the invention. Also provided are methods of using the

polynucleotides and polypeptides of the invention to identify the entire coding region of the invention, non-coding regions of the invention, regulatory sequences of the invention, and secreted, mature, pro-, prepro-, forms of the invention (as applicable).

In preferred embodiments, the invention provides methods for identifying the glycosylation sites inherent in the polynucleotides and polypeptides of the invention, and the subsequent alteration, deletion, and/or addition of said sites for a number of desirable characteristics which include, but are not limited to, augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

In further preferred embodiments, methods are provided for evolving the polynucleotides and polypeptides of the present invention using molecular evolution techniques in an effort to create and identify novel variants with desired structural, functional, and/or physical characteristics.

The present invention further provides for other experimental methods and procedures currently available to derive functional assignments. These procedures include but are not limited to spotting of clones on arrays, micro-array technology, PCR based methods (e.g., quantitative PCR), anti-sense methodology, gene knockout experiments, and other procedures that could use sequence information from clones to build a primer or a hybrid partner.

Polynucleotides and Polypeptides of the Invention

Features of the Polypeptide Encoded by Gene No:1

The polypeptide of this gene provided as SEQ ID NO:2 (Figures 1A-C), encoded by the polynucleotide sequence according to SEQ ID NO:1 (Figures 1A-C), and/or encoded by the polynucleotide contained within the deposited clone, K+alphaM1, has significant homology at the nucleotide and amino acid level to the human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gil2815899; SEQ ID NO:3), the rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gil7514119; SEQ ID NO:4), and the human Kv8.1 neuronal potassium channel alpha subunit (Kv8.1; Genbank

Accession No: gil6604550; SEQ ID NO:5). An alignment of the K+alphaM1 polypeptide with these proteins is provided in Figure 2.

The K+alphaM1 polypeptide was determined to have 41% identity and 52% similarity with the human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gil2815899; SEQ ID NO:3); 40.59% identity and 51.7% similarity to the rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gil7514119; SEQ ID NO:4); and 41.35% identity and 51.2% similarity to the human Kv8.1 neuronal potassium channel alpha subunit (Kv8.1; Genbank Accession No: gil6604550; SEQ ID NO:5).

The human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gil2815899; SEQ ID NO:3) has been shown to slow deactivation and inactivation kinetics of hKv2.1 when coexpressed with hKv2.l, compared with hKv2. 1 expressed alone (Am. J. Physiol. 277 (3), C412-C424 (1999)). This channel is also referred to as the human ortholog of the rat Kv9.3 protein.

The rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gil7514119; SEQ ID NO:4) has been described by Patel, A. J., et al., EMBO, 16 (22): 6615 (1997), and in Biochem. Biophys. Res. Commun. 248 (3), 927-934 (1998). The rKv9.3 Shab-like subunit in rat PA myocytes is an electrically silent subunit which associates with Kv2.1, for example, and modulates its biophysical properties. The rKv9.3 heteromultimer, unlike Kv2.1 alone, opens in the voltage range of the resting membrane potential of PA myocytes. Patel, et al., demonstrate that the activity of rKv2.1/rKv9.3 is tightly controlled by internal ATP and is reversibly inhibited by hypoxia. Metabolic regulation of the Kv2.1/rKv9.3 heteromultimer appears to play an important role in hypoxic pulmonary arterial vasoconstriction and in the possible development of pulmonary arterial hypertension. EMBO, 16 (22): 6615 (1997). As described elsewhere herein, potassium channel alpha subunits do not express potassium channel current by themselves, but induce profound changes in the properties of the Shab channels Kv2.1 and Kv2.2, among others. Most interestingly, these silent subunits have the ability to create a diverse range of effects, since Kv8.1 acts as a dominant inhibitory subunit while rKv9.3 behaves as a stimulatory one. Examination of the single-channel properties of Kv2.1 and Kv2.1/rKv9.3 clearly revealed that rKv9.3 alters the single-channel conductance

of Kv2.1. The ability of rKv9.3 to'drag'the Kv2.1 activation voltage threshold into the range of PA myocytes RMP suggests that the channel complex contributes to the setting of the RMP (-54 4 mV) and, consequently, in the setting of the resting pulmonary arterial pressure. Rat Kv9.3 also speeded up Kv2.1 activation, for instance, and dramatically slowed down deactivation.

The K+alphaM1 polypeptide was determined to have a conserved domain comprising six amino acid residues. These residues are highlighted in the alignment in Figure 2.

In preferred embodiments, the following K+alphaM1 polypeptides are encompassed by the present invention: DMYPETHLGRFFAFLCIAFGIILNGMPISILYNKFSDYYS (SEQ ID NO:11). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1 polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

Expression profiling designed to measure the steady state mRNA levels encoding the K+alphaM1 polypeptide showed predominately high expression levels in testicular tissue, and to a lesser extent, in brain tissue (See Figure 4).

Based upon the observed homology, the polypeptide of the present invention may share at least some biological activity with potassium channel subunits, specifically with potassium channel alpha subunits.

As described elsewhere herein, potassium channel alpha subunits have been implicated in inhibiting the activity of potassium channels. Such inhibition typically is manifested by potassium channels forming heteromultimer complexes with a potassium channel alpha subunit. As a result of the inhibition potential of alpha subunits, they are often referred to as a potassium channel antagonists.

Potassium channel antagonists are useful for a number of physiological disorders in mammals, including humans. Ion channels, including potassium channels, are found in all mammalian cells and are involved in the modulation of various physiological processes and normal cellular homeostasis. Potassium channels generally control the resting membrane potential, and the efflux of potassium ions causes repolarization of the plasma membrane after cell depolarization. Potassium

channel antagonists prevent repolarization and cause the cell to stay in the depolarized, excited state.

There are a number of potassium channel subtypes. Physiologically, one important subtype is the maxi-K channel, defined as high -conductance calcium-activated potassium channel, which is present in neuronal tissue and smooth muscle. Intracellular calcium concentration (Ca.sup.2+.sub.i) and membrane potential gate these channels. For example, maxi-K channels are opened to enable efflux of potassium ions by an increase in the intracellular Ca.sub.2+ concentration or by membrane depolarization (change in potential). Elevation of intracellular calcium concentration is required for neurotransmitter release, smooth muscle contraction, proliferation of some cell types and other processes. Modulation of maxi-K channel activity therefore affects cellular processes that depend on influx of calcium through voltage-dependent pathways, such as transmitter release from the nerve terminals and smooth muscle contraction.

A number of marketed drugs function as potassium channel antagonists. The most important of these include the compounds Glyburide, Glipizide and Tolbutamide. These potassium channel antagonists are useful as antidiabetic agents. Potassium channel antagonists are also utilized as Class III antiarrhythmic agents and to treat acute infractions in humans. A number of naturally occurring toxins are known to block potassium channels including apamin, iberiotoxin, charybdotoxin, margatoxin, noxiustoxin, kaliotoxin, dendrotoxin(s), mast cell degranuating (MCD) peptide, and beta.-bungarotoxin (.beta.-BTX).

Depression is related to a decrease in neurotransmitter release. Current treatments of depression include blockers of neurotransmitter uptake, and inhibitors of enzymes involved in neurotransmitter degradation which act to prolong the lifetime of neurotransmitters.

It is believed that certain diseases such as depression, memory disorders and Alzheimer's disease are the result of an impairment in neurotransmitter release.

Potassium channel antagonists may therefore be utilized as cell excitants which may stimulate release of neurotransmitters such as acetylcholine, serotonin and dopamine. Enhanced neurotransmitter release may reverse the symptoms associated with depression and Alzheimer's disease.

The K+alphaM1 polynucleotides and polypeptides of the present invention, including agonists and/or fragments thereof, have uses that include modulating potassium channel activity in various cells, tissues, and organisms, and particularly in mammalian testicular and brain tissue, preferably human. K+alphaM1 polynucleotides and polypeptides of the present invention, including agonists and/or fragments thereof, may be useful in diagnosing, treating, prognosing, and/or preventing neural, reproductive (particularly male reproductive), metabolic, and/or proliferative diseases or disorders.

The strong homology to potassium channel alpha subunits, combined with the predominate localized expression in testis tissue further emphasizes the potential utility for K+alphaM1 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing testicular, in addition to reproductive disorders.

In preferred embodiments, K+alphaM1 polynucleotides and polypeptides including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing the following, non-limiting, diseases or disorders of the testis: spermatogenesis, infertility, Klinefelter's syndrome, XX male, germinal cell aplasia, cryptorchidism, varicocele, immotile cilia syndrome, and viral orchitis. The K+alphaM1 polynucleotides and polypeptides including agonists and fragments thereof, may also have uses related to modulating testicular development, embryogenesis, reproduction, and in ameliorating, treating, and/or preventing testicular proliferative disorders (e.g., cancers, which include, for example, choriocarcinoma, Nonseminoma, seminona, and testicular germ cell tumors).

Likewise, the predominate localized expression in testis tissue also emphasizes the potential utility for K+alphaM1 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing metabolic diseases and disorders which include the following, not limiting examples: premature puberty, incomplete puberty, Kallman syndrome, Cushing's syndrome, hyperprolactinemia, hemochromatosis, congenital adrenal hyperplasia, FSH deficiency, and granulomatous disease, for example.

In addition, the strong homology to potassium channel alpha subunits, combined with the localized expression in brain tissue further emphasizes the

potential utility for K+alphaM1 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing neuronal disorders.

In preferred embodiments, K+alphaM1 polynucleotides and polypeptides, including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing certain neuronal disorders. Epileptic seizures can be induced by agents (e.g., pentylenetetrazol) which block potassium channels, most likely due to the loss of regulation of cellular membrane potentials. A potential role for potassium channels in Alzheimer's disease has been suggested by studies demonstrating that a significant component of senile plaques, beta amyloid or A beta, also blocks voltage-gated potassium channels in hippocampal neurons. (Antes, L. M. et al. (1998) Seminar Nephrol 18:31-45; Stoffel, M. and Jan, L. Y. (1998) Nat. Genet. 18:6-8; Madeja, M. et al. (1997) Eur. J. Neurosci. 9:390-395; and Good, T. A. et al. (1996) Biophys. J. 70:296-304.).

In addition, antagonists of the K+alphaM1 polynucleotides and polypeptides may have uses that include diagnosing, treating, prognosing, and/or preventing diseases or disorders related to hyper potassium channel alpha subunit activity, which may include neural, reproductive (particularly male reproductive), metabolic, and/or proliferative diseases or disorders.

Alternatively, K+alphaM1 polypeptides of the invention, or agonists thereof, are administered to treat, prevent, prognose, and/or diagnose disorders involving excessive smooth muscle tone or excitability, which include, but are not limited to asthma, angina, hypertension, incontinence, pre-term labor, migraine, cerebral ischemia, and irratible bowel syndrome.

Moreover, K+alphaM1 polynucleotides and polypeptides, including fragments and agonists thereof, may have uses which include treating, diagnosing, prognosing, and/or preventing some classes of disorders that may be affected by effective manipulation of Shaker-like potassium ion channels, which include neurological disorders, tumor driven diseases, metabolic diseases, cardiac diseases, and autoimmune diseases. Examples of disease states and conditions from these and other classes, as well as affected normal body functions, encompass: hypoglycemia, anoxia/hypoxia, renal disease, osteoporosis, hyperkalemia, hypokalemia, hypertension, Addison's disease, abnormal apoptosis, induced apoptosis, clotting,

modulation of acetylcholine function, and modulation of monoaminesepilepsy, allergic encephalomyelitis, multiple sclerosis (any demylelinating disease), acute traverse myelitis, neurofibromatosis, cardioplegia, cardiomyopathy, ischemia, ischemia reperfusion, cerebral ischemia, sickle cell anemia, cardiac arrythmias, peripheral monocuropathy, polynucuropathy, Gullain-Barre' Syndrome, peroneal muscular dystrophy, neuropathies, Parkinson's disease, palsies, cerebral palsy, progressive supranuclear palsy, pseudobubar palsy, Huntington's disease, dystonia, dyskinesias, chorea, althetosis, choreothetosis, tics, memory degeneration, taste perception, smooth muscle function, skeletal muscle function, sleep disorders, modulation of neurotransmitters, acute disseminated encephalomyelitis, optic neuromyelitis, muscular dystrophy, myasthenia gravis, multiple sclerosis, and cerebral vasospasm, hypertension, angina pectoris, asthma, congestive heart failure, ischemia related disorders, cardiac dysrhythmias, diabetes, carcinomas, neurocarcinomas, autoimmune-hypertrophy, neuromyotonia (Isaac's Syndrome) muscular disorders associated with drug abuse, and treatment for poisoning.

K+alphaM1 polypeptides and polynucleotides have additional uses which include diagnosing diseases related to the over and/or under expression of K+alphaM1 by identifying mutations in the K+alphaM1 gene by using K+alphaM1 sequences as probes or by determining K+alphaM1 protein or mRNA expression levels. K+alphaM1 polypeptides may be useful for screening compounds that affect the activity of the protein. K+alphaM1 peptides can also be used for the generation of specific antibodies and as bait in yeast two hybrid screens to find proteins the specifically interact with K+alphaM1 (described elsewhere herein). Based on the expression pattern of this novel sequence, diseases that can be treated with agonists and/or antagonists for K+alphaM1 include various forms of generalized epilepsy.

Although it is believed the encoded polypeptide may share at least some biological activities with potassium channel alpha subunits, a number of methods of determining the exact biological function of this clone are either known in the art or are described elsewhere herein. Briefly, the function of this clone may be determined by applying microarray methodology. Nucleic acids corresponding to the K+alphaM1 polynucleotides, in addition to, other clones of the present invention, may be arrayed

on microchips for expression profiling. Depending on which polynucleotide probe is used to hybridize to the slides, a change in expression of a specific gene may provide additional insight into the function of this gene based upon the conditions being studied. For example, an observed increase or decrease in expression levels when the polynucleotide probe used comes from tissue that has been treated with known potassium channel inhibitors, which include, but are not limited to the drugs listed above, might indicate a function in modulating potassium channel function, for example. In the case of K+alphaM1, testicular and/or brain tissue should be used to extract RNA to prepare the probe.

In addition, the function of the protein may be assessed by applying quantitative PCR methodology, for example. Real time quantitative PCR would provide the capability of following the expression of the K+alphaM1 gene throughout development, for example. Quantitative PCR methodology requires only a nominal amount of tissue from each developmentally important step is needed to perform such experiements. Therefore, the application of quantitative PCR methodology to refining the biological function of this polypeptide is encompassed by the present invention. Also encompassed by the present invention are quantitative PCR probes corresponding to the polynucleotide sequence provided as SEQ ID NO:1 (Figures 1A-C).

The function of the protein may also be assessed through complementation assays in yeast. For example, in the case of the K+alphaM1, transforming yeast deficient in potassium channel alpha subunit activity and assessing their ability to grow would provide convincing evidence the K+alphaM1 polypeptide has potassium channel alpha subunit activity activity. Additional assay conditions and methods that may be used in assessing the function of the polynucletides and polypeptides of the present invention are known in the art, some of which are disclosed elsewhere herein.

Alternatively, the biological function of the encoded polypeptide may be determined by disrupting a homologue of this polypeptide in Mice and/or rats and observing the resulting phenotype.

Moreover, the biological function of this polypeptide may be determined by the application of antisense and/or sense methodology and the resulting generation of transgenic mice and/or rats. Expressing a particular gene in either sense or antisense

orientation in a transgenic mouse or rat could lead to respectively higher or lower expression levels of that particular gene. Altering the endogenous expression levels of a gene can lead to the obervation of a particular phenotype that can then be used to derive indications on the function of the gene. The gene can be either over-expressed or under expressed in every cell of the organism at all times using a strong ubiquitous promoter, or it could be expressed in one or more discrete parts of the organism using a well characterized tissue-specific promoter (e.g., a testis specific promoter or a brain specific promoter), or it can be expressed at a specified time of development using an inducible and/or a developmentally regulated promoter.

In the case of K+alphaM1 transgenic mice or rats, if no phenotype is apparent in normal growth conditions, observing the organism under diseased conditions (neural or testicular disorders, depression, testicular or brain cancer, etc.) may lead to understanding the function of the gene. Therefore, the application of antisense and/or sense methodology to the creation of transgenic mice or rats to refine the biological function of the polypeptide is encompassed by the present invention.

In preferred embodiments, the following N-terminal deletion mutants are encompassed by the present invention: M1-N545, L2-N545, K3-N545, Q4-N545, S5-N545, E6-N545, R7-N545, R8-N545, R9-N545, S10-N545, W11-N545, S12-N545, Y13-N545, R14-N545, P15-N545, W16-N545, N17-N545, T18-N545, T19-N545, E20-N545, N21-N545, E22-N545, G23-N545, S24-N545, Q25-N545, H26-N545, R27-N545, R28-N545, S29-N545, I30-N545, C31-N545, S32-N545, L33-N545, G34-N545, A35-N545, R36-N545, S37-N545, G38-N545, S39-N545, Q40-N545, A41-N545, S42-N545, I43-N545, H44-N545, G45-N545, W46-N545, T47-N545, E48-N545, G49-N545, N50-N545, Y51-N545, N52-N545, Y53-N545, Y54-N545, I55-N545, E56-N545, E57-N545, D58-N545, E59-N545, D60-N545, G61-N545, E62-N545, E63-N545, E64-N545, D65-N545, Q66-N545, W67-N545, K68-N545, D69-N545, D70-N545, L71-N545, A72-N545, E73-N545, E74-N545, D75-N545, Q76-N545, Q77-N545, A78-N545, G79-N545, E80-N545, V81-N545, T82-N545, T83-N545, A84-N545, K85-N545, P86-N545, E87-N545, G88-N545, P89-N545, S90-N545, D91-N545, P92-N545, P93-N545, A94-N545, L95-N545, L96-N545, S97-N545, T98-N545, L99-N545, N100-N545, V101-N545, N102-N545, V103-N545, G104-N545, G105-N545, H106-N545, S107-N545, Y108-N545, Q109-N545, L110-

N545, D111-N545, Y112-N545, C113-N545, E114-N545, L115-N545, A116-N545, G117-N545, F118-N545, P119-N545, K120-N545, T121-N545, R122-N545, L123-N545, G124-N545, R125-N545, L126-N545, A127-N545, T128-N545, S129-N545, T130-N545, S131-N545, R132-N545, S133-N545, R134-N545, Q135-N545, L136-N545, S137-N545, L138-N545, C139-N545, D140-N545, D141-N545, Y142-N545, E143-N545, E144-N545, Q145-N545, T146-N545, D147-N545, E148-N545, Y149-N545, F150-N545, F151-N545, D152-N545, R153-N545, D154-N545, P155-N545, A156-N545, V157-N545, F158-N545, Q159-N545, L160-N545, V161-N545, Y162-N545, N163-N545, F164-N545, Y165-N545, L166-N545, S167-N545, G168-N545, V169-N545, L170-N545, L171-N545, V172-N545, L173-N545, D174-N545, G175-N545, L176-N545, C177-N545, P178-N545, R179-N545, R180-N545, F181-N545, L182-N545, E183-N545, E184-N545, L185-N545, G186-N545, Y187-N545, W188-N545, G189-N545, V190-N545, R191-N545, L192-N545, K193-N545, Y194-N545, T195-N545, P196-N545, R197-N545, C198-N545, C199-N545, R200-N545, I201-N545, C202-N545, F203-N545, E204-N545, E205-N545, R206-N545, R207-N545, D208-N545, E209-N545, L210-N545, S211-N545, E212-N545, R213-N545, L214-N545, K215-N545, I216-N545, Q217-N545, H218-N545, E219-N545, L220-N545, R221-N545, A222-N545, Q223-N545, A224-N545, Q225-N545, V226-N545, E227-N545, E228-N545, A229-N545, E230-N545, E231-N545, L232-N545, F233-N545, R234-N545, D235-N545, M236-N545, R237-N545, F238-N545, Y239-N545, G240-N545, P241-N545, Q242-N545, R243-N545, R244-N545, R245-N545, L246-N545, W247-N545, N248-N545, L249-N545, M250-N545, E251-N545, K252-N545, P253-N545, F254-N545, S255-N545, S256-N545, V257-N545, A258-N545, A259-N545, K260-N545, A261-N545, I262-N545, G263-N545, V264-N545, A265-N545, S266-N545, S267-N545, T268-N545, F269-N545, V270-N545, L271-N545, V272-N545, S273-N545, V274-N545, V275-N545, A276-N545, L277-N545, A278-N545, L279-N545, N280-N545, T281-N545, V282-N545, E283-N545, E284-N545, M285-N545, Q286-N545, Q287-N545, H288-N545, S289-N545, G290-N545, Q291-N545, G292-N545, E293-N545, G294-N545, G295-N545, P296-N545, D297-N545, L298-N545, R299-N545, P300-N545, I301-N545, L302-N545, E303-N545, H304-N545, V305-N545, E306-N545, M307-N545, L308-N545, C309-N545, M310-N545, G311-N545, F312-N545, F313-N545, T314-N545, L315-N545, E316-N545, Y317-N545, L318-

N545, L319-N545, R320-N545, L321-N545, A322-N545, S323-N545, T324-N545, P325-N545, D326-N545, L327-N545, R328-N545, R329-N545, F330-N545, A331-N545, R332-N545, S333-N545, A334-N545, L335-N545, N336-N545, L337-N545, V338-N545, D339-N545, L340-N545, V341-N545, A342-N545, I343-N545, L344-N545, P345-N545, L346-N545, Y347-N545, L348-N545, Q349-N545, L350-N545, L351-N545, L352-N545, E353-N545, C354-N545, F355-N545, T356-N545, G357-N545, E358-N545, G359-N545, H360-N545, Q361-N545, R362-N545, G363-N545, Q364-N545, T365-N545, V366-N545, G367-N545, S368-N545, V369-N545, G370-N545, K371-N545, V372-N545, G373-N545, Q374-N545, V375-N545, L376-N545, R377-N545, V378-N545, M379-N545, R380-N545, L381-N545, M382-N545, R383-N545, I384-N545, F385-N545, R386-N545, I387-N545, L388-N545, K389-N545, L390-N545, A391-N545, R392-N545, H393-N545, S394-N545, T395-N545, G396-N545, L397-N545, R398-N545, A399-N545, F400-N545, G401-N545, F402-N545, T403-N545, L404-N545, R405-N545, Q406-N545, C407-N545, Y408-N545, Q409-N545, Q410-N545, V411-N545, G412-N545, C413-N545, L414-N545, L415-N545, L416-N545, F417-N545, I418-N545, A419-N545, M420-N545, G421-N545, I422-N545, F423-N545, T424-N545, F425-N545, S426-N545, A427-N545, A428-N545, V429-N545, Y430-N545, S431-N545, V432-N545, E433-N545, H434-N545, D435-N545, V436-N545, P437-N545, S438-N545, T439-N545, N440-N545, F441-N545, T442-N545, T443-N545, I444-N545, P445-N545, H446-N545, S447-N545, W448-N545, W449-N545, W450-N545, A451-N545, A452-N545, V453-N545, S454-N545, I455-N545, S456-N545, T457-N545, V458-N545, G459-N545, Y460-N545, G461-N545, D462-N545, M463-N545, Y464-N545, P465-N545, E466-N545, T467-N545, H468-N545, L469-N545, G470-N545, R471-N545, F472-N545, F473-N545, A474-N545, F475-N545, L476-N545, C477-N545, I478-N545, A479-N545, F480-N545, G481-N545, I482-N545, I483-N545, L484-N545, N485-N545, G486-N545, M487-N545, P488-N545, I489-N545, S490-N545, I491-N545, L492-N545, Y493-N545, N494-N545, K495-N545, F496-N545, S497-N545, D498-N545, Y499-N545, Y500-N545, S501-N545, K502-N545, L503-N545, K504-N545, A505-N545, Y506-N545, E507-N545, Y508-N545, T509-N545, T510-N545, I511-N545, R512-N545, R513-N545, E514-N545, R515-N545, G516-N545, E517-N545, V518-N545, N519-N545, F520-N545, M521-N545, Q522-N545, R523-N545, A524-N545, R525-N545, K526-

N545, K527-N545, I528-N545, A529-N545, E530-N545, C531-N545, L532-N545, L533-N545, G534-N545, S535-N545, N536-N545, P537-N545, Q538-N545, L539-N545, of SEQ ID NO:2. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these N-terminal K+alphaM1 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal deletion mutants are encompassed by the present invention: M1-N545, M1-E544, M1-Q543, M1-R542, M1-P541, M1-T540, M1-L539, M1-Q538, M1-P537, M1-N536, M1-S535, M1-G534, M1-L533, M1-L532, M1-C531, M1-E530, M1-A529, M1-I528, M1-K527, M1-K526, M1-R525, M1-A524, M1-R523, M1-Q522, M1-M521, M1-F520, M1-N519, M1-V518, M1-E517, M1-G516, M1-R515, M1-E514, M1-R513, M1-R512, M1-I511, M1-T510, M1-T509, M1-Y508, M1-E507, M1-Y506, M1-A505, M1-K504, M1-L503, M1-K502, M1-S501, M1-Y500, M1-Y499, M1-D498, M1-S497, M1-F496, M1-K495, M1-N494, M1-Y493, M1-L492, M1-I491, M1-S490, M1-I489, M1-P488, M1-M487, M1-G486, M1-N485, M1-L484, M1-I483, M1-I482, M1-G481, M1-F480, M1-A479, M1-I478, M1-C477, M1-L476, M1-F475, M1-A474, M1-F473, M1-F472, M1-R471, M1-G470, M1-L469, M1-H468, M1-T467, M1-E466, M1-P465, M1-Y464, M1-M463, M1-D462, M1-G461, M1-Y460, M1-G459, M1-V458, M1-T457, M1-S456, M1-I455, M1-S454, M1-V453, M1-A452, M1-A451, M1-W450, M1-W449, M1-W448, M1-S447, M1-H446, M1-P445, M1-I444, M1-T443, M1-T442, M1-F441, M1-N440, M1-T439, M1-S438, M1-P437, M1-V436, M1-D435, M1-H434, M1-E433, M1-V432, M1-S431, M1-Y430, M1-V429, M1-A428, M1-A427, M1-S426, M1-F425, M1-T424, M1-F423, M1-I422, M1-G421, M1-M420, M1-A419, M1-I418, M1-F417, M1-L416, M1-L415, M1-L414, M1-C413, M1-G412, M1-V411, M1-Q410, M1-Q409, M1-Y408, M1-C407, M1-Q406, M1-R405, M1-L404, M1-T403, M1-F402, M1-G401, M1-F400, M1-A399, M1-R398, M1-L397, M1-G396, M1-T395, M1-S394, M1-H393, M1-R392, M1-A391, M1-L390, M1-K389, M1-L388, M1-I387, M1-R386, M1-F385, M1-I384, M1-R383, M1-M382, M1-L381, M1-R380, M1-M379, M1-V378, M1-R377, M1-L376, M1-V375, M1-Q374, M1-G373, M1-V372, M1-K371, M1-G370, M1-V369, M1-S368, M1-G367, M1-V366, M1-T365, M1-Q364, M1-G363, M1-R362, M1-Q361, M1-H360, M1-G359, M1-E358,

M1-G357, M1-T356, M1-F355, M1-C354, M1-E353, M1-L352, M1-L351, M1-L350, M1-Q349, M1-L348, M1-Y347, M1-L346, M1-P345, M1-L344, M1-I343, M1-A342, M1-V341, M1-L340, M1-D339, M1-V338, M1-L337, M1-N336, M1-L335, M1-A334, M1-S333, M1-R332, M1-A331, M1-F330, M1-R329, M1-R328, M1-L327, M1-D326, M1-P325, M1-T324, M1-S323, M1-A322, M1-L321, M1-R320, M1-L319, M1-L318, M1-Y317, M1-E316, M1-L315, M1-T314, M1-F313, M1-F312, M1-G311, M1-M310, M1-C309, M1-L308, M1-M307, M1-E306, M1-V305, M1-H304, M1-E303, M1-L302, M1-I301, M1-P300, M1-R299, M1-L298, M1-D297, M1-P296, M1-G295, M1-G294, M1-E293, M1-G292, M1-Q291, M1-G290, M1-S289, M1-H288, M1-Q287, M1-Q286, M1-M285, M1-E284, M1-E283, M1-V282, M1-T281, M1-N280, M1-L279, M1-A278, M1-L277, M1-A276, M1-V275, M1-V274, M1-S273, M1-V272, M1-L271, M1-V270, M1-F269, M1-T268, M1-S267, M1-S266, M1-A265, M1-V264, M1-G263, M1-I262, M1-A261, M1-K260, M1-A259, M1-A258, M1-V257, M1-S256, M1-S255, M1-F254, M1-P253, M1-K252, M1-E251, M1-M250, M1-L249, M1-N248, M1-W247, M1-L246, M1-R245, M1-R244, M1-R243, M1-Q242, M1-P241, M1-G240, M1-Y239, M1-F238, M1-R237, M1-M236, M1-D235, M1-R234, M1-F233, M1-L232, M1-E231, M1-E230, M1-A229, M1-E228, M1-E227, M1-V226, M1-Q225, M1-A224, M1-Q223, M1-A222, M1-R221, M1-L220, M1-E219, M1-H218, M1-Q217, M1-I216, M1-K215, M1-L214, M1-R213, M1-E212, M1-S211, M1-L210, M1-E209, M1-D208, M1-R207, M1-R206, M1-E205, M1-E204, M1-F203, M1-C202, M1-I201, M1-R200, M1-C199, M1-C198, M1-R197, M1-P196, M1-T195, M1-Y194, M1-K193, M1-L192, M1-R191, M1-V190, M1-G189, M1-W188, M1-Y187, M1-G186, M1-L185, M1-E184, M1-E183, M1-L182, M1-F181, M1-R180, M1-R179, M1-P178, M1-C177, M1-L176, M1-G175, M1-D174, M1-L173, M1-V172, M1-L171, M1-L170, M1-V169, M1-G168, M1-S167, M1-L166, M1-Y165, M1-F164, M1-N163, M1-Y162, M1-V161, M1-L160, M1-Q159, M1-F158, M1-V157, M1-A156, M1-P155, M1-D154, M1-R153, M1-D152, M1-F151, M1-F150, M1-Y149, M1-E148, M1-D147, M1-T146, M1-Q145, M1-E144, M1-E143, M1-Y142, M1-D141, M1-D140, M1-C139, M1-L138, M1-S137, M1-L136, M1-Q135, M1-R134, M1-S133, M1-R132, M1-S131, M1-T130, M1-S129, M1-T128, M1-A127, M1-L126, M1-R125, M1-G124, M1-L123, M1-R122, M1-T121, M1-K120, M1-P119, M1-F118, M1-G117, M1-A116, M1-L115, M1-E114, M1-C113,

M1-Y112, M1-D111, M1-L110, M1-Q109, M1-Y108, M1-S107, M1-H106, M1-G105, M1-G104, M1-V103, M1-N102, M1-V101, M1-N100, M1-L99, M1-T98, M1-S97, M1-L96, M1-L95, M1-A94, M1-P93, M1-P92, M1-D91, M1-S90, M1-P89, M1-G88, M1-E87, M1-P86, M1-K85, M1-A84, M1-T83, M1-T82, M1-V81, M1-E80, M1-G79, M1-A78, M1-Q77, M1-Q76, M1-D75, M1-E74, M1-E73, M1-A72, M1-L71, M1-D70, M1-D69, M1-K68, M1-W67, M1-Q66, M1-D65, M1-E64, M1-E63, M1-E62, M1-G61, M1-D60, M1-E59, M1-D58, M1-E57, M1-E56, M1-I55, M1-Y54, M1-Y53, M1-N52, M1-Y51, M1-N50, M1-G49, M1-E48, M1-T47, M1-W46, M1-G45, M1-H44, M1-I43, M1-S42, M1-A41, M1-Q40, M1-S39, M1-G38, M1-S37, M1-R36, M1-A35, M1-G34, M1-L33, M1-S32, M1-C31, M1-I30, M1-S29, M1-R28, M1-R27, M1-H26, M1-Q25, M1-S24, M1-G23, M1-E22, M1-N21, M1-E20, M1-T19, M1-T18, M1-N17, M1-W16, M1-P15, M1-R14, M1-Y13, M1-S12, M1-W11, M1-S10, M1-R9, M1-R8, M1-R7, of SEQ ID NO:2. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these C-terminal K+alphaM1 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

Alternatively, preferred polypeptides of the present invention may comprise polypeptide sequences corresponding to, for example, internal regions of the K+alphaM1 polypeptide (e.g., any combination of both N- and C- terminal K+alphaM1 polypeptide deletions) of SEQ ID NO:2. For example, internal regions could be defined by the equation: amino acid NX to amino acid CX, wherein NX refers to any N-terminal deletion polypeptide amino acid of K+alphaM1 (SEQ ID NO:2), and where CX refers to any C-terminal deletion polypeptide amino acid of K+alphaM1 (SEQ ID NO:2). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these polypeptides as an immunogenic and/or antigenic epitope as described elsewhere herein.

The K+alphaM1 polypeptides of the present invention were determined to comprise several phosphorylation sites based upon the Motif algorithm (Genetics Computer Group, Inc.). The phosphorylation of such sites may regulate some biological activity of the K+alphaM1 polypeptide. For example, phosphorylation at specific sites may be involved in regulating the proteins ability to associate or bind to other molecules (e.g., proteins, ligands, substrates, DNA, etc.). In the present case,

phosphorylation may modulate the ability of the K+alphaM1 polypeptide to associate with other potassium channel alpha subunits, beta subunits, or its ability to modulate potassium channel function.

Specifically, the K+alphaM1 polypeptide was predicted to comprise two tyrosine phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). Such sites are phosphorylated at the tyrosine amino acid residue. The consensus pattern for tyrosine phosphorylation sites are as follows: [RK]-x(2)-[DE]-x(3)-Y, or [RK]-x(3)-[DE]-x(2)-Y, where Y represents the phosphorylation site and 'x' represents an intervening amino acid residue. Additional information specific to tyrosine phosphorylation sites can be found in Patschinsky T., Hunter T., Esch F.S., Cooper J.A., Sefton B.M., Proc. Natl. Acad. Sci. U.S.A. 79:973-977(1982); Hunter T., J. Biol. Chem... 257:4843-4848(1982), and Cooper J.A., Esch F.S., Taylor S.S., Hunter T., J. Biol. Chem... 259:7835-7841(1984), which are hereby incorporated herein by reference.

In preferred embodiments, the following tyrosine phosphorylation site polypeptides are encompassed by the present invention: DGLCPRRFLEELGYWGVRL (SEQ ID NO:12) and GLCPRRFLEELGYWGVRL (SEQ ID NO:13). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1 tyrosine phosphorylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The K+alphaM1 polypeptide was predicted to comprise nine PKC phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). In vivo, protein kinase C exhibits a preference for the phosphorylation of serine or threonine residues. The PKC phosphorylation sites have the following consensus pattern: [ST]-x-[RK], where S or T represents the site of phosphorylation and 'x' an intervening amino acid residue. Additional information regarding PKC phosphorylation sites can be found in Woodget J.R., Gould K.L., Hunter T., Eur. J. Biochem. 161:177-184(1986), and Kishimoto A., Nishiyama K., Nakanishi H., Uratsuji Y., Nomura H., Takeyama Y., Nishizuka Y., J. Biol. Chem... 260:12492-12499(1985); which are hereby incorporated by reference herein.

In preferred embodiments, the following PKC phosphorylation site polypeptides are encompassed by the present invention: MLKQSERRRSWS (SEQ ID NO:14), RRRSWSYRPWNTT (SEQ ID NO:15), AGEVTTAKPEGPS (SEQ ID NO:16), RLATSTSRSRQLS (SEQ ID NO:17), VRLKYTPRCCRIC (SEQ ID NO:18), RRDELSERLKIQH (SEQ ID NO:19), RAFGFTLRQCYQQ (SEQ ID NO:20), AYEYTTIRRERGE (SEQ ID NO:21), and SNPQLTPRQEN (SEQ ID NO:22). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1 PKC phosphorylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The K+alphaM1 polypeptide has been shown to comprise two glycosylation sites according to the Motif algorithm (Genetics Computer Group, Inc.). As discussed more specifically herein, rotein glycosylation is thought to serve a variety of functions including: augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

In preferred embodiments, the following asparagine glycosylation site polypeptides are encompassed by the present invention: SYRPWNTTENEGSQ (SEQ ID NO:23), and/or DVPSTNFTTIPHSW (SEQ ID NO:24). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1 asparagine glycosylation polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

Moreover, a comparison of two independent cDNA sequences used in the determination of the consensus polynucleotide sequence of K+alphaM1 (SEQ ID NO:1), revealed 3 single base pair polymorphisms. These polymorphisms are labeled on in Figures 1A-C as 'S', in bold letters. Either a 'C' or a 'G' can be found at nucleotide position 841, 883 and 1065 of SEQ ID NO:1 (Figures 1A-C). The last two polymorphisms occur in the coding region but are silent with respect to the amino acid code. These polymorphisms are useful as genetic markers for any study that attempts to look for linkage between K+alphaM1 and a disease or disease state.

Additional K+alphaM1 polymorphisms have been identified by comparing the K+alphaM1 polynucleotide to the K+alphaM1.v1 and K+alphaM1.v2 polynucleotides

(see Figures 10A-E) located at nucleotide position 894, 1937, and 2197 of SEQ ID NO:1. The present invention encompasses the presence of either a "G" or a "T" at nucleotide position 894; the presence of either a "T" or a "C" at nucleotide position 1937; and/or the presence of either an "A" or a "G" at nucleotide position 2197 of SEQ ID NO:1. These polymorphisms are useful as genetic markers for any study that attempts to look for linkage between K+alphaM1 and a disease or disease state.

In preferred embodiments, the following single nucleotide polymorphism polynucleotides are encompassed by the present invention: GTGAGGGACCCCTACGACAGCCAGGAGGAAA (SEQ ID NO:25), GTGAGGGACCCCTACCACAGCCAGGAGGAAA (SEQ ID NO:26), GGAAGACGAAGACGGGGAGGAGGAGGACCAG (SEQ ID NO:27), GGAAGACGAAGACGGCGAGGAGGAGGACCAG (SEQ ID NO:28), GGCCATCGGGGTGGCGTCCAGCACCTTCGTG (SEQ ID NO:29), GGCCATCGGGGTGGCCTCCAGCACCTTCGTG (SEQ ID NO:30), CACGATGTGCCCAGC**A**CCAACTTCACTACCA (SEQ ID NO:77), CACGATGTGCCCAGC**G**CCAACTTCACTACCA (SEQ ID NO:78) AGCCATGCTCAAACA**G**AGTGAGAGGAGACGG (SEQ ID NO:79), AGCCATGCTCAAACA**T**AGTGAGAGGAGACGG (SEQ ID NO:80), ACCTTCAGCTGCTGC**T**CGAGTGCTTCACGGG (SEQ ID NO:81), and/or ACCTTCAGCTGCTGC**C**CGAGTGCTTCACGGG (SEQ ID NO:82). Polypeptides encoded by these polynucleotides are also provided.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 841 of SEQ ID NO:1 is a non-coding mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'G' to 'T' polynucleotide polymorphism located at nucleic acid 894 of SEQ ID NO:1 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 1065 of SEQ ID NO:1 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 1677 of SEQ ID NO:1 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'T' to 'C' polynucleotide polymorphism located at nucleic acid 1937 of SEQ ID NO:1 is a missense mutation resulting in a change in an encoding amino acid from 'L' to 'P' at amino acid position 352 of SEQ ID NO:2.

The predicted 'A' to 'G' polynucleotide polymorphism located at nucleic acid 2197 of SEQ ID NO:1 is a missense mutation resulting in a change in an encoding amino acid from 'T' to 'A' at amino acid position 439 of SEQ ID NO:2.

The present invention relates to isolated nucleic acid molecules comprising, or alternatively, consisting of all or a portion of the variant allele of the human K+alphaM1 potassium channel alpha subunit gene (e.g., wherein reference or wildtype human K+alphaM1 potassium channel alpha subunit gene is exemplified by SEQ ID NO:1). Preferred portions are at least 10, preferably at least 20, preferably at least 40, preferably at least 100, contiguous polynucleotides comprising anyone of the human K+alphaM1 potassium channel alpha subunit gene alleles described herein and exemplified in Figures 11A-C (SEQ ID NO:115).

In one embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with the reference allele at nucleotide position 841, 894, 1065, 1677, 1937, and/or 2197 of SEQ ID NO:1 (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 841, 894, 1065, 1677, 1937, and/or 2197 of SEQ ID NO:1. The presence of the variant allele at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having the reference allele at that position, or a greater likelihood of having more severe symptoms.

Conversely, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with the variant allele at nucleotide position 841, 894, 1065, 1677, 1937, and/or 2197 of SEQ ID NO:1 (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at

position 841, 894, 1065, 1677, 1937, and/or 2197 of SEQ ID NO:1. The presence of the variant allele at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having the reference allele at that position, or a greater likelihood of having more severe symptoms.

The present invention further relates to isolated proteins or polypeptides comprising, or alternatively, consisting of all or a portion of the encoded variant amino acid sequence of the human K+alphaM1 potassium channel alpha subunit polypeptide (e.g., wherein reference or wildtype human K+alphaM1 potassium channel alpha subunit polypeptide is exemplified by SEQ ID NO:6). Preferred portions are at least 10, preferably at least 20, preferably at least 40, preferably at least 100, contiguous polypeptides and comprises a "R" at the amino acid position corresponding to amino acid 317 of the human K+alphaM1 potassium channel alpha subunit polypeptide, or a portion of SEQ ID NO:8. Alternatively, preferred portions are at least 10, preferably at least 20, preferably at least 40, preferably at least 100, contiguous polypeptides and comprises a "Q" at the amino acid position corresponding to amino acid 317 of the human K+alphaM1 potassium channel alpha subunit protein, or a portion of SEQ ID NO:8. The invention further relates to isolated nucleic acid molecules encoding such polypeptides or proteins, as well as to antibodies that bind to such proteins or polypeptides.

The present invention also encompasses immunogenic and/or antigenic epitopes of the K+alphaM1 polypeptide.

In preferred embodiments, the following immunogenic and/or antigenic epitope polypeptide is encompassed by the present invention: amino acid residues from about amino acid 211 to about amino acid 228, from about amino acid 211 to about amino acid 219, from about amino acid 220 to about amino acid 228, from about amino acid 319 to about amino acid 334, from about amino acid 319 to about amino acid 327, from about amino acid 326 to about amino acid 334, from about amino acid 496 to about amino acid 504, from about amino acid 501 to about amino acid 509 of SEQ ID NO:2 (Figures 1A-C). In this context, the term "about" may be construed to mean 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids beyond the N-terminus and/or C-terminus of the above referenced polypeptide. Polynucleotides encoding this polypeptide are also provided.

As referenced elsewhere herein, the K+alphaM1 polypeptide was predicted to comprise 6 transmembrane domains using the Tmphred program within the Vector NTI suite of programs. The predicted transmembrane domains have been termed TM1 thru TM6 and are located at about amino acid 155 to about amino acid 180 (TM1); from about amino acid 254 to about amino acid 282 (TM2), from about amino acid 301 to about amino acid 322 (TM3), from about amino acid 333 to about amino acid 356 (TM4), from about amino acid 406 to about amino acid 432 (TM5), and from about amino acid 469 to about amino acid 492 (TM6) of SEQ ID NO:2 (Figures 1A-C). In this context, the term "about" may be construed to mean 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids beyond the N-Terminus and/or C-terminus of the above referenced polypeptide.

In preferred embodiments, the following transmembrane domain polypeptides are encompassed by the present invention: PAVFQLVYNFYLSGVLLVLDGLCPRR (SEQ ID NO:31), FSSVAAKAIGVASSTFVLVSVVALALNTV (SEQ ID NO:32), ILEHVEMLCMGFFTLEYLLRLA (SEQ ID NO:107), SALNLVDLVAILPLYLQLLLECFT (SEQ ID NO:108), QCYQQVGCLLLFIAMGIFTFSAAVYSV (SEQ ID NO:109), and/or LGRFFAFLCIAFGIILNGMPISIL (SEQ ID NO:110). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1 transmembrane domain polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The present invention also encompasses the polypeptide sequences that intervene between each of the predicted K+alphaM1 transmembrane domains. Since these regions are solvent accessible either extracellularly or intracellularly, they are particularly useful for designing antibodies specific to each region. Such antibodies may be useful as antagonists or agonists of the K+alphaM1 full-length polypeptide and may modulate its activity.

In preferred embodiments, the following inter-transmembrane domain polypeptides are encompassed by the present invention: FLEELGYWGVRLKYTPRCCRICFEERRDELSERLKIQHELRAQAQVEEAEELFRDMRFYGPQRRRLWNL MEKP (SEQ ID NO:121), EEMQQHSGQGEGGPDLRP (SEQ ID NO:122), STPDLRRFAR (SEQ ID NO:123), GEGHQRGQTVGSVGKVGQVLRVMRLMRIFRILKLARHSTGLRAFGFTLR (SEQ ID NO:124), and/or EHDVPSTNFTTIPHSWWWAAVSISTVGYGDMYPETH (SEQ ID

NO:125). The present invention also encompasses the use of these K+alphaM1 intertransmembrane domain polypeptides, and fragments thereof, as immunogenic and/or antigenic epitopes as described elsewhere herein.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO: 1 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2836 of SEQ ID NO:1, b is an integer between 15 to 2850, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1, and where b is greater than or equal to a+14.

Features of the Polypeptide Encoded by Gene No:2

The polypeptide of this gene provided as SEQ ID NO:34 (Figures 6A-C), encoded by the polynucleotide sequence according to SEQ ID NO:33 (Figures 6A-C), and/or encoded by the polynucleotide contained within the deposited clone, K+alphaM1.v1, has significant homology at the nucleotide and amino acid level to the human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gi|2815899; SEQ ID NO:3), the rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gi|7514119; SEQ ID NO:4), and the human Kv8.1 neuronal potassium channel alpha subunit (Kv8.1; Genbank Accession No: gi|6604550; SEQ ID NO:5). An alignment of the K+alphaM1.v1 polypeptide with these proteins is provided in Figures 9A-B.

The K+alphaM1.v1 polypeptide was determined to have 41% identity and 52% similarity with the human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gi|2815899; SEQ ID NO:3); 39% identity and 49.88% similarity to the rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gi|7514119; SEQ ID NO:4); and 39.8% identity and

49% similarity to the human Kv8.1 neuronal potassium channel alpha subunit (Kv8.1; Genbank Accession No: gil6604550; SEQ ID NO:5).

The human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gil2815899; SEQ ID NO:3) has been shown to slow deactivation and inactivation kinetics of hKv2.1 when coexpressed with hKv2.l, compared with hKv2. 1 expressed alone (Am. J. Physiol. 277 (3), C412-C424 (1999)). This channel is also referred to as the human ortholog of the rat Kv9.3 protein.

The rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gil7514119; SEQ ID NO:4) has been described by Patel, A. J., et al., EMBO, 16 (22): 6615 (1997), and in Biochem. Biophys. Res. Commun. 248 (3), 927-934 (1998). The rKv9.3 Shab-like subunit in rat PA myocytes is an electrically silent subunit which associates with Kv2.1, for example, and modulates its biophysical properties. The rKv9.3 heteromultimer, unlike Kv2.1 alone, opens in the voltage range of the resting membrane potential of PA myocytes. Patel, et al., demonstrate that the activity of rKv2.1/rKv9.3 is tightly controlled by internal ATP and is reversibly inhibited by hypoxia. Metabolic regulation of the Kv2.1/rKv9.3 heteromultimer appears to play an important role in hypoxic pulmonary arterial vasoconstriction and in the possible development of pulmonary arterial hypertension. EMBO, 16 (22): 6615 (1997). As described elsewhere herein, potassium channel alpha subunits do not express potassium channel current by themselves, but induce profound changes in the properties of the Shab channels Kv2.1 and Kv2.2, among others. Most interestingly, these silent subunits have the ability to create a diverse range of effects, since Kv8.1 acts as a dominant inhibitory subunit while rKv9.3 behaves as a stimulatory one. Examination of the single-channel properties of Kv2.1 and Kv2.1/rKv9.3 clearly revealed that rKv9.3 alters the single-channel conductance of Kv2.1. The ability of rKv9.3 to'drag'the Kv2.1 activation voltage threshold into the range of PA myocytes RMP suggests that the channel complex contributes to the setting of the RMP (-54 4 mV) and, consequently, in the setting of the resting pulmonary arterial pressure. Rat Kv9.3 also speeded up Kv2.1 activation, for instance, and dramatically slowed down deactivation.

The K+alphaM1.v1 polypeptide was determined to have a conserved domain comprising six amino acid residues. These residues are highlighted in the alignment in Figure 9.

In preferred embodiments, the following K+alphaM1.v1 polypeptides are encompassed by the present invention: DMHPETHLGRFFAFLCIAFGIILNGMPISILYNKFSDYYS (SEQ ID NO:37). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of this K+alphaM1.v1 polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

Expression profiling designed to measure the steady state mRNA levels encoding the K+alphaM1 polypeptide showed predominately high expression levels in particular tissue, and to a lesser extent, in brain tissue (See Figure 4).

Based upon the observed homology, the polypeptide of the present invention may share at least some biological activity with potassium channel subunits, specifically with potassium channel alpha subunits.

As described elsewhere herein, potassium channel alpha subunits have been implicated in inhibiting the activity of potassium channels. Such inhibition typically is manifested by potassium channels forming heteromultimer complexes with a potassium channel alpha subunit. As a result of the inhibition potential of alpha subunits, they are often referred to as a potassium channel antagonists.

Potassium channel antagonists are useful for a number of physiological disorders in mammals, including humans. Ion channels, including potassium channels, are found in all mammalian cells and are involved in the modulation of various physiological processes and normal cellular homeostasis. Potassium channels generally control the resting membrane potential, and the efflux of potassium ions causes repolarization of the plasma membrane after cell depolarization. Potassium channel antagonists prevent repolarization and cause the cell to stay in the depolarized, excited state.

There are a number of potassium channel subtypes. Physiologically, one important subtype is the maxi-K channel, defined as high -conductance calcium-activated potassium channel, which is present in neuronal tissue and smooth muscle. Intracellular calcium concentration (Ca.sup.2+.sub.i) and membrane potential gate

these channels. For example, maxi-K channels are opened to enable efflux of potassium ions by an increase in the intracellular Ca.sub.2+ concentration or by membrane depolarization (change in potential). Elevation of intracellular calcium concentration is required for neurotransmitter release, smooth muscle contraction, proliferation of some cell types and other processes. Modulation of maxi-K channel activity therefore affects cellular processes that depend on influx of calcium through voltage-dependent pathways, such as transmitter release from the nerve terminals and smooth muscle contraction.

A number of marketed drugs function as potassium channel antagonists. The most important of these include the compounds Glyburide, Glipizide and Tolbutamide. These potassium channel antagonists are useful as antidiabetic agents. Potassium channel antagonists are also utilized as Class III antiarrhythmic agents and to treat acute infractions in humans. A number of naturally occurring toxins are known to block potassium channels including apamin, iberiotoxin, charybdotoxin, margatoxin, noxiustoxin, kaliotoxin, dendrotoxin(s), mast cell degranuating (MCD) peptide, and beta.-bungarotoxin (.beta.-BTX).

Depression is related to a decrease in neurotransmitter release. Current treatments of depression include blockers of neurotransmitter uptake, and inhibitors of enzymes involved in neurotransmitter degradation which act to prolong the lifetime of neurotransmitters.

It is believed that certain diseases such as depression, memory disorders and Alzheimer's disease are the result of an impairment in neurotransmitter release.

Potassium channel antagonists may therefore be utilized as cell excitants which may stimulate release of neurotransmitters such as acetylcholine, serotonin and dopamine. Enhanced neurotransmitter release may reverse the symptoms associated with depression and Alzheimer's disease.

The K+alphaM1.v1 polynucleotides and polypeptides of the present invention, including agonists and/or fragments thereof, have uses that include modulating potassium channel activity in various cells, tissues, and organisms, and particularly in mammalian testicular and brain tissue, preferably human. K+alphaM1.v1 polynucleotides and polypeptides of the present invention, including agonists and/or fragments thereof, may be useful in diagnosing, treating, prognosing, and/or

preventing neural, reproductive (particularly male reproductive), metabolic, and/or proliferative diseases or disorders.

The strong homology to potassium channel alpha subunits, combined with the predominate localized expression of K+alphaM1 in testis tissue emphasizes the potential utility for K+alphaM1.v1 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing testicular, in addition to reproductive disorders.

In preferred embodiments, K+alphaM1.v1 polynucleotides and polypeptides including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing the following, non-limiting, diseases or disorders of the testis: spermatogenesis, infertility, Klinefelter's syndrome, XX male, germinal cell aplasia, cryptorchidism, varicocele, immotile cilia syndrome, and viral orchitis. The K+alphaM1.v1 polynucleotides and polypeptides including agonists and fragments thereof, may also have uses related to modulating testicular development, embryogenesis, reproduction, and in ameliorating, treating, and/or preventing testicular proliferative disorders (e.g., cancers, which include, for example, choriocarcinoma, Nonseminoma, seminona, and testicular germ cell tumors).

Likewise, the predominate localized expression in testis tissue also emphasizes the potential utility for K+alphaM1.v1 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing metabolic diseases and disorders which include the following, not limiting examples: premature puberty, incomplete puberty, Kallman syndrome, Cushing's syndrome, hyperprolactinemia, hemochromatosis, congenital adrenal hyperplasia, FSH deficiency, and granulomatous disease, for example.

In addition, the strong homology to potassium channel alpha subunits, combined with the localized expression of K+alphaM1 in brain tissue further emphasizes the potential utility for K+alphaM1.v1 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing neuronal disorders.

In preferred embodiments, K+alphaM1.v1 polynucleotides and polypeptides, including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing certain neuronal disorders. Epileptic seizures can be induced by agents (e.g., pentylenetetrazol) which block potassium

channels, most likely due to the loss of regulation of cellular membrane potentials. A potential role for potassium channels in Alzheimer's disease has been suggested by studies demonstrating that a significant component of senile plaques, beta amyloid or A beta, also blocks voltage-gated potassium channels in hippocampal neurons. (Antes, L. M. et al. (1998) Seminar Nephrol 18:31-45; Stoffel, M. and Jan, L. Y. (1998) Nat. Genet. 18:6-8; Madeja, M. et al. (1997) Eur. J. Neurosci. 9:390-395; and Good, T. A. et al. (1996) Biophys. J. 70:296-304.).

In addition, antagonists of the K+alphaM1.v1 polynucleotides and polypeptides may have uses that include diagnosing, treating, prognosing, and/or preventing diseases or disorders related to hyper potassium channel alpha subunit activity, which may include neural, reproductive (particularly male reproductive), metabolic, and/or proliferative diseases or disorders.

Alternatively, K+alphaM1.v1 polypeptides of the invention, or agonists thereof, are administered to treat, prevent, prognose, and/or diagnose disorders involving excessive smooth muscle tone or excitability, which include, but are not limited to asthma, angina, hypertension, incontinence, pre-term labor, migraine, cerebral ischemia, and irratible bowel syndrome.

Moreover, K+alphaM1.v1 polynucleotides and polypeptides, including fragments and agonists thereof, may have uses which include treating, diagnosing, prognosing, and/or preventing some classes of disorders that may be affected by effective manipulation of Shaker-like potassium ion channels, which include neurological disorders, tumor driven diseases, metabolic diseases, cardiac diseases, and autoimmune diseases. Examples of disease states and conditions from these and other classes, as well as affected normal body functions, encompass: hypoglycemia, anoxia/hypoxia, renal disease, osteoporosis, hyperkalemia, hypokalemia, hypertension, Addison's disease, abnormal apoptosis, induced apoptosis, clotting, modulation of acetylcholine function, and modulation of monoaminesepilepsy, allergic encephalomyelitis, multiple sclerosis (any demylelinating disease), acute traverse myelitis, neurofibromatosis, cardioplegia, cardiomyopathy, ischemia, ischemia reperfusion, cerebral ischemia, sickle cell anemia, cardiac arrythmias, peripheral monocuropathy, polynucuropathy, Gullain-Barre' Syndrome, peroneal muscular dystrophy, neuropathies, Parkinson's disease, palsies, cerebral palsy,

progressive supranuclear palsy, pseudobubar palsy, Huntington's disease, dystonia, dyskinesias, chorea, althetosis, choreothetosis, tics, memory degeneration, taste perception, smooth muscle function, skeletal muscle function, sleep disorders, modulation of neurotransmitters, acute disseminated encephalomyelitis, optic neuromyelitis, muscular dystrophy, myasthenia gravis, multiple sclerosis, and cerebral vasospasm, hypertension, angina pectoris, asthma, congestive heart failure, ischemia related disorders, cardiac dysrhythmias, diabetes, carcinomas, neurocarcinomas, autoimmune-hypertrophy, neuromyotonia (Isaac's Syndrome) muscular disorders associated with drug abuse, and treatment for poisoning.

K+alphaM1.v1 polypeptides and polynucleotides have additional uses which include diagnosing diseases related to the over and/or under expression of K+alphaM1.v1 by identifying mutations in the K+alphaM1.v1 gene by using K+alphaM1.v1 sequences as probes or by determining K+alphaM1.v1 protein or mRNA expression levels. K+alphaM1.v1 polypeptides may be useful for screening compounds that affect the activity of the protein. K+alphaM1.v1 peptides can also be used for the generation of specific antibodies and as bait in yeast two hybrid screens to find proteins the specifically interact with K+alphaM1.v1 (described elsewhere herein). Based on the expression pattern of this novel sequence, diseases that can be treated with agonists and/or antagonists for K+alphaM1.v1 include various forms of generalized epilepsy.

Although it is believed the encoded polypeptide may share at least some biological activities with potassium channel alpha subunits, a number of methods of determining the exact biological function of this clone are either known in the art or are described elsewhere herein. Briefly, the function of this clone may be determined by applying microarray methodology. Nucleic acids corresponding to the K+alphaM1.v1 polynucleotides, in addition to, other clones of the present invention, may be arrayed on microchips for expression profiling. Depending on which polynucleotide probe is used to hybridize to the slides, a change in expression of a specific gene may provide additional insight into the function of this gene based upon the conditions being studied. For example, an observed increase or decrease in expression levels when the polynucleotide probe used comes from tissue that has been

treated with known potassium channel inhibitors, which include, but are not limited to the drugs listed above, might indicate a function in modulating potassium channel function, for example. In the case of K+alphaM1.v1, testicular and/or brain tissue should be used to extract RNA to prepare the probe.

In addition, the function of the protein may be assessed by applying quantitative PCR methodology, for example. Real time quantitative PCR would provide the capability of following the expression of the K+alphaM1.v1 gene throughout development, for example. Quantitative PCR methodology requires only a nominal amount of tissue from each developmentally important step is needed to perform such experiements. Therefore, the application of quantitative PCR methodology to refining the biological function of this polypeptide is encompassed by the present invention. Also encompassed by the present invention are quantitative PCR probes corresponding to the polynucleotide sequence provided as SEQ ID NO:33 (Figures 6A-C).

The function of the protein may also be assessed through complementation assays in yeast. For example, in the case of the K+alphaM1.v1, transforming yeast deficient in potassium channel alpha subunit activity and assessing their ability to grow would provide convincing evidence the K+alphaM1.v1 polypeptide has potassium channel alpha subunit activity activity. Additional assay conditions and methods that may be used in assessing the function of the polynucletides and polypeptides of the present invention are known in the art, some of which are disclosed elsewhere herein.

Alternatively, the biological function of the encoded polypeptide may be determined by disrupting a homologue of this polypeptide in Mice and/or rats and observing the resulting phenotype.

Moreover, the biological function of this polypeptide may be determined by the application of antisense and/or sense methodology and the resulting generation of transgenic mice and/or rats. Expressing a particular gene in either sense or antisense orientation in a transgenic mouse or rat could lead to respectively higher or lower expression levels of that particular gene. Altering the endogenous expression levels of a gene can lead to the obervation of a particular phenotype that can then be used to derive indications on the function of the gene. The gene can be either over-expressed

or under expressed in every cell of the organism at all times using a strong ubiquitous promoter, or it could be expressed in one or more discrete parts of the organism using a well characterized tissue-specific promoter (e.g., a testis specific promoter or a brain specific promoter), or it can be expressed at a specified time of development using an inducible and/or a developmentally regulated promoter.

In the case of K+alphaM1.v1 transgenic mice or rats, if no phenotype is apparent in normal growth conditions, observing the organism under diseased conditions (neural or testicular disorders, depression, testicular or brain cancer, etc.) may lead to understanding the function of the gene. Therefore, the application of antisense and/or sense methodology to the creation of transgenic mice or rats to refine the biological function of the polypeptide is encompassed by the present invention.

In preferred embodiments, the following N-terminal K+alphaM1.v1 deletion polypeptides are encompassed by the present invention: M1-N545, L2-N545, K3-N545, Q4-N545, S5-N545, E6-N545, R7-N545, R8-N545, R9-N545, S10-N545, W11-N545, S12-N545, Y13-N545, R14-N545, P15-N545, W16-N545, N17-N545, T18-N545, T19-N545, E20-N545, N21-N545, E22-N545, G23-N545, S24-N545, Q25-N545, H26-N545, R27-N545, R28-N545, S29-N545, I30-N545, C31-N545, S32-N545, L33-N545, G34-N545, A35-N545, R36-N545, S37-N545, G38-N545, S39-N545, Q40-N545, A41-N545, S42-N545, I43-N545, H44-N545, G45-N545, W46-N545, T47-N545, E48-N545, G49-N545, N50-N545, Y51-N545, N52-N545, Y53-N545, Y54-N545, I55-N545, E56-N545, E57-N545, D58-N545, E59-N545, D60-N545, G61-N545, E62-N545, E63-N545, E64-N545, D65-N545, Q66-N545, W67-N545, K68-N545, D69-N545, D70-N545, L71-N545, A72-N545, E73-N545, E74-N545, D75-N545, Q76-N545, Q77-N545, A78-N545, G79-N545, E80-N545, V81-N545, T82-N545, T83-N545, A84-N545, K85-N545, P86-N545, E87-N545, G88-N545, P89-N545, S90-N545, D91-N545, P92-N545, P93-N545, A94-N545, L95-N545, L96-N545, S97-N545, T98-N545, L99-N545, N100-N545, V101-N545, N102-N545, V103-N545, G104-N545, G105-N545, H106-N545, S107-N545, Y108-N545, Q109-N545, L110-N545, D111-N545, Y112-N545, C113-N545, E114-N545, L115-N545, A116-N545, G117-N545, F118-N545, P119-N545, K120-N545, T121-N545, R122-N545, L123-N545, G124-N545, R125-N545, L126-N545, A127-N545, T128-N545, S129-N545, T130-N545, S131-N545, R132-N545, S133-N545, R134-N545,

Q135-N545, L136-N545, S137-N545, L138-N545, C139-N545, D140-N545, D141-N545, Y142-N545, E143-N545, E144-N545, Q145-N545, T146-N545, D147-N545, E148-N545, Y149-N545, F150-N545, F151-N545, D152-N545, R153-N545, D154-N545, P155-N545, A156-N545, V157-N545, F158-N545, Q159-N545, L160-N545, V161-N545, Y162-N545, N163-N545, F164-N545, Y165-N545, L166-N545, S167-N545, G168-N545, V169-N545, L170-N545, L171-N545, V172-N545, L173-N545, D174-N545, G175-N545, L176-N545, C177-N545, P178-N545, R179-N545, R180-N545, F181-N545, L182-N545, E183-N545, E184-N545, L185-N545, G186-N545, Y187-N545, W188-N545, G189-N545, V190-N545, R191-N545, L192-N545, K193-N545, Y194-N545, T195-N545, P196-N545, R197-N545, C198-N545, C199-N545, R200-N545, I201-N545, C202-N545, F203-N545, E204-N545, E205-N545, R206-N545, R207-N545, D208-N545, E209-N545, L210-N545, S211-N545, E212-N545, R213-N545, L214-N545, K215-N545, I216-N545, Q217-N545, H218-N545, E219-N545, L220-N545, R221-N545, A222-N545, Q223-N545, A224-N545, Q225-N545, V226-N545, E227-N545, E228-N545, A229-N545, E230-N545, E231-N545, L232-N545, F233-N545, R234-N545, D235-N545, M236-N545, R237-N545, F238-N545, Y239-N545, G240-N545, P241-N545, Q242-N545, R243-N545, R244-N545, R245-N545, L246-N545, W247-N545, N248-N545, L249-N545, M250-N545, E251-N545, K252-N545, P253-N545, F254-N545, S255-N545, S256-N545, V257-N545, A258-N545, A259-N545, K260-N545, A261-N545, I262-N545, G263-N545, V264-N545, A265-N545, S266-N545, S267-N545, T268-N545, F269-N545, V270-N545, L271-N545, V272-N545, S273-N545, V274-N545, V275-N545, A276-N545, L277-N545, A278-N545, L279-N545, N280-N545, T281-N545, V282-N545, E283-N545, E284-N545, M285-N545, Q286-N545, Q287-N545, H288-N545, S289-N545, G290-N545, Q291-N545, G292-N545, E293-N545, G294-N545, G295-N545, P296-N545, D297-N545, L298-N545, R299-N545, P300-N545, I301-N545, L302-N545, E303-N545, H304-N545, V305-N545, E306-N545, M307-N545, L308-N545, C309-N545, M310-N545, G311-N545, F312-N545, F313-N545, T314-N545, L315-N545, E316-N545, Y317-N545, L318-N545, L319-N545, R320-N545, L321-N545, A322-N545, S323-N545, T324-N545, P325-N545, D326-N545, L327-N545, R328-N545, R329-N545, F330-N545, A331-N545, R332-N545, S333-N545, A334-N545, L335-N545, N336-N545, L337-N545, V338-N545, D339-N545, L340-N545, V341-N545, A342-N545,

I343-N545, L344-N545, P345-N545, L346-N545, Y347-N545, L348-N545, Q349-N545, L350-N545, L351-N545, P352-N545, E353-N545, C354-N545, F355-N545, T356-N545, G357-N545, E358-N545, G359-N545, H360-N545, Q361-N545, R362-N545, G363-N545, Q364-N545, T365-N545, V366-N545, G367-N545, S368-N545, V369-N545, G370-N545, K371-N545, V372-N545, G373-N545, Q374-N545, V375-N545, L376-N545, R377-N545, V378-N545, M379-N545, R380-N545, L381-N545, M382-N545, R383-N545, I384-N545, F385-N545, R386-N545, I387-N545, L388-N545, K389-N545, L390-N545, A391-N545, R392-N545, H393-N545, S394-N545, T395-N545, G396-N545, L397-N545, R398-N545, A399-N545, S400-N545, A401-N545, S402-N545, R403-N545, C404-N545, A405-N545, S406-N545, A407-N545, T408-N545, S409-N545, R410-N545, W411-N545, A412-N545, C413-N545, L414-N545, L415-N545, L416-N545, F417-N545, I418-N545, A419-N545, M420-N545, G421-N545, I422-N545, F423-N545, T424-N545, F425-N545, S426-N545, A427-N545, A428-N545, V429-N545, Y430-N545, S431-N545, V432-N545, E433-N545, H434-N545, D435-N545, V436-N545, P437-N545, S438-N545, T439-N545, N440-N545, F441-N545, T442-N545, T443-N545, I444-N545, P445-N545, H446-N545, S447-N545, W448-N545, W449-N545, W450-N545, A451-N545, A452-N545, V453-N545, S454-N545, I455-N545, S456-N545, T457-N545, V458-N545, G459-N545, Y460-N545, G461-N545, D462-N545, M463-N545, Y464-N545, P465-N545, E466-N545, T467-N545, H468-N545, L469-N545, G470-N545, R471-N545, F472-N545, F473-N545, A474-N545, F475-N545, L476-N545, C477-N545, I478-N545, A479-N545, F480-N545, G481-N545, I482-N545, I483-N545, L484-N545, N485-N545, G486-N545, M487-N545, P488-N545, I489-N545, S490-N545, I491-N545, L492-N545, Y493-N545, N494-N545, K495-N545, F496-N545, S497-N545, D498-N545, Y499-N545, Y500-N545, S501-N545, K502-N545, L503-N545, K504-N545, A505-N545, Y506-N545, E507-N545, Y508-N545, T509-N545, T510-N545, I511-N545, R512-N545, R513-N545, E514-N545, R515-N545, G516-N545, E517-N545, V518-N545, N519-N545, F520-N545, M521-N545, Q522-N545, R523-N545, A524-N545, R525-N545, K526-N545, K527-N545, I528-N545, A529-N545, E530-N545, C531-N545, L532-N545, L533-N545, G534-N545, S535-N545, N536-N545, P537-N545, Q538-N545, and/or L539-N545 of SEQ ID NO:34. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also

encompasses the use of these N-terminal K+alphaM1.v1 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal K+alphaM1.v1 deletion polypeptides are encompassed by the present invention: M1-N545, M1-E544, M1-Q543, M1-R542, M1-P541, M1-T540, M1-L539, M1-Q538, M1-P537, M1-N536, M1-S535, M1-G534, M1-L533, M1-L532, M1-C531, M1-E530, M1-A529, M1-I528, M1-K527, M1-K526, M1-R525, M1-A524, M1-R523, M1-Q522, M1-M521, M1-F520, M1-N519, M1-V518, M1-E517, M1-G516, M1-R515, M1-E514, M1-R513, M1-R512, M1-I511, M1-T510, M1-T509, M1-Y508, M1-E507, M1-Y506, M1-A505, M1-K504, M1-L503, M1-K502, M1-S501, M1-Y500, M1-Y499, M1-D498, M1-S497, M1-F496, M1-K495, M1-N494, M1-Y493, M1-L492, M1-I491, M1-S490, M1-I489, M1-P488, M1-M487, M1-G486, M1-N485, M1-L484, M1-I483, M1-I482, M1-G481, M1-F480, M1-A479, M1-I478, M1-C477, M1-L476, M1-F475, M1-A474, M1-F473, M1-F472, M1-R471, M1-G470, M1-L469, M1-H468, M1-T467, M1-E466, M1-P465, M1-Y464, M1-M463, M1-D462, M1-G461, M1-Y460, M1-G459, M1-V458, M1-T457, M1-S456, M1-I455, M1-S454, M1-V453, M1-A452, M1-A451, M1-W450, M1-W449, M1-W448, M1-S447, M1-H446, M1-P445, M1-I444, M1-T443, M1-T442, M1-F441, M1-N440, M1-T439, M1-S438, M1-P437, M1-V436, M1-D435, M1-H434, M1-E433, M1-V432, M1-S431, M1-Y430, M1-V429, M1-A428, M1-A427, M1-S426, M1-F425, M1-T424, M1-F423, M1-I422, M1-G421, M1-M420, M1-A419, M1-I418, M1-F417, M1-L416, M1-L415, M1-L414, M1-C413, M1-A412, M1-W411, M1-R410, M1-S409, M1-T408, M1-A407, M1-S406, M1-A405, M1-C404, M1-R403, M1-S402, M1-A401, M1-S400, M1-A399, M1-R398, M1-L397, M1-G396, M1-T395, M1-S394, M1-H393, M1-R392, M1-A391, M1-L390, M1-K389, M1-L388, M1-I387, M1-R386, M1-F385, M1-I384, M1-R383, M1-M382, M1-L381, M1-R380, M1-M379, M1-V378, M1-R377, M1-L376, M1-V375, M1-Q374, M1-G373, M1-V372, M1-K371, M1-G370, M1-V369, M1-S368, M1-G367, M1-V366, M1-T365, M1-Q364, M1-G363, M1-R362, M1-Q361, M1-H360, M1-G359, M1-E358, M1-G357, M1-T356, M1-F355, M1-C354, M1-E353, M1-P352, M1-L351, M1-L350, M1-Q349, M1-L348, M1-Y347, M1-L346, M1-P345, M1-L344, M1-I343, M1-A342, M1-V341, M1-L340, M1-D339, M1-V338, M1-L337, M1-N336, M1-L335, M1-A334, M1-S333, M1-R332, M1-A331, M1-F330, M1-R329,

M1-R328, M1-L327, M1-D326, M1-P325, M1-T324, M1-S323, M1-A322, M1-L321, M1-R320, M1-L319, M1-L318, M1-Y317, M1-E316, M1-L315, M1-T314, M1-F313, M1-F312, M1-G311, M1-M310, M1-C309, M1-L308, M1-M307, M1-E306, M1-V305, M1-H304, M1-E303, M1-L302, M1-I301, M1-P300, M1-R299, M1-L298, M1-D297, M1-P296, M1-G295, M1-G294, M1-E293, M1-G292, M1-Q291, M1-G290, M1-S289, M1-H288, M1-Q287, M1-Q286, M1-M285, M1-E284, M1-E283, M1-V282, M1-T281, M1-N280, M1-L279, M1-A278, M1-L277, M1-A276, M1-V275, M1-V274, M1-S273, M1-V272, M1-L271, M1-V270, M1-F269, M1-T268, M1-S267, M1-S266, M1-A265, M1-V264, M1-G263, M1-I262, M1-A261, M1-K260, M1-A259, M1-A258, M1-V257, M1-S256, M1-S255, M1-F254, M1-P253, M1-K252, M1-E251, M1-M250, M1-L249, M1-N248, M1-W247, M1-L246, M1-R245, M1-R244, M1-R243, M1-Q242, M1-P241, M1-G240, M1-Y239, M1-F238, M1-R237, M1-M236, M1-D235, M1-R234, M1-F233, M1-L232, M1-E231, M1-E230, M1-A229, M1-E228, M1-E227, M1-V226, M1-Q225, M1-A224, M1-Q223, M1-A222, M1-R221, M1-L220, M1-E219, M1-H218, M1-Q217, M1-I216, M1-K215, M1-L214, M1-R213, M1-E212, M1-S211, M1-L210, M1-E209, M1-D208, M1-R207, M1-R206, M1-E205, M1-E204, M1-F203, M1-C202, M1-I201, M1-R200, M1-C199, M1-C198, M1-R197, M1-P196, M1-T195, M1-Y194, M1-K193, M1-L192, M1-R191, M1-V190, M1-G189, M1-W188, M1-Y187, M1-G186, M1-L185, M1-E184, M1-E183, M1-L182, M1-F181, M1-R180, M1-R179, M1-P178, M1-C177, M1-L176, M1-G175, M1-D174, M1-L173, M1-V172, M1-L171, M1-L170, M1-V169, M1-G168, M1-S167, M1-L166, M1-Y165, M1-F164, M1-N163, M1-Y162, M1-V161, M1-L160, M1-Q159, M1-F158, M1-V157, M1-A156, M1-P155, M1-D154, M1-R153, M1-D152, M1-F151, M1-F150, M1-Y149, M1-E148, M1-D147, M1-T146, M1-Q145, M1-E144, M1-E143, M1-Y142, M1-D141, M1-D140, M1-C139, M1-L138, M1-S137, M1-L136, M1-Q135, M1-R134, M1-S133, M1-R132, M1-S131, M1-T130, M1-S129, M1-T128, M1-A127, M1-L126, M1-R125, M1-G124, M1-L123, M1-R122, M1-T121, M1-K120, M1-P119, M1-F118, M1-G117, M1-A116, M1-L115, M1-E114, M1-C113, M1-Y112, M1-D111, M1-L110, M1-Q109, M1-Y108, M1-S107, M1-H106, M1-G105, M1-G104, M1-V103, M1-N102, M1-V101, M1-N100, M1-L99, M1-T98, M1-S97, M1-L96, M1-L95, M1-A94, M1-P93, M1-P92, M1-D91, M1-S90, M1-P89, M1-G88, M1-E87, M1-P86, M1-K85, M1-A84,

M1-T83, M1-T82, M1-V81, M1-E80, M1-G79, M1-A78, M1-Q77, M1-Q76, M1-D75, M1-E74, M1-E73, M1-A72, M1-L71, M1-D70, M1-D69, M1-K68, M1-W67, M1-Q66, M1-D65, M1-E64, M1-E63, M1-E62, M1-G61, M1-D60, M1-E59, M1-D58, M1-E57, M1-E56, M1-I55, M1-Y54, M1-Y53, M1-N52, M1-Y51, M1-N50, M1-G49, M1-E48, M1-T47, M1-W46, M1-G45, M1-H44, M1-I43, M1-S42, M1-A41, M1-Q40, M1-S39, M1-G38, M1-S37, M1-R36, M1-A35, M1-G34, M1-L33, M1-S32, M1-C31, M1-I30, M1-S29, M1-R28, M1-R27, M1-H26, M1-Q25, M1-S24, M1-G23, M1-E22, M1-N21, M1-E20, M1-T19, M1-T18, M1-N17, M1-W16, M1-P15, M1-R14, M1-Y13, M1-S12, M1-W11, M1-S10, M1-R9, M1-R8, and/or M1-R7 of SEQ ID NO:34. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these C-terminal K+alphaM1.v1 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

Alternatively, preferred polypeptides of the present invention may comprise polypeptide sequences corresponding to, for example, internal regions of the K+alphaM1.v1 polypeptide (e.g., any combination of both N- and C- terminal K+alphaM1.v1 polypeptide deletions) of SEQ ID NO:34. For example, internal regions could be defined by the equation: amino acid NX to amino acid CX, wherein NX refers to any N-terminal deletion polypeptide amino acid of K+alphaM1.v1 (SEQ ID NO:34), and where CX refers to any C-terminal deletion polypeptide amino acid of K+alphaM1.v1 (SEQ ID NO:34). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these polypeptides as an immunogenic and/or antigenic epitope as described elsewhere herein.

The K+alphaM1.v1 polypeptides of the present invention were determined to comprise several phosphorylation sites based upon the Motif algorithm (Genetics Computer Group, Inc.). The phosphorylation of such sites may regulate some biological activity of the K+alphaM1.v1 polypeptide. For example, phosphorylation at specific sites may be involved in regulating the proteins ability to associate or bind to other molecules (e.g., proteins, ligands, substrates, DNA, etc.). In the present case, phosphorylation may modulate the ability of the K+alphaM1.v1 polypeptide to associate with other potassium channel alpha subunits, beta subunits, or its ability to modulate potassium channel function.

Specifically, the K+alphaM1.v1 polypeptide was predicted to comprise two tyrosine phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). Such sites are phosphorylated at the tyrosine amino acid residue. The consensus pattern for tyrosine phosphorylation sites are as follows: [RK]-x(2)-[DE]-x(3)-Y, or [RK]-x(3)-[DE]-x(2)-Y, where Y represents the phosphorylation site and 'x' represents an intervening amino acid residue. Additional information specific to tyrosine phosphorylation sites can be found in Patschinsky T., Hunter T., Esch F.S., Cooper J.A., Sefton B.M., Proc. Natl. Acad. Sci. U.S.A. 79:973-977(1982); Hunter T., J. Biol. Chem... 257:4843-4848(1982), and Cooper J.A., Esch F.S., Taylor S.S., Hunter T., J. Biol. Chem... 259:7835-7841(1984), which are hereby incorporated herein by reference.

In preferred embodiments, the following tyrosine phosphorylation site polypeptides are encompassed by the present invention: DGLCPRRFLEELGYWGVRL (SEQ ID NO:49), and/or GLCPRRFLEELGYWGVRL (SEQ ID NO:50). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1.v1 tyrosine phosphorylation polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The K+alphaM1.v1 polypeptide was predicted to comprise nine PKC phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). In vivo, protein kinase C exhibits a preference for the phosphorylation of serine or threonine residues. The PKC phosphorylation sites have the following consensus pattern: [ST]-x-[RK], where S or T represents the site of phosphorylation and 'x' an intervening amino acid residue. Additional information regarding PKC phosphorylation sites can be found in Woodget J.R., Gould K.L., Hunter T., Eur. J. Biochem. 161:177-184(1986), and Kishimoto A., Nishiyama K., Nakanishi H., Uratsuji Y., Nomura H., Takeyama Y., Nishizuka Y., J. Biol. Chem... 260:12492-12499(1985); which are hereby incorporated by reference herein.

In preferred embodiments, the following PKC phosphorylation site polypeptides are encompassed by the present invention: MLKQSERRRSWS (SEQ ID NO:40), RRRSWSYRPWNTT (SEQ ID NO:41), AGEVTTAKPEGPS (SEQ ID NO:42), RLATSTSRSRQLS (SEQ ID NO:43), VRLKYTPRCCRIC (SEQ ID

NO:44), RRDELSERLKIQH (SEQ ID NO:45), RCASATSRWACLL (SEQ ID NO:46), AYEYTTIRRERGE (SEQ ID NO:47), and/or SNPQLTPRQEN (SEQ ID NO:48). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1.v1 PKC phosphorylation polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The K+alphaM1.v1 polypeptide has been shown to comprise two glycosylation sites according to the Motif algorithm (Genetics Computer Group, Inc.). As discussed more specifically herein, rotein glycosylation is thought to serve a variety of functions including: augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

In preferred embodiments, the following asparagine glycosylation site polypeptides are encompassed by the present invention: SYRPWNTTENEGSQ (SEQ ID NO:38), and/or DVPSTNFTTIPHSW (SEQ ID NO:39). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1.v1 asparagine glycosylation polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

Moreover, a comparison of two independent cDNA sequences used in the determination of the consensus polynucleotide sequence of K+alphaM1.v1 (SEQ ID NO:33), revealed 3 single base pair polymorphisms. These polymorphisms are labeled in bold in Figures 6A-C . Either a 'C' or a 'G' can be found at nucleotide position 37, 261, and 873 of SEQ ID NO:33 (Figures 6A-C). The last two polymorphisms occur in the coding region but are silent with respect to the amino acid code. These polymorphisms are useful as genetic markers for any study that attempts to look for linkage between K+alphaM1.v1 and a disease or disease state.

Additional K+alphaM1.v1 polymorphisms have been identified by comparing the K+alphaM1.v1 polynucleotide to the K+alphaM1 and K+alphaM1.v2 polynucleotides (see Figures 10A-E) located at nucleotide position 90, 1133, and 1393 of SEQ ID NO:33. The present invention encompasses the presence of either a "G" or a "T" at nucleotide position 90; the presence of either a "T" or a "C" at nucleotide position 1133; and/or the presence of either an "A" or a "G" at nucleotide

position 1393 of SEQ ID NO:33. These polymorphisms are useful as genetic markers for any study that attempts to look for linkage between K+alphaM1.v1 and a disease or disease state.

In preferred embodiments, the following single nucleotide polymorphism polynucleotides are encompassed by the present invention: AGCCATGCTCAAACA**G**AGTGAGAGGAGACGG (SEQ ID NO:83), AGCCATGCTCAAACA**T**AGTGAGAGGAGACGG (SEQ ID NO:84), GGAAGACGAAGACGG**C**GAGGAGGAGGACCAG (SEQ ID NO:85), GGAAGACGAAGACGG**G**GAGGAGGAGGACCAG (SEQ ID NO:86), GGCCATCGGGGTGGC**C**TCCAGCACCTTCGTG (SEQ ID NO:87), GGCCATCGGGGTGGC**G**TCCAGCACCTTCGTG (SEQ ID NO:88) ACCTTCAGCTGCTGC**C**CGAGTGCTTCACGGG (SEQ ID NO:89), ACCTTCAGCTGCTGC**T**CGAGTGCTTCACGGG (SEQ ID NO:90), CACGATGTGCCCAGC**A**CCAACTTCACTACCA (SEQ ID NO:91), CACGATGTGCCCAGC**G**CCAACTTCACTACCA (SEQ ID NO:92), AATTCGCCCTTCTAC**C**ACAGCCAGGAGGAAA (SEQ ID NO:93), and/or AATTCGCCCTTCTAC**G**ACAGCCAGGAGGAAA (SEQ ID NO:93). Polypeptides encoded by these polynucleotides are also provided.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 37 of SEQ ID NO:33 is a non-coding mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'G' to 'T' polynucleotide polymorphism located at nucleic acid 894 of SEQ ID NO:33 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 261 of SEQ ID NO:33 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 873 of SEQ ID NO:33 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'T' to 'C' polynucleotide polymorphism located at nucleic acid 1133 of SEQ ID NO:33 is a missense mutation resulting in a change in an encoding amino acid from 'L' to 'P' at amino acid position 352 of SEQ ID NO:34.

The predicted 'A' to 'G' polynucleotide polymorphism located at nucleic acid 1393 of SEQ ID NO:33 is a missense mutation resulting in a change in an encoding amino acid from 'T' to 'A' at amino acid position 439 of SEQ ID NO:34.

The present invention relates to isolated nucleic acid molecules comprising, or alternatively, consisting of all or a portion of the variant allele of the human K+alphaM1.v1 potassium channel alpha subunit gene (e.g., wherein reference or wildtype human K+alphaM1.v1 potassium channel alpha subunit gene is exemplified by SEQ ID NO:33). Preferred portions are at least 10, preferably at least 20, preferably at least 40, preferably at least 100, contiguous polynucleotides comprising anyone of the human K+alphaM1.v1 potassium channel alpha subunit gene alleles described herein and exemplified in Figures 12A-C (SEQ ID NO:117).

In one embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with the reference allele at nucleotide position 37, 90, 261, 873, 1133, and/or 1393 of SEQ ID NO:33 (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 37, 90, 261, 873, 1133, and/or 1393 of SEQ ID NO:33. The presence of the variant allele at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having the reference allele at that position, or a greater likelihood of having more severe symptoms.

Conversely, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with the variant allele at nucleotide position 37, 90, 261, 873, 1133, and/or 1393 of SEQ ID NO:33 (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 37, 90, 261, 873, 1133, and/or 1393 of SEQ ID NO:33. The presence of the variant allele at this position indicates that the individual has a greater likelihood of

having a disorder associated therewith than an individual having the reference allele at that position, or a greater likelihood of having more severe symptoms.

The present invention also encompasses immunogenic and/or antigenic epitopes of the K+alphaM1.v1 polypeptide.

In preferred embodiments, the following immunogenic and/or antigenic epitope polypeptide is encompassed by the present invention: amino acid residues from about amino acid 211 to about amino acid 228, from about amino acid 211 to about amino acid 219, from about amino acid 220 to about amino acid 228, from about amino acid 319 to about amino acid 334, from about amino acid 319 to about amino acid 327, from about amino acid 326 to about amino acid 334, from about amino acid 496 to about amino acid 504, from about amino acid 501 to about amino acid 509 of SEQ ID NO:34 (Figures 6A-C). In this context, the term "about" may be construed to mean 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids beyond the N-terminus and/or C-terminus of the above referenced polypeptide. Polynucleotides encoding this polypeptide are also provided.

As referenced elsewhere herein, the K+alphaM1.v1 polypeptide was predicted to comprise 6 transmembrane domains using the Tmphred program within the Vector NTI suite of programs. The predicted transmembrane domains have been termed TM1 thru TM6 and are located at about amino acid 156 to about amino acid 178 (TM1); from about amino acid 261 to about amino acid 282 (TM2), from about amino acid 333 to about amino acid 355 (TM3), from about amino acid 411 to about amino acid 429 (TM4), from about amino acid 441 to about amino acid 461 (TM5), and from about amino acid 472 to about amino acid 492 (TM6) of SEQ ID NO:34 (Figures 6A-C). In this context, the term "about" may be construed to mean 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids beyond the N-Terminus and/or C-terminus of the above referenced polypeptide.

In preferred embodiments, the following transmembrane domain polypeptides are encompassed by the present invention: AVFQLVYNFYLSGVLLVLDGLCP (SEQ ID NO:52), AIGVASSTFVLVSVVALALNTV (SEQ ID NO:53), SALNLVDLVAILPLYLQLLPECF (SEQ ID NO:54), WACLLLFIAMGIFTFSAAV (SEQ ID NO:55), FTTIPHSWWWAAVSISTVGY (SEQ ID NO:56), and/or FFAFLCIAFGIILNGMPISIL (SEQ ID NO:57). Polynucleotides encoding these

polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1.v1 transmembrane domain polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The present invention also encompasses the polypeptide sequences that intervene between each of the predicted K+alphaM1.v1 transmembrane domains. Since these regions are solvent accessible either extracellularly or intracellularly, they are particularly useful for designing antibodies specific to each region. Such antibodies may be useful as antagonists or agonists of the K+alphaM1.v1 full-length polypeptide and may modulate its activity.

In preferred embodiments, the following inter-transmembrane domain polypeptides are encompassed by the present invention: RRFLEELGYWGVRLKYTPRCCRICFEERRDELSERLKIQHELRAQAQVEEAEELFRDMRFYGP QRRRLWNLMEKPFSSVAAK (SEQ ID NO:126), EEMQQHSGQGEGGPDLRPILEHVEMLCMGFFTLEYLLRLASTPDLRRFAR (SEQ ID NO:127), TGEGHQRGQTVGSVGKVGQVLRVMRLMRIFRILKLARHSTGLRASASRCASATSR (SEQ ID NO:128), YSVEHDVPSTN (SEQ ID NO:129), and/or GDMYPETHLGR (SEQ ID NO:130). The present invention also encompasses the use of these K+alphaM1.v1 intertransmembrane domain polypeptides, and fragments thereof, as immunogenic and/or antigenic epitopes as described elsewhere herein.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1857 of SEQ ID NO:1, b is an integer between 15 to 1871, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a+14.

Features of the Polypeptide Encoded by Gene No:3

The polypeptide of this gene provided as SEQ ID NO:36 (Figures 7A-C), encoded by the polynucleotide sequence according to SEQ ID NO:35 (Figures 7A-C),

and/or encoded by the polynucleotide contained within the deposited clone, K+alphaM1.v2, has significant homology at the nucleotide and amino acid level to the human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gi|2815899; SEQ ID NO:3), the rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gi|7514119; SEQ ID NO:4), and the human Kv8.1 neuronal potassium channel alpha subunit (Kv8.1; Genbank Accession No: gi|6604550; SEQ ID NO:5). An alignment of the K+alphaM1.v2 polypeptide with these proteins is provided in Figures 9A-B.

The K+alphaM1.v2 polypeptide was determined to have 41.1% identity and 52% similarity with the human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gi|2815899; SEQ ID NO:3); 40.6% identity and 51.7% similarity to the rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gi|7514119; SEQ ID NO:4); and 41.1% identity and 51% similarity to the human Kv8.1 neuronal potassium channel alpha subunit (Kv8.1; Genbank Accession No: gi|6604550; SEQ ID NO:5).

The human Shab-related delayed rectifier K+ channel alpha subunit (Shab-related; Genbank Accession No: gi|2815899; SEQ ID NO:3) has been shown to slow deactivation and inactivation kinetics of hKv2.1 when coexpressed with hKv2.l, compared with hKv2. 1 expressed alone (Am. J. Physiol. 277 (3), C412-C424 (1999)). This channel is also referred to as the human ortholog of the rat Kv9.3 protein.

The rat Kv9.3 voltage-gated K+ channel alpha chain (Kv9.3; Genbank Accession No. gi|7514119; SEQ ID NO:4) has been described by Patel, A. J., et al., EMBO, 16 (22): 6615 (1997), and in Biochem. Biophys. Res. Commun. 248 (3), 927-934 (1998). The rKv9.3 Shab-like subunit in rat PA myocytes is an electrically silent subunit which associates with Kv2.1, for example, and modulates its biophysical properties. The rKv9.3 heteromultimer, unlike Kv2.1 alone, opens in the voltage range of the resting membrane potential of PA myocytes. Patel, et al., demonstrate that the activity of rKv2.1/rKv9.3 is tightly controlled by internal ATP and is reversibly inhibited by hypoxia. Metabolic regulation of the Kv2.1/rKv9.3 heteromultimer appears to play an important role in hypoxic pulmonary arterial vasoconstriction and in the possible development of pulmonary arterial hypertension. EMBO, 16 (22): 6615 (1997). As described elsewhere herein, potassium channel

alpha subunits do not express potassium channel current by themselves, but induce profound changes in the properties of the Shab channels Kv2.1 and Kv2.2, among others. Most interestingly, these silent subunits have the ability to create a diverse range of effects, since Kv8.1 acts as a dominant inhibitory subunit while rKv9.3 behaves as a stimulatory one. Examination of the single-channel properties of Kv2.1 and Kv2.1/rKv9.3 clearly revealed that rKv9.3 alters the single-channel conductance of Kv2.1. The ability of rKv9.3 to'drag'the Kv2.1 activation voltage threshold into the range of PA myocytes RMP suggests that the channel complex contributes to the setting of the RMP (-54 4 mV) and, consequently, in the setting of the resting pulmonary arterial pressure. Rat Kv9.3 also speeded up Kv2.1 activation, for instance, and dramatically slowed down deactivation.

The K+alphaM1.v2 polypeptide was determined to have a conserved domain comprising six amino acid residues. These residues are highlighted in the alignment in Figure 9.

In preferred embodiments, the following K+alphaM1.v2 polypeptides are encompassed by the present invention: DMYPETHLGRFFAFLCIAFGIILNGMPISILYNKFSDYYS (SEQ ID NO:51). Polynucleotides encoding these polypeptides are also provided.

Expression profiling designed to measure the steady state mRNA levels encoding the K+alphaM1 polypeptide showed predominately high expression levels in testicular tissue, and to a lesser extent, in brain tissue (See Figure 4).

Based upon the observed homology, the polypeptide of the present invention may share at least some biological activity with potassium channel subunits, specifically with potassium channel alpha subunits.

As described elsewhere herein, potassium channel alpha subunits have been implicated in inhibiting the activity of potassium channels. Such inhibition typically is manifested by potassium channels forming heteromultimer complexes with a potassium channel alpha subunit. As a result of the inhibition potential of alpha subunits, they are often referred to as a potassium channel antagonists.

Potassium channel antagonists are useful for a number of physiological disorders in mammals, including humans. Ion channels, including potassium channels, are found in all mammalian cells and are involved in the modulation of

various physiological processes and normal cellular homeostasis. Potassium channels generally control the resting membrane potential, and the efflux of potassium ions causes repolarization of the plasma membrane after cell depolarization. Potassium channel antagonists prevent repolarization and cause the cell to stay in the depolarized, excited state.

There are a number of potassium channel subtypes. Physiologically, one important subtype is the maxi-K channel, defined as high -conductance calcium-activated potassium channel, which is present in neuronal tissue and smooth muscle. Intracellular calcium concentration (Ca.sup.2+.sub.i) and membrane potential gate these channels. For example, maxi-K channels are opened to enable efflux of potassium ions by an increase in the intracellular Ca.sub.2+ concentration or by membrane depolarization (change in potential). Elevation of intracellular calcium concentration is required for neurotransmitter release, smooth muscle contraction, proliferation of some cell types and other processes. Modulation of maxi-K channel activity therefore affects cellular processes that depend on influx of calcium through voltage-dependent pathways, such as transmitter release from the nerve terminals and smooth muscle contraction.

A number of marketed drugs function as potassium channel antagonists. The most important of these include the compounds Glyburide, Glipizide and Tolbutamide. These potassium channel antagonists are useful as antidiabetic agents. Potassium channel antagonists are also utilized as Class III antiarrhythmic agents and to treat acute infractions in humans. A number of naturally occurring toxins are known to block potassium channels including apamin, iberiotoxin, charybdotoxin, margatoxin, noxiustoxin, kaliotoxin, dendrotoxin(s), mast cell degranuating (MCD) peptide, and beta.-bungarotoxin (.beta.-BTX).

Depression is related to a decrease in neurotransmitter release. Current treatments of depression include blockers of neurotransmitter uptake, and inhibitors of enzymes involved in neurotransmitter degradation which act to prolong the lifetime of neurotransmitters.

It is believed that certain diseases such as depression, memory disorders and Alzheimer's disease are the result of an impairment in neurotransmitter release.

Potassium channel antagonists may therefore be utilized as cell excitants which may stimulate release of neurotransmitters such as acetylcholine, serotonin and dopamine. Enhanced neurotransmitter release may reverse the symptoms associated with depression and Alzheimer's disease.

The K+alphaM1.v2 polynucleotides and polypeptides of the present invention, including agonists and/or fragments thereof, have uses that include modulating potassium channel activity in various cells, tissues, and organisms, and particularly in mammalian testicular and brain tissue, preferably human. K+alphaM1.v2 polynucleotides and polypeptides of the present invention, including agonists and/or fragments thereof, may be useful in diagnosing, treating, prognosing, and/or preventing neural, reproductive (particularly male reproductive), metabolic, and/or proliferative diseases or disorders.

The strong homology to potassium channel alpha subunits, combined with the predominate localized expression of K+alphaM1 in testis tissue further emphasizes the potential utility for K+alphaM1.v2 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing testicular, in addition to reproductive disorders.

In preferred embodiments, K+alphaM1.v2 polynucleotides and polypeptides including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing the following, non-limiting, diseases or disorders of the testis: spermatogenesis, infertility, Klinefelter's syndrome, XX male, germinal cell aplasia, cryptorchidism, varicocele, immotile cilia syndrome, and viral orchitis. The K+alphaM1.v2 polynucleotides and polypeptides including agonists and fragments thereof, may also have uses related to modulating testicular development, embryogenesis, reproduction, and in ameliorating, treating, and/or preventing testicular proliferative disorders (e.g., cancers, which include, for example, choriocarcinoma, Nonseminoma, seminona, and testicular germ cell tumors).

Likewise, the predominate localized expression in testis tissue also emphasizes the potential utility for K+alphaM1.v2 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing metabolic diseases and disorders which include the following, not limiting examples: premature puberty, incomplete puberty, Kallman syndrome, Cushing's syndrome, hyperprolactinemia, hemochromatosis,

congenital adrenal hyperplasia, FSH deficiency, and granulomatous disease, for example.

In addition, the strong homology to potassium channel alpha subunits, combined with the localized expression of K+alphaM1 in brain tissue further emphasizes the potential utility for K+alphaM1.v2 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing neuronal disorders.

In preferred embodiments, K+alphaM1.v2 polynucleotides and polypeptides, including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing certain neuronal disorders. Epileptic seizures can be induced by agents (e.g., pentylenetetrazol) which block potassium channels, most likely due to the loss of regulation of cellular membrane potentials. A potential role for potassium channels in Alzheimer's disease has been suggested by studies demonstrating that a significant component of senile plaques, beta amyloid or A beta, also blocks voltage-gated potassium channels in hippocampal neurons. (Antes, L. M. et al. (1998) Seminar Nephrol 18:31-45; Stoffel, M. and Jan, L. Y. (1998) Nat. Genet. 18:6-8; Madeja, M. et al. (1997) Eur. J. Neurosci. 9:390-395; and Good, T. A. et al. (1996) Biophys. J. 70:296-304.).

In addition, antagonists of the K+alphaM1.v2 polynucleotides and polypeptides may have uses that include diagnosing, treating, prognosing, and/or preventing diseases or disorders related to hyper potassium channel alpha subunit activity, which may include neural, reproductive (particularly male reproductive), metabolic, and/or proliferative diseases or disorders.

Alternatively, K+alphaM1.v2 polypeptides of the invention, or agonists thereof, are administered to treat, prevent, prognose, and/or diagnose disorders involving excessive smooth muscle tone or excitability, which include, but are not limited to asthma, angina, hypertension, incontinence, pre-term labor, migraine, cerebral ischemia, and irratible bowel syndrome.

Moreover, K+alphaM1.v2 polynucleotides and polypeptides, including fragments and agonists thereof, may have uses which include treating, diagnosing, prognosing, and/or preventing some classes of disorders that may be affected by effective manipulation of Shaker-like potassium ion channels, which include neurological disorders, tumor driven diseases, metabolic diseases, cardiac diseases,

and autoimmune diseases. Examples of disease states and conditions from these and other classes, as well as affected normal body functions, encompass: hypoglycemia, anoxia/hypoxia, renal disease, osteoporosis, hyperkalemia, hypokalemia, hypertension, Addison's disease, abnormal apoptosis, induced apoptosis, clotting, modulation of acetylcholine function, and modulation of monoaminesepilepsy, allergic encephalomyelitis, multiple sclerosis (any demylelinating disease), acute traverse myelitis, neurofibromatosis, cardioplegia, cardiomyopathy, ischemia, ischemia reperfusion, cerebral ischemia, sickle cell anemia, cardiac arrythmias, peripheral monocuropathy, polynucuropathy, Gullain-Barre' Syndrome, peroneal muscular dystrophy, neuropathies, Parkinson's disease, palsies, cerebral palsy, progressive supranuclear palsy, pseudobubar palsy, Huntington's disease, dystonia, dyskinesias, chorea, althetosis, choreothetosis, tics, memory degeneration, taste perception, smooth muscle function, skeletal muscle function, sleep disorders, modulation of neurotransmitters, acute disseminated encephalomyelitis, optic neuromyelitis, muscular dystrophy, myasthenia gravis, multiple sclerosis, and cerebral vasospasm, hypertension, angina pectoris, asthma, congestive heart failure, ischemia related disorders, cardiac dysrhythmias, diabetes, carcinomas, neurocarcinomas, autoimmune-hypertrophy, neuromyotonia (Isaac's Syndrome) muscular disorders associated with drug abuse, and treatment for poisoning.

K+alphaM1.v2 polypeptides and polynucleotides have additional uses which include diagnosing diseases related to the over and/or under expression of K+alphaM1.v2 by identifying mutations in the K+alphaM1.v2 gene by using K+alphaM1.v2 sequences as probes or by determining K+alphaM1.v2 protein or mRNA expression levels. K+alphaM1.v2 polypeptides may be useful for screening compounds that affect the activity of the protein. K+alphaM1.v2 peptides can also be used for the generation of specific antibodies and as bait in yeast two hybrid screens to find proteins the specifically interact with K+alphaM1.v2 (described elsewhere herein). Based on the expression pattern of this novel sequence, diseases that can be treated with agonists and/or antagonists for K+alphaM1.v2 include various forms of generalized epilepsy.

Although it is believed the encoded polypeptide may share at least some biological activities with potassium channel alpha subunits, a number of methods of determining the exact biological function of this clone are either known in the art or are described elsewhere herein. Briefly, the function of this clone may be determined by applying microarray methodology. Nucleic acids corresponding to the K+alphaM1.v2 polynucleotides, in addition to, other clones of the present invention, may be arrayed on microchips for expression profiling. Depending on which polynucleotide probe is used to hybridize to the slides, a change in expression of a specific gene may provide additional insight into the function of this gene based upon the conditions being studied. For example, an observed increase or decrease in expression levels when the polynucleotide probe used comes from tissue that has been treated with known potassium channel inhibitors, which include, but are not limited to the drugs listed above, might indicate a function in modulating potassium channel function, for example. In the case of K+alphaM1.v2, testicular and/or brain tissue should be used to extract RNA to prepare the probe.

In addition, the function of the protein may be assessed by applying quantitative PCR methodology, for example. Real time quantitative PCR would provide the capability of following the expression of the K+alphaM1.v2 gene throughout development, for example. Quantitative PCR methodology requires only a nominal amount of tissue from each developmentally important step is needed to perform such experiements. Therefore, the application of quantitative PCR methodology to refining the biological function of this polypeptide is encompassed by the present invention. Also encompassed by the present invention are quantitative PCR probes corresponding to the polynucleotide sequence provided as SEQ ID NO:35 (Figures 7A-C).

The function of the protein may also be assessed through complementation assays in yeast. For example, in the case of the K+alphaM1.v2, transforming yeast deficient in potassium channel alpha subunit activity and assessing their ability to grow would provide convincing evidence the K+alphaM1.v2 polypeptide has potassium channel alpha subunit activity activity. Additional assay conditions and methods that may be used in assessing the function of the polynucletides and

polypeptides of the present invention are known in the art, some of which are disclosed elsewhere herein.

Alternatively, the biological function of the encoded polypeptide may be determined by disrupting a homologue of this polypeptide in Mice and/or rats and observing the resulting phenotype.

Moreover, the biological function of this polypeptide may be determined by the application of antisense and/or sense methodology and the resulting generation of transgenic mice and/or rats. Expressing a particular gene in either sense or antisense orientation in a transgenic mouse or rat could lead to respectively higher or lower expression levels of that particular gene. Altering the endogenous expression levels of a gene can lead to the obervation of a particular phenotype that can then be used to derive indications on the function of the gene. The gene can be either over-expressed or under expressed in every cell of the organism at all times using a strong ubiquitous promoter, or it could be expressed in one or more discrete parts of the organism using a well characterized tissue-specific promoter (e.g., a testis specific promoter or a brain specific promoter), or it can be expressed at a specified time of development using an inducible and/or a developmentally regulated promoter.

In the case of K+alphaM1.v2 transgenic mice or rats, if no phenotype is apparent in normal growth conditions, observing the organism under diseased conditions (neural or testicular disorders, depression, testicular or brain cancer, etc.) may lead to understanding the function of the gene. Therefore, the application of antisense and/or sense methodology to the creation of transgenic mice or rats to refine the biological function of the polypeptide is encompassed by the present invention.

In preferred embodiments, the following N-terminal K+alphaM1.v2 deletion polypeptides are encompassed by the present invention: M1-N545, L2-N545, K3-N545, H4-N545, S5-N545, E6-N545, R7-N545, R8-N545, R9-N545, S10-N545, W11-N545, S12-N545, Y13-N545, R14-N545, P15-N545, W16-N545, N17-N545, T18-N545, T19-N545, E20-N545, N21-N545, E22-N545, G23-N545, S24-N545, Q25-N545, H26-N545, R27-N545, R28-N545, S29-N545, I30-N545, C31-N545, S32-N545, L33-N545, G34-N545, A35-N545, R36-N545, S37-N545, G38-N545, S39-N545, Q40-N545, A41-N545, S42-N545, I43-N545, H44-N545, G45-N545, W46-N545, T47-N545, E48-N545, G49-N545, N50-N545, Y51-N545, N52-N545, Y53-

N545, Y54-N545, I55-N545, E56-N545, E57-N545, D58-N545, E59-N545, D60-N545, G61-N545, E62-N545, E63-N545, E64-N545, D65-N545, Q66-N545, W67-N545, K68-N545, D69-N545, D70-N545, L71-N545, A72-N545, E73-N545, E74-N545, D75-N545, Q76-N545, Q77-N545, A78-N545, G79-N545, E80-N545, V81-N545, T82-N545, T83-N545, A84-N545, K85-N545, P86-N545, E87-N545, G88-N545, P89-N545, S90-N545, D91-N545, P92-N545, P93-N545, A94-N545, L95-N545, L96-N545, S97-N545, T98-N545, L99-N545, N100-N545, V101-N545, N102-N545, V103-N545, G104-N545, G105-N545, H106-N545, S107-N545, Y108-N545, Q109-N545, L110-N545, D111-N545, Y112-N545, C113-N545, E114-N545, L115-N545, A116-N545, G117-N545, F118-N545, P119-N545, K120-N545, T121-N545, R122-N545, L123-N545, G124-N545, R125-N545, L126-N545, A127-N545, T128-N545, S129-N545, T130-N545, S131-N545, R132-N545, S133-N545, R134-N545, Q135-N545, L136-N545, S137-N545, L138-N545, C139-N545, D140-N545, D141-N545, Y142-N545, E143-N545, E144-N545, Q145-N545, T146-N545, D147-N545, E148-N545, Y149-N545, F150-N545, F151-N545, D152-N545, R153-N545, D154-N545, P155-N545, A156-N545, V157-N545, F158-N545, Q159-N545, L160-N545, V161-N545, Y162-N545, N163-N545, F164-N545, Y165-N545, L166-N545, S167-N545, G168-N545, V169-N545, L170-N545, L171-N545, V172-N545, L173-N545, D174-N545, G175-N545, L176-N545, C177-N545, P178-N545, R179-N545, R180-N545, F181-N545, L182-N545, E183-N545, E184-N545, L185-N545, G186-N545, Y187-N545, W188-N545, G189-N545, V190-N545, R191-N545, L192-N545, K193-N545, Y194-N545, T195-N545, P196-N545, R197-N545, C198-N545, C199-N545, R200-N545, I201-N545, C202-N545, F203-N545, E204-N545, E205-N545, R206-N545, R207-N545, D208-N545, E209-N545, L210-N545, S211-N545, E212-N545, R213-N545, L214-N545, K215-N545, I216-N545, Q217-N545, H218-N545, E219-N545, L220-N545, R221-N545, A222-N545, Q223-N545, A224-N545, Q225-N545, V226-N545, E227-N545, E228-N545, A229-N545, E230-N545, E231-N545, L232-N545, F233-N545, R234-N545, D235-N545, M236-N545, R237-N545, F238-N545, Y239-N545, G240-N545, P241-N545, Q242-N545, R243-N545, R244-N545, R245-N545, L246-N545, W247-N545, N248-N545, L249-N545, M250-N545, E251-N545, K252-N545, P253-N545, F254-N545, S255-N545, S256-N545, V257-N545, A258-N545, A259-N545, K260-N545, A261-N545, I262-N545, G263-N545, V264-N545,

A265-N545, S266-N545, S267-N545, T268-N545, F269-N545, V270-N545, L271-N545, V272-N545, S273-N545, V274-N545, V275-N545, A276-N545, L277-N545, A278-N545, L279-N545, N280-N545, T281-N545, V282-N545, E283-N545, E284-N545, M285-N545, Q286-N545, Q287-N545, H288-N545, S289-N545, G290-N545, Q291-N545, G292-N545, E293-N545, G294-N545, G295-N545, P296-N545, D297-N545, L298-N545, R299-N545, P300-N545, I301-N545, L302-N545, E303-N545, H304-N545, V305-N545, E306-N545, M307-N545, L308-N545, C309-N545, M310-N545, G311-N545, F312-N545, F313-N545, T314-N545, L315-N545, E316-N545, Y317-N545, L318-N545, L319-N545, R320-N545, L321-N545, A322-N545, S323-N545, T324-N545, P325-N545, D326-N545, L327-N545, R328-N545, R329-N545, F330-N545, A331-N545, R332-N545, S333-N545, A334-N545, L335-N545, N336-N545, L337-N545, V338-N545, D339-N545, L340-N545, V341-N545, A342-N545, I343-N545, L344-N545, P345-N545, L346-N545, Y347-N545, L348-N545, Q349-N545, L350-N545, L351-N545, L352-N545, E353-N545, C354-N545, F355-N545, T356-N545, G357-N545, E358-N545, G359-N545, H360-N545, Q361-N545, R362-N545, G363-N545, Q364-N545, T365-N545, V366-N545, G367-N545, S368-N545, V369-N545, G370-N545, K371-N545, V372-N545, G373-N545, Q374-N545, V375-N545, L376-N545, R377-N545, V378-N545, M379-N545, R380-N545, L381-N545, M382-N545, R383-N545, I384-N545, F385-N545, R386-N545, I387-N545, L388-N545, K389-N545, L390-N545, A391-N545, R392-N545, H393-N545, S394-N545, T395-N545, G396-N545, L397-N545, R398-N545, A399-N545, F400-N545, G401-N545, F402-N545, T403-N545, L404-N545, R405-N545, Q406-N545, C407-N545, Y408-N545, Q409-N545, Q410-N545, V411-N545, G412-N545, C413-N545, L414-N545, L415-N545, L416-N545, F417-N545, I418-N545, A419-N545, M420-N545, G421-N545, I422-N545, F423-N545, T424-N545, F425-N545, S426-N545, A427-N545, A428-N545, V429-N545, Y430-N545, S431-N545, V432-N545, E433-N545, H434-N545, D435-N545, V436-N545, P437-N545, S438-N545, A439-N545, N440-N545, F441-N545, T442-N545, T443-N545, I444-N545, P445-N545, H446-N545, S447-N545, W448-N545, W449-N545, W450-N545, A451-N545, A452-N545, V453-N545, S454-N545, I455-N545, S456-N545, T457-N545, V458-N545, G459-N545, Y460-N545, G461-N545, D462-N545, M463-N545, Y464-N545, P465-N545, E466-N545, T467-N545, H468-N545, L469-N545, G470-N545, R471-N545, F472-

N545, F473-N545, A474-N545, F475-N545, L476-N545, C477-N545, I478-N545, A479-N545, F480-N545, G481-N545, I482-N545, I483-N545, L484-N545, N485-N545, G486-N545, M487-N545, P488-N545, I489-N545, S490-N545, I491-N545, L492-N545, Y493-N545, N494-N545, K495-N545, F496-N545, S497-N545, D498-N545, Y499-N545, Y500-N545, S501-N545, K502-N545, L503-N545, K504-N545, A505-N545, Y506-N545, E507-N545, Y508-N545, T509-N545, T510-N545, I511-N545, R512-N545, R513-N545, E514-N545, R515-N545, G516-N545, E517-N545, V518-N545, N519-N545, F520-N545, M521-N545, Q522-N545, R523-N545, A524-N545, R525-N545, K526-N545, K527-N545, I528-N545, A529-N545, E530-N545, C531-N545, L532-N545, L533-N545, G534-N545, S535-N545, N536-N545, P537-N545, Q538-N545, and/or L539-N545 of SEQ ID NO:36. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these N-terminal K+alphaM1.v2 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal K+alphaM1.v2 deletion polypeptides are encompassed by the present invention: M1-N545, M1-E544, M1-Q543, M1-R542, M1-P541, M1-T540, M1-L539, M1-Q538, M1-P537, M1-N536, M1-S535, M1-G534, M1-L533, M1-L532, M1-C531, M1-E530, M1-A529, M1-I528, M1-K527, M1-K526, M1-R525, M1-A524, M1-R523, M1-Q522, M1-M521, M1-F520, M1-N519, M1-V518, M1-E517, M1-G516, M1-R515, M1-E514, M1-R513, M1-R512, M1-I511, M1-T510, M1-T509, M1-Y508, M1-E507, M1-Y506, M1-A505, M1-K504, M1-L503, M1-K502, M1-S501, M1-Y500, M1-Y499, M1-D498, M1-S497, M1-F496, M1-K495, M1-N494, M1-Y493, M1-L492, M1-I491, M1-S490, M1-I489, M1-P488, M1-M487, M1-G486, M1-N485, M1-L484, M1-I483, M1-I482, M1-G481, M1-F480, M1-A479, M1-I478, M1-C477, M1-L476, M1-F475, M1-A474, M1-F473, M1-F472, M1-R471, M1-G470, M1-L469, M1-H468, M1-T467, M1-E466, M1-P465, M1-Y464, M1-M463, M1-D462, M1-G461, M1-Y460, M1-G459, M1-V458, M1-T457, M1-S456, M1-I455, M1-S454, M1-V453, M1-A452, M1-A451, M1-W450, M1-W449, M1-W448, M1-S447, M1-H446, M1-P445, M1-I444, M1-T443, M1-T442, M1-F441, M1-N440, M1-A439, M1-S438, M1-P437, M1-V436, M1-D435, M1-H434, M1-E433, M1-V432, M1-S431, M1-Y430, M1-V429, M1-A428, M1-A427, M1-S426, M1-F425, M1-T424, M1-F423, M1-I422, M1-G421, M1-

M420, M1-A419, M1-I418, M1-F417, M1-L416, M1-L415, M1-L414, M1-C413, M1-G412, M1-V411, M1-Q410, M1-Q409, M1-Y408, M1-C407, M1-Q406, M1-R405, M1-L404, M1-T403, M1-F402, M1-G401, M1-F400, M1-A399, M1-R398, M1-L397, M1-G396, M1-T395, M1-S394, M1-H393, M1-R392, M1-A391, M1-L390, M1-K389, M1-L388, M1-I387, M1-R386, M1-F385, M1-I384, M1-R383, M1-M382, M1-L381, M1-R380, M1-M379, M1-V378, M1-R377, M1-L376, M1-V375, M1-Q374, M1-G373, M1-V372, M1-K371, M1-G370, M1-V369, M1-S368, M1-G367, M1-V366, M1-T365, M1-Q364, M1-G363, M1-R362, M1-Q361, M1-H360, M1-G359, M1-E358, M1-G357, M1-T356, M1-F355, M1-C354, M1-E353, M1-L352, M1-L351, M1-L350, M1-Q349, M1-L348, M1-Y347, M1-L346, M1-P345, M1-L344, M1-I343, M1-A342, M1-V341, M1-L340, M1-D339, M1-V338, M1-L337, M1-N336, M1-L335, M1-A334, M1-S333, M1-R332, M1-A331, M1-F330, M1-R329, M1-R328, M1-L327, M1-D326, M1-P325, M1-T324, M1-S323, M1-A322, M1-L321, M1-R320, M1-L319, M1-L318, M1-Y317, M1-E316, M1-L315, M1-T314, M1-F313, M1-F312, M1-G311, M1-M310, M1-C309, M1-L308, M1-M307, M1-E306, M1-V305, M1-H304, M1-E303, M1-L302, M1-I301, M1-P300, M1-R299, M1-L298, M1-D297, M1-P296, M1-G295, M1-G294, M1-E293, M1-G292, M1-Q291, M1-G290, M1-S289, M1-H288, M1-Q287, M1-Q286, M1-M285, M1-E284, M1-E283, M1-V282, M1-T281, M1-N280, M1-L279, M1-A278, M1-L277, M1-A276, M1-V275, M1-V274, M1-S273, M1-V272, M1-L271, M1-V270, M1-F269, M1-T268, M1-S267, M1-S266, M1-A265, M1-V264, M1-G263, M1-I262, M1-A261, M1-K260, M1-A259, M1-A258, M1-V257, M1-S256, M1-S255, M1-F254, M1-P253, M1-K252, M1-E251, M1-M250, M1-L249, M1-N248, M1-W247, M1-L246, M1-R245, M1-R244, M1-R243, M1-Q242, M1-P241, M1-G240, M1-Y239, M1-F238, M1-R237, M1-M236, M1-D235, M1-R234, M1-F233, M1-L232, M1-E231, M1-E230, M1-A229, M1-E228, M1-E227, M1-V226, M1-Q225, M1-A224, M1-Q223, M1-A222, M1-R221, M1-L220, M1-E219, M1-H218, M1-Q217, M1-I216, M1-K215, M1-L214, M1-R213, M1-E212, M1-S211, M1-L210, M1-E209, M1-D208, M1-R207, M1-R206, M1-E205, M1-E204, M1-F203, M1-C202, M1-I201, M1-R200, M1-C199, M1-C198, M1-R197, M1-P196, M1-T195, M1-Y194, M1-K193, M1-L192, M1-R191, M1-V190, M1-G189, M1-W188, M1-Y187, M1-G186, M1-L185, M1-E184, M1-E183, M1-L182, M1-F181, M1-R180, M1-R179, M1-P178,

M1-C177, M1-L176, M1-G175, M1-D174, M1-L173, M1-V172, M1-L171, M1-L170, M1-V169, M1-G168, M1-S167, M1-L166, M1-Y165, M1-F164, M1-N163, M1-Y162, M1-V161, M1-L160, M1-Q159, M1-F158, M1-V157, M1-A156, M1-P155, M1-D154, M1-R153, M1-D152, M1-F151, M1-F150, M1-Y149, M1-E148, M1-D147, M1-T146, M1-Q145, M1-E144, M1-E143, M1-Y142, M1-D141, M1-D140, M1-C139, M1-L138, M1-S137, M1-L136, M1-Q135, M1-R134, M1-S133, M1-R132, M1-S131, M1-T130, M1-S129, M1-T128, M1-A127, M1-L126, M1-R125, M1-G124, M1-L123, M1-R122, M1-T121, M1-K120, M1-P119, M1-F118, M1-G117, M1-A116, M1-L115, M1-E114, M1-C113, M1-Y112, M1-D111, M1-L110, M1-Q109, M1-Y108, M1-S107, M1-H106, M1-G105, M1-G104, M1-V103, M1-N102, M1-V101, M1-N100, M1-L99, M1-T98, M1-S97, M1-L96, M1-L95, M1-A94, M1-P93, M1-P92, M1-D91, M1-S90, M1-P89, M1-G88, M1-E87, M1-P86, M1-K85, M1-A84, M1-T83, M1-T82, M1-V81, M1-E80, M1-G79, M1-A78, M1-Q77, M1-Q76, M1-D75, M1-E74, M1-E73, M1-A72, M1-L71, M1-D70, M1-D69, M1-K68, M1-W67, M1-Q66, M1-D65, M1-E64, M1-E63, M1-E62, M1-G61, M1-D60, M1-E59, M1-D58, M1-E57, M1-E56, M1-I55, M1-Y54, M1-Y53, M1-N52, M1-Y51, M1-N50, M1-G49, M1-E48, M1-T47, M1-W46, M1-G45, M1-H44, M1-I43, M1-S42, M1-A41, M1-Q40, M1-S39, M1-G38, M1-S37, M1-R36, M1-A35, M1-G34, M1-L33, M1-S32, M1-C31, M1-I30, M1-S29, M1-R28, M1-R27, M1-H26, M1-Q25, M1-S24, M1-G23, M1-E22, M1-N21, M1-E20, M1-T19, M1-T18, M1-N17, M1-W16, M1-P15, M1-R14, M1-Y13, M1-S12, M1-W11, M1-S10, M1-R9, M1-R8, and/or M1-R7 of SEQ ID NO:36. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these C-terminal K+alphaM1.v2 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

Alternatively, preferred polypeptides of the present invention may comprise polypeptide sequences corresponding to, for example, internal regions of the K+alphaM1.v2 polypeptide (e.g., any combination of both N- and C- terminal K+alphaM1.v2 polypeptide deletions) of SEQ ID NO:36. For example, internal regions could be defined by the equation: amino acid NX to amino acid CX, wherein NX refers to any N-terminal deletion polypeptide amino acid of K+alphaM1.v2 (SEQ ID NO:36), and where CX refers to any C-terminal deletion polypeptide amino acid

of K+alphaM1.v2 (SEQ ID NO:36). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these polypeptides as an immunogenic and/or antigenic epitope as described elsewhere herein.

The K+alphaM1.v2 polypeptides of the present invention were determined to comprise several phosphorylation sites based upon the Motif algorithm (Genetics Computer Group, Inc.). The phosphorylation of such sites may regulate some biological activity of the K+alphaM1.v2 polypeptide. For example, phosphorylation at specific sites may be involved in regulating the proteins ability to associate or bind to other molecules (e.g., proteins, ligands, substrates, DNA, etc.). In the present case, phosphorylation may modulate the ability of the K+alphaM1.v2 polypeptide to associate with other potassium channel alpha subunits, beta subunits, or its ability to modulate potassium channel function.

Specifically, the K+alphaM1.v2 polypeptide was predicted to comprise two tyrosine phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). Such sites are phosphorylated at the tyrosine amino acid residue. The consensus pattern for tyrosine phosphorylation sites are as follows: [RK]-x(2)-[DE]-x(3)-Y, or [RK]-x(3)-[DE]-x(2)-Y, where Y represents the phosphorylation site and 'x' represents an intervening amino acid residue. Additional information specific to tyrosine phosphorylation sites can be found in Patschinsky T., Hunter T., Esch F.S., Cooper J.A., Sefton B.M., Proc. Natl. Acad. Sci. U.S.A. 79:973-977(1982); Hunter T., J. Biol. Chem... 257:4843-4848(1982), and Cooper J.A., Esch F.S., Taylor S.S., Hunter T., J. Biol. Chem... 259:7835-7841(1984), which are hereby incorporated herein by reference.

In preferred embodiments, the following tyrosine phosphorylation site polypeptides are encompassed by the present invention: DGLCPRRFLEELGYWGVRL (SEQ ID NO:75), and/or GLCPRRFLEELGYWGVRL (SEQ ID NO:76). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1.v2 tyrosine phosphorylation polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The K+alphaM1.v2 polypeptide was predicted to comprise nine PKC phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). In

vivo, protein kinase C exhibits a preference for the phosphorylation of serine or threonine residues. The PKC phosphorylation sites have the following consensus pattern: [ST]-x-[RK], where S or T represents the site of phosphorylation and 'x' an intervening amino acid residue. Additional information regarding PKC phosphorylation sites can be found in Woodget J.R., Gould K.L., Hunter T., Eur. J. Biochem. 161:177-184(1986), and Kishimoto A., Nishiyama K., Nakanishi H., Uratsuji Y., Nomura H., Takeyama Y., Nishizuka Y., J. Biol. Chem... 260:12492-12499(1985); which are hereby incorporated by reference herein.

In preferred embodiments, the following PKC phosphorylation site polypeptides are encompassed by the present invention: MLKHSERRRSWS (SEQ ID NO:66), RRRSWSYRPWNTT (SEQ ID NO:67), AGEVTTAKPEGPS (SEQ ID NO:68), RLATSTSRSRQLS (SEQ ID NO:69), VRLKYTPRCCRIC (SEQ ID NO:70), RRDELSERLKIQH (SEQ ID NO:71), RAFGFTLRQCYQQ (SEQ ID NO:72), AYEYTTIRRERGE (SEQ ID NO:73), and/or SNPQLTPRQEN (SEQ ID NO:74). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1.v2 PKC phosphorylation polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The K+alphaM1.v2 polypeptide has been shown to comprise two glycosylation sites according to the Motif algorithm (Genetics Computer Group, Inc.). As discussed more specifically herein, rotein glycosylation is thought to serve a variety of functions including: augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

In preferred embodiments, the following asparagine glycosylation site polypeptides are encompassed by the present invention: SYRPWNTTENEGSQ (SEQ ID NO:64), and/or DVPSANFTTIPHSW (SEQ ID NO:65). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1.v2 asparagine glycosylation polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

Moreover, a comparison of two independent cDNA sequences used in the determination of the consensus polynucleotide sequence of K+alphaM1.v2 (SEQ ID NO:35),

revealed 3 single base pair polymorphisms. These polymorphisms are labeled in bold in Figures 7A-C . Either a 'C' or a 'G' can be found at nucleotide position 37, 261, and 873 of SEQ ID NO:35 (Figures 7A-C). The last two polymorphisms occurs in the coding region but are silent with respect to the amino acid code. These polymorphisms are useful as genetic markers for any study that attempts to look for linkage between K+alphaM1.v2 and a disease or disease state.

Additional K+alphaM1.v2 polymorphisms have been identified by comparing the K+alphaM1.v2 polynucleotide to the K+alphaM1 and K+alphaM1.v2 polynucleotides (see Figures 10A-E) located at nucleotide position 90, 1133, and 1393 of SEQ ID NO:35. The present invention encompasses the presence of either a "G" or a "T" at nucleotide position 90; the presence of either a "T" or a "C" at nucleotide position 1133; and/or the presence of either an "A" or a "G" at nucleotide position 1393 of SEQ ID NO:35. These polymorphisms are useful as genetic markers for any study that attempts to look for linkage between K+alphaM1.v2 and a disease or disease state.

In preferred embodiments, the following single nucleotide polymorphism polynucleotides are encompassed by the present invention:
AGCCATGCTCAAACA**G**AGTGAGAGGAGACGG (SEQ ID NO:95),
AGCCATGCTCAAACA**T**AGTGAGAGGAGACGG (SEQ ID NO:96),
GGAAGACGAAGACGG**C**GAGGAGGAGGACCAG (SEQ ID NO:97),
GGAAGACGAAGACGG**G**GAGGAGGAGGACCAG (SEQ ID NO:98),
GGCCATCGGGGTGGC**C**TCCAGCACCTTCGTG (SEQ ID NO:99),
GGCCATCGGGGTGGC**G**TCCAGCACCTTCGTG (SEQ ID NO:100)
ACCTTCAGCTGCTGC**C**CGAGTGCTTCACGGG (SEQ ID NO:101),
ACCTTCAGCTGCTGC**T**CGAGTGCTTCACGGG (SEQ ID NO:102),
CACGATGTGCCCAGC**A**CCAACTTCACTACCA (SEQ ID NO:103),
CACGATGTGCCCAGC**G**CCAACTTCACTACCA (SEQ ID NO:104),
AATTCGCCCTTCTAC**C**ACAGCCAGGAGGAAA (SEQ ID NO:105), and/or
AATTCGCCCTTCTAC**G**ACAGCCAGGAGGAAA (SEQ ID NO:106).
Polypeptides encoded by these polynucleotides are also provided.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 37 of SEQ ID NO:35 is a non-coding mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'G' to 'T' polynucleotide polymorphism located at nucleic acid 894 of SEQ ID NO:35 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 261 of SEQ ID NO:35 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'C' to 'G' polynucleotide polymorphism located at nucleic acid 873 of SEQ ID NO:35 is a silent mutation and does not change the amino acid sequence of the encoded polypeptide.

The predicted 'T' to 'C' polynucleotide polymorphism located at nucleic acid 1133 of SEQ ID NO:35 is a missense mutation resulting in a change in an encoding amino acid from 'L' to 'P' at amino acid position 352 of SEQ ID NO:36.

The predicted 'A' to 'G' polynucleotide polymorphism located at nucleic acid 1393 of SEQ ID NO:35 is a missense mutation resulting in a change in an encoding amino acid from 'T' to 'A' at amino acid position 439 of SEQ ID NO:36.

The present invention relates to isolated nucleic acid molecules comprising, or alternatively, consisting of all or a portion of the variant allele of the human K+alphaM1.v2 potassium channel alpha subunit gene (e.g., wherein reference or wildtype human K+alphaM1.v2 potassium channel alpha subunit gene is exemplified by SEQ ID NO:35). Preferred portions are at least 10, preferably at least 20, preferably at least 40, preferably at least 100, contiguous polynucleotides comprising anyone of the human K+alphaM1.v2 potassium channel alpha subunit gene alleles described herein and exemplified in Figures 13A-C (SEQ ID NO:119).

In one embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with the reference allele at nucleotide position 37, 90, 261, 873, 1133, and/or 1393 of SEQ ID NO:35 (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 37, 90, 261, 873, 1133, and/or 1393 of SEQ ID NO:35. The presence of the variant allele at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having

the reference allele at that position, or a greater likelihood of having more severe symptoms.

Conversely, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with the variant allele at nucleotide position 37, 90, 261, 873, 1133, and/or 1393 of SEQ ID NO:35 (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 37, 90, 261, 873, 1133, and/or 1393 of SEQ ID NO:35. The presence of the variant allele at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having the reference allele at that position, or a greater likelihood of having more severe symptoms.

The present invention also encompasses immunogenic and/or antigenic epitopes of the K+alphaM1.v2 polypeptide.

In preferred embodiments, the following immunogenic and/or antigenic epitope polypeptide is encompassed by the present invention: amino acid residues from about amino acid 211 to about amino acid 228, from about amino acid 211 to about amino acid 219, from about amino acid 220 to about amino acid 228, from about amino acid 319 to about amino acid 334, from about amino acid 319 to about amino acid 327, from about amino acid 326 to about amino acid 334, from about amino acid 496 to about amino acid 504, from about amino acid 501 to about amino acid 509 of SEQ ID NO:36 (Figures 7A-C). In this context, the term "about" may be construed to mean 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids beyond the N-terminus and/or C-terminus of the above referenced polypeptide. Polynucleotides encoding this polypeptide are also provided.

As referenced elsewhere herein, the K+alphaM1.v2 polypeptide was predicted to comprise 6 transmembrane domains using the Tmphred program within the Vector NTI suite of programs. The predicted transmembrane domains have been termed TM1 thru TM6 and are located at about amino acid 156 to about amino acid 178 (TM1); from about amino acid 261 to about amino acid 279 (TM2), from about amino acid 333 to about amino acid 352 (TM3), from about amino acid 410 to about amino acid 430 (TM4), from about amino acid 443 to about amino acid 461 (TM5), and from about amino acid 472 to about amino acid 491 (TM6) of SEQ ID NO:36 (Figures 7A-

C). In this context, the term "about" may be construed to mean 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids beyond the N-Terminus and/or C-terminus of the above referenced polypeptide.

In preferred embodiments, the following transmembrane domain polypeptides are encompassed by the present invention: AVFQLVYNFYLSGVLLVLDGLCP (SEQ ID NO:58), AIGVASSTFVLVSVVALAL (SEQ ID NO:59), SALNLVDLVAILPLYLQLLL (SEQ ID NO:60), QVGCLLLFIAMGIFTFSAAVY (SEQ ID NO:61), TIPHSWWWAAVSISTVGYG (SEQ ID NO:62), and/or FFAFLCIAFGIILNGMPISI (SEQ ID NO:63). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these K+alphaM1.v2 transmembrane domain polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The present invention also encompasses the polypeptide sequences that intervene between each of the predicted K+alphaM1.v2 transmembrane domains. Since these regions are solvent accessible either extracellularly or intracellularly, they are particularly useful for designing antibodies specific to each region. Such antibodies may be useful as antagonists or agonists of the K+alphaM1.v2 full-length polypeptide and may modulate its activity.

In preferred embodiments, the following inter-transmembrane domain polypeptides are encompassed by the present invention: RRFLEELGYWGVRLKYTPRCCRICFEERRDELSERLKIQHELRAQAQVEEAEE LFRDMRFYGPQRRRLWNLMEKPFSSVAAK (SEQ ID NO:131), NTVEEMQQHSGQGEGGPDLRPILEHVEMLCMGFFTLEYLLRLASTPDLRRFA R (SEQ ID NO:132), ECFTGEGHQRGQTVGSVGKVGQVLRVMRLMRIFRILKLARHSTGLRAFGFTL RQCYQ (SEQ ID NO:133), SVEHDVPSANFT (SEQ ID NO:134), and/or DMYPETHLGR (SEQ ID NO:135). The present invention also encompasses the use of these K+alphaM1.v2 intertransmembrane domain polypeptides, and fragments thereof, as immunogenic and/or antigenic epitopes as described elsewhere herein.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1857 of SEQ ID NO:35, b is an integer between 15 to 1871, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a+14.

Table I

Gene No.	CDNA CloneID	ATCC Deposit No. Z and Date	Vector	NT SEQ ID. No. X	Total NT Seq of Clone	5' NT of Start Codon of ORF	3' NT of ORF	AA Seq ID No. Y	Total AA of ORF
1.	K+alphaM1 (BAC15, clone E1, clone Bb1-E3)	PTA-2766 12/08/00	PSport1	1	2850	883	2517	2	545
2.	K+alphaM1. v1 (BAC15-FL2A)	PTA-2966 01/24/01	PSport1	33	1871	79	1713	34	545
3.	K+alphaM1. v2 (BAC15-FL2B)	N/A	PSport1	35	1871	79	1713	36	545

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X"was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually several overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC deposit No:PTA-2766 and Date." "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq. Of Clone" refers to the total number of nucleotides in the clone contig identified by "Gene No." The deposited clone may contain all or most of

the sequence of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon of ORF."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The total number of amino acids within the open reading frame of SEQ ID NO:Y is identified as "Total AA of ORF".

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further herein. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the proteins encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides may cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted

translated amino acid sequence identified as SEQ ID NO:Y,but also a sample of plasmid DNA containing a cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited cDNA, collecting the protein, and determining its sequence.

The present invention also relates to the genes corresponding to SEQ ID NO:X,SEQ ID NO:Y,or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are species homologs, allelic variants, and/or orthologs. The skilled artisan could, using procedures well-known in the art, obtain the polynucleotide sequence corresponding to full-length genes (including, but not limited to the full-length coding region), allelic variants, splice variants, orthologs, and/or species homologues of genes corresponding to SEQ ID NO:X,SEQ ID NO:Y,or a deposited clone, relying on the sequence from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologues may be isolated and identified by making suitable probes or primers which correspond to the 5', 3', or internal regions of the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the protein, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using protocols described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the full-length form of the protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the sequence identified as SEQ ID NO:X, and/or a cDNA provided in ATCC Deposit No. Z:. The present invention also provides a polypeptide comprising, or alternatively consisting of, the sequence identified as SEQ ID NO:Y, and/or a polypeptide encoded by the cDNA provided in ATCC deposit No:PTA-2766. The present invention also provides polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y, and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit No:PTA-2766.

Preferably, the present invention is directed to a polynucleotide comprising, or alternatively consisting of, the sequence identified as SEQ ID NO:X, and/or a cDNA provided in ATCC Deposit No.: that is less than, or equal to, a polynucleotide sequence that is 5 mega basepairs, 1 mega basepairs, 0.5 mega basepairs, 0.1 mega basepairs, 50,000 basepairs, 20,000 basepairs, or 10,000 basepairs in length.

The present invention encompasses polynucleotides with sequences complementary to those of the polynucleotides of the present invention disclosed herein. Such sequences may be complementary to the sequence disclosed as SEQ ID NO:X, the sequence contained in a deposit, and/or the nucleic acid sequence encoding the sequence disclosed as SEQ ID NO:2.

The present invention also encompasses polynucleotides capable of hybridizing, preferably under reduced stringency conditions, more preferably under stringent conditions, and most preferably under highly stingent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in Table 2 below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

TABLE 2

Stringency Condition	Polynucleotide Hybrid±	Hybrid Length (bp) ‡	Hyridization Temperature and Buffer†	Wash Temperature and Buffer †
A	DNA:DNA	> or equal to 50	65°C; 1xSSC –or- 42°C; 1xSSC, 50% formamide	65°C; 0.3xSSC
B	DNA:DNA	< 50	Tb*; 1xSSC	Tb*; 1xSSC
C	DNA:RNA	> or equal to 50	67°C; 1xSSC –or- 45°C; 1xSSC, 50% formamide	67°C; 0.3xSSC
D	DNA:RNA	< 50	Td*; 1xSSC	Td*; 1xSSC
E	RNA:RNA	> or equal to 50	70°C; 1xSSC –or- 50°C; 1xSSC, 50% formamide	70°C; 0.3xSSC
F	RNA:RNA	< 50	Tf*; 1xSSC	Tf*; 1xSSC
G	DNA:DNA	> or equal to 50	65°C; 4xSSC –or- 45°C; 4xSSC, 50% formamide	65°C; 1xSSC
H	DNA:DNA	< 50	Th*; 4xSSC	Th*; 4xSSC
I	DNA:RNA	> or equal to 50	67°C; 4xSSC –or- 45°C; 4xSSC, 50% formamide	67°C; 1xSSC
J	DNA:RNA	< 50	Tj*; 4xSSC	Tj*; 4xSSC
K	RNA:RNA	> or equal to 50	70°C; 4xSSC –or- 40°C; 6xSSC, 50% formamide	67°C; 1xSSC
L	RNA:RNA	< 50	Tl*; 2xSSC	Tl*; 2xSSC
M	DNA:DNA	> or equal to 50	50°C; 4xSSC –or- 40°C 6xSSC, 50% formamide	50°C; 2xSSC

Stringency Condition	Polynucleotide Hybrid±	Hybrid Length (bp) ‡	Hyridization Temperature and Buffer†	Wash Temperature and Buffer †
N	DNA:DNA	< 50	Tn*; 6xSSC	Tn*; 6xSSC
O	DNA:RNA	> or equal to 50	55°C; 4xSSC –or- 42°C; 6xSSC, 50% formamide	55°C; 2xSSC
P	DNA:RNA	< 50	Tp*; 6xSSC	Tp*; 6xSSC
Q	RNA:RNA	> or equal to 50	60°C; 4xSSC –or- 45°C; 6xSSC, 50% formamide	60°C; 2xSSC
R	RNA:RNA	< 50	Tr*; 4xSSC	Tr*; 4xSSC

5

‡: The "hybrid length" is the anticipated length for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucletotide of unknown sequence, the hybrid is assumed to be that of the hybridizing polynucleotide of the present invention. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity. Methods of aligning two or more polynucleotide sequences and/or determining the percent identity between two polynucleotide sequences are well known in the art (e.g., MegAlign program of the DNA*Star suite of programs, etc).

†: SSPE (1xSSPE is 0.15M NaCl, 10mM NaH2PO4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl anmd 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete. The hydridizations and washes may additionally include 5X Denhardt's reagent, .5-1.0% SDS, 100ug/ml denatured, fragmented salmon sperm DNA, 0.5% sodium pyrophosphate, and up to 50% formamide.

*Tb – Tr: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature Tm of the hybrids there Tm is determined according to the following equations. For hybrids less than 18 base pairs in length, Tm(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, $Tm(°C) = 81.5 + 16.6(\log_{10}[Na+]) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and [Na+] is the concentration of sodium ions in the hybridization buffer ([NA+] for 1xSSC = .165 M).

±: The present invention encompasses the substitution of any one, or more DNA or RNA hybrid partners with either a PNA, or a modified polynucleotide. Such modified polynucleotides are known in the art and are more particularly described elsewhere herein.

Additional examples of stringency conditions for polynucleotide hybridization are provided, for example, in Sambrook, J., E.F. Fritsch, and T.Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Current Protocols in Molecular

Biology, 1995, F.M., Ausubel et al., eds, John Wiley and Sons, Inc., sections 2.10 and 6.3-6.4, which are hereby incorporated by reference herein.

Preferably, such hybridizing polynucleotides have at least 70% sequence identity (more preferably, at least 80% identity; and most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which they hybridize, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps. The determination of identity is well known in the art, and discussed more specifically elsewhere herein.

The invention encompasses the application of PCR methodology to the polynucleotide sequences of the present invention, the clone deposited with the ATCC, and/or the cDNA encoding the polypeptides of the present invention. PCR techniques for the amplification of nucleic acids are described in US Patent No. 4, 683, 195 and Saiki et al., Science, 239:487-491 (1988). PCR, for example, may include the following steps, of denaturation of template nucleic acid (if double-stranded), annealing of primer to target, and polymerization. The nucleic acid probed or used as a template in the amplification reaction may be genomic DNA, cDNA, RNA, or a PNA. PCR may be used to amplify specific sequences from genomic DNA, specific RNA sequence, and/or cDNA transcribed from mRNA. References for the general use of PCR techniques, including specific method parameters, include Mullis et al., Cold Spring Harbor Symp. Quant. Biol., 51:263, (1987), Ehrlich (ed), PCR Technology, Stockton Press, NY, 1989; Ehrlich et al., Science, 252:1643-1650, (1991); and "PCR Protocols, A Guide to Methods and Applications", Eds., Innis et al., Academic Press, New York, (1990).

Signal Sequences

The present invention also encompasses mature forms of the polypeptide comprising, or alternatively consisting of, the polypeptide sequence of SEQ ID NO:Y, the polypeptide encoded by the polynucleotide described as SEQ ID NO:X, and/or the polypeptide sequence encoded by a cDNA in the deposited clone. The present invention also encompasses polynucleotides encoding mature forms of the present

invention, such as, for example the polynucleotide sequence of SEQ ID NO:X, and/or the polynucleotide sequence provided in a cDNA of the deposited clone.

According to the signal hypothesis, proteins secreted by eukaryotic cells have a signal or secretary leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most eukaryotic cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

The established method for identifying the location of signal sequences, in addition, to their cleavage sites has been the SignalP program (v1.1) developed by Henrik Nielsen et al., Protein Engineering 10:1-6 (1997). The program relies upon the algorithm developed by von Heinje, though provides additional parameters to increase the prediction accuracy.

More recently, a hidden Markov model has been developed (H. Neilson, et al., Ismb 1998;6:122-30), which has been incorporated into the more recent SignalP (v2.0). This new method increases the ability to identify the cleavage site by discriminating between signal peptides and uncleaved signal anchors. The present invention encompasses the application of the method disclosed therein to the

prediction of the signal peptide location, including the cleavage site, to any of the polypeptide sequences of the present invention.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the polypeptide of the present invention may contain a signal sequence. Polypeptides of the invention which comprise a signal sequence have an N-terminus beginning within 5 residues (i.e., + or - 5 residues, or Preferably at the –5, -4, -3, -2, -1, +1, +2, +3, +4, or +5 residue) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as desribed below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

The present invention also encompases variants (e.g., allelic variants, orthologs, etc.) of the polynucleotide sequence disclosed herein in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in the deposited clone.

The present invention also encompasses variants of the polypeptide sequence, and/or fragments therein, disclosed in SEQ ID NO:Y, a polypeptide encoded by the polunucleotide sequence in SEQ ID NO:X, and/or a polypeptide encoded by a cDNA in the deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a K+alphaM1 related polypeptide having an amino acid sequence as shown in the sequence listing and described in SEQ ID NO:X or the cDNA contained in ATCC deposit No:PTA-2766; (b) a nucleotide sequence encoding a mature K+alphaM1 related polypeptide having the amino acid sequence as shown in the sequence listing and described in SEQ ID NO:X or the cDNA contained in ATCC deposit No:PTA-2766; (c) a nucleotide sequence encoding a biologically active fragment of a K+alphaM1 related polypeptide having an amino acid sequence shown in the sequence listing and described in SEQ ID NO:X or the cDNA contained in ATCC deposit No:PTA-2766; (d) a nucleotide sequence encoding an antigenic fragment of a K+alphaM1 related polypeptide having an amino acid sequence sown in the sequence listing and described in SEQ ID NO:X or the cDNA contained in ATCC deposit No:PTA-2766; (e) a nucleotide sequence encoding a K+alphaM1 related polypeptide comprising the complete amino acid sequence encoded by a human cDNA plasmid containined in SEQ ID NO:X or the cDNA contained in ATCC deposit No:PTA-2766; (f) a nucleotide sequence encoding a mature K+alphaM1 realted polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA contained in ATCC deposit No:PTA-2766; (g) a nucleotide sequence encoding a biologically active fragement of a K+alphaM1 related polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA contained in ATCC deposit No:PTA-2766; (h) a nucleotide sequence encoding an antigenic fragment of a K+alphaM1 related polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA contained in ATCC deposit No:PTA-2766; (I) a nucleotide sequence complimentary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h), above.

The present invention is also directed to polynucleotide sequences which comprise, or alternatively consist of, a polynucleotide sequence which is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h), above. Polynucleotides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecule which comprise, or alternatively, consist of a polynucleotide which hybridizes under stringent conditions, or alternatively, under lower stringency conditions, to a polynucleotide in (a), (b), (c), (d), (e), (f), (g), or (h), above. Polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polypeptides.

Another aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively, consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a K+alphaM1 related polypeptide having an amino acid sequence as shown in the sequence listing and described in Table 1; (b) a nucleotide sequence encoding a mature K+alphaM1 related polypeptide having the amino acid sequence as shown in the sequence listing and described in Table 1; (c) a nucleotide sequence encoding a biologically active fragment of a K+alphaM1 related polypeptide having an amino acid sequence as shown in the sequence listing and described in Table 1; (d) a nucleotide sequence encoding an antigenic fragment of a K+alphaM1 related polypeptide having an amino acid sequence as shown in the sequence listing and described in Table 1; (e) a nucleotide sequence encoding a K+alphaM1 related polypeptide comprising the complete amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (f) a nucleotide sequence encoding a mature K+alphaM1 related polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1: (g) a nucleotide sequence encoding a biologically active fragment of a K+alphaM1 related polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table 1; (h) a nucleotide sequence encoding an antigenic

fragment of a K+alphaM1 related polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC deposit and described in Table 1; (i) a nucleotide sequence complimentary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h) above.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively, consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h), above.

The present invention encompasses polypeptide sequences which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 98%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, the following non-limited examples, the polypeptide sequence identified as SEQ ID NO:Y, the polypeptide sequence encoded by a cDNA provided in the deposited clone, and/or polypeptide fragments of any of the polypeptides provided herein. Polynucleotides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecule which comprise, or alternatively, consist of a polynucleotide which hybridizes under stringent conditions, or alternatively, under lower stringency conditions, to a polynucleotide in (a), (b), (c), (d), (e), (f), (g), or (h), above. Polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polypeptides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 98%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X,a polypeptide sequence encoded by the cDNA in cDNA plasmid:Z, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein). Polynucleotides which hybridize to the complement of the nucleic acid molecules encoding these polypeptides under stringent hybridization conditions or alternatively, under lower stringency conditions,

are also encompasses by the present invention, as are the polypeptides encoded by these polynucleotides.

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence referenced in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the CLUSTALW computer program (Thompson, J.D., et al., Nucleic Acids Research, 2(22):4673-4680, (1994)), which is based on the algorithm of Higgins, D.G., et al., Computer Applications in the Biosciences (CABIOS), 8(2):189-191, (1992). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. However, the CLUSTALW algorithm automatically converts U's to T's when comparing RNA sequences to DNA sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a CLUSTALW alignment of DNA sequences to calculate percent identity via pairwise alignments are: Matrix=IUB, k-tuple=1, Number of Top Diagonals=5, Gap Penalty=3, Gap Open Penalty 10, Gap Extension Penalty=0.1, Scoring Method=Percent, Window Size=5 or the length of the subject nucleotide sequence, whichever is shorter. For multiple

alignments, the following CLUSTALW parameters are preferred: Gap Opening Penalty=10; Gap Extension Parameter=0.05; Gap Separation Penalty Range=8; End Gap Separation Penalty=Off; % Identity for Alignment Delay=40%; Residue Specific Gaps:Off; Hydrophilic Residue Gap=Off; and Transition Weighting=0. The pairwise and multple alignment parameters provided for CLUSTALW above represent the default parameters as provided with the AlignX software program (Vector NTI suite of programs, version 6.0).

The present invention encompasses the application of a manual correction to the percent identity results, in the instance where the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions. If only the local pairwise percent identity is required, no manual correction is needed. However, a manual correction may be applied to determine the global percent identity from a global polynucleotide alignment. Percent identity calculations based upon global polynucleotide alignments are often preferred since they reflect the percent identity between the polynucleotide molecules as a whole (i.e., including any polynucleotide overhangs, not just overlapping regions), as opposed to, only local matching polynucleotides. Manual corrections for global percent identity determinations are required since the CLUSTALW program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the CLUSTALW sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above CLUSTALW program using the specified parameters, to arrive at a final percent identity score. This corrected score may be used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the CLUSTALW alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the

subject sequence and therefore, the CLUSTALW alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the CLUSTALW program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by CLUSTALW is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are required for the purposes of the present invention.

In addition to the above method of aligning two or more polynucleotide or polypeptide sequences to arrive at a percent identity value for the aligned sequences, it may be desirable in some circumstances to use a modified version of the CLUSTALW algorithm which takes into account known structural features of the sequences to be aligned, such as for example, the SWISS-PROT designations for each sequence. The result of such a modifed CLUSTALW algorithm may provide a more accurate value of the percent identity for two polynucleotide or polypeptide sequences. Support for such a modified version of CLUSTALW is provided within the CLUSTALW algorithm and would be readily appreciated to one of skill in the art of bioinformatics.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the mRNA to those preferred by a bacterial host such as E. coli).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem... 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein (Dobeli et al., J. Biotechnology 7:199-216 (1988)).

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem.. 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the protein will likely be retained when less than the majority of the residues of the protein are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can

readily be determined by routine methods described herein and otherwise known in the art.

Alternatively, such N-terminus or C-terminus deletions of a polypeptide of the present invention may, in fact, result in a significant increase in one or more of the biological activities of the polypeptide(s). For example, biological activity of many polypeptides are governed by the presence of regulatory domains at either one or both termini. Such regulatory domains effectively inhibit the biological activity of such polypeptides in lieu of an activation event (e.g., binding to a cognate ligand or receptor, phosphorylation, proteolytic processing, etc.). Thus, by eliminating the regulatory domain of a polypeptide, the polypeptide may effectively be rendered biologically active in the absence of an activation event.

Thus, the invention further includes polypeptide variants that show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved.

The invention encompasses polypeptides having a lower degree of identity but having sufficient similarity so as to perform one or more of the same functions performed by the polypeptide of the present invention. Similarity is determined by conserved amino acid substitution. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics (e.g., chemical properties). According to Cunningham et al above, such conservative substitutions are likely to be phenotypically silent. Additional guidance concerning which amino acid changes are likely to be phenotypically silent are found in Bowie et al., Science 247:1306-1310 (1990).

Tolerated conservative amino acid substitutions of the present invention involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Both identity and similarity can be readily calculated by reference to the following publications: Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Informatics Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press,New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991.

In addition, the present invention also encompasses substitution of amino acids based upon the probability of an amino acid substitution resulting in conservation of function. Such probabilities are determined by aligning multiple

genes with related function and assessing the relative penalty of each substitution to proper gene function. Such probabilities are often described in a matrix and are used by some algorithms (e.g., BLAST, CLUSTALW, GAP, etc.) in calculating percent similarity wherein similarity refers to the degree by which one amino acid may substitute for another amino acid without lose of function. An example of such a matrix is the PAM250 or BLOSUM62 matrix.

Aside from the canonical chemically conservative substitutions referenced above, the invention also encompasses substitutions which are typically not classified as conservative, but that may be chemically conservative under certain circumstances. Analysis of enzymatic catalysis for proteases, for example, has shown that certain amino acids within the active site of some enzymes may have highly perturbed pKa's due to the unique microenvironment of the active site. Such perturbed pKa's could enable some amino acids to substitute for other amino acids while conserving enzymatic structure and function. Examples of amino acids that are known to have amino acids with perturbed pKa's are the Glu-35 residue of Lysozyme, the Ile-16 residue of Chymotrypsin, the His-159 residue of Papain, etc. The conservation of function relates to either anomalous protonation or anomalous deprotonation of such amino acids, relative to their canonical, non-perturbed pKa. The pKa perturbation may enable these amino acids to actively participate in general acid-base catalysis due to the unique ionization environment within the enzyme active site. Thus, substituting an amino acid capable of serving as either a general acid or general base within the microenvironment of an enzyme active site or cavity, as may be the case, in the same or similar capacity as the wild-type amino acid, would effectively serve as a conservative amino substitution.

Besides conservative amino acid substitution, variants of the present invention include, but are not limited to, the following: (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion

region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

Moreover, the invention further includes polypeptide variants created through the application of molecular evolution ("DNA Shuffling") methodology to the polynucleotide disclosed as SEQ ID NO:X, the sequence of the clone submitted in a deposit, and/or the cDNA encoding the polypeptide disclosed as SEQ ID NO:Y. Such DNA Shuffling technology is known in the art and more particularly described elsewhere herein (e.g., WPC, Stemmer, PNAS, 91:10747, (1994)), and in the Examples provided herein).

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is directed to polynucleotide fragments of the polynucleotides of the invention, in addition to polypeptides encoded therein by said polynucleotides and/or fragments.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context “about” includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus, or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X,or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More

preferably, these polynucleotides can be used as probes or primers as discussed herein. Also encompassed by the present invention are polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions, as are the polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the full-length protein. Further preferred polypeptide fragments include the full-length protein having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of the full-length polypeptide. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the full-length protein. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions,

surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

In a preferred embodiment, the functional activity displayed by a polypeptide encoded by a polynucleotide fragment of the invention may be one or more biological activities typically associated with the full-length polypeptide of the invention. Illustrative of these biological activities includes the fragments ability to bind to at least one of the same antibodies which bind to the full-length protein, the fragments ability to interact with at lease one of the same proteins which bind to the full-length, the fragments ability to elicit at least one of the same immune responses as the full-length protein (i.e., to cause the immune system to create antibodies specific to the same epitope, etc.), the fragments ability to bind to at least one of the same polynucleotides as the full-length protein, the fragments ability to bind to a receptor of the full-length protein, the fragments ability to bind to a ligand of the full-length protein, and the fragments ability to multimerize with the full-length protein. However, the skilled artisan would appreciate that some fragments may have biological activities which are desirable and directly inapposite to the biological activity of the full-length protein. The functional activity of polypeptides of the invention, including fragments, variants, derivatives, and analogs thereof can be determined by numerous methods available to the skilled artisan, some of which are described elsewhere herein.

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y,or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that

hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:1), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100

amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free

or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991,

Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment,

or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, monovalent, bispecific, heteroconjugate, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Moreover, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules, as well as, antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation of the animal or plant, and may have less non-specific tissue binding than an intact antibody (Wahl et al., J. Nucl. Med.. 24:316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human,

murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homologue of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologues of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than

85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10-2 M, 10-2 M, 5 X 10-3 M, 10-3 M, 5 X 10-4 M, 10-4 M, 5 X 10-5 M, 10-5 M, 5 X 10-6 M, 10-6M, 5 X 10-7 M, 107 M, 5 X 10-8 M, 10-8 M, 5 X 10-9 M, 10-9 M, 5 X 10-10 M, 10-10 M, 5 X 10-11 M, 10-11 M, 5 X 10-12 M, 10-12 M, 5 X 10-13 M, 10-13 M, 5 X 10-14 M, 10-14 M, 5 X 10-15 M, or 10-15 M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or

serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem... 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring

levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionucleotides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art.

The antibodies of the present invention may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan (Harlow, et al., Antibodies: A Laboratory Manual, (Cold spring Harbor Laboratory Press, 2nd ed. (1988), which is hereby incorporated herein by reference in its entirety). For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. The administration of the

polypeptides of the present invention may entail one or more injections of an immunizing agent and, if desired, an adjuvant. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art. For the purposes of the invention, "immunizing agent" may be defined as a polypeptide of the invention, including fragments, variants, and/or derivatives thereof, in addition to fusions with heterologous polypeptides and other forms of the polypeptides described herein.

Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections, though they may also be given intramuscularly, and/or through IV). The immunizing agent may include polypeptides of the present invention or a fusion protein or variants thereof. Depending upon the nature of the polypeptides (i.e., percent hydrophobicity, percent hydrophilicity, stability, net charge, isoelectric point etc.), it may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Such conjugation includes either chemical conjugation by derivitizing active chemical functional groups to both the polypeptide of the present invention and the immunogenic protein such that a covalent bond is formed, or through fusion-protein based methodology, or other methods known to the skilled artisan. Examples of such immunogenic proteins include, but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum. Additional examples of adjuvants which may be employed includes the MPL-TDM adjuvant

(monophosphoryl lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

The antibodies of the present invention may comprise monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975) and U.S. Pat. No. 4,376,110, by Harlow, et al., Antibodies: A Laboratory Manual, (Cold spring Harbor Laboratory Press, 2nd ed. (1988), by Hammerling, et al., Monoclonal Antibodies and T-Cell Hybridomas (Elsevier, N.Y., (1981)), or other methods known to the artisan. Other examples of methods which may be employed for producing monoclonal antibodies includes, but are not limited to, the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

In a hybridoma method, a mouse, a humanized mouse, a mouse with a human immune system, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized in vitro.

The immunizing agent will typically include polypeptides of the present invention or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986), pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably

contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. As inferred throughout the specification, human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the polypeptides of the present invention. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbant assay (ELISA). Such techniques are known in the art and within the skill of the artisan. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollart, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, supra). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640. Alternatively, the hybridoma cells may be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-sepharose, hydroxyapatite

chromatography, gel exclusion chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The skilled artisan would acknowledge that a variety of methods exist in the art for the production of monoclonal antibodies and thus, the invention is not limited to their sole production in hydridomas. For example, the monoclonal antibodies may be made by recombinant DNA methods, such as those described in US patent No. 4, 816, 567. In this context, the term "monoclonal antibody" refers to an antibody derived from a single eukaryotic, phage, or prokaryotic clone. The DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies, or such chains from human, humanized, or other sources). The hydridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transformed into host cells such as Simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (US Patent No. 4, 816, 567; Morrison et al, supra) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies.

Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples herein. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to

recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988).

For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101;

and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332). Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the methods of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Reichmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (US Patent No. 4, 816, 567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possible some FR residues are substituted from analogous sites in rodent antibodies.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988)l and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety. The techniques of cole et al., and Boerder et al., are also available for the preparation of human monoclonal

antibodies (cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Riss, (1985); and Boerner et al., J. Immunol., 147(1):86-95, (1991)).

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA), Genpharm (San Jose, CA), and Medarex, Inc. (Princeton, NJ) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and creation of an antibody repertoire. This approach is described, for example, in US patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,106, and in the following scientific publications: Marks et al., Biotechnol., 10:779-783 (1992); Lonberg et al., Nature 368:856-859 (1994); Fishwild et al., Nature Biotechnol., 14:845-51 (1996); Neuberger, Nature Biotechnol., 14:826 (1996); Lonberg and Huszer, Intern. Rev. Immunol., 13:65-93 (1995).

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that “mimic” polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that “mimic” the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

The antibodies of the present invention may be bispecific antibodies. Bispecific antibodies are monoclonal, Preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present invention, one of the binding specificities may be directed towards a polypeptide of the present

invention, the other may be for any other antigen, and preferably for a cell-surface protein, receptor, receptor subunit, tissue-specific antigen, virally derived protein, virally encoded envelope protein, bacterially derived protein, or bacterial surface protein, etc.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transformed into a suitable host organism. For further details of generating bispecific antibodies see, for example Suresh et al., Meth. In Enzym., 121:210 (1986).

Heteroconjugate antibodies are also contemplated by the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4, 676, 980), and for the treatment of HIV infection (WO 91/00360; WO 92/20373; and EP03089). It is contemplated that the antibodies may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioester bond.

Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in US Patent No. 4,676,980.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:2.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the

antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes

from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example,

in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells)

harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem... 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable

expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY

(1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to

a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their

entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem... 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson

et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine,

cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon, ß-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., Int. Immunol., 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents

In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

The present invention also encompasses the creation of synthetic antibodies directed against the polypeptides of the present invention. One example of synthetic antibodies is described in Radrizzani, M., et al., Medicina, (Aires), 59(6):753-8, (1999)). Recently, a new class of synthetic antibodies has been described and are referred to as molecularly imprinted polymers (MIPs) (Semorex, Inc.). Antibodies, peptides, and enzymes are often used as molecular recognition elements in chemical and biological sensors. However, their lack of stability and signal transduction mechanisms limits their use as sensing devices. Molecularly imprinted polymers (MIPs) are capable of mimicking the function of biological receptors but with less stability constraints. Such polymers provide high sensitivity and selectivity while maintaining excellent thermal and mechanical stability. MIPs have the ability to bind to small molecules and to target molecules such as organics and proteins' with equal or greater potency than that of natural antibodies. These "super" MIPs have higher affinities for their target and thus require lower concentrations for efficacious binding.

During synthesis, the MIPs are imprinted so as to have complementary size, shape, charge and functional groups of the selected target by using the target molecule itself (such as a polypeptide, antibody, etc.), or a substance having a very similar structure, as its "print" or "template." MIPs can be derivatized with the same reagents afforded to antibodies. For example, fluorescent 'super' MIPs can be coated onto beads or wells for use in highly sensitive separations or assays, or for use in high throughput screening of proteins.

Moreover, MIPs based upon the structure of the polypeptide(s) of the present invention may be useful in screening for compounds that bind to the polypeptide(s) of the invention. Such a MIP would serve the role of a synthetic "receptor" by minimicking the native architecture of the polypeptide. In fact, the ability of a MIP to serve the role of a synthetic receptor has already been demonstrated for the estrogen receptor (Ye, L., Yu, Y., Mosbach, K, Analyst., 126(6):760-5, (2001); Dickert, F, L., Hayden, O., Halikias, K, P, Analyst., 126(6):766-71, (2001)). A synthetic receptor may either be mimicked in its entirety (e.g., as the entire protein), or mimicked as a series of short peptides corresponding to the protein (Rachkov, A., Minoura, N, Biochim, Biophys, Acta., 1544(1-2):255-66, (2001)). Such a synthetic receptor MIPs may be employed in any one or more of the screening methods described elsewhere herein.

MIPs have also been shown to be useful in "sensing" the presence of its mimicked molecule (Cheng, Z., Wang, E., Yang, X, Biosens, Bioelectron., 16(3):179-85, (2001) ; Jenkins, A, L., Yin, R., Jensen, J. L, Analyst., 126(6):798-802, (2001) ; Jenkins, A, L., Yin, R., Jensen, J. L, Analyst., 126(6):798-802, (2001)). For example, a MIP designed using a polypeptide of the present invention may be used in assays designed to identify, and potentially quantitate, the level of said polypeptide in a sample. Such a MIP may be used as a substitute for any component described in the assays, or kits, provided herein (e.g., ELISA, etc.).

A number of methods may be employed to create MIPs to a specific receptor, ligand, polypeptide, peptide, organic molecule. Several preferred methods are described by Esteban et al in J. Anal, Chem., 370(7):795-802, (2001), which is hereby

incorporated herein by reference in its entirety in addition to any references cited therein. Additional methods are known in the art and are encompassed by the present invention, such as for example, Hart, B, R., Shea, K, J. J. Am. Chem, Soc., 123(9):2072-3, (2001); and Quaglia, M., Chenon, K., Hall, A, J., De, Lorenzi, E., Sellergren, B, J. Am. Chem, Soc., 123(10):2146-54, (2001); which are hereby incorporated by reference in their entirety herein.

Uses for Antibodies directed against polypeptides of the invention

The antibodies of the present invention have various utilities. For example, such antibodies may be used in diagnostic assays to detect the presence or quantification of the polypeptides of the invention in a sample. Such a diagnostic assay may be comprised of at least two steps. The first, subjecting a sample with the antibody, wherein the sample is a tissue (e.g., human, animal, etc.), biological fluid (e.g., blood, urine, sputum, semen, amniotic fluid, saliva, etc.), biological extract (e.g., tissue or cellular homogenate, etc.), a protein microchip (e.g., See Arenkov P, et al., Anal Biochem., 278(2):123-131 (2000)), or a chromatography column, etc. And a second step involving the quantification of antibody bound to the substrate. Alternatively, the method may additionally involve a first step of attaching the antibody, either covalently, electrostatically, or reversibly, to a solid support, and a second step of subjecting the bound antibody to the sample, as defined above and elsewhere herein.

Various diagnostic assay techniques are known in the art, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogenous phases (Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc., (1987), pp147-158). The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as 2H, 14C, 32P, or 125I, a florescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase, green fluorescent protein, or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety

may be employed, including those methods described by Hunter et al., Nature, 144:945 (1962); Dafvid et al., Biochem., 13:1014 (1974); Pain et al., J. Immunol. Metho., 40:219(1981); and Nygren, J. Histochem. And Cytochem., 30:407 (1982).

Antibodies directed against the polypeptides of the present invention are useful for the affinity purification of such polypeptides from recombinant cell culture or natural sources. In this process, the antibodies against a particular polypeptide are immobilized on a suitable support, such as a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the polypeptides to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except for the desired polypeptides, which are bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the desired polypeptide from the antibody.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison et al., Cell, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein

sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the

incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses Of Antibodies

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of

the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10-2 M, 10-2 M, 5 X 10-3 M, 10-3 M, 5 X 10-4 M, 10-4 M, 5 X 10-5 M, 10-5 M, 5 X 10-6 M, 10-6 M, 5 X 10-7 M, 10-7 M, 5 X 10-8 M, 10-8 M, 5 X 10-9 M, 10-9 M, 5 X 10-10 M, 10-10 M, 5 X 10-11 M, 10-11 M, 5 X 10-12 M, 10-12 M, 5 X 10-13 M, 10- 13 M, 5 X 10-14 M, 10-14 M, 5 X 10-15 M, and 10-15 M.

Antibodies directed against polypeptides of the present invention are useful for inhibiting allergic reactions in animals. For example, by administering a therapeutically acceptable dose of an antibody, or antibodies, of the present invention, or a cocktail of the present antibodies, or in combination with other antibodies of varying sources, the animal may not elicit an allergic response to antigens.

Likewise, one could envision cloning the gene encoding an antibody directed against a polypeptide of the present invention, said polypeptide having the potential to elicit an allergic and/or immune response in an organism, and transforming the organism with said antibody gene such that it is expressed (e.g., constitutively, inducibly, etc.) in the organism. Thus, the organism would effectively become resistant to an allergic response resulting from the ingestion or presence of such an immune/allergic reactive polypeptide. Moreover, such a use of the antibodies of the present invention may have particular utility in preventing and/or ameliorating autoimmune diseases and/or disorders, as such conditions are typically a result of antibodies being directed against endogenous proteins. For example, in the instance where the polypeptide of the present invention is responsible for modulating the immune response to auto-antigens, transforming the organism and/or individual with a construct comprising any of the promoters disclosed herein or otherwise known in the art, in addition, to a polynucleotide encoding the antibody directed against the polypeptide of the present invention could effective inhibit the organisms immune system from eliciting an immune response to the auto-antigen(s). Detailed descriptions of therapeutic and/or gene therapy applications of the present invention are provided elsewhere herein.

Alternatively, antibodies of the present invention could be produced in a plant (e.g., cloning the gene of the antibody directed against a polypeptide of the present invention, and transforming a plant with a suitable vector comprising said gene for constitutive expression of the antibody within the plant), and the plant subsequently ingested by an animal, thereby conferring temporary immunity to the animal for the specific antigen the antibody is directed towards (See, for example, US Patent Nos. 5,914,123 and 6,034,298).

In another embodiment, antibodies of the present invention, preferably polyclonal antibodies, more preferably monoclonal antibodies, and most preferably single-chain antibodies, can be used as a means of inhibiting gene expression of a particular gene, or genes, in a human, mammal, and/or other organism. See, for example, International Publication Number WO 00/05391, published 2/3/00, to Dow Agrosciences LLC. The application of such methods for the antibodies of the present invention are known in the art, and are more particularly described elsewhere herein.

In yet another embodiment, antibodies of the present invention may be useful for multimerizing the polypeptides of the present invention. For example, certain proteins may confer enhanced biological activity when present in a multimeric state (i.e., such enhanced activity may be due to the increased effective concentration of such proteins whereby more protein is available in a localized location).

Antibody-based Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other

desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem... 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl.

Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Compositions

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical

composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem.. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material,

including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g.,

Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with

routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other

species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging With Antibodies

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of

actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of

99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive

with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a

second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because certain proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. Similarly, peptide cleavage sites can be introduced in-between such peptide moieties, which could additionally be subjected to protease activity to remove said peptide(s) from the protein of the present invention. The addition of peptide moieties, including peptide cleavage sites, to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric

secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of the constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem... 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences (also referred to as “tags”). Due to the availability of antibodies specific to such “tags”, purification of the fused polypeptide of the invention, and/or its identification is significantly facilitated since antibodies specific to the polypeptides of the invention are not required. Such purification may be in the form of an affinity purification whereby an anti-tag antibody or another type of affinity matrix (e.g., anti-tag antibody attached to the matrix of a flow-thru column) that binds to the epitope tag is present. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984)).

The skilled artisan would acknowledge the existence of other “tags” which could be readily substituted for the tags referred to supra for purification and/or identification of polypeptides of the present invention (Jones C., et al., J Chromatogr

A. 707(1):3-22 (1995)). For example, the c-myc tag and the 8F9, 3C7, 6E10, G4m B7 and 9E10 antibodies thereto (Evan et al., Molecular and Cellular Biology 5:3610-3616 (1985)); the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky et al., Protein Engineering, 3(6):547-553 (1990), the Flag–peptide – i.e., the octapeptide sequence DYKDDDDK (SEQ ID NO:9), (Hopp et al., Biotech. 6:1204-1210 (1988); the KT3 epitope peptide (Martin et al., Science, 255:192-194 (1992)); a-tubulin epitope peptide (Skinner et al., J. Biol. Chem..., 266:15136-15166, (1991)); the T7 gene 10 protein peptide tag (Lutz-Freyermuth et al., Proc. Natl. Sci. USA, 87:6363-6397 (1990)), the FITC epitope (Zymed, Inc.), the GFP epitope (Zymed, Inc.), and the Rhodamine epitope (Zymed, Inc.).

The present invention also encompasses the attachment of up to nine codons encoding a repeating series of up to nine arginine amino acids to the coding region of a polynucleotide of the present invention. The invention also encompasses chemically derivitizing a polypeptide of the present invention with a repeating series of up to nine arginine amino acids. Such a tag, when attached to a polypeptide, has recently been shown to serve as a universal pass, allowing compounds access to the interior of cells without additional derivitization or manipulation (Wender, P., et al., unpublished data).

Protein fusions involving polypeptides of the present invention, including fragments and/or variants thereof, can be used for the following, non-limiting examples, subcellular localization of proteins, determination of protein-protein interactions via immunoprecipitation, purification of proteins via affinity chromatography, functional and/or structural characterization of protein. The present invention also encompasses the application of hapten specific antibodies for any of the uses referenced above for epitope fusion proteins. For example, the polypeptides of the present invention could be chemically derivatized to attach hapten molecules (e.g., DNP, (Zymed, Inc.)). Due to the availability of monoclonal antibodies specific to such haptens, the protein could be readily purified using immunoprecipation, for example.

Polypeptides of the present invention, including fragments and/or variants thereof, in addition to, antibodies directed against such polypeptides, fragments, and/or variants, may be fused to any of a number of known, and yet to be determined,

toxins, such as ricin, saporin (Mashiba H, et al., Ann. N. Y. Acad. Sci. 1999;886:233-5), or HC toxin (Tonukari NJ, et al., Plant Cell. 2000 Feb;12(2):237-248), for example. Such fusions could be used to deliver the toxins to desired tissues for which a ligand or a protein capable of binding to the polypeptides of the invention exists.

The invention encompasses the fusion of antibodies directed against polypeptides of the present invention, including variants and fragments thereof, to said toxins for delivering the toxin to specific locations in a cell, to specific tissues, and/or to specific species. Such bifunctional antibodies are known in the art, though a review describing additional advantageous fusions, including citations for methods of production, can be found in P.J. Hudson, Curr. Opp. In. Imm. 11:548-557, (1999); this publication, in addition to the references cited therein, are hereby incorporated by reference in their entirety herein. In this context, the term "toxin" may be expanded to include any heterologous protein, a small molecule, radionucleotides, cytotoxic drugs, liposomes, adhesion molecules, glycoproteins, ligands, cell or tissue-specific ligands, enzymes, of bioactive agents, biological response modifiers, anti-fungal agents, hormones, steroids, vitamins, peptides, peptide analogs, anti-allergenic agents, anti-tubercular agents, anti-viral agents, antibiotics, anti-protozoan agents, chelates, radioactive particles, radioactive ions, X-ray contrast agents, monoclonal antibodies, polyclonal antibodies and genetic material. In view of the present disclosure, one skilled in the art could determine whether any particular "toxin" could be used in the compounds of the present invention. Examples of suitable "toxins" listed above are exemplary only and are not intended to limit the "toxins" that may be used in the present invention.

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host

cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are

not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of

the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast Pichia pastoris is used to express the polypeptide of the present invention in a eukaryotic system. Pichia pastoris is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, Pichia pastoris must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (AOX1) is highly active. In the presence of methanol, alcohol oxidase produced from the AOX1 gene comprises up to approximately 30% of the total soluble protein in Pichia pastoris. See, Ellis, S.B., et al., Mol. Cell. Biol. 5:1111-21 (1985); Koutz, P.J, et al., Yeast 5:167-77 (1989); Tschopp, J.F., et al., Nucl. Acids Res. 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the AOX1 regulatory sequence is expressed at exceptionally high levels in Pichia yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a Pichea yeast system essentially as described in "Pichia Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong AOX1 promoter linked to the Pichia pastoris alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG, as required.

In another embodiment, high-level expression of a heterologous coding

sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., Nature, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-

butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein, the addition of epitope tagged peptide fragments (e.g., FLAG, HA, GST, thioredoxin, maltose binding protein, etc.), attachment of affinity tags such as biotin and/or streptavidin, the covalent attachment of chemical moieties to the amino acid backbone, N- or C-terminal processing of the polypeptides ends (e.g., proteolytic processing), deletion of the N-terminal methionine residue, etc.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and

may include one, two, three or more attached chemical moieties.

The invention further encompasses chemical derivitization of the polypeptides of the present invention, preferably where the chemical is a hydrophilic polymer residue. Exemplary hydrophilic polymers, including derivatives, may be those that include polymers in which the repeating units contain one or more hydroxy groups (polyhydroxy polymers), including, for example, poly(vinyl alcohol); polymers in which the repeating units contain one or more amino groups (polyamine polymers), including, for example, peptides, polypeptides, proteins and lipoproteins, such as albumin and natural lipoproteins; polymers in which the repeating units contain one or more carboxy groups (polycarboxy polymers), including, for example, carboxymethylcellulose, alginic acid and salts thereof, such as sodium and calcium alginate, glycosaminoglycans and salts thereof, including salts of hyaluronic acid, phosphorylated and sulfonated derivatives of carbohydrates, genetic material, such as interleukin-2 and interferon, and phosphorothioate oligomers; and polymers in which the repeating units contain one or more saccharide moieties (polysaccharide polymers), including, for example, carbohydrates.

The molecular weight of the hydrophilic polymers may vary, and is generally about 50 to about 5,000,000, with polymers having a molecular weight of about 100 to about 50,000 being preferred. The polymers may be branched or unbranched. More preferred polymers have a molecular weight of about 150 to about 10,000, with molecular weights of 200 to about 8,000 being even more preferred.

For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

Additional preferred polymers which may be used to derivatize polypeptides of the invention, include, for example, poly(ethylene glycol) (PEG), poly(vinylpyrrolidine), polyoxomers, polysorbate and poly(vinyl alcohol), with PEG

polymers being particularly preferred. Preferred among the PEG polymers are PEG polymers having a molecular weight of from about 100 to about 10,000. More preferably, the PEG polymers have a molecular weight of from about 200 to about 8,000, with PEG 2,000, PEG 5,000 and PEG 8,000, which have molecular weights of 2,000, 5,000 and 8,000, respectively, being even more preferred. Other suitable hydrophilic polymers, in addition to those exemplified above, will be readily apparent to one skilled in the art based on the present disclosure. Generally, the polymers used may include polymers that can be attached to the polypeptides of the invention via alkylation or acylation reactions.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the

N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminus) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As with the various polymers exemplified above, it is contemplated that the polymeric residues may contain functional groups in addition, for example, to those typically involved in linking the polymeric residues to the polypeptides of the present invention. Such functionalities include, for example, carboxyl, amine, hydroxy and thiol groups. These functional groups on the polymeric residues can be further reacted, if desired, with materials that are generally reactive with such functional groups and which can assist in targeting specific tissues in the body including, for example, diseased tissue. Exemplary materials which can be reacted with the additional functional groups include, for example, proteins, including antibodies, carbohydrates, peptides, glycopeptides, glycolipids, lectins, and nucleosides.

In addition to residues of hydrophilic polymers, the chemical used to derivatize the polypeptides of the present invention can be a saccharide residue. Exemplary saccharides which can be derived include, for example, monosaccharides or sugar alcohols, such as erythrose, threose, ribose, arabinose, xylose, lyxose, fructose, sorbitol, mannitol and sedoheptulose, with preferred monosaccharides being fructose, mannose, xylose, arabinose, mannitol and sorbitol; and disaccharides, such as lactose, sucrose, maltose and cellobiose. Other saccharides include, for example, inositol and ganglioside head groups. Other suitable saccharides, in addition to those exemplified above, will be readily apparent to one skilled in the art based on the present disclosure. Generally, saccharides which may be used for derivitization include saccharides that can be attached to the polypeptides of the invention via alkylation or acylation reactions.

Moreover, the invention also encompasses derivitization of the polypeptides of the present invention, for example, with lipids (including cationic, anionic,

polymerized, charged, synthetic, saturated, unsaturated, and any combination of the above, etc.). stabilizing agents.

The invention encompasses derivitization of the polypeptides of the present invention, for example, with compounds that may serve a stabilizing function (e.g., to increase the polypeptides half-life in solution, to make the polypeptides more water soluble, to increase the polypeptides hydrophilic or hydrophobic character, etc.). Polymers useful as stabilizing materials may be of natural, semi-synthetic (modified natural) or synthetic origin. Exemplary natural polymers include naturally occurring polysaccharides, such as, for example, arabinans, fructans, fucans, galactans, galacturonans, glucans, mannans, xylans (such as, for example, inulin), levan, fucoidan, carrageenan, galatocarolose, pectic acid, pectins, including amylose, pullulan, glycogen, amylopectin, cellulose, dextran, dextrin, dextrose, glucose, polyglucose, polydextrose, pustulan, chitin, agarose, keratin, chondroitin, dermatan, hyaluronic acid, alginic acid, xanthin gum, starch and various other natural homopolymer or heteropolymers, such as those containing one or more of the following aldoses, ketoses, acids or amines: erythose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, dextrose, mannose, gulose, idose, galactose, talose, erythrulose, ribulose, xylulose, psicose, fructose, sorbose, tagatose, mannitol, sorbitol, lactose, sucrose, trehalose, maltose, cellobiose, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, glucuronic acid, gluconic acid, glucaric acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, and neuraminic acid, and naturally occurring derivatives thereof Accordingly, suitable polymers include, for example, proteins, such as albumin, polyalginates, and polylactide-coglycolide polymers. Exemplary semi-synthetic polymers include carboxymethylcellulose, hydroxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose, and methoxycellulose. Exemplary synthetic polymers include polyphosphazenes, hydroxyapatites, fluoroapatite polymers, polyethylenes (such as, for example, polyethylene glycol (including for example, the class of compounds referred to as Pluronics.RTM., commercially available from BASF, Parsippany, N.J.), polyoxyethylene, and polyethylene terephthlate), polypropylenes (such as, for example, polypropylene glycol), polyurethanes (such as, for example, polyvinyl

alcohol (PVA), polyvinyl chloride and polyvinylpyrrolidone), polyamides including nylon, polystyrene, polylactic acids, fluorinated hydrocarbon polymers, fluorinated carbon polymers (such as, for example, polytetrafluoroethylene), acrylate, methacrylate, and polymethylmethacrylate, and derivatives thereof. Methods for the preparation of derivatized polypeptides of the invention which employ polymers as stabilizing compounds will be readily apparent to one skilled in the art, in view of the present disclosure, when coupled with information known in the art, such as that described and referred to in Unger, U.S. Pat. No. 5,205,290, the disclosure of which is hereby incorporated by reference herein in its entirety.

Moreover, the invention encompasses additional modifications of the polypeptides of the present invention. Such additional modifications are known in the art, and are specifically provided, in addition to methods of derivitization, etc., in US Patent No. 6,028,066, which is hereby incorporated in its entirety herein.

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing

polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described

herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions

between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse

orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

In addition, the polynucleotide insert of the present invention could be operatively linked to "artificial" or chimeric promoters and transcription factors. Specifically, the artificial promoter could comprise, or alternatively consist, of any combination of cis-acting DNA sequence elements that are recognized by trans-acting transcription factors. Preferably, the cis acting DNA sequence elements and trans-acting transcription factors are operable in mammals. Further, the trans-acting transcription factors of such “artificial” promoters could also be "artificial" or chimeric in design themselves and could act as activators or repressors to said “artificial” promoter.

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic

cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. Disease mapping data are known in the art. Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected organisms can be examined. First, visible structural alterations in the chromosomes, such as deletions or

translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected organisms, but not in normal organisms, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal organisms is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected organisms as compared to unaffected organisms can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an organism and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

By "measuring the expression level of a polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of organisms not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an organism, body fluids, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as the following non-limiting examples, sputum, amniotic fluid, urine, saliva, breast milk, secretions, interstitial fluid, blood, serum, spinal fluid, etc.) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from organisms are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may Preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including proliferative diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and

tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the stronger binding characteristics of PNA:DNA hybrids. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T.sub.m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

The present invention encompasses the addition of a nuclear localization signal, operably linked to the 5' end, 3' end, or any location therein, to any of the oligonucleotides, antisense oligonucleotides, triple helix oligonucleotides, ribozymes, PNA oligonucleotides, and/or polynucleotides, of the present invention. See, for example, G. Cutrona, et al., Nat. Biotech., 18:300-303, (2000); which is hereby incorporated herein by reference.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell. In one example, polynucleotide sequences of the present invention may be used to construct chimeric RNA/DNA oligonucleotides corresponding to said sequences, specifically designed to induce host cell mismatch repair mechanisms in an organism upon systemic injection, for example (Bartlett, R.J., et al., Nat. Biotech, 18:615-622 (2000), which is hereby incorporated by reference herein in its entirety). Such RNA/DNA oligonucleotides could be designed to correct genetic defects in certain host strains, and/or to introduce desired phenotypes in the host (e.g., introduction of a specific polymorphism within an endogenous gene corresponding to a polynucleotide of the present invention that may ameliorate and/or prevent a disease symptom and/or disorder, etc.). Alternatively, the polynucleotide sequence of the present invention may be used to construct duplex oligonucleotides corresponding to said sequence, specifically designed to correct genetic defects in certain host strains, and/or to introduce desired phenotypes into the host (e.g., introduction of a specific polymorphism within an endogenous gene corresponding to a polynucleotide of the present invention that may ameliorate and/or prevent a disease symptom and/or disorder, etc). Such methods of using duplex oligonucleotides are known in the art and are encompassed by the present invention (see EP1007712, which is hereby incorporated by reference herein in its entirety).

The polynucleotides are also useful for identifying organisms from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel.

In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an organisms genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, organisms can be identified because each organism will have a unique set of DNA sequences. Once an unique ID database is established for an organism, positive identification of that organism, living or dead, can be made from extremely small tissue samples. Similarly, polynucleotides of the present invention can be used as polymorphic markers, in addition to, the identification of transformed or non-transformed cells and/or tissues.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination. Moreover, as mentioned above, such reagents can be used to screen and/or identify transformed and non-transformed cells and/or tissues.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, 131I, 112In, 99mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of

Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be

delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the

polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA

constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem..., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc.

Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology , 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a

suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca2+-EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta, 394:483 (1975); Wilson et al., Cell , 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA , 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem..., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA , 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis

virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, ex vivo or in vivo, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartzet al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science, 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA , 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993);

Rosenfeld et al., Cell , 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature , 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper

viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either ex vivo or in vivo. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5′ end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5′ and 3′ ends. Preferably, the 3′ end of the first targeting sequence contains the same restriction enzyme site as the 5′ end of the amplified promoter and the 5′ end of the second targeting sequence contains the same restriction site as the 3′ end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as

liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5′ end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., “gene guns”), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated

plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA , 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise

condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

Biological Activities

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

Immune Activity

The polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer or some autoimmune diseases, disorders, and/or conditions, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders,

and/or conditions of hematopoietic cells. A polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies), blood platelet diseases, disorders, and/or conditions (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating, preventing, and/or diagnosing autoimmune diseases, disorders, and/or conditions. Many autoimmune diseases, disorders, and/or conditions result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-

cells, may be an effective therapy in preventing autoimmune diseases, disorders, and/or conditions.

Examples of autoimmune diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide or agonists or antagonist may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and

malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

Hyperproliferative Disorders

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders, and/or conditions, including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative diseases, disorders, and/or conditions can be treated, prevented, and/or diagnosed. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating, preventing, and/or diagnosing hyperproliferative diseases, disorders, and/or conditions, such as a chemotherapeutic agent.

Examples of hyperproliferative diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative diseases, disorders, and/or conditions can also be treated, prevented, and/or diagnosed by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative

diseases, disorders, and/or conditions include, but are not limited to: hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating or preventing cell proliferative diseases, disorders, and/or conditions by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating or preventing cell-proliferative diseases, disorders, and/or conditions in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more Preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or

disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating, preventing, and/or diagnosing one or more of the described diseases, disorders, and/or conditions. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating, preventing, and/or diagnosing a subject having or developing cell proliferative and/or differentiation diseases, disorders, and/or conditions as

described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of diseases, disorders, and/or conditions related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5X10-6M, 10-6M, 5X10-7M, 10-7M, 5X10-8M, 10-8M, 5X10-9M, 10-9M, 5X10-10M, 10-10M, 5X10-11M, 10-11M, 5X10-12M, 10-12M, 5X10-13M, 10-13M, 5X10-14M, 10-14M, 5X10-15M, and 10-15M.

Moreover, polypeptides of the present invention may be useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-

domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat. Res. 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem. Biol. Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int. J. Tissue React. 20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present

invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Cardiovascular Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia.

Cardiovascular diseases, disorders, and/or conditions include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular diseases, disorders, and/or conditions also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial

block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular diseases, disorders, and/or conditions, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular diseases, disorders, and/or conditions include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subaraxhnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromoboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle

accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides of the invention may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides of the invention are described in more detail herein.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad et al., Cell 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye diseases, disorders, and/or conditions, and psoriasis. See, e.g., reviews by Moses et al., Biotech. 9:630-634 (1991); Folkman et al., N. Engl. J. Med., 333:1757-1763 (1995); Auerbach et al., J. Microvasc. Res. 29:401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, Am. J. Opthalmol. 94:715-743 (1982); and Folkman et al., Science 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, Science 235:442-447 (1987).

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can

be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman et al., Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).Thus, the present invention provides a method of treating, preventing, and/or diagnosing an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat or prevent a cancer or tumor. Cancers which may be treated, prevented, and/or diagnosed with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non- small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat or prevent cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating, preventing, and/or diagnosing other diseases, disorders, and/or conditions, besides cancers, which involve angiogenesis. These diseases, disorders, and/or conditions include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas;

artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating, preventing, and/or diagnosing hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating, preventing, and/or diagnosing neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular diseases, disorders, and/or conditions associated with neovascularization which can be treated, prevented, and/or diagnosed with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris

neovascularization. See, e.g., reviews by Waltman et al., Am. J. Ophthal. 85:704-710 (1978) and Gartner et al., Surv. Ophthal. 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of diseases, disorders, and/or conditions can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected

directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating or preventing neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat or prevent early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating or preventing retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreous injection and/or via intraocular implants.

Additionally, diseases, disorders, and/or conditions which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, diseases, disorders, and/or conditions and/or states, which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uvietis, delayed wound healing, endometriosis, vascluogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (Helicobacter pylori), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and

fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly

preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates,

sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem... 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer,

melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated, prevented or diagnosed by the polynucleotides or polypeptides, or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder

carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative diseases, disorders, and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and

antimetabolites. Polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are a non-exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral

infections.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to treat gastric and doudenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflamamatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associate with the under expression of the polynucleotides of the invention.

Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and

alveoli could be effectively treated, prevented, and/or diagnosed using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

In addition, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neurological Diseases

Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1)

ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases, disorders, and/or conditions, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In a preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides, or

agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral ischemia. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral infarction. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture; (2) increased sprouting of neurons in culture or in vivo; (3) increased production of a neuron-associated molecule in culture or in vivo, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction in vivo. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that may be treated, prevented, and/or diagnosed according to the invention include,

but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Infectious Disease

A polypeptide or polynucleotide and/or agonist or antagonist of the present invention can be used to treat, prevent, and/or diagnose infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated, prevented, and/or diagnosed. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polypeptide or polynucleotide and/or agonist or antagonist of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a

variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi), Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella),

Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., mengitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: tetanus, Diptheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparium, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used totreat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to

treat, prevent, and/or diagnose malaria.

Preferably, treatment or prevention using a polypeptide or polynucleotide and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a

polynucleotide or polypeptide and/or agonist or antagonist of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated, prevented, and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stoke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated, prevented, and/or diagnosed using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

Chemotaxis

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat, prevent, and/or diagnose wounds.

It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used to treat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the

present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors),or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a

standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which a polypeptide of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labeled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of polypeptides of the invention thereby

effectively generating agonists and antagonists of polypeptides of the invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, S. Trends Biotechnol. 16(2):76-82 (1998); Hansson, L. O., et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide sequence of the invention molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides of the invention may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptides of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention.

An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and 3[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of 3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat, prevent, and/or diagnose disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to the polypeptides of the invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with the polypeptide, (b) assaying a biological activity , and (b) determining if a biological activity of the polypeptide has been

altered.

Also, one could identify molecules bind a polypeptide of the invention experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, any combination or all of contained in the polypeptide sequences of the invention. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta pleated sheet regions in one of the polypeptide sequences of the invention. Additional embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions in one of the polypeptide sequences of the invention.

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific

destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, Pseudomonas exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic

or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

The human K+alphaM1 polypeptides and/or peptides of the present invention, or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic drugs or compounds in a variety of drug screening techniques. The fragment employed in such a screening assay may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The reduction or abolition of activity of the formation of binding complexes between the ion channel protein and the agent being tested can be measured. Thus, the present invention provides a method for screening or assessing a plurality of compounds for their specific binding affinity with a K+alphaM1 polypeptide, or a bindable peptide fragment, of this invention, comprising providing a plurality of compounds, combining the K+alphaM1 polypeptide, or a bindable peptide fragment, with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions and detecting binding of the K+alphaM1 polypeptide or peptide to each of the plurality of test compounds, thereby identifying the compounds that specifically bind to the K+alphaM1 polypeptide or peptide.

Methods of identifying compounds that modulate the activity of the novel human K+alphaM1 polypeptides and/or peptides are provided by the present invention and comprise combining a potential or candidate compound or drug modulator of calpain biological activity with an K+alphaM1 polypeptide or peptide, for example, the K+alphaM1 amino acid sequence as set forth in SEQ ID NOS:2, and measuring an effect of the candidate compound or drug modulator on the biological activity of the K+alphaM1 polypeptide or peptide. Such measurable effects include, for example, physical binding interaction; the ability to cleave a suitable calpain substrate; effects on native and cloned K+alphaM1-expressing cell line; and effects of modulators or other calpain-mediated physiological measures.

Another method of identifying compounds that modulate the biological activity of the novel K+alphaM1 polypeptides of the present invention comprises combining a potential or candidate compound or drug modulator of a calpain biological activity with a host cell that expresses the K+alphaM1 polypeptide and measuring an effect of the candidate compound or drug modulator on the biological activity of the K+alphaM1 polypeptide. The host cell can also be capable of being induced to express the K+alphaM1 polypeptide, e.g., via inducible expression.

Physiological effects of a given modulator candidate on the K+alphaM1 polypeptide can also be measured. Thus, cellular assays for particular calpain modulators may be either direct measurement or quantification of the physical biological activity of the K+alphaM1 polypeptide, or they may be measurement or quantification of a physiological effect. Such methods preferably employ a K+alphaM1 polypeptide as described herein, or an overexpressed recombinant K+alphaM1 polypeptide in suitable host cells containing an expression vector as described herein, wherein the K+alphaM1 polypeptide is expressed, overexpressed, or undergoes upregulated expression.

Another aspect of the present invention embraces a method of screening for a compound that is capable of modulating the biological activity of a K+alphaM1 polypeptide, comprising providing a host cell containing an expression vector harboring a nucleic acid sequence encoding a K+alphaM1 polypeptide, or a functional peptide or portion thereof (e.g., SEQ ID NOS:2); determining the biological activity of the expressed K+alphaM1 polypeptide in the absence of a modulator compound; contacting the cell with the modulator compound and determining the biological activity of the expressed K+alphaM1 polypeptide in the presence of the modulator compound. In such a method, a difference between the activity of the K+alphaM1 polypeptide in the presence of the modulator compound and in the absence of the modulator compound indicates a modulating effect of the compound.

Essentially any chemical compound can be employed as a potential modulator or ligand in the assays according to the present invention. Compounds tested as calpain modulators can be any small chemical compound, or biological entity (e.g., protein, sugar, nucleic acid, lipid). Test compounds will typically be small chemical molecules and peptides. Generally, the compounds used as potential modulators can be dissolved in aqueous or organic (e.g., DMSO-based) solutions. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source. Assays are typically run in parallel, for example, in microtiter formats on microtiter plates in robotic assays. There are many suppliers of chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica

Analytika (Buchs, Switzerland), for example. Also, compounds may be synthesized by methods known in the art.

High throughput screening methodologies are particularly envisioned for the detection of modulators of the novel K+alphaM1 polynucleotides and polypeptides described herein. Such high throughput screening methods typically involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (e.g., ligand or modulator compounds). Such combinatorial chemical libraries or ligand libraries are then screened in one or more assays to identify those library members (e.g., particular chemical species or subclasses) that display a desired characteristic activity. The compounds so identified can serve as conventional lead compounds, or can themselves be used as potential or actual therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds generated either by chemical synthesis or biological synthesis, by combining a number of chemical building blocks (i.e., reagents such as amino acids). As an example, a linear combinatorial library, e.g., a polypeptide or peptide library, is formed by combining a set of chemical building blocks in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide or peptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

The preparation and screening of combinatorial chemical libraries is well known to those having skill in the pertinent art. Combinatorial libraries include, without limitation, peptide libraries (e.g. U.S. Patent No. 5,010,175; Furka, 1991, *Int. J. Pept. Prot. Res.*, 37:487-493; and Houghton et al., 1991, *Nature*, 354:84-88). Other chemistries for generating chemical diversity libraries can also be used. Nonlimiting examples of chemical diversity library chemistries include, peptoids (PCT Publication No. WO 91/019735), encoded peptides (PCT Publication No. WO 93/20242), random bio-oligomers (PCT Publication No. WO 92/00091), benzodiazepines (U.S. Patent No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:6909-6913), vinylogous polypeptides (Hagihara et al., 1992, *J. Amer. Chem. Soc.*, 114:6568), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., 1992, *J. Amer. Chem.*

Soc., 114:9217-9218), analogous organic synthesis of small compound libraries (Chen et al., 1994, *J. Amer. Chem. Soc.*, 116:2661), oligocarbamates (Cho et al., 1993, *Science*, 261:1303), and/or peptidyl phosphonates (Campbell et al., 1994, *J. Org. Chem.*, 59:658), nucleic acid libraries (see Ausubel, Berger and Sambrook, all supra), peptide nucleic acid libraries (U.S. Patent No. 5,539,083), antibody libraries (e.g., Vaughn et al., 1996, *Nature Biotechnology*, 14(3):309-314) and PCT/US96/10287), carbohydrate libraries (e.g., Liang et al., 1996, *Science*, 274-1520-1522) and U.S. Patent No. 5,593,853), small organic molecule libraries (e.g., benzodiazepines, Baum C&EN, Jan. 18, 1993, page 33; and U.S. Patent No. 5,288,514; isoprenoids, U.S. Patent No. 5,569,588; thiazolidinones and metathiazanones, U.S. Patent No. 5,549,974; pyrrolidines, U.S. Patent Nos. 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent No. 5,506,337; and the like).

Devices for the preparation of combinatorial libraries are commercially available (e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY; Symphony, Rainin, Woburn, MA; 433A Applied Biosystems, Foster City, CA; 9050 Plus, Millipore, Bedford, MA). In addition, a large number of combinatorial libraries are commercially available (e.g., ComGenex, Princeton, NJ; Asinex, Moscow, Russia; Tripos, Inc., St. Louis, MO; ChemStar, Ltd., Moscow, Russia; 3D Pharmaceuticals, Exton, PA; Martek Biosciences, Columbia, MD, and the like).

In one embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the cell or tissue expressing an ion channel is attached to a solid phase substrate. In such high throughput assays, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well of a microtiter plate can be used to perform a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 96 modulators. If 1536 well plates are used, then a single plate can easily assay from about 100 to about 1500 different compounds. It is possible to assay several different plates per day; thus, for example, assay screens for up to about 6,000-20,000 different compounds are possible using the described integrated systems.

In another of its aspects, the present invention encompasses screening and small molecule (e.g., drug) detection assays which involve the detection or identification of small molecules that can bind to a given protein, i.e., a K+alphaM1 polypeptide or peptide. Particularly preferred are assays suitable for high throughput screening methodologies.

In such binding-based detection, identification, or screening assays, a functional assay is not typically required. All that is needed is a target protein, preferably substantially purified, and a library or panel of compounds (e.g., ligands, drugs, small molecules) or biological entities to be screened or assayed for binding to the protein target. Preferably, most small molecules that bind to the target protein will modulate activity in some manner, due to preferential, higher affinity binding to functional areas or sites on the protein.

An example of such an assay is the fluorescence based thermal shift assay (3-Dimensional Pharmaceuticals, Inc., 3DP, Exton, PA) as described in U.S. Patent Nos. 6,020,141 and 6,036,920 to Pantoliano et al.; see also, J. Zimmerman, 2000, *Gen. Eng. News*, 20(8)). The assay allows the detection of small molecules (e.g., drugs, ligands) that bind to expressed, and preferably purified, ion channel polypeptide based on affinity of binding determinations by analyzing thermal unfolding curves of protein-drug or ligand complexes. The drugs or binding molecules determined by this technique can be further assayed, if desired, by methods, such as those described herein, to determine if the molecules affect or modulate function or activity of the target protein.

To purify a K+alphaM1 polypeptide or peptide to measure a biological binding or ligand binding activity, the source may be a whole cell lysate that can be prepared by successive freeze-thaw cycles (e.g., one to three) in the presence of standard protease inhibitors. The K+alphaM1 polypeptide may be partially or completely purified by standard protein purification methods, e.g., affinity chromatography using specific antibody described *infra*, or by ligands specific for an epitope tag engineered into the recombinant K+alphaM1 polypeptide molecule, also as described herein. Binding activity can then be measured as described.

Compounds which are identified according to the methods provided herein, and which modulate or regulate the biological activity or physiology of the

K+alphaM1 polypeptides according to the present invention are a preferred embodiment of this invention. It is contemplated that such modulatory compounds may be employed in treatment and therapeutic methods for treating a condition that is mediated by the novel K+alphaM1 polypeptides by administering to an individual in need of such treatment a therapeutically effective amount of the compound identified by the methods described herein.

In addition, the present invention provides methods for treating an individual in need of such treatment for a disease, disorder, or condition that is mediated by the K+alphaM1 polypeptides of the invention, comprising administering to the individual a therapeutically effective amount of the K+alphaM1-modulating compound identified by a method provided herein.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X,or the complementary strand thereof, and/or to nucleotide sequences contained a deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of

oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5 end and a HindIII site on the 3 end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl2, 10MM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature, 29:304-310 (1981), the promoter contained in the 3´ long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A., 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., Nature, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest.

However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5´ end of the message, e.g., the 5´ untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3´ untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., Nature, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5´ - or 3´ - non-translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5´ untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5´ -, 3´ - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g.,

for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556 (1989); Lemaitre et al., Proc. Natl. Acad. Sci., 84:648-652 (1987); PCT Publication NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res., 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5´-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5´-UG-3´. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5´ end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA

transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

Biotic Associations

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase the organisms ability, either directly or indirectly, to initiate and/or maintain biotic associations with other organisms. Such associations may be symbiotic, nonsymbiotic, endosymbiotic, macrosymbiotic, and/or microsymbiotic in nature. In general, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase the organisms ability to form biotic associations with any member of the fungal, bacterial, lichen, mycorrhizal, cyanobacterial, dinoflaggellate, and/or algal, kingdom, phylums, families, classes, genuses, and/or species.

The mechanism by which a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase the host organisms ability, either directly or indirectly, to initiate and/or maintain biotic associations is variable, though may include, modulating osmolarity to desirable levels for the symbiont, modulating pH to desirable levels for the symbiont, modulating secretions of organic acids, modulating the secretion of specific proteins, phenolic compounds, nutrients, or the increased expression of a protein required for host-biotic organisms interactions (e.g., a receptor, ligand, etc.). Additional mechanisms are known in the art and are encompassed by the invention (see, for example, "Microbial Signalling and Communication", eds., R. England, G. Hobbs, N. Bainton, and D. McL. Roberts, Cambridge University Press, Cambridge, (1999); which is hereby incorporated herein by reference).

In an alternative embodiment, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may decrease the host organisms ability to form biotic associations with another organism, either directly or indirectly. The mechanism by which a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may decrease the host organisms ability, either directly or indirectly, to initiate and/or maintain biotic associations with another organism is variable, though may include, modulating osmolarity to undesirable levels,

modulating pH to undesirable levels, modulating secretions of organic acids, modulating the secretion of specific proteins, phenolic compounds, nutrients, or the decreased expression of a protein required for host-biotic organisms interactions (e.g., a receptor, ligand, etc.). Additional mechanisms are known in the art and are encompassed by the invention (see, for example, "Microbial Signalling and Communication", eds., R. England, G. Hobbs, N. Bainton, and D. McL. Roberts, Cambridge University Press, Cambridge, (1999); which is hereby incorporated herein by reference).

The hosts ability to maintain biotic associations with a particular pathogen has significant implications for the overall health and fitness of the host. For example, human hosts have symbiosis with enteric bacteria in their gastrointestinal tracts, particularly in the small and large intestine. In fact, bacteria counts in feces of the distal colon often approach 10^{12} per milliliter of feces. Examples of bowel flora in the gastrointestinal tract are members of the Enterobacteriaceae, Bacteriodes, in addition to a-hemolytic streptococci, E. coli, Bifobacteria, Anaerobic cocci, Eubacteria, Costridia, lactobacilli, and yeasts. Such bacteria, among other things, assist the host in the assimilation of nutrients by breaking down food stuffs not typically broken down by the hosts digestive system, particularly in the hosts bowel. Therefore, increasing the hosts ability to maintain such a biotic association would help assure proper nutrition for the host.

Aberrations in the enteric bacterial population of mammals, particularly humans, has been associated with the following disorders: diarrhea, ileus, chronic inflammatory disease, bowel obstruction, duodenal diverticula, biliary calculous disease, and malnutrition. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention are useful for treating, detecting, diagnosing, prognosing, and/or ameliorating, either directly or indirectly, and of the above mentioned diseases and/or disorders associated with aberrant enteric flora population.

The composition of the intestinal flora, for example, is based upon a variety of factors, which include, but are not limited to, the age, race, diet, malnutrition, gastric acidity, bile salt excretion, gut motility, and immune mechanisms. As a result, the polynucleotides and polypeptides, including agonists, antagonists, and fragments

thereof, may modulate the ability of a host to form biotic associations by affecting, directly or indirectly, at least one or more of these factors.

Although the predominate intestinal flora comprises anaerobic organisms, an underlying percentage represents aerobes (e.g., E. coli). This is significant as such aerobes rapidly become the predominate organisms in intraabdominal infections – effectively becoming opportunistic early in infection pathogenesis. As a result, there is an intrinsic need to control aerobe populations, particularly for immune compromised individuals.

In a preferred embodiment, a polynucleotides and polypeptides, including agonists, antagonists, and fragments thereof, are useful for inhibiting biotic associations with specific enteric symbiont organisms in an effort to control the population of such organisms.

Biotic associations occur not only in the gastrointestinal tract, but also on an in the integument. As opposed to the gastrointestinal flora, the cutaneous flora is comprised almost equally with aerobic and anaerobic organisms. Examples of cutaneous flora are members of the gram-positive cocci (e.g., S. aureus, coagulase-negative staphylococci, micrococcus, M.sedentarius), gram-positive bacilli (e.g., Corynebacterium species, C. minutissimum, Brevibacterium species, Propoionibacterium species, P.acnes), gram-negative bacilli (e.g., Acinebacter species), and fungi (Pityrosporum orbiculare). The relatively low number of flora associated with the integument is based upon the inability of many organisms to adhere to the skin. The organisms referenced above have acquired this unique ability. Therefore, the polynucleotides and polypeptides of the present invention may have uses which include modulating the population of the cutaneous flora, either directly or indirectly.

Aberrations in the cutaneous flora are associated with a number of significant diseases and/or disorders, which include, but are not limited to the following: impetigo, ecthyma, blistering distal dactulitis, pustules, folliculitis, cutaneous abscesses, pitted keratolysis, trichomycosis axcillaris, dermatophytosis complex, axillary odor, erthyrasma, cheesy foot odor, acne, tinea versicolor, seborrheic dermititis, and Pityrosporum folliculitis, to name a few. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention are useful for

treating, detecting, diagnosing, prognosing, and/or ameliorating, either directly or indirectly, and of the above mentioned diseases and/or disorders associated with aberrant cutaneous flora population.

Additional biotic associations, including diseases and disorders associated with the aberrant growth of such associations, are known in the art and are encompassed by the invention. See, for example, "Infectious Disease", Second Edition, Eds., S.L., Gorbach, J.G., Bartlett, and N.R., Blacklow, W.B. Saunders Company, Philadelphia, (1998); which is hereby incorporated herein by reference).

Pheromones

In another embodiment, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase the organisms ability to synthesize and/or release a pheromone. Such a pheromone may, for example, alter the organisms behavior and/or metabolism.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may modulate the biosynthesis and/or release of pheromones, the organisms ability to respond to pheromones (e.g., behaviorally, and/or metabolically), and/or the organisms ability to detect pheromones. Preferably, any of the pheromones, and/or volatiles released from the organism, or induced, by a polynucleotide or polypeptide and/or agonist or antagonist of the invention have behavioral effects the organism.

Other Activities

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate

neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, caricadic rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine

levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to increase the efficacy of a pharmaceutical composition, either directly or indirectly. Such a use may be administered in simultaneous conjunction with said pharmaceutical, or separately through either the same or different route of administration (e.g., intravenous for the polynucleotide or polypeptide of the present invention, and orally for the pharmaceutical, among others described herein.).

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to prepare individuals for extraterrestrial travel, low gravity environments, prolonged exposure to extraterrestrial radiation levels, low oxygen levels, reduction of metabolic activity, exposure to extraterrestrial pathogens, etc. Such a use may be administered either prior to an extraterrestrial event, during an extraterrestrial event, or both. Moreover, such a use may result in a number of beneficial changes in the recipient, such as, for example, any one of the following, non-limiting, effects: an increased level of hematopoietic cells, particularly red blood cells which would aid the recipient in coping with low oxygen levels; an increased level of B-cells, T-cells, antigen presenting cells, and/or macrophages, which would aid the recipient in coping with exposure to extraterrestrial pathogens, for example; a temporary (i.e., reversible) inhibition of hematopoietic cell production which would aid the recipient in coping with exposure to extraterrestrial radiation levels; increase and/or stability of bone mass which would aid the recipient in coping with low gravity environments; and/or decreased metabolism which would effectively facilitate the recipients ability to prolong their extraterrestrial travel by any one of the following, non-limiting means: (i) aid the recipient by decreasing their basal daily energy requirements; (ii) resulting in a lower level of oxidative and/or metabolic stress (i.e., to enable recipient to cope with increased extraterrestial radiation levels by decreasing the level of internal oxidative/metabolic damage acquired during normal basal energy

requirements; and/or (iii) enabling recipient to subsist at a lower metabolic temperature (i.e., cryogenic, and/or sub-cryogenic environment).

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the "5´ NT of Start Codon of ORF" and ending with the nucleotide at about the position of the "3´ NT of ORF" as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:1.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:1.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the "5´ NT of ORF" and ending with the nucleotide at about the position of the "3´ NT of ORF" as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:1.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and

determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a cDNA clone identified by a cDNA Clone

Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions "Total AA of the Open Reading Frame (ORF)" as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:2.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:2.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:2.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of the protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a

cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein

encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing a pathological condition associated with an organism with abnormal structure or expression of a gene encoding a protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule(s) into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the "Total AA of ORF" of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

References:

Ackerman, M. J., and Clapham, D. E. (1997). Ion channels--basic science and clinical disease. N. Engl. J. Med. *336*, 1575-1586.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. L. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acid Res. *25*, 3389-3402.

Bateman, A., Birney, E. R., Durbin, S. R., Eddy, S. R., Howe, K. L., and Sonnhammer, E. L. L. (2000). The Pfam protein families database. Nucleic Acids Research *28*, 263-266.

Jan, L. Y., and Jan, Y. N. (1997). Cloned potassium channels from eukaryotes and prokaryotes. Annu. Rev. Neurosci. *20*, 91-123.

Salians, M., Duprat, F., Heurteaux, C., Hugnot, J. P., and Lazdunski, M. (1997). New modulatory aplha subunits for mammalian Shab K+ channels. J. Biol. Chem... *272*, 24371-24379.

Shepard, A. R., and Rae, J. L. (1999). Electrically silent potassium channel subunits from human lens epithelium. American Journal of physiology *277*, 412-424.

Examples

Description of the Preferred Embodiments

Example 1 – Bioinformatics Analysis

Ion channel sequences were used as probes to search the human genomic sequence database. The search program used was gapped BLAST (Altschul et al., 1997). Ion channel specific Hidden Markov Models (HMMs) built in-house or obtained from the public PFAM databases were also used as probes (Bateman et al., 2000). The search program used for HMMs was the Genewise/Wise2 package (http://www.sanger.ac.uk/Software/Wise2/index.shtml). The top genomic exon hits from the results were searched back against the non-redundant protein and patent sequence databases. From this analysis, exons encoding potential novel ion channels

were identified based on sequence homology. Also, the genomic region surrounding the matching exons were analyzed. Based on this analysis, partial sequence of a novel human ion channel related gene was identified directly from the genomic sequence. The full-length clone of this novel ion channel gene was experimentally obtained by using the sequence from genomic data.

Example 2 - Method for Constructing a size fractionated brain cDNA Library

Brain poly A + RNA was purchased from Clontech and converted into double stranded cDNA using the SuperScript™ Plasmid System for cDNA Synthesis and Plasmid Cloning (Life Technologies) except that no radioisotope was incorporated in either of the cDNA synthesis steps and that the cDNA was fractionated by HPLC. This was accomplished on a TransGenomics HPLC system equipped with a size exclusion column (TosoHass) with dimensions of 7.8mm x 30cm and a particle size of 10µm. Tris buffered saline was used as the mobile phase and the column was run at a flow rate of 0.5 mL/min.

The resulting chromatograms were analyzed to determine which fractions should be pooled to obtain the largest cDNA's; generally fractions that eluted in the range of 12 to 15 minutes were pooled. The cDNA was precipitated prior to ligation into the Sal I / Not I sites in the pSport vector supplied with the kit. Using a combination of PCR with primers to the ends of the vector and Sal I/Not I restriction enzyme digestion of mini-prep DNA, it was determined that the average insert size of the library was greater the 3.5 Kb. The overall complexity of the library was greater that 10^7 independent clones. The library was amplified in semi-solid agar for 2 days at 30° C. An aliquot (200 microliters) of the amplified library was inoculated into a 200 ml culture for single-stranded DNA isolation by super-infection with a f1 helper phage. After overnight growth, the released phage particles with precipitated with PEG and the DNA isolated with proteinase K, SDS and phenol extractions. The single stranded circular DNA was concentrated by ethanol precipitation and used for the cDNA capture experiments.

Example 3 - Cloning of the Novel Human Potassium Channel

Using the predict exon genomic sequence from bac AC019222, an antisense 80 bp oligo with biotin on the 5' end was designed with the following sequence;

5'-bTAGCCCAGCTCCTCCAGGAAGCGGCGCGTACACAGCCCGTCGAGCAC CAGCAGCACCCCGGACAGGTAGAAATTGTAGAC-3' (SEQ ID NO:6)

One microliter (one hundred and fifty nanograms) of the biotinylated oligo was added to six microliters (six micrograms) of a single-stranded covalently closed circular brain cDNA library (see Example 2) and seven microliters of 100% formamide in a 0.5 ml PCR tube. The mixture was heated in a thermal cycler to 95° C for 2 mins. Fourteen microliters of 2X hybridization buffer (50% formamide, 1.5 M NaCl, 0.04 M $NaPO_4$, pH 7.2, 5 mM EDTA, 0.2% SDS) was added to the heated probe/cDNA library mixture and incubated at 42° C for 26 hours. Hybrids between the biotinylated oligo and the circular cDNA were isolated by diluting the hybridization mixture to 220 microliters in a solution containing 1 M NaCl, 10 mM Tris-HCl pH 7.5, 1mM EDTA, pH 8.0 and adding 125 microliters of streptavidin magnetic beads. This solution was incubated at 42° C for 60 mins, mixing every 5 mins to resuspend the beads. The beads were separated from the solution with a magnet and the beads washed three times in 200 microliters of 0.1 X SSPE, 0.1% SDS at 45° C.

The single stranded cDNAs were release from the biotinlyated oligo/streptavidin magnetic bead complex by adding 50 microliters of 0.1 N NaOH and incubating at room temperature for 10 mins. Six microliters of 3 M Sodium Acetate was added along with 15 micrograms of glycogen and the solution ethanol precipitated with 120 microliters of 100% ethanol. The DNA was resuspend in 12 microliters of TE (10 mM Tris-HCl, pH 8.0), 1mM EDTA, pH 8.0). The single stranded cDNA was converted into double strands in a thermal cycler by mixing 5 microliters of the captured DNA with 1.5 microliters 10 micromolar standard SP6 primer (homologous to a sequence on the cDNA cloning vector) and 1.5 microliters of 10 X PCR buffer. The mixture was heated to 95° C for 20 seconds, then ramped down to 59 ° C. At this time 15 microliters of a repair mix, that was preheated to 70° C

(Repair mix contains 4 microliters of 5 mM dNTPs (1.25 mM each), 1.5 microliters of 10X PCR buffer, 9.25 microliters of water, and 0.25 microliters of Taq polymerase). The solution was ramped back to 73° C and incubated for 23 mins. The repaired DNA was ethanol precipitate and resuspended in 10 microliters of TE. Two microliters were electroporated in E. coli DH12S cells and resulting colonies were screen by PCR, using a primer pair designed from the genomic exonic sequence to identify the proper cDNAs.

Oligos used to identity the cDNA by PCR.

AC019222.1 5'-ACCCCGGACAGGTAGAAATTG-3' (SEQ ID NO:7)
s
AC019222.1 5'-TTCCCCAAGACGCCTAGGT-3' (SEQ ID NO:8)
a

Those cDNA clones that were positive by PCR had the inserts sized and two clones were chosen for DNA sequencing. Both clones had identical sequence. The sequence is presented in Figures 1A-C (SEQ ID NO:1).

Example 4 – Expression profiling of novel human potassium channel modulatory beta subunit K+alphaM1

The same PCR primer pair (SEQ ID NO:8 and 9) that was used to identify the K+alphaM1 cDNA clones was used to measure the steady state levels of mRNA by quantitative PCR. Briefly, first strand cDNA was made from commercially available mRNA. The relative amount of cDNA used in each assay was determined by performing a parallel experiment using a primer pair for a gene expressed in equal amounts in all tissues, cyclophilin. The cyclophilin primer pair detected small variations in the amount of cDNA in each sample and these data were used for normalization of the data obtained with the primer pair for K+alphaM1. The PCR data was converted into a relative assessment of the difference in transcript abundance

amongst the tissues tested and the data is presented below. Transcripts corresponding to K+alphaM1 is expressed highly in the lung, pancreas , prostate and small intestine.

Example 5 – Method of assessing ability of K+alphaM1 polypeptides to associate with potassium channel subunits using the yeast two-hybrid system.

In an effort to determine whether the K+alphaM1 polypeptides of the present invention are capable of functioning as potassium channel alpha subunits, it would be important to effectively test the interaction between K+alphaM1 and various portions of other potassium channel alpha or beta subunits, in a yeast two-hybrid system. Such a system could be created using methods known in the art (see, for example, S. Fields and O. Song, Nature, 340:245-246 (1989); and Gaston-SM and Loughlin-KR, Urology, 53(4): 835-42 (1999); which are hereby incorporated herein by reference in their entirety, including the articles referenced therein).

Cytoplasmic NH and COOH terminal domains of different potassium channel alpha- or beta-subunits could be subcloned and expressed as fusion proteins of the GAL4 DNA binding (DB) domain using molecular biology techniques within the skill of the artisan.

Exemplary subunits which could be used in the two-hybrid system to assess K+alphaM1s ability to associate with other alpha or beta subunits include, but are not limited to, the NH-terminal domain of human Kv1, Kv2, Kv3, Kv4 or Kv7, in addition to, the rat Kv9.3, human Shab-related subunit, the human Kv8.1, the Drosophila Shab11 of the Kv2 subfamily, the Shaw2 of the Kv3 subfamily, or the Shal2 of the Kv4 subfamily. Additional alpha subunits could be used in the two-hybrid system. Such subunits are known in the art and are encompassed by the present invention.

Additional subunits which may be employed in assessing the ability of K+alphaM1 to associate with other alpha or beta subunits are provided, for example, Kv 1. 1, Kv 1.2, Kv 1.3, Kv 1.4, Kv 1.5, Kv 1.6, Kv 1.7, Kv2.1, Kv2.2, Kv2.3, Kv3.1, Kv3.2, Kv3.3, Kv3.4, Kv4.1, Kv4.2, Kv4.3, Kv5.1, Kv6.1, Kv7.1, Kv8.1, Kv9.1, Kv9.2, Kv9.3, KQT1, KQT2, KQT3, KCNQ2, KCNQ3, ISK, HERG1, HERG2, ELK1, ELK2, all inward rectifier potassium channel subunits, and 2-pore K channels

subunits. Any K channel subunit may be used in the methods of the present invention. See, e. g., Chandy, K. G. and Gutman, G. A., Handbook of Receptors and Channels, CRC Press, Boca Raton, FL (1995); Wei, A., et al., Neuropharmacology, 35 (7): 805 (1996).

Example 6 – Method of assessing ability of K+alphaM1 polypeptides to form oligomeric complexes with itself or other potassium channel subunits in solution.

Aside from determining whether the K+alphaM1 polypeptides are capable of interacting with other potassium channel alpha and/or beta subunits in a yeast two-hybrid assay, it would be an important next step to assess its ability to form oligomeric complexes with itself, in addition to other alpha or beta subunits in solution. Such a finding would be significant as it would provide convincing evidence that K+alphaM1 could serve as a potassium channel alpha subunit and may modulate potassium channel function.

A number of methods could be used that are known in the art, for example, the method described by Sanguinetti, M.C., et al., Nature, 384:80-83 (1996) could be adapted using methods within the skill of the artisan.

Example 7 – Method of assessing whether the formation of K+alphaM1/potassium channel subunits has any effect on inhibiting potassium channel function.

Once the K+alphaM1 polypeptides are determined to form oligomeric and/or heteromultimeric complexes with other alpha or beta subunits, it would be important to determine whether such an interaction is physiologically relevant. Alternatively, this experiment could be performed prior to the oligomerization and yeast-two hybrid experiments described above.

Expression constructs comprising the coding region of the K+alphaM1 polypeptide under the control of a constitutive or inducible promoter could be created and used to transiently or stably transfect a cell line lacking endogenous potassium channel alpha expression (e.g., K+alphaM1). Once transfected, the ability of the cells

to transduce K+ could be assessed using techniques known in the art. Alternatively, any cell line could be transfected with K+alphaM1 polypeptides and the potassium channel function of the cell assessed. Alternatively, oocytes from the South African clawed frog X. laevis could be used to assess the ability of expressed K+alphaM1 polypeptides to modulate endogenous or transfected potassium channel function (for example, Wagner-CA; Friedrich-B; Setiawan-I; Lang-F; Broer, Cell-Physiol-Biochem., 10(1-2):1-12 (2000); which is hereby incorporated herein by reference in its entirety, including the references cited therein). Additional methods could be applied for assessing the ability of K+alphaM1 to modulate potassium channel activity. For example, the method described by McDonald, T.V., et al., Nature, 388:289-292 (1997) could be adapted using methods within the skill of the artisan.

Example 8 – 86Rb Efflux Method of assessing whether K+alphaM1 has any effect on inhibiting potassium channel function.

Depolarization of human neuroblastoma cells by high concentrations of extracellular potassium ions, leads to the activation of the voltage-gated potassium channels. The activity of such potassium channels is demonstrated to be effectively and rapidly monitored by tracking the efflux of 86Rb from pre-loaded target cells in response to the depolarizing stimulus. The transformation of neuroblastoma cells with vectors comprising the encoding K+alphaM1 polynucleotides and testing their efflux relative to control (non-transformed cells) would enable a definitive means to assess the ability of K+alphaM1 to modulate potassium channel function. Detailed methods relative to this technique may be found in Toral, J., et al., Use of Cultured Human Neuroblastoma Cells in Rapid Discovery of the Voltage-gated Potassium-channel Blockers, J. Pharm, Pharmacol., 46:731(1994) (which is hereby incorporated herein by reference). The blocking of individual K+ channels by a K+alphaM1 would result in a significant decrease in 86Rb efflux which can be readily detected by this assay.

Toral, J. et al. have successfully used this assay to discover a number of novel chemical structures capable of blocking the voltage-gated potassium channels in neurons and cardiocytes. The potassium-channel blocking activity of these compounds has been verified by electrophysiological techniques, as well as by 86Rb

efflux from cultured mammalian cells transfected with nucleic acids which encode potassium channel subunits.

Briefly, the functional high-volume 86Rb efflux assay is performed in 96-well microtitre plates, it represents a rapid and high-volume primary screening method for the detection and identification of potassium-channel modulators.

Highly purified human NT2 neuron cells and hNT post mitotic cenral nervous system cells for differentiation toward neuronal phenotype. are available from STRATAGENE, La Jolla, CA, for transfection to allow the study of potassium channel genes and assays described herein.

Buffers and reagents

Buffer

MOPS-PSS, pH 7.4 (NaCl 120 mM; KC1 7.0 mM; CaCI2 2.0 mM; MgC12 1.0 mM; ouabain 10pom; 4-morpholinepropanesulphonic acid, MOPS 20mM).

Depolarizing Solution

MOPS-PSS containing KC1 (80mM) replacing the equivalent concentrations of NaCl.

Candidate compounds are dissolved at a stock concentration of 10-100 mM, either in MOPS-PSS or dimethylsulphoxide (DMSO), and are subsequently diluted in the incubation buffer to the desired concentration. Candidate compounds are dissolved in MOPS-PSS containing bovine serum albumin (0.1 @ w/v) at 50-500pM stock concentration.

Cell Transfections.

Neuroblastoma cells may be transfected using methods well known in the art, or otherwise disclosed herein (e.g., electroporation, DEAE dextrane, liposome, viral vector, biolistics, etc.).

Cell culture and 86Rb loading

Human neuroblastoma cells TE671 are obtained from American Type Culture Collection (HTB 139) and are maintained at 370C in Dulbecco's Modified Eagle's

Medium (DMEM) supplemented with 10% foetal bovine serum, 4.5 gL-l glucose and 2.0 mM L-glutamine.

Cells are plated and loaded with 86Rb in 96-well microtitre plates as described by Daniel, S., et al., J. Pharmacol. Methods, 25:185(1991).

86Rb Efflux Assay Procedure

The growth medium in the microtitre plate is discarded by a sharp flicking of the plate. The adherent cell layer is washed three times with 200uL MOPS-PSS using a 12-channel pipetter.

The cells are incubated for 30 min at room temperature either with 200 RL MOPS-PSS, or 20 uL of the depolarizing solutions, in the presence or absence of a candidate compound potassium-channel blocker. Supernatant (150 KILL) from each well is removed and counted.

Cell layer is solubilized in 200 uL 0.1% Tween 20 in water and 150 uL is also counted in a Packard 2200 CA liquid scintillation counter. All supernatants are counted in 7.0 mL distilled water.

The percent efflux is calculated as follows: % total efflux = (counts minx in supernatant) (counts min in supernatant + counts mint in cell extract) x 100 and the value of percent net efflux is calculated as: % net efflux = % total efflux - Wo basal efflux where % total efflux is that induced by the depolarizing solution containing 100 mM KCI.

The basal efflux is the efflux (leak) of 86Rb observed in the physiological saline, MOPS-PSS.

Example 9 – Method of identifying the cognate ligand of the K+alphaM1 polypeptide.

A number of methods are known in the art for identifying the cognate binding partner of a particular polypeptide. For example, the encoding K+alphaM1 polynucleotide could be engineered to comprise an epitope tag. The epitope could be any epitope known in the art or disclosed elsewhere herein. Once created, the epitope tagged K+alphaM1 encoding polynucleotide could be cloned into an expression vector and used to transfect a variety of cell lines representing different tissue origins

(e.g., brain, testis, etc.). The transfected cell lines could then be induced to overexpress the K+alphaM1 polypeptide. Since other electrically silent channels appear to remain in the endoplasmic reticulum in the absence of their cognate binding partner, evidence for a cell type expressing the proper conducing channel would be the observed cell surface expression of K+alphaM1. The presence of the K+alphaM1 polypeptide on the cell surface could be determined by fractionating whole cell lysates into cellular and membrane protein fractions and performing immunoprecipitation using the antibody directed against the epitope engineered into the K+alphaM1 polypeptide. Monoclonal or polyclonal antibodies directed against the K+alphaM1 polypeptide could be created and used in place of the antibodies directed against the epitope.

Alternatively, the cell surface proteins could be distinguished from cellular proteins by biotinylating the surface proteins and then performing immunoprecipitations with antibody specific to the K+alphaM1 protein. After electrophoretic separation, the biotinylated protein could be detected with streptavidin-HRP (using standard methods known to those skilled in the art). Identification of the proteins bound to K+alphaM1 could be made in those cells by immunoprecipation, followed by one-dimensional electrophoresis, followed by various versions of mass spectrometry. Such mass-spectrometry methods are known in the art, such as for example the methods taught by Ciphergen Biosystems Inc. (see US Patent No. 5,792,664; which is hereby incorporated herein by reference).

Example 10 – Method Of Discovering Additional Single Nucleotide Polymorphisms (SNPs) Of The Present Invention

Additional SNPs may be discovered in the polynucleotides of the present invention based on comparative DNA sequencing of PCR products derived from genomic DNA from multiple individuals. The genomic DNA samples may be purchased from Coriell Institute (Collingswood, NJ). PCR amplicons may be designed to cover the entire coding region of the exons using the Primer3 program (Rozen S 2000). Exon-intron structure of candidate genes and intron sequences may be obtained by blastn search of Genbank cDNA sequences against the human genome draft sequences. The sizes of these PCR amplicons will vary according to the exon-

intron structure. All the samples may be amplified from genomic DNA (20 ng) in reactions (50 ul) containing 10 mM Tris-Cl pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 150 uM dNTPs, 3 uM PCR primers, and 3.75 U TaqGold DNA polymerase (PE Biosystems).

PCR is performed in MJ Research Tetrad machines under a cycling condition of 94 degrees 10 min, 30 cycles of 94 degrees 30 sec, 60 degrees 30sec, and 72 degrees 30 sec, followed by 72 degrees 7 min. PCR products may be purified using QIAquick PCR purification kit (Qiagen), and may be sequenced by the dye-terminator method using PRISM 3700 automated DNA sequencer (Applied Biosystems, Foster City, CA) following the manufacturer's instruction outlined in the Owner's Manual (which is hereby incorporated herein by reference in its entirety). Sequencing results may be analyzed for the presence of polymorphisms using PolyPhred software(Nickerson DA 1997; Rieder MJ 1999). All the sequence traces of potential polymorphisms may be visually inspected to confirm the presence of SNPs.

Alternative methods for identifying SNPs of the present invention are known in the art. One such method involves resequencing of target sequences from individuals of diverse ethnic and geographic backgrounds by hybridization to probes immobilized to microfabricated arrays. The strategy and principles for the design and use of such arrays are generally described in WO 95/11995.

A typical probe array used in such an analysis would have two groups of four sets of probes that respectively tile both strands of a reference sequence. A first probe set comprises a plurality of probes exhibiting perfect complementarily with one of the reference sequences. Each probe in the first probe set has an interrogation position that corresponds to a nucleotide in the reference sequence. That is, the interrogation position is aligned with the corresponding nucleotide in the reference sequence, when the probe and reference sequence are aligned to maximize complementarily between the two. For each probe in the first set, there are three corresponding probes from three additional probe sets. Thus, there are four probes corresponding to each nucleotide in the reference sequence. The probes from the three additional probe sets would be identical to the corresponding probe from the first probe set except at the interrogation position, which occurs in the same position in each of the four corresponding probes from the four probe sets, and is occupied by a different

nucleotide in the four probe sets. In the present analysis, probes may be nucleotides long. Arrays tiled for multiple different references sequences may be included on the same substrate.

Publicly available sequences for a given gene can be assembled into Gap4 (http:/ /www .biozentrum. unibas.ch/-biocomp/staden/Overview .html). PCR primers covering each exon, could be designed, for example, using Primer 3 (httP://www-genome.wi.mit.edu/cgi- bin/primer/primer3.cgi). Primers would not be designed in regions where there are sequence discrepancies between reads. Genomic DNA could be amplified from at least two individuals using 2.5 pmol each primer, 1.5 mM MgC12, 100 ~M dNTPs, 0.75 ~M AmpliTaq GOLD polymerase, and about 19ng DNA in a 15 ul reaction. Reactions could be assembled using a PACKARD MultiPROBE robotic pipetting station and then puṫ in MJ 96-well tetrad thermocyclers (96°C for minutes, followed by cycles of 96°C for seconds, 59°C for 2 minutes, and 72°C for 2 minutes). A subset of the PCR assays for each individual could then be run on 3% NuSieve gels in 0.5X TBE to confirm that the reaction worked.

For a given DNA, 5ul (about 50 ng) of each PCR or RT -PCR product could be pooled (Final volume = 150-200 ul). The products can be purified using QiaQuick PCR purification from Qiagen. The samples would then be eluted once in 35ul sterile water and 4 ul lOX One-Phor-All buffer (Pharmacia). The pooled samples are then digested with 0.2u DNaseI (Promega) for 10 minutes at 37°C and then labeled with 0.5 nmols biotin-N6- ddATP and 15u Terminal Transferase (GibcoBRL Life Technology) for 60 minutes at 37°C. Both fragmentation and labeling reactions could be terminated by incubating the pooled sample for 15 minutes at 100°C.

Low-density DNA chips {Affymetrix,CA) may be hybridized following the manufacturer's instructions. Briefly, the hybridization cocktail consisted of 3M TMACI, mM Tris pH 7.8, 0.01% Triton X-100, 100 mg/ml herring sperm DNA {Gibco BRL), 200 pM control biotin-labeled oligo. The processed PCR products are then denatured for 7 minutes at 100°C and then added to prewarmed {37°C) hybridization solution. The chips are hybridized overnight at 44°C. Chips are ished in 1X SSPET and 6X SSPET followed by staining with 2 ug/ml SARPE and 0.5 mg/ml

acetylated BSA in 200 ul of 6X SSPET for 8 minutes at room temperature. Chips are scanned using a Molecular Dynamics scanner.

Chip image files may be analyzed using Ulysses {Affymetrix, CA) which uses four algorithms to identify potential polymorphisms. Candidate polymorphisms may be visually inspected and assigned a confidence value: where high confidence candidates display all three genotypes, while likely candidates show only two genotypes {homozygous for reference sequence and heterozygous for reference and variant). Some of the candidate polymorphisms may be confirmed by ABI sequencing. Identified polymorphisms could then be compared to several databases to determine if they are novel.

Example 11 – Method Of Determining The Allele Frequency For Each SNP Of The Present Invention.

Allele frequencies of these polymorphisms may be determined by genotyping various DNA samples (Coriell Institute, Collingswood, NJ) using FP-TDI assay (Chen X 1999).. Automated genotyping calls may be made with an allele calling software developed by Joel Hirschorn (Whitehead Institute/MIT Center for Genome Research, personal communication).

Briefly, the no template controls (NTCs) may be labeled accordingly in column C. The appropriate cells may be completed in column L indicating whether REF (homozygous ROX) or VAR (homozygous TAMRA) are expected to be rare genotypes (<10% of all samples) – the latter is important in helping the program to identify rare homozygotes. The number of 96 well plates genotyped in cell P2 are noted (generally between 0.5 and 4) - the program works best if this is accurate. No more than 384 samples can be analyzed at a time. The pairs of mP values from the LJL may be pasted into columns E and F; making sure there may be no residual data is left at the bottom fewer than 384 data points are provided. The DNA names may be provided in columns A, B or C; column I will be a concatenation of columns A, B and C. In addition, the well numbers for each sample may be also provided in column D.

With the above information provided, the program should automatically cluster the points and identify genotypes. The program works by converting the mP values into polar coordinates (distance from origin and angle from origin) with the

angle being on a scale from 0 to 2; heterozygotes are placed as close to 1 as possible.

The cutoff values in columns L and M may be adjusted as desired.

Expert parameters: The most important parameters are the maximum angle for REF and minimum angle for VAR. These parameters may need to be changed in a particularly skewed assay which may be observed when an REF or VAR cluster is close to an angle of 1 and has called as a failed or HETs.

Other parameters are low and high cutoffs that are used to determine which points are considered for the determination of edges of the clusters. With small numbers of data points, the high cutoff may need to be increased (to 500 or so). This may be the right thing to do for every assay, but certainly when the program fails to identify a small cluster with high signal.

NTC TAMRA and ROX indicate the position of the no template control or failed samples as estimated by the computer algorithm.

No signal = mP< is the threshold below which points are automatically considered failures. "Throw out points with signal above" is the TAMRA or ROX mP value above which points are considered failures. The latter may occasionally need to be adjusted from 250 to 300, but caveat emptor for assays with signals >250. 'Lump' or 'split' describes a subtle difference in the way points are grouped into clusters. Lump generally is better. 'HETs expected' in the rare case where only homozygotes of either class are expected (e.g. a study of X chromosome SNPs in males), change this to "N".

Notes on method of clustering: The origin is defined by the NTCs or other low signal points (the position of the origin is shown as "NTC TAMRA" and "NTC ROX"); the points with very low or high signal are not considered initially. The program finds the point farthest from the origin and calls that a HET; the ROX/TAMRA ratio is calculated from this point, placing the heterozygotes at 45 degrees from the origin (an angle of "1"). The angles from the origin are calculated (the scale ranges from 0 to 2) and used to define clusters. A histogram of angles is generated. The cluster boundaries are defined by an algorithm that takes into account the shape of the histogram. The homozygote clusters are defined as the leftmost and rightmost big clusters (unless the allele is specified as being rare, in which case the

cluster need not be big). The heterozygote is the biggest cluster in between the REF and VAR. If there are two equal clusters, the one best-separated from REF and VAR is called HET. All other clusters are failed. Some fine tuning is applied to lump in scattered points on the edges of the clusters (if "Lump" is selected). The boundaries of the clusters are "Angles" in column L.

Once the clusters are defined, the interquartile distance of signal intensity is defined for each cluster. Points falling more than 3 or 4 interquartiles from the mean are excluded. (These are the "Signal cutoffs" in column M)

Allele frequency of the B1 receptor R317Q variant (AE103s1) is as follows. 7% in African Americans (7/94), 0% in Caucasians (0/94), 0% in Asians (0/60), and 0% in Amerindians (0/20). Higher frequency of this form in African Americans than in Caucasians matches the profile of a potential genetic risk factor for angioedema, which is observed more frequently in African Americans than in Caucasians (Brown NJ 1996; Brown NJ 1998; Agostoni A 1999; Coats 2000).

The invention encompasses additional methods of determinig the allelic frequency of the SNPs of the present invention. Such methods may be known in the art, some of which are described elsewhere herein.

Example 12 – Alternative Methods of Detecting Polymorphisms Encompassed By The Present Invention.

A. Preparation of Samples

Polymorphisms are detected in a target nucleic acid from an individual being analyzed. For assay of genomic DNA, virtually any biological sample (other than pure red blood cells) is suitable. For example, convenient tissue samples include whole blood, semen, saliva, tears, urine, fecal material, sweat, buccal, skin and hair. For assay of cDNA or mRNA, the tissue sample must be obtained from an organ in which the target nucleic acid is expressed. For example, if the target nucleic acid is a cytochrome P450, the liver is a suitable source.

Many of the methods described below require amplification of DNA from target samples. This can be accomplished by e.g., PCR. See generally PCR Technology: Principles and Applications for DNA Amplification (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); PCR Protocols: A Guide to Methods and

Applications (eds. Innis, et al., Academic Press, San Diego, CA, 1990); Mattila et al., Nucleic Acids Res. 19, 4967 (1991); Eckert et al., PCR Methods and Applications 1, (1991); PCR (eds. McPherson et al., IRL Press, Oxford); and U.S. Patent 4,683,202.

Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu and Wallace, Genomics 4:560 (1989), Landegren et al., Science 241:1077 (1988), transcription amplification (Kwoh et al., Proc. Natl. Acad. Sci. USA 86, 1173 (1989), and self-sustained sequence replication (Guatelli et al., Proc. Nat. Acad. Sci. USA, 87:1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

Additional methods of amplification are known in the art or are described elsewhere herein.

B. Detection of Polymorphisms in Target DNA

There are two distinct types of analysis of target DNA for detecting polymorphisms. The first type of analysis, sometimes referred to as de novo characterization, is carried out to identify polymorphic sites not previously characterized (i.e., to identify new polymorphisms). This analysis compares target sequences in different individuals to identify points ofvariation, i.e., polymorphic sites. By analyzing groups of individuals representing the greatest ethnic diversity among humans and greatest breed and species variety in plants and animals, patterns characteristic of the most common alleles/haplotypes of the locus can be identified, and the frequencies of such alleles/haplotypes in the population can be determined. Additional allelic frequencies can be determined for subpopulations characterized by criteria such as geography, race, or gender. The de novo identification ofpolymorphisms of the invention is described in the Examples section.

The second type of analysis determines which form(s) ofa characterized (known) polymorphism are present in individuals under test. Additional methods of analysis are known in the art or are described elsewhere herein.

1. Allele-Specific Probes

The design and use of allele-specific probes for analyzing polymorphisms is described by e.g., Saiki et al., Nature 324,163-166 (1986); Dattagupta, EP 235,726, Saiki, WO 89/11548. Allele-specific probes can be designed that hybridize to a segment of target DNA from one individual but do not hybridize to the corresponding segment from another individual due to the presence of different polymorphic forms in the respective segments from the two individuals. Hybridization conditions should be sufficiently stringent that there is a significant difference in hybridization intensity between alleles, and preferably an essentially binary response, whereby a probe hybridizes to only one of the alleles. Some probes are designed to hybridize to a segment of target DNA such that the polymorphic site aligns with a central position (e.g., in a 15-mer at the 7 position; in a 16-mer, at either the 8 or 9 position) of the probe. This design of probe achieves good discrimination in hybridization between different allelic forms.

A1lele-specific probes are often used in pairs, one member of a pair showing a perfect match to a reference form of a target sequence and the other member showing a perfect match to a variant form. Several pairs of probes can then be immobilized on the same support for simultaneous analysis of multiple polymorphisms within the same target sequence.

2. Tiling Arrays

The polymorphisms can also be identified by hybridization to nucleic acid arrays, some examples of which are described in WO 95/11995. The same arrays or different arrays can be used for analysis of characterized polymorphisms. -WO 95/11995 also describes sub arrays that are optimized for detection of a variant form of a precharacterized polymorphism. Such a sub array contains probes designed to be complementary to a second reference sequence, which is an allelic variant of the first reference sequence. The second group of probes is designed by the same principles as described, except that the probes exhibit complementarity to the second reference sequence. The inclusion of a second group (or further groups) can be particularly useful for analyzing short subsequences of the primary reference sequence in which

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 275

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 275

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO:1 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: PTA-2766, which is hybridizable to SEQ ID NO:1;

(b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:2 or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No: PTA-2766, which is hybridizable to SEQ ID NO:1;

(c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:2 or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No: PTA-2766, which is hybridizable to SEQ ID NO:1;

(d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:2 or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No: PTA-2766, which is hybridizable to SEQ ID NO:1;

(e) a polynucleotide encoding a polypeptide of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: PTA-2766, which is hybridizable to SEQ ID NO:1, having biological activity;

(f) a polynucleotide which is a variant of SEQ ID NO:1;

(g) a polynucleotide which is an allelic variant of SEQ ID NO:1;

(h) an isolated polynucleotide comprising nucleotides 886 to 2517 of SEQ ID NO:1, wherein said nucleotides encode a polypeptide of SEQ ID NO:2 minus the start codon;

(i) an isolated polynucleotide comprising nucleotides 883 to 2517 of SEQ ID NO:1, wherein said nucleotides encode a polypeptide of SEQ ID NO:2 including the start codon;

(j) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:1; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(j), wherein said polynucleotide does not

hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a potassium channel alpha subunit protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:2 or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No: PTA-2766, which is hybridizable to SEQ ID NO:1.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:1 or the cDNA sequence included in ATCC Deposit No: PTA-2766, which is hybridizable to SEQ ID NO:1.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: PTA-2766;

(b) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: PTA-2766, having biological activity;

(c) a polypeptide domain of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: PTA-2766;

(d) a polypeptide epitope of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: PTA-2766;

(e) a full length protein of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: PTA-2766;

(f) a variant of SEQ ID NO:2;

(g) an allelic variant of SEQ ID NO:2;

(h) a species homologue of SEQ ID NO:2;

(i) a polypeptide comprising amino acids 2 to 545 of SEQ ID NO:2, wherein said amino acids 2 to 545 comprise a polypeptide of SEQ ID NO:2 minus the start methionine;

(j) a polypeptide comprising amino acids 1 to 545 of SEQ ID NO:2; and

(k) a polypeptide encoded by the cDNA contained in ATCC Deposit No. PTA-2766.

12. The isolated polypeptide of claim 11, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:2.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:1 in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.

24. A process for making polynucleotide sequences encoding gene products having altered activity selected from the group consisting of SEQ ID NO:2 activity comprising,

a) shuffling a nucleotide sequence of claim 1,

b) expressing the resulting shuffled nucleotide sequences and,

c) selecting for altered biological activity of SEQ ID NO:2 activity as compared to the activity of the gene product of said unmodified nucleotide sequence.

25. The process of claim 24, wherein the nucleotide sequence is any one of the sequences selected from the group consisting of SEQ ID NO:1, 33, and 35.

26. A shuffled polynucleotide sequence produced from the process of claim 25.

27. An isolated nucleic acid molecule consisting of a polynucleotide having a nucleotide sequence selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide of SEQ ID NO:2;

(b) a polynucleotide comprising nucleotides 886 to 2517 of SEQ ID NO:1, wherein said nucleotides encode a polypeptide of SEQ ID NO:2 minus the start codon;

(c) a polynucleotide comprising nucleotides 883 to 2517 of SEQ ID NO:1, wherein said nucleotides encode a polypeptide of SEQ ID NO:2 including the start codon;

(d) a polynucleotide encoding the K+alphaM1 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. PTA-2766;

(e) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:1;

(f) a polynucleotide comprising a polymorphic allele at nucleotide position selected from the group consisting of 841, 894, 1065, 1937, 1677, and 2197 of SEQ ID NO:1; and

(g) a polynucleotide comprising a polymorphic allele at nucleotide position selected from the group consisting of 841, 894, 1065, 1937, 1677, and 2197, wherein the nucleotide at the polymorphic allele is selected from the group consisting of 841C, 841G, 894G, 894T, 1065C, 1065G, 1677C, 1677G, 1937T, 1937C, 2197A, and 2197G of SEQ ID NO:1.

28. The isolated nucleic acid molecule of claim 27, wherein the polynucleotide comprises a nucleotide sequence encoding a potassium channel alpha subunit protein.

29. The isolated nucleic acid molecule of claim 27, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the polypeptide sequence identified as SEQ ID NO:2.

30. The isolated nucleic acid molecule of claim 28, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

31. A recombinant vector comprising the isolated nucleic acid molecule of claim 28.

32. A recombinant host cell comprising the recombinant vector of claim 31.

33. An isolated polypeptide consisting of an amino acid sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:2 having potassium channel modulatory activity;

(b) a polypeptide domain of SEQ ID NO:2 having potassium channel modulatory activity;

(c) a full length protein of SEQ ID NO:2;

(d) a variant of SEQ ID NO:2 having potassium channel modulatory activity;

(e) an allelic variant of SEQ ID NO:2;

(f) a polypeptide corresponding to amino acids 2 to 545 of SEQ ID NO:2, wherein said amino acids 2 to 545 comprise a polypeptide of SEQ ID NO:2 minus the start methionine;

(g) a polypeptide corresponding to amino acids 1 to 545 of SEQ ID NO:2; and

(h) a polypeptide encoded by the cDNA contained in ATCC Deposit No. PTA-2766.

34. A method of screening for candidate compounds capable of binding to and/or modulating activity of a potassium channel alpha subunit, comprising:

a.) contacting a test compound with a substantially or partially purified polypeptide according to claim 28; and

b.) selecting as candidate compounds those test compounds that bind to and/or modulate activity of the polypeptide.

35. The method according to claim 34, wherein the candidate compounds are small molecules.

36. An isolated nucleic acid molecule consisting of a polynucleotide having a nucleotide sequence selected from the group consisting of:

(a) a polynucleotide comprising nucleotides 82 to 1713 of SEQ ID NO:33, wherein said nucleotides encode a polypeptide of SEQ ID NO:34 minus the start codon;

(b) a polynucleotide comprising nucleotides 79 to 1713 of SEQ ID NO:33, wherein said nucleotides encode a polypeptide of SEQ ID NO:34 including the start codon;

(c) a polynucleotide encoding the K+alphaM1.v1 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. PTA-2766;

(d) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:33;

(e) a polynucleotide comprising a polymorphic allele at nucleotide position selected from the group consisting of 37, 90, 261, 873, 1133, and 1393 of SEQ ID NO:33; and

(f) a polynucleotide comprising a polymorphic allele at nucleotide position selected from the group consisting of 37, 90, 261, 873, 1133, and 1393, wherein the nucleotide at the polymorphic allele is selected from the group consisting of 37C, 37G, 90G, 90T, 261C, 261G, 873C, 873G, 1133T, 1133C, 1393A, and 1393G of SEQ ID NO:33.

37. An isolated nucleic acid molecule consisting of a polynucleotide having a nucleotide sequence selected from the group consisting of:

(a) a polynucleotide having the nucleic acid sequence according to nucleotides 82 to 1713 of SEQ ID NO:35, wherein said nucleotides encode a polypeptide of SEQ ID NO:36 minus the start codon;

(b) a polynucleotide having the nucleic acid sequence according to nucleotides 79 to 1713 of SEQ ID NO:35, wherein said nucleotides encode a polypeptide of SEQ ID NO:36 including the start codon;

(c) a polynucleotide encoding the K+alphaM1.v2 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. PTA-2766;

(d) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:36; a polynucleotide comprising a polymorphic allele at nucleotide position selected from the group consisting of 37, 90, 261, 873, 1133, and 1393 of SEQ ID NO:35;

(e) a polynucleotide comprising a polymorphic allele at nucleotide position selected from the group consisting of 37, 90, 261, 873, 1133, and 1393 of SEQ ID NO:35, wherein the nucleotide at the polymorphic allele is selected from the group consisting of 37C, 37G, 90G, 90T, 261C, 261G, 873C, 873G, 1133T, 1133C, 1393A, and 1393G of SEQ ID NO:35.

Figure 1A

```
                .         .         .         .         .         .
   1  AAGTCAGGCTCCCTTTAAATATGGCCTAATATTGCTGAGCAGGGTTGTGAGGCCCTAAAA   60

                .         .         .         .         .         .
  61  ACGTCTTCCTACCATTCCTGGAATTCTACCTTGAAACATGTCTCTATCCTTTAAGAGAAA  120

                .         .         .         .         .         .
 121  GGGAGGAGATAAAAAGGAGAGAGAGAAGCTGAAGCTGACTCAAAGATCCGACTGGATCTG  180

                .         .         .         .         .         .
 181  AACAGTGCCCCAGGGAGAATCCATTTGAAAAAAAAAAAAAAATGTGATCATGTGAATGGA  240

                .         .         .         .         .         .
 241  CAAGAAGGAGATGGCTTTAGATCTTATATGCTCTAAACGAAGAGTTACGCTGAGAGGGAA  300

                .         .         .         .         .         .
 301  ACTGACTTGTCATGAAGTCAGCTTTGTTCCGTTGCTATGTGTCATCCCTGCTAATGGTGA  360

                .         .         .         .         .         .
 361  GTTTACCTAAGGGCAGAGGCTACCATCTCAACCATGAAGCTGAAGACACAGGCATCCGTA  420

                .         .         .         .         .         .
 421  TTCTATAGCTAATTCAGTTGATTTCATCTCAGCACACATACACTGAGCGCTTCCTAAGAG  480

                .         .         .         .         .         .
 481  CGAGGTTGACCGACATTTTTATTAGCAATAATCTCTGCCTTCTTCTGATTACCTAGAGAT  540

                .         .         .         .         .         .
 541  TTAAGACCACATAATCATCCTCTACCTCACAGGGTCAAGGGAGTGGGGGAGGAAATGGGC  600

                .         .         .         .         .         .
 601  TAAGAGGTTCTAAATCCCTCCTAACACTTGCTTCTTCCAAATCAGCAAGATTAGAGCAGT  660

                .         .         .         .         .         .
 661  CAACAGCTGACTGCGTTCAGACCCTGCAGGCTGGGCTGGCCTGCCCAGGACCTGAGAAGG  720

                .         .         .         .         .         .
 721  GGCAGCTCCGGTGGCAATGTCTGAGCCCCTAGCTGTGCTGGTCCGGGCTGGCCTCTCTAA  780

                .         .         .         .         .         .
 781  GACAGTGCAGGCCACGTGATCCATCCTCCTAGAGGCAGTGAGCAGGTGAGGGACCCCTAC  840

                .         .         .         .         .         .
 841  SACAGCCAGGAGGAAAAAGCTAGGCGTCCACTTTCCGCAGCCATGCTCAAACAGAGTGAG  900
   1                                          M   L   K   Q   S   E      6

                .         .         .         .         .         .
 901  AGGAGACGGTCCTGGAGCTACAGGCCCTGGAACACGACGGAGAATGAGGGCAGCCAACAC  960
   7  R   R   R   S   W   S   Y   R   P   W   N   T   T   E   N   E   G   S   Q   H     26

                .         .         .         .         .         .
 961  CGCAGGAGCATTTGCTCCCTGGGTGCCCGTTCCGGCTCCCAGGCCAGCATCCACGGCTGG  1020
  27  R   R   S   I   C   S   L   G   A   R   S   G   S   Q   A   S   I   H   G   W     46

                .         .         .         .         .         .
1021  ACAGAGGGCAACTATAACTACTACATCGAGGAAGACGAAGACGGSGAGGAGGAGGACCAG  1080
  47  T   E   G   N   Y   N   Y   Y   I   E   E   D   E   D   G   E   E   E   D   Q     66
```

Figure 1B

```
1081  TGGAAGGACGACCTGGCAGAAGAGGACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCC  1140
  67  W  K  D  D  L  A  E  E  D  Q  Q  A  G  E  V  T  T  A  K  P    86

1141  GAGGGCCCCAGCGACCCTCCGGCCCTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCAC  1200
  87  E  G  P  S  D  P  P  A  L  L  S  T  L  N  V  N  V  G  G  H   106

1201  AGCTACCAGCTGGACTACTGCGAGCTGGCCGGCTTCCCCAAGACGCGCCTAGGTCGCCTG  1260
 107  S  Y  Q  L  D  Y  C  E  L  A  G  F  P  K  T  R  L  G  R  L   126

1261  GCCACCTCCACCAGCCGCAGCCGCCAGCTAAGCCTGTGCGACGACTACGAGGAGCAGACA  1320
 127  A  T  S  T  S  R  S  R  Q  L  S  L  C  D  D  Y  E  E  Q  T   146

1321  GACGAATACTTCTTCGACCGCGACCCGGCCGTCTTCCAGCTGGTCTACAATTTCTACCTG  1380
 147  D  E  Y  F  F  D  R  D  P  A  V  F  Q  L  V  Y  N  F  Y  L   166

1381  TCCGGGGTGCTGCTGGTGCTCGACGGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGC  1440
 167  S  G  V  L  L  V  L  D  G  L  C  P  R  R  F  L  E  E  L  G   186

1441  TACTGGGGCGTGCGGCTCAAGTACACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGG  1500
 187  Y  W  G  V  R  L  K  Y  T  P  R  C  C  R  I  C  F  E  E  R   206

1501  CGCGACGAGCTGAGCGAACGGCTCAAGATCCAGCACGAGCTGCGCGCGCAGGCGCAGGTC  1560
 207  R  D  E  L  S  E  R  L  K  I  Q  H  E  L  R  A  Q  A  Q  V   226

1561  GAGGAGGCGGAGGAACTCTTCCGCGACATGCGCTTCTACGGCCCGCAGCGGCGCCGCCTC  1620
 227  E  E  A  E  E  L  F  R  D  M  R  F  Y  G  P  Q  R  R  R  L   246

1621  TGGAACCTCATGGAGAAGCCATTCTCCTCGGTGGCCGCCAAGGCCATCGGGGTGGCSTCC  1680
 247  W  N  L  M  E  K  P  F  S  S  V  A  A  K  A  I  G  V  A  S   266

1681  AGCACCTTCGTGCTCGTCTCCGTGGTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAG  1740
 267  S  T  F  V  L  V  S  V  V  A  L  A  L  N  T  V  E  E  M  Q   286

1741  CAGCACTCGGGGCAGGGCGAGGGCGGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAG  1800
 287  Q  H  S  G  Q  G  E  G  G  P  D  L  R  P  I  L  E  H  V  E   306

1801  ATGCTGTGCATGGGCTTCTTCACGCTCGAGTACCTGCTGCGCCTAGCCTCCACGCCCGAC  1860
 307  M  L  C  M  G  F  F  T  L  E  Y  L  L  R  L  A  S  T  P  D   326

1861  CTGAGGCGCTTCGCGCGCAGCGCCCTCAACCTGGTGGACCTGGTGGCCATCCTGCCGCTC  1920
 327  L  R  R  F  A  R  S  A  L  N  L  V  D  L  V  A  I  L  P  L   346

1921  TACCTTCAGCTGCTGCTCGAGTGCTTCACGGGCGAGGGCCACCAACGCGGCCAGACGGTG  1980
 347  Y  L  Q  L  L  L  E  C  F  T  G  E  G  H  Q  R  G  Q  T  V   366
```

Figure 1C

```
1981  GGCAGCGTGGGTAAGGTGGGTCAGGTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGC  2040
 367  G  S  V  G  K  V  G  Q  V  L  R  V  M  R  L  M  R  I  F  R    386

2041  ATCCTCAAGCTGGCGCGCCACTCCACCGGACTGCGTGCCTTCGGCTTCACGCTGCGCCAG  2100
 387  I  L  K  L  A  R  H  S  T  G  L  R  A  F  G  F  T  L  R  Q    406

2101  TGCTACCAGCAGGTGGGCTGCCTGCTGCTCTTCATCGCCATGGGCATCTTCACTTTCTCT  2160
 407  C  Y  Q  Q  V  G  C  L  L  L  F  I  A  M  G  I  F  T  F  S    426

2161  GCGGCTGTCTACTCTGTGGAGCACGATGTGCCCAGCACCAACTTCACTACCATCCCCCAC  2220
 427  A  A  V  Y  S  V  E  H  D  V  P  S  T  N  F  T  T  I  P  H    446

2221  TCCTGGTGGTGGGCCGCGGTGAGCATCTCCACCGTGGGCTACGGAGACATGTACCCAGAG  2280
 447  S  W  W  W  A  A  V  S  I  S  T  V  G  Y  G  D  M  Y  P  E    466

2281  ACCCACCTGGGCAGGTTTTTTGCCTTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGG  2340
 467  T  H  L  G  R  F  F  A  F  L  C  I  A  F  G  I  I  L  N  G    486

2341  ATGCCCATTTCCATCCTCTACAACAAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTAT  2400
 487  M  P  I  S  I  L  Y  N  K  F  S  D  Y  Y  S  K  L  K  A  Y    506

2401  GAGTATACCACCATACGCAGGGAGAGGGGAGAGGTGAACTTCATGCAGAGAGCCAGAAAG  2460
 507  E  Y  T  T  I  R  R  E  R  G  E  V  N  F  M  Q  R  A  R  K    526

2461  AAGATAGCTGAGTGTTTGCTTGGAAGCAACCCACAGCTCACCCCAAGACAAGAGAATTAG  2520
 527  K  I  A  E  C  L  L  G  S  N  P  Q  L  T  P  R  Q  E  N  *    546

2521  TATTTTATAGGACATGTGGCTGGTAGATTCCATGAACTTCAAGGCTTCATTGCTCTTTTT  2580

2581  TTAATCATTATGATTGGCAGCAAAAGGAAATGTGAAGCAGACATACACAAAGGCCATTTC  2640

2641  GTTCACAAAGTACTGCCTCTAGAAATACTCATTTTGGCCCAAACTCAGAATGTCTCATAG  2700

2701  TTGCTCTGTGTTGTGTGAAACATCTGACCTTCTCAATGACGTTGATATTGAAAACCTGAG  2760

2761  GGGAGCAACAGCTTAGATTTTACTTGTAGCTTCTCGTGGCATCTAGCTCAATAAATATTT  2820

2821  TTGGACTTGAAAAAAAAAAAAAAAAAAAA  2850
```

Figure 2.

```
Shab-related      ------------------------------------------------------------
Kv9.3             ------------------------------------------------------------
Kv8.1             ------------------------------------------------------MPSS
K+alphaM1         MLKQSERRRSWSYRPWNTTENEGSQHRRSICSLGARSGSQASIHGWTEGNYNYYIEEDED
                    ::.:.... . .  :  ::: .:.... .   .. .: . .   : : :   :  ..

Shab-related      ---------------MVFGEFFHR--PGQDE-----ELVNLNVGGFKQSVDQSTLLRFPH
Kv9.3             ---------------MVFGEFFHR--PGQDE-----ELVNLNVGGFKQSVDQSTLLRFPH
Kv8.1             GRALLDSPLDSGSLTSLDSSVFCS--EGEGEPLALGDCFTVNVGGSRFVLSQQALSCFPH
K+alphaM1         GEEEDQWKDDLAEEDQQAGEVTTAKPEGPSDPPALLSTLNVNVGGHSYQLDYCELAGFPK
                   .   :   :  .     ...   :  * .:     . ..:****    :.   *  **:

Shab-related      TRLGKLLTCHSEE----------AILELCDDYSVADKEYYFDRNPSLFRYVLNFYYTGKL
Kv9.3             TRLGKLLTCHSEE----------AILELCDDYSVADKEYYFDRNPSLFRYVLNFYYTGKL
Kv8.1             TRLGKLAVVVASYRRPGALAAVPSPLELCDDANPVDNEYFFDRSSQAFRYVLHYYRTGRL
K+alphaM1         TRLGRLATSTSRS----------RQLSLCDDYEEQTDEYFFDRDPAVFQLVYNFYLSGVL
                  ****:* .  :              *.**** .   .**:***..  *: * ::* :* *

Shab-related      HVMEELCVFSFCQEIEYWGINELFIDSCCSNRYQERK-EENHEKDWDQKSHDVSTDSSFE
Kv9.3             HVMEELCVFSFCDEIEYWGINELFIDSCCSSRYQERK-EESHEKDWDQKSNDVSTDSSFE
Kv8.1             HVMEQLCALSFLQEIQYWGIDELSIDSCCRDRYFRRK-ELSETLDFKKDTEDQESQHESE
K+alphaM1         LVLDGLCPRRFLEELGYWGVRLKYTPRCCRICFEERRDELSERLKIQHELRAQAQVEEAE
                   *:: **   * :*: ***:       **   : .*::* ..  . .:. .      . *

Shab-related      ESSLFEKELEKFDTLR-FGQLRKKIWIRMENPAYCLSAKLIAISSLSVVLASIVAMCVHS
Kv9.3             ESSLFEKELEKFDELR-FGQLRKKIWIRMENPAYCLSAKLIAISSLSVVLASIVAMCVHS
Kv8.1             QD---------FSQGP-CPTVRQKLWNILEKPGSSTAARIFGVISIIFVVVSIINMALMS
K+alphaM1         EL---------FRDMRFYGPQRRRLWNLMEKPFSSVAAKAIGVASSTFVLVSVVALALNT
                  :          *         *:::*  :*:*  . :*: :.: *  .*:.*:: :.: :

Shab-related      MSEFQN-----EDGEVDDPVLEGVEIACIAWFTGELAVRLAAAPCQKKFWKNPLNIIDFV
Kv9.3             MSEFQN-----EDGEVDDPVLEGVEIACIAWFTGELAIRLVAAPSQKKFWKNPLNIIDFV
Kv8.1             A----------ELSWLDLQLLEILEYVCISWFTGEFVLRFLCVRDRCRFLRKVPNIIDLL
K+alphaM1         VEEMQQHSGQGEGGPDLRPILEHVEMLCMGFFTLEYLLRLASTPDLRRFARSALNLVDLV
                   .: ::.. : * .     :** :*  *:.:** *  :*: ..    :* :.  *::*::

Shab-related      SIIPFYATLAVDTKE----EESEDIENMGKVVQIL---RLMRIFRILKLARHSVGLRSLG
Kv9.3             SIIPFYATLAVDTKE----EESEDIENMGKVVQIL---RLMRIFRILKLARHSVGLRSLG
Kv8.1             AILPFYITLLVESLSGS--QTTQELENVGRIVQVL---RLLRALRMLKLGRHSTGLRSLG
K+alphaM1         AILPLYLQLLLECFTGEGHQRGQTVGSVGKVGQVLRVMRLMRIFRILKLARHSTGLRAFG
                  :*:*:*  * ::    . .:  : : .:*:: *:*.  **:* :*:***.***.***::*

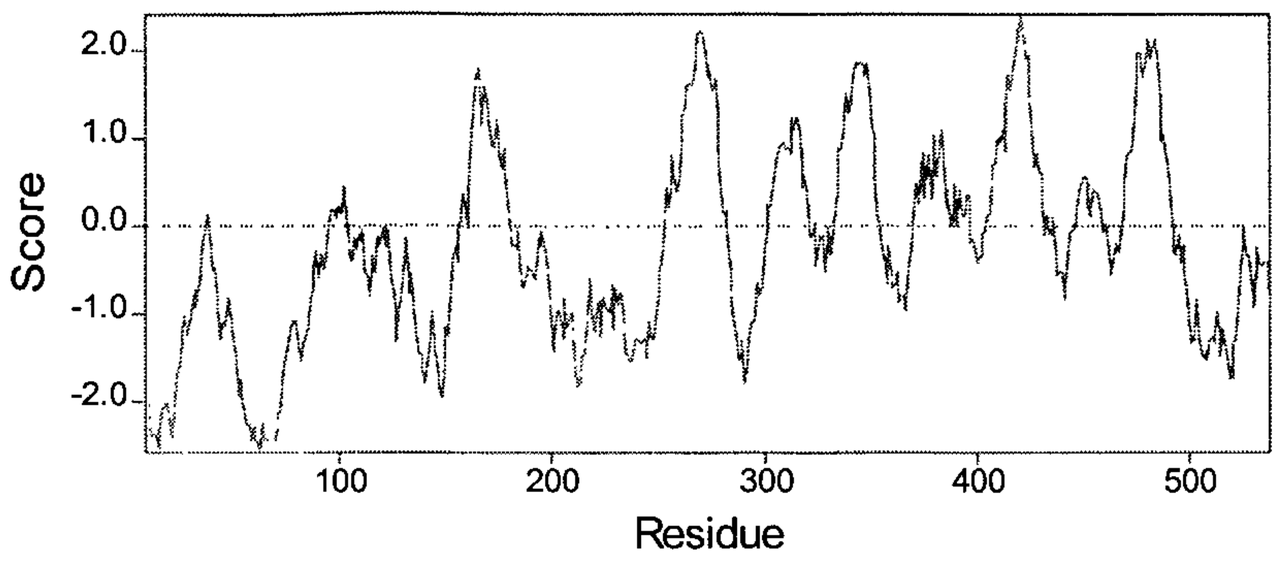
Shab-related      ATLRHSYHEVGLLLLFLSVGISIFSVLIYSVEKDDHTSSLTSIPICWWWATISMTTVGYG
Kv9.3             ATLRHSYHEVGLLLLFLSVGISIFSVLIYSVEKDELASSLTSIPICWWWATISMTTVGYG
Kv8.1             MTITQCYEEVGLLLLFLSVGISIFSTVEYFAEQSIPDTTFTSVPCAWWWATTSMTTVGYG
K+alphaM1         FTLRQCYQQVGCLLLFIAMGIFTFSAAVYSVEHDVPSTNFTTIPHSWWWAAVSISTVGYG
                   *: :.*.:** ****:::**  **.  * .*:.   :.:*::* .****: *::*****

Shab-related      DTHPVTLAGKLIASTCICGILVVALPITIIFNKFSKYYQ--KQKDIDVDQ-CSEDAPEK
Kv9.3             DTHPVTLAGKIIASTCICGILVVALPITIIFNKFSKYYQ--KQKDMDVDQ-CSEDPPEK
Kv8.1             DIRPDTTTGKIVAFMCILSGILVLALPIAIINDRFSACYFTLKLKEAAVRQREALKKLTK
K+alphaM1         DMYPETHLGRFFAFLCIAFGILILNGMPISILYNKFSDYYS--KLKAYEYTT------IRR
                  *  * *  *::.*  **  **:: .:**:*: ::**  *   * *              :

Shab-related      ---CHELPYFNIRDIYAQRMHAFITSLSSVGIVVSDPDSTDASSIEDNEDICNTTSLENC
Kv9.3             ---CHELPYFNIRDVYAQQVHAFITSLSSIGIVVSDPDSTDASSVEDNEDAYNTASLENC
Kv8.1             NIATDSYISVNLRDVYARSIMEMLR-LKGR---ER--ASTRSSGG-D--DFWF-------
K+alphaM1         -----ERGEVNFMQRARKKIAECLLGSNPQ-------LTPRQEN----------------
                       .   .*: :   : :   :   .           :.  ..

Shab-related      TAK
Kv9.3             TAK
Kv8.1             ---
K+alphaM1         ---
```

Figure 3.


Profile View
Score
2.0
1.0
0.0
-1.0
-2.0
100
200
300
400
500
Residue
Key
Kyte-Doolittle hydropathy: bac15

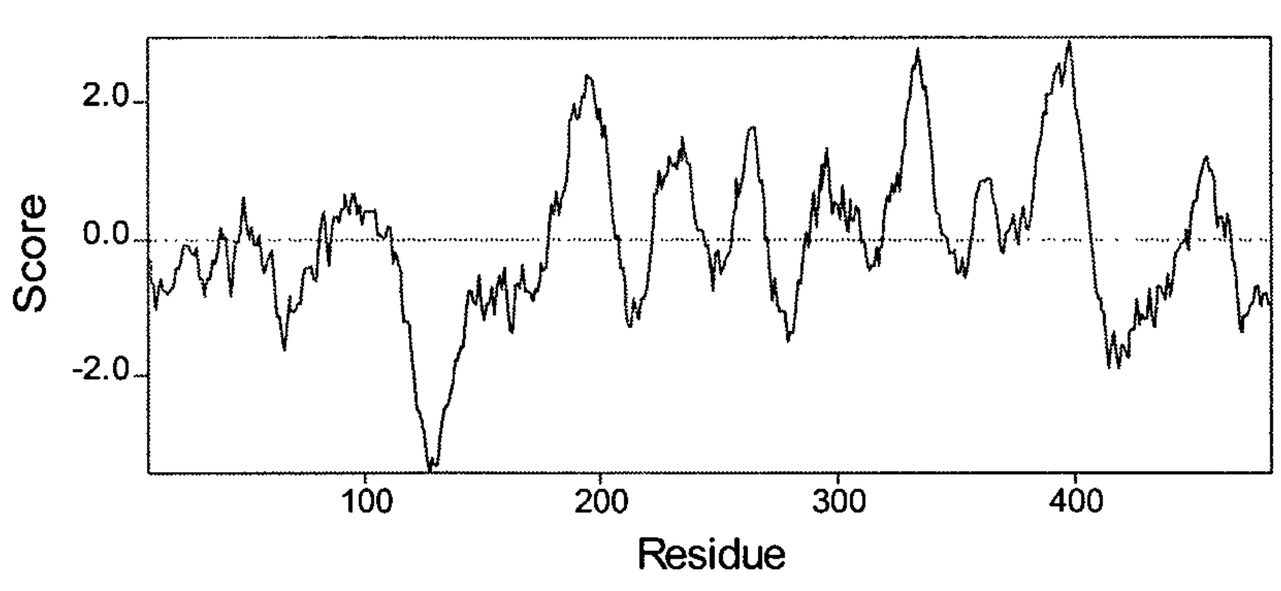

Profile View
Score
2.0
0.0
-2.0
100
200
300
400
Residue
Key
Kyte-Doolittle hydropathy: SHAB-R

Figure 4.

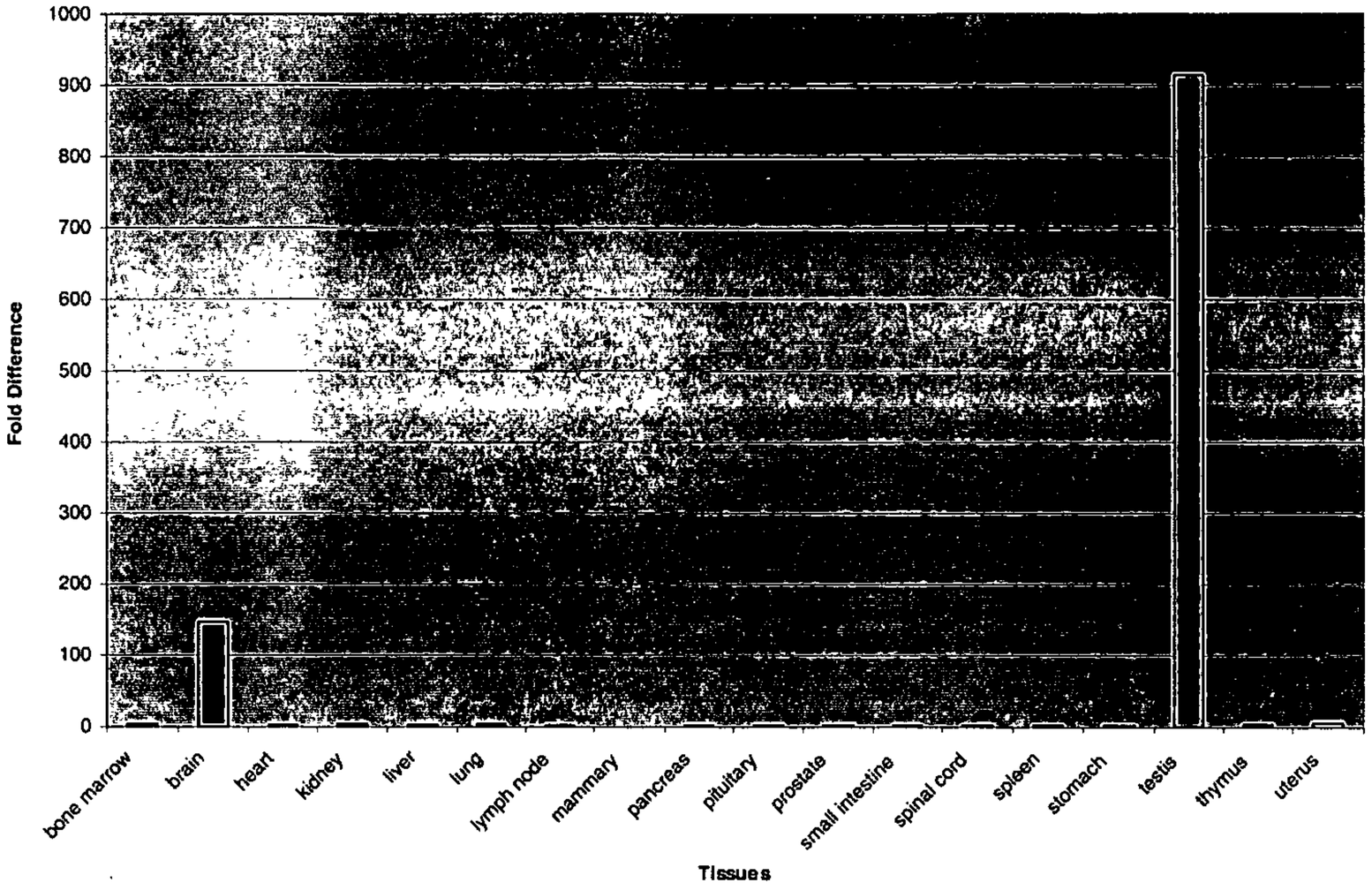

1000
900
800
700
600
500
400
300
200
100
0
Fold Difference
bone marrow
brain
heart
kidney
liver
lung
lymph node
mammary
pancreas
pituitary
prostate
small intestine
spinal cord
spleen
stomach
testis
thymus
uterus
Tissues

Figure 5.

K+aplhaM1

Gene Name	Genbank Accession No.	Percent Identity	Percent Similarity
Human shab-related K+ channel	gi|2815899	41%	52%
Rat Kv9.3	gi|7514119	40.59%	51.7%
Human Kv8.1	gi|6604550	41.35%	51.2%

K+aplhaM1.v1

Gene Name	Genbank Accession No.	Percent Identity	Percent Similarity
Human shab-related K+ channel	gi|2815899	41%	52%
Rat Kv9.3	gi|7514119	39%	49.88%
Human Kv8.1	gi|6604550	39.8%	49%

K+aplhaM1.v2

Gene Name	Genbank Accession No.	Percent Identity	Percent Similarity
Human shab-related K+ channel	gi|2815899	41.1%	52%
Rat Kv9.3	gi|7514119	40.6%	51.7%
Human Kv8.1	gi|6604550	41.1%	51%

Figure 6A

```
                .         .         .         .         .         .
  1  CTAGTCCTGCAGGTTTAAACGAATTCGCCCTTCTACCACAGCCAGGAGGAAAAAGCTAGG    60

                .         .         .         .         .         .
 61  CGTCCACTTTCCGCAGCCATGCTCAAACAGAGTGAGAGGAGACGGTCCTGGAGCTACAGG    120
  1                      M   L   K   Q   S   E   R   R   R   S   W   S   Y   R     14

                .         .         .         .         .         .
121  CCCTGGAACACGACGGAGAATGAGGGCAGCCAACACCGCAGGAGCATTTGCTCCCTGGGT    180
 15  P   W   N   T   T   E   N   E   G   S   Q   H   R   R   S   I   C   S   L   G     34

                .         .         .         .         .         .
181  GCCCGTTCCGGCTCCCAGGCCAGCATCCACGGCTGGACAGAGGGCAACTATAACTACTAC    240
 35  A   R   S   G   S   Q   A   S   I   H   G   W   T   E   G   N   Y   N   Y   Y     54

                .         .         .         .         .         .
241  ATCGAGGAAGACGAAGACGGCGAGGAGGAGGACCAGTGGAAGGACGACCTGGCAGAAGAG    300
 55  I   E   E   D   E   D   G   E   E   E   D   Q   W   K   D   D   L   A   E   E     74

                .         .         .         .         .         .
301  GACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCCGAGGGCCCCAGCGACCCTCCGGCC    360
 75  D   Q   Q   A   G   E   V   T   T   A   K   P   E   G   P   S   D   P   P   A     94

                .         .         .         .         .         .
361  CTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCACAGCTACCAGCTGGACTACTGCGAG    420
 95  L   L   S   T   L   N   V   N   V   G   G   H   S   Y   Q   L   D   Y   C   E    114

                .         .         .         .         .         .
421  CTGGCCGGCTTCCCCAAGACGCGCCTAGGTCGCCTGGCCACCTCCACCAGCCGCAGCCGC    480
115  L   A   G   F   P   K   T   R   L   G   R   L   A   T   S   T   S   R   S   R    134

                .         .         .         .         .         .
481  CAGCTAAGCCTGTGCGACGACTACGAGGAGCAGACAGACGAATACTTCTTCGACCGCGAC    540
135  Q   L   S   L   C   D   D   Y   E   E   Q   T   D   E   Y   F   F   D   R   D    154

                .         .         .         .         .         .
541  CCGGCCGTCTTCCAGCTGGTCTACAATTTCTACCTGTCCGGGGTGCTGCTGGTGCTCGAC    600
155  P   A   V   F   Q   L   V   Y   N   F   Y   L   S   G   V   L   L   V   L   D    174

                .         .         .         .         .         .
601  GGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGCTACTGGGGCGTGCGGCTCAAGTAC    660
175  G   L   C   P   R   R   F   L   E   E   L   G   Y   W   G   V   R   L   K   Y    194

                .         .         .         .         .         .
661  ACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGGCGCGACGAGCTGAGCGAACGGCTC    720
195  T   P   R   C   C   R   I   C   F   E   E   R   R   D   E   L   S   E   R   L    214

                .         .         .         .         .         .
721  AAGATCCAGCACGAGCTGCGCGCGCAGGCGCAGGTCGAGGAGGCGGAGGAACTCTTCCGC    780
215  K   I   Q   H   E   L   R   A   Q   A   Q   V   E   E   A   E   E   L   F   R    234

                .         .         .         .         .         .
781  GACATGCGCTTCTACGGCCCGCAGCGGCGCCGCCTCTGGAACCTCATGGAGAAGCCATTC    840
235  D   M   R   F   Y   G   P   Q   R   R   R   L   W   N   L   M   E   K   P   F    254

                .         .         .         .         .         .
841  TCCTCGGTGGCCGCCAAGGCCATCGGGGTGGCCTCCAGCACCTTCGTGCTCGTCTCCGTG    900
255  S   S   V   A   A   K   A   I   G   V   A   S   S   T   F   V   L   V   S   V    274
```

Figure 6B

```
901   GTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAGCAGCACTCGGGGCAGGGCGAGGGC   960
275   V  A  L  A  L  N  T  V  E  E  M  Q  Q  H  S  G  Q  G  E  G     294

961   GGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAGATGCTGTGCATGGGCTTCTTCACG   1020
295   G  P  D  L  R  P  I  L  E  H  V  E  M  L  C  M  G  F  F  T     314

1021  CTCGAGTACCTGCTGCGCCTAGCCTCCACGCCCGACCTGAGGCGCTTCGCGCGCAGCGCC   1080
315   L  E  Y  L  L  R  L  A  S  T  P  D  L  R  R  F  A  R  S  A     334

1081  CTCAACCTGGTGGACCTGGTGGCCATCCTGCCGCTCTACCTTCAGCTGCTGCCCGAGTGC   1140
335   L  N  L  V  D  L  V  A  I  L  P  L  Y  L  Q  L  L  P  E  C     354

1141  TTCACGGGCGAGGGCCACCAACGCGGCCAGACGGTGGGCAGCGTGGGTAAGGTGGGTCAG   1200
355   F  T  G  E  G  H  Q  R  G  Q  T  V  G  S  V  G  K  V  G  Q     374

1201  GTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGCATCCTCAAGCTGGCGCGCCACTCC   1260
375   V  L  R  V  M  R  L  M  R  I  F  R  I  L  K  L  A  R  H  S     394

1261  ACCGGACTGCGTGCTTCGGCTTCACGCTGCGCCAGTGCTACCAGCAGGTGGGCGTGCCTG   1320
395   T  G  L  R  A  S  A  S  R  C  A  S  A  T  S  R  W  A  C  L     414

1321  CTGCTCTTCATCGCCATGGGCATCTTCACTTTCTCTGCGGCTGTCTACTCTGTGGAGCAC   1380
415   L  L  F  I  A  M  G  I  F  T  F  S  A  A  V  Y  S  V  E  H     434

1381  GATGTGCCCAGCACCAACTTCACTACCATCCCCCACTCCTGGTGGTGGGCCGCGGTGAGC   1440
435   D  V  P  S  T  N  F  T  T  I  P  H  S  W  W  W  A  A  V  S     454

1441  ATCTCCACCGTGGGCTACGGAGACATGTACCCAGAGACCCACCTGGGCAGGTTTTTTGCC   1500
455   I  S  T  V  G  Y  G  D  M  Y  P  E  T  H  L  G  R  F  F  A     474

1501  TTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGGATGCCCATTTCCATCCTCTACAAC   1560
475   F  L  C  T  A  F  G  T  I  L  N  G  M  P  I  S  I  L  Y  N     494

1561  AAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTATGAGTATACCACCATACGCAGGGAG   1620
495   K  F  S  D  Y  Y  S  K  L  K  A  Y  E  Y  T  T  I  R  R  E     514

1621  AGGGGAGAGGTGAACTTCATGCAGAGAGCCAGAAAGAAGATAGCTGAGTGTTTGCTTGGA   1680
515   R  G  E  V  N  F  M  Q  R  A  R  K  K  I  A  E  C  L  L  G     534

1681  AGCAACCCACAGCTCACCCCAAGACAAGAGAATTAGTATTTTATAGGACATGTGGCTGGT   1740
535   S  N  P  Q  L  T  P  R  Q  E  N                               545

1741  AGATTCCATGAACTTCAAGGCTTCATTGCTCTTTTTTTAATCATTATGATTGGCAGCAAA   1800
```

Figure 6C

```
                    .         .         .         .         .         .
1801  AGGAAATGTGAAGCAGACATACACAAAGGCCATTTCGTTCACAAAGAAGGGCGAATTCGC  1860

                    .
1861  GGCCGCTAAAT  1871
```

Figure 7A

```
   1  CTAGTCCTGCAGGTTTAAACGAATTCGCCCTTCTACCACAGCCAGGAGGAAAAAGCTAGG    60

  61  CGTCCACTTTCCGCAGCCATGCTCAAACATAGTGAGAGGAGACGGTCCTGGAGCTACAGG    120
   1                      M  L  K  H  S  E  R  R  R  S  W  S  Y  R     14

 121  CCCTGGAACACGACGGAGAATGAGGGCAGCCAACACCGCAGGAGCATTTGCTCCCTGGGT    180
  15  P  W  N  T  T  E  N  E  G  S  Q  H  R  R  S  I  C  S  L  G     34

 181  GCCCGTTCCGGCTCCCAGGCCAGCATCCACGGCTGGACAGAGGGCAACTATAACTACTAC    240
  35  A  R  S  G  S  Q  A  S  I  H  G  W  T  E  G  N  Y  N  Y  Y     54

 241  ATCGAGGAAGACGAAGACGGCGAGGAGGAGGACCAGTGGAAGGACGACCTGGCAGAAGAG    300
  55  I  E  E  D  E  D  G  E  E  E  D  Q  W  K  D  D  L  A  E  E     74

 301  GACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCCGAGGGCCCCAGCGACCCTCCGGCC    360
  75  D  Q  Q  A  G  E  V  T  T  A  K  P  E  G  P  S  D  P  P  A     94

 361  CTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCACAGCTACCAGCTGGACTACTGCGAG    420
  95  L  L  S  T  L  N  V  N  V  G  G  H  S  Y  Q  L  D  Y  C  E    114

 421  CTGGCCGGCTTCCCCAAGACGCGCCTAGGTCGCCTGGCCACCTCCACCAGCCGCAGCCGC    480
 115  L  A  G  F  P  K  T  R  L  G  R  L  A  T  S  T  S  R  S  R    134

 481  CAGCTAAGCCTGTGCGACGACTACGAGGAGCAGACAGACGAATACTTCTTCGACCGCGAC    540
 135  Q  L  S  L  C  D  D  Y  E  E  Q  T  D  E  Y  F  F  D  R  D    154

 541  CCGGCCGTCTTCCAGCTGGTCTACAATTTCTACCTGTCCGGGGTGCTGCTGGTGCTCGAC    600
 155  P  A  V  F  Q  L  V  Y  N  F  Y  L  S  G  V  L  L  V  L  D    174

 601  GGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGCTACTGGGGCGTGCGGCTCAAGTAC    660
 175  G  L  C  P  R  R  F  L  E  E  L  G  Y  W  G  V  R  L  K  Y    194

 661  ACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGGCGCGACGAGCTGAGCGAACGGCTC    720
 195  T  P  R  C  C  R  I  C  F  E  E  R  R  D  E  L  S  E  R  L    214

 721  AAGATCCAGCACGAGCTGCGCGCGCAGGCGCAGGTCGAGGAGGCGGAGGAACTCTTCCGC    780
 215  K  I  Q  H  E  L  R  A  Q  A  Q  V  E  E  A  E  E  L  F  R    234

 781  GACATGCGCTTCTACGGCCCGCAGCGGCGCCGCCTCTGGAACCTCATGGAGAAGCCATTC    840
 235  D  M  R  F  Y  G  P  Q  R  R  R  L  W  N  L  M  E  K  P  F    254

 841  TCCTCGGTGGCCGCCAAGGCCATCGGGGTGGCCTCCAGCACCTTCGTGCTCGTCTCCGTG    900
 255  S  S  V  A  A  K  A  I  G  V  A  S  S  T  F  V  L  V  S  V    274
```

Figure 7B

```
901  GTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAGCAGCACTCGGGGCAGGGCGAGGGC  960
275  V  A  L  A  L  N  T  V  E  E  M  Q  Q  H  S  G  Q  G  E  G    294

961  GGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAGATGCTGTGCATGGGCTTCTTCACG  1020
295  G  P  D  L  R  P  I  L  E  H  V  E  M  L  C  M  G  F  F  T    314

1021 CTCGAGTACCTGCTGCGCCTAGCCTCCACGCCCGACCTGAGGCGCTTCGCGCGCAGCGCC  1080
315  L  E  Y  L  L  R  L  A  S  T  P  D  L  R  R  F  A  R  S  A    334

1081 CTCAACCTGGTGGACCTGGTGGCCATCCTGCCGCTCTACCTTCAGCTGCTGCTCGAGTGC  1140
335  L  N  L  V  D  L  V  A  I  L  P  L  Y  L  Q  L  L  L  E  C    354

1141 TTCACGGGCGAGGGCCACCAACGCGGCCAGACGGTGGGCAGCGTGGGTAAGGTGGGTCAG  1200
355  F  T  G  E  G  H  Q  R  G  Q  T  V  G  S  V  G  K  V  G  Q    374

1201 GTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGCATCCTCAAGCTGGCGCGCCACTCC  1260
375  V  L  R  V  M  R  L  M  R  I  F  R  I  L  K  L  A  R  H  S    394

1261 ACCGGACTGCGTGCCTTCGGCTTCACGCTGCGCCAGTGCTACCAGCAGGTGGGCTGCCTG  1320
395  T  G  L  R  A  F  G  F  T  L  R  Q  C  Y  Q  Q  V  G  C  L    414

1321 CTGCTCTTCATCGCCATGGGCATCTTCACTTTCTCTGCGGCTGTCTACTCTGTGGAGCAC  1380
415  L  L  F  I  A  M  G  I  F  T  F  S  A  A  V  Y  S  V  E  H    434

1381 GATGTGCCCAGCGCCAACTTCACTACCATCCCCCACTCCTGGTGGTGGGCCGCGGTGAGC  1440
435  D  V  P  S  A  N  F  T  T  I  P  H  S  W  W  W  A  A  V  S    454

1441 ATCTCCACCGTGGGCTACGGAGACATGTACCCAGAGACCCACCTGGGCAGGTTTTTTGCC  1500
455  I  S  T  V  G  Y  G  D  M  Y  P  E  T  H  L  G  R  F  F  A    474

1501 TTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGGATGCCCATTTCCATCCTCTACAAC  1560
475  F  L  C  I  A  F  G  I  I  L  N  G  M  P  I  S  I  L  V  N    494

1561 AAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTATGAGTATACCACCATACGCAGGGAG  1620
495  K  F  S  D  Y  Y  S  K  L  K  A  Y  E  Y  T  T  I  R  R  E    514

1621 AGGGGAGAGGTGAACTTCATGCAGAGAGCCAGAAAGAAGATAGCTGAGTGTTTGCTTGGA  1680
515  R  G  E  V  N  F  M  Q  R  A  R  K  K  I  A  E  C  L  L  G    534

1681 AGCAACCCACAGCTCACCCCAAGACAAGAGAATTAGTATTTTATAGGACATGTGGCTGGT  1740
535  S  N  P  Q  L  T  P  R  Q  E  N                              545

1741 AGATTCCATGAACTTCAAGGCTTCATTGCTCTTTTTTTAATCATTATGATTGGCAGCAAA  1800
```

Figure 7C

```
                .         .         .         .         .         .
1801  AGGAAATGTGAAGCAGACATACACAAAGGCCATTTCGTTCACAAAGAAGGGCGAATTCGC   1860

                .
1861  GGCCGCTAAAT  1871
```

Figure 8.

```
                          1                                                  50
K+alphaM1          (1)    MLKQSERRRSWSYRPWNTTENEGSQHRRSICSLGARSGSQASIHGWTEGN
K+alpha1_FL2_a     (1)    MLKQSERRRSWSYRPWNTTENEGSQHRRSICSLGARSGSQASIHGWTEGN
K+alphaM1_FL2_b    (1)    MLKHSERRRSWSYRPWNTTENEGSQHRRSICSLGARSGSQASIHGWTEGN

                         51                                                 100
K+alphaM1          (51)   YNYYIEEDEDGEEEDQWKDDLAEEDQQAGEVTTAKPEGPSDPPALLSTLN
K+alpha1_FL2_a     (51)   YNYYIEEDEDGEEEDQWKDDLAEEDQQAGEVTTAKPEGPSDPPALLSTLN
K+alphaM1_FL2_b    (51)   YNYYIEEDEDGEEEDQWKDDLAEEDQQAGEVTTAKPEGPSDPPALLSTLN

                        101                                                 150
K+alphaM1          (101)  VNVGGHSYQLDYCELAGFPKTRLGRLATSTSRSRQLSLCDDYEEQTDEYF
K+alpha1_FL2_a     (101)  VNVGGHSYQLDYCELAGFPKTRLGRLATSTSRSRQLSLCDDYEEQTDEYF
K+alphaM1_FL2_b    (101)  VNVGGHSYQLDYCELAGFPKTRLGRLATSTSRSRQLSLCDDYEEQTDEYF

                        151                                                 200
K+alphaM1          (151)  FDRDPAVFQLVYNFYLSGVLLVLDGLCPRRFLEELGYWGVRLKYTPRCCR
K+alpha1_FL2_a     (151)  FDRDPAVFQLVYNFYLSGVLLVLDGLCPRRFLEELGYWGVRLKYTPRCCR
K+alphaM1_FL2_b    (151)  FDRDPAVFQLVYNFYLSGVLLVLDGLCPRRFLEELGYWGVRLKYTPRCCR

                        201                                                 250
K+alphaM1          (201)  ICFEERRDELSERLKIQHELRAQAQVEEAEELFRDMRFYGPQRRRLWNLM
K+alpha1_FL2_a     (201)  ICFEERRDELSERLKIQHELRAQAQVEEAEELFRDMRFYGPQRRRLWNLM
K+alphaM1_FL2_b    (201)  ICFEERRDELSERLKIQHELRAQAQVEEAEELFRDMRFYGPQRRRLWNLM

                        251                                                 300
K+alphaM1          (251)  EKPFSSVAAKAIGVASSTFVLVSVVALALNTVEEMQQHSGQGEGGPDLRP
K+alpha1_FL2_a     (251)  EKPFSSVAAKAIGVASSTFVLVSVVALALNTVEEMQQHSGQGEGGPDLRP
K+alphaM1_FL2_b    (251)  EKPFSSVAAKAIGVASSTFVLVSVVALALNTVEEMQQHSGQGEGGPDLRP

                        301                                                 350
K+alphaM1          (301)  ILEHVEMLCMGFFTLEYLLRLASTPDLRRFARSALNLVDLVAILPLYLQL
K+alpha1_FL2_a     (301)  ILEHVEMLCMGFFTLEYLLRLASTPDLRRFARSALNLVDLVAILPLYLQL
K+alphaM1_FL2_b    (301)  ILEHVEMLCMGFFTLEYLLRLASTPDLRRFARSALNLVDLVAILPLYLQL

                        351                                                 400
K+alphaM1          (351)  LLECFTGEGHQRGQTVGSVGKVGQVLRVMRLMRIFRILKLARHSTGLRAF
K+alpha1_FL2_a     (351)  LPECFTGEGHQRGQTVGSVGKVGQVLRVMRLMRIFRILKLARHSTGLRAS
K+alphaM1_FL2_b    (351)  LLECFTGEGHQRGQTVGSVGKVGQVLRVMRLMRIFRILKLARHSTGLRAF

                        401                                                 450
K+alphaM1          (401)  GFTLRQCYQQVGCLLLFIAMGIFTFSAAVYSVEHDVPSTNFTTIPHSWWW
K+alpha1_FL2_a     (401)  ASRCASATSRWACLLLFIAMGIFTFSAAVYSVEHDVPSTNFTTIPHSWWW
K+alphaM1_FL2_b    (401)  GFTLRQCYQQVGCLLLFIAMGIFTFSAAVYSVEHDVPSANFTTIPHSWWW

                        451                                                 500
K+alphaM1          (451)  AAVSISTVGYGDMYPETHLGRFFAFLCIAFGIILNGMPISILYNKFSDYY
K+alpha1_FL2_a     (451)  AAVSISTVGYGDMYPETHLGRFFAFLCIAFGIILNGMPISILYNKFSDYY
K+alphaM1_FL2_b    (451)  AAVSISTVGYGDMYPETHLGRFFAFLCIAFGIILNGMPISILYNKFSDYY

                        501                                         545
K+alphaM1          (501)  SKLKAYEYTTIRRERGEVNFMQRARKKIAECLLGSNPQLTPRQEN
K+alpha1_FL2_a     (501)  SKLKAYEYTTIRRERGEVNFMQRARKKIAECLLGSNPQLTPRQEN
K+alphaM1_FL2_b    (501)  SKLKAYEYTTIRRERGEVNFMQRARKKIAECLLGSNPQLTPRQEN
```

Figure 9A

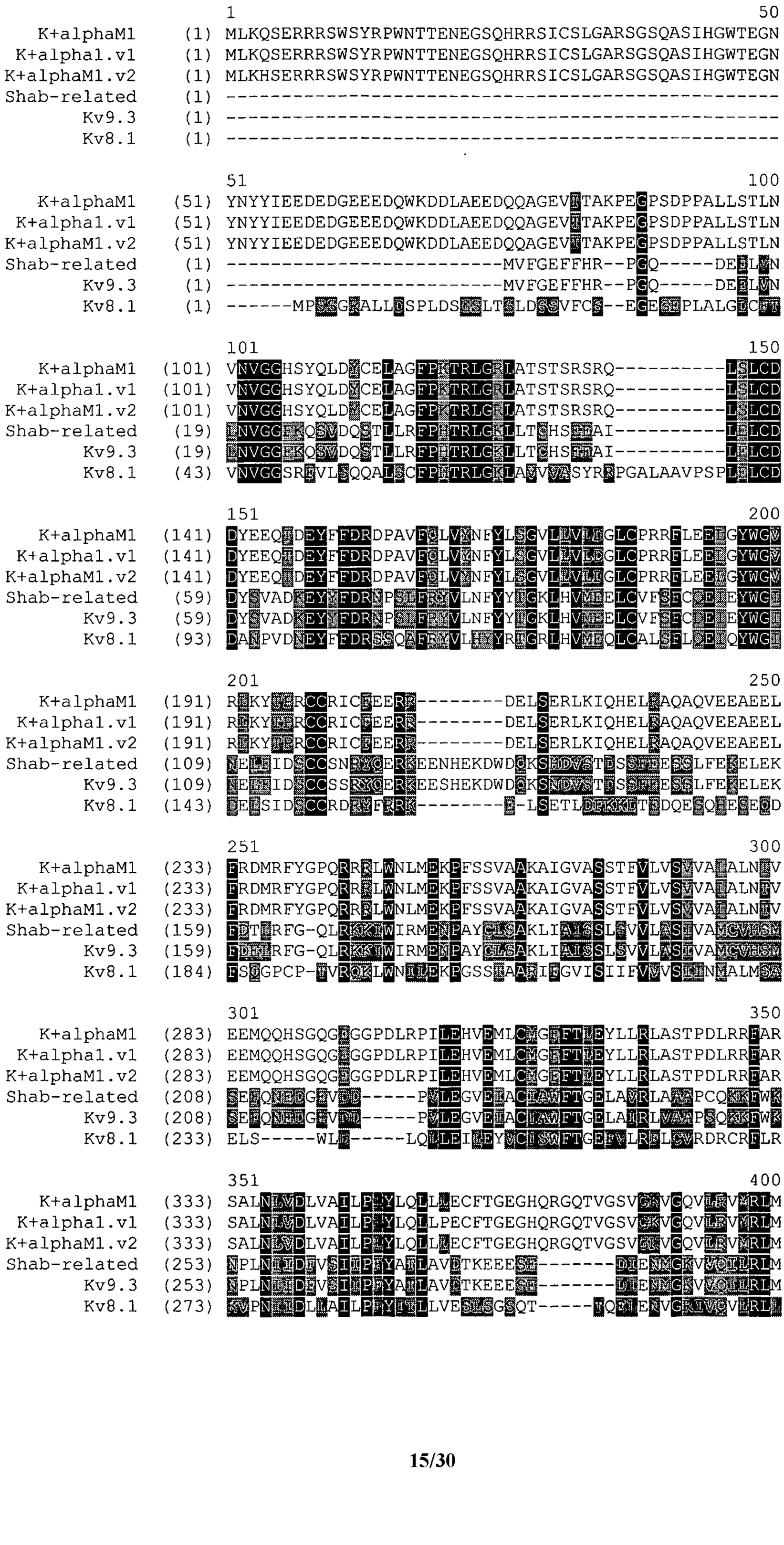

1 50
K+alphaM1 (1) MLKQSERRRSWSYRPWNTTENEGSQHRRSICSLGARSGSQASIHGWTEGN
K+alpha1.v1 (1) MLKQSERRRSWSYRPWNTTENEGSQHRRSICSLGARSGSQASIHGWTEGN
K+alphaM1.v2 (1) MLKHSERRRSWSYRPWNTTENEGSQHRRSICSLGARSGSQASIHGWTEGN
Shab-related (1) ----------
Kv9.3 (1) ----------
Kv8.1 (1) ----------
51 100
K+alphaM1 (51) YNYYIEEDEDGEEEDQWKDDLAEEDQQAGEVMTAKPEGPSDPPALLSTLN
K+alpha1.v1 (51) YNYYIEEDEDGEEEDQWKDDLAEEDQQAGEVMTAKPEGPSDPPALLSTLN
K+alphaM1.v2 (51) YNYYIEEDEDGEEEDQWKDDLAEEDQQAGEVMTAKPEGPSDPPALLSTLN
Shab-related (1) ----------MVFGEFFHR--PGQ-----DEELVN
Kv9.3 (1) ----------MVFGEFFHR--PGQ-----DEELVN
Kv8.1 (1) ------MPSSGRALLDSPLDSGSLTSLDSSVFCS--EGEGSPLALGDCFT
101 150
K+alphaM1 (101) VNVGGHSYQLDYCELAGFPKTRLGRLATSTSRSRQ----------LSLCD
K+alpha1.v1 (101) VNVGGHSYQLDYCELAGFPKTRLGRLATSTSRSRQ----------LSLCD
K+alphaM1.v2 (101) VNVGGHSYQLDYCELAGFPKTRLGRLATSTSRSRQ----------LSLCD
Shab-related (19) LNVGGLKQSVDQSTLLRFPHTRLGKLLTCHSEEAI----------LELCD
Kv9.3 (19) LNVGGLKQSVDQSTLLRFPHTRLGKLLTCHSEEAI----------LELCD
Kv8.1 (43) VNVGGSRLVLSQQALSCFPETRLGKLAVVVASYRRPGALAAVPSPLELCD
151 200
K+alphaM1 (141) DYEEQTDEYFFDRDPAVFQLVYNFYLSGVLLVLDGLCPRRFLEELGYWGV
K+alpha1.v1 (141) DYEEQTDEYFFDRDPAVFQLVYNFYLSGVLLVLDGLCPRRFLEELGYWGV
K+alphaM1.v2 (141) DYEEQTDEYFFDRDPAVFQLVYNFYLSGVLLVLDGLCPRRFLEELGYWGV
Shab-related (59) DYSVADKEYYFDRNPSLFRYVLNFYYTGKLHVMEELCVFSFCQELEYWGI
Kv9.3 (59) DYSVADKEYYFDRNPSLFRYVLNFYYTGKLHVMEELCVFSFCQELEYWGI
Kv8.1 (93) DANPVDNEYFFDRSSQAFRYVLHYYRTGRLHVMEQLCALSFLQELQYWGI
201 250
K+alphaM1 (191) RLKYLERCCRICFEERR--------DELSERLKIQHELRAQAQVEEAEEL
K+alpha1.v1 (191) RLKYLERCCRICFEERR--------DELSERLKIQHELRAQAQVEEAEEL
K+alphaM1.v2 (191) RLKYLERCCRICFEERR--------DELSERLKIQHELRAQAQVEEAEEL
Shab-related (109) NELLEIDSCCSNRYQERKEENHEKDWDQKSNDVSTLSSFEESSLFEKELEK
Kv9.3 (109) NELLEIDSCCSSRYQERKEESHEKDWDQKSNDVSTLSSFEESSLFEKELEK
Kv8.1 (143) DELSIDSCCRDRYFRRK--------E-LSETLDFKKLTFDQESQHESEQD
251 300
K+alphaM1 (233) FRDMRFYGPQRRRLWNLMEKPFSSVAAKAIGVASSTFVLVSVVAVIALNIV
K+alpha1.v1 (233) FRDMRFYGPQRRRLWNLMEKPFSSVAAKAIGVASSTFVLVSVVAVIALNFV
K+alphaM1.v2 (233) FRDMRFYGPQRRRLWNLMEKPFSSVAAKAIGVASSTFVLVSVVAVIALNIV
Shab-related (159) FDTIRFG-QLRKKIWIRMENPAYCLSAKLIAISSLSVVLASILVAMCVHSM
Kv9.3 (159) FDEIRFG-QLRKKIWIRMENPAYCLSAKLIAISSLSVVLASILVAMCVHSM
Kv8.1 (184) FSQGPCP-IVRQKLWNILEKPGSSIAARIFGVISIIFVVVSIIINMALMSA
301 350
K+alphaM1 (283) EEMQQHSGQGEGGPDLRPILEHVEMLCMGFFTLEYLLRLASTPDLRRFAR
K+alpha1.v1 (283) EEMQQHSGQGEGGPDLRPILEHVEMLCMGFFTLEYLLRLASTPDLRRFAR
K+alphaM1.v2 (283) EEMQQHSGQGEGGPDLRPILEHVEMLCMGFFTLEYLLRLASTPDLRRFAR
Shab-related (208) SEFQNEDGEVDD-----PVLEGVELACLAWFTGELAVRLAAVPCQKKFWK
Kv9.3 (208) SEFQNEDGEVDD-----PVLEGVELACLAWFTGELAIRLVAAPSQKKFWK
Kv8.1 (233) ELS-----WLD-----LQLLEICEYVCISWFTGEFVLREICVRDRCRFLR
351 400
K+alphaM1 (333) SALNLVDLVAILPFYLQLLLECFTGEGHQRGQTVGSVGKVGQVLRVMRLM
K+alpha1.v1 (333) SALNLVDLVAILPFYLQLLLPECFTGEGHQRGQTVGSVGKVGQVLRVMRLM
K+alphaM1.v2 (333) SALNLVDLVAILPFYLQLLLECFTGEGHQRGQTVGSVGLVGQVLRVMRLM
Shab-related (253) NPLNIIDFVSIIPFYATLLAVDTKEEESE-------DIENMGKVVQILRLM
Kv9.3 (253) NPLNIIDFVSIIPFYATLLAVDTKEEESE-------DIENMGSVVQILRLM
Kv8.1 (273) KVPNILDLLAILPFYITLLVESLSGSQT-----TQEIENVGRIVQVLRLL

Figure 9B

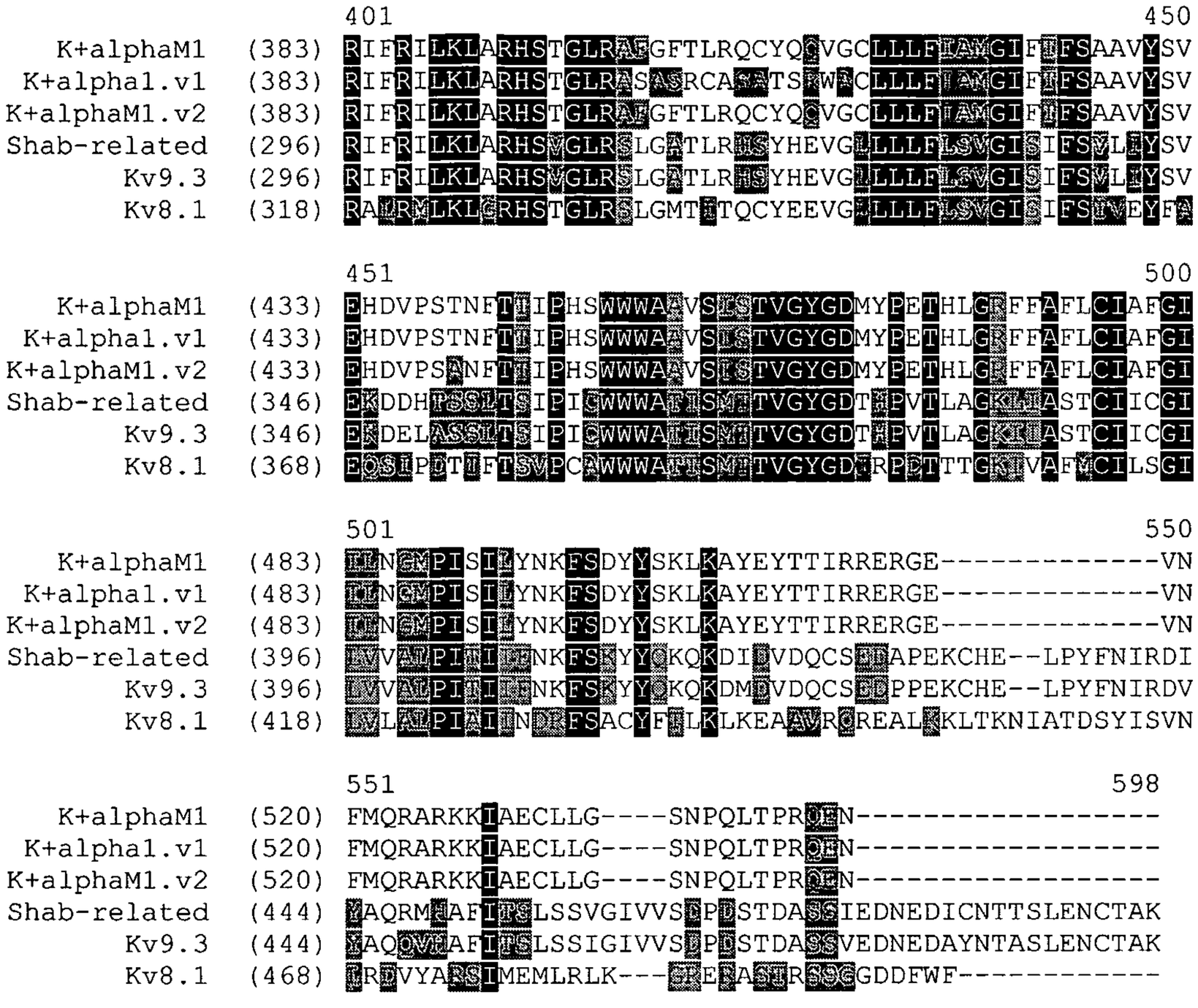

401
450
K+alphaM1 (383) RIFRILKLARHSTGLRAFGFTLRQCYQGVGCLLLFLAMGIFTFSAAVYSV
K+alpha1.v1 (383) RIFRILKLARHSTGLRASASRCASATSRWACLLLFLAMGIFTFSAAVYSV
K+alphaM1.v2 (383) RIFRILKLARHSTGLRAFGFTLRQCYQGVGCLLLFLAMGIFTFSAAVYSV
Shab-related (296) RIFRILKLARHSVGLRSLGATLRHSYHEVGLLLLFLSVGISIFSVLIYSV
Kv9.3 (296) RIFRILKLARHSVGLRSLGATLRHSYHEVGLLLLFLSVGISIFSVLIYSV
Kv8.1 (318) RALRVLKLGRHSTGLRSLGMTITQCYEEVGLLLLFLSVGISIFSIVEYFA
451
500
K+alphaM1 (433) EHDVPSTNFTTIPHSWWWAVVSLSTVGYGDMYPETHLGRFFAFLCIAFGI
K+alpha1.v1 (433) EHDVPSTNFTTIPHSWWWAVVSLSTVGYGDMYPETHLGRFFAFLCIAFGI
K+alphaM1.v2 (433) EHDVPSANFTTIPHSWWWAVVSLSTVGYGDMYPETHLGRFFAFLCIAFGI
Shab-related (346) EKDDHPSSLTSIPIGWWWATISMITVGYGDTHPVTLAGKLIASTCIICGI
Kv9.3 (346) EKDELASSLTSIPICWWWATISMITVGYGDTHPVTLAGKIIASTCIICGI
Kv8.1 (368) EQSIPDTIFTSVPCAWWWATISMITVGYGDIRPVTTTGKIVAFMCILSGI
501
550
K+alphaM1 (483) LLNGMPISILYNKFSDYYSKLKAYEYTTIRRERGE----------VN
K+alpha1.v1 (483) LLNGMPISILYNKFSDYYSKLKAYEYTTIRRERGE----------VN
K+alphaM1.v2 (483) LLNGMPISILYNKFSDYYSKLKAYEYTTIRRERGE----------VN
Shab-related (396) LVVALPIIIIFENKFSKYYQKQKDIDVDQCSEDAPEKCHE--LPYFNIRDI
Kv9.3 (396) LVVALPIIIIFENKFSKYYQKQKDMDVDQCSEDPPEKCHE--LPYFNIRDV
Kv8.1 (418) LVLALPIAIINDKFSACYFELKLKEAAVRGREALKKLTKNIATDSYISVN
551
598
K+alphaM1 (520) FMQRARKKIAECLLG----SNPQLTPRQEN----------
K+alpha1.v1 (520) FMQRARKKIAECLLG----SNPQLTPRQEN----------
K+alphaM1.v2 (520) FMQRARKKIAECLLG----SNPQLTPRQEN----------
Shab-related (444) YAQRMFAFITSLSSVGIVVSLDPDSTDASSIEDNEDICNTTSLENCTAK
Kv9.3 (444) YAQQVHAFITSLSSIGIVVSLDPDSTDASSVEDNEDAYNTASLENCTAK
Kv8.1 (468) FRDVYARSIMEMLRLK---GRELASTRSSGGDDFWF----------

Figure 10A

```
                      1                                                  50
      K+alphaM1     (1) AAGTCAGGCTCCCTTTAAATATGGCCTAATATTGCTGAGCAGGGTTGTGA
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                     51                                                 100
      K+alphaM1    (51) GGCCCTAAAAACGTCTTCCTACCATTCCTGGAATTCTACCTTGAAACATG
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    101                                                 150
      K+alphaM1   (101) TCTCTATCCTTTAAGAGAAAGGGAGGAGATAAAAAGGAGAGAGAGAAGCT
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    151                                                 200
      K+alphaM1   (151) GAAGCTGACTCAAAGATCCGACTGGATCTGAACAGTGCCCCAGGGAGAAT
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    201                                                 250
      K+alphaM1   (201) CCATTTGAAAAAAAAAAAAAAAATGTGATCATGTGAATGGACAAGAAGGAG
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    251                                                 300
      K+alphaM1   (251) ATGGCTTTAGATCTTATATGCTCTAAACGAAGAGTTACGCTGAGAGGGAA
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    301                                                 350
      K+alphaM1   (301) ACTGACTTGTCATGAAGTCAGCTTTGTTCCGTTGCTATGTGTCATCCCTG
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    351                                                 400
      K+alphaM1   (351) CTAATGGTGAGTTTACCTAAGGGCAGAGGCTACCATCTCAACCATGAAGC
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    401                                                 450
      K+alphaM1   (401) TGAAGACACAGGCATCCGTATTCTATAGCTAATTCAGTTGATTTCATCTC
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    451                                                 500
      K+alphaM1   (451) AGCACACATACACTGAGCGCTTCCTAAGAGCGAGGTTGACCGACATTTTT
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    501                                                 550
      K+alphaM1   (501) ATTAGCAATAATCTCTGCCTTCTTCTGATTACCTAGAGATTTAAGACCAC
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    551                                                 600
      K+alphaM1   (551) ATAATCATCCTCTACCTCACAGGGTCAAGGGAGTGGGGGAGGAAATGGGC
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------

                    601                                                 650
      K+alphaM1   (601) TAAGAGGTTCTAAATCCCTCCTAACACTTGCTTCTTCCAAATCAGCAAGA
K+alphaM1.v1        (1) --------------------------------------------------
K+alphaM1.v2        (1) --------------------------------------------------
```

Figure 10B

```
                          651                                                700
      K+alphaM1     (651) TTAGAGCAGTCAACAGCTGACTGCGTTCAGACCCTGCAGGCTGGGCTGGC
K+alphaM1.v1          (1) ------------------------------------------------------
K+alphaM1.v2          (1) ------------------------------------------------------

                          701                                                750
      K+alphaM1     (701) CTGCCCAGGACCTGAGAAGGGGCAGCTCCGGTGGCAATGTCTGAGCCCCT
K+alphaM1.v1          (1) ------------------------------------------------------
K+alphaM1.v2          (1) ------------------------------------------------------

                          751                                                800
      K+alphaM1     (751) AGCTGTGCTGGTCCGGGCTGGCCTCTCTAAGACAGTGCAGGCCACGTGAT
K+alphaM1.v1          (1) ------------------------------------------------------
K+alphaM1.v2          (1) ------------------------------------------------------

                          801                                                850
      K+alphaM1     (801) CCATCCTCCTAGAGGCAGTGAGCAGGTGAGGGACCCCTACSACAGCCAGG
K+alphaM1.v1          (1) --CTAGTCCTGCAGG--TTTAAACGAATTCGCCCTTCTACCACAGCCAGG
K+alphaM1.v2          (1) --CTAGTCCTGCAGG--TTTAAACGAATTCGCCCTTCTACCACAGCCAGG

                          851                                                900
      K+alphaM1     (851) AGGAAAAAGCTAGGCGTCCACTTTCCGCAGCCATGCTCAAACAGAGTGAG
K+alphaM1.v1         (47) AGGAAAAAGCTAGGCGTCCACTTTCCGCAGCCATGCTCAAACAGAGTGAG
K+alphaM1.v2         (47) AGGAAAAAGCTAGGCGTCCACTTTCCGCAGCCATGCTCAAACATAGTGAG

                          901                                                950
      K+alphaM1     (901) AGGAGACGGTCCTGGAGCTACAGGCCCTGGAACACGACGGAGAATGAGGG
K+alphaM1.v1         (97) AGGAGACGGTCCTGGAGCTACAGGCCCTGGAACACGACGGAGAATGAGGG
K+alphaM1.v2         (97) AGGAGACGGTCCTGGAGCTACAGGCCCTGGAACACGACGGAGAATGAGGG

                          951                                               1000
      K+alphaM1     (951) CAGCCAACACCGCAGGAGCATTTGCTCCCTGGGTGCCCGTTCCGGCTCCC
K+alphaM1.v1         (147) CAGCCAACACCGCAGGAGCATTTGCTCCCTGGGTGCCCGTTCCGGCTCCC
K+alphaM1.v2         (147) CAGCCAACACCGCAGGAGCATTTGCTCCCTGGGTGCCCGTTCCGGCTCCC

                         1001                                               1050
      K+alphaM1    (1001) AGGCCAGCATCCACGGCTGGACAGAGGGCAACTATAACTACTACATCGAG
K+alphaM1.v1         (197) AGGCCAGCATCCACGGCTGGACAGAGGGCAACTATAACTACTACATCGAG
K+alphaM1.v2         (197) AGGCCAGCATCCACGGCTGGACAGAGGGCAACTATAACTACTACATCGAG

                         1051                                               1100
      K+alphaM1    (1051) GAAGACGAAGACGGSGAGGAGGAGGACCAGTGGAAGGACGACCTGGCAGA
K+alphaM1.v1         (247) GAAGACGAAGACGGCGAGGAGGAGGACCAGTGGAAGGACGACCTGGCAGA
K+alphaM1.v2         (247) GAAGACGAAGACGGCGAGGAGGAGGACCAGTGGAAGGACGACCTGGCAGA

                         1101                                               1150
      K+alphaM1    (1101) AGAGGACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCCGAGGGCCCCA
K+alphaM1.v1         (297) AGAGGACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCCGAGGGCCCCA
K+alphaM1.v2         (297) AGAGGACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCCGAGGGCCCCA

                         1151                                               1200
      K+alphaM1    (1151) GCGACCCTCCGGCCCTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCAC
K+alphaM1.v1         (347) GCGACCCTCCGGCCCTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCAC
K+alphaM1.v2         (347) GCGACCCTCCGGCCCTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCAC

                         1201                                               1250
      K+alphaM1    (1201) AGCTACCAGCTGGACTACTGCGAGCTGGCCGGCTTCCCCAAGACGCGCCT
K+alphaM1.v1         (397) AGCTACCAGCTGGACTACTGCGAGCTGGCCGGCTTCCCCAAGACGCGCCT
K+alphaM1.v2         (397) AGCTACCAGCTGGACTACTGCGAGCTGGCCGGCTTCCCCAAGACGCGCCT

                         1251                                               1300
      K+alphaM1    (1251) AGGTCGCCTGGCCACCTCCACCAGCCGCAGCCGCCAGCTAAGCCTGTGCG
K+alphaM1.v1         (447) AGGTCGCCTGGCCACCTCCACCAGCCGCAGCCGCCAGCTAAGCCTGTGCG
K+alphaM1.v2         (447) AGGTCGCCTGGCCACCTCCACCAGCCGCAGCCGCCAGCTAAGCCTGTGCG
```

Figure 10C

```
                                1301                                1350
      K+alphaM1   (1301) ACGACTACGAGGAGCAGACAGACGAATACTTCTTCGACCGCGACCCGGCC
K+alphaM1.v1      (497) ACGACTACGAGGAGCAGACAGACGAATACTTCTTCGACCGCGACCCGGCC
K+alphaM1.v2      (497) ACGACTACGAGGAGCAGACAGACGAATACTTCTTCGACCGCGACCCGGCC

                                1351                                1400
      K+alphaM1   (1351) GTCTTCCAGCTGGTCTACAATTTCTACCTGTCCGGGGTGCTGCTGGTGCT
K+alphaM1.v1      (547) GTCTTCCAGCTGGTCTACAATTTCTACCTGTCCGGGGTGCTGCTGGTGCT
K+alphaM1.v2      (547) GTCTTCCAGCTGGTCTACAATTTCTACCTGTCCGGGGTGCTGCTGGTGCT

                                1401                                1450
      K+alphaM1   (1401) CGACGGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGCTACTGGGGCG
K+alphaM1.v1      (597) CGACGGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGCTACTGGGGCG
K+alphaM1.v2      (597) CGACGGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGCTACTGGGGCG

                                1451                                1500
      K+alphaM1   (1451) TGCGGCTCAAGTACACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGG
K+alphaM1.v1      (647) TGCGGCTCAAGTACACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGG
K+alphaM1.v2      (647) TGCGGCTCAAGTACACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGG

                                1501                                1550
      K+alphaM1   (1501) CGCGACGAGCTGAGCGAACGGCTCAAGATCCAGCACGAGCTGCGCGCGCA
K+alphaM1.v1      (697) CGCGACGAGCTGAGCGAACGGCTCAAGATCCAGCACGAGCTGCGCGCGCA
K+alphaM1.v2      (697) CGCGACGAGCTGAGCGAACGGCTCAAGATCCAGCACGAGCTGCGCGCGCA

                                1551                                1600
      K+alphaM1   (1551) GGCGCAGGTCGAGGAGGCGGAGGAACTCTTCCGCGACATGCGCTTCTACG
K+alphaM1.v1      (747) GGCGCAGGTCGAGGAGGCGGAGGAACTCTTCCGCGACATGCGCTTCTACG
K+alphaM1.v2      (747) GGCGCAGGTCGAGGAGGCGGAGGAACTCTTCCGCGACATGCGCTTCTACG

                                1601                                1650
      K+alphaM1   (1601) GCCCGCAGCGGCGCCGCCTCTGGAACCTCATGGAGAAGCCATTCTCCTCG
K+alphaM1.v1      (797) GCCCGCAGCGGCGCCGCCTCTGGAACCTCATGGAGAAGCCATTCTCCTCG
K+alphaM1.v2      (797) GCCCGCAGCGGCGCCGCCTCTGGAACCTCATGGAGAAGCCATTCTCCTCG

                                1651                                1700
      K+alphaM1   (1651) GTGGCCGCCAAGGCCATCGGGGTGGCSTCCAGCACCTTCGTGCTCGTCTC
K+alphaM1.v1      (847) GTGGCCGCCAAGGCCATCGGGGTGGCCTCCAGCACCTTCGTGCTCGTCTC
K+alphaM1.v2      (847) GTGGCCGCCAAGGCCATCGGGGTGGCCTCCAGCACCTTCGTGCTCGTCTC

                                1701                                1750
      K+alphaM1   (1701) CGTGGTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAGCAGCACTCGG
K+alphaM1.v1      (897) CGTGGTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAGCAGCACTCGG
K+alphaM1.v2      (897) CGTGGTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAGCAGCACTCGG

                                1751                                1800
      K+alphaM1   (1751) GGCAGGGCGAGGGCGGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAG
K+alphaM1.v1      (947) GGCAGGGCGAGGGCGGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAG
K+alphaM1.v2      (947) GGCAGGGCGAGGGCGGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAG

                                1801                                1850
      K+alphaM1   (1801) ATGCTGTGCATGGGCTTCTTCACGCTCGAGTACCTGCTGCGCCTAGCCTC
K+alphaM1.v1      (997) ATGCTGTGCATGGGCTTCTTCACGCTCGAGTACCTGCTGCGCCTAGCCTC
K+alphaM1.v2      (997) ATGCTGTGCATGGGCTTCTTCACGCTCGAGTACCTGCTGCGCCTAGCCTC

                                1851                                1900
      K+alphaM1   (1851) CACGCCCGACCTGAGGCGCTTCGCGCGCAGCGCCCTCAACCTGGTGGACC
K+alphaM1.v1      (1047) CACGCCCGACCTGAGGCGCTTCGCGCGCAGCGCCCTCAACCTGGTGGACC
K+alphaM1.v2      (1047) CACGCCCGACCTGAGGCGCTTCGCGCGCAGCGCCCTCAACCTGGTGGACC

                                1901                                1950
      K+alphaM1   (1901) TGGTGGCCATCCTGCCGCTCTACCTTCAGCTGCTGCTCGAGTGCTTCACG
K+alphaM1.v1      (1097) TGGTGGCCATCCTGCCGCTCTACCTTCAGCTGCTGCCCGAGTGCTTCACG
K+alphaM1.v2      (1097) TGGTGGCCATCCTGCCGCTCTACCTTCAGCTGCTGCTCGAGTGCTTCACG
```

Figure 10D

```
                          1951                                                2000
      K+alphaM1   (1951)  GGCGAGGGCCACCAACGCGGCCAGACGGTGGGCAGCGTGGGTAAGGTGGG
K+alphaM1.v1      (1147)  GGCGAGGGCCACCAACGCGGCCAGACGGTGGGCAGCGTGGGTAAGGTGGG
K+alphaM1.v2      (1147)  GGCGAGGGCCACCAACGCGGCCAGACGGTGGGCAGCGTGGGTAAGGTGGG

                          2001                                                2050
      K+alphaM1   (2001)  TCAGGTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGCATCCTCAAGC
K+alphaM1.v1      (1197)  TCAGGTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGCATCCTCAAGC
K+alphaM1.v2      (1197)  TCAGGTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGCATCCTCAAGC

                          2051                                                2100
      K+alphaM1   (2051)  TGGCGCGCCACTCCACCGGACTGCGTGCCTTCGGCTTCACGCTGCGCCAG
K+alphaM1.v1      (1247)  TGGCGCGCCACTCCACCGGACTGCGTGC-TTCGGCTTCACGCTGCGCCAG
K+alphaM1.v2      (1247)  TGGCGCGCCACTCCACCGGACTGCGTGCCTTCGGCTTCACGCTGCGCCAG

                          2101                                                2150
      K+alphaM1   (2101)  TGCTACCAGCAGGTGGGC-TGCCTGCTGCTCTTCATCGCCATGGGCATCT
K+alphaM1.v1      (1296)  TGCTACCAGCAGGTGGGCGTGCCTGCTGCTCTTCATCGCCATGGGCATCT
K+alphaM1.v2      (1297)  TGCTACCAGCAGGTGGGC-TGCCTGCTGCTCTTCATCGCCATGGGCATCT

                          2151                                                2200
      K+alphaM1   (2150)  TCACTTTCTCTGCGGCTGTCTACTCTGTGGAGCACGATGTGCCCAGCACC
K+alphaM1.v1      (1346)  TCACTTTCTCTGCGGCTGTCTACTCTGTGGAGCACGATGTGCCCAGCACC
K+alphaM1.v2      (1346)  TCACTTTCTCTGCGGCTGTCTACTCTGTGGAGCACGATGTGCCCAGCGCC

                          2201                                                2250
      K+alphaM1   (2200)  AACTTCACTACCATCCCCCACTCCTGGTGGTGGGCCGCGGTGAGCATCTC
K+alphaM1.v1      (1396)  AACTTCACTACCATCCCCCACTCCTGGTGGTGGGCCGCGGTGAGCATCTC
K+alphaM1.v2      (1396)  AACTTCACTACCATCCCCCACTCCTGGTGGTGGGCCGCGGTGAGCATCTC

                          2251                                                2300
      K+alphaM1   (2250)  CACCGTGGGCTACGGAGACATGTACCCAGAGACCCACCTGGGCAGGTTTT
K+alphaM1.v1      (1446)  CACCGTGGGCTACGGAGACATGTACCCAGAGACCCACCTGGGCAGGTTTT
K+alphaM1.v2      (1446)  CACCGTGGGCTACGGAGACATGTACCCAGAGACCCACCTGGGCAGGTTTT

                          2301                                                2350
      K+alphaM1   (2300)  TTGCCTTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGGATGCCCATT
K+alphaM1.v1      (1496)  TTGCCTTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGGATGCCCATT
K+alphaM1.v2      (1496)  TTGCCTTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGGATGCCCATT

                          2351                                                2400
      K+alphaM1   (2350)  TCCATCCTCTACAACAAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTA
K+alphaM1.v1      (1546)  TCCATCCTCTACAACAAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTA
K+alphaM1.v2      (1546)  TCCATCCTCTACAACAAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTA

                          2401                                                2450
      K+alphaM1   (2400)  TGAGTATACCACCATACGCAGGGAGAGGGGAGAGGTGAACTTCATGCAGA
K+alphaM1.v1      (1596)  TGAGTATACCACCATACGCAGGGAGAGGGGAGAGGTGAACTTCATGCAGA
K+alphaM1.v2      (1596)  TGAGTATACCACCATACGCAGGGAGAGGGGAGAGGTGAACTTCATGCAGA

                          2451                                                2500
      K+alphaM1   (2450)  GAGCCAGAAAGAAGATAGCTGAGTGTTTGCTTGGAAGCAACCCACAGCTC
K+alphaM1.v1      (1646)  GAGCCAGAAAGAAGATAGCTGAGTGTTTGCTTGGAAGCAACCCACAGCTC
K+alphaM1.v2      (1646)  GAGCCAGAAAGAAGATAGCTGAGTGTTTGCTTGGAAGCAACCCACAGCTC

                          2501                                                2550
      K+alphaM1   (2500)  ACCCCAAGACAAGAGAATTAGTATTTTATAGGACATGTGGCTGGTAGATT
K+alphaM1.v1      (1696)  ACCCCAAGACAAGAGAATTAGTATTTTATAGGACATGTGGCTGGTAGATT
K+alphaM1.v2      (1696)  ACCCCAAGACAAGAGAATTAGTATTTTATAGGACATGTGGCTGGTAGATT

                          2551                                                2600
      K+alphaM1   (2550)  CCATGAACTTCAAGGCTTCATTGCTCTTTTTTTAATCATTATGATTGGCA
K+alphaM1.v1      (1746)  CCATGAACTTCAAGGCTTCATTGCTCTTTTTTTAATCATTATGATTGGCA
K+alphaM1.v2      (1746)  CCATGAACTTCAAGGCTTCATTGCTCTTTTTTTAATCATTATGATTGGCA
```

Figure 10E

```
                        2601                                                2650
      K+alphaM1   (2600) GCAAAAGGAAATGTGAAGCAGACATACACAAAGGCCATTTCGTTCACAAA
K+alphaM1.v1      (1796) GCAAAAGGAAATGTGAAGCAGACATACACAAAGGCCATTTCGTTCACAAA
K+alphaM1.v2      (1796) GCAAAAGGAAATGTGAAGCAGACATACACAAAGGCCATTTCGTTCACAAA

                        2651                                                2700
      K+alphaM1   (2650) GTACTGCCTCTAGAAATACTCATTTTGGCCCAAACTCAGAATGTCTCATA
K+alphaM1.v1      (1846) GAAGGGCG---A---ATTCGCG----GCCGCTAAAT--------------
K+alphaM1.v2      (1846) GAAGGGCG---A---ATTCGCG----GCCGCTAAAT--------------

                        2701                                                2750
      K+alphaM1   (2700) GTTGCTCTGTGTTGTGTGAAACATCTGACCTTCTCAATGACGTTGATATT
K+alphaM1.v1      (1872) ----------.-----------------------------------------
K+alphaM1.v2      (1872) ------------------------------------------------

                        2751                                                2800
      K+alphaM1   (2750) GAAAACCTGAGGGGAGCAACAGCTTAGATTTTACTTGTAGCTTCTCGTGG
K+alphaM1.v1      (1872) ------------------------------------------------
K+alphaM1.v2      (1872) ------------------------------------------------

                        2801                                                2850
      K+alphaM1   (2800) CATCTAGCTCAATAAATATTTTTGGACTTGAAAAAAAAAAAAAAAAAAAA
K+alphaM1.v1      (1872) ------------------------------------------------
K+alphaM1.v2      (1872) ------------------------------------------------

                        2851
      K+alphaM1   (2850) A
K+alphaM1.v1      (1872) -
K+alphaM1.v2      (1872) -
```

Figure 11A

```
                .         .         .         .         .         .
   1  AAGTCAGGCTCCCTTTAAATATGGCCTAATATTGCTGAGCAGGGTTGTGAGGCCCTAAAA   60

                .         .         .         .         .         .
  61  ACGTCTTCCTACCATTCCTGGAATTCTACCTTGAAACATGTCTCTATCCTTTAAGAGAAA  120

                .         .         .         .         .         .
 121  GGGAGGAGATAAAAAGGAGAGAGAAGCTGAAGCTGACTCAAAGATCCGACTGGATCTG   180

                .         .         .         .         .         .
 181  AACAGTGCCCCAGGGAGAATCCATTTGAAAAAAAAAAAAAAATGTGATCATGTGAATGGA  240

                .         .         .         .         .         .
 241  CAAGAAGGAGATGGCTTTAGATCTTATATGCTCTAAACGAAGAGTTACGCTGAGAGGGAA  300

                .         .         .         .         .         .
 301  ACTGACTTGTCATGAAGTCAGCTTTGTTCCGTTGCTATGTGTCATCCCTGCTAATGGTGA  360

                .         .         .         .         .         .
 361  GTTTACCTAAGGGCAGAGGCTACCATCTCAACCATGAAGCTGAAGACACAGGCATCCGTA  420

                .         .         .         .         .         .
 421  TTCTATAGCTAATTCAGTTGATTTCATCTCAGCACACATACACTGAGCGCTTCCTAAGAG  480

                .         .         .         .         .         .
 481  CGAGGTTGACCGACATTTTTATTAGCAATAATCTCTGCCTTCTTCTGATTACCTAGAGAT  540

                .         .         .         .         .         .
 541  TTAAGACCACATAATCATCCTCTACCTCACAGGGTCAAGGGAGTGGGGGAGGAAATGGGC  600

                .         .         .         .         .         .
 601  TAAGAGGTTCTAAATCCCTCCTAACACTTGCTTCTTCCAAATCAGCAAGATTAGAGCAGT  660

                .         .         .         .         .         .
 661  CAACAGCTGACTGCGTTCAGACCCTGCAGGCTGGGCTGGCCTGCCCAGGACCTGAGAAGG  720

                .         .         .         .         .         .
 721  GGCAGCTCCGGTGGCAATGTCTGAGCCCCTAGCTGTGCTGGTCCGGGCTGGCCTCTCTAA  780

                .         .         .         .         .         .
 781  GACAGTGCAGGCCACGTGATCCATCCTCCTAGAGGCAGTGAGCAGGTGAGGGACCCCTAC  840

                .         .         .         .         .         .
 841  NACAGCCAGGAGGAAAAAGCTAGGCGTCCACTTTCCGCAGCCATGCTCAAACANAGTGAG  900
   1                                          M   L   K   Q   S   E      6

                .         .         .         .         .         .
 901  AGGAGACGGTCCTGGAGCTACAGGCCCTGGAACACGACGGAGAATGAGGGCAGCCAACAC  960
   7  R   R   R   S   W   S   Y   R   P   W   N   T   T   E   N   E   G   S   Q   H     26

                .         .         .         .         .         .
 961  CGCAGGAGCATTTGCTCCCTGGGTGCCCGTTCCGGCTCCCAGGCCAGCATCCACGGCTGG  1020
  27  R   R   S   I   C   S   L   G   A   R   S   G   S   Q   A   S   I   H   G   W     46

                .         .         .         .         .         .
1021  ACAGAGGGCAACTATAACTACTACATCGAGGAAGACGAAGACGGNGAGGAGGAGGACCAG  1080
  47  T   E   G   N   Y   N   Y   Y   I   E   E   D   E   D   G   E   E   E   D   Q     66
```

Figure 11B

```
1081  TGGAAGGACGACCTGGCAGAAGAGGACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCC  1140
  67  W  K  D  D  L  A  E  E  D  Q  Q  A  G  E  V  T  T  A  K  P    86

1141  GAGGGCCCCAGCGACCCTCCGGCCCTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCAC  1200
  87  E  G  P  S  D  P  P  A  L  L  S  T  L  N  V  N  V  G  G  H   106

1201  AGCTACCAGCTGGACTACTGCGAGCTGGCCGGCTTCCCCAAGACGCGCCTAGGTCGCCTG  1260
 107  S  Y  Q  L  D  Y  C  E  L  A  G  F  P  K  T  R  L  G  R  L   126

1261  GCCACCTCCACCAGCCGCAGCCGCCAGCTAAGCCTGTGCGACGACTACGAGGAGCAGACA  1320
 127  A  T  S  T  S  R  S  R  Q  L  S  L  C  D  D  Y  E  E  Q  T   146

1321  GACGAATACTTCTTCGACCGCGACCCGGCCGTCTTCCAGCTGGTCTACAATTTCTACCTG  1380
 147  D  E  Y  F  F  D  R  D  P  A  V  F  Q  L  V  Y  N  F  Y  L   166

1381  TCCGGGGTGCTGCTGGTGCTCGACGGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGC  1440
 167  S  G  V  L  L  V  L  D  G  L  C  P  R  R  F  L  E  E  L  G   186

1441  TACTGGGGCGTGCGGCTCAAGTACACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGG  1500
 187  Y  W  G  V  R  L  K  Y  T  P  R  C  C  R  I  C  F  E  E  R   206

1501  CGCGACGAGCTGAGCGAACGGCTCAAGATCCAGCACGAGCTGCGCGCGCAGGCGCAGGTC  1560
 207  R  D  E  L  S  E  R  L  K  I  Q  H  E  L  R  A  Q  A  Q  V   226

1561  GAGGAGGCGGAGGAACTCTTCCGCGACATGCGCTTCTACGGCCCGCAGCGGCGCCGCCTC  1620
 227  E  E  A  E  E  L  F  R  D  M  R  F  Y  G  P  Q  R  R  R  L   246

1621  TGGAACCTCATGGAGAAGCCATTCTCCTCGGTGGCCGCCAAGGCCATCGGGGTGGCNTCC  1680
 247  W  N  L  M  E  K  P  F  S  S  V  A  A  K  A  I  G  V  A  S   266

1681  AGCACCTTCGTGCTCGTCTCCGTGGTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAG  1740
 267  S  T  F  V  L  V  S  V  V  A  L  A  L  N  T  V  E  E  M  Q   286

1741  CAGCACTCGGGGCAGGGCGAGGGCGGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAG  1800
 287  Q  H  S  G  Q  G  E  G  G  P  D  L  R  P  I  L  E  H  V  E   306

1801  ATGCTGTGCATGGGCTTCTTCACGCTCGAGTACCTGCTGCGCCTAGCCTCCACGCCCGAC  1860
 307  M  L  C  M  G  F  F  T  L  E  Y  L  L  R  L  A  S  T  P  D   326

1861  CTGAGGCGCTTCGCGCGCAGCGCCCTCAACCTGGTGGACCTGGTGGCCATCCTGCCGCTC  1920
 327  L  R  R  F  A  R  S  A  L  N  L  V  D  L  V  A  I  L  P  L   346

1921  TACCTTCAGCTGCTGCNCGAGTGCTTCACGGGCGAGGGCCACCAACGCGGCCAGACGGTG  1980
 347  Y  L  Q  L  L  X  E  C  F  T  G  E  G  H  Q  R  G  Q  T  V   366
```

Figure 11C

```
1981  GGCAGCGTGGGTAAGGTGGGTCAGGTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGC  2040
 367  G  S  V  G  K  V  G  Q  V  L  R  V  M  R  L  M  R  I  F  R    386

2041  ATCCTCAAGCTGGCGCGCCACTCCACCGGACTGCGTGCCTTCGGCTTCACGCTGCGCCAG  2100
 387  I  L  K  L  A  R  H  S  T  G  L  R  A  F  G  F  T  L  R  Q    406

2101  TGCTACCAGCAGGTGGGCTGCCTGCTGCTCTTCATCGCCATGGGCATCTTCACTTTCTCT  2160
 407  C  Y  Q  Q  V  G  C  L  L  L  F  I  A  M  G  I  F  T  F  S    426

2161  GCGGCTGTCTACTCTGTGGAGCACGATGTGCCCAGCNCCAACTTCACTACCATCCCCCAC  2220
 427  A  A  V  Y  S  V  E  H  D  V  P  S  X  N  F  T  T  I  P  H    446

2221  TCCTGGTGGTGGGCCGCGGTGAGCATCTCCACCGTGGGCTACGGAGACATGTACCCAGAG  2280
 447  S  W  W  W  A  A  V  S  I  S  T  V  G  Y  G  D  M  Y  P  E    466

2281  ACCCACCTGGGCAGGTTTTTTGCCTTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGG  2340
 467  T  H  L  G  R  F  F  A  F  L  C  I  A  F  G  I  I  L  N  G    486

2341  ATGCCCATTTCCATCCTCTACAACAAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTAT  2400
 487  M  P  I  S  I  L  Y  N  K  F  S  D  Y  Y  S  K  L  K  A  Y    506

2401  GAGTATACCACCATACGCAGGGAGAGGGGAGAGGTGAACTTCATGCAGAGAGCCAGAAAG  2460
 507  E  Y  T  T  I  R  R  E  R  G  E  V  N  F  M  Q  R  A  R  K    526

2461  AAGATAGCTGAGTGTTTGCTTGGAAGCAACCCACAGCTCACCCCAAGACAAGAGAATTAG  2520
 527  K  I  A  E  C  L  L  G  S  N  P  Q  L  T  P  R  Q  E  N  *    546

2521  TATTTTATAGGACATGTGGCTGGTAGATTCCATGAACTTCAAGGCTTCATTGCTCTTTTT  2580

2581  TTAATCATTATGATTGGCAGCAAAAGGAAATGTGAAGCAGACATACACAAAGGCCATTTC  2640

2641  GTTCACAAAGTACTGCCTCTAGAAATACTCATTTTGGCCCAAACTCAGAATGTCTCATAG  2700

2701  TTGCTCTGTGTTGTGTGAAACATCTGACCTTCTCAATGACGTTGATATTGAAAACCTGAG  2760

2761  GGGAGCAACAGCTTAGATTTTACTTGTAGCTTCTCGTGGCATCTAGCTCAATAAATATTT  2820

2821  TTGGACTTGAAAAAAAAAAAAAAAAAAAA  2850
```

Figure 12A

```
                .         .         .         .         .         .
   1  CTAGTCCTGCAGGTTTAAACGAATTCGCCCTTCTACNACAGCCAGGAGGAAAAAGCTAGG    60

                .         .         .         .         .         .
  61  CGTCCACTTTCCGCAGCCATGCTCAAACANAGTGAGAGGAGACGGTCCTGGAGCTACAGG    120
   1                      M  L  K  Q  S  E  R  R  R  S  W  S  Y  R     14

                .         .         .         .         .         .
 121  CCCTGGAACACGACGGAGAATGAGGGCAGCCAACACCGCAGGAGCATTTGCTCCCTGGGT    180
  15  P  W  N  T  T  E  N  E  G  S  Q  H  R  R  S  I  C  S  L  G     34

                .         .         .         .         .         .
 181  GCCCGTTCCGGCTCCCAGGCCAGCATCCACGGCTGGACAGAGGGCAACTATAACTACTAC    240
  35  A  R  S  G  S  Q  A  S  I  H  G  W  T  E  G  N  Y  N  Y  Y     54

                .         .         .         .         .         .
 241  ATCGAGGAAGACGAAGACGGNGAGGAGGAGGACCAGTGGAAGGACGACCTGGCAGAAGAG    300
  55  I  E  E  D  E  D  G  E  E  E  D  Q  W  K  D  D  L  A  E  E     74

                .         .         .         .         .         .
 301  GACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCCGAGGGCCCCAGCGACCCTCCGGCC    360
  75  D  Q  Q  A  G  E  V  T  T  A  K  P  E  G  P  S  D  P  P  A     94

                .         .         .         .         .         .
 361  CTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCACAGCTACCAGCTGGACTACTGCGAG    420
  95  L  L  S  T  L  N  V  N  V  G  G  H  S  Y  Q  L  D  Y  C  E    114

                .         .         .         .         .         .
 421  CTGGCCGGCTTCCCCAAGACGCGCCTAGGTCGCCTGGCCACCTCCACCAGCCGCAGCCGC    480
 115  L  A  G  F  P  K  T  R  L  G  R  L  A  T  S  T  S  R  S  R    134

                .         .         .         .         .         .
 481  CAGCTAAGCCTGTGCGACGACTACGAGGAGCAGACAGACGAATACTTCTTCGACCGCGAC    540
 135  Q  L  S  L  C  D  D  Y  E  E  Q  T  D  E  Y  F  F  D  R  D    154

                .         .         .         .         .         .
 541  CCGGCCGTCTTCCAGCTGGTCTACAATTTCTACCTGTCCGGGGTGCTGCTGGTGCTCGAC    600
 155  P  A  V  F  Q  L  V  Y  N  F  Y  L  S  G  V  L  L  V  L  D    174

                .         .         .         .         .         .
 601  GGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGCTACTGGGGCGTGCGGCTCAAGTAC    660
 175  G  L  C  P  R  R  F  L  E  E  L  G  Y  W  G  V  R  L  K  Y    194

                .         .         .         .         .         .
 661  ACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGGCGCGACGAGCTGAGCGAACGGCTC    720
 195  T  P  R  C  C  R  I  C  F  E  E  R  R  D  E  L  S  E  R  L    214

                .         .         .         .         .         .
 721  AAGATCCAGCACGAGCTGCGCGCGCAGGCGCAGGTCGAGGAGGCGGAGGAACTCTTCCGC    780
 215  K  I  Q  H  E  L  R  A  Q  A  Q  V  E  E  A  E  E  L  F  R    234

                .         .         .         .         .         .
 781  GACATGCGCTTCTACGGCCCGCAGCGGCGCCGCCTCTGGAACCTCATGGAGAAGCCATTC    840
 235  D  M  R  F  Y  G  P  Q  R  R  R  L  W  N  L  M  E  K  P  F    254

                .         .         .         .         .         .
 841  TCCTCGGTGGCCGCCAAGGCCATCGGGGTGGCNTCCAGCACCTTCGTGCTCGTCTCCGTG    900
 255  S  S  V  A  A  K  A  I  G  V  A  S  S  T  F  V  L  V  S  V    274
```

Figure 12B

```
                .         .         .         .         .         .
 901  GTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAGCAGCACTCGGGGCAGGGCGAGGGC   960
 275  V  A  L  A  L  N  T  V  E  E  M  Q  Q  H  S  G  Q  G  E  G     294

                .         .         .         .         .         .
 961  GGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAGATGCTGTGCATGGGCTTCTTCACG   1020
 295  G  P  D  L  R  P  I  L  E  H  V  E  M  L  C  M  G  F  F  T     314

                .         .         .         .         .         .
1021  CTCGAGTACCTGCTGCGCCTAGCCTCCACGCCCGACCTGAGGCGCTTCGCGCGCAGCGCC   1080
 315  L  E  Y  L  L  R  L  A  S  T  P  D  L  R  R  F  A  R  S  A     334

                .         .         .         .         .         .
1081  CTCAACCTGGTGGACCTGGTGGCCATCCTGCCGCTCTACCTTCAGCTGCTGCNCGAGTGC   1140
 335  L  N  L  V  D  L  V  A  I  L  P  L  Y  L  Q  L  L  X  E  C     354

                .         .         .         .         .         .
1141  TTCACGGGCGAGGGCCACCAACGCGGCCAGACGGTGGGCAGCGTGGGTAAGGTGGGTCAG   1200
 355  F  T  G  E  G  H  Q  R  G  Q  T  V  G  S  V  G  K  V  G  Q     374

                .         .         .         .         .         .
1201  GTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGCATCCTCAAGCTGGCGCGCCACTCC   1260
 375  V  L  R  V  M  R  L  M  R  I  F  R  I  L  K  L  A  R  H  S     394

                .         .         .         .         .         .
1261  ACCGGACTGCGTGCTTCGGCTTCACGCTGCGCCAGTGCTACCAGCAGGTGGGCGTGCCTG   1320
 395  T  G  L  R  A  S  A  S  R  C  A  S  A  T  S  R  W  A  C  L     414

                .         .         .         .         .         .
1321  CTGCTCTTCATCGCCATGGGCATCTTCACTTTCTCTGCGGCTGTCTACTCTGTGGAGCAC   1380
 415  L  L  F  I  A  M  G  I  F  T  F  S  A  A  V  Y  S  V  E  H     434

                .         .         .         .         .         .
1381  GATGTGCCCAGCNCCAACTTCACTACCATCCCCCACTCCTGGTGGTGGGCCGCGGTGAGC   1440
 435  D  V  P  S  X  N  F  T  T  I  P  H  S  W  W  W  A  A  V  S     454

                .         .         .         .         .         .
1441  ATCTCCACCGTGGGCTACGGAGACATGTACCCAGAGACCCACCTGGGCAGGTTTTTTGCC   1500
 455  I  S  T  V  G  Y  G  D  M  Y  P  E  T  H  L  G  R  F  F  A     474

                .         .         .         .         .         .
1501  TTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGGATGCCCATTTCCATCCTCTACAAC   1560
 475  F  L  C  I  A  F  G  I  I  L  N  G  M  P  I  S  I  L  Y  N     494

                .         .         .         .         .         .
1561  AAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTATGAGTATACCACCATACGCAGGGAG   1620
 495  K  F  S  D  Y  Y  S  K  L  K  A  Y  E  Y  T  T  I  R  R  E     514

                .         .         .         .         .         .
1621  AGGGGAGAGGTGAACTTCATGCAGAGAGCCAGAAAGAAGATAGCTGAGTGTTTGCTTGGA   1680
 515  R  G  E  V  N  F  M  Q  R  A  R  K  K  I  A  E  C  L  L  G     534

                .         .         .         .         .         .
1681  AGCAACCCACAGCTCACCCCAAGACAAGAGAATTAGTATTTTATAGGACATGTGGCTGGT   1740
 535  S  N  P  Q  L  T  P  R  Q  E  N                               545

                .         .         .         .         .         .
1741  AGATTCCATGAACTTCAAGGCTTCATTGCTCTTTTTTTAATCATTATGATTGGCAGCAAA   1800
```

Figure 12C

```
                .         .         .         .         .         .
1801  AGGAAATGTGAAGCAGACATACACAAAGGCCATTTCGTTCACAAAGAAGGGCGAATTCGC   1860

                .
1861  GGCCGCTAAAT  1871
```

Figure 13A

```
   1  CTAGTCCTGCAGGTTTAAACGAATTCGCCCTTCTACNACAGCCAGGAGGAAAAAGCTAGG    60

  61  CGTCCACTTTCCGCAGCCATGCTCAAACANAGTGAGAGGAGACGGTCCTGGAGCTACAGG    120
   1                      M  L  K  H  S  E  R  R  R  S  W  S  Y  R     14

 121  CCCTGGAACACGACGGAGAATGAGGGCAGCCAACACCGCAGGAGCATTTGCTCCCTGGGT    180
  15  P  W  N  T  T  E  N  E  G  S  Q  H  R  R  S  I  C  S  L  G     34

 181  GCCCGTTCCGGCTCCCAGGCCAGCATCCACGGCTGGACAGAGGGCAACTATAACTACTAC    240
  35  A  R  S  G  S  Q  A  S  I  H  G  W  T  E  G  N  Y  N  Y  Y     54

 241  ATCGAGGAAGACGAAGACGGNGAGGAGGAGGACCAGTGGAAGGACGACCTGGCAGAAGAG    300
  55  I  E  E  D  E  D  G  E  E  E  D  Q  W  K  D  D  L  A  E  E     74

 301  GACCAGCAGGCAGGGGAGGTCACCACCGCCAAGCCCGAGGGCCCCAGCGACCCTCCGGCC    360
  75  D  Q  Q  A  G  E  V  T  T  A  K  P  E  G  P  S  D  P  P  A     94

 361  CTGCTGTCCACGCTGAATGTGAACGTGGGTGGCCACAGCTACCAGCTGGACTACTGCGAG    420
  95  L  L  S  T  L  N  V  N  V  G  G  H  S  Y  Q  L  D  Y  C  E    114

 421  CTGGCCGGCTTCCCCAAGACGCGCCTAGGTCGCCTGGCCACCTCCACCAGCCGCAGCCGC    480
 115  L  A  G  F  P  K  T  R  L  G  R  L  A  T  S  T  S  R  S  R    134

 481  CAGCTAAGCCTGTGCGACGACTACGAGGAGCAGACAGACGAATACTTCTTCGACCGCGAC    540
 135  Q  L  S  L  C  D  D  Y  E  E  Q  T  D  E  Y  F  F  D  R  D    154

 541  CCGGCCGTCTTCCAGCTGGTCTACAATTTCTACCTGTCCGGGGTGCTGCTGGTGCTCGAC    600
 155  P  A  V  F  Q  L  V  Y  N  F  Y  L  S  G  V  L  L  V  L  D    174

 601  GGGCTGTGTCCGCGCCGCTTCCTGGAGGAGCTGGGCTACTGGGGCGTGCGGCTCAAGTAC    660
 175  G  L  C  P  R  R  F  L  E  E  L  G  Y  W  G  V  R  L  K  Y    194

 661  ACGCCACGCTGCTGCCGCATCTGCTTCGAGGAGCGGCGCGACGAGCTGAGCGAACGGCTC    720
 195  T  P  R  C  C  R  I  C  F  E  E  R  R  D  E  L  S  E  R  L    214

 721  AAGATCCAGCACGAGCTGCGCGCGCAGGCGCAGGTCGAGGAGGCGGAGGAACTCTTCCGC    780
 215  K  I  Q  H  E  L  R  A  Q  A  Q  V  E  E  A  E  E  L  F  R    234

 781  GACATGCGCTTCTACGGCCCGCAGCGGCGCCGCCTCTGGAACCTCATGGAGAAGCCATTC    840
 235  D  M  R  F  Y  G  P  Q  R  R  R  L  W  N  L  M  E  K  P  F    254

 841  TCCTCGGTGGCCGCCAAGGCCATCGGGGTGGCNTCCAGCACCTTCGTGCTCGTCTCCGTG    900
 255  S  S  V  A  A  K  A  I  G  V  A  S  S  T  F  V  L  V  S  V    274
```

Figure 13B

```
                .         .         .         .         .         .
901   GTGGCGCTGGCGCTCAACACCGTGGAGGAGATGCAGCAGCACTCGGGGCAGGGCGAGGGC   960
275   V  A  L  A  L  N  T  V  E  E  M  Q  Q  H  S  G  Q  G  E  G    294

                .         .         .         .         .         .
961   GGCCCAGACCTGCGGCCCATCCTGGAGCACGTGGAGATGCTGTGCATGGGCTTCTTCACG   1020
295   G  P  D  L  R  P  I  L  E  H  V  E  M  L  C  M  G  F  F  T    314

                .         .         .         .         .         .
1021  CTCGAGTACCTGCTGCGCCTAGCCTCCACGCCCGACCTGAGGCGCTTCGCGCGCAGCGCC   1080
315   L  E  Y  L  L  R  L  A  S  T  P  D  L  R  R  F  A  R  S  A    334

                .         .         .         .         .         .
1081  CTCAACCTGGTGGACCTGGTGGCCATCCTGCCGCTCTACCTTCAGCTGCTGCNCGAGTGC   1140
335   L  N  L  V  D  L  V  A  I  L  P  L  Y  L  Q  L  L  X  E  C    354

                .         .         .         .         .         .
1141  TTCACGGGCGAGGGCCACCAACGCGGCCAGACGGTGGGCAGCGTGGGTAAGGTGGGTCAG   1200
355   F  T  G  E  G  H  Q  R  G  Q  T  V  G  S  V  G  K  V  G  Q    374

                .         .         .         .         .         .
1201  GTGTTGCGCGTCATGCGCCTCATGCGCATCTTCCGCATCCTCAAGCTGGCGCGCCACTCC   1260
375   V  L  R  V  M  R  L  M  R  I  F  R  I  L  K  L  A  R  H  S    394

                .         .         .         .         .         .
1261  ACCGGACTGCGTGCCTTCGGCTTCACGCTGCGCCAGTGCTACCAGCAGGTGGGCTGCCTG   1320
395   T  G  L  R  A  F  G  F  T  L  R  Q  C  Y  Q  Q  V  G  C  L    414

                .         .         .         .         .         .
1321  CTGCTCTTCATCGCCATGGGCATCTTCACTTTCTCTGCGGCTGTCTACTCTGTGGAGCAC   1380
415   L  L  F  I  A  M  G  I  F  T  F  S  A  A  V  Y  S  V  E  H    434

                .         .         .         .         .         .
1381  GATGTGCCCAGCNCCAACTTCACTACCATCCCCCACTCCTGGTGGTGGGCCGCGGTGAGC   1440
435   D  V  P  S  X  N  F  T  T  I  P  H  S  W  W  W  A  A  V  S    454

                .         .         .         .         .         .
1441  ATCTCCACCGTGGGCTACGGAGACATGTACCCAGAGACCCACCTGGGCAGGTTTTTTGCC   1500
455   I  S  T  V  G  Y  G  D  M  Y  P  E  T  H  L  G  R  F  F  A    474

                .         .         .         .         .         .
1501  TTCCTCTGCATTGCTTTTGGGATCATTCTCAACGGGATGCCCATTTCCATCCTCTACAAC   1560
475   F  L  C  I  A  F  G  I  I  L  N  G  M  P  I  S  I  L  Y  N    494

                .         .         .         .         .         .
1561  AAGTTTTCTGATTACTACAGCAAGCTGAAGGCTTATGAGTATACCACCATACGCAGGGAG   1620
495   K  F  S  D  Y  Y  S  K  L  K  A  Y  E  Y  T  T  I  R  R  E    514

                .         .         .         .         .         .
1621  AGGGGAGAGGTGAACTTCATGCAGAGAGCCAGAAAGAAGATAGCTGAGTGTTTGCTTGGA   1680
515   R  G  E  V  N  F  M  Q  R  A  R  K  K  I  A  E  C  L  L  G    534

                .         .         .         .         .         .
1681  AGCAACCCACAGCTCACCCCAAGACAAGAGAATTAGTATTTTATAGGACATGTGGCTGGT   1740
535   S  N  P  Q  L  T  P  R  Q  E  N                              545

                .         .         .         .         .         .
1741  AGATTCCATGAACTTCAAGGCTTCATTGCTCTTTTTTTAATCATTATGATTGGCAGCAAA   1800
```

Figure 13C

```
                .         .         .         .         .         .
1801  AGGAAATGTGAAGCAGACATACACAAAGGCCATTTCGTTCACAAAGAAGGGCGAATTCGC  1860

                .
1861  GGCCGCTAAAT  1871
```