



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07H 21/04, C07K 14/00, 16/00, C12N 15/63, 15/85, 15/86, C12P 21/00	A1	(11) International Publication Number: WO 99/58548 (43) International Publication Date: 18 November 1999 (18.11.99)
(21) International Application Number: PCT/US99/09969 (22) International Filing Date: 7 May 1999 (07.05.99) (30) Priority Data: 60/084,740 8 May 1998 (08.05.98) US (71) Applicant: MYRIAD GENETICS, INC. [US/US]; 320 Wakara Way, Salt Lake City, UT 84108 (US). (72) Inventors: BARTEL, Paul, L.; 2042 Michigan Avenue, Salt Lake City, UT 84108 (US). TAVTIGIAN, Sean, V.; 557 E. 1st Avenue #3, Salt Lake City, UT 84103 (US). (74) Agents: IHNEN, Jeffrey, L. et al.; Rothwell, Figg, Ernst & Kurz, Suite 701 East, 555 13th Street N.W., Columbia Square, Washington, DC 20004 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, 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, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, 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), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MMSC2 – AN MMACI INTERACTING PROTEIN (57) Abstract The present invention is directed to the <i>MMSC2</i> gene, its protein product and the use of the protein to (i) detect mutant <i>MMAC1</i> proteins, (ii) screen for drugs which can be used for suppressing tumor growth and (iii) identify proteins which interact with the <i>MMAC1</i> gene or are involved in the tumor suppression pathway of the <i>MMAC1</i> gene.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

TITLE OF THE INVENTION

MMSC2 - AN MMAC1 INTERACTING PROTEIN

5 BACKGROUND OF THE INVENTION

The present invention is directed to the *MMSC2* gene, its protein product and the use of the protein to (i) detect mutant MMAC1 proteins, (ii) screen for drugs which can be used for suppressing tumor growth and (iii) identify proteins which interact with the *MMAC1* gene or are involved in the tumor suppression pathway of the *MMAC1* gene.

10 The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference, and for convenience are respectively grouped in the appended List of References.

A number of genetic alterations are involved in the oncogenesis of glioblastoma multiforme, including inactivation of *p53*, *p16*, *RB*, amplification of the gene encoding
15 epidermal growth factor receptor and several other molecular alterations (Louis & Gusella, 1995). However the most common genetic alteration is the deletion of large regions or an entire copy of chromosome 10 (Fulst et al., 1990; Rahseed et al., 1992). Recently, the tumor suppressor gene *MMAC1* (Steck et al., 1997), also known as *PTEN* (Li et al., 1997) or *TEP1* (Li & Sun, 1997) was mapped to 10q23 and shown to be mutated in 17-24% of xenografted and
20 primary glioblastomas, 14% of breast cancer samples and 25% of kidney carcinomas (Steck et al., 1997). The mutation frequency in established cell lines of these tumor types is somewhat higher. In addition to this predicted involvement in sporadic cancer, germ-line *MMAC1* mutations have been detected in two autosomal dominant disorders, Cowden disease (Nelen et al., 1997; Liaw et al., 1997), a syndrome that confers an elevated risk for tumors of breast,
25 thyroid and skin, and Bannayan-Zonana syndrome (Marsh et al., 1997), a condition characterized by macrocephaly, lipomas, intestinal hamartomatous polyps, vascular malformations and some skin disorders. Mutations of *MMAC1* in primary endometrial carcinomas (Kong et al., 1997) and in juvenile polyposis coli (Olschwang et al., 1998) have also been seen.

30 The predicted protein product of the *MMAC1* gene has several regions of homology with other proteins. The MMAC1 protein has an amino terminal domain with extensive homology to tensin, a protein that interacts with actin filaments at focal adhesions, and with auxilin, a protein involved in synaptic vesicle transport. The MMAC1 protein also has a region with extensive

homology to protein tyrosine phosphatases (Steck et al., 1997; Li et al., 1997). Mutations of *MMAC1* in tumors, its cytoplasmic localization (Li & Sun, 1997) and its intrinsic phosphatase activity (Li & Sun, 1997; Myers et al., 1997) suggested that its activity could be important in some aspect of tumor progression, possibly to counteract the oncogenic effect of a specific protein tyrosine kinase. In addition, *MMAC1* is rapidly down-regulated by TGF β in cells sensitive to its cell growth and cell adhesion regulatory properties (Li & Sun, 1997).

Experiments on glioma cell growth have shown that MMAC1 is a protein phosphatase that exhibits functional and specific growth-suppressing activity. In such experiments, the introduction of HA-tagged MMAC1 into glioma cells containing endogenous mutant alleles caused growth suppression, but was without effect in cells containing wild-type MMAC1 (Furnari et al., 1997). The ectopic expression of *MMAC1* alleles, which carried mutations found in primary tumors and have been shown or are expected to inactivate its phosphatase activity, caused little growth suppression (Furnari et al., 1997). Although these activities of *MMAC1* are known, the mechanisms of tumor suppression by *MMAC1* and the interaction of the MMAC1 protein with other proteins are not well understood.

Many cytosolic signaling proteins and cytoskeletal proteins are composed of modular units of small protein-protein interactive domains that allow reversible and regulated assembly into larger protein complexes. These domains include the Src-homology SH2 and SH3 domains (Schlessinger, 1994; Pawson, 1994), pleckstrin-homology (PH) domains (Lemmon et al., 1996; Shaw, 1996), phosphotyrosine-binding (PTB) domains (Harrison, 1996; van der Greer & Pawson, 1995; Kavanaugh et al., 1995) and postsynaptic density protein, disc-large, zo-1 (PDZ) domains (Woods & Bryant, 1991; Dho et al., 1992; Woods & Bryant, 1993; Kennedy, 1995; Kornau et al., 1995). So far, PDZ domains have been found in more than 50 proteins (Tsunoda et al., 1997), and many proteins have multiple PDZ domains (Pawson & Scott, 1997). For a review of PDZ domains, as well as the other protein-protein interactive domains, see Pawson & Scott (1997).

A distinguishing feature of PDZ domains is their recognition of short peptides at the carboxyl terminal end of proteins. For example, one family of PDZ domains selected peptides with the consensus motif Glu-(Ser/Thr)-Xaa-(Val/Ile) (SEQ ID NO:1) at the carboxy terminus, whereas a second family of PDZ domains selected peptides with hydrophobic or aromatic side chains at the carboxy terminal three residues (Songyang et al., 1997). The presence of multiple PDZ domains in proteins may have at least two important consequences. An individual PDZ-

containing protein could bind several subunits of a particular channel thereby inducing channel aggregations. Furthermore, the individual domains of a protein can have distinct binding specificities thereby inducing the formation of clusters that contain heterogeneous groups of proteins.

5 One example of this latter consequence of multiple PDZ domains is the InaD protein which contains five PDZ domains and acts as a scaffolding protein to organize the light-activated signaling events in *Drosophila* (Shieh & Zhu, 1996; Tsunoda et al., 1997). InaD associates through distinct PDZ domains with a calcium channel (TRP), phospholipase C- β (the target of rhodopsin-activated heterotrimeric guanine nucleotide-binding protein (Gq α) and
10 protein kinase C.

Two further properties of PDZ domains or proteins which contain them may expand their potential activities. First, some PDZ domains may bind internal peptide sequences and, indeed, have a propensity to undergo homotypic or heterotypic interactions with other PDZ domains (Brenman et al., 1996). Second, proteins with PDZ domains frequently contain other interaction
15 modules, including SH3 and LIM domains, and catalytic elements such as a tyrosine phosphatase or nitric oxide synthase domains. PDZ domains may therefore both coordinate the localization and clustering of receptors and channels, and provide a bridge to the cytoskeleton or intracellular signaling pathways.

It is desired to determine the mechanisms of tumor suppression for *MMAC1* and to
20 identify proteins which interact with the MMAC1 protein. Such proteins can be used to assay for mutated MMAC1 proteins and/or screen potential drugs for suppressing tumor growth and/or identify additional proteins which interact with MMAC1.

SUMMARY OF THE INVENTION

25 The present invention is directed to the *MMSC2* gene, its protein product and the use of the protein to (i) detect mutant MMAC1 proteins, (ii) screen for drugs which can be used for suppressing tumor growth and (iii) identify proteins which interact with the *MMAC1* gene or are involved in the tumor suppression pathway of the *MMAC1* gene.

Using yeast two-hybrid screening, it has been found MMAC1 binds to a protein herein
30 named MMSC2. The nucleotide sequence is set forth as SEQ ID NO:2, and the amino acid sequence is set forth as SEQ ID NO:3. It has been found that MMSC2 has 11 PDZ domains and that one or more of these domains interacts specifically with the three carboxyl terminal amino

acids of MMAC1. Specifically, it has been found that PDZ domain numbers 7,10 and 13 interact with MMAC1, with 7 appearing stronger. Since MMSC2 contains 11 PDZ domains and interacts with MMAC1, a known tumor suppressor having a region of homology with protein tyrosine phosphatases, MMSC2 acts as a scaffolding protein in a common biochemical pathway with MMAC1. These characteristics indicate that the interaction between MMAC1 and MMSC2 is required for the tumor suppressor activity of *MMAC1*.

BRIEF DESCRIPTION OF THE FIGURES AND TABLES

Figure 1 shows a diagram of *MMSC2* indicating the position of ORF and the positions of the 13 PDZ domains.

Figure 2 shows a diagram of the key clones used to assemble the full length *MMSC2* sequence, the probes used to identify those clones, and the relative position of the partially sequenced mouse ortholog 9BP-1 (GenBank Accession # AF000168).

SUMMARY OF SEQUENCE LISTING

SEQ ID NO:1 is a consensus motif to which one family of PDZ domains interacts. SEQ ID NO:2 is the nucleotide sequence for the *MMSC2* gene. SEQ ID NO:3 is the amino acid sequence for the MMSC2 protein. SEQ ID NO:4 is the 15 C-terminal amino acids of MMAC1. SEQ ID NO:5 is primer 9BP-1 F1. SEQ ID NO:6 is primer 9BP-1 R4. SEQ ID NO:7 is primer 9BP-1 #1. SEQ ID NO:8 is primer 9BP-1 #2. SEQ ID NO:9 is primer 9BP-1 #5. SEQ ID NO:10 is primer 9BP-1 #7. SEQ ID NO:11 is the SH3 binding peptide. SEQ ID NO:12 is the MMAC1 binding peptide. SEQ ID NOs:13-72 are primers for PCR amplification of the *MMSC2* gene.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to the *MMSC2* gene, its protein product and the use of the protein to (i) detect mutant MMAC1 proteins, (ii) screen for drugs which can be used for suppressing tumor growth and (iii) identify proteins which interact with the *MMAC1* gene or are involved in the tumor suppression pathway of the *MMAC1* gene.

Using yeast two-hybrid screening, it has been found MMAC1 binds to a protein herein named MMSC2. The nucleotide sequence is set forth as SEQ ID NO:2, and the amino acid sequence is set forth as SEQ ID NO:3. It has been found MMSC2 has 11 PDZ domains and that

one or more of these domains interacts specifically with the three carboxyl terminal amino acids of MMAC1. Specifically, it has been found that PDZ domain numbers 7, 10 and 13 interact with MMAC1 with 7 appearing stronger. Since MMSC2 contains 11 PDZ domains and interacts with MMAC1, a known tumor suppressor having a region of homology with protein tyrosine phosphatases, MMSC2 acts as a scaffolding protein in a common biochemical pathway with MMAC1. These characteristics indicate that the interaction between MMAC1 and MMSC2 is required for the tumor suppressor activity of *MMAC1*.

The evidence presented herein shows that the function of MMSC2 is to make a scaffold that binds to MMAC1, the phosphatase substrate(s), and the (probably oncogene) tyrosine kinase(s). Thus, a valuable drug will be one that can prevent binding of either the substrate(s) or the tyrosine kinases(s) to MMSC2.

The yeast two-hybrid screening assay described herein identified five clones encoding bona fide MMAC1-interacting proteins. These clones were named PDZBN2B, PDZBN3A, PDZBN5B, PDZBN18D, and pdzk4. Comparison of the sequences of these clones suggested that they were all partial cDNAs derived from the same novel gene. A search of GenBank with these sequences revealed strong sequence similarity with a partial mouse cDNA sequence called 9ORF binding protein 1 (9BP-1)(GenBank Accession # AF000168).

Several rounds of cDNA library screening were required to identify cDNA clones that could be assembled into the full length *MMSC2* sequence. In the first round, a 509 base pair(bp) probe was developed from the 5' end of clone PDZBN2B using the primers 9BP-1 F1 and 9BP-1 R4. This probe was used to screen a human placental cDNA library and a human prostate cDNA library. Two of the informative clones obtained were p118a(placental) and pr63(prostate). A search of GenBank with this additional sequence yielded an additional human EST (GenBank Accession # C75629). For the second round of cDNA library screening, a 202bp probe was developed from the 5' end of this EST using primers 9BP-1 #1 and 9BP-1 #2. This probe was used to screen a human prostate cDNA library; two of the informative clones obtained were clone #10 and clone #3. For the third round of cDNA library screening, a 172bp probe was developed from the 5' end of clone #3 using primers 9BP-1 #5 and 9BP-1 #7 and used to screen a human prostate cDNA library. One of the resulting clones, clone # 6, yielded the start codon and part of the 5' UTR, including in-frame upstream stop codons. The nucleotide sequence for *MMSC2* is set forth in SEQ ID NO:2 with the amino acid sequence of the encoded protein set forth in SEQ ID NO:3.

As previously noted, SEQ ID NO:2 sets forth the nucleotide sequence for *MMSC2*. However, it has been found that the mRNA for *MMSC2* is subject to alternate splicing. On the basis of the sequence for *MMSC2*, genomic clones have been isolated and are being analyzed to determine splice junctions and alternate splicing for the mRNA. In addition, the PDZ domains of *MMSC2* are analyzed in the yeast two-hybrid assay to identify other proteins which interact with *MMSC2* and consequently are involved in the *MMAC1* tumor suppressor pathway.

Since *MMSC2* is an *MMAC1* interacting protein that is involved in tumor suppression activity in the *MMAC1* pathway, mutations in the *MMSC2* gene which affect the interaction of *MMSC2* with *MMAC1* or affect the interaction of other proteins with *MMAC1* as a result of the scaffolding effect of *MMSC2* will interfere with the *MMAC1* tumor suppressor pathway and lead to tumorigenesis. Thus, an additional aspect of the present invention is the screening of *MMSC2* for such mutations using conventional techniques. Such methods may further comprise the step of amplifying a portion of the *MMSC2* gene, and may further include a step of providing a set of polynucleotides which are primers for amplification of said portion of the *MMSC2* gene. The method is useful for identifying mutations for use in either diagnosis of cancer or prognosis of cancer. Since such variants can now be detected earlier, i.e., before symptoms appear, and more definitively, better treatment options will be available in those individuals identified as having harmful mutations in *MMSC2*.

The present invention is directed to the determination that the *MMSC2* binds to the C-terminal region of *MMAC1* and is involved in a common pathway with *MMAC1* which is a known tumor suppressor. Since many of the mutations in *MMAC1* are frameshift or nonsense mutations which consequently alter the C-terminus of *MMAC1*, *MMSC2* can be used to assay for normal or mutated *MMAC1* proteins using conventional techniques.

Finally, the present invention is directed to a method for screening drug candidates to identify drugs useful for treating or preventing cancer. Drug screening is performed by expressing mutant *MMSC2* and assaying the effect of a drug candidate on the binding of *MMSC2* with *MMAC1*. Similarly, one can test the effect of a drug candidate on the binding of wild-type *MMSC2* with a mutant *MMAC1*. Such assays can be performed *in vitro* or *in vivo*, such as in oocytes, mammalian cells or transgenic animals. Other assays may test the ability of a drug, wherein the drug may be, e.g., a peptide, to replace the activity of *MMSC2* such that the drug plus *MMAC1* will work in concert similar to the normal wild-type interactions of *MMSC2* and *MMAC1*. Again, similar assays may be performed to screen for drugs which replace a

mutant MMAC1 and will bind to wild-type MMSC2 to replace the MMAC1 function which is lacking as a result of a mutated MMAC1.

According to the diagnostic and prognostic method of the present invention, alteration of the wild-type *MMSC2* gene is detected. In addition, the method can be performed by detecting the wild-type *MMSC2* gene and confirming the lack of a cause of cancer as a result of this locus. "Alteration of a wild-type gene" encompasses all forms of mutations including deletions, insertions and point mutations in the coding and noncoding regions. Deletions may be of the entire gene or of only a portion of the gene. Point mutations may result in stop codons, frameshift mutations or amino acid substitutions. Somatic mutations are those which occur only in certain tissues and are not inherited in the germline. Germline mutations can be found in any of a body's tissues and are inherited. Point mutational events may occur in regulatory regions, such as in the promoter of the gene, leading to loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the *MMSC2* gene product, or to a decrease in mRNA stability or translation efficiency.

Useful diagnostic techniques include, but are not limited to fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded conformation analysis (SSCA), RNase protection assay, allele-specific oligonucleotide (ASO), dot blot analysis, hybridization using nucleic acid modified with gold nanoparticles and PCR-SSCP, as discussed in detail further below. Also useful is the recently developed technique of DNA microchip technology.

The presence of cancer due to a germline mutation at this locus may be ascertained by testing any tissue of a human for mutations of the *MMSC2* gene. For example, a person who has inherited a germline *MMSC2* mutation, especially one which alters the interaction of MMSC2 with MMAC1, would be prone to develop cancer. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the *MMSC2* gene. Alteration of a wild-type *MMSC2* allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing can detect sequence variation. Another approach is the single-stranded conformation polymorphism assay

(SSCP) (Orita et al., 1989). This method does not detect all sequence changes, especially if the DNA fragment size is greater than 200 bp, but can be optimized to detect most DNA sequence variation. The reduced detection sensitivity is a disadvantage, but the increased throughput possible with SSCP makes it an attractive, viable alternative to direct sequencing for mutation detection on a research basis. The fragments which have shifted mobility on SSCP gels are then sequenced to determine the exact nature of the DNA sequence variation. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield et al., 1991), heteroduplex analysis (HA) (White et al., 1992) and chemical mismatch cleavage (CMC) (Grompe et al., 1989). None of the methods described above will detect large deletions, duplications or insertions, nor will they detect a regulatory mutation which affects transcription or translation of the protein. Other methods which might detect these classes of mutations such as a protein truncation assay or the asymmetric assay, detect only specific types of mutations and would not detect missense mutations. A review of currently available methods of detecting DNA sequence variation can be found in a recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific oligonucleotide (ASO) hybridization can be utilized to rapidly screen large numbers of other samples for that same mutation. Such a technique can utilize probes which are labeled with gold nanoparticles to yield a visual color result (Elghanian et al., 1997).

A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably with a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of cancer cases. Southern blots displaying hybridizing fragments differing in length from control DNA when probed with sequences near or including the *MMSC2* locus indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis (PFGE) is employed.

Detection of point mutations may be accomplished amplification, e.g., PCR, from genomic or cDNA and sequencing the amplified nucleic acid or by molecular cloning of the *MMSC2* allele and sequencing the allele using techniques well known in the art.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis (SSCP) (Orita et al., 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell et al.,

1990; Sheffield et al., 1989); 3) RNase protection assays (Finkelstein et al., 1990; Kinszler et al., 1991); 4) allele-specific oligonucleotides (ASOs) (Conner et al., 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and 6) allele-specific PCR (Rano and Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular *MMSC2* mutation. If the particular mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

In the first three methods (SSCP, DGGE and RNase protection assay), a new electrophoretic band appears. SSCP detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of samples. An example of a mismatch cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type *MMSC2* gene coding sequence. The riboprobe and either mRNA or DNA isolated from the person are annealed (hybridized) together and subsequently digested with the

enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., 1988; Shenk et al., 1975; Novack et al., 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the *MMSC2* gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the *MMSC2* gene which have been amplified by use of PCR may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the gene. Hybridization of allele-specific probes with amplified *MMSC2* sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under high stringency hybridization conditions indicates the presence of the same mutation in the tissue as in the allele-specific probe.

The newly developed technique of nucleic acid analysis via microchip technology is also applicable to the present invention. In this technique, literally thousands of distinct oligonucleotide probes are built up in an array on a silicon chip. Nucleic acid to be analyzed is fluorescently labeled and hybridized to the probes on the chip. It is also possible to study nucleic acid-protein interactions using these nucleic acid microchips. Using this technique one can determine the presence of mutations or even sequence the nucleic acid being analyzed or one can measure expression levels of a gene of interest. The method is one of parallel processing of

many, even thousands, of probes at once and can tremendously increase the rate of analysis. Several papers have been published which use this technique. Some of these are Hacia et al., 1996; Shoemaker et al., 1996; Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1996; Lipshutz et al., 1995. This method has already been used to screen people for mutations in the breast cancer gene BRCA1 (Hacia et al., 1996). This new technology has been reviewed in a news article in Chemical and Engineering News (Borman, 1996) and been the subject of an editorial (Nature Genetics, 1996). Also see Fodor (1997).

The most definitive test for mutations in a candidate locus is to directly compare genomic *MMSC2* sequences from patients with those from a control population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

Mutations from patients falling outside the coding region of *MMSC2* can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the genes. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in patients as compared to control individuals.

Alteration of *MMSC2* mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type gene. Alteration of wild-type genes can also be detected by screening for alteration of wild-type *MMSC2* protein. For example, monoclonal antibodies immunoreactive with *MMSC2* can be used to screen a tissue. Lack of cognate antigen would indicate a mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered *MMSC2* protein can be used to detect alteration of the wild-type *MMSC2* gene. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect *MMSC2* biochemical function. Finding a mutant *MMSC2* gene product indicates alteration of a wild-type *MMSC2* gene. One such binding assay is the binding of *MMSC2* with wild-type *MMAC1*. Conversely, wild-type *MMSC2* or the PDZ domain interacting with *MMAC1* can be used in a protein binding assay or biochemical function assay to detect normal or mutant *MMAC1* proteins, where the mutant proteins are proteins lacking a wild-type C-terminus.

A mutant *MMSC2* gene or gene product or a mutant *MMAC1* can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant genes or gene products in tissues can be applied to other body samples. By screening such body samples, a simple early diagnosis can be achieved for cancer
5 resulting from a mutation in the *MMSC2* gene.

The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular *MMSC2* allele using PCR. The pairs of single-stranded DNA primers for *MMSC2* can be annealed to sequences within or surrounding the *MMSC2* gene in order to prime amplifying DNA synthesis of the gene itself. A complete set of these primers allows
10 synthesis of all of the nucleotides of the gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele-specific primers can also be used. Such primers anneal only to particular *MMSC2* mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

In order to facilitate subsequent cloning of amplified sequences, primers may have
15 restriction enzyme site sequences appended to their 5' ends. Alternatively, primers can also be prepared with 5' phosphoryl groups which will allow for blunt end cloning of amplified sequences. Thus, all nucleotides of the primers are derived from *MMSC2* sequence or sequences adjacent to *MMSC2*, except for the few nucleotides necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. The primers themselves can be synthesized
20 using techniques which are well known in the art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. Given the sequence of *MMSC2*, design of particular primers is well within the skill of the art.

The nucleic acid probes provided by the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase
25 protection method for detecting point mutations already discussed above. The probes can be used to detect PCR amplification products. They may also be used to detect mismatches with the *MMSC2* gene or mRNA using other techniques.

Mutations which interfere with the function of the *MMSC2* gene product are involved in the pathogenesis of cancer. Thus, the presence of an altered (or a mutant) *MMSC2* gene which
30 produces a protein having a loss of function, or altered function, directly increases the risk of cancer. In order to detect a *MMSC2* gene mutation, a biological sample is prepared and analyzed for a difference between the sequence of the allele being analyzed and the sequence of the wild-

type allele. Mutant *MMSC2* alleles can be initially identified by any of the techniques described above. The mutant alleles are then sequenced to identify the specific mutation of the particular mutant allele. Alternatively, mutant alleles can be initially identified by identifying mutant (altered) proteins, using conventional techniques. The mutant alleles are then sequenced to identify the specific mutation for each allele. The mutations, especially those which lead to an altered function of the protein, are then used for the diagnostic and prognostic methods of the present invention.

Definitions

The present invention employs the following definitions.

"Amplification of Polynucleotides" utilizes methods such as the polymerase chain reaction (PCR), ligation amplification (or ligase chain reaction, LCR) and amplification methods based on the use of Q-beta replicase. Also useful are strand displacement amplification (SDA), thermophilic SDA, nucleic acid sequence based amplification (3SR or NASBA) and repair chain reaction (RCR). These methods are well known and widely practiced in the art. See, e.g., U.S. Patents 4,683,195 and 4,683,202 and Innis *et al.*, 1990 (for PCR); Wu *et al.*, 1989a and EP 320,308A (for LCR); U.S. Patents 5,270,184 and 5,455,166 and Walker *et al.*, 1992 (for SDA); Spargo *et al.*, 1996 (for thermophilic SDA) and U.S. Patent 5,409,818, Fahy *et al.*, 1991 and Compton, 1991 for 3SR and NASBA. Reagents and hardware for conducting PCR are commercially available. Primers useful to amplify sequences from the *MMSC2* region are preferably complementary to, and hybridize specifically to sequences in the *MMSC2* region or in regions that flank a target region therein. *MMSC2* sequences generated by amplification may be sequenced directly. Alternatively, but less desirably, the amplified sequence(s) may be cloned prior to sequence analysis. A method for the direct cloning and sequence analysis of enzymatically amplified genomic segments has been described by Scharf, 1986.

"Analyte polynucleotide" and **"analyte strand"** refer to a single- or double-stranded polynucleotide which is suspected of containing a target sequence, and which may be present in a variety of types of samples, including biological samples.

"Antibodies." The present invention also provides polyclonal and/or monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof, which are capable of specifically binding to the *MMSC2* polypeptide and fragments thereof or to polynucleotide sequences from the *MMSC2* region. The term **"antibody"** is used both to refer to

a homogeneous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Polypeptides may be prepared synthetically in a peptide synthesizer and coupled to a carrier molecule (e.g., keyhole limpet hemocyanin) and injected over several months into rabbits. Rabbit sera is tested for immunoreactivity to the MMSC2 polypeptide or
5 fragment. Monoclonal antibodies may be made by injecting mice with the protein polypeptides, fusion proteins or fragments thereof. Monoclonal antibodies will be screened by ELISA and tested for specific immunoreactivity with MMSC2 polypeptide or fragments thereof. See, Harlow and Lane, 1988. These antibodies will be useful in assays as well as pharmaceuticals.

Once a sufficient quantity of desired polypeptide has been obtained, it may be used for
10 various purposes. A typical use is the production of antibodies specific for binding. These antibodies may be either polyclonal or monoclonal, and may be produced by *in vitro* or *in vivo* techniques well known in the art. For production of polyclonal antibodies, an appropriate target immune system, typically mouse or rabbit, is selected. Substantially purified antigen is presented to the immune system in a fashion determined by methods appropriate for the animal
15 and by other parameters well known to immunologists. Typical sites for injection are in footpads, intramuscularly, intraperitoneally, or intradermally. Of course, other species may be substituted for mouse or rabbit. Polyclonal antibodies are then purified using techniques known in the art, adjusted for the desired specificity.

An immunological response is usually assayed with an immunoassay. Normally, such
20 immunoassays involve some purification of a source of antigen, for example, that produced by the same cells and in the same fashion as the antigen. A variety of immunoassay methods are well known in the art. See, e.g., Harlow and Lane, 1988, or Goding, 1986.

Monoclonal antibodies with affinities of 10^{-8} M⁻¹ or preferably 10^{-9} to 10^{-10} M⁻¹ or stronger will typically be made by standard procedures as described, e.g., in Harlow and Lane,
25 1988 or Goding, 1986. Briefly, appropriate animals will be selected and the desired immunization protocol followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the
30 desired region of the antigen.

Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors.

See Huse *et al.*, 1989. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include U.S. Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced (see U.S. Patent 4,816,567).

"Binding partner" refers to a molecule capable of binding a ligand molecule with high specificity, as for example, an antigen and an antigen-specific antibody or an enzyme and its inhibitor. In general, the specific binding partners must bind with sufficient affinity to immobilize the analyte copy/complementary strand duplex (in the case of polynucleotide hybridization) under the isolation conditions. Specific binding partners are known in the art and include, for example, biotin and avidin or streptavidin, IgG and protein A, the numerous, known receptor-ligand couples, and complementary polynucleotide strands. In the case of complementary polynucleotide binding partners, the partners are normally at least about 15 bases in length, and may be at least 40 bases in length. It is well recognized by those of skill in the art that lengths shorter than 15 (e.g., 8 bases), between 15 and 40, and greater than 40 bases may also be used. The polynucleotides may be composed of DNA, RNA, or synthetic nucleotide analogs. In addition, as disclosed herein, MMAC1 and PDZ binding peptides, as well as several other proteins, bind to or interact with MMSC2. Each of these proteins are also considered binding partners herein. Further binding partners can be identified using, e.g., the two-hybrid yeast screening assay as described herein.

A **"biological sample"** refers to a sample of tissue or fluid suspected of containing an analyte polynucleotide or polypeptide from an individual including, but not limited to, e.g., plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, blood cells, tumors, organs, tissue and samples of *in vitro* cell culture constituents.

"Encode". A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof. The anti-

sense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

"Isolated" or "substantially pure". An "isolated" or "substantially pure" nucleic acid (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native human sequence or protein, e.g., ribosomes, polymerases, many other human genome sequences and proteins. The term embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

"MMSC2 Allele" refers to normal alleles of the *MMSC2* locus that interact with MMAC1 as well as alleles of *MMSC2* carrying variations that affect the interaction with MMAC1 and that cause cancer.

"MMSC2 Locus", "MMSC2 Gene", "MMSC2 Nucleic Acids" or "MMSC2 Polynucleotide" each refer to polynucleotides, all of which are in the *MMSC2* region, that are likely to be expressed in normal tissue, certain alleles of which adversely affect the interaction with MMAC1 and result in cancer. The *MMSC2* locus is intended to include coding sequences, intervening sequences and regulatory elements controlling transcription and/or translation. The *MMSC2* locus is intended to include all allelic variations of the DNA sequence.

These terms, when applied to a nucleic acid, refer to a nucleic acid which encodes a human *MMSC2* polypeptide, fragment, homolog or variant, including, e.g., protein fusions or deletions. The nucleic acids of the present invention will possess a sequence which is either derived from, or substantially similar to a natural *MMSC2*-encoding gene or one having substantial homology with a natural *MMSC2*-encoding gene or a portion thereof.

The *MMSC2* gene or nucleic acid includes normal alleles of the *MMSC2* gene, both silent alleles having no effect on the amino acid sequence of the *MMSC2* polypeptide and alleles leading to amino acid sequence variants of the *MMSC2* polypeptide that do not substantially affect its function. These terms also include alleles having one or more mutations which adversely affect the function of the *MMSC2* polypeptide. A mutation may be a change in the *MMSC2* nucleic acid sequence which produces a deleterious change in the amino acid sequence of the *MMSC2* polypeptide, resulting in partial or complete loss of *MMSC2* function, or may be a change in the nucleic acid sequence which results in the loss of effective *MMSC2* expression or the production of aberrant forms of the *MMSC2* polypeptide.

The *MMSC2* nucleic acid may be that shown in SEQ ID NO:2, or it may be an allele as described above, or a variant or derivative differing from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to the nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

Thus, nucleic acid according to the present invention may include a sequence different from the sequence shown in SEQ ID NO:2 yet encode a polypeptide with the same amino acid sequence as shown in SEQ ID NO:3. That is, nucleic acids of the present invention include sequences which are degenerate as a result of the genetic code. On the other hand, the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in SEQ ID NO:3. Nucleic acid encoding a polypeptide which is an amino acid sequence variant, derivative or allele of the amino acid sequence shown in SEQ ID NO:3 is also provided by the present invention.

The *MMSC2* gene also refers to (a) any DNA sequence that (i) hybridizes to the complement of the DNA sequences that encode the amino acid sequence set forth in SEQ ID NO:3 under highly stringent conditions (Ausubel et al.) (ii) and encodes a gene product functionally equivalent to *MMSC2*, or (b) any DNA sequence that (i) hybridizes to the complement of the DNA sequences that encode the amino acid sequence set forth in SEQ ID NO:3 under less stringent conditions, such as moderately stringent conditions (Ausubel et al.), and (ii) encodes a gene product functionally equivalent to *MMSC2*. The invention also includes nucleic acid molecules that are the complements of the sequences described herein.

The polynucleotide compositions of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other

chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The present invention provides recombinant nucleic acids comprising all or part of the *MMSC2* region. The recombinant construct may be capable of replicating autonomously in a host cell. Alternatively, the recombinant construct may become integrated into the chromosomal DNA of the host cell. Such a recombinant polynucleotide comprises a polynucleotide of genomic, cDNA, RNA, semi-synthetic, or synthetic origin which, by virtue of its origin or manipulation, 1) is not associated with all or a portion of a polynucleotide with which it is associated in nature; 2) is linked to a polynucleotide other than that to which it is linked in nature; or 3) does not occur in nature. Where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

Therefore, recombinant nucleic acids comprising sequences otherwise not naturally occurring are provided by this invention. Although the wild-type sequence may be employed, it will often be altered, e.g., by deletion, substitution or insertion. cDNA or genomic libraries of various types may be screened as natural sources of the nucleic acids of the present invention, or such nucleic acids may be provided by amplification of sequences resident in genomic DNA or other natural sources, e.g., by PCR. The choice of cDNA libraries normally corresponds to a tissue source which is abundant in mRNA for the desired proteins. Phage libraries are normally preferred, but other types of libraries may be used. Clones of a library are spread onto plates, transferred to a substrate for screening, denatured and probed for the presence of desired sequences.

The DNA sequences used in this invention will usually comprise at least about five codons (15 nucleotides), more usually at least about 7-15 codons, and most preferably, at least about 35 codons. One or more introns may also be present. This number of nucleotides is usually about the minimal length required for a successful probe that would hybridize specifically with a *MMSC2*-encoding sequence. In this context, oligomers of as low as 8 nucleotides, more generally 8-17 nucleotides, can be used for probes, especially in connection with chip technology.

Techniques for nucleic acid manipulation are described generally, for example, in Sambrook *et al.*, 1989 or Ausubel *et al.*, 1992. Reagents useful in applying such techniques, such as restriction enzymes and the like, are widely known in the art and commercially available

from such vendors as New England BioLabs, Boehringer Mannheim, Amersham, Promega, U. S. Biochemicals, New England Nuclear, and a number of other sources. The recombinant nucleic acid sequences used to produce fusion proteins of the present invention may be derived from natural or synthetic sequences. Many natural gene sequences are obtainable from various cDNA or from genomic libraries using appropriate probes. See, GenBank, National Institutes of Health.

As used herein, a "**portion**" of the *MMSC2* locus or region or allele is defined as having a minimal size of at least about eight nucleotides, or preferably about 15 nucleotides, or more preferably at least about 25 nucleotides, and may have a minimal size of at least about 40 nucleotides. This definition includes all sizes in the range of 8-40 nucleotides as well as greater than 40 nucleotides. Thus, this definition includes nucleic acids of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400, 500 nucleotides, or nucleic acids having any number of nucleotides within these values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc, nucleotides), or nucleic acids having more than 500 nucleotides, or any number of nucleotides between 500 and the number shown in SEQ ID NO:2. The present invention includes all novel nucleic acids having at least 8 nucleotides derived from SEQ ID NO:2, its complement or functionally equivalent nucleic acid sequences. The present invention does not include nucleic acids which exist in the prior art. That is, the present invention includes all nucleic acids having at least 8 nucleotides derived from SEQ ID NO:2 with the proviso that it does not include nucleic acids existing in the prior art.

"**MMSC2 protein**" or "**MMSC2 polypeptide**" refers to a protein or polypeptide encoded by the *MMSC2* locus, variants or fragments thereof. The term "polypeptide" refers to a polymer of amino acids and its equivalent and does not refer to a specific length of the product; thus, peptides, oligopeptides and proteins are included within the definition of a polypeptide. This term also does not refer to, or exclude modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages as well as other modifications known in the art, both naturally and non-naturally occurring. Ordinarily, such polypeptides will be at least about 50% homologous to the native *MMSC2* sequence, preferably in excess of about 90%, and more preferably at least about 95% homologous. Also included are proteins encoded by DNA which hybridize under high or low stringency conditions, to *MMSC2*-

encoding nucleic acids and closely related polypeptides or proteins retrieved by antisera to the MMSC2 protein(s).

The MMSC2 polypeptide may be that shown in SEQ ID NO:3 which may be in isolated and/or purified form, free or substantially free of material with which it is naturally associated.

5 The polypeptide may, if produced by expression in a prokaryotic cell or produced synthetically, lack native post-translational processing, such as glycosylation. Alternatively, the present invention is also directed to polypeptides which are sequence variants, alleles or derivatives of the MMSC2 polypeptide. Such polypeptides may have an amino acid sequence which differs from that set forth in SEQ ID NO:3 by one or more of addition, substitution, deletion or
10 insertion of one or more amino acids. Preferred such polypeptides have MMSC2 function.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, such as stability against proteolytic cleavage, without the loss of other functions or properties. Amino acid substitutions may be made on the basis of similarity in polarity,
15 charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. Preferred substitutions are ones which are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine,
20 arginine; and tyrosine, phenylalanine.

Certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules or binding sites on proteins interacting with the MMSC2 polypeptide. Since it is the interactive capacity and nature
25 of a protein which defines that protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. In making such changes, the hydrophathic index of amino acids may be considered. The importance of the hydrophobic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte & Doolittle, 1982). Alternatively, the substitution of like amino acids can be made effectively
30 on the basis of hydrophilicity. The importance of hydrophilicity in conferring interactive biological function of a protein is generally understood in the art (U.S. Patent 4,554,101). The

use of the hydrophobic index or hydrophilicity in designing polypeptides is further discussed in U.S. Patent 5,691,198.

The length of polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression.

The term **"peptide mimetic"** or **"mimetic"** is intended to refer to a substance which has the essential biological activity of the *MMSC2* polypeptide. A peptide mimetic may be a peptide-containing molecule that mimics elements of protein secondary structure (Johnson et al., 1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen, enzyme and substrate or scaffolding proteins. A peptide mimetic is designed to permit molecular interactions similar to the natural molecule. A mimetic may not be a peptide at all, but it will retain the essential biological activity of natural *MMSC2* polypeptide.

"Probes". Polynucleotide polymorphisms associated with *MMSC2* alleles which predispose to cancer are detected by hybridization with a polynucleotide probe which forms a stable hybrid with that of the target sequence, under highly stringent to moderately stringent hybridization and wash conditions. If it is expected that the probes will be perfectly complementary to the target sequence, high stringency conditions will be used. Hybridization stringency may be lessened if some mismatching is expected, for example, if variants are expected with the result that the probe will not be completely complementary. Conditions are chosen which rule out nonspecific/adventitious bindings, that is, which minimize noise. (It should be noted that throughout this disclosure, if it is simply stated that "stringent" conditions are used that is meant to be read as "high stringency" conditions are used.) Since such indications identify neutral DNA polymorphisms as well as mutations, these indications need further analysis to demonstrate detection of a *MMSC2* susceptibility allele.

Probes for *MMSC2* alleles may be derived from the sequences of the *MMSC2* region, its cDNA, functionally equivalent sequences, or the complements thereof. The probes may be of any suitable length, which span all or a portion of the *MMSC2* region, and which allow specific

hybridization to the region. If the target sequence contains a sequence identical to that of the probe, the probes may be short, e.g., in the range of about 8-30 base pairs, since the hybrid will be relatively stable under even highly stringent conditions. If some degree of mismatch is expected with the probe, i.e., if it is suspected that the probe will hybridize to a variant region, a longer probe may be employed which hybridizes to the target sequence with the requisite specificity.

The probes will include an isolated polynucleotide attached to a label or reporter molecule and may be used to isolate other polynucleotide sequences, having sequence similarity by standard methods. For techniques for preparing and labeling probes see, e.g., Sambrook et al., 1989 or Ausubel et al., 1992. Other similar polynucleotides may be selected by using homologous polynucleotides. Alternatively, polynucleotides encoding these or similar polypeptides may be synthesized or selected by use of the redundancy in the genetic code. Various codon substitutions may be introduced, e.g., by silent changes (thereby producing various restriction sites) or to optimize expression for a particular system. Mutations may be introduced to modify the properties of the polypeptide, perhaps to change the polypeptide degradation or turnover rate.

Probes comprising synthetic oligonucleotides or other polynucleotides of the present invention may be derived from naturally occurring or recombinant single- or double-stranded polynucleotides, or be chemically synthesized. Probes may also be labeled by nick translation, Klenow fill-in reaction, or other methods known in the art.

Portions of the polynucleotide sequence having at least about eight nucleotides, usually at least about 15 nucleotides, and fewer than about 9 kb, usually fewer than about 1.0 kb, from a polynucleotide sequence encoding *MMSC2* are preferred as probes. This definition therefore includes probes of sizes 8 nucleotides through 9000 nucleotides. Thus, this definition includes probes of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400, 500 nucleotides, or probes having any number of nucleotides within these values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc, nucleotides), or probes having more than 500 nucleotides, or any number of nucleotides between 500 and the number shown in SEQ ID NO:2. The probes may also be used to determine whether mRNA encoding *MMSC2* is present in a cell or tissue. The present invention includes all novel probes having at least 8 nucleotides derived from SEQ ID NO:2, its complement or functionally equivalent nucleic acid sequences. The present invention does not include probes which exist in the prior art. That is, the present invention includes all probes having at least 8 nucleotides

derived from SEQ ID NO:2 with the proviso that it does not include probes existing in the prior art.

Similar considerations and nucleotide lengths are also applicable to primers which may be used for the amplification of all or part of the *MMSC2* gene. Thus, a definition for primers includes primers of 8, 12, 15, 20, 25, 40, 60, 80, 100, 200, 300, 400, 500 nucleotides, or primers having any number of nucleotides within these values (e.g., 9, 10, 11, 16, 23, 30, 38, 50, 72, 121, etc, nucleotides), or primers having more than 500 nucleotides, or any number of nucleotides between 500 and 9000. The primers may also be used to determine whether mRNA encoding *MMSC2* is present in a cell or tissue. The present invention includes all novel primers having at least 8 nucleotides derived from the *MMSC2* locus for amplifying the *MMSC2* gene, its complement or functionally equivalent nucleic acid sequences. The present invention does not include primers which exist in the prior art. That is, the present invention includes all primers having at least 8 nucleotides with the proviso that it does not include primers existing in the prior art.

"Protein modifications or fragments" are provided by the present invention for *MMSC2* polypeptides or fragments thereof which are substantially homologous to primary structural sequence but which include, e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, e.g., with radionuclides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as ^{32}P , ligands which bind to labeled antiligands (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods of labeling polypeptides are well known in the art. See Sambrook *et al.*, 1989 or Ausubel *et al.*, 1992.

Besides substantially full-length polypeptides, the present invention provides for biologically active fragments of the polypeptides. Significant biological activities include ligand-binding, immunological activity and other biological activities characteristic of *MMSC2* polypeptides. Immunological activities include both immunogenic function in a target immune

system, as well as sharing of immunological epitopes for binding, serving as either a competitor or substitute antigen for an epitope of the MMSC2 protein. As used herein, "epitope" refers to an antigenic determinant of a polypeptide. An epitope could comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least
5 five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods of determining the spatial conformation of such amino acids are known in the art.

For immunological purposes, tandem-repeat polypeptide segments may be used as immunogens, thereby producing highly antigenic proteins. Alternatively, such polypeptides will serve as highly efficient competitors for specific binding. Production of antibodies specific for
10 MMSC2 polypeptides or fragments thereof is described below.

The present invention also provides for fusion polypeptides, comprising MMSC2 polypeptides and fragments. Homologous polypeptides may be fusions between two or more MMSC2 polypeptide sequences or between the sequences of MMSC2 and a related protein. Likewise, heterologous fusions may be constructed which would exhibit a combination of
15 properties or activities of the derivative proteins. For example, ligand-binding or other domains may be "swapped" between different new fusion polypeptides or fragments. Such homologous or heterologous fusion polypeptides may display, for example, altered strength or specificity of binding. Fusion partners include immunoglobulins, bacterial β -galactosidase, trpE, protein A, β -lactamase, α -amylase, alcohol dehydrogenase and yeast alpha mating factor. See Godowski *et*
20 *al.*, 1988.

Fusion proteins will typically be made by either recombinant nucleic acid methods, as described below, or may be chemically synthesized. Techniques for the synthesis of polypeptides are described, for example, in Merrifield, 1963.

"Protein purification" refers to various methods for the isolation of the MMSC2
25 polypeptides from other biological material, such as from cells transformed with recombinant nucleic acids encoding MMSC2, and are well known in the art. For example, such polypeptides may be purified by immunoaffinity chromatography employing, e.g., the antibodies provided by the present invention. Various methods of protein purification are well known in the art, and include those described in Deutscher, 1990 and Scopes, 1982.

30 The terms **"isolated"**, **"substantially pure"**, and **"substantially homogeneous"** are used interchangeably to describe a protein or polypeptide which has been separated from components which accompany it in its natural state. A monomeric protein is substantially pure when at least

about 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art which are utilized for purification.

A MMSC2 protein is substantially free of naturally associated components when it is separated from the native contaminants which accompany it in its natural state. Thus, a polypeptide which is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

A polypeptide produced as an expression product of an isolated and manipulated genetic sequence is an "isolated polypeptide," as used herein, even if expressed in a homologous cell type. Synthetically made forms or molecules expressed by heterologous cells are inherently isolated molecules.

"Recombinant nucleic acid" is a nucleic acid which is not naturally occurring, or which is made by the artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions.

"Regulatory sequences" refers to those sequences normally within 100 kb of the coding region of a locus, but they may also be more distant from the coding region, which affect the expression of the gene (including transcription of the gene, and translation, splicing, stability or the like of the messenger RNA).

"Substantial homology or similarity". A nucleic acid or fragment thereof is "substantially homologous" ("or substantially similar") to another if, when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its

complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least about 95-98% of the nucleotide bases.

Alternatively, substantial homology or (similarity) exists when a nucleic acid or fragment thereof will hybridize to another nucleic acid (or a complementary strand thereof) under selective hybridization conditions, to a strand, or to its complement. Selectivity of hybridization exists when hybridization which is substantially more selective than total lack of specificity occurs. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, Kanehisa, 1984. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides.

Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. The stringency conditions are dependent on the length of the nucleic acid and the base composition of the nucleic acid and can be determined by techniques well known in the art. See, e.g., Wetmur and Davidson, 1968.

Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art.

The terms "**substantial homology**" or "**substantial identity**", when referring to polypeptides, indicate that the polypeptide or protein in question exhibits at least about 30% identity with an entire naturally-occurring protein or a portion thereof, usually at least about 70%

identity, more usually at least about 80% identity, preferably at least about 90% identity, and more preferably at least about 95% identity.

Homology, for polypeptides, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wisconsin 53705. Protein analysis software matches similar sequences using measure of homology assigned to various substitutions, deletions and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

"**Substantially similar function**" refers to the function of a modified nucleic acid or a modified protein, with reference to the wild-type *MMSC2* nucleic acid or wild-type *MMSC2* polypeptide. The modified polypeptide will be substantially homologous to the wild-type *MMSC2* polypeptide and will have substantially the same function. The modified polypeptide may have an altered amino acid sequence and/or may contain modified amino acids. In addition to the similarity of function, the modified polypeptide may have other useful properties, such as a longer half-life. The similarity of function (activity) of the modified polypeptide may be substantially the same as the activity of the wild-type *MMSC2* polypeptide. Alternatively, the similarity of function (activity) of the modified polypeptide may be higher than the activity of the wild-type *MMSC2* polypeptide. The modified polypeptide is synthesized using conventional techniques, or is encoded by a modified nucleic acid and produced using conventional techniques. The modified nucleic acid is prepared by conventional techniques. A nucleic acid with a function substantially similar to the wild-type *MMSC2* gene function produces the modified protein described above.

A polypeptide "**fragment**," "**portion**" or "**segment**" is a stretch of amino acid residues of at least about five to seven contiguous amino acids, often at least about seven to nine contiguous amino acids, typically at least about nine to 13 contiguous amino acids and, most preferably, at least about 20 to 30 or more contiguous amino acids.

The polypeptides of the present invention, if soluble, may be coupled to a solid-phase support, e.g., nitrocellulose, nylon, column packing materials (e.g., Sepharose beads), magnetic beads, glass wool, plastic, metal, polymer gels, cells, or other substrates. Such supports may take the form, for example, of beads, wells, dipsticks, or membranes.

"Target region" refers to a region of the nucleic acid which is amplified and/or detected. The term "target sequence" refers to a sequence with which a probe or primer will form a stable hybrid under desired conditions.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, and immunology. See, e.g., Maniatis *et al.*, 1982; Sambrook *et al.*, 1989; Ausubel *et al.*, 1992; Glover, 1985; Anand, 1992; Guthrie and Fink, 1991. A general discussion of techniques and materials for human gene mapping, including mapping of human chromosome 1, is provided, e.g., in White and Lalouel, 1988.

Preparation of recombinant or chemically synthesized nucleic acids; vectors, transformation, host cells

Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natural or synthetic polynucleotide fragments coding for a desired fragment will be incorporated into recombinant polynucleotide constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian, plant, insect or other eukaryotic cell lines. The purification of nucleic acids produced by the methods of the present invention are described, e.g., in Sambrook *et al.*, 1989 or Ausubel *et al.*, 1992.

The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Carruthers, 1981 or the triester method according to Matteucci and Caruthers, 1981, and may be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding

segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences.

5 Such vectors may be prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Sambrook et al., 1989 or Ausubel et al., 1992.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may include, when appropriate, those naturally associated with the *MMSC2* gene. Examples of workable combinations of cell lines and expression vectors are
10 described in Sambrook et al., 1989 or Ausubel et al., 1992; see also, e.g., Metzger et al., 1988. Many useful vectors are known in the art and may be obtained from such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. Promoters such as the *trp*, *lac* and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include promoter regions for metallothionein, 3-phosphoglycerate kinase
15 or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, enzymes responsible for maltose and galactose utilization, and others. Vectors and promoters suitable for use in yeast expression are further described in Hitzeman et al. (EP 73,675A). Appropriate non-native mammalian promoters might include the early and late promoters from SV40 (Fiers et al., 1978) or promoters derived from murine Molony leukemia virus, mouse
20 tumor virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. Insect promoters may be derived from baculovirus. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1983). See also, e.g.,
25 U.S. Patent No. 5,691,198.

While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence
30 of this gene ensures growth of only those host cells which express the inserts. Typical selection genes encode proteins that a) confer resistance to antibiotics or other toxic substances, e.g. ampicillin, neomycin, methotrexate, etc., b) complement auxotrophic deficiencies, or c) supply

critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art.

The vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the
5 resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al., 1988), or the vectors can be introduced directly into host cells by methods well known in the art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an
10 infectious agent, such as a retroviral genome); and other methods. See generally, Sambrook et al., 1989 and Ausubel et al., 1992. The introduction of the polynucleotides into the host cell by any method known in the art, including, *inter alia*, those described above and in U.S. Patent No. 5,691,198, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

15 Large quantities of the nucleic acids and polypeptides of the present invention may be prepared by expressing the *MMSC2* nucleic acid or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used.

20 Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. Propagation of mammalian cells in culture is *per se* well known. See, Jakoby and Pastan (eds.), 1979. Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines. An
25 example of a commonly used insect cell line is SF9. However, it will be appreciated by the skilled practitioner that other cell lines may be appropriate, e.g., to provide higher expression, desirable glycosylation patterns, or other features.

Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA
30 molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

Prokaryotic or eukaryotic cells transformed with the polynucleotides of the present invention will be useful not only for the production of the nucleic acids and polypeptides of the present invention, but also, for example, in studying the characteristics of MMSC2 polypeptide.

The probes and primers based on the *MMSC2* gene sequence disclosed herein are used to
5 identify homologous *MMSC2* gene sequences and proteins in other species. These gene sequences and proteins are used in the diagnostic/prognostic, therapeutic and drug screening methods described herein for the species from which they have been isolated.

Methods of Use: Drug Screening

10 This invention is particularly useful for screening compounds by using the MMSC2 polypeptide or binding fragment thereof in any of a variety of drug screening techniques, such as those described herein and in published PCT application WO 97/02048. Since MMSC2 acts as a scaffold that binds to MMAC1, the phosphatase substrate(s) and the (probably oncogene) tyrosine kinase(s), a valuable drug candidate will be a drug that can prevent binding of either the
15 substrate(s) or the tyrosine kinase(s) to MMSC2.

The MMSC2 polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, or borne on a cell surface. One method of drug screening utilizes eukaryotic or procaryotic host cells which are stably transformed with recombinant polynucleotides expressing the polypeptide or fragment, preferably in competitive binding
20 assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, for the formation of complexes between a MMSC2 polypeptide or fragment and the agent being tested, or examine the degree to which the formation of a complex between a MMSC2 polypeptide or fragment and a known ligand, e.g., MMAC1, is aided or interfered with by the agent being tested.

25 Thus, the present invention provides methods of screening for drugs comprising contacting such an agent with a MMSC2 polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the MMSC2 polypeptide or fragment, or (ii) for the presence of a complex between the MMSC2 polypeptide or fragment and a ligand, by methods well known in the art. In such competitive binding assays the MMSC2 polypeptide or
30 fragment is typically labeled. Free MMSC2 polypeptide or fragment is separated from that present in a protein:protein complex, and the amount of free (i.e., uncomplexed) label is a measure of the binding of the agent being tested to MMSC2 or its interference with or promotion

of MMSC2:ligand binding, respectively. One may also measure the amount of bound, rather than free, MMSC2. It is also possible to label the ligand rather than the MMSC2 and to measure the amount of ligand binding to MMSC2 in the presence and in the absence of the drug being tested.

5 Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the MMSC2 polypeptides and is described in detail in Geysen (published PCT application WO 84/03564). 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 MMSC2 polypeptide and washed. Bound
10 MMSC2 polypeptide is then detected by methods well known in the art.

Purified MMSC2 can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to the polypeptide can be used to capture antibodies to immobilize the MMSC2 polypeptide on the solid phase.

This invention also contemplates the use of competitive drug screening assays in which
15 neutralizing antibodies capable of specifically binding the MMSC2 polypeptide compete with a test compound for binding to the MMSC2 polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants of the MMSC2 polypeptide.

The above screening methods are not limited to assays employing only MMSC2 but are
20 also applicable to studying MMSC2-protein complexes, e.g., the complex which occurs between MMSC2 and MMAC1. The effect of drugs on the activity of this complex, especially when either the MMSC2 or the MMSC2 binding protein (e.g., MMAC1) contains a mutation, is analyzed.

In accordance with these methods, the following assays are examples of assays which
25 can be used for screening for drug candidates.

A mutant MMSC2 (*per se* or as part of a fusion protein) is mixed with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type MMSC2 binds. This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the mutant MMSC2 with the wild-type protein is measured. If the amount of the binding is
30 more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in *MMSC2*. This assay is useful where the wild-type protein is a tumor suppressor, such as MMAC1.

A wild-type MMSC2 (*per se* or as part of a fusion protein) is mixed with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type MMSC2 binds. This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the wild-type MMSC2 with the wild-type protein is measured. If the amount of the binding is more in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in *MMSC2*. This assay is useful where the wild-type protein is a tumor suppressor, such as *MMAC1*.

A mutant MMSC2 (*per se* or as part of a fusion protein) is mixed with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type MMSC2 binds. This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the mutant MMSC2 with the wild-type protein is measured. If the amount of the binding is less in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in *MMSC2*. This assay is useful if the protein is an oncoprotein or a substrate of the oncoprotein.

A wild-type MMSC2 (*per se* or as part of a fusion protein) is mixed with a wild-type protein (*per se* or as part of a fusion protein) to which wild-type MMSC2 binds. This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the wild-type MMSC2 with the wild-type protein is measured. If the amount of the binding is less in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in *MMSC2* or a cancer resulting from a mutation in *MMAC1*. This assay is useful if the protein is an oncoprotein or a substrate of the oncoprotein.

A mutant protein, which as a wild-type protein binds to MMSC2 (*per se* or as part of a fusion protein) is mixed with a wild-type MMSC2 (*per se* or as part of a fusion protein). This mixing is performed in both the presence of a drug and the absence of the drug, and the amount of binding of the mutant protein with the wild-type MMSC2 is measured. If the amount of the binding is less in the presence of said drug than in the absence of said drug, the drug is a drug candidate for treating cancer resulting from a mutation in the gene encoding the protein.

The polypeptide of the invention may also be used for screening compounds developed as a result of combinatorial library technology. Combinatorial library technology provides an efficient way of testing a potential vast number of different substances for ability to modulate activity of a polypeptide. Such libraries and their use are known in the art. The use of peptide libraries is preferred. See, for example, WO 97/02048.

Briefly, a method of screening for a substance which modulates activity of a polypeptide may include contacting one or more test substances with the polypeptide in a suitable reaction medium, testing the activity of the treated polypeptide and comparing that activity with the activity of the polypeptide in comparable reaction medium untreated with the test substance or substances. A difference in activity between the treated and untreated polypeptides is indicative of a modulating effect of the relevant test substance or substances.

Prior to or as well as being screened for modulation of activity, test substances may be screened for ability to interact with the polypeptide, e.g., in a yeast two-hybrid system (e.g., Bartel et al., 1993). This system may be used as a coarse screen prior to testing a substance for actual ability to modulate activity of the polypeptide. Alternatively, the screen could be used to screen test substances for binding to a MMSC2 specific binding partner, such as MMAC1, or to find mimetics of the MMSC2 polypeptide.

Following identification of a substance which modulates or affects polypeptide activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e., manufacture or formulation, or a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

Thus, the present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of polypeptide activity, in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a method comprising administration of such a composition comprising such a substance, a method comprising administration of such a composition to a patient, e.g., for treatment (which may include preventative treatment) of cancer, use of such a substance in the manufacture of a composition for administration, e.g., for treatment of cancer, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients. A substance identified using as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many in vivo pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use.

The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is

unsuitable for a particular method of administration, e.g., peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large numbers of molecules for a target property.

- 5 There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g., by substituting each residue in turn. Alanine scans of peptide are commonly used to refine such peptide motifs.
- 10 These parts or residues constituting the active region of the compound are known as its "pharmacophore".

 Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g., stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g., spectroscopic techniques, x-ray diffraction data and NMR. Computational

15 analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

 In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner

20 change conformation on binding, allowing the model to take account of this in the design of the mimetic.

 A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted onto it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be

25 pharmacologically acceptable, and does not degrade in vivo, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimization or modification can then be

30 carried out to arrive at one or more final mimetics for in vivo or clinical testing.

Methods of Use: Nucleic Acid Diagnosis and Diagnostic Kits

In order to detect the presence of a *MMSC2* allele predisposing an individual to cancer, a biological sample such as blood is prepared and analyzed for the presence or absence of susceptibility alleles of *MMSC2*. In order to detect the presence of cancer or as a prognostic indicator, a biological sample is prepared and analyzed for the presence or absence of mutant alleles of *MMSC2*. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic laboratories, or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

Initially, the screening method involves amplification of the relevant *MMSC2* sequences. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such screening methods include two-step label amplification methodologies that are well known in the art. Both PCR and non-PCR based screening strategies can detect target sequences with a high level of sensitivity.

The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is the polymerase chain reaction (PCR). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes.

When the probes are used to detect the presence of the target sequences the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence, e.g., denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely

complementary to the targeted region for *MMSC2*. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. These factors are outlined in, for example, Maniatis et al., 1982 and Sambrook et al., 1989. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadraplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies, gold nanoparticles and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these variations are reviewed in, e.g., Matthews and Kricka, 1988; Landegren et al., 1988; U.S. Patent 4,868,105; and in EP 225,807A.

As noted above, non-PCR based screening assays are also contemplated in this invention. This procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output resulting in a 10^3 - 10^6 increase in sensitivity. For an example relating to the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes, see Jablonski et al., 1986.

Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic

acid probe capable of specifically binding *MMSC2*. Allele specific probes are also contemplated within the scope of this example and exemplary allele specific probes include probes encompassing the predisposing mutations of this disclosure.

In one example, the small ligand attached to the nucleic acid probe is specifically
5 recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. For methods for labeling nucleic acid probes according to this embodiment see Martin et al., 1990. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of
10 specifically complexing to the first ligand. A well known embodiment of this example is the biotin-avidin type of interactions. For methods for labeling nucleic acid probes and their use in biotin-avidin based assays see Rigby et al., 1977 and Nguyen et al., 1992.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes capable of detecting
15 *MMSC2*. Thus, in one example to detect the presence of *MMSC2* in a cell sample, more than one probe complementary to the gene is employed and in particular the number of different probes is alternatively two, three, or five different nucleic acid probe sequences. In another example, to detect the presence of mutations in the *MMSC2* gene sequence in a patient, more than one probe complementary to these genes is employed where the cocktail includes probes
20 capable of binding to the allele-specific mutations identified in populations of patients with alterations in *MMSC2*. In this embodiment, any number of probes can be used, and will preferably include probes corresponding to the major gene mutations identified as predisposing an individual to cancer.

25 Methods of Use: Peptide Diagnosis and Diagnostic Kits

The presence of cancer can also be detected on the basis of the alteration of wild-type *MMSC2* polypeptide. Such alterations can be determined by sequence analysis in accordance with conventional techniques. More preferably, antibodies (polyclonal or monoclonal) are used to detect differences in, or the absence of *MMSC2* peptides. Techniques for raising and
30 purifying antibodies are well known in the art and any such techniques may be chosen to achieve the preparations claimed in this invention. In a preferred embodiment of the invention, antibodies will immunoprecipitate *MMSC2* proteins from solution as well as react with these

proteins on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, antibodies will detect MMSC2 proteins in paraffin or frozen tissue sections, using immunocytochemical techniques.

Preferred embodiments relating to methods for detecting MMSC2 or its mutations include enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies. Exemplary sandwich assays are described by David et al., in U.S. Patent Nos. 4,376,110 and 4,486,530, hereby incorporated by reference.

Alternatively, alterations in the MMSC2 sequence can be determined by detecting alterations in the interaction of MMSC2 with MMAC1 or the C-terminus of MMAC1. Wild-type MMAC1 or its C-terminus can be bound to a solid phase and the interaction with MMSC2 assayed by conventional techniques. Analogously, alterations in MMAC1 which affect its interaction with MMSC2 can be detected using wild-type MMSC2 or its PDZ domain which interacts with MMAC1 bound to a solid phase.

Methods of Use: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide *in vivo*. See, e.g., Hodgson, 1991. In one approach, one first determines the three-dimensional structure of a protein of interest (e.g., MMSC2 polypeptide) by x-ray crystallography, by computer modeling or most typically, by a combination of approaches. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. An example of rational drug design is the development of HIV protease inhibitors (Erickson *et al.*, 1990). In addition, peptides (e.g., MMSC2 polypeptide) are analyzed by an alanine scan (Wells, 1991). In this technique, an amino acid residue is replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is analyzed in this manner to determine the important regions of the peptide.

It is also possible to isolate a target-specific antibody, selected by a functional assay, and then to solve its crystal structure. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacore.

Thus, one may design drugs which have, e.g., improved *MMSC2* polypeptide activity or stability or which act as inhibitors, agonists, antagonists, etc. of *MMSC2* polypeptide activity. By virtue of the availability of cloned *MMSC2* sequence, sufficient amounts of the *MMSC2* polypeptide may be made available to perform such analytical studies as x-ray crystallography. In addition, the knowledge of the *MMSC2* protein sequence provided herein will guide those employing computer modeling techniques in place of, or in addition to x-ray crystallography.

Methods of Use: Gene Therapy

According to the present invention, a method is also provided of supplying wild-type *MMSC2* function to a cell which carries a mutant *MMSC2* allele. Supplying such a function should allow normal functioning of the recipient cells. The wild-type gene or a part of the gene may be introduced into the cell in a vector such that the gene remains extrachromosomal. In such a situation, the gene will be expressed by the cell from the extrachromosomal location. More preferred is the situation where the wild-type gene or a part thereof is introduced into the mutant cell in such a way that it recombines with the endogenous mutant gene present in the cell. Such recombination requires a double recombination event which results in the correction of the gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation and viral transduction are known in the art, and the choice of method is within the competence of the practitioner.

As generally discussed above, the *MMSC2* gene or fragment, where applicable, may be employed in gene therapy methods in order to increase the amount of the expression products of such gene in cells. It may also be useful to increase the level of expression of the *MMSC2* gene

even in those persons in which the mutant gene is expressed at a "normal" level, but the gene product is not fully functional.

Gene therapy would be carried out according to generally accepted methods, for example, as described by Friedman (1991) or Culver (1996). Cells from a patient would be first analyzed
5 by the diagnostic methods described above, to ascertain the production of MMSC2 polypeptide in the cells. A virus or plasmid vector (see further details below), containing a copy of the *MMSC2* gene linked to expression control elements, is prepared. The vector may be capable of replicating inside the cells. Alternatively, the vector may be replication deficient and is replicated in helper cells for use in gene therapy. Suitable vectors are known, such as disclosed
10 in U.S. Patent 5,252,479, published PCT application WO 93/07282 and U.S. Patent No. 5,691,198. The vector is then injected into the patient. If the transfected gene is not permanently incorporated into the genome of each of the targeted cells, the treatment may have to be repeated periodically.

Gene transfer systems known in the art may be useful in the practice of the gene therapy
15 methods of the present invention. These include viral and nonviral transfer methods. A number of viruses have been used as gene transfer vectors or as the basis for preparing gene transfer vectors, including papovaviruses (e.g., SV40, Madzak et al., 1992), adenovirus (Berkner, 1992; Berkner et al., 1988; Gorziglia and Kapikian, 1992; Quantin et al., 1992; Rosenfeld et al., 1992; Wilkinson et al., 1992; Stratford-Perricaudet et al., 1990; Schneider et al., 1998), vaccinia virus
20 (Moss, 1992), adeno-associated virus (Muzyczka, 1992; Ohi et al., 1990; Russell & Hirata, 1998), herpesviruses including HSV and EBV (Margolskee, 1992; Johnson et al., 1992; Fink et al., 1992; Breakfield and Geller, 1987; Freese et al., 1990; Fink et al., 1996), lentiviruses (Naldini et al., 1996), vaccinia virus (Moss, 1996), Sindbis and Semliki Forest virus (Berglund et al., 1993), and retroviruses of avian (Bradyopadhyay and Temin, 1984; Petropoulos et al.,
25 1992), murine (Miller, 1992; Miller et al., 1985; Sorge et al., 1984; Mann and Baltimore, 1985; Miller et al., 1988), and human origin (Shimada et al., 1991; Helseth et al., 1990; Page et al., 1990; Buchschacher and Panganiban, 1992). Most human gene therapy protocols have been based on disabled murine retroviruses, although adenovirus and adeno-associated virus are also being used.

Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham and van der Eb, 1973; Pellicer et al., 1980); mechanical techniques, for example microinjection (Anderson et al., 1980; Gordon et al., 1980;

Brinster et al., 1981; Constantini and Lacy, 1981); membrane fusion-mediated transfer via liposomes (Felgner et al., 1987; Wang and Huang, 1989; Kaneda et al., 1989; Stewart et al., 1992; Nabel et al., 1990; Lim et al., 1992); and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990; Wu et al., 1991; Zenke et al., 1990; Wu et al., 1989b; Wolff et al., 1991; Wagner et al., 1990; Wagner et al., 1991; Cotten et al., 1990; Curiel et al., 1991a; Curiel et al., 1991b).

In an approach which combines biological and physical gene transfer methods, plasmid DNA of any size is combined with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged. For other techniques for the delivery of adenovirus based vectors Schneider et al. (1998) and U.S. Patent No. 5,691,198.

Liposome/DNA complexes have been shown to be capable of mediating direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer process is nonspecific, localized *in vivo* uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992).

Expression vectors in the context of gene therapy are meant to include those constructs containing sequences sufficient to express a polynucleotide that has been cloned therein. In viral expression vectors, the construct contains viral sequences sufficient to support packaging of the construct. If the polynucleotide encodes MMSC2, expression will produce MMSC2. If the polynucleotide encodes an antisense polynucleotide or a ribozyme, expression will produce the antisense polynucleotide or ribozyme. Thus in this context, expression does not require that a protein product be synthesized. In addition to the polynucleotide cloned into the expression vector, the vector also contains a promoter functional in eukaryotic cells. The cloned polynucleotide sequence is under control of this promoter. Suitable eukaryotic promoters include those described above. The expression vector may also include sequences, such as selectable markers and other sequences described herein.

Gene transfer techniques which target DNA directly to brain tissue is preferred. Receptor-mediated gene transfer, for example, is accomplished by the conjugation of DNA (usually in the form of covalently closed supercoiled plasmid) to a protein ligand via polylysine. Ligands are chosen on the basis of the presence of the corresponding ligand receptors on the cell surface of

the target cell/tissue type. These ligand-DNA conjugates can be injected directly into the blood if desired and are directed to the target tissue where receptor binding and internalization of the DNA-protein complex occurs. To overcome the problem of intracellular destruction of DNA, coinfection with adenovirus can be included to disrupt endosome function.

- 5 The therapy is as follows: patients who carry a *MMSC2* susceptibility allele are treated with a gene delivery vehicle such that some or all of their brain precursor cells receive at least one additional copy of a functional normal *MMSC2* allele, respectively. In this step, the treated individuals have reduced risk of cancer to the extent that the effect of the susceptible allele has been countered by the presence of the normal allele.

10

Methods of Use: Peptide Therapy

- Peptides which have *MMSC2* activity can be supplied to cells which carry a mutant or missing *MMSC2* allele. Protein can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors. Alternatively, *MMSC2* polypeptide can be extracted from *MMSC2*-producing mammalian cells. In addition, the techniques of synthetic chemistry can be employed to synthesize *MMSC2* protein. Any of such techniques can provide the preparation of the present invention which comprises the *MMSC2* protein. The preparation is substantially free of other human proteins. This is most readily accomplished by synthesis in a microorganism or *in vitro*.

- 20 Active *MMSC2* molecules can be introduced into cells by microinjection or by use of liposomes, for example. Alternatively, some active molecules may be taken up by cells, actively or by diffusion. Supply of molecules with *MMSC2* activity should lead to inhibition of cancer. Other molecules with *MMSC2* activity (for example, peptides, drugs or organic compounds) may also be used to effect such an inhibition. Modified polypeptides having substantially similar function are also used for peptide therapy.

25

Methods of Use: Transformed Hosts

- Animals for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant *MMSC2* alleles, usually from a second animal species, as well as insertion of disrupted homologous genes. Alternatively, the endogenous *MMSC2* gene of the animals may be disrupted by insertion or deletion mutation or other genetic alterations using conventional

30

techniques (Capecchi, 1989; Valancius and Smithies, 1991; Hasty *et al.*, 1991; Shinkai *et al.*, 1992; Mombaerts *et al.*, 1992; Philpott *et al.*, 1992; Snouwaert *et al.*, 1992; Donehower *et al.*, 1992). After test substances have been administered to the animals, the presence of cancer must be assessed. If the test substance prevents or suppresses the appearance of cancer, then the test substance is a candidate therapeutic agent for treatment of cancer. These animal models provide an extremely important testing vehicle for potential therapeutic products.

Methods of Use: Transgenic/Knockout Animals and Models

In one embodiment of the invention, transgenic animals are produced which contain a functional transgene encoding a functional MMSC2 polypeptide or variants thereof. Transgenic animals expressing MMSC2 transgenes, recombinant cell lines derived from such animals and transgenic embryos may be useful in methods for screening for and identifying agents that induce or repress function of MMSC2. Transgenic animals of the present invention also can be used as models for studying indications such as cancers.

In one embodiment of the invention, a MMSC2 transgene is introduced into a non-human host to produce a transgenic animal expressing a human or murine MMSC2 gene. The transgenic animal is produced by the integration of the transgene into the genome in a manner that permits the expression of the transgene. Methods for producing transgenic animals are generally described by Wagner and Hoppe (U.S. Patent No. 4,873,191; which is incorporated herein by reference), Brinster *et al.* 1985; which is incorporated herein by reference in its entirety) and in "Manipulating the Mouse Embryo; A Laboratory Manual" 2nd edition (eds., Hogan, Beddington, Costantini and Long, Cold Spring Harbor Laboratory Press, 1994; which is incorporated herein by reference in its entirety).

It may be desirable to replace the endogenous MMSC2 by homologous recombination between the transgene and the endogenous gene; or the endogenous gene may be eliminated by deletion as in the preparation of "knock-out" animals. Typically, a MMSC2 gene flanked by genomic sequences is transferred by microinjection into a fertilized egg. The microinjected eggs are implanted into a host female, and the progeny are screened for the expression of the transgene. Transgenic animals may be produced from the fertilized eggs from a number of animals including, but not limited to reptiles, amphibians, birds, mammals, and fish. Within a particularly preferred embodiment, transgenic mice are generated which overexpress MMSC2 or express a mutant form of the polypeptide. Alternatively, the absence of a MMSC2 in "knock-

out" mice permits the study of the effects that loss of MMSC2 protein has on a cell *in vivo*. Knock-out mice also provide a model for the development of MMSC2-related cancers.

Methods for producing knockout animals are generally described by Shastry (1995, 1998) and Osterrieder and Wolf (1998). The production of conditional knockout animals, in which the gene is active until knocked out at the desired time is generally described by Feil et al. (1996), Gagneten et al. (1997) and Lobe and Nagy (1998). Each of these references is incorporated herein by reference.

As noted above, transgenic animals and cell lines derived from such animals may find use in certain testing experiments. In this regard, transgenic animals and cell lines capable of expressing wild-type or mutant MMSC2 may be exposed to test substances. These test substances can be screened for the ability to enhance wild-type MMSC2 expression and or function or impair the expression or function of mutant MMSC2.

Pharmaceutical Compositions and Routes of Administration

The MMSC2 polypeptides, antibodies, peptides and nucleic acids of the present invention can be formulated in pharmaceutical compositions, which are prepared according to conventional pharmaceutical compounding techniques. See, for example, *Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, PA). The composition may contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may comprise, in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral, intrathecal, epineural or parenteral.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and

capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, WO 96/11698.

For parenteral administration, the compound may dissolved in a pharmaceutical carrier and administered as either a solution of a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

The active agent is preferably administered in an therapeutically effective amount. The actual amount administered, and the rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc., is within the responsibility of general practitioners or spealists, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in *Remington's Parmaceutical Sciences*.

Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

Instead of administering these agents directly, they could be produced in the target cell, e.g. in a viral vector such as described above or in a cell based delivery system such as described in U.S. Patent No. 5,550,050 and published PCT application Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635. designed for implantation in a patient. The vector could be targeted to the specific cells to be treated, or it could contain regulatory elements which are more tissue specific to the target cells. The cell based delivery system is designed to be implanted in a patient's body at the desired target site and contains a coding sequence for the active agent. Alternatively, the agent could be administered in a precursor form for conversion to the active form by an

activating agent produced in, or targeted to, the cells to be treated. See for example, EP 425,731A and WO 90/07936.

The identification of the association between the *MMSC2* gene mutations and cancer permits the early presymptomatic screening of individuals to identify those at risk for developing cancer. To identify such individuals, *MMSC2* alleles are screened for mutations either directly or after cloning the alleles. The alleles are tested for the presence of nucleic acid sequence differences from the normal allele using any suitable technique, including but not limited to, one of the following methods: fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded conformation analysis (SSCP), linkage analysis, RNase protection assay, allele specific oligonucleotide (ASO), dot blot analysis and PCR-SSCP analysis. Also useful is the recently developed technique of DNA microchip technology. For example, either (1) the nucleotide sequence of both the cloned alleles and normal *MMSC2* gene or appropriate fragment (coding sequence or genomic sequence) are determined and then compared, or (2) the RNA transcripts of the *MMSC2* gene or gene fragment are hybridized to single stranded whole genomic DNA from an individual to be tested, and the resulting heteroduplex is treated with Ribonuclease A (RNase A) and run on a denaturing gel to detect the location of any mismatches. Two of these methods can be carried out according to the following procedures.

The alleles of the *MMSC2* gene in an individual to be tested are cloned using conventional techniques. For example, a blood sample is obtained from the individual. The genomic DNA isolated from the cells in this sample is partially digested to an average fragment size of approximately 20 kb. Fragments in the range from 18-21 kb are isolated. The resulting fragments are ligated into an appropriate vector. The sequences of the clones are then determined and compared to the normal *MMSC2* gene.

Alternatively, polymerase chain reactions (PCRs) are performed with primer pairs for the 5' region or the exons of the *MMSC2* gene. PCRs can also be performed with primer pairs based on any sequence of the normal *MMSC2* gene. For example, primer pairs for one of the introns can be prepared and utilized. Finally, RT-PCR can also be performed on the mRNA. The amplified products are then analyzed by single stranded conformation polymorphisms (SSCP) using conventional techniques to identify any differences and these are then sequenced and compared to the normal gene sequence.

Individuals can be quickly screened for common *MMSC2* gene variants by amplifying the individual's DNA using suitable primer pairs and analyzing the amplified product, e.g., by dot-blot hybridization using allele-specific oligonucleotide probes.

The second method employs RNase A to assist in the detection of differences between the normal *MMSC2* gene and defective genes. This comparison is performed in steps using small (~500 bp) restriction fragments of the *MMSC2* gene as the probe. First, the *MMSC2* gene is digested with a restriction enzyme(s) that cuts the gene sequence into fragments of approximately 500 bp. These fragments are separated on an electrophoresis gel, purified from the gel and cloned individually, in both orientations, into an SP6 vector (e.g., pSP64 or pSP65). The SP6-based plasmids containing inserts of the *MMSC2* gene fragments are transcribed *in vitro* using the SP6 transcription system, well known in the art, in the presence of [α -³²P]GTP, generating radiolabeled RNA transcripts of both strands of the gene.

Individually, these RNA transcripts are used to form heteroduplexes with the allelic DNA using conventional techniques. Mismatches that occur in the RNA:DNA heteroduplex, owing to sequence differences between the *MMSC2* fragment and the *MMSC2* allele subclone from the individual, result in cleavage in the RNA strand when treated with RNase A. Such mismatches can be the result of point mutations or small deletions in the individual's allele. Cleavage of the RNA strand yields two or more small RNA fragments, which run faster on the denaturing gel than the RNA probe itself.

Any differences which are found, will identify an individual as having a molecular variant of the *MMSC2* gene and the consequent presence of cancer. These variants can take a number of forms. The most severe forms would be frame shift mutations or large deletions which would cause the gene to code for an abnormal protein or one which would significantly alter protein expression. Less severe disruptive mutations would include small in-frame deletions and nonconservative base pair substitutions which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic to an acidic amino acid or vice versa, from a hydrophobic to hydrophilic amino acid or vice versa, or other mutations which would affect secondary or tertiary protein structure. Silent mutations or those resulting in conservative amino acid substitutions would not generally be expected to disrupt protein function.

Genetic testing will enable practitioners to identify individuals at risk for cancer at, or even before, birth. Finally, this invention changes our understanding of the cause and treatment of cancer.

5

EXAMPLES

The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

10

EXAMPLE 1

Identification of *MMSC2*

A yeast two-hybrid assay was performed using conventional techniques, such as described by Fields and Song (1989), Chevray and Nathans (1992), Bartel et al. (1993) and Lee et al. (1995). Sequence encoding the C-terminal 15 amino acids of MMAC1 (NEPFDEDQHTQITKV; SEQ ID NO:4) plus its stop codon was generated using an oligonucleotide synthesizer and was ligated to plasmid pGBT.C such that the coding sequence of *MMAC1* was in-frame with coding sequence for the Gal4p DNA-binding domain. This plasmid construct was introduced into the yeast reporter strain J692 along with a library of activation domain fusion plasmids prepared from human kidney cDNA (Clontech). Transformants were spread onto 20 - 150 mm plates of yeast minimal media lacking leucine, tryptophan, and histidine, and containing 25 mM 3-amino-1,2,4-triazole (Gietz et al., 1995; Bartel and Fields, 1995). After one week incubation at 30°C, yeast colonies were assayed for expression of the *lacZ* reporter gene by beta-galactosidase filter assay (Breen and Naysmyth, 1985). Colonies that both grew in the absence of histidine and were positive for production of beta-galactosidase were chosen for further characterization.

25

The activation domain plasmid was purified from positive colonies by the smash-and-grab technique. These plasmids were introduced into *E. coli* DH10B (Gibco BRL) by electroporation and purified from *E. coli* by the alkaline lysis method. To test for the specificity of the interaction, specific activation domain plasmids were cotransformed into strain J692 with plasmids encoding various DNA-binding domain fusion proteins, including fusions to C-terminal segments of MMAC1 and human lamin C. Transformants from these experiments were assayed for expression of the *HIS3* and *lacZ* reporter genes. Positives that expressed reporter

30

genes with *MMAC1* constructs and not with lamin C constructs encode bona fide MMAC1-interacting proteins. These proteins were identified and characterized by sequence analysis of the insert of the appropriate activation domain plasmid.

Five of the clones encoding bona fide MMAC1-interacting proteins were named PDZBN2B, PDZBN3A, PDZBN5B, PDZBN18D, and pdzk4. Comparison of the sequences of these clones suggested that they were all partial cDNAs derived from the same novel gene. A search of GenBank with these sequences revealed strong sequence similarity with a partial mouse cDNA sequence called 9ORF binding protein 1 (9BP-1)(GenBank Accession # AF000168).

Several rounds of cDNA library screening were required to identify cDNA clones that could be assembled into the full length *MMSC2* sequence. In the first round, a 509 base pair(bp) probe was developed from the 5' end of clone PDZBN2B using primers

9BP-1 F1: AGACAGCAAAGATGACAGTAA (SEQ ID NO:5) and

9BP-1 R4: CTCCTCCTCTTTGTATGGG (SEQ ID NO:6).

This probe was used to screen a human placental cDNA library and a human prostate cDNA library. Two of the informative clones obtained were p118a(placental) and pr63(prostate). A search of GenBank with this additional sequence yielded an additional human EST (GenBank Accession # C75629). For the second round of cDNA library screening, a 202bp probe was developed from the 5' end of this EST using primers

9BP-1 #1: GCTTTTGCCGAAATGGGTTAGT (SEQ ID NO:7) and

9BP-1 #2: GATCGGTCTTTGTTCCCAGCA (SEQ ID NO:8).

This probe was used to screen a human prostate cDNA library; two of the informative clones obtained were clone #10 and clone #3. For the third round of cDNA library screening, a 172bp probe was developed from the 5' end of clone #3 using primers

9BP-1 #5: TGTGAGCAAGTTTAGTGAG (SEQ ID NO:9) and

9BP-1 #7: GGTGATTTTCCCAAGTAA (SEQ ID NO:10)

and used to screen a human prostate cDNA library. One of the resulting clones, clone # 6, yielded the start codon and part of the 5' UTR, including in-frame upstream stop codons. The nucleotide sequence for *MMSC2* is set forth in SEQ ID NO:2 with the amino acid sequence of the encoded protein set forth in SEQ ID NO:3.

Figure 1 shows a diagram of *MMSC2* indicating the position of ORF and the positions of the 13 PDZ domains. Figure 2 shows a diagram of the key clones used to assemble the full

length *MMSC2* sequence, the probes used to identify those clones, and the relative position of the partially sequenced mouse ortholog 9BP-1 (Accession # AF000168). Within the 5' UTR, the in-frame upstream stop codon at nucleotide 42 demonstrates that the start codon at nucleotide 57 has been correctly identified. The 13 PDZ domains correspond to the amino acids of *MMSC2* as shown in Table 1.

TABLE 1
Sequence Correspondence of 13 PDZ Domains

	<u>Domain Number</u>	<u>Domain Name</u>	<u>Amino Acid Span</u>
10	1	P15	136 - 222
	2	P14	256 - 335
	3	P13	376 - 461
	4	P10	555 - 632
	5	P12	699 - 785
15	6	P11	1007 - 1090
	7	P9	1150 - 1241
	8	P8	1316 - 1398
	9	P7	1439 - 1529
	10	P1	1595 - 1677
20	11	P2	1691 - 1772
	12	P3	1828 - 1913
	13	P4	1953 - 2037

EXAMPLE 2

Identification of *MMSC2*-interacting proteins by two-hybrid analysis

DNA fragments encoding all or portions of *MMSC2* are ligated to a two-hybrid DNA-binding domain vector such as pGBT.C such that the coding sequence of *MMSC2* is in-frame with coding sequence for the Gal4p DNA-binding domain. These DNA fragments may encode specific PDZ domains of *MMSC2* plus the 5 to 10 amino acids N- and C-terminal of each specific PDZ. A plasmid that encodes a DNA-binding domain fusion to a fragment of *MMSC2* PDZ is introduced into the yeast reporter strain (such as J692) along with a library of cDNAs fused to an activation domain. Transformants are spread onto 20 - 150 mm plates of selective media, such as yeast minimal media lacking leucine, tryptophan, and histidine, and containing 25 mM 3-amino-1,2,4-triazole. After one week incubation at 30° C, yeast colonies are assayed for expression of the *lacZ* reporter gene by beta-galactosidase filter assay. Colonies that both

grow in the absence of histidine and are positive for production of beta-galactosidase are chosen for further characterization.

The activation domain plasmid is purified from positive colonies by the smash-and-grab technique. These plasmids are introduced into *E. coli* (e.g., DH10B (Gibco BRL)) by electroporation and purified from *E. coli* by the alkaline lysis method. To test for the specificity of the interaction, specific activation domain plasmids are cotransformed into strain J692 with plasmids encoding various DNA-binding domain fusion proteins, including fusions to segments of MMSC2 and human lamin C. Transformants from these experiments are assayed for expression of the *HIS3* and *lacZ* reporter genes. Positives that express reporter genes with MMSC2 constructs and not with lamin C constructs encode bona fide MMSC2-interacting proteins. These proteins are identified and characterized by sequence analysis of the insert of the appropriate activation domain plasmid.

EXAMPLE 3

Characterization of the Binding Specificity of MMSC2 PDZ Domains by Two-Hybrid Analysis

DNA fragments encoding specific PDZ domains of MMSC2 plus the 5 to 10 amino acids N- and C-terminal of each specific PDZ domain are generated by PCR amplification. These fragments are ligated to a two-hybrid DNA-binding domain vector such as pGBT.C such that the coding sequence of MMSC2 is in-frame with coding sequence for the Gal4p DNA-binding domain. An activation domain library is prepared that encodes an activation domain fused in-frame to random peptide sequences that end with a stop codon. An example of this type of library is the Clontech random peptide library. A plasmid that encodes a DNA-binding domain fusion to a specific MMSC2 PDZ domain is introduced into the yeast reporter strain (such as J692) along with a library of random peptides fused to an activation domain. Transformants are spread onto 20 - 150 mm plates of selective media, such as yeast minimal media lacking leucine, tryptophan, and histidine, and containing 25 mM 3-amino-1,2,4-triazole. After one week incubation at 30° C, yeast colonies are assayed for expression of the *lacZ* reporter gene by beta-galactosidase filter assay. Colonies that both grow in the absence of histidine and are positive for production of beta-galactosidase are chosen for sequence analysis. The insert of the activation domain construct is characterized by sequence analysis. The sequence of the peptide that binds to the MMSC2 PDZ domain is obtained by conceptual translation of the nucleotide

sequence. Peptide sequences from multiple isolates are aligned to determine a consensus binding motif. This motif can be used to identify cellular proteins that bind MMSC2 and to develop small molecules that inhibit binding to these specific PDZ domains.

5

EXAMPLE 4

In vitro Protein-Protein Interaction Assay

cDNAs encoding each of the MMSC2 PDZ domains (amino acid residues identified in Table 1), and any desired control proteins, were generated by PCR and subcloned as glutathione S-transferase (GST) fusions in pGEX vectors (Pharmacia). After sequencing to confirm expression construct integrity, the resulting clones were expressed in *E. coli* and the desired fusion proteins isolated with glutathione-agarose and recovered with glutathione elution. These fusion proteins or control proteins were then adsorbed to different wells of a 96-well ELISA plate and remaining sites blocked with BSA. Synthetic commercially synthesized peptides encoding the desired PDZ-binding domain (i.e., the 16 C-terminal amino acids of MMAC1, or the C-terminal peptide sequences of interacting proteins identified by the approach of Example 2, or the C-terminal peptide sequences identified by the approach of Example 3), or a control peptide, and biotinylated at the amino-terminus, were pre-bound to streptavidin-alkaline phosphatase in a 4:1 molar ratio. The biotinylated peptide streptavidin-alkaline phosphatase complexes were then blocked with free biotin. These pre-bound peptide streptavidin-alkaline phosphatase complexes were then incubated with the immobilized PDZ domains in wash buffer containing PBS, BSA and triton-X100. Unbound material was removed with repeated washes. Bound peptide/streptavidin-alkaline phosphatase complex was then quantitated by a colorimetric phosphatase assay read on a 96-well plate reader. The following peptides were used in the initial study:

25

SH3 binding peptide: biotin-SGSGILAPPVPPRNTR-COOH (SEQ ID NO:11)

MMAC1.388-403: biotin-ENEPFDEDQHTQITKV-COOH (SEQ ID NO:12).

The relative affinity of each of the 13 PDZ domains encoded by MMSC2 for an MMAC1 C-terminal PDZ peptide as measured by an ELISA assay is set forth in Table 2.

TABLE 2

PDZ Binding Assay

<u>Domain Number</u>	<u>Domain Name</u>	<u>Peptide</u>	<u>A405</u>
5	1	P15	MMAC1
		SH3	0.011
	2	P14	MMAC1
		SH3	0.007
	3	P13	MMAC1
		SH3	0.001
10	4	P10	MMAC1
		SH3	0.006
	5	P12	MMAC1
		SH3	0.006
15	6	P11	MMAC1
		SH3	0.008
	7	P9	MMAC1
		SH3	0.024
	8	P8	MMAC1
		SH3	0.010
20	9	P7	MMAC1
		SH3	0.000
	10	P1	MMAC1
		SH3	0.005
25	11	P2	MMAC1
		SH3	0.016
	12	P3	MMAC1
		SH3	0.017
30	13	P4	MMAC1
		SH3	0.667
			SH3
			0.006
			MMAC1
			0.008
			SH3
			0.009
			MMAC1
			0.017
			SH3
			0.009
			MMAC1
			0.174
			SH3
			0.002
			MMAC1
			0.012
			SH3
			0.009
			MMAC1
			0.003
			SH3
			0.011
			MMAC1
			0.456
			SH3
			0.006

The GST-affinity pull down assay is a complementary *in vitro* method for investigating protein-protein interactions. PDZ domain-GST fusion proteins are incubated with synthetic biotinylated peptides in wash buffer (these peptides were described above). Streptavidin magnetic beads are then added to recover the biotinylated peptide, then unbound material removed by washing. The beads are then incubated with SDS/DTT loading buffer at 100° C and bound protein detected by SDS/PAGE and coomassie blue staining.

EXAMPLE 5

Mutation screening of MMSC2

Nested PCR amplifications were performed on cDNA from tumor cell lines. Total cell line RNAs were reverse transcribed with Superscript II (Life Technologies) and random

hexamers. Using the outer primer pair from each amplicon (i.e. 9BP.1A and 9BP.1P or 9BP.2A and 9BP.2P), approximately 10 ng of cDNA from each cell line was amplified for 26 cycles. Products were diluted 60 fold and then reamplified for 22-26 cycles using nested M13 tailed primers (i.e. 9BP.1B and 9BP.1Q or 9BP.2B and 9BP.2Q). Typical primary amplification
5 cycling conditions were an initial denaturation at 95° C for 60s, followed by 26 cycles of 96° C (12s), 58° C (15s) and 72° C (90s). Typical secondary amplification cycling conditions were an initial denaturation at 95° C for 60s, followed by 22-26 cycles of 96° C (12s), 58° C (15s) and 72° C (40s). The resulting RT-PCR products were sequenced with dye-primer chemistry on ABI
10 Sequencer.

The primers used are set forth in Table 3. The sequence variants are set forth in Table 4.

TABLE 3
Table of Primers

Primer	SEQ ID	Sequence
9BP.1A	13	GCCACCGCGGGATTAAGTTTCT
9BP.1P	14	TGTAGCCAGCAATGGTAATTCCT
9BP.1B	15	GTTTTCCCAGTCACGACGGTTCCATTTTAATTGCTGTTAAT
9BP.1Q	16	AGGAAACAGCTATGACCATGGGGATAATAAAAACGATTCATTT
9BP.1C	17	GTTTTCCCAGTCACGACGTTGAATATGCCCCACGTTCCCTC
9BP.1R	18	AGGAAACAGCTATGACCATTCTTTCAATCTTCCATCTCTATG
9BP.1D	19	GTTTTCCCAGTCACGACGAACAGAGGAGAGCTGGGAATA
9BP.1S	20	AGGAAACAGCTATGACCATCAAACCAGATCCATCATTACC
9BP.1E	21	GTTTTCCCAGTCACGACGGCACAATTTAGCTCACTCTAA
9BP.1T	22	AGGAAACAGCTATGACCATGGATGAGGAGAGGGTGATGC
9BP.2A	23	TCTAGCAGGAATGAGCAGTGAG
9BP.2P	24	GATCCTGATAATCTAAAATGCTAA
9BP.2B	25	GTTTTCCCAGTCACGACGAAGTTGATGATTGCAAGAGGTG
9BP.2Q	26	AGGAAACAGCTATGACCATGGTTTGTGCCATCTACTGCTAT
9BP.2C	27	GTTTTCCCAGTCACGACGAAAGCAGTGCCGTTGAGCATG
9BP.2R	28	AGGAAACAGCTATGACCATGCTGACAGTAATGGATACCTT
9BP.2D	29	GTTTTCCCAGTCACGACGGATTTTTTATCTTCGACGAGAAA
9BP.2S	30	AGGAAACAGCTATGACCATTCCCCAAGTAAAGTTATGCCAT

9BP.2E	31	GTTTTCCCAGTCACGACGTCCTGTTGGACACAGCGGGA
9BP.2T	32	AGGAAACAGCTATGACCATCATGGCCAAAGGTGCTTGAA
9BP.3A	33	CCACCCACCACCCAATCAGAAT
9BP.3P	34	CATCTCGACTAATGGCACCTCC
9BP.3B	35	GTTTTCCCAGTCACGACGGAGACAGAGGATCCAGTGCT
9BP.3Q	36	AGGAAACAGCTATGACCATCCCTGACGGTGCTCCCTTCA
9BP.3C	37	GTTTTCCCAGTCACGACGTTAACTTGGAAAACAGCAGTCT
9BP.3R	38	AGGAAACAGCTATGACCATCATCACCACAAGAACTGCCATG
9BP.3D	39	GTTTTCCCAGTCACGACGACTCTCCTGAAAATGACAGCAT
9BP.3S	40	AGGAAACAGCTATGACCATTAAATGAGATTGAGTCCACACT
9BP.3E	41	GTTTTCCCAGTCACGACGATAAATGACTACACACCTGCAA
9BP.3T	42	AGGAAACAGCTATGACCATAACGATCATCCCCAAGCCATCT
9BP.4A	43	CTGAGTACCTGCTTGAACAGAG
9BP.4P	44	GACCATTGATCTCTAGAAGCTC
9BP.4B	45	GTTTTCCCAGTCACGACGGGACTATTAATATAGCAAAAGGC
9BP.4Q	46	AGGAAACAGCTATGACCATCAGTGCCATTACTCTTCCAGA
9BP.4C	47	GTTTTCCCAGTCACGACGTACTTATGTGCCTGCAGAACA
9BP.4R	48	AGGAAACAGCTATGACCATCATGTTTGATGAAAATGCCCC
9BP.4D	49	GTTTTCCCAGTCACGACGATTGTTGGTGGACGAGGGATG
9BP.4S	50	AGGAAACAGCTATGACCATCCATTTTCGGCAAAGGCTGAAG
9BP.4E	51	GTTTTCCCAGTCACGACGCAGAGTCAGAGCCAGAGAAGG
9BP.4T	52	AGGAAACAGCTATGACCATAGAAGCTCATCTGCAATTTGC
9BP.5A	53	CAGGCGAGCTGCATATGATTG
9BP.5P	54	CCTCCTTTGACAATGTCTGACAC
9BP.5B	55	GTTTTCCCAGTCACGACGGTGTCTTCATAGTGGGGATTGAT
9BP.5Q	56	AGGAAACAGCTATGACCATGAAGCTCCAGATGTTGCACAT
9BP.5C	57	GTTTTCCCAGTCACGACGAGAGCCAACTGTTACTACTTC
9BP.5R	58	AGGAAACAGCTATGACCATTGAAGGAACAGCCTGGGAATC
9BP.5D	59	GTTTTCCCAGTCACGACGTTAGCCTTCTGAAGACAGCAA
9BP.5S	60	AGGAAACAGCTATGACCATCATGGATAATAATGGCACCCA
9BP.5E	61	GTTTTCCCAGTCACGACGTTTCCAAAGGGCGAACAGGGC
9BP.5T	62	AGGAAACAGCTATGACCATCCAACAATACTTAATCCTAGGC
9BP.6A	63	TGGAATTGACTTGAGAAAGGCCA
9BP.6P	64	CCCCCTACAGTTTTGAAGACCC
9BP.6B	65	GTTTTCCCAGTCACGACGAAGAGGAGGAAGTGTGTGACAC

9BP.6Q	66	AGGAAACAGCTATGACCATGACAGGCTGCCTTCACTCACC
9BP.6C	67	GTTTTCCCAGTCACGACGTCAAAGCTGGTCCATTCCATT
9BP.6R	68	AGGAAACAGCTATGACCATGGATGTGCCACAGATGGTGAC
9BP.6D	69	GTTTTCCCAGTCACGACGATGATGCACCCAACTGGAGTT
9BP.6S	70	AGGAAACAGCTATGACCATGGCTGCCATATCCTCCAACATA
9BP.6E	71	GTTTTCCCAGTCACGACGGGACCTCCTCAATGTAAGTCT
9BP.6T	72	AGGAAACAGCTATGACCATATTGTGACGACCAAGTGCATTC

TABLE 4

Sequence Variants

5	<u>Cell line</u>	<u>Type</u>	<u>nt variant</u>	<u>aa change</u>	<u>note</u>
	LNCAP.FGC	prostatic	G163A	val->ile	heterozygous
	OV-1063	ovarian	G343T	gly->trp	non-het*
	UACC812	breast	A1074G	thr->thr	heterozygous
	UACC8933	breast	G5624A	arg->lys	non-het*
10	HS776T	pancreatic	G5624A	arg->lys	non-het*

* In mutation screening from cDNA, a non-heterozygous call for a sequence variant is consistent with non-expression of one allele, hemizyosity at that position, or homozygosity at that position.

15

EXAMPLE 6

Generation of Polyclonal Antibody Against MMSC2

Segments of *MMSC2* coding sequence are expressed as fusion protein in *E. coli*. The overexpressed protein is purified by gel elution and used to immunize rabbits and mice using a procedure similar to the one described by Harlow and Lane, 1988. This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

Briefly, a stretch of *MMSC2* coding sequence is cloned as a fusion protein in plasmid PET5A (Novagen, Inc., Madison, WI). After induction with IPTG, the overexpression of a fusion protein with the expected molecular weight is verified by SDS/PAGE. Fusion protein is purified from the gel by electroelution. Identification of the protein as the *MMSC2* fusion product is verified by protein sequencing at the N-terminus. Next, the purified protein is used as immunogen in rabbits. Rabbits are immunized with 100 µg of the protein in complete Freund's adjuvant and boosted twice in 3 week intervals, first with 100 µg of immunogen in incomplete

Freund's adjuvant followed by 100 µg of immunogen in PBS. Antibody containing serum is collected two weeks thereafter. This procedure is repeated to generate antibodies against the mutant forms of the *MMSC2* gene product. These antibodies, in conjunction with antibodies to wild type *MMSC2*, are used to detect the presence and the relative level of the mutant forms in various tissues and biological fluids.

EXAMPLE 7

Generation of Polyclonal Antibody Against MMSC2-MMSC2 Interacting Protein Complex

MMSC2 is capable of binding to certain proteins, e.g., *MMAC1*. A complex of the two proteins is prepared, e.g., by mixing purified preparations of each of the two proteins. If desired, the protein complex can be stabilized by cross-linking the proteins in the complex by methods known to those of skill in the art. The protein complex is used to immunize rabbits and mice using a procedure similar to the one described by Harlow and Lane, 1988. This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

Briefly, the purified protein complex is used as immunogen in rabbits. Rabbits are immunized with 100 µg of the protein in complete Freund's adjuvant and boosted twice in 3 week intervals, first with 100 µg of immunogen in incomplete Freund's adjuvant followed by 100 µg of immunogen in PBS. Antibody containing serum is collected two weeks thereafter.

This procedure is repeated to generate antibodies against forms of the complex which comprise mutant *MMSC2* or mutant *MMSC2* interacting protein (e.g., mutant *MMAC1*). These antibodies, in conjunction with antibodies to wild type *MMSC2* or *MMSC2* interacting protein (e.g., *MMAC1*), are used to detect the presence and the relative level of the mutant forms in various tissues and biological fluids.

EXAMPLE 8

Generation of Monoclonal Antibodies Specific for MMSC2

Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising intact *MMSC2* or *MMSC2* peptides (wild type or mutant) conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known.

The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 µg of immunogen and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

Spleens are removed from immune mice and a single cell suspension is prepared (see Harlow and Lane, 1988). Cell fusions are performed essentially as described by Kohler and Milstein, 1975. Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) are fused with immune spleen cells using polyethylene glycol as described by Harlow and Lane, 1988. Cells are plated at a density of 2×10^5 cells/well in 96 well tissue culture plates. Individual wells are examined for growth and the supernatants of wells with growth are tested for the presence of MMSC2 specific antibodies by ELISA or RIA using wild type or mutant MMSC2 target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibody for characterization and assay development.

EXAMPLE 9

Generation of Monoclonal Antibodies Specific for MMSC2-MMSC2 Interacting Protein Complex

Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising MMSC2-MMSC2 interacting protein complexes (wild type or mutant), such as MMAC1, conjugated to keyhole limpet hemocyanin using glutaraldehyde or EDC as is well known. The complexes may be stabilized by cross-linking.

The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 µg of immunogen and after the fourth injection blood samples are taken from the mice to determine if the serum contains antibody to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

Spleens are removed from immune mice and a single cell suspension is prepared (see Harlow and Lane, 1988). Cell fusions are performed essentially as described by Kohler and

Milstein, 1975. Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, MD) are fused with immune spleen cells using polyethylene glycol as described by Harlow and Lane, 1988. Cells are plated at a density of 2×10^5 cells/well in 96 well tissue culture plates. Individual wells are examined for growth and the supernatants of wells with growth are tested for the presence of MMSC2-MMSC2 interacting protein complex specific antibodies by ELISA or RIA using wild type or mutant MMSC2-MMSC2 interacting protein complexes as target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibody for characterization and assay development. Antibodies are tested for binding to MMSC2 alone or to MMSC2 interacting protein alone to determine which are specific for the complex as opposed to binding to the individual proteins.

EXAMPLE 10

Sandwich Assay for MMSC2

Monoclonal antibody is attached to a solid surface such as a plate, tube, bead or particle. Preferably, the antibody is attached to the well surface of a 96-well ELISA plate. 100 μ L sample (e.g., serum, urine, tissue cytosol) containing the MMSC2 peptide/protein (wild-type or mutants) is added to the solid phase antibody. The sample is incubated for 2 hrs at room temperature. Next the sample fluid is decanted, and the solid phase is washed with buffer to remove unbound material. 100 μ L of a second monoclonal antibody (to a different determinant on the MMSC2 peptide/protein) is added to the solid phase. This antibody is labeled with a detector molecule (e.g., ^{125} I, enzyme, fluorophore, or a chromophore) and the solid phase with the second antibody is incubated for two hrs at room temperature. The second antibody is decanted and the solid phase is washed with buffer to remove unbound material.

The amount of bound label, which is proportional to the amount of MMSC2 peptide/protein present in the sample, is quantified. Separate assays are performed using monoclonal antibodies which are specific for the wild-type MMSC2 as well as monoclonal antibodies specific for each of the mutations identified in MMSC2.

EXAMPLE 11

Sandwich Assay for MMAC1 Using MMSC2

MMSC2 or PDZ domain 7 of MMSC2 is attached to a solid surface such as a plate, tube, bead or particle. Preferably, MMSC2 or its PDZ domain is attached to the well surface of a 96-well ELISA plate. 100 μ L sample (e.g., serum, urine, tissue cytosol) containing the MMAC1 peptide/protein (wild-type or mutants) is added to the solid phase MMSC2. The sample is incubated for 2 hrs at room temperature. Next the sample fluid is decanted, and the solid phase is washed with buffer to remove unbound material. 100 μ L of a monoclonal antibody to MMAC1 is added to the solid phase. The antibody is labeled with a detector molecule (e.g., ^{125} I, enzyme, fluorophore, or a chromophore) and the solid phase with the antibody is incubated for two hrs at room temperature. The antibody is decanted and the solid phase is washed with buffer to remove unbound material. The amount of bound label, which is proportional to the amount of wild-type MMAC1 present in the sample, is quantified.

EXAMPLE 12

Drug Screening

The invention is useful in screening for drugs which can overcome mutations in MMSC2 and also mutations in MMAC1. The knowledge that MMSC2 and MMAC1 form a complex is useful in designing such assays. If a mutation is present in either MMSC2 or in MMAC1 which prevents the MMSC2-MMAC1 complex from forming, drugs may be screened which will overcome the mutation and allow the protein complex to form and to be active. Such screening assays can be, e.g., a yeast two hybrid assay which is dependent upon two proteins interacting. In such an assay, the presence of a mutant protein may show no activity or low activity in such an assay, while the presence of a useful drug will result in formation of a proper complex which results in activity in the assay.

A simple binding assay which shows the binding, i.e., formation of a complex, can similarly be used as outlined above. Useful drugs will increase the formation of MMSC2-MMAC1 complexes. Antibodies may also be used to monitor the amount of complex present. Antibodies specific for the complex are especially useful. If the presence of a drug increases the amount of complex present then the drug is a good candidate for treating the cancer which is a result of the mutation in either the MMSC2 or the MMAC1.

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

LIST OF REFERENCES

- Anand, R. (1992). *Techniques for the Analysis of Complex Genomes*, (Academic Press).
- Anderson, et al. (1980). *Proc. Natl. Acad. Sci. USA* **77**:5399-5403.
- 10 Ausubel, F.M., et al. (1992). *Current Protocols in Molecular Biology*, (J. Wiley and Sons, N.Y.)
- Bartel, P.L. and Fields, S. (1995). *Methods in Enzymology* **254**:241-263.
- Bartel, P.L. et al. (1993). "Using the 2-hybrid system to detect protein-protein interactions." In *Cellular Interactions in Development: A Practical Approach*, Oxford University Press, pp. 153-179.
- 15 Beaucage and Carruthers (1981). *Tetra. Letts.* **22**:1859-1862.
- Berglund, P. et al. (1993) *Biotechnology* **11**:916-920
- Berkner, et al. (1988). *BioTechniques* **6**:616-629.
- Berkner (1992). *Curr. Top. Microbiol. Immunol.* **158**:39-61.
- Borman, S. (1996). *Chemical & Engineering News*, December 9 issue, pp. 42-43.
- 20 Brandyopadhyay and Temin (1984). *Mol. Cell. Biol.* **4**:749-754.
- Breakfield and Geller (1987). *Mol. Neurobiol.* **1**:337-371.
- Breeden, L., and Naysmyth, K. (1985). *Cold Spring Harbor Symp. Quant. Biol.* **50**:643-650.
- Brenman, J.E. et al. (1996). *Cell* **84**:757-767.
- Brinster, et al. (1981). *Cell* **27**:223-231.
- 25 Buchschacher and Panganiban (1992). *J. Virol.* **66**:2731-2739.
- Capecchi, M.R. (1989). *Science* **244**:1288.
- Cariello (1988). *Human Genetics* **42**:726.
- Chee, M., et al. (1996). *Science* **274**:610-614.
- Chevray, P.M. & Nathans, D.N. (1992). *Proc. Natl. Acad. Sci. USA* **89**:5789- 5793.
- 30 Cho, K.O. et al. (1992). *Neuron* **9**:929-942.
- Compton, J. (1991). "Nucleic acid sequence-based amplification." *Nature* **350**:91-92.
- Conner, B.J., et al. (1983). *Proc. Natl. Acad. Sci. USA* **80**:278-282.
- Constantini and Lacy (1981). *Nature* **294**:92-94.
- Cotten, M., et al. (1990). *Proc. Natl. Acad. Sci. USA* **87**:4033-4037.

- Cotton, R.G., et al. (1988). *Proc. Natl. Acad. Sci. USA* **85**:4397-4401.
- Culver, K. (1996). *Gene Therapy: A Primer for Physicians*, 2nd Ed., Mary Ann Liebert.
- Curiel, et al. (1991a). *Hum. Gene Ther.* **3**:147-154.
- Curiel, et al. (1991b). *Proc. Natl. Acad. Sci. USA* **88**:8850-8854.
- 5 Deutscher, M. (1990). *Meth. Enzymology* **182** (Academic Press, San Diego, Cal.).
- Donehower, L.A., et al. (1992). *Nature* **356**:215.
- Editorial (1996). *Nature Genetics* **14**:367-370.
- Elghanian, R., et al. (1997). *Science* **277**:1078-1081.
- Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, Cold Spring Harbor,
 10 New York (1983).
- Erickson, J., et al. (1990). *Science* **249**:527-533.
- Fahy, E., et al. (1991). *PCR Methods Appl.* **1**:25-33.
- Felgner, et al. (1987). *Proc. Natl. Acad. Sci. USA* **84**:7413-7417.
- Fields, S. & Song, O.-K. (1989). *Nature* **340**:245-246.
- 15 Fiers, et al. (1978). *Nature* **273**:113.
- Fink, et al. (1992). *Hum. Gene Ther.* **3**:11-19.
- Fink, D.J. et al. (1996). *Ann. Rev. Neurosci.* **19**:265-287.
- Finkelstein, J., et al. (1990). *Genomics* **7**:167-172.
- Fodor, S.P.A. (1997). DNA Sequencing. Massively Parallel Genomics. *Science* **277**:393-395.
- 20 Freese, et al. (1990). *Biochem. Pharmacol.* **40**:2189-2199.
- Friedman, T. (1991). In *Therapy for Genetic Diseases*, T. Friedman, ed., Oxford University Press, pp. 105-121.
- Furnari, F.B. et al. (1997). *Proc. Natl. Acad. Sci. USA* **94**:12479-12484.
- Gietz, R.D., et al. (1995). *Yeast* **11**:355-360.
- 25 Glover, D. (1985). *DNA Cloning, I and II* (Oxford Press).
- Goding (1986). *Monoclonal Antibodies: Principles and Practice*, 2d ed. (Academic Press, N.Y.).
- Godowski, et al. (1988). *Science* **241**:812-816.
- Gordon, et al. (1980). *Proc. Natl. Acad. Sci. USA* **77**:7380-7384.
- 30 Gorziglia and Kapikian (1992). *J. Virol.* **66**:4407-4412.
- Graham and van der Eb (1973). *Virology* **52**:456-467.
- Grompe, M. (1993). *Nature Genetics* **5**:111-117.
- Grompe, M., et al. (1989). *Proc. Natl. Acad. Sci. USA* **86**:5855-5892.
- Guthrie, G. and Fink, G.R. (1991). *Guide to Yeast Genetics and Molecular Biology* (Academic
 35 Press).

- Hacia, J.G., et al. (1996). *Nature Genetics* **14**:441-447.
- Harlow and Lane (1988). *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
- Harrison, S.C. (1996). *Cell* **86**:341-343.
- 5 Hasty, P., K., et al. (1991). *Nature* **350**:243.
- Helseth, et al. (1990). *J. Virol.* **64**:2416-2420.
- Hodgson, J. (1991). *Bio/Technology* **9**:19-21.
- Huse, et al. (1989). *Science* **246**:1275-1281.
- 10 Innis, et al. (1990). *PCR Protocols: A Guide to Methods and Applications* (Academic Press, San Diego, Cal.).
- Jablonski, E., et al. (1986). *Nucl. Acids Res.* **14**:6115-6128.
- Jakoby, W.B. and Pastan, I.H. (eds.) (1979). *Cell Culture. Methods in Enzymology*, volume 58 (Academic Press, Inc., Harcourt Brace Jovanovich (New York)).
- Johnson, et al. (1992). *J. Virol.* **66**:2952-2965.
- 15 Johnson, et al. (1993). "Peptide Turn Mimetics" in *Biotechnology and Pharmacy*, Pezzuto et al., eds., Chapman and Hall, New York.
- Kaneda, et al. (1989). *J. Biol. Chem.* **264**:12126-12129.
- Kanehisa (1984). *Nucl. Acids Res.* **12**:203-213.
- Kavanaugh, W.M. et al. (1995). *Science* **268**:1177-1179.
- 20 Kennedy, M.B. (1995). *Trends Biochem. Sci.* **20**:350.
- Kinszler, K.W., et al. (1991). *Science* **251**:1366-1370.
- Kohler, G. and Milstein, C. (1975). *Nature* **256**:495-497.
- Kong, D. et al. (1997). *Nature Genetics* **17**:143-144.
- Kornau, H.C. et al. (1995). *Science* **269**:1737-1740.
- 25 Kraemer, F.B. et al. (1993). *J. Lipid Res.* **34**:663-672.
- Kubo, T., et al. (1988). *FEBS Letts.* **241**:119.
- Kyte, J. & Dolittle, R.F. (1982). *J. Mol. Bio.* **157**:105-132.
- Landegren, et al. (1988). *Science* **242**:229.
- Lee, J.E. et al. (1995). *Science* **268**:836-844.
- 30 Lemmon, M.A. et al. (1996). *Cell* **85**:621-624.
- Li, D.M. & Sun, H. (1997). *Cancer Res.* **57**:2124-2129.
- Li, J. et al. (1997). *Science* **275**:1943-1947.
- Liaw, D. et al. (1997). *Nature Genetics* **16**:64-67.
- Lim, et al. (1992). *Circulation* **83**:2007-2011.
- 35 Lipshutz, R.J., et al. (1995). *Biotechniques* **19**:442-447.

- Lockhart, D.J., et al. (1996). *Nature Biotechnology* **14**:1675-1680.
- Louis, D.N. & Gusella, J.F. (1995). *Trends. Genet.* **11**:412-415.
- Madzak, et al. (1992). *J. Gen. Virol.* **73**:1533-1536.
- Maldonado, E., et al. (1996). *Nature* **381**:86-89.
- 5 Maniatis, T. et al. (1982). *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
- Mann and Baltimore (1985). *J. Virol.* **54**:401-407.
- Margolskee (1992). *Curr. Top. Microbiol. Immunol.* **158**:67-90.
- Marsh, D.J. et al. (1997). *Nature Genetics* **16**:333-334.
- 10 Martin, R., et al. (1990). *BioTechniques* **9**:762-768.
- Matteucci, M.D. and Caruthers, M.H. (1981). *J. Am. Chem. Soc.* **103**:3185.
- Matthews and Kricka (1988). *Anal. Biochem.* **169**:1.
- Merrifield (1963). *J. Am. Chem. Soc.* **85**:2149-2156.
- Metzger, et al. (1988). *Nature* **334**:31-36.
- 15 Miller (1992). *Curr. Top. Microbiol. Immunol.* **158**:1-24.
- Miller, et al. (1985). *Mol. Cell. Biol.* **5**:431-437.
- Miller, et al. (1988). *J. Virol.* **62**:4337-4345.
- Modrich, P. (1991). *Ann. Rev. Genet.* **25**:229-253.
- Mombaerts, P., et al. (1992). *Cell* **68**:869.
- 20 Moss (1992). *Curr. Top. Microbiol. Immunol.* **158**:25-38.
- Moss, B. (1996). *Proc. Natl. Acad. Sci. USA* **93**:11341-11348.
- Muzyczka (1992). *Curr. Top. Microbiol. Immunol.* **158**:97-123.
- Nabel, et al. (1990). *Science* **249**:1285-1288.
- Nabel (1992). *Hum. Gene Ther.* **3**:399-410.
- 25 Naldini, L. et al. (1996). *Science* **272**:263-267.
- Nelen, M.R. et al. (1997). *Hum. Mol. Genet.* **6**:1383-1387.
- Newton, C.R., et al. (1989). *Nucl. Acids Res.* **17**:2503-2516.
- Nguyen, Q., et al. (1992). *BioTechniques* **13**:116-123.
- Novack, et al. (1986). *Proc. Natl. Acad. Sci. USA* **83**:586.
- 30 Ohi, et al. (1990). *Gene* **89**:279-282.
- Olschwang, S. et al. (1998). *Nature Genetics* **18**:12-13.
- Orita, M., et al. (1989). *Proc. Natl. Acad. Sci. USA* **86**:2766-2770.
- Page, et al. (1990). *J. Virol.* **64**:5370-5276.
- Pawson, T. (1994). *Adv. Cancer Res.* **64**:87-110.

- Pawson, T. & Scott, J.D. (1997). *Science* **278**:2075-2080.
- Pellicer, et al. (1980). *Science* **209**:1414-1422.
- Petropoulos, et al. (1992). *J. Virol.* **66**:3391-3397.
- Philpott, K.L., et al. (1992). *Science* **256**:1448.
- 5 Quantin, et al. (1992). *Proc. Natl. Acad. Sci. USA* **89**:2581-2584.
- Rano and Kidd (1989). *Nucl. Acids Res.* **17**:8392.
- Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, PA).
- Rigby, P.W.J., et al. (1977). *J. Mol. Biol.* **113**:237-251.
- Rosenfeld, et al. (1992). *Cell* **68**:143-155.
- 10 Russell, D. & Hirata, R. (1998). *Nature Genetics* **18**:323-328.
- Sambrook, J., et al. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
- Sande, S., and Privalsky, M.L. (1996). *Molecular Endocrin.* **10**:813-825.
- Scharf (1986). *Science* **233**:1076.
- 15 Schlessinger, J. (1994). "SH2/SH3 signaling proteins." *Curr. Opin. Genet. Dev.* **4**:25-30.
- Schneider, G. et al. (1998). *Nature Genetics* **18**:180-183.
- Scopes, R. (1982). *Protein Purification: Principles and Practice*, (Springer-Verlag, N.Y.).
- Shaw, G. (1996). *Bioessays* **18**:35-46.
- Sheffield, V.C., et al. (1989). *Proc. Natl. Acad. Sci. USA* **86**:232-236.
- 20 Sheffield, V.C., et al., (1991). *Am. J. Hum. Genet.* **49**:699-706.
- Shenk, T.E., et al. (1975). *Proc. Natl. Acad. Sci. USA* **72**:989-993.
- Shieh, B. & Zhu, M. (1996). *Neuron* **16**:991-998.
- Shimada, et al. (1991). *J. Clin. Invest.* **88**:1043-1047.
- Shinkai, Y., et al. (1992). *Cell* **68**:855.
- 25 Shoemaker, D.D., et al. (1996). *Nature Genetics* **14**:450-456.
- Snouwaert, J.N., et al. (1992). *Science* **257**:1083.
- Songyang, Z. et al. (1997). *Science* **275**:73-77.
- Sorge, et al. (1984). *Mol. Cell. Biol.* **4**:1730-1737.
- Spargo, C.A., et al. (1996). *Mol. Cell. Probes* **10**:247-256.
- 30 Steck, P.A. et al. (1997). *Nature Genetics* **15**:356-362.
- Stewart, et al. (1992). *Hum. Gene Ther.* **3**:267-275.
- Stratford-Perricaudet, et al. (1990). *Hum. Gene Ther.* **1**:241-256.
- Tsunoda, S. et al. (1997). *Nature* **388**:243-249.
- Valancius, V. and Smithies, O. (1991). *Mol. Cell Biol.* **11**:1402.

- van der Greer, P. & Pawson, T. (1995). *Trends Biochem. Sci.* **20**:277-280.
- Wagner, et al. (1991). *Proc. Natl. Acad. Sci. USA* **88**:4255-4259.
- Wagner, et al. (1990). *Proc. Natl. Acad. Sci. USA* **87**:3410-3414.
- Walker, G.T., et al. (1992). *Nucl. Acids Res.* **20**:1691-1696.
- 5 Wang and Huang (1989). *Biochemistry* **28**:9508-9514.
- Wartell, R.M., et al. (1990). *Nucl. Acids Res.* **18**:2699-2705.
- Wells, J.A. (1991). *Methods in Enzymol.* **202**:390-411.
- Wetmur and Davidson (1968). *J. Mol. Biol.* **31**:349-370.
- White, M.B., et al. (1992). *Genomics* **12**:301-306.
- 10 White and Lalouel (1988). *Ann. Rev. Genet.* **22**:259-279.
- Wilkinson, et al. (1992). *Nucleic Acids Res.* **20**:2233-2239.
- Wolff, et al. (1990). *Science* **247**:1465-1468.
- Wolff, et al. (1991). *BioTechniques* **11**:474-485.
- Woods, D.F. & Bryant, P.J. (1991). *Cell* **66**:451-464.
- 15 Woods, D.F. & Bryant, P.J. (1993). *Mech. Dev.* **44**:5-89.
- Wu, et al. (1989a). *Genomics* **4**:560-569.
- Wu, et al. (1989b). *J. Biol. Chem.* **264**:16985-16987.
- Wu, et al. (1991). *J. Biol. Chem.* **266**:14338-14342.
- Zenke, et al. (1990). *Proc. Natl. Acad. Sci. USA* **87**:3655-3659.

20

Patents and Patent Applications:

Geysen, H., published PCT application WO 84/03564, published 13 September 1984

Hitzeman et al., EP 73,675A.

EP 225,807A.

25 EP 332,435A.

EP 425,731 A

U.S. Patent No. 3,817,837

U.S. Patent No. 3,850,752

U.S. Patent No. 3,939,350

30 U.S. Patent No. 3,996,345

U.S. Patent No. 4,275,149

U.S. Patent No. 4,277,437

U.S. Patent No. 4,366,241

U.S. Patent No. 4,376,110.

- U.S. Patent No. 4,486,530.
U.S. Patent No. 4,554,101
U.S. Patent No. 4,683,195
U.S. Patent No. 4,683,202
5 U.S. Patent No. 4,816,567
U.S. Patent No. 4,868,105.
U.S. Patent No. 5,252,479.
U.S. Patent No. 5,270,184.
U.S. Patent No. 5,409,818.
10 U.S. Patent No. 5,455,166.
U.S. Patent No. 5,550,050
U.S. Patent No. 5,691,198
WO 90/07936
WO 92/19195
15 WO 93/07282.
WO 94/25503
WO 95/01203
WO 95/05452
WO 96/02286
20 WO 96/02646
WO 96/40871
WO 96/40959
WO 97/02048
WO 97/12635

WHAT IS CLAIMED IS:

1. An isolated nucleic acid coding for a MMSC2 polypeptide, said polypeptide having the amino acid sequence set forth in SEQ ID NO:3 or a modified form which is functionally equivalent in its interaction with MMAC1.
- 5 2. The isolated nucleic acid of claim 1, wherein said DNA has the nucleotide sequence set forth in SEQ ID NO:2, its complement or a corresponding RNA.
- 10 3. The isolated nucleic acid of claim 1, which is a DNA comprising an allelic variant of the nucleotide sequence set forth in SEQ ID NO:2, its complement or a corresponding RNA.
4. The isolated nucleic acid of claim 1 coding for a mutated form of the MMSC2 polypeptide set forth in SEQ ID NO:3.
- 15 5. The isolated nucleic acid of claim 4, which is a DNA comprising a mutated form of the nucleotide sequence set forth in SEQ ID NO:2, its complement or a corresponding RNA.
- 20 6. The isolated nucleic acid of claim 5, wherein the mutation is selected from the group consisting of a deletion mutation, a nonsense mutation, an insertion mutation, a frameshift mutation and a missense mutation.
- 25 7. An isolated nucleic acid having at least 15 contiguous nucleotides of a nucleic acid as claimed in any one of claims 1 to 6 wherein the nucleic acid sequence suitable for use as a hybridization probe to detect in a sample (i) a DNA having a nucleotide sequence selected from the nucleotide sequence set forth in SEQ ID NO:2, allelic variants thereof and mutated forms thereof or (ii) an RNA corresponding to said DNA.
- 30 8. A set of nucleic acid probes for use in a microchip assay, wherein each of said nucleic acid probes comprises at least 8 contiguous nucleotides of a nucleic acid as claimed in any one of claims 1 to 6 and said set encompasses part or all of said nucleic acid.

9. A replicative cloning vector which comprises an isolated nucleic acid as claimed in any one of claims 1 to 8 and a replicon operative in a host cell.
10. An expression vector which comprises an isolated nucleic acid of any one of claims 1 to 8 wherein the coding sequence for the MMSC2 polypeptide or modified form thereof is operably linked to suitable control sequences capable of directing expression of said coding sequence in host cells for said vector.
11. Host cells transformed with a vector as claimed in claim 9 or 10.
12. A method of producing a polypeptide which is the MMSC2 polypeptide having the amino acid sequence set forth in SEQ ID NO:3 or a modified form of said polypeptide as defined in claim 1 which comprises (i) culturing the host cells of claim 11 containing an expression vector encoding said polypeptide under conditions suitable for the production of said MMSC2 polypeptide and (ii) recovering said polypeptide.
13. A method as claimed in claim 12 which further comprises labeling the recovered polypeptide.
14. A preparation of human MMSC2 polypeptide substantially free of other human proteins, said polypeptide having the amino acid sequence set forth in SEQ ID NO:3.
15. A preparation of human MMSC2 polypeptide substantially free of other human proteins, the amino acid sequence of said polypeptide having substantial sequence homology with the wild-type MMSC2 polypeptide having the amino acid sequence set forth in SEQ ID NO:3, and said polypeptide having substantially similar function as the wild-type MMSC2 polypeptide.
16. A preparation of a polypeptide substantially free of other proteins, said polypeptide being a mutated human MMSC2 polypeptide obtainable by expression of a mutated form of the nucleic acid sequence set forth in SEQ ID NO:2.

17. A preparation as claimed in any one of claims 14 to 16 wherein said polypeptide is labeled.
18. An antibody capable of specifically binding one or more polypeptides as claimed in any one of claims 14 to 16.
19. An antigenic fragment of a poly peptide as defined in any one of claims 14 to 16 suitable for use as an immunogen to obtain an antibody as claimed in claim 18.
20. A polypeptide as defined in any one of claims 14 to 16 and 19 in the form of a fusion protein.
21. Use of a polypeptide as defined in any one of claims 14 to 16, 19 and 20 as an immunogen for antibody production.
22. A use as claimed in claim 21, wherein one or more antibodies products are subsequently labeled or bound to a solid support.
23. A pair of single-stranded oligonucleotide primers for determination of a nucleotide sequence of a *MMSC2* gene by a nucleic acid amplification reaction, the sequence of said primers being derived from genomic clones for *MMSC2*, and the use of said primers in a nucleic acid amplification reaction resulting in the synthesis of DNA or RNA corresponding to all or part of the sequence of the *MMSC2* gene.
24. A pair of primers as claimed in claim 23 for determination of all or part of the sequence of the *MMSC2* gene having the nucleotide sequence set forth in SEQ ID NO:2, allelic variant or a mutated form thereof.
25. A method for identifying a mutant *MMSC2* nucleotide sequence in a suspected mutant *MMSC2* allele which comprises comparing the nucleotide sequence of the suspected mutant *MMSC2* allele with a wild-type *MMSC2* nucleotide sequence, wherein a

difference between the suspected mutant and the wild-type sequence identifies a mutant *MMSC2* nucleotide sequence.

26. A method for detecting an alteration in *MMSC2* wherein said alteration is associated with cancer in a human, wherein if said alteration is in germline it is associated with predisposition to said cancer and if said alteration is in somatic tissue it indicates that said somatic tissue is cancerous, wherein said method comprises analyzing a *MMSC2* gene or a *MMSC2* gene expression product from a tissue of said human.
27. A method as claimed in claim 26 wherein the sequence of the *MMSC2* gene in said sample is compared with the sequence of one or more wild-type *MMSC2* gene sequences selected from the sequence set forth in SEQ ID NO:2 and wild-type allelic variants thereof.
28. The method of claim 26 wherein said expression product is selected from the group consisting of mRNA of the *MMSC2* gene and a *MMSC2* polypeptide encoded by the *MMSC2* gene.
29. The method of any one of claims 26-28 wherein one or more of the following procedures is carried out:
- (a) observing shifts in electrophoretic mobility of single-stranded DNA from said sample on non-denaturing polyacrylamide gels;
 - (b) hybridizing a *MMSC2* gene probe to genomic DNA isolated from said sample under conditions suitable for hybridization of said probe to said gene;
 - (c) determining hybridization of an allele-specific probe to genomic DNA from said sample;
 - (d) amplifying all or part of the *MMSC2* gene from said sample to produce an amplified sequence and sequencing the amplified sequence;
 - (e) determining by nucleic acid amplification the presence of a specific *MMSC2* mutant allele in said sample;
 - (f) molecularly cloning all or part of the *MMSC2* gene from said sample to produce a cloned sequence and sequencing the cloned sequence;

(g) determining whether there is a mismatch between molecules (1) *MMSC2* gene genomic DNA or *MMSC2* mRNA isolated from said sample, and (2) a nucleic acid probe complementary to the human wild-type *MMSC2* gene DNA, when molecules (1) and (2) are hybridized to each other to form a duplex;

5 (h) amplification of *MMSC2* gene sequences in said sample and hybridization of the amplified sequences to nucleic acid probes which comprise wild-type *MMSC2* gene sequences;

(i) amplification of *MMSC2* gene sequences in said tissue and hybridization of the amplified sequences to nucleic acid probes which comprise mutant *MMSC2* gene sequences;

10 (j) screening for a deletion mutation;

(k) screening for a point mutation;

(l) screening for an insertion mutation;

(m) determining *in situ* hybridization of the *MMSC2* gene in said sample with one or more nucleic acid probes which comprise the *MMSC2* gene sequence or a mutant *MMSC2* gene sequence;

(n) immunoblotting;

(o) immunocytochemistry;

(p) assaying for binding interactions between *MMSC2* protein isolated from said tissue and a binding partner capable of specifically binding the polypeptide expression product of a *MMSC2* mutant allele and/or a binding partner for the *MMSC2* polypeptide having the amino acid sequence set forth in SEQ ID NO:3; and

(q) assaying for the inhibition of biochemical activity of said binding partner.

25 30. The method of claim 29 wherein said alteration of *MMSC2* protein is detected by assaying for binding interactions between said *MMSC2* protein isolated from said tissue and *MMAC1* protein.

31. A method for detecting an alteration in *MMAC1* wherein said alteration is associated with cancer in a human, wherein if said alteration is in germline it is associated with predisposition to said cancer and if said alteration is in somatic tissue it indicates that said somatic tissue is cancerous, wherein said method comprises analyzing an *MMAC1*

polypeptide from a tissue of said human by assaying for binding interactions between said MMAC1 polypeptide and MMSC2 or PDZ domain number 7 of said MMSC2.

32. A transgenic animal which carries an altered *MMSC2* allele in its genome.

5

33. An isolated mutant MMSC2 which cannot form a complex with a wild-type protein with which wild-type MMSC2 does form a complex.

34. The isolated mutant MMSC2 of claim 33 wherein said wild-type protein is MMAC1.

10

35. An isolated protein complex comprising MMSC2 and MMAC1.

36. The isolated protein complex of claim 35 wherein said MMSC2 contains an alteration.

15 37. The isolated protein complex of claim 35 wherein said MMAC1 contains an alteration.

38. A protein complex comprising a fragment of MMSC2 and a fragment of MMAC1.

20 39. The protein complex of claim 38 wherein said fragment of MMSC2 comprises PDZ domain number 7.

40. The protein complex of claim 38 wherein said MMSC2 comprises an alteration.

41. The protein complex of claim 38 wherein said MMAC1 comprises an alteration.

25

42. An isolated antibody immunoreactive with the protein complex of any one of claims 35 to 41.

30 43. The antibody of claim 42 wherein said antibody is not immunoreactive with either pure MMSC2 or pure MMAC1.

44. The antibody of claim 42 or 43 wherein said antibody is a monoclonal antibody.

45. A method for supplying a wild-type *MMSC2* gene function or a *MMSC2* function substantially similar to said wild-type function to a cell which has lost said gene function or has altered gene function by virtue of a mutation in said *MMSC2* gene, wherein said method comprises introducing into the cell a nucleic acid which suppresses a transformed state of said cell, said nucleic acid selected from the group consisting of a wild-type *MMSC2* gene nucleic acid or a nucleic acid substantially homologous to said wild-type *MMSC2* gene nucleic acid, such that said nucleic acid is expressed in said cell.
46. The method of claim 45 wherein said nucleic acid is a portion of wild-type *MMSC2* gene, said portion encoding a part of said wild-type gene polypeptide which is required for non-neoplastic growth of said cell.
47. A method for supplying a wild-type *MMSC2* gene function or a *MMSC2* function substantially similar to wild-type to a cell which has lost said gene function or has altered gene function by virtue of a mutation in said *MMSC2* gene, wherein said method comprises introducing into said cell a molecule which suppresses a transformed state of said cell, said molecule selected from the group consisting of all or a part of a wild-type *MMSC2* polypeptide which is required for non-neoplastic growth of said cell, a polypeptide substantially homologous to said wild-type *MMSC2* polypeptide and a molecule which mimics the function of said wild-type *MMSC2* polypeptide.
48. A method for diagnosing a predisposition for cancer in a human wherein said method comprises assaying for the ability of *MMSC2* or a fragment of *MMSC2* from said human to form a complex with a protein to which wild-type *MMSC2* binds wherein an inability to form said complex is indicative of a predisposition to cancer.
49. The method of claim 48 wherein said protein is *MMAC1*.
50. The method of claim 48 wherein said assay comprises a yeast two-hybrid assay.

51. The method of claim 48 wherein said assay comprises measuring *in vitro* a complex formed by mixing said protein and MMSC2 purified from said human.
52. The method of claim 48 wherein said assay comprises measuring *in vitro* a complex formed by mixing MMSC2 and said protein purified from said human.
53. The method of claim 48 wherein said complex is measured by binding with an antibody specific for a MMSC2-said protein complex.
54. The method of claim 48 wherein said assay comprises mixing an antibody specific for a MMSC2-said protein complex with a tissue extract from said person, wherein the lack of formation of a MMSC2-said protein-antibody complex between said antibody and said tissue extract is indicative of a predisposition to cancer.
55. A method for determining whether a mutation in a protein to which MMSC2 binds is predispositive for cancer wherein said method comprises binding said protein with said mutation to a wild-type MMSC2 and determining whether a complex forms, wherein the lack of a complex indicates said mutation is predispositive.
56. A method for determining whether a mutation in MMSC2 is predispositive for cancer wherein said method comprises binding a MMSC2 with said mutation to a protein to which wild-type MMSC2 binds and determining whether a complex forms, wherein the lack of a complex indicates said mutation is predispositive.
57. A method for treating a human with cancer resulting from a mutation in a protein to which MMSC2 binds and wherein said mutation prevents binding of said protein to MMSC2 wherein said method comprises treating said person with a complex of wild-type MMSC2 and wild-type of said protein.
58. The method of claim 57 wherein said protein is MMAC1.

59. A method of screening for drug candidates useful in treating a cancer resulting from a mutation in *MMSC2*, wherein said method involves mixing a mutant *MMSC2* with a wild- type protein, to which wild-type *MMSC2* binds, in both the presence of a drug and the absence of said drug and measuring the amount of binding of said mutant *MMSC2* with said wild-type protein, wherein if the amount of said binding is greater in the presence of said drug than in the absence of said drug then said drug is a drug candidate for treating said cancer.
60. The method of claim 59 wherein said mutant *MMSC2* is a fusion protein and/or said wild- type protein is a fusion protein.
61. The method of claim 59 or 60 wherein said wild-type protein is *MMAC1*.
62. A method for screening for drug candidates useful in treating a cancer resulting from a mutation in a protein, which protein when wild-type binds with wild-type *MMSC2*, wherein said method involves mixing said protein containing said mutation with wild-type *MMSC2* in both the presence of a drug and the absence of said drug and measuring the amount of binding of said protein containing said mutation with said wild-type *MMSC2*, wherein if the amount of said binding is greater in the presence of said drug than in the absence of said drug then said drug is a drug candidate for treating said cancer.
63. The method of claim 62 wherein said wild-type *MMSC2* is a fusion protein and/or said mutant protein is a fusion protein.
64. The method of claim 62 or 63 wherein said protein is *MMAC1*.
65. A method of screening for drug candidates useful in treating a cancer resulting from a mutation in *MMSC2*, wherein said method involves mixing a mutant *MMSC2* with a wild- type protein, to which wild-type *MMSC2* binds, in both the presence of a drug and the absence of said drug and measuring the amount of binding of said mutant *MMSC2* with said wild-type protein, wherein if the amount of said binding is less in the presence

of said drug than in the absence of said drug then said drug is a drug candidate for treating said cancer.

66. The method of claim 65 wherein said wild-type MMSC2 is a fusion protein and/or said wild-type protein is a fusion protein.

67. The method of claim 65 or 66 wherein said wild-type protein is MMAC1.

68. A method of screening for drug candidates useful in treating a cancer resulting from a mutation in *MMSC2*, wherein said method involves mixing a wild-type MMSC2 with a wild-type protein, to which wild-type MMSC2 binds, in both the presence of a drug and the absence of said drug and measuring the amount of binding of said mutant MMSC2 with said wild-type protein, wherein if the amount of said binding is less in the presence of said drug than in the absence of said drug then said drug is a drug candidate for treating said cancer.

69. The method of claim 68 wherein said wild-type MMSC2 is a fusion protein and/or said wild-type protein is a fusion protein.

70. The method of claim 68 or 69 wherein said wild-type protein is MMAC1.

71. A method of screening for drug candidates useful in treating a cancer resulting from a mutation in *MMAC1*, wherein said method involves mixing a wild-type MMSC2 with a wild-type protein, to which wild-type MMSC2 binds, in both the presence of a drug and the absence of said drug and measuring the amount of binding of said mutant MMSC2 with said wild-type protein, wherein if the amount of said binding is less in the presence of said drug than in the absence of said drug then said drug is a drug candidate for treating said cancer.

72. The method of claim 71 wherein said wild-type MMSC2 is a fusion protein and/or said wild-type protein is a fusion protein.

73. The method of claim 71 or 72 wherein said wild-type protein is MMAC1.

74. The method of any one of claims 59 to 73, wherein said wild-type protein which binds to MMSC2 binds to one or more of the PDZ domains of MMSC2.

5

75. A method of screening for drug candidates useful in treating a cancer resulting from a mutation in *MMSC2*, wherein said method comprises treating an animal which is homozygous for *MMSC2* containing said mutation with a drug wherein if said animal does not develop cancer said drug is a drug candidate for treating said cancer.

10

76. A method of screening for drug candidates useful in treating a cancer resulting from a mutation in *MMSC2*, wherein said method comprises treating an animal which has a tumor and which is homozygous for *MMSC2* containing said mutation with a drug wherein if said tumor regresses said drug is a drug candidate for treating said cancer.

15

77. The method of claim 75 or 76 wherein said animal is transgenic for *MMSC2* with said mutation.

78. A method of screening for drug candidates useful in treating a cancer resulting from a mutation in *MMSC2*, wherein said method comprises the steps of:

20

(a) growing a cell culture of cells which are homozygous for *MMSC2* containing said mutation in the presence of a drug,

(b) growing a cell culture of cells which contain a wild-type *MMSC2* gene, and

(c) growing a cell culture of cells which are homozygous for *MMSC2* containing said mutation in the absence of said drug,

25

wherein if the cells in step (a) behave more like the cells in step (b) than like the cells in step (c) then said drug is a drug candidate for treating said cancer.

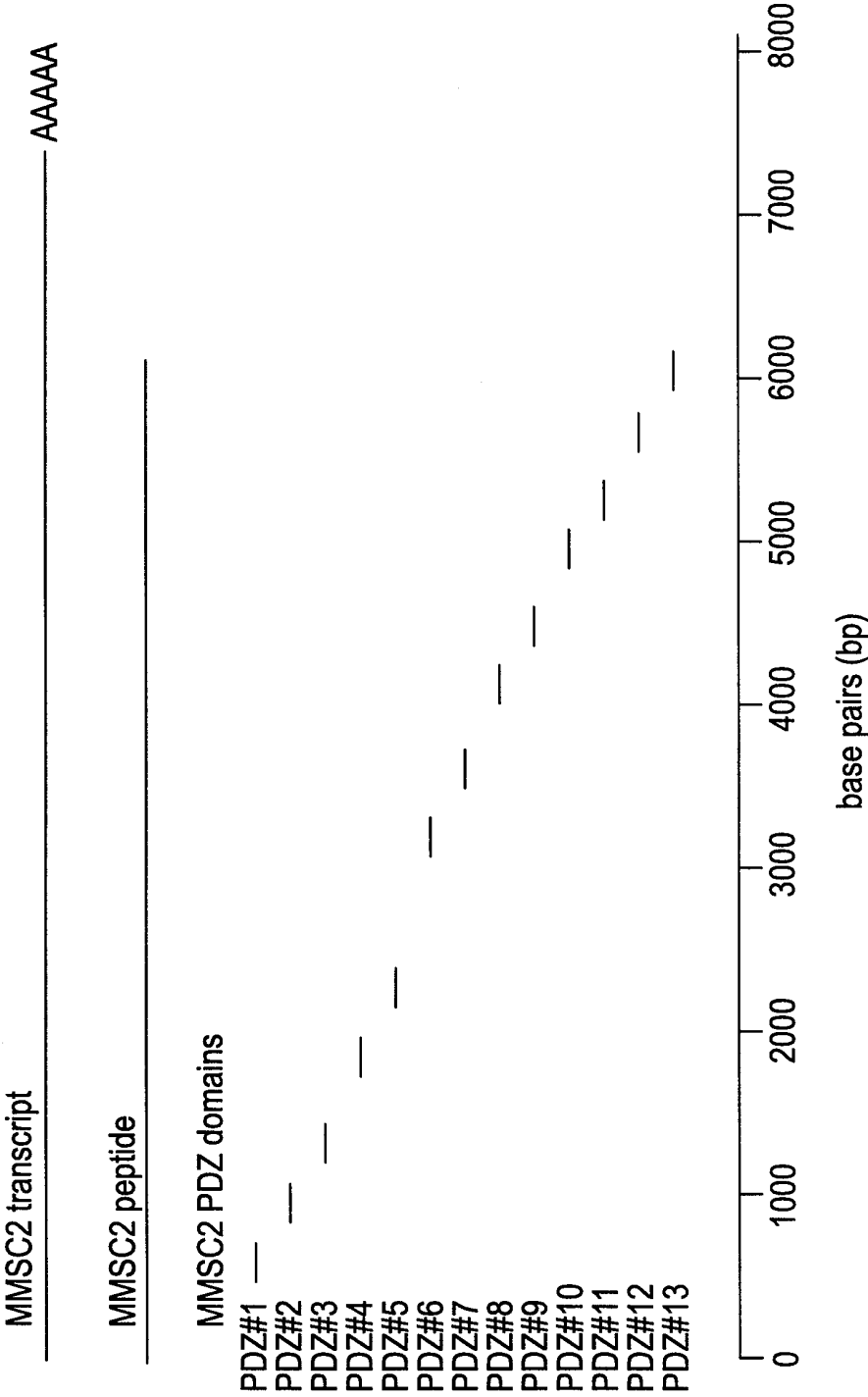


FIG. 1

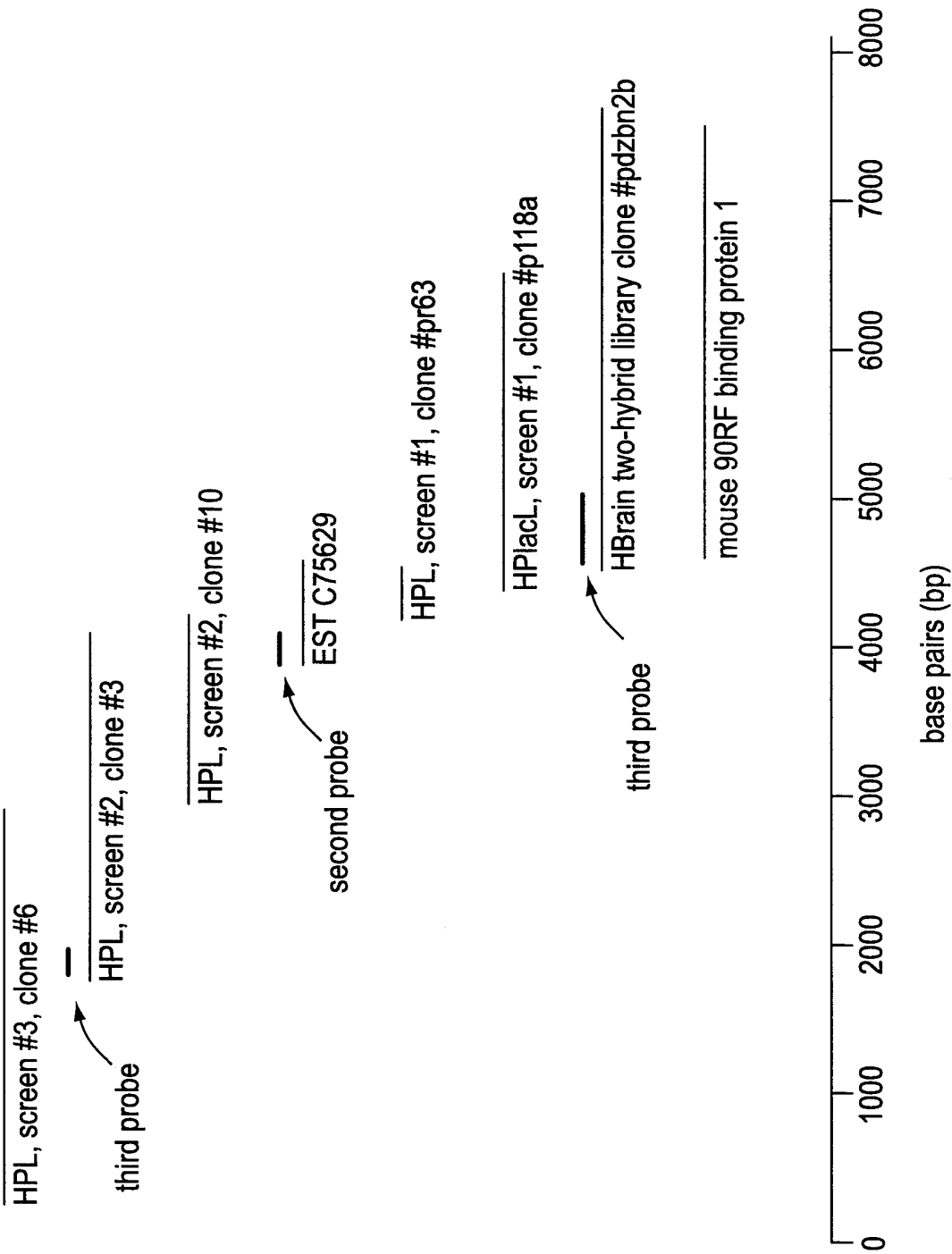


FIG. 2

SEQUENCE LISTING

<110> Myriad Genetics, Inc.
 <120> MMSC2- An MMAC1 Interacting Protein
 <130> MMSC2
 <140>
 <141>
 <150> US 60/084,740
 <151> 1998-05-08
 <160> 72
 <170> PatentIn Ver. 2.0
 <210> 1
 <211> 4
 <212> PRT
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence:Consensus motif
 for interaction of PDZ domains.
 <220>
 <221> PEPTIDE
 <222> (2)..(4)
 <223> Xaa at residue 2 may be Ser or Thr; Xaa at residue
 3 may be any amino acid; Xaa at residue 4 may be
 Val or Ile.
 <400> 1
 Glu Xaa Xaa Xaa
 1
 <210> 2
 <211> 7431
 <212> DNA
 <213> Homo sapiens
 <220>
 <221> CDS
 <222> (57)..(6167)
 <400> 2
 gttccatttt aattgctgtt aatcatttca gagaagaaca ctgaactttg aaaaaa atg 59
 Met
 1
 ttg gaa gcc att gac aaa aat cgg gcc ctg cat gca gca gag cgc ttg 107
 Leu Glu Ala Ile Asp Lys Asn Arg Ala Leu His Ala Ala Glu Arg Leu
 5 10 15
 caa acc aag ctg cga gaa cgt ggg gat gta gca aat gaa gac aaa ctg 155
 Gln Thr Lys Leu Arg Glu Arg Gly Asp Val Ala Asn Glu Asp Lys Leu
 20 25 30
 agc ctt ctg aag tca gtc ctg cag agc cct ctc ttc agt cag att ctg 203
 Ser Leu Leu Lys Ser Val Leu Gln Ser Pro Leu Phe Ser Gln Ile Leu
 35 40 45

agc ctt cag act tct gta cag cag ctg aaa gac cag gta aat att gca	251
Ser Leu Gln Thr Ser Val Gln Gln Leu Lys Asp Gln Val Asn Ile Ala	
50 55 60 65	
act tca gca act tca aat att gaa tat gcc cac gtt cct cat ctc agc	299
Thr Ser Ala Thr Ser Asn Ile Glu Tyr Ala His Val Pro His Leu Ser	
70 75 80	
cca gct gtg att cct act ctg caa aat gaa tcg ttt tta tta tcc cca	347
Pro Ala Val Ile Pro Thr Leu Gln Asn Glu Ser Phe Leu Leu Ser Pro	
85 90 95	
aac aat ggg aat ctg gaa gca ctt aca gga cct ggt att cca cac att	395
Asn Asn Gly Asn Leu Glu Ala Leu Thr Gly Pro Gly Ile Pro His Ile	
100 105 110	
aat ggg aaa cct gct tgt gat gaa ttt gat cag ctt atc aaa aat atg	443
Asn Gly Lys Pro Ala Cys Asp Glu Phe Asp Gln Leu Ile Lys Asn Met	
115 120 125	
gcc cag ggt cgc cat gta gaa gtt ttt gag ctc ctc aaa cct cca tct	491
Ala Gln Gly Arg His Val Glu Val Phe Glu Leu Leu Lys Pro Pro Ser	
130 135 140 145	
gga ggc ctt ggg ttt agt gtt gtg gga cta aga agt gaa aac aga gga	539
Gly Gly Leu Gly Phe Ser Val Val Gly Leu Arg Ser Glu Asn Arg Gly	
150 155 160	
gag ctg gga ata ttt gtt caa gag ata caa gag ggc agt gtg gcc cat	587
Glu Leu Gly Ile Phe Val Gln Glu Ile Gln Glu Gly Ser Val Ala His	
165 170 175	
aga gat gga aga ttg aaa gaa act gat caa att ctt gct atc aat gga	635
Arg Asp Gly Arg Leu Lys Glu Thr Asp Gln Ile Leu Ala Ile Asn Gly	
180 185 190	
cag gct ctt gat cag aca att aca cat cag cag gct atc agc atc ctg	683
Gln Ala Leu Asp Gln Thr Ile Thr His Gln Gln Ala Ile Ser Ile Leu	
195 200 205	
cag aaa gcc aaa gat act gtc cag cta gtt att gcc aga ggc tca ttg	731
Gln Lys Ala Lys Asp Thr Val Gln Leu Val Ile Ala Arg Gly Ser Leu	
210 215 220 225	
cct cag ctt gtc agc ccc ata gtt tcc cgt tct cca tct gca gcc agc	779
Pro Gln Leu Val Ser Pro Ile Val Ser Arg Ser Pro Ser Ala Ala Ser	
230 235 240	
aca att tca gct cac tct aat ccg gtt cac tgg caa cac atg gaa acg	827
Thr Ile Ser Ala His Ser Asn Pro Val His Trp Gln His Met Glu Thr	
245 250 255	
att gaa ttg gtg aat gat gga tct ggt ttg gga ttt ggc atc ata gga	875
Ile Glu Leu Val Asn Asp Gly Ser Gly Leu Gly Phe Gly Ile Ile Gly	
260 265 270	
gga aaa gca act ggt gtg ata gta aaa acc att ctg cct gga gga gta	923
Gly Lys Ala Thr Gly Val Ile Val Lys Thr Ile Leu Pro Gly Gly Val	
275 280 285	
gct gat cag cat ggg cgt tta tgc agt gga gac cac att cta aag att	971
Ala Asp Gln His Gly Arg Leu Cys Ser Gly Asp His Ile Leu Lys Ile	
290 295 300 305	
ggt gac aca gat cta gca gga atg agc agt gag caa gta gca caa gtc	1019

Gly	Asp	Thr	Asp	Leu	Ala	Gly	Met	Ser	Ser	Glu	Gln	Val	Ala	Gln	Val		
				310					315					320			
ctt	agg	caa	tgt	gga	aat	aga	gtt	aag	ttg	atg	att	gca	aga	ggc	gcc	1067	
Leu	Arg	Gln	Cys	Gly	Asn	Arg	Val	Lys	Leu	Met	Ile	Ala	Arg	Gly	Ala		
			325					330					335				
ata	gaa	gaa	cgt	aca	gca	ccc	act	gct	ttg	ggc	atc	acc	ctc	tcc	tca	1115	
Ile	Glu	Glu	Arg	Thr	Ala	Pro	Thr	Ala	Leu	Gly	Ile	Thr	Leu	Ser	Ser		
			340					345					350				
tcc	cca	act	tca	aca	cca	gag	ttg	cgg	gtt	gat	gct	tct	act	cag	aaa	1163	
Ser	Pro	Thr	Ser	Thr	Pro	Glu	Leu	Arg	Val	Asp	Ala	Ser	Thr	Gln	Lys		
			355					360					365				
ggc	gaa	gaa	agt	gag	aca	ttt	gat	gta	gaa	ctc	act	aaa	aat	gtc	caa	1211	
Gly	Glu	Glu	Ser	Glu	Thr	Phe	Asp	Val	Glu	Leu	Thr	Lys	Asn	Val	Gln		
						375									385		
gga	tta	gga	att	acc	att	gct	ggc	tac	att	gga	gat	aaa	aaa	ttg	gaa	1259	
Gly	Leu	Gly	Ile	Thr	Ile	Ala	Gly	Tyr	Ile	Gly	Asp	Lys	Lys	Leu	Glu		
						390									400		
cct	tca	gga	atc	ttt	gta	aag	agc	att	aca	aaa	agc	agt	gcc	gtt	gag	1307	
Pro	Ser	Gly	Ile	Phe	Val	Lys	Ser	Ile	Thr	Lys	Ser	Ser	Ala	Val	Glu		
cat	gat	gga	aga	atc	caa	att	gga	gac	caa	att	ata	gca	gta	gat	ggc	1355	
His	Asp	Gly	Arg	Ile	Gln	Ile	Gly	Asp	Gln	Ile	Ile	Ala	Val	Asp	Gly		
aca	aac	ctt	cag	ggt	ttt	act	aat	cag	caa	gca	gta	gag	gta	ttg	cga	1403	
Thr	Asn	Leu	Gln	Gly	Phe	Thr	Asn	Gln	Gln	Ala	Val	Glu	Val	Leu	Arg		
cat	aca	gga	caa	act	gtg	ctc	ctg	aca	cta	atg	agg	aga	gga	atg	aag	1451	
His	Thr	Gly	Gln	Thr	Val	Leu	Leu	Thr	Leu	Met	Arg	Arg	Gly	Met	Lys		
cag	gaa	gcc	gag	ctc	atg	tca	agg	gaa	gac	gtc	aca	aaa	gat	gca	gat	1499	
Gln	Glu	Ala	Glu	Leu	Met	Ser	Arg	Glu	Asp	Val	Thr	Lys	Asp	Ala	Asp		
ttg	tct	cct	gtt	aat	gcc	agc	ata	atc	aaa	gaa	aat	tat	gaa	aaa	gat	1547	
Leu	Ser	Pro	Val	Asn	Ala	Ser	Ile	Ile	Lys	Glu	Asn	Tyr	Glu	Lys	Asp		
gaa	gat	ttt	tta	tct	tcg	acg	aga	aac	acc	aac	ata	tta	cca	act	gaa	1595	
Glu	Asp	Phe	Leu	Ser	Ser	Thr	Arg	Asn	Thr	Asn	Ile	Leu	Pro	Thr	Glu		
gaa	gaa	ggg	tat	cca	tta	ctg	tca	gct	gag	ata	gaa	gaa	ata	gaa	gat	1643	
Glu	Glu	Gly	Tyr	Pro	Leu	Leu	Ser	Ala	Glu	Ile	Glu	Glu	Ile	Glu	Asp		
gca	caa	aaa	caa	gaa	gct	gct	ctg	ctg	aca	aaa	tgg	caa	agg	att	atg	1691	
Ala	Gln	Lys	Gln	Glu	Ala	Ala	Leu	Leu	Thr	Lys	Trp	Gln	Arg	Ile	Met		
gga	att	aac	tat	gaa	ata	gtg	gtg	gcc	cat	gtg	agc	aag	ttt	agt	gag	1739	
Gly	Ile	Asn	Tyr	Glu	Ile	Val	Val	Ala	His	Val	Ser	Lys	Phe	Ser	Glu		
aac	agt	gga	ttg	ggg	ata	agc	ctg	gaa	gcg	aca	gtg	gga	cat	cat	ttt	1787	
Asn	Ser	Gly	Leu	Gly	Ile	Ser	Leu	Glu	Ala	Thr	Val	Gly	His	His	Phe		

565				570				575								
atc	cga	tct	gtt	cta	cca	gag	ggt	cct	gtt	gga	cac	agc	ggg	aag	ctc	1835
Ile	Arg	Ser	Val	Leu	Pro	Glu	Gly	Pro	Val	Gly	His	Ser	Gly	Lys	Leu	
		580					585					590				
ttc	agt	gga	gac	gag	cta	ttg	gaa	gta	aat	ggc	ata	act	tta	ctt	ggg	1883
Phe	Ser	Gly	Asp	Glu	Leu	Leu	Glu	Val	Asn	Gly	Ile	Thr	Leu	Leu	Gly	
	595					600					605					
gaa	aat	cac	caa	gat	gtg	gtg	aat	atc	tta	aaa	gaa	ctg	cct	ata	gaa	1931
Glu	Asn	His	Gln	Asp	Val	Val	Asn	Ile	Leu	Lys	Glu	Leu	Pro	Ile	Glu	
610					615					620					625	
gtg	aca	atg	gtg	tgc	tgt	cgt	cga	act	gtg	cca	ccc	acc	acc	caa	tca	1979
Val	Thr	Met	Val	Cys	Cys	Arg	Arg	Thr	Val	Pro	Pro	Thr	Thr	Gln	Ser	
				630					635					640		
gaa	ttg	gat	agc	ctg	gac	tta	tgt	gat	att	gag	cta	aca	gaa	aag	cct	2027
Glu	Leu	Asp	Ser	Leu	Asp	Leu	Cys	Asp	Ile	Glu	Leu	Thr		Lys	Pro	
			645					650					655			
cac	gta	gat	cta	ggg	gag	ttc	atc	ggg	tca	tca	gag	aca	gag	gat	cca	2075
His	Val	Asp	Leu	Gly	Glu	Phe	Ile	Gly	Ser	Ser	Glu	Thr	Glu	Asp	Pro	
		660					665					670				
gtg	ctg	gcg	atg	act	gat	gcg	ggg	cag	agt	aca	gaa	gag	gtt	caa	gca	2123
Val	Leu	Ala	Met	Thr	Asp	Ala	Gly	Gln	Ser	Thr	Glu	Glu	Val	Gln	Ala	
	675					680					685					
cct	ttg	gcc	atg	tgg	gag	gct	ggc	att	cag	cac	ata	gag	ctg	gag	aaa	2171
Pro	Leu	Ala	Met	Trp	Glu	Ala	Gly	Ile	Gln	His	Ile	Glu	Leu	Glu	Lys	
690					695					700					705	
ggg	agc	aaa	gga	ctt	ggg	ttt	agc	att	tta	gat	tat	cag	gat	cca	att	2219
Gly	Ser	Lys	Gly	Leu	Gly	Phe	Ser	Ile	Leu	Asp	Tyr	Gln	Asp	Pro	Ile	
			710						715					720		
gat	cca	gca	agc	act	gtg	att	ata	att	cgt	tct	ttg	gtg	cct	ggc	ggc	2267
Asp	Pro	Ala	Ser	Thr	Val	Ile	Ile	Ile	Arg	Ser	Leu	Val	Pro	Gly	Gly	
			725					730					735			
att	gct	gaa	aag	gat	gga	cga	ctt	ctt	cct	ggg	gac	cga	ctc	atg	ttt	2315
Ile	Ala	Glu	Lys	Asp	Gly	Arg	Leu	Leu	Pro	Gly	Asp	Arg	Leu	Met	Phe	
		740					745					750				
gta	aac	gat	gtt	aac	ttg	gaa	aac	agc	agt	ctt	gag	gaa	gct	gta	gaa	2363
Val	Asn	Asp	Val	Asn	Leu	Glu	Asn	Ser	Ser	Leu	Glu	Glu	Ala	Val	Glu	
	755					760					765					
gca	ctg	aag	gga	gca	ccg	tca	ggg	act	gtg	aga	ata	gga	gtt	gct	aag	2411
Ala	Leu	Lys	Gly	Ala	Pro	Ser	Gly	Thr	Val	Arg	Ile	Gly	Val	Ala	Lys	
770					775					780					785	
cct	tta	ccc	ctt	tca	cca	gaa	gaa	ggg	tat	gtt	tct	gct	aag	gag	gat	2459
Pro	Leu	Pro	Leu	Ser	Pro	Glu	Glu	Gly	Tyr	Val	Ser	Ala	Lys	Glu	Asp	
			790						795					800		
tcc	ttt	ctc	tac	cca	cca	cac	tcc	tgt	gag	gaa	gca	ggg	ctg	gct	gac	2507
Ser	Phe	Leu	Tyr	Pro	Pro	His	Ser	Cys	Glu	Glu	Ala	Gly	Leu	Ala	Asp	
			805					810					815			
aaa	ccc	ctc	ttc	agg	gct	gac	ttg	gct	ctg	gtg	ggc	aca	aat	gat	gct	2555
Lys	Pro	Leu	Phe	Arg	Ala	Asp	Leu	Ala	Leu	Val	Gly	Thr	Asn	Asp	Ala	
		820					825					830				

gac tta gta gat gaa tcc aca ttt gag tct cca tac tct cct gaa aat	2603
Asp Leu Val Asp Glu Ser Thr Phe Glu Ser Pro Tyr Ser Pro Glu Asn	
835 840 845	
gac agc atc tac tct act caa gcc tct att tta tct ctt cat ggc agt	2651
Asp Ser Ile Tyr Ser Thr Gln Ala Ser Ile Leu Ser Leu His Gly Ser	
850 855 860 865	
tct tgt ggt gat ggc ctg aac tat ggt tct tcc ctt cca tca tct cct	2699
Ser Cys Gly Asp Gly Leu Asn Tyr Gly Ser Ser Leu Pro Ser Ser Pro	
870 875 880	
cct aag gat gtt att gaa aat tct tgt gat cca gta ctt gat ctg cat	2747
Pro Lys Asp Val Ile Glu Asn Ser Cys Asp Pro Val Leu Asp Leu His	
885 890 895	
atg tct ctg gag gaa cta tat acc cag aat ctc ctg caa aga cag gat	2795
Met Ser Leu Glu Glu Leu Tyr Thr Gln Asn Leu Leu Gln Arg Gln Asp	
900 905 910	
gag aat aca cct tcg gtg gac ata agt atg ggg cct gct tct ggc ttt	2843
Glu Asn Thr Pro Ser Val Asp Ile Ser Met Gly Pro Ala Ser Gly Phe	
915 920 925	
act ata aat gac tac aca cct gca aat gct att gaa caa caa tat gaa	2891
Thr Ile Asn Asp Tyr Thr Pro Ala Asn Ala Ile Glu Gln Gln Tyr Glu	
930 935 940 945	
tgt gaa aac aca ata gtg tgg act gaa tct cat tta cca agt gaa gtt	2939
Cys Glu Asn Thr Ile Val Trp Thr Glu Ser His Leu Pro Ser Glu Val	
950 955 960	
ata tca agt gca gaa ctt cct tct gtg cta ccc gat tca gct gga aag	2987
Ile Ser Ser Ala Glu Leu Pro Ser Val Leu Pro Asp Ser Ala Gly Lys	
965 970 975	
ggc tct gag tac ctg ctt gaa cag agc tcc ctg gcc tgt aat gct gag	3035
Gly Ser Glu Tyr Leu Leu Glu Gln Ser Ser Leu Ala Cys Asn Ala Glu	
980 985 990	
tgt gtc atg ctt caa aat gta tct aaa gaa tct ttt gaa agg act att	3083
Cys Val Met Leu Gln Asn Val Ser Lys Glu Ser Phe Glu Arg Thr Ile	
995 1000 1005	
aat ata gca aaa ggc aat tct agc cta gga atg aca gtt agt gct aat	3131
Asn Ile Ala Lys Gly Asn Ser Ser Leu Gly Met Thr Val Ser Ala Asn	
1010 1015 1020 1025	
aaa gat ggc ttg ggg atg atc gtt cga agc att att cat gga ggt gcc	3179
Lys Asp Gly Leu Gly Met Ile Val Arg Ser Ile Ile His Gly Gly Ala	
1030 1035 1040	
att agt cga gat ggc cgg att gcc att ggg gac tgc atc ttg tcc att	3227
Ile Ser Arg Asp Gly Arg Ile Ala Ile Gly Asp Cys Ile Leu Ser Ile	
1045 1050 1055	
aat gaa gag tct acc atc agt gta acc aat gcc cag gca cga gct atg	3275
Asn Glu Glu Ser Thr Ile Ser Val Thr Asn Ala Gln Ala Arg Ala Met	
1060 1065 1070	
ttg aga aga cat tct ctc att ggc cct gac ata aaa att act tat gtg	3323
Leu Arg Arg His Ser Leu Ile Gly Pro Asp Ile Lys Ile Thr Tyr Val	
1075 1080 1085	

cct gca gaa cat ttg gaa gag ttc aaa ata agc ttg gga caa caa tct	3371
Pro Ala Glu His Leu Glu Glu Phe Lys Ile Ser Leu Gly Gln Gln Ser	
1090 1095 1100 1105	
gga aga gta atg gca ctg gat att ttt tct tca tac act ggc aga gac	3419
Gly Arg Val Met Ala Leu Asp Ile Phe Ser Ser Tyr Thr Gly Arg Asp	
1110 1115 1120	
att cca gaa tta cca gag cga gaa gag gga gag ggt gaa gaa agc gaa	3467
Ile Pro Glu Leu Pro Glu Arg Glu Glu Gly Glu Gly Glu Glu Ser Glu	
1125 1130 1135	
ctt caa aac aca gca tat agc aat tgg aat cag ccc agg cgg gtg gaa	3515
Leu Gln Asn Thr Ala Tyr Ser Asn Trp Asn Gln Pro Arg Arg Val Glu	
1140 1145 1150	
ctc tgg aga gaa cca agc aaa tcc tta ggc atc agc att gtt ggt gga	3563
Leu Trp Arg Glu Pro Ser Lys Ser Leu Gly Ile Ser Ile Val Gly Gly	
1155 1160 1165	
cga ggg atg ggg agt cgg cta agc aat gga gaa gtg atg agg ggc att	3611
Arg Gly Met Gly Ser Arg Leu Ser Asn Gly Glu Val Met Arg Gly Ile	
1170 1175 1180 1185	
ttc atc aaa cat gtt ctg gaa gat agt cca gct ggc aaa aat gga acc	3659
Phe Ile Lys His Val Leu Glu Asp Ser Pro Ala Gly Lys Asn Gly Thr	
1190 1195 1200	
ttg aaa cct gga gat aga atc gta gag gtg gat gga atg gac ctc aga	3707
Leu Lys Pro Gly Asp Arg Ile Val Glu Val Asp Gly Met Asp Leu Arg	
1205 1210 1215	
gat gca agc cat gaa caa gct gtg gaa gcc att cgg aaa gca ggc aac	3755
Asp Ala Ser His Glu Gln Ala Val Glu Ala Ile Arg Lys Ala Gly Asn	
1220 1225 1230	
cct gta gtc ttt atg gta cag agc att ata aac aga cca agg gca ccc	3803
Pro Val Val Phe Met Val Gln Ser Ile Ile Asn Arg Pro Arg Ala Pro	
1235 1240 1245	
agt cag tca gag tca gag cca gag aag gct cca ttg tgc agt gtg ccc	3851
Ser Gln Ser Glu Ser Glu Pro Glu Lys Ala Pro Leu Cys Ser Val Pro	
1250 1255 1260 1265	
cca ccc cct cct tca gcc ttt gcc gaa atg ggt agt gat cac aca cag	3899
Pro Pro Pro Pro Ser Ala Phe Ala Glu Met Gly Ser Asp His Thr Gln	
1270 1275 1280	
tca tct gca agc aaa atc tca caa gat gtg gac aaa gag gat gag ttt	3947
Ser Ser Ala Ser Lys Ile Ser Gln Asp Val Asp Lys Glu Asp Glu Phe	
1285 1290 1295	
ggt tac agc tgg aaa aat atc aga gag cgt tat gga acc cta aca ggc	3995
Gly Tyr Ser Trp Lys Asn Ile Arg Glu Arg Tyr Gly Thr Leu Thr Gly	
1300 1305 1310	
gag ctg cat atg att gaa ctg gag aaa ggt cat agt ggt ttg ggc cta	4043
Glu Leu His Met Ile Glu Leu Glu Lys Gly His Ser Gly Leu Gly Leu	
1315 1320 1325	
agt ctt gct ggg aac aaa gac cga tcc agg atg agt gtc ttc ata gtg	4091
Ser Leu Ala Gly Asn Lys Asp Arg Ser Arg Met Ser Val Phe Ile Val	
1330 1335 1340 1345	
ggg att gat cca aat gga gct gca gga aaa gat ggt cga ttg caa att	4139

Gly Ile Asp Pro Asn Gly Ala Ala Gly Lys Asp Gly Arg Leu Gln Ile
 1350 1355 1360

gca gat gag ctt cta gag atc aat ggt cag att tta tat gga aga agt 4187
 Ala Asp Glu Leu Leu Glu Ile Asn Gly Gln Ile Leu Tyr Gly Arg Ser
 1365 1370 1375

cat cag aat gcc tca tca atc att aaa tgt gcc cct tct aaa gtg aaa 4235
 His Gln Asn Ala Ser Ser Ile Ile Lys Cys Ala Pro Ser Lys Val Lys
 1380 1385 1390

ata att ttt atc aga aat aaa gat gca gtg aat cag atg gcc gta tgt 4283
 Ile Ile Phe Ile Arg Asn Lys Asp Ala Val Asn Gln Met Ala Val Cys
 1395 1400 1405

cct gga aat gca gta gaa cct ttg cct tct aac tca gaa aat ctt caa 4331
 Pro Gly Asn Ala Val Glu Pro Leu Pro Ser Asn Ser Glu Asn Leu Gln
 1410 1415 1420 1425

aat aag gag aca gag cca act gtt act act tct gat gca gct gtg gac 4379
 Asn Lys Glu Thr Glu Pro Thr Val Thr Thr Ser Asp Ala Ala Val Asp
 1430 1435 1440

ctc agt tca ttt aaa aat gtg caa cat ctg gag ctt ccc aag gat cag 4427
 Leu Ser Ser Phe Lys Asn Val Gln His Leu Glu Leu Pro Lys Asp Gln
 1445 1450 1455

ggg ggt ttg ggt att gct atc agc gaa gaa gat aca ctc agt gga gtc 4475
 Gly Gly Leu Gly Ile Ala Ile Ser Glu Glu Asp Thr Leu Ser Gly Val
 1460 1465 1470

atc ata aag agc tta aca gag cat ggg gta gca gcc acg gat gga cga 4523
 Ile Ile Lys Ser Leu Thr Glu His Gly Val Ala Ala Thr Asp Gly Arg
 1475 1480 1485

ctc aaa gtc gga gat cag ata ctg gct gta gat gat gaa att gtt gtt 4571
 Leu Lys Val Gly Asp Gln Ile Leu Ala Val Asp Asp Glu Ile Val Val
 1490 1495 1500 1505

ggt tac cct att gaa aag ttt att agc ctt ctg aag aca gca aag atg 4619
 Gly Tyr Pro Ile Glu Lys Phe Ile Ser Leu Leu Lys Thr Ala Lys Met
 1510 1515 1520

aca gta aaa ctt acc atc cat gct gag aat cca gat tcc cag gct gtt 4667
 Thr Val Lys Leu Thr Ile His Ala Glu Asn Pro Asp Ser Gln Ala Val
 1525 1530 1535

cct tca gca gct ggt gca gcc agt gga gaa aaa aag aac agc tcc cag 4715
 Pro Ser Ala Ala Gly Ala Ala Ser Gly Glu Lys Lys Asn Ser Ser Gln
 1540 1545 1550

tct ctg atg gtc cca cag tct ggc tcc cca gaa ccg gag tcc atc cga 4763
 Ser Leu Met Val Pro Gln Ser Gly Ser Pro Glu Pro Glu Ser Ile Arg
 1555 1560 1565

aat aca agc aga tca tca aca cca gca att ttt gct tct gat cct gca 4811
 Asn Thr Ser Arg Ser Ser Thr Pro Ala Ile Phe Ala Ser Asp Pro Ala
 1570 1575 1580 1585

acc tgc ccc att atc cct ggc tgc gaa aca acc atc gag att tcc aaa 4859
 Thr Cys Pro Ile Ile Pro Gly Cys Glu Thr Thr Ile Glu Ile Ser Lys
 1590 1595 1600

ggg cga aca ggg ctg ggc ctg agc atc gtt ggg ggt tca gac acg ctg 4907
 Gly Arg Thr Gly Leu Gly Leu Ser Ile Val Gly Gly Ser Asp Thr Leu

1605	1610	1615	
ctg ggt gcc att att atc cat gaa gtt tat gaa gaa gga gca gca tgt			4955
Leu Gly Ala Ile Ile Ile His Glu Val Tyr Glu Glu Gly Ala Ala Cys			
1620	1625	1630	
aaa gat gga aga ctc tgg gct gga gat cag atc tta gag gtg aat gga			5003
Lys Asp Gly Arg Leu Trp Ala Gly Asp Gln Ile Leu Glu Val Asn Gly			
1635	1640	1645	
att gac ttg aga aag gcc aca cat gat gaa gca atc aat gtc ctg aga			5051
Ile Asp Leu Arg Lys Ala Thr His Asp Glu Ala Ile Asn Val Leu Arg			
1650	1655	1660	1665
cag acg cca cag aga gtg cgc ctg aca ctc tac aga gat gag gcc cca			5099
Gln Thr Pro Gln Arg Val Arg Leu Thr Leu Tyr Arg Asp Glu Ala Pro			
1670	1675	1680	
tac aaa gag gag gaa gtg tgt gac acc ctc act att gag ctg cag aag			5147
Tyr Lys Glu Glu Glu Val Cys Asp Thr Leu Thr Ile Glu Leu Gln Lys			
1685	1690	1695	
aag ccg gga aaa ggc cta gga tta agt att gtt ggt aaa aga aac gat			5195
Lys Pro Gly Lys Gly Leu Gly Leu Ser Ile Val Gly Lys Arg Asn Asp			
1700	1705	1710	
act gga gta ttt gtg tca gac att gtc aaa gga gga att gca gat gcc			5243
Thr Gly Val Phe Val Ser Asp Ile Val Lys Gly Gly Ile Ala Asp Ala			
1715	1720	1725	
gat gga aga ctg atg cag gga gac cag ata tta atg gtg aat ggg gaa			5291
Asp Gly Arg Leu Met Gln Gly Asp Gln Ile Leu Met Val Asn Gly Glu			
1730	1735	1740	1745
gac gtt cgt aat gcc acc caa gaa gcg gtt gcc gct ttg cta aag tgt			5339
Asp Val Arg Asn Ala Thr Gln Glu Ala Val Ala Ala Leu Leu Lys Cys			
1750	1755	1760	
tcc cta ggc aca gta acc ttg gaa gtt gga aga atc aaa gct ggt cca			5387
Ser Leu Gly Thr Val Thr Leu Glu Val Gly Arg Ile Lys Ala Gly Pro			
1765	1770	1775	
ttc cat tca gag agg agg cca tct caa agc agc cag gtg agt gaa ggc			5435
Phe His Ser Glu Arg Arg Pro Ser Gln Ser Ser Gln Val Ser Glu Gly			
1780	1785	1790	
agc ctg tca tct ttc act ttt cca ctc tct gga tcc agt aca tct gag			5483
Ser Leu Ser Ser Phe Thr Phe Pro Leu Ser Gly Ser Ser Thr Ser Glu			
1795	1800	1805	
tca ctg gaa agt agc tca aag aag aat gca ttg gca tct gaa ata cag			5531
Ser Leu Glu Ser Ser Ser Lys Lys Asn Ala Leu Ala Ser Glu Ile Gln			
1810	1815	1820	1825
gga tta aga aca gtc gaa atg aaa aag ggc cct act gac tca ctg gga			5579
Gly Leu Arg Thr Val Glu Met Lys Lys Gly Pro Thr Asp Ser Leu Gly			
1830	1835	1840	
atc agc atc gct gga gga gta ggc agc cca ctt ggt gat gtg cct ata			5627
Ile Ser Ile Ala Gly Gly Val Gly Ser Pro Leu Gly Asp Val Pro Ile			
1845	1850	1855	
ttt att gca atg atg cac cca act gga gtt gca gca cag acc caa aaa			5675
Phe Ile Ala Met Met His Pro Thr Gly Val Ala Ala Gln Thr Gln Lys			
1860	1865	1870	

ctc aga gtt ggg gat agg att gtc acc atc tgt ggc aca tcc act gag 5723
 Leu Arg Val Gly Asp Arg Ile Val Thr Ile Cys Gly Thr Ser Thr Glu
 1875 1880 1885

ggc atg act cac acc caa gca gtt aac cta ctg aaa aat gca tct ggc 5771
 Gly Met Thr His Thr Gln Ala Val Asn Leu Leu Lys Asn Ala Ser Gly
 1890 1895 1900 1905

tcc att gaa atg cag gtg gtt gct gga gga gac gtg agt gtg gtc aca 5819
 Ser Ile Glu Met Gln Val Val Ala Gly Gly Asp Val Ser Val Val Thr
 1910 1915 1920

ggt cat cag cag gag cct gca agt tcc agt ctt tct ttc act ggg ctg 5867
 Gly His Gln Gln Glu Pro Ala Ser Ser Ser Leu Ser Phe Thr Gly Leu
 1925 1930 1935

acg tca agc agt ata ttt cag gat gat tta gga cct cct caa tgt aag 5915
 Thr Ser Ser Ser Ile Phe Gln Asp Asp Leu Gly Pro Pro Gln Cys Lys
 1940 1945 1950

tct att aca cta gag cga gga cca gat ggc tta ggc ttc agt ata gtt 5963
 Ser Ile Thr Leu Glu Arg Gly Pro Asp Gly Leu Gly Phe Ser Ile Val
 1955 1960 1965

gga gga tat ggc agc cct cat gga gac tta ccc att tat gtt aaa aca 6011
 Gly Gly Tyr Gly Ser Pro His Gly Asp Leu Pro Ile Tyr Val Lys Thr
 1970 1975 1980 1985

gtg ttt gca aag gga gca gcc tct gaa gac gga cgt ctg aaa agg ggc 6059
 Val Phe Ala Lys Gly Ala Ala Ser Glu Asp Gly Arg Leu Lys Arg Gly
 1990 1995 2000

gat cag atc att gct gtc aat ggg cag agt cta gaa gga gtc acc cat 6107
 Asp Gln Ile Ile Ala Val Asn Gly Gln Ser Leu Glu Gly Val Thr His
 2005 2010 2015

gaa gaa gct gtt gcc atc ctt aaa cgg aca aaa ggc act gtc act ttg 6155
 Glu Glu Ala Val Ala Ile Leu Lys Arg Thr Lys Gly Thr Val Thr Leu
 2020 2025 2030

atg gtt ctc tct tgaattggct gccagaattg aaccaacca acccctagct 6207
 Met Val Leu Ser
 2035

cacctcctac tgtaaagaga atgcactggc cctgacaatt tttatgctgt gttcagccgg 6267

gtctttcaaaa ctgtaggggg gaaataacac ttaagtttct ttttctcatc tagaaatgct 6327

ttccttactg acaacctaac atcatttttc ttttcttctt gcattttgtg aacttaaaga 6387

gaaggaatat ttgtgtaggt gaatctcggt tttatttgtg gagatatcta atgttttgta 6447

gtcacatggg caagaattat tacatgctaa gctggttagt ataaagaaag ataattctaa 6507

agctaaccac agaaaatggc ttcagtaaata taggatgaaa aatgaaaata taaaataaag 6567

aagaaaatct cggggagttt aaaaaaatg cctcaatttg gcaatctacc tcctctcccc 6627

accccaaact aaaaaagaa aaaaagggtt tctaataaaa atctttaaaa atactgtcag 6687

tattttaaaa ttttcaacag tattataaaa acattgcac tccccacctc taatatgcat 6747

atatattttt cctgctaaaa ttggtttcta caattgagta aatggcaaat acatgaagca 6807

atgtccctaa attttataaa gaaattatat ttaatgcaca tttcaatttt cattcttatt 6867
 tttgaccttt tataaaatat tttcatgttg ctataagtaa atgatgatgc caccocatgt 6927
 tgactatggt ttttctagaa agcaactatg ctgctaacca tagaggaaca tagaagggtt 6987
 ccagaatctt tagtgctggt ttttaacaacc gatgcaacat taaaaatgtg ttagtgtgct 7047
 gtgcaattgg ttttcaattc atattaatct taatgacaga gaacaatgtg ttactaatta 7107
 ttttggttgt atgccattag taaattgata gaaaaattaa ggggattaac ataacttcat 7167
 ttcattgcct tatattaaca tcttataata caatagttta agactaaggg aaacagatgg 7227
 agctgtttat tgagacaact ggtgaggaat tatcatgtgt tcattcccat tttagagcgt 7287
 gaaactccta cattagaata tataaagtca ctttaaatat ctatatttgt aacagaagta 7347
 gtgtacagat attttattac agcatttttg tgtaaatgca gaattaaagt gaataaataa 7407
 gaattttcag tgggtgcaaaa aaaa 7431

<210> 3

<211> 2037

<212> PRT

<213> Homo sapiens

<400> 3

Met Leu Glu Ala Ile Asp Lys Asn Arg Ala Leu His Ala Ala Glu Arg
 1 5 10 15

Leu Gln Thr Lys Leu Arg Glu Arg Gly Asp Val Ala Asn Glu Asp Lys
 20 25 30

Leu Ser Leu Leu Lys Ser Val Leu Gln Ser Pro Leu Phe Ser Gln Ile
 35 40 45

Leu Ser Leu Gln Thr Ser Val Gln Gln Leu Lys Asp Gln Val Asn Ile
 50 55 60

Ala Thr Ser Ala Thr Ser Asn Ile Glu Tyr Ala His Val Pro His Leu
 65 70 75 80

Ser Pro Ala Val Ile Pro Thr Leu Gln Asn Glu Ser Phe Leu Leu Ser
 85 90 95

Pro Asn Asn Gly Asn Leu Glu Ala Leu Thr Gly Pro Gly Ile Pro His
 100 105 110

Ile Asn Gly Lys Pro Ala Cys Asp Glu Phe Asp Gln Leu Ile Lys Asn
 115 120 125

Met Ala Gln Gly Arg His Val Glu Val Phe Glu Leu Leu Lys Pro Pro
 130 135 140

Ser Gly Gly Leu Gly Phe Ser Val Val Gly Leu Arg Ser Glu Asn Arg
 145 150 155 160

Gly Glu Leu Gly Ile Phe Val Gln Glu Ile Gln Glu Gly Ser Val Ala
 165 170 175

His Arg Asp Gly Arg Leu Lys Glu Thr Asp Gln Ile Leu Ala Ile Asn
 180 185 190

Gly Gln Ala Leu Asp Gln Thr Ile Thr His Gln Gln Ala Ile Ser Ile
 195 200 205
 Leu Gln Lys Ala Lys Asp Thr Val Gln Leu Val Ile Ala Arg Gly Ser
 210 215 220
 Leu Pro Gln Leu Val Ser Pro Ile Val Ser Arg Ser Pro Ser Ala Ala
 225 230 235 240
 Ser Thr Ile Ser Ala His Ser Asn Pro Val His Trp Gln His Met Glu
 245 250 255
 Thr Ile Glu Leu Val Asn Asp Gly Ser Gly Leu Gly Phe Gly Ile Ile
 260 265 270
 Gly Gly Lys Ala Thr Gly Val Ile Val Lys Thr Ile Leu Pro Gly Gly
 275 280 285
 Val Ala Asp Gln His Gly Arg Leu Cys Ser Gly Asp His Ile Leu Lys
 290 295 300
 Ile Gly Asp Thr Asp Leu Ala Gly Met Ser Ser Glu Gln Val Ala Gln
 305 310 315 320
 Val Leu Arg Gln Cys Gly Asn Arg Val Lys Leu Met Ile Ala Arg Gly
 325 330 335
 Ala Ile Glu Glu Arg Thr Ala Pro Thr Ala Leu Gly Ile Thr Leu Ser
 340 345 350
 Ser Ser Pro Thr Ser Thr Pro Glu Leu Arg Val Asp Ala Ser Thr Gln
 355 360 365
 Lys Gly Glu Glu Ser Glu Thr Phe Asp Val Glu Leu Thr Lys Asn Val
 370 375 380
 Gln Gly Leu Gly Ile Thr Ile Ala Gly Tyr Ile Gly Asp Lys Lys Leu
 385 390 395 400
 Glu Pro Ser Gly Ile Phe Val Lys Ser Ile Thr Lys Ser Ser Ala Val
 405 410 415
 Glu His Asp Gly Arg Ile Gln Ile Gly Asp Gln Ile Ile Ala Val Asp
 420 425 430
 Gly Thr Asn Leu Gln Gly Phe Thr Asn Gln Gln Ala Val Glu Val Leu
 435 440 445
 Arg His Thr Gly Gln Thr Val Leu Leu Thr Leu Met Arg Arg Gly Met
 450 455 460
 Lys Gln Glu Ala Glu Leu Met Ser Arg Glu Asp Val Thr Lys Asp Ala
 465 470 475 480
 Asp Leu Ser Pro Val Asn Ala Ser Ile Ile Lys Glu Asn Tyr Glu Lys
 485 490 495
 Asp Glu Asp Phe Leu Ser Ser Thr Arg Asn Thr Asn Ile Leu Pro Thr
 500 505 510
 Glu Glu Glu Gly Tyr Pro Leu Leu Ser Ala Glu Ile Glu Glu Ile Glu
 515 520 525
 Asp Ala Gln Lys Gln Glu Ala Ala Leu Leu Thr Lys Trp Gln Arg Ile
 530 535 540

Met Gly Ile Asn Tyr Glu Ile Val Val Ala His Val Ser Lys Phe Ser
 545 550 555 560
 Glu Asn Ser Gly Leu Gly Ile Ser Leu Glu Ala Thr Val Gly His His
 565 570 575
 Phe Ile Arg Ser Val Leu Pro Glu Gly Pro Val Gly His Ser Gly Lys
 580 585 590
 Leu Phe Ser Gly Asp Glu Leu Leu Glu Val Asn Gly Ile Thr Leu Leu
 595 600 605
 Gly Glu Asn His Gln Asp Val Val Asn Ile Leu Lys Glu Leu Pro Ile
 610 615 620
 Glu Val Thr Met Val Cys Cys Arg Arg Thr Val Pro Pro Thr Thr Gln
 625 630 635 640
 Ser Glu Leu Asp Ser Leu Asp Leu Cys Asp Ile Glu Leu Thr Glu Lys
 645 650 655
 Pro His Val Asp Leu Gly Glu Phe Ile Gly Ser Ser Glu Thr Glu Asp
 660 665 670
 Pro Val Leu Ala Met Thr Asp Ala Gly Gln Ser Thr Glu Glu Val Gln
 675 680 685
 Ala Pro Leu Ala Met Trp Glu Ala Gly Ile Gln His Ile Glu Leu Glu
 690 695 700
 Lys Gly Ser Lys Gly Leu Gly Phe Ser Ile Leu Asp Tyr Gln Asp Pro
 705 710 715 720
 Ile Asp Pro Ala Ser Thr Val Ile Ile Ile Arg Ser Leu Val Pro Gly
 725 730 735
 Gly Ile Ala Glu Lys Asp Gly Arg Leu Leu Pro Gly Asp Arg Leu Met
 740 745 750
 Phe Val Asn Asp Val Asn Leu Glu Asn Ser Ser Leu Glu Glu Ala Val
 755 760 765
 Glu Ala Leu Lys Gly Ala Pro Ser Gly Thr Val Arg Ile Gly Val Ala
 770 775 780
 Lys Pro Leu Pro Leu Ser Pro Glu Glu Gly Tyr Val Ser Ala Lys Glu
 785 790 795 800
 Asp Ser Phe Leu Tyr Pro Pro His Ser Cys Glu Glu Ala Gly Leu Ala
 805 810 815
 Asp Lys Pro Leu Phe Arg Ala Asp Leu Ala Leu Val Gly Thr Asn Asp
 820 825 830
 Ala Asp Leu Val Asp Glu Ser Thr Phe Glu Ser Pro Tyr Ser Pro Glu
 835 840 845
 Asn Asp Ser Ile Tyr Ser Thr Gln Ala Ser Ile Leu Ser Leu His Gly
 850 855 860
 Ser Ser Cys Gly Asp Gly Leu Asn Tyr Gly Ser Ser Leu Pro Ser Ser
 865 870 875 880
 Pro Pro Lys Asp Val Ile Glu Asn Ser Cys Asp Pro Val Leu Asp Leu

885										890					895				
His	Met	Ser	Leu	Glu	Glu	Leu	Tyr	Thr	Gln	Asn	Leu	Leu	Gln	Arg	Gln				
			900					905					910						
Asp	Glu	Asn	Thr	Pro	Ser	Val	Asp	Ile	Ser	Met	Gly	Pro	Ala	Ser	Gly				
		915					920					925							
Phe	Thr	Ile	Asn	Asp	Tyr	Thr	Pro	Ala	Asn	Ala	Ile	Glu	Gln	Gln	Tyr				
	930					935					940								
Glu	Cys	Glu	Asn	Thr	Ile	Val	Trp	Thr	Glu	Ser	His	Leu	Pro	Ser	Glu				
945					950					955					960				
Val	Ile	Ser	Ser	Ala	Glu	Leu	Pro	Ser	Val	Leu	Pro	Asp	Ser	Ala	Gly				
				965					970					975					
Lys	Gly	Ser	Glu	Tyr	Leu	Leu	Glu	Gln	Ser	Ser	Leu	Ala	Cys	Asn	Ala				
			980					985					990						
Glu	Cys	Val	Met	Leu	Gln	Asn	Val	Ser	Lys	Glu	Ser	Phe	Glu	Arg	Thr				
		995					1000					1005							
Ile	Asn	Ile	Ala	Lys	Gly	Asn	Ser	Ser	Leu	Gly	Met	Thr	Val	Ser	Ala				
	1010					1015					1020								
Asn	Lys	Asp	Gly	Leu	Gly	Met	Ile	Val	Arg	Ser	Ile	Ile	His	Gly	Gly				
025					1030					1035				1040					
Ala	Ile	Ser	Arg	Asp	Gly	Arg	Ile	Ala	Ile	Gly	Asp	Cys	Ile	Leu	Ser				
			1045					1050					1055						
Ile	Asn	Glu	Glu	Ser	Thr	Ile	Ser	Val	Thr	Asn	Ala	Gln	Ala	Arg	Ala				
		1060						1065					1070						
Met	Leu	Arg	Arg	His	Ser	Leu	Ile	Gly	Pro	Asp	Ile	Lys	Ile	Thr	Tyr				
	1075						1080					1085							
Val	Pro	Ala	Glu	His	Leu	Glu	Glu	Phe	Lys	Ile	Ser	Leu	Gly	Gln	Gln				
	1090					1095					1100								
Ser	Gly	Arg	Val	Met	Ala	Leu	Asp	Ile	Phe	Ser	Ser	Tyr	Thr	Gly	Arg				
105					1110					1115				1120					
Asp	Ile	Pro	Glu	Leu	Pro	Glu	Arg	Glu	Glu	Gly	Glu	Gly	Glu	Glu	Ser				
			1125					1130					1135						
Glu	Leu	Gln	Asn	Thr	Ala	Tyr	Ser	Asn	Trp	Asn	Gln	Pro	Arg	Arg	Val				
		1140						1145					1150						
Glu	Leu	Trp	Arg	Glu	Pro	Ser	Lys	Ser	Leu	Gly	Ile	Ser	Ile	Val	Gly				
	1155						1160					1165							
Gly	Arg	Gly	Met	Gly	Ser	Arg	Leu	Ser	Asn	Gly	Glu	Val	Met	Arg	Gly				
	1170					1175					1180								
Ile	Phe	Ile	Lys	His	Val	Leu	Glu	Asp	Ser	Pro	Ala	Gly	Lys	Asn	Gly				
185					1190					1195				1200					
Thr	Leu	Lys	Pro	Gly	Asp	Arg	Ile	Val	Glu	Val	Asp	Gly	Met	Asp	Leu				
			1205					1210					1215						
Arg	Asp	Ala	Ser	His	Glu	Gln	Ala	Val	Glu	Ala	Ile	Arg	Lys	Ala	Gly				
		1220						1225					1230						

Asn Pro Val Val Phe Met Val Gln Ser Ile Ile Asn Arg Pro Arg Ala
 1235 1240 1245
 Pro Ser Gln Ser Glu Ser Glu Pro Glu Lys Ala Pro Leu Cys Ser Val
 1250 1255 1260
 Pro Pro Pro Pro Pro Ser Ala Phe Ala Glu Met Gly Ser Asp His Thr
 1265 1270 1275 1280
 Gln Ser Ser Ala Ser Lys Ile Ser Gln Asp Val Asp Lys Glu Asp Glu
 1285 1290 1295
 Phe Gly Tyr Ser Trp Lys Asn Ile Arg Glu Arg Tyr Gly Thr Leu Thr
 1300 1305 1310
 Gly Glu Leu His Met Ile Glu Leu Glu Lys Gly His Ser Gly Leu Gly
 1315 1320 1325
 Leu Ser Leu Ala Gly Asn Lys Asp Arg Ser Arg Met Ser Val Phe Ile
 1330 1335 1340
 Val Gly Ile Asp Pro Asn Gly Ala Ala Gly Lys Asp Gly Arg Leu Gln
 1345 1350 1355 1360
 Ile Ala Asp Glu Leu Leu Glu Ile Asn Gly Gln Ile Leu Tyr Gly Arg
 1365 1370 1375
 Ser His Gln Asn Ala Ser Ser Ile Ile Lys Cys Ala Pro Ser Lys Val
 1380 1385 1390
 Lys Ile Ile Phe Ile Arg Asn Lys Asp Ala Val Asn Gln Met Ala Val
 1395 1400 1405
 Cys Pro Gly Asn Ala Val Glu Pro Leu Pro Ser Asn Ser Glu Asn Leu
 1410 1415 1420
 Gln Asn Lys Glu Thr Glu Pro Thr Val Thr Thr Ser Asp Ala Ala Val
 1425 1430 1435 1440
 Asp Leu Ser Ser Phe Lys Asn Val Gln His Leu Glu Leu Pro Lys Asp
 1445 1450 1455
 Gln Gly Gly Leu Gly Ile Ala Ile Ser Glu Glu Asp Thr Leu Ser Gly
 1460 1465 1470
 Val Ile Ile Lys Ser Leu Thr Glu His Gly Val Ala Ala Thr Asp Gly
 1475 1480 1485
 Arg Leu Lys Val Gly Asp Gln Ile Leu Ala Val Asp Asp Glu Ile Val
 1490 1495 1500
 Val Gly Tyr Pro Ile Glu Lys Phe Ile Ser Leu Leu Lys Thr Ala Lys
 1505 1510 1515 1520
 Met Thr Val Lys Leu Thr Ile His Ala Glu Asn Pro Asp Ser Gln Ala
 1525 1530 1535
 Val Pro Ser Ala Ala Gly Ala Ala Ser Gly Glu Lys Lys Asn Ser Ser
 1540 1545 1550
 Gln Ser Leu Met Val Pro Gln Ser Gly Ser Pro Glu Pro Glu Ser Ile
 1555 1560 1565
 Arg Asn Thr Ser Arg Ser Ser Thr Pro Ala Ile Phe Ala Ser Asp Pro
 1570 1575 1580

Ala Thr Cys Pro Ile Ile Pro Gly Cys Glu Thr Thr Ile Glu Ile Ser
 585 1590 1595 1600
 Lys Gly Arg Thr Gly Leu Gly Leu Ser Ile Val Gly Gly Ser Asp Thr
 1605 1610 1615
 Leu Leu Gly Ala Ile Ile Ile His Glu Val Tyr Glu Glu Gly Ala Ala
 1620 1625 1630
 Cys Lys Asp Gly Arg Leu Trp Ala Gly Asp Gln Ile Leu Glu Val Asn
 1635 1640 1645
 Gly Ile Asp Leu Arg Lys Ala Thr His Asp Glu Ala Ile Asn Val Leu
 1650 1655 1660
 Arg Gln Thr Pro Gln Arg Val Arg Leu Thr Leu Tyr Arg Asp Glu Ala
 665 1670 1675 1680
 Pro Tyr Lys Glu Glu Glu Val Cys Asp Thr Leu Thr Ile Glu Leu Gln
 1685 1690 1695
 Lys Lys Pro Gly Lys Gly Leu Gly Leu Ser Ile Val Gly Lys Arg Asn
 1700 1705 1710
 Asp Thr Gly Val Phe Val Ser Asp Ile Val Lys Gly Gly Ile Ala Asp
 1715 1720 1725
 Ala Asp Gly Arg Leu Met Gln Gly Asp Gln Ile Leu Met Val Asn Gly
 1730 1735 1740
 Glu Asp Val Arg Asn Ala Thr Gln Glu Ala Val Ala Ala Leu Leu Lys
 745 1750 1755 1760
 Cys Ser Leu Gly Thr Val Thr Leu Glu Val Gly Arg Ile Lys Ala Gly
 1765 1770 1775
 Pro Phe His Ser Glu Arg Arg Pro Ser Gln Ser Ser Gln Val Ser Glu
 1780 1785 1790
 Gly Ser Leu Ser Ser Phe Thr Phe Pro Leu Ser Gly Ser Ser Thr Ser
 1795 1800 1805
 Glu Ser Leu Glu Ser Ser Ser Lys Lys Asn Ala Leu Ala Ser Glu Ile
 1810 1815 1820
 Gln Gly Leu Arg Thr Val Glu Met Lys Lys Gly Pro Thr Asp Ser Leu
 825 1830 1835 1840
 Gly Ile Ser Ile Ala Gly Gly Val Gly Ser Pro Leu Gly Asp Val Pro
 1845 1850 1855
 Ile Phe Ile Ala Met Met His Pro Thr Gly Val Ala Ala Gln Thr Gln
 1860 1865 1870
 Lys Leu Arg Val Gly Asp Arg Ile Val Thr Ile Cys Gly Thr Ser Thr
 1875 1880 1885
 Glu Gly Met Thr His Thr Gln Ala Val Asn Leu Leu Lys Asn Ala Ser
 1890 1895 1900
 Gly Ser Ile Glu Met Gln Val Val Ala Gly Gly Asp Val Ser Val Val
 905 1910 1915 1920
 Thr Gly His Gln Gln Glu Pro Ala Ser Ser Ser Leu Ser Phe Thr Gly

16

	1925		1930		1935
Leu Thr Ser Ser Ser Ile Phe Gln Asp Asp Leu Gly Pro Pro Gln Cys					
	1940		1945		1950
Lys Ser Ile Thr Leu Glu Arg Gly Pro Asp Gly Leu Gly Phe Ser Ile					
	1955		1960		1965
Val Gly Gly Tyr Gly Ser Pro His Gly Asp Leu Pro Ile Tyr Val Lys					
	1970		1975		1980
Thr Val Phe Ala Lys Gly Ala Ala Ser Glu Asp Gly Arg Leu Lys Arg					
985		1990		1995	2000
Gly Asp Gln Ile Ile Ala Val Asn Gly Gln Ser Leu Glu Gly Val Thr					
	2005		2010		2015
His Glu Glu Ala Val Ala Ile Leu Lys Arg Thr Lys Gly Thr Val Thr					
	2020		2025		2030
Leu Met Val Leu Ser					
	2035				

<210> 4
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 4
 Asn Glu Pro Phe Asp Glu Asp Gln His Thr Gln Ile Thr Lys Val
 1 5 10 15

<210> 5
 <211> 21
 <212> DNA
 <213> Homo sapiens

<400> 5
 agacagcaaa gatgacagta a 21

<210> 6
 <211> 20
 <212> DNA
 <213> Homo sapiens

<400> 6
 cttcctcctc tttgtatggg 20

<210> 7
 <211> 21
 <212> DNA
 <213> Homo sapiens

<400> 7
 gcttttgccg aaatgggtag t 21

<210> 8
 <211> 21
 <212> DNA
 <213> Homo sapiens

<400> 8
gatcggtcctt tggtcccagc a

21

<210> 9
<211> 19
<212> DNA
<213> Homo sapiens

<400> 9
tgtgagcaag ttttagtgag

19

<210> 10
<211> 19
<212> DNA
<213> Homo sapiens

<400> 10
ggtgattttc cccaagtaa

19

<210> 11
<211> 16
<212> PRT
<213> Homo sapiens

<400> 11
Ser Gly Ser Gly Ile Leu Ala Pro Pro Val Pro Pro Arg Asn Thr Arg
1 5 10 15

<210> 12
<211> 16
<212> PRT
<213> Homo sapiens

<400> 12
Glu Asn Glu Pro Phe Asp Glu Asp Gln His Thr Gln Ile Thr Lys Val
1 5 10 15

<210> 13
<211> 22
<212> DNA
<213> Homo sapiens

<400> 13
gccaccgcgg gattaagttt ct

22

<210> 14
<211> 23
<212> DNA
<213> Homo sapiens

<400> 14
tgtagccagc aatggtaatt cct

23

<210> 15
<211> 41
<212> DNA
<213> Homo sapiens

<400> 15
gttttcccag tcacgacggt tccattttaa ttgctgttaa t 41

<210> 16
<211> 43
<212> DNA
<213> Homo sapiens

<400> 16
aggaaacagc tatgaccatg gggataataa aaacgattca ggg 43

<210> 17
<211> 39
<212> DNA
<213> Homo sapiens

<400> 17
gttttcccag tcacgacggt gaatatgcc acgttcctc 39

<210> 18
<211> 42
<212> DNA
<213> Homo sapiens

<400> 18
aggaaacagc tatgaccatt ctttcaatct tccatctcta tg 42

<210> 19
<211> 39
<212> DNA
<213> Homo sapiens

<400> 19
gttttcccag tcacgacggt gaatatgcc acgttcctc 39

<210> 20
<211> 41
<212> DNA
<213> Homo sapiens

<400> 20
aggaaacagc tatgaccatc aaaccagatc catcattcac c 41

<210> 21
<211> 40
<212> DNA
<213> Homo sapiens

<400> 21
gttttcccag tcacgacggc acaatttcag ctcaactctaa 40

<210> 22
<211> 39
<212> DNA
<213> Homo sapiens

<400> 22

19

aggaaacagc tatgaccatg gatgaggaga gggatgatgc 39

<210> 23
<211> 22
<212> DNA
<213> Homo sapiens

<400> 23
tctagcagga atgagcagtg ag 22

<210> 24
<211> 24
<212> DNA
<213> Homo sapiens

<400> 24
gatcctgata atctaaaatg ctaa 24

<210> 25
<211> 40
<212> DNA
<213> Homo sapiens

<400> 25
gttttcccag tcacgacgaa gttgatgatt gcaagaggtg 40

<210> 26
<211> 41
<212> DNA
<213> Homo sapiens

<400> 26
aggaaacagc tatgaccatg gtttgtgcca tctactgcta t 41

<210> 27
<211> 39
<212> DNA
<213> Homo sapiens

<400> 27
gttttcccag tcacgacgaa acgagtgccg ttgagcatg 39

<210> 28
<211> 40
<212> DNA
<213> Homo sapiens

<400> 28
aggaaacagc tatgaccatg ctgacagtaa tggataccct 40

<210> 29
<211> 41
<212> DNA
<213> Homo sapiens

<400> 29
gttttcccag tcacgacgga ttttttatct tcgacgagaa a 41

<210> 30
<211> 42
<212> DNA
<213> Homo sapiens

<400> 30
aggaaacagc tatgaccatt tccccaagta aagttatgcc at 42

<210> 31
<211> 38
<212> DNA
<213> Homo sapiens

<400> 31
gttttcccag tcacgacgtc ctggttgaca cagcggga 38

<210> 32
<211> 39
<212> DNA
<213> Homo sapiens

<400> 32
aggaaacagc tatgaccatc atggccaaag gtgcttgaa 39

<210> 33
<211> 22
<212> DNA
<213> Homo sapiens

<400> 33
ccaccacca cccaatcaga at 22

<210> 34
<211> 22
<212> DNA
<213> Homo sapiens

<400> 34
catctcgact aatggcacct cc 22

<210> 35
<211> 38
<212> DNA
<213> Homo sapiens

<400> 35
gttttcccag tcacgacgga gacagaggat ccagtgct 38

<210> 36
<211> 39
<212> DNA
<213> Homo sapiens

<400> 36
aggaaacagc tatgaccatc cctgacggtg ctcccttca 39

<210> 37

<211> 40
<212> DNA
<213> Homo sapiens

<400> 37
gttttcccag tcacgacgtt aacttggaac acagcagctc 40

<210> 38
<211> 41
<212> DNA
<213> Homo sapiens

<400> 38
aggaaacagc tatgaccatc atcaccacaa gaactgccat g 41

<210> 39
<211> 40
<212> DNA
<213> Homo sapiens

<400> 39
gttttcccag tcacgacgac tctcctgaaa atgacagcat 40

<210> 40
<211> 41
<212> DNA
<213> Homo sapiens

<400> 40
aggaaacagc tatgaccatt aaatgagatt cagtccacac t 41

<210> 41
<211> 40
<212> DNA
<213> Homo sapiens

<400> 41
gttttcccag tcacgacgat aaatgactac acacctgcaa 40

<210> 42
<211> 41
<212> DNA
<213> Homo sapiens

<400> 42
aggaaacagc tatgaccata acgatcatcc ccaagccatc t 41

<210> 43
<211> 22
<212> DNA
<213> Homo sapiens

<400> 43
ctgagtacct gcttgaacag ag 22

<210> 44
<211> 22
<212> DNA

<213> Homo sapiens

<400> 44

gaccattgat ctctagaagc tc

22

<210> 45

<211> 41

<212> DNA

<213> Homo sapiens

<400> 45

gttttcccag tcacgacggg actattaata tagcaaaagg c

41

<210> 46

<211> 40

<212> DNA

<213> Homo sapiens

<400> 46

aggaaacagc tatgaccatc agtgccatta ctcttcaga

40

<210> 47

<211> 39

<212> DNA

<213> Homo sapiens

<400> 47

gttttcccag tcacgacgta cttatgtgcc tgcagaaca

39

<210> 48

<211> 40

<212> DNA

<213> Homo sapiens

<400> 48

aggaaacagc tatgaccatc atgtttgatg aaaatgcccc

40

<210> 49

<211> 39

<212> DNA

<213> Homo sapiens

<400> 49

gttttcccag tcacgacgat tggttggtgga cgagggatg

39

<210> 50

<211> 40

<212> DNA

<213> Homo sapiens

<400> 50

aggaaacagc tatgaccatc catttcggca aaggctgaag

40

<210> 51

<211> 39

<212> DNA

<213> Homo sapiens

<400> 51 gttttcccag tcacgacgca gagtcagagc cagagaagg	39
<210> 52 <211> 40 <212> DNA <213> Homo sapiens	
<400> 52 aggaaacagc tatgaccata gaagctcagc tgcaatttgc	40
<210> 53 <211> 21 <212> DNA <213> Homo sapiens	
<400> 53 caggcgagct gcatatgatt g	21
<210> 54 <211> 23 <212> DNA <213> Homo sapiens	
<400> 54 cctcctttga caatgtctga cac	23
<210> 55 <211> 41 <212> DNA <213> Homo sapiens	
<400> 55 gttttcccag tcacgacggt gtcttcatag tggggattga t	41
<210> 56 <211> 40 <212> DNA <213> Homo sapiens	
<400> 56 aggaaacagc tatgaccatg aagctccaga tgttgacat	40
<210> 57 <211> 39 <212> DNA <213> Homo sapiens	
<400> 57 gttttcccag tcacgacgag agccaactgt tactacttc	39
<210> 58 <211> 40 <212> DNA <213> Homo sapiens	
<400> 58 aggaaacagc tatgaccatt gaaggaacag cctgggaatc	40

<210> 59
<211> 39
<212> DNA
<213> Homo sapiens

<400> 59
gttttccag tcacgacgtt agccttctga agacagcaa 39

<210> 60
<211> 40
<212> DNA
<213> Homo sapiens

<400> 60
aggaaacagc tatgaccatc atggataata atggcaccca 40

<210> 61
<211> 39
<212> DNA
<213> Homo sapiens

<400> 61
gttttccag tcacgacgtt tccaaagggc gaacagggc 39

<210> 62
<211> 41
<212> DNA
<213> Homo sapiens

<400> 62
aggaaacagc tatgaccatc caacaatact taatcctagg c 41

<210> 63
<211> 23
<212> DNA
<213> Homo sapiens

<400> 63
tggaattgac ttgagaaagg cca 23

<210> 64
<211> 22
<212> DNA
<213> Homo sapiens

<400> 64
ccccctacag ttttgaagac cc 22

<210> 65
<211> 40
<212> DNA
<213> Homo sapiens

<400> 65
gttttccag tcacgacgaa gaggaggaag tgtgtgacac 40

<210> 66
<211> 40
<212> DNA
<213> Homo sapiens

<400> 66
aggaaacagc tatgaccatg acaggctgcc ttcactcacc

40

<210> 67
<211> 39
<212> DNA
<213> Homo sapiens

<400> 67
gttttcccag tcacgacgtc aaagctggtc cattccatt

39

<210> 68
<211> 40
<212> DNA
<213> Homo sapiens

<400> 68
aggaaacagc tatgaccatg gatgtgccac agatggtgac

40

<210> 69
<211> 39
<212> DNA
<213> Homo sapiens

<400> 69
gttttcccag tcacgacgat gatgcaccca actggagtt

39

<210> 70
<211> 40
<212> DNA
<213> Homo sapiens

<400> 70
aggaaacagc tatgaccatg gctgccatat cctccaacta

40

<210> 71
<211> 39
<212> DNA
<213> Homo sapiens

<400> 71
gttttcccag tcacgacggg acctcctcaa tgtaagtct

39

<210> 72
<211> 40
<212> DNA
<213> Homo sapiens

<400> 72
aggaaacagc tatgaccata ttgtcaggac cagtgcattc

40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09969

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.
US CL : 435/70.1, 320.1, 325; 530/350, 387.1; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/70.1, 320.1, 325; 530/350, 387.1; 536/23.5

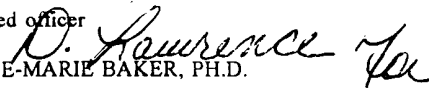
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
STN (file: medicine)
search terms: mmasc2, mmac1, interact?(10a)protein, cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEN et al. A study of the PTEN/MMAC1 gene in 136 breast cancer families. Human Genetics. January 1998, Vol. 102, No. 1, pages 124-125, entire document.	1-8, 12-18, 23-42, 45-73, 75-78
A	DAHIA et al. A highly conserved processed PTEN pseudogene is located on chromosome band 9p21. Oncogene. 07 May 1998, Vol. 16, No. 18, pages 2403-2406, entire document.	1-8, 12-18, 23-42, 45-73, 75-78
A	DUERR et al. PTEN mutations in gliomas and glioneuronal tumors. Oncogene. 30 April 1998, Vol. 16, No. 17, pages 2259-2264, entire document.	1-8, 12-18, 23-42, 45-73, 75-78

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.	
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
Date of the actual completion of the international search 21 JUNE 1999	Date of mailing of the international search report 04 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer  ANNE-MARIE BAKER, PH.D. Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09969

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	GRONBAEK et al. Alterations of the MMAC1/PTEN gene in lymphoid malignancies. Blood. 15 June 1998, Vol. 91, No. 12, pages 4388-4390, entire document.	1-8, 12-18, 23-42, 45-73, 75-78
A	WHANG et al. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. Proc. Natl. Acad. Sci. USA. April 1998, Vol. 95, No. 9, pages 5246-5250, entire document.	1-8, 12-18, 23-42, 45-73, 75-78

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09969

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 21/04; C07K 14/00, 16/00; C12N 15/63, 15/85, 15/86; C12P 21/00