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(54) **BINDING POLYPEPTIDES AND METHODS
BASED THEREON**

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(57) **ABSTRACT**

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Binding polypeptides that specifically bind BLYS protein or BLYS-like polypeptides can be used in methods of the invention for detecting, diagnosing, or prognosing a disease or disorder associated with aberrant BLYS or BLYS receptor expression or inappropriate function of BLYS or BLYS receptor, comprising BLYS binding polypeptides or fragments or variants thereof, that specifically bind to BLYS. The present invention further relates to methods and compositions for preventing, treating or ameliorating a disease or disorder associated with aberrant BLYS or BLYS receptor expression or inappropriate BLYS function or BLYS receptor function, comprising administering to an animal, preferably a human, an effective amount of one or more BLYS binding polypeptides or fragments or variants thereof, that specifically bind to BLYS.

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BINDING POLYPEPTIDES AND METHODS BASED THEREON

FIELD OF THE INVENTION

[0001] The present invention relates to therapeutic and diagnostic uses for molecules that bind to B lymphocyte stimulator protein (BLyS). In particular, the present invention also relates to methods and compositions for detecting, diagnosing, or prognosing a disease or disorder associated with aberrant BLyS or BLyS receptor expression or inappropriate function of BLyS or BLyS receptor, comprising BLyS binding polypeptides or fragments or variants thereof, that specifically bind to BLyS. The present invention further relates to methods and compositions for preventing, treating or ameliorating a disease or disorder associated with aberrant BLyS or BLyS receptor expression or inappropriate BLyS function or BLyS receptor function, comprising administering to an animal, preferably a human, an effective amount of one or more BLyS binding polypeptides or fragments or variants thereof, that specifically bind to BLyS.

BACKGROUND OF THE INVENTION

[0002] B lymphocyte stimulator (BLyS) is a member of the tumor necrosis factor ("TNF") superfamily that induces both in vivo and in vitro B cell proliferation and differentiation (Moore et al., *Science*, 285: 260-263 (1999)). BLyS is distinguishable from other B cell growth and differentiation factors such as IL-2, IL-4, IL-5, IL-6, IL-7, IL-13, IL-15, CD40L, or CD27L (CD70) by its monocyte-specific gene and protein expression pattern and its specific receptor distribution and biological activity on B lymphocytes. BLyS expression is not detected on natural killer ("NK") cells, T cells or B cells, but is restricted to cells of myeloid origin. BLyS expression on resting monocytes is upregulated by interferon-gamma (IFN-gamma). The gene encoding BLyS has been mapped to chromosome 13q34.

[0003] BLyS is expressed as a 285 amino acid type II membrane-bound polypeptide and a soluble 152 amino acid polypeptide (Moore et al., 1999, supra). The membrane-bound form of BLyS has a predicted transmembrane spanning domain between amino acid residues 47 and 73. The NH₂-terminus of the soluble form of BLyS begins at Ala¹³⁴ of the membrane-bound form of BLyS. Both the soluble and membrane-bound forms of the protein form homotrimers. Soluble recombinant BLyS has been shown to induce in vitro proliferation of murine splenic B cells and to bind to a cell-surface receptor on these cells (Moore et al., 1999, supra). Soluble BLyS administration to mice has been shown to result in an increase in the proportion of CD45R^{dull}, Ly6 D^{bright} (also known as ThB) B cells and an increase in serum IgM and IgA levels (Moore et al., 1999, supra). Thus, BLyS displays a B cell tropism in both its receptor distribution and biological activity.

[0004] Based on its expression pattern and biological activity, BLyS has been suggested to be involved in the exchange of signals between B cells and monocytes or their differentiated progeny. The restricted expression patterns of BLyS receptor and ligand suggest that BLyS may function as a regulator of T cell-independent responses in a manner analogous to that of CD40 and CD40L in T cell-dependent antigen activation.

[0005] Accordingly, molecules that specifically bind BLyS would find a variety of uses in the study of the BLyS

cytokine, in the manufacture and purification of BLyS in commercial and medically pure quantities, and in the development new therapeutic or diagnostic reagents. BLyS binding polypeptides may also find medical utility in, for example, the treatment of B cell and/or monocyte disorders associated with autoimmunity, neoplasia, or immunodeficiency syndromes.

SUMMARY OF THE INVENTION

[0006] New polypeptides that specifically bind to B lymphocyte stimulator protein (BLyS) and/or BLyS-like polypeptides have been discovered, and the therapeutic and diagnostic applications for such polypeptides are disclosed herein. Particular polypeptides useful in the methods of this invention specifically bind to a polypeptide or polypeptide fragment of human BLyS (SEQ ID NOs:173 and/or 174) or BLyS expressed on human monocytes; murine BLyS (SEQ ID NOs:175 and/or 176) or BLyS expressed on murine monocytes; rat BLyS (either the soluble forms as given in SEQ ID NOs:177, 178, 179 and/or 180 or in a membrane associated form, e.g., on the surface of rat monocytes); or monkey BLyS (e.g., the monkey BLyS polypeptides of SEQ ID NOS:181 and/or 182, the soluble form of monkey BLyS, or BLyS expressed on monkey monocytes), preferably human BLyS.

[0007] In preferred methods of the invention, BLyS binding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of SEQ ID NOs:1-12, 20-172, and 186-444, preferably SEQ ID NOs:163-172 and 436-444 as referred to herein and in Tables 1-8, 13 and 14, and fragments and variants thereof, will be used.

[0008] In specific preferred embodiments, the BLyS binding polypeptides bind BLyS and/or BLyS-like polypeptides with high affinity. In other embodiments, the BLyS binding polypeptides reversibly bind BLyS and/or BLyS-like polypeptides. In still other embodiments, the BLyS binding polypeptides irreversibly bind BLyS and/or BLyS-like polypeptides.

[0009] The cysteine residues in certain polypeptides useful in the methods of the invention are believed to form a disulfide bond, which would cause the polypeptide containing these cysteine residues to form a stable loop structure under non-reducing conditions. Especially preferred BLyS binding polypeptides useful in the methods of the invention are polypeptide molecules that comprise amino acid sequences that form stable loop structures or other stable structures that bind BLyS or BLyS-like polypeptides.

[0010] Analysis of the sequences of the BLyS binding polypeptides described herein shows a strong selection for polypeptides containing the tetrapeptide Asp-Xaa-Leu-Thr (SEQ ID NO:446), and therefore in its broadest aspects, the present invention relates to methods for using polypeptides capable of binding to BLyS comprising the polypeptide Asp-Xaa-Leu-Thr (SEQ ID NO:446), where Xaa is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala (preferably Pro or Ser).

[0011] In addition, seven consensus sequences (SEQ ID NOs:1-7) are disclosed for peptides useful in the methods of the invention, based on the specific BLyS binding polypeptides shown in Tables 1-8. In preferred methods according to the invention, BLyS binding polypeptides comprising one or

more of these sequences are used. Such preferred methods utilize BLYS binding polypeptides including polypeptides with the potential to form a cyclic or loop structure between invariant Cys residues comprising, or alternatively consisting of, an amino acid sequence selected from A-E (SEQ ID NOs:1-5):

X₁₂ is Asp, Gln, Glu, Gly, Ile, Leu, Lys, Phe, Ser, Trp, Tyr, or Val;

X₁₃ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; and

(A) X₁-X₂-X₃-Cys-X₅-Phe-X₇-Trp-Glu-Cys-X₁₁-X₁₂-X₁₃, (SEQ ID NO:1)

wherein

X₁ is Ala, Asn, Lys, or Ser;

X₂ is Ala, Glu, Met, Ser, or Val;

X₁₄ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Trp, Tyr, Val, or is absent,

[0013] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

(C) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₃-X₁₄-X₁₅, (SEQ ID NO:3)

X₃ is Ala, Asn, Lys, or Pro (preferably Lys);

X₅ is Phe, Trp, or Tyr (preferably Tyr);

X₇ is Pro or Tyr (preferably Pro);

X₁₁ is Ala, Gln, His, Phe, or Val;

X₁₂ is Asn, Gln, Gly, His, Ser, or Val; and

X₁₃ is Ala, Asn, Gly, Ile, Pro, or Ser,

[0012] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

wherein

X₁ is Ala, Arg, Asn, Asp, Leu, Lys, Phe, Pro, Ser, or Thr;

X₂ is Asn, Asp, Gln, His, Ile, Lys, Pro, Thr, or Trp;

X₃ is Ala, Arg, Asn, Gln, Glu, His, Phe, Pro, or Thr (preferably Ala);

X₅ is Asn, Asp, Pro, Ser, or Thr (preferably Asp);

X₆ is Arg, Asp, Ile, Leu, Met, Pro, or Val (preferably Ile);

(B) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-Cys-X₁₂-X₁₃-X₁₄, (SEQ ID NO:2)

wherein

X₁ is Ala, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, or is absent;

X₂ is Ala, Asn, Asp, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val;

X₃ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val (preferably Asp);

X₅ is Asp, Ile, Leu, or Tyr (preferably Asp or Leu);

X₆ is Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Pro, Tyr, or Val (preferably Glu or Leu);

X₇ is His, Leu, Lys, or Phe (preferably His or Leu);

X₈ is Leu, Pro, or Thr (preferably Thr or Pro);

X₉ is Arg, Asn, Gly, His, Ile, Lys, Met, or Trp (preferably Lys);

X₁₀ is Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Trp, Tyr, or Val;

X₇ is Ala, Ile, Leu, Pro, Thr, or Val (preferably Val or Leu);

X₈ is Asn, His, Ile, Leu, Lys, Phe, or Thr (preferably Thr);

X₉ is Asn, Glu, Gly, His, Leu, Lys, Met, Pro, or Thr (preferably Leu);

X₁₀ is Arg, Asn, Asp, Gln, Glu, Gly, Ile, Lys, Met, Pro, Ser, or Trp;

X₁₁ is Arg, Glu, Gly, Lys, Phe, Ser, Trp, or Tyr (preferably Ser);

X₁₃ is Gln, Glu, Ile, Leu, Phe, Pro, Ser, Tyr, or Val (preferably Val);

X₁₄ is Asn, Gly, Ile, Phe, Pro, Thr, Trp, or Tyr; and

X₁₅ is Asn, Asp, Glu, Leu, Lys, Met, Pro, or Thr (preferably Glu or Pro),

[0014] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

(D) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-Cys-X₁₄-X₁₅-X₁₆, (SEQ ID NO:4)

wherein

X₁ is Asn, Asp, His, Leu, Phe, Pro, Ser, Tyr, or is absent (preferably Ser);

X₂ is Arg, Asn, Asp, His, Phe, Ser, or Trp (preferably Arg);

X₃ is Asn, Asp, Leu, Pro, Ser, or Val (preferably Asn or Asp);

X₅ is Asp, Gln, His, Ile, Leu, Lys, Met, Phe, or Thr;

X₆ is His, Ile, Leu, Met, Phe, Pro, Trp, or Tyr;

X₇ is Asp, His, Leu, or Ser (preferably Asp);

X₈ is Ala, Arg, Asp, Glu, Leu, Phe, Pro, or Thr (preferably Glu or Pro);

X₉ is Ala, Arg, Asn, or Leu (preferably Leu);

X₁₀ is Ile, Leu, Met, Pro, Ser, or Thr (preferably Thr);

X₁₁ is Ala, Arg, Asn, Gly, His, Lys, Ser, or Tyr;

X₁₂ is Ala, Arg, Asn, Gln, Leu, Met, Ser, Trp, Tyr, or Val;

X₁₄ is Asp, Gly, Leu, Phe, Tyr, or Val (preferably Leu);

X₁₅ is Asn, His, Leu, Pro, or Tyr (preferably His, Leu or Pro); and

X₁₆ is Asn, Asp, His, Phe, Ser, or Tyr, (preferably Asp or Ser),

[0015] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

X₉ is Asp, Leu, Pro, Thr, or Val (preferably Leu or Thr);

X₁₀ is Arg, Gln, His, Ile, Leu, Lys, Met, Phe, Thr, Trp or Tyr (preferably Lys or Thr);

X₁₁ is Ala, Arg, Asn, Gln, Glu, His, Leu, Lys, Met, or Thr (preferably Arg or Leu);

X₁₂ is Ala, Asn, Gln, Gly, Leu, Lys, Phe, Pro, Thr, Trp, or Tyr (preferably Thr or Trp);

X₁₃ is Ala, Arg, Gln, His, Lys, Met, Phe, Pro, Thr, Trp, or Tyr (preferably Met or Phe);

X₁₄ is Arg, Gln, Glu, Gly, His, Leu, Met, Phe, Pro, Ser, Thr, Tyr, or Val (preferably Val);

X₁₆ is Arg, Asp, Gly, His, Lys, Met, Phe, Pro, Ser, or Trp (preferably Met);

X₁₇ is Arg, Asn, Asp, Gly, His, Phe, Pro, Ser, Trp or Tyr, (preferably Arg, His, or Tyr); and

X₁₈ is Ala, Arg, Asn, Asp, His, Leu, Phe, or Trp (preferably His or Asn),

wherein said polypeptide binds BLYS and/or BLYS-like polypeptides.

(E) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃-X₁₄-Cys-X₁₆-X₁₇-X₁₈, (SEQ ID NO:5)

wherein

X₁ is Arg, Asp, Gly, His, Leu, Phe, Pro, Ser, Trp, Tyr, or is absent (preferably Arg);

[0016] Additional preferred embodiments include methods utilizing linear BLYS binding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from F and G (SEQ ID NOs:6 and 7):

(F) X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂, (SEQ ID NO:6)

X₂ is Ala, Arg, Asn, Asp, Gly, Pro, Ser, or is absent (preferably Asn, Asp, Gly, or Pro);

X₃ is Arg, Asn, Gln, Glu, Gly, Lys, Met, Pro, Trp or Val (preferably Gly or Met);

X₅ is Arg, Asn, Gln, Glu, His, Leu, Phe, Pro, Trp, Tyr, or Val (preferably Trp, Tyr, or Val);

X₆ is Arg, Asp, Gln, Gly, Ile, Lys, Phe, Thr, Trp or Tyr (preferably Asp);

X₇ is Ala, Arg, Asp, Glu, Gly, Leu, Ser, or Tyr (preferably Asp);

X₈ is Asp, Gln, Glu, Leu, Met, Phe, Pro, Ser, or Tyr (preferably Leu);

wherein

X₁ is Ala, Arg, Gly, His, Leu, Lys, Met, Phe, Trp, Tyr, or Val (preferably Gly, Tyr, or Val);

X₂ is Ala, Arg, Gln, His, Ile, Leu, Phe, Thr, Trp, or Tyr (preferably His or Tyr);

X₃ is Ala, Asp, Lys, Phe, Thr, Trp or Tyr (preferably Asp or Tyr);

X₄ is Arg, Asp, Gln, Lys, Met, Phe, Pro, Ser, Tyr, or Val (preferably Asp or Gln);

X₅ is Asp, Leu, Lys, Phe, Pro, Ser, or Val (preferably Leu or Ser);

X₆ is His, Ile, Leu, Pro, Ser, or Thr (preferably Leu or Thr);

X₇ is Arg, Gly, His, Leu, Lys, Met, or Thr (preferably Lys or Thr);

X₈ is Ala, Arg, Asn, Ile, Leu, Lys, Met, or Thr (preferably Leu or Lys);

X₉ is Ala, Asn, Arg, Asp, Glu, Gly, His, Leu, Met, Ser, Trp, Tyr, or Val (preferably Met or Ser);

X₁₀ is Ile, Leu, Phe, Ser, Thr, Trp, Tyr, or Val (preferably Thr or Leu);

X₁₁ is Ala, Arg, Gly, His, Ile, Leu, Lys, Pro, Ser, Thr, Trp, Tyr, or Val (preferably Pro or Thr); and

X₁₂ is Arg, Asp, His, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val (preferably Arg or Pro),

[0017] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

(G) X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃, (SEQ ID NO:7)

wherein

X₁ is Asp, Gln, Glu, Gly, His, Lys, Met, or Trp (preferably Glu, Lys);

X₂ is Arg, Gln, His, Ile, Leu, or Pro (preferably His or Pro);

X₃ is Asp, Gly, Ile, Lys, Thr, Tyr or Val (preferably Tyr);

X₄ is Asn, Asp, Gln, Glu, Met, Pro, Ser, or Tyr (preferably Asp or Gln);

X₅ is Asn, Asp, His, Ile, Leu, Met, Pro, Thr or Val (preferably Asn or Thr);

X₆ is Asp, Glu, His, Leu, Lys, Pro, or Val (preferably Asp or Pro);

X₇ is Arg, Asn, Gln, His, Ile, Leu, Met, Pro, or Thr (preferably Ile or Pro);

X₈ is Gln, Gly, His, Leu, Met, Ser, or Thr (preferably Leu or Thr);

X₉ is Asn, Gln, Gly, His, Leu, Lys, Ser, or Thr (preferably Lys);

X₁₀ is Ala, Gly, Ile, Leu, Lys, Met, or Phe (preferably Gly or Met);

X₁₁ is Ala, Glu, His, Ile, Leu, Met, Ser, Thr, Trp, Tyr, or Val (preferably Ala or Thr);

X₁₂ is Arg, Gln, Glu, Gly, His, Ile, Lys, Tyr, or Val (preferably Arg or His); and

X₁₃ is Arg, Asn, Glu, His, Ile, Ser, Thr, Trp, or Val (preferably His),

wherein said polypeptide binds BLYS and/or BLYS-like polypeptides.

[0018] Additional polypeptides useful in the methods of the invention include polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from H-L (SEQ ID NOs:8-12):

(H) Cys-X₂-Phe-X₄-Trp-Glu-Cys, (SEQ ID NO:8)

wherein

X₂ is Phe, Trp, or Tyr (preferably Tyr); and

[0019] X₄ is Pro or Tyr (preferably Pro); or

(I) Cys-X₂-X₃-X₄-X₅-X₆-X₇-Cys, (SEQ ID NO:9)

wherein

X₂ is Asp, Ile, Leu, or Tyr (preferably Asp or Leu);

X₃ is Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Pro, Tyr, or Val (preferably Glu or Leu);

X₄ is His, Leu, Lys, or Phe (preferably His or Leu);

X₅ is Leu, Pro, or Thr (preferably Thr or Pro);

X₆ is Arg, Asn, Gly, His, Ile, Lys, Met, or Trp (preferably Lys); and

[0020] X₇ is Ala, Asn, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Trp, Tyr, or Val; or

(J) Cys-X₂-X₃-X₄-X₅-X₆-X₇-X₈-Cys, (SEQ ID NO:10)

wherein

X₂ is Asn, Asp, Pro, Ser, or Thr (preferably Asp);

X₃ is Arg, Asp, Ile, Leu, Met, Pro, or Val (preferably Ile);

X₄ is Ala, Ile, Leu, Pro, Thr, or Val (preferably Val or Leu);

X₅ is Asn, His, Ile, Leu, Lys, Phe, or Thr (preferably Thr);

X₆ is Asn, Glu, Gly, His, Leu, Lys, Met, Pro, or Thr (preferably Leu);

X₇ is Arg, Asn, Asp, Gln, Glu, Gly, Ile, Lys, Met, Pro, Ser, or Trp;

[0021] X₈ is Arg, Glu, Gly, Lys, Phe, Ser, Trp, or Tyr (preferably Ser); or

(K) Cys-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-Cys, (SEQ ID NO:11)

wherein

X₂ is Asp, Gln, His, Ile, Leu, Lys, Met, Phe, or Thr;

X₃ is His, Ile, Leu, Met, Phe, Pro, Trp, or Tyr;

X₄ is Asp, His, Leu, or Ser (preferably Asp);

X₅ is Ala, Arg, Asp, Glu, Leu, Phe, Pro, or Thr (preferably Glu or Pro);

X₆ is Ala, Arg, Asn, or Leu (preferably Leu);

X₇ is Ile, Leu, Met, Pro, Ser, or Thr (preferably Thr);

X₈ is Ala, Arg, Asn, Gly, His, Lys, Ser, or Tyr;

[0022] X₉ is Ala, Arg, Asn, Gln, Leu, Met, Ser, Trp, Tyr, or Val; or

(L) Cys-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys, (SEQ ID NO:12)

wherein

X₂ is Arg, Asn, Gln, Glu, His, Leu, Phe, Pro, Trp, Tyr, or Val (preferably Trp, Tyr, or Val);

X₃ is Arg, Asp, Gln, Gly, Ile, Lys, Phe, Thr, Trp or Tyr (preferably Asp);

X₄ is Ala, Arg, Asp, Glu, Gly, Leu, Ser, or Tyr (preferably Asp);

X₁₄ is Asp, Glu, Asn, Val, His, Gln, Arg, Gly, Ser, Tyr, Ala, Cys, Lys, Ile, Thr or Leu (i.e., not Phe, Met, Pro, or Trp; preferably Asp, Val or Glu).

[0024] Preferred methods will utilize polypeptides comprising a core sequence of the formula N:

(N) X₁-X₂-Asp-X₄-Leu-Thr-X₇-Leu-X₉-X₁₀, (SEQ ID NO:448)

X₅ is Asp, Gln, Glu, Leu, Met, Phe, Pro, Ser, or Tyr (preferably Leu);

X₆ is Asp, Leu, Pro, Thr, or Val (preferably Leu or Thr);

X₇ is Arg, Gln, His, Ile, Leu, Lys, Met, Phe, Thr, Trp or Tyr (preferably Lys or Thr);

X₈ is Ala, Arg, Asn, Gln, Glu, His, Leu, Lys, Met, or Thr (preferably Arg or Leu);

X₉ is Ala, Asn, Gln, Gly, Leu, Lys, Phe, Pro, Thr, Trp, or Tyr (preferably Thr or Trp);

X₁₀ is Ala, Arg, Gln, His, Lys, Met, Phe, Pro, Thr, Trp, or Tyr (preferably Met or Phe);

X₁₁ is Arg, Gln, Glu, Gly, His, Leu, Met, Phe, Pro, Ser, Thr, Tyr, or Val (preferably Val);

wherein said polypeptides bind BLYS and/or BLYS-like polypeptides.

[0023] In preferred embodiments of the present invention, BLYS binding polypeptides are used which comprise the following amino acid sequence M (SEQ ID NO:447):

(M) Ala-X₂-X₃-X₄-Asp-X₆-Leu-Thr-X₉-Leu-X₁₁-X₁₂-X₁₃-X₁₄, (SEQ ID NO:447)

wherein

X₂ is Asn, Ser, Tyr, Asp, Phe, Ile, Gln, His, Pro, Lys, Leu, Met, Thr, Val, Glu, Ala, Gly, Cys, or Trp (i.e., any amino acid except Arg; preferably Asn);

X₃ is Trp, Glu, Lys, Cys, Leu, Ala, Arg, Gly, or Ser (preferably Trp);

X₄ is Tyr, Phe, Glu, Cys, Asn (preferably Tyr);

X₆ is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala (preferably Pro or Ser);

X₉ is Lys, Asn, Gln, Gly, or Arg (preferably Lys);

X₁₁ is Trp, Ser, Thr, Arg, Cys, Tyr, or Lys (preferably Trp);

X₁₂ is Leu, Phe, Val, Ile, or His (preferably Leu);

X₁₃ is Pro, Leu, His, Ser, Arg, Asn, Gln, Thr, Val, Ala, Cys, Ile, Phe, or Tyr (i.e., not Asp, Glu, Gly, Lys, Met, or Trp; preferably Pro); and

wherein

X₁ is Trp, Glu, Lys, Cys, Leu, Ala, Arg, Gly, or Ser (preferably Trp);

X₂ is Tyr, Phe, Glu, Cys, Asn (preferably Tyr);

X₄ is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala (preferably Pro or Ser);

X₇ is Lys, Asn, Gln, Gly, or Arg (preferably Lys);

X₉ is Trp, Ser, Thr, Arg, Cys, Tyr, or Lys (preferably Trp); and

X₁₀ is Leu, Phe, Val, Ile, or His (preferably Leu).

[0025] Especially preferred methods according to the invention will utilize BLYS binding polypeptides which comprise the core peptide Trp-Tyr-Asp-Pro-Leu-Thr-Lys-Leu-Trp-Leu (SEQ ID NO:436).

[0026] BLYS binding polypeptides used in the methods of the present invention may also have an amino terminal (N-terminal) capping or functional group, such as an acetyl group, which, for example, blocks the amino terminal amino group from undesirable reactions or is useful in linking the BLYS binding polypeptide to another molecule, matrix,

resin, or solid support. BLYS binding polypeptides may also have a carboxy terminal (C-terminal) capping or functional group, such as an amide group, which, for example, blocks the C-terminal carboxyl group from undesirable reactions or provides a functional group useful in conjugating the binding polypeptide to other molecules, matrices, resins, or solid supports. Preferably, the N- and/or C-terminal capping groups are polypeptide linker molecules. An especially preferred C-terminal linker molecule that is useful for immobilizing a BLYS binding polypeptide to a solid support or chromatographic matrix material comprises the amino acid sequence Pro-Gly-Pro-Glu-Gly-Gly-Gly-Lys (SEQ ID NO:13). Another useful C-terminal linker, e.g., for fluoresceinating peptides, is Gly-Gly-Lys (see Table 14).

[0027] In the methods of the present invention, it may be advantageous to use BLYS binding polypeptides that have

been modified, for example, to increase or decrease the stability of the molecule, while retaining the ability to bind BLYS and/or BLYS-like polypeptides. An example of a modified BLYS binding polypeptide is a polypeptide in which one of two cysteine residues is substituted with a non-naturally occurring amino acid that is capable of condensing with the remaining cysteine side chain to form a stable thioether bridge, thereby generating a cyclic BLYS binding polypeptide. Such cyclic thioether molecules of synthetic peptides may be routinely generated using techniques known in the art, e.g., as described in PCT publication WO 97/46251, incorporated herein by reference.

[0028] Some of the methods provided herein utilize BLYS binding polypeptides that have been attached, coupled, linked or adhered to a matrix or resin or solid support. Techniques for attaching, linking or adhering polypeptides to matrices, resins and solid supports are well known in the art. Suitable matrices, resins or solid supports for these materials may be any composition known in the art to which a BLYS binding polypeptide could be attached, coupled, linked, or adhered, including but not limited to, a chromatographic resin or matrix, such as SEPHAROSE-4 FF agarose beads, the wall or floor of a well in a plastic microtiter dish, such as used in an enzyme-linked immunosorbent assay (ELISA), or a silica based biochip. Materials useful as solid supports on which to immobilize binding polypeptides for use in the methods include, but are not limited to, polyacrylamide, agarose, silica, nitrocellulose, paper, plastic, nylon, metal, and combinations thereof. A BLYS binding polypeptide may be immobilized on a matrix, resin or solid support material by a non-covalent association or by covalent bonding, using techniques known in the art.

[0029] In certain embodiments of the present invention, it is preferred to utilize BLYS binding polypeptides or phage displaying such binding polypeptides that irreversibly bind the BLYS protein in its native, soluble trimeric form.

[0030] In certain embodiments of the present, it is preferred to utilize BLYS binding polypeptides of the present invention or phage displaying such binding polypeptides that reversibly bind the BLYS protein in its native, soluble trimeric form.

[0031] In further embodiments of the present invention, a method may call for the use of a composition of matter comprising isolated nucleic acids, preferably DNA, encoding a BLYS binding polypeptide. In specific embodiments, nucleic acid molecules encode a BLYS binding polypeptide comprising the amino acid sequence of SEQ ID NOs:1-12, 20-172, or 186-444. In additional embodiments, the nucleic acid molecules encode a polypeptide variant or fragment of a polypeptide comprising an amino acid sequence of SEQ ID NOs:1-12, 20-172, or 186-444. In a further additional embodiment, such nucleic acid molecules encode a BLYS binding polypeptide, the complementary strand of which nucleic acid hybridizes to a polynucleotide sequence encoding a polypeptide described in Tables 1-8 and 13 and in Examples 2, 5 and 6 (SEQ ID NOs:1-12, 20-172 and 186-444), under stringent conditions, e.g., hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45° C. followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65° C., under highly stringent conditions, e.g., hybridization to filter-bound nucleic acid in 6xSSC at about 45° C. followed by one or more

washes in 0.1xSSC/0.2% SDS at about 68° C., or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F. M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. 1, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3).

[0032] In further embodiments of the invention, recombinant bacteriophage are utilized which display BLYS binding polypeptides on their surfaces. Such phage may be routinely generated using techniques known in the art and are useful, for example, as screening reagents and reagents for detecting BLYS.

[0033] In other methods according to the invention, a BLYS binding polypeptide is used to detect or isolate BLYS or BLYS-like polypeptides in a solution. Such solutions include, but are not limited to, BLYS or BLYS-like polypeptides suspended or dissolved in water or a buffer solution as well as any fluid and/or cell obtained from an individual, biological fluid, body tissue, body cell, cell line, tissue culture, or other source which may contain BLYS or BLYS-like polypeptides, such as, cell culture medium, cell extracts, and tissue homogenates. Biological fluids include, but are not limited to, sera, plasma, lymph, blood, blood fractions, urine, synovial fluid, spinal fluid, saliva, and mucous.

[0034] Methods according to the present invention may advantageously utilize panels of BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants) wherein the panel members correspond to one, two, three, four, five, ten, fifteen, twenty, or more different BLYS binding polypeptides. Methods according to the present invention may alternatively use mixtures of BLYS binding polypeptides, wherein the mixture corresponds to one, two, three, four, five, ten, fifteen, twenty, or more different BLYS binding polypeptides. The present invention also provides methods of using compositions comprising, or alternatively consisting of, one, two, three, four, five, ten, fifteen, twenty, or more BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof). Alternatively, a method according to the invention may utilize a composition comprising, or alternatively consisting of, nucleic acid molecules encoding one or more BLYS binding polypeptides.

[0035] The methods of the present invention also provides for the use of fusion proteins comprising a BLYS binding polypeptide (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof), and a heterologous polypeptide. A composition useful in methods of the present invention may comprise, or alternatively consist of, one, two, three, four, five, ten, fifteen, twenty or more fusion proteins capable of binding to BLYS. Alternatively, a composition useful in methods of the invention may comprise, or alternatively consist of, nucleic acid molecules encoding one, two, three, four, five, ten, fifteen, twenty or more such fusion proteins.

[0036] The present invention encompasses methods and compositions for detecting, diagnosing, prognosing, and/or monitoring diseases or disorders associated with aberrant BLYS or BLYS receptor expression or inappropriate BLYS or BLYS receptor function in an animal, preferably a mammal, and most preferably a human, comprising, or alternatively consisting of, use of BLYS binding polypeptides (including

molecules which comprise, or alternatively consist of, BLYS binding polypeptide fragments or variants thereof) that specifically bind to BLYS. Diseases and disorders which can be detected, diagnosed, prognosed and/or monitored with the BLYS binding polypeptides include, but are not limited to, immune system diseases or disorders (e.g., autoimmune diseases or disorders, immunodeficiencies, lupus, glomerular nephritis, rheumatoid arthritis, multiple sclerosis, graft vs. host disease, myasthenia gravis, Hashimoto's disease, and immunodeficiency syndrome), proliferative diseases or disorders (e.g., cancer, premalignant conditions, benign tumors, hyperproliferative disorders, benign proliferative disorders, leukemia, lymphoma, chronic lymphocytic leukemia, multiple myeloma, Hodgkin's lymphoma, Hodgkin's disease, T cell proliferative diseases and disorders, B cell proliferative diseases and disorders, monocytic proliferative diseases or disorders, acute myelogenous leukemia, macrophage proliferative diseases and disorders, and carcinoma), infectious diseases (e.g., AIDS), and inflammatory disorders (e.g., asthma, allergic disorders, and rheumatoid arthritis).

[0037] In specific embodiments, the present invention encompasses methods and compositions for detecting, diagnosing, prognosing and/or monitoring diseases or disorders associated with hypergammaglobulinemia (e.g., AIDS, autoimmune diseases, and some immunodeficiencies). In other specific embodiments, the present invention encompasses methods and compositions for detecting, diagnosing, prognosing and/or monitoring diseases or disorders associated with hypogammaglobulinemia (e.g., an immunodeficiency).

[0038] The present invention further encompasses methods and compositions for preventing, treating and/or ameliorating diseases or disorders associated with aberrant BLYS or BLYS receptor expression or inappropriate BLYS or BLYS receptor function in an animal, preferably a mammal, and most preferably a human, comprising, or alternatively consisting of, administering to an animal in which such treatment, prevention or amelioration is desired one or more BLYS binding polypeptides (including molecules which comprise, or alternatively consist of, BLYS binding polypeptide fragments or variants thereof) in an amount effective to treat, prevent or ameliorate the disease or disorder. Diseases and disorders which can be prevented, treated, and/or ameliorated with the BLYS binding polypeptides include, but are not limited to, immune system diseases or disorders (e.g., autoimmune diseases or disorders, immunodeficiencies, lupus, glomerular nephritis, rheumatoid arthritis, multiple sclerosis, graft vs. host disease, myasthenia gravis, Hashimoto's disease, immunodeficiency syndrome, hypogammaglobulinemia, and hypergammaglobulinemia), proliferative diseases or disorders (e.g., cancer, premalignant conditions, benign tumors, hyperproliferative disorders, benign proliferative disorders, leukemia, lymphoma, chronic lymphocytic leukemia, multiple myeloma, Hodgkin's lymphoma, Hodgkin's disease, T cell proliferative diseases and disorders, B cell proliferative diseases and disorders, monocytic proliferative diseases or disorders, acute myelogenous leukemia, macrophage proliferative diseases and disorders, and carcinoma), infectious diseases (e.g., AIDS), and inflammatory disorders (e.g., asthma, allergic disorders, and rheumatoid arthritis).

[0039] In specific embodiments, the present invention encompasses methods and compositions (e.g., BLYS binding polypeptides that antagonize BLYS activity) for preventing, treating and/or ameliorating diseases or disorders associated with hypergammaglobulinemia (e.g., AIDS, autoimmune diseases, and some immunodeficiency syndromes). In other specific embodiments, the present invention encompasses methods and compositions (e.g., BLYS binding polypeptides that enhance BLYS activity) for preventing, treating or ameliorating diseases or disorders associated with hypogammaglobulinemia (e.g., an immunodeficiency syndrome).

[0040] In specific embodiments, the present invention encompasses methods and compositions (e.g., BLYS binding polypeptides that antagonize BLYS activity) for preventing, treating and/or ameliorating immune system diseases or disorders, comprising, or alternatively consisting of, administering to an animal in which such treatment, prevention, and/or amelioration is desired, a BLYS binding polypeptide in an amount effective to treat, prevent and/or ameliorate the disease or disorder.

[0041] In specific embodiments, the present invention encompasses methods and compositions (e.g., BLYS binding polypeptides that antagonize BLYS activity) for preventing, treating and/or ameliorating diseases or disorders of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such treatment, prevention, and/or amelioration is desired, a BLYS binding polypeptide in an amount effective to treat, prevent and/or ameliorate the disease or disorder.

[0042] Autoimmune disorders, diseases, or conditions that may be detected, diagnosed, prognosed, monitored, treated, prevented, and/or ameliorated using the BLYS binding polypeptides include, but are not limited to, autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmune neutropenia, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, gluten-sensitive enteropathy, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g., IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henoch-Schoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, myocarditis, IgA glomerulonephritis, dense deposit disease, rheumatic heart disease, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis), systemic lupus erythematosus, discoid lupus, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rheumatoid arthritis, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiomyopathy syndrome, urticaria, atopic

dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulomatous, degenerative, and atrophic disorders).

[0043] Immunodeficiencies that may be detected, diagnosed, prognosed, monitored, treated, prevented, and/or ameliorated using the BLYS binding polypeptides include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0044] The present invention further encompasses methods and compositions for inhibiting or reducing immunoglobulin production, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide inhibits or reduces BLYS mediated immunoglobulin production.

[0045] The present invention further encompasses methods and compositions for inhibiting or reducing immunoglobulin production, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a BLYS binding polypeptide in an amount effective to inhibit or reduce immunoglobulin production.

[0046] The present invention further encompasses methods and compositions for inhibiting or reducing B cell proliferation, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide inhibits or reduces BLYS mediated B cell proliferation.

[0047] The present invention further encompasses methods and compositions for inhibiting or reducing B cell proliferation comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a BLYS binding polypeptide in an amount effective to inhibit or reduce B cell proliferation.

[0048] The present invention further encompasses methods and compositions for inhibiting or reducing activation of

B cells, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide inhibits or reduces BLYS mediated B cell activation.

[0049] The present invention further encompasses methods and compositions for inhibiting or reducing activation of B cells, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a BLYS binding polypeptide in an amount effective to inhibit or reduce B cell activation.

[0050] The present invention further encompasses methods and compositions for decreasing lifespan of B cells, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide inhibits or reduces BLYS regulated lifespan of B cells.

[0051] The present invention further encompasses methods and compositions for decreasing lifespan of B cells, comprising, or alternatively consisting of, administering to an animal in which such decrease is desired, a BLYS binding polypeptide in an amount effective to decrease B cell lifespan.

[0052] The present invention further encompasses methods and compositions for inhibiting or reducing graft rejection, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a BLYS binding polypeptide in an amount effective to inhibit or reduce graft rejection.

[0053] The present invention further encompasses methods and compositions for killing cells of hematopoietic origin, comprising, or alternatively consisting of, contacting BLYS binding polypeptides with BLYS to form a complex; and contacting the complex with cells of hematopoietic origin.

[0054] The present invention further encompasses methods and compositions for killing cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such killing is desired, a BLYS binding polypeptide in an amount effective to kill cells of hematopoietic origin.

[0055] The present invention further encompasses methods and compositions for stimulating immunoglobulin production, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of the BLYS binding polypeptide stimulates BLYS mediated immunoglobulin production.

[0056] The present invention further encompasses methods and compositions for stimulating immunoglobulin production comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a BLYS binding polypeptide in an amount effective to stimulate immunoglobulin production.

[0057] The present invention further encompasses methods and compositions for stimulating B cell proliferation, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide stimulates BLYS mediated B cell proliferation.

[0058] The present invention further encompasses methods and compositions for stimulating B cell proliferation, comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a BLYS binding polypeptide in an amount effective to stimulate B cell proliferation.

[0059] The present invention further encompasses methods and compositions for increasing activation of B cells, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide increases BLYS mediated activation of B cells.

[0060] The present invention further encompasses methods and compositions for increasing activation of B cells, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a BLYS binding polypeptide in an amount effective to increase B cell activation.

[0061] The present invention further encompasses methods and compositions for increasing lifespan of B cells, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide increases BLYS regulated lifespan of B cells.

[0062] The present invention further encompasses methods and compositions for increasing lifespan of B cells, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a BLYS binding polypeptide in an amount effective to increase lifespan of B cells.

Definitions

[0063] In order that the invention may be clearly understood, the following terms are defined:

[0064] The term “recombinant” is used to describe non-naturally altered or manipulated nucleic acids, host cells transfected with exogenous nucleic acids, or polypeptide molecules that are expressed non-naturally, through manipulation of isolated nucleic acid (typically, DNA) and transformation or transfection of host cells. “Recombinant” is a term that specifically encompasses nucleic acid molecules that have been constructed in vitro using genetic engineering techniques, and use of the term “recombinant” as an adjective to describe a molecule, construct, vector, cell, polypeptide or polynucleotide specifically excludes naturally occurring such molecules, constructs, vectors, cells, polypeptides or polynucleotides.

[0065] The term “bacteriophage” is defined as a bacterial virus containing a nucleic acid core and a protective shell built up by the aggregation of a number of different protein molecules. The terms “bacteriophage” and “phage” are synonymous and are used herein interchangeably.

[0066] The term “affinity ligand” is sometimes used herein and is synonymous with BLYS binding polypeptides.

[0067] The term “BLYS protein” as used herein encompasses both the membrane (e.g., SEQ ID NOS:173 and 174) and soluble forms (e.g., amino acids 134-285 of SEQ ID NO:173) of BLYS. BLYS protein may be monomeric, dimeric, or trimeric or multivalent. Preferably, BLYS proteins are homotrimeric.

[0068] The term “BLYS-like polypeptide” as used herein encompasses natural BLYS or full-length recombinant BLYS as well as fragments and variants thereof, such as, a modified or truncated form of natural BLYS or full-length recombinant BLYS, which BLYS and BLYS-like polypeptide retain a BLYS functional activity. BLYS or BLYS fragments that may be specifically bound by the compositions useful according to the invention include, but are not limited to, human BLYS (SEQ ID NOS:173 and/or 174) or BLYS expressed on human monocytes; murine BLYS (SEQ ID NOS:175 and/or 176) or BLYS expressed on murine monocytes; rat BLYS (either the soluble forms as given in SEQ ID NOS:177, 178, 179 and/or 180 or in a membrane associated form, e.g., on the surface of rat monocytes); or monkey BLYS (e.g., the monkey BLYS polypeptides of SEQ ID NOS:181 and/or 182, the soluble form of monkey BLYS, or BLYS expressed on monkey monocytes) or fragments thereof. Preferably compositions useful according to the invention bind human BLYS (SEQ ID NOS:173 and/or 174) or fragments thereof. BLYS and BLYS-like polypeptides retain at least one functional activity of the natural or full-length BLYS, including but not limited to the following activities: binding to BLYS receptor (e.g., TACI (GenBank accession number AAC51790), and BCMA (GenBank accession number NP_001183)), stimulating B cell proliferation, stimulating immunoglobulin secretion by B cells, stimulating the BLYS receptor signaling cascade and/or being bound by an anti-BLYS antibody or other BLYS binding polypeptide. Assays that can be used to determine the functional activities of BLYS or BLYS like polypeptides can readily be determined by one skilled in the art (e.g., see assays disclosed in Moore et al., 1999, supra) “BLYS-like polypeptides” also include fusion polypeptides in which all or a portion of BLYS is fused or conjugated to another polypeptide. BLYS-like polypeptides that are fusion polypeptides retain at least one functional activity of BLYS, preferably the ability to stimulate B lymphocytes (see, for example, Moore et al., *Science*, 285: 260-263 (1999)), to bind the BLYS receptors (e.g., TACI or BCMA), and/or to be bound by an anti-BLYS antibody or other BLYS binding polypeptide. BLYS fusion polypeptides may be made by recombinant DNA techniques in which a gene or other polynucleotide coding sequence for BLYS or a fragment thereof is ligated in-frame (recombined) with the coding sequence of another protein or polypeptide. The resulting recombinant DNA molecule is then inserted into any of a variety of plasmid or phage expression vectors, which enable expression of the fusion protein molecule in an appropriate eukaryotic or prokaryotic host cell. BLYS fusion polypeptides may be generated by synthetic or semi-synthetic procedures as well.

[0069] The terms “BLYS target” or “BLYS target protein” are sometimes used herein and encompass BLYS and/or BLYS-like polypeptides. Thus, the BLYS binding polypeptides used according to the methods of the invention bind “BLYS target proteins” and can be used to bind, detect, remove, and/or purify “BLYS target proteins.”

[0070] The term “binding polypeptide” is used herein to refer to any polypeptide capable of forming a binding complex with another molecule, polypeptide, peptidomimetic or transformant.

[0071] A “BLYS binding polypeptide” is a molecule that can bind BLYS target protein. Non-limiting examples of BLYS binding polypeptides useful in the methods of the

invention are the polypeptide molecules having an amino acid sequence described herein (see SEQ ID NOs:1-12, 20-172, and 186-444). The term BLYS binding polypeptide also encompasses BLYS binding fragments and variants (including derivatives) of polypeptides having the specific amino acid sequences described herein (SEQ ID NOs:1-12, 20-172, and 186-444). By "variant" of an amino acid sequence as described herein is meant a polypeptide that binds BLYS, but does not necessarily comprise an identical or similar amino acid sequence of a BLYS binding polypeptide specified herein. BLYS binding polypeptides useful according to the invention which are variants of a BLYS binding polypeptide specified herein satisfy at least one of the following: (a) a polypeptide comprising, or alternatively consisting of, an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% least 99%, or 100% identical to the amino acid sequence of a BLYS binding polypeptide sequence disclosed herein (SEQ ID NOs:1-12, 20-172, and 186-444), (b) a polypeptide encoded by a nucleotide sequence, the complementary sequence of which hybridizes under stringent conditions to a nucleotide sequence encoding a BLYS binding polypeptide disclosed herein (e.g., a nucleic acid sequence encoding the amino acid sequence of SEQ ID NOs:1-12, 20-172, and 186-444), and/or a fragment of a BLYS binding polypeptide disclosed herein, of at least 5 amino acid residues, at least 10 amino acid residues, at least 15 amino acid residues, or at least 20 amino acid residues. BLYS binding polypeptides useful according to the invention also encompass polypeptide sequences that have been modified for various applications provided that such modifications do not eliminate the ability to bind a BLYS target. Specific, non-limiting examples of modifications contemplated include C-terminal or N-terminal amino acid substitutions or peptide chain elongations for the purpose of linking the BLYS binder to a chromatographic material or other solid support. Other substitutions contemplated herein include substitution of one or both of a pair of cysteine residues that normally form disulfide links, for example with non-naturally occurring amino acid residues having reactive side chains, for the purpose of forming a more stable bond between those amino acid positions than the former disulfide bond. All such modified binding polypeptides are also considered BLYS binding polypeptides so long as the modified polypeptides retain the ability to bind BLYS and/or BLYS-like polypeptides, and therefore, may be used in one or more of the various methods described herein, such as, to detect, purify, or isolate BLYS or BLYS-like polypeptides in or from a solution. BLYS binding polypeptides also include variants of the specific BLYS binding polypeptide sequences disclosed herein (e.g., SEQ ID NOs:1-12, 20-172, and 186-444) which have an amino acid sequence corresponding to one of these polypeptide sequences, but in which the polypeptide sequence is altered by substitutions, additions or deletions that provide for molecules that bind BLYS. Thus, the BLYS binding polypeptides include polypeptides containing, as a primary amino acid sequence, all or part of the particular BLYS binding polypeptide sequence including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence, resulting in a peptide which is functionally active. For example, one or more amino acid residues within the

sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such BLYS binding polypeptides can be made either by chemical peptide synthesis or by recombinant production from a nucleic acid encoding the BLYS binding polypeptide which nucleic acid has been mutated. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis, in vitro site-directed mutagenesis (Hutchinson et al., *J. Biol. Chem.*, 253:6551 (1978)), use of TAB.RTM. linkers (Pharmacia), etc.

[0072] As used and understood herein, percent homology or percent identity of two amino acid sequences or of two nucleic acid sequences is determined using the algorithm of Karlin and Atschul (*Proc. Natl. Acad. Sci. USA*, 87: 2264-2268 (1990)), modified as in Karlin and Altschul (*Proc. Natl. Acad. Sci. USA*, 90: 5873-5877 (1993)). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (*J. Mol. Biol.*, 215: 403-410 (1990)). BLAST nucleotide searches are performed with the NBLAST program to obtain nucleotide sequences homologous to a nucleic acid molecule described herein. BLAST protein searches are performed with the XBLAST program to obtain amino acid sequences homologous to a reference polypeptide. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al. (*Nucleic Acids Res.*, 25: 3389-3402 (1997)). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used. See, <http://www.ncbi.nlm.nih.gov>. Alternatively, the percent identity of two amino acid sequences or of two nucleic acid sequences can be determined once the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide at the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical overlapping positions/total number of positions × 100%). In one embodiment, the two sequences are the same length.

[0073] The term "polypeptide", as used herein, refers to a linear, branched, or cyclic (e.g., containing a loop structure) polymer of two or more amino acid residues linked with a peptide bond. The term "polypeptide" is not restricted to any particular upper limit of amino acid residues. Thus, the BLYS affinity ligands that comprise an amino acid sequence described herein are properly referred to as "BLYS binding

polypeptides” because such binding polypeptides contain at least two amino acid residues held together by a peptide bond, even though such molecules may also contain one or more additional moieties or groups that are not amino acids, such as N-terminal and/or C-terminal capping or functional groups, and that may or may not be involved in a peptide bond. The polypeptides may be monovalent, divalent, trivalent, or multivalent and may comprise one or more of the BLYS binding polypeptides having the amino acid sequence of SEQ ID NOs:1-12, 20-172, and 186-444 and/or fragments or variants thereof. The term “peptide” is used herein to have the same meaning as “polypeptide.”

[0074] The term “antibody,” as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. Examples of molecules which are described by the term “antibody” in this application include, but are not limited to: single chain Fvs (scFvs), Fab fragments, Fab' fragments, F(ab')₂, disulfide linked Fvs (sdFvs), Fvs, and fragments comprising or alternatively consisting of, either a VL or a VH domain. The term “single chain Fv” or “scFv” as used herein refers to a polypeptide comprising a VL domain of antibody linked to a VH domain of an antibody.

[0075] “Feed stream”: BLYS and BLYS-like polypeptides that are bound by a BLYS binding polypeptide of this invention may be produced by any method known in the art, including, but not limited to, chemical synthesis; production in transformed host cells; secretion into culture medium by naturally occurring cells or recombinantly transformed bacteria, yeasts, fungi, insect cells, plant cells, and mammalian cells; production in genetically engineered organisms (for example, transgenic mammals); and production in non-genetically engineered organisms. The solution, sample, or mixture that contains a BLYS or BLYS-like polypeptide as it is produced or is found present in a production solution will sometimes be referred to as the “feed stream”.

[0076] The term “binding” refers to the determination by standard techniques that a binding polypeptide recognizes and binds to a given target. Such standard techniques include, but are not limited to, affinity chromatography, equilibrium dialysis, gel filtration, enzyme linked immunosorbent assay (ELISA), FACS analysis, and the monitoring of spectroscopic changes that result from binding, e.g., using fluorescence anisotropy, either by direct binding measurements or competition assays with another binder.

[0077] The term “specificity” refers to a binding polypeptide useful according to the invention that has a higher binding affinity for one target over another. Thus, the term “BLYS target protein specificity” refers to a molecule having a higher affinity for BLYS target protein as compared with another molecule that is not a BLYS target protein.

[0078] The term “epitopes” as used herein refers to portions of BLYS having antigenic or immunogenic activity in an animal, preferably a mammal. An epitope having immunogenic activity is a portion of BLYS that elicits an antibody response in an animal. An epitope having antigenic activity is a portion of BLYS to which an antibody or BLYS binding

polypeptide specifically binds as determined by any method known in the art, for example, by the immunoassays described herein. Antigenic epitopes need not necessarily be immunogenic.

[0079] The term “fragment” as used herein refers to a polypeptide comprising an amino acid sequence of at least 5 amino acid residues, at least 6 amino acid residues, at least 7 amino acid residues, at least 8 amino acid residues, at least 9 amino acid residues, at least 10 amino acid residues, at least 11 amino acid residues, at least 12 amino acid residues, at least 13 amino acid residues, at least 14 amino acid residues, at least 15 amino acid residues, at least 16 amino acid residues, at least 17 amino acid residues, at least 18 amino acid residues, at least 19 amino acid residues, at least 20 amino acid residues, at least 21 amino acid residues, at least 22 amino acid residues, at least 23 amino acid residues, at least 24 amino acid residues, or at least 25 amino acid residues of the amino acid sequence of BLYS, or a BLYS binding polypeptide (including molecules that comprise, or alternatively consist of, BLYS binding polypeptide fragments or variants thereof).

[0080] The term “fusion protein” as used herein refers to a polypeptide that comprises, or alternatively consists of, an amino acid sequence of a BLYS binding polypeptide and an amino acid sequence of a heterologous polypeptide (i.e., a polypeptide unrelated to the BLYS binding polypeptide).

[0081] The term “host cell” as used herein refers to the particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0082] Other terms are defined as necessary in the text below.

DETAILED DESCRIPTION OF THE INVENTION

[0083] The present invention provides methods and compositions for detecting, diagnosing, prognosing, and/or monitoring diseases or disorders associated with aberrant BLYS or BLYS receptor expression or inappropriate BLYS or BLYS receptor function in an animal, preferably a mammal, and most preferably a human, comprising, or alternatively consisting of, use of BLYS binding polypeptides (including molecules which comprise, or alternatively consist of, BLYS binding polypeptide fragments or variants thereof) that specifically bind to BLYS. Diseases and disorders which can be detected, diagnosed, prognosed and/or monitored with the BLYS binding polypeptides include, but are not limited to, immune system diseases or disorders (e.g., autoimmune diseases or disorders, immunodeficiencies, lupus, glomerular nephritis, rheumatoid arthritis, multiple sclerosis, graft vs. host disease, myasthenia gravis, Hashimoto's disease, and immunodeficiency syndrome), proliferative diseases or disorders (e.g., cancer, premalignant conditions, benign tumors, hyperproliferative disorders, benign proliferative disorders, leukemia, lymphoma, chronic lymphocytic leukemia, multiple myeloma, Hodgkin's lymphoma, Hodgkin's disease, T cell proliferative diseases and disorders, B cell proliferative diseases and disorders, monocytic proliferative

diseases or disorders, acute myelogenous leukemia, macrophage proliferative diseases and disorders, and carcinoma), infectious diseases (e.g., AIDS), and inflammatory disorders (e.g., asthma, allergic disorders, and rheumatoid arthritis).

[0084] The present invention further encompasses methods and compositions for preventing, treating and/or ameliorating diseases or disorders associated with aberrant BLYS or BLYS receptor expression or inappropriate BLYS or BLYS receptor function in an animal, preferably a mammal, and most preferably a human, comprising, or alternatively consisting of, administering to an animal in which such treatment, prevention or amelioration is desired one or more BLYS binding polypeptides (including molecules which comprise, or alternatively consist of, BLYS binding polypeptide fragments or variants thereof) in an amount effective to treat, prevent or ameliorate the disease or disorder. Diseases and disorders which can be prevented, treated, and/or ameliorated with the BLYS binding polypeptides include, but are not limited to, immune system diseases or disorders (e.g., autoimmune diseases or disorders, immunodeficiencies, lupus, glomerular nephritis, rheumatoid arthritis, multiple sclerosis, graft vs. host disease, myasthenia gravis, Hashimoto's disease, immunodeficiency syndrome, hypogammaglobulinemia, and hypergammaglobulinemia), proliferative diseases or disorders (e.g., cancer, premalignant conditions, benign tumors, hyperproliferative disorders, benign proliferative disorders, leukemia, lymphoma, chronic lymphocytic leukemia, multiple myeloma, Hodgkin's lymphoma, Hodgkin's disease, T cell proliferative diseases and disorders, B cell proliferative diseases and disorders, monocytic proliferative diseases or disorders, acute myelogenous leukemia, macrophage proliferative diseases and disorders, and

[0086] In preferred embodiments, the BLYS binding polypeptides used according to the present invention (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof), specifically bind to BLYS and do not cross-react with any other antigens. In more preferred embodiments, the BLYS binding polypeptides specifically bind to BLYS and do not cross-react with TRAIL (Hahne et al., *J. Exp. Med.*, 188(6):1185-90 (1998)), APRIL (Wilet et al., *Immunity*, 3(6):673-82 (1995)), Endokine-alpha (Kwon et al., *J. Biol. Chem.*, 274(10):6056-61 (1999)), TNF-alpha, TNF-beta (Nedwin et al., *J. Immunol.*, 135(4):2492-7 (1985)), Fas-L (Suda et al., *Cell*, 75(6):1169-78 (1993)), or LIGHT (Mauri et al., *Immunity*, 8(1):21-30 (1998)).

[0087] Many BLYS binding polypeptides have been discovered which may be used in the methods of the present invention. Specific BLYS binding polypeptides for use in the present invention comprise, or alternatively consist of, an amino acid sequence selected from the group consisting of SEQ ID NOs:1-12, 20-172, and 186-444, preferably SEQ ID NOs:163-172 or 436-444 as referred to above and in Tables 1-8, 13 and 14. In its broadest aspects, the methods of the present invention may be carried out using a polypeptide capable of binding to BLYS and comprising the polypeptide Asp-Xaa-Leu-Thr (SEQ ID NO:446), where Xaa is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala (preferably Pro or Ser).

[0088] Additional polypeptides for use in the methods described herein include polypeptides with the potential to form a cyclic or loop structure between invariant Cys residues comprising, or alternatively consisting of, an amino acid sequence selected from A-E (SEQ ID NOs:1-5):

(A) X₁-X₂-X₃-Cys-X₅-Phe-X₇-Trp-Glu-Cys-X₁₁-X₁₂-X₁₃, (SEQ ID NO:1)

carcinoma), infectious diseases (e.g., AIDS), and inflammatory disorders (e.g., asthma, allergic disorders, and rheumatoid arthritis).

BLYS Binding Polypeptides

[0085] The methods of the present invention may be performed utilizing new polypeptides and families of polypeptides that specifically bind to B lymphocyte stimulator protein (BLYS) and/or BLYS-like polypeptides. In particular, the invention encompasses diagnostic and therapeutic uses for polypeptides that specifically bind to a polypeptide or polypeptide fragment of human BLYS (SEQ ID NOs:173 and/or 174) or BLYS expressed on human monocytes; murine BLYS (SEQ ID NOs:175 and/or 176) or BLYS expressed on murine monocytes; rat BLYS (either the soluble forms as given in SEQ ID NOs:177, 178, 179 and/or

wherein

X₁ is Ala, Asn, Lys, or Ser;

X₂ is Ala, Glu, Met, Ser, or Val;

X₃ is Ala, Asn, Lys, or Pro (preferably Lys);

X₅ is Phe, Trp, or Tyr (preferably Tyr);

X₇ is Pro or Tyr (preferably Pro);

X₁₁ is Ala, Gln, His, Phe, or Val;

X₁₂ is Asn, Gln, Gly, His, Ser, or Val; and

X₁₃ is Ala, Asn, Gly, Ile, Pro, or Ser,

[0089] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

(B) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-Cys-X₁₂-X₁₃-X₁₄, (SEQ ID NO:2)

180 or in a membrane associated form, e.g., on the surface of rat monocytes); or monkey BLYS (e.g., the monkey BLYS polypeptides of SEQ ID NOs:181 and/or 182, the soluble form of monkey BLYS, or BLYS expressed on monkey monocytes); preferably human BLYS.

wherein

X₁ is Ala, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, or is absent;

X₂ is Ala, Asn, Asp, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val;

X₃ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val (preferably Asp);

X₅ is Asp, Ile, Leu, or Tyr (preferably Asp or Leu);

X₆ is Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Pro, Tyr, or Val (preferably Glu or Leu);

X₇ is His, Leu, Lys, or Phe (preferably His or Leu);

X₈ is Leu, Pro, or Thr (preferably Thr or Pro);

X₉ is Arg, Asn, Gly, His, Ile, Lys, Met, or Trp (preferably Lys);

X₁₃ is Gln, Glu, Ile, Leu, Phe, Pro, Ser, Tyr, or Val (preferably Val);

X₁₄ is Asn, Gly, Ile, Phe, Pro, Thr, Trp, or Tyr; and

X₁₅ is Asn, Asp, Glu, Leu, Lys, Met, Pro, or Thr (preferably Glu or Pro),

[0091] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

(D) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-Cys-X₁₄-X₁₅-X₁₆, (SEQ ID NO:4)

X₁₀ is Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Trp, Tyr, or Val;

X₁₂ is Asp, Gln, Glu, Gly, Ile, Leu, Lys, Phe, Ser, Trp, Tyr, or Val;

X₁₃ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; and

X₁₄ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Trp, Tyr, Val, or is absent,

[0090] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

wherein

X₁ is Asn, Asp, His, Leu, Phe, Pro, Ser, Tyr, or is absent (preferably Ser);

X₂ is Arg, Asn, Asp, His, Phe, Ser, or Trp (preferably Arg);

X₃ is Asn, Asp, Leu, Pro, Ser, or Val (preferably Asn or Asp);

X₅ is Asp, Gln, His, Ile, Leu, Lys, Met, Phe, or Thr;

X₆ is His, Ile, Leu, Met, Phe, Pro, Trp, or Tyr;

X₇ is Asp, His, Leu, or Ser (preferably Asp);

(C) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₃-X₁₄-X₁₅, (SEQ ID NO:3)

wherein

X₁ is Ala, Arg, Asn, Asp, Leu, Lys, Phe, Pro, Ser, or Thr;

X₂ is Asn, Asp, Gln, His, Ile, Lys, Pro, Thr, or Trp;

X₃ is Ala, Arg, Asn, Gln, Glu, His, Phe, Pro, or Thr (preferably Ala);

X₅ is Asn, Asp, Pro, Ser, or Thr (preferably Asp);

X₆ is Arg, Asp, Ile, Leu, Met, Pro, or Val (preferably Ile);

X₇ is Ala, Ile, Leu, Pro, Thr, or Val (preferably Val or Leu);

X₈ is Asn, His, Ile, Leu, Lys, Phe, or Thr (preferably Thr);

X₉ is Asn, Glu, Gly, His, Leu, Lys, Met, Pro, or Thr (preferably Leu);

X₈ is Ala, Arg, Asp, Glu, Leu, Phe, Pro, or Thr (preferably Glu or Pro);

X₉ is Ala, Arg, Asn, or Leu (preferably Leu);

X₁₀ is Ile, Leu, Met, Pro, Ser, or Thr (preferably Thr);

X₁₁ is Ala, Arg, Asn, Gly, His, Lys, Ser, or Tyr;

X₁₂ is Ala, Arg, Asn, Gln, Leu, Met, Ser, Trp, Tyr, or Val;

X₁₄ is Asp, Gly, Leu, Phe, Tyr, or Val (preferably Leu);

X₁₅ is Asn, His, Leu, Pro, or Tyr (preferably His, Leu or Pro); and

X₁₆ is Asn, Asp, His, Phe, Ser, or Tyr, (preferably Asp or Ser),

[0092] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

(E) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃-X₁₄-Cys-X₁₆-X₁₇-X₁₈, (SEQ ID NO:5)

X₁₀ is Arg, Asn, Asp, Gln, Glu, Gly, Ile, Lys, Met, Pro, Ser, or Trp;

X₁₁ is Arg, Glu, Gly, Lys, Phe, Ser, Trp, or Tyr (preferably Ser);

wherein

X₁ is Arg, Asp, Gly, His, Leu, Phe, Pro, Ser, Trp, Tyr, or is absent (preferably Arg);

X₂ is Ala, Arg, Asn, Asp, Gly, Pro, Ser, or is absent (preferably Asn, Asp, Gly, or Pro);

X₃ is Arg, Asn, Gln, Glu, Gly, Lys, Met, Pro, Trp or Val (preferably Gly or Met);

X₅ is Arg, Asn, Gln, Glu, His, Leu, Phe, Pro, Trp, Tyr, or Val (preferably Trp, Tyr, or Val);

X₆ is Arg, Asp, Gln, Gly, Ile, Lys, Phe, Thr, Trp or Tyr (preferably Asp);

X₇ is Ala, Arg, Asp, Glu, Gly, Leu, Ser, or Tyr (preferably Asp);

X₈ is Asp, Gln, Glu, Leu, Met, Phe, Pro, Ser, or Tyr (preferably Leu);

X₉ is Asp, Leu, Pro, Thr, or Val (preferably Leu or Thr);

X₁₀ is Arg, Gln, His, Ile, Leu, Lys, Met, Phe, Thr, Trp or Tyr (preferably Lys or Thr);

X₁₁ is Ala, Arg, Asn, Gln, Glu, His, Leu, Lys, Met, or Thr (preferably Arg or Leu);

X₅ is Asp, Leu, Lys, Phe, Pro, Ser, or Val (preferably Leu or Ser);

X₆ is His, Ile, Leu, Pro, Ser, or Thr (preferably Leu or Thr);

X₇ is Arg, Gly, His, Leu, Lys, Met, or Thr (preferably Lys or Thr);

X₈ is Ala, Arg, Asn, Ile, Leu, Lys, Met, or Thr (preferably Leu or Lys);

X₉ is Ala, Asn, Arg, Asp, Glu, Gly, His, Leu, Met, Ser, Trp, Tyr, or Val (preferably Met or Ser);

X₁₀ is Ile, Leu, Phe, Ser, Thr, Trp, Tyr, or Val (preferably Thr or Leu);

X₁₁ is Ala, Arg, Gly, His, Ile, Leu, Lys, Pro, Ser, Thr, Trp, Tyr, or Val (preferably Pro or Thr); and

X₁₂ is Arg, Asp, His, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val (preferably Arg or Pro),

[0094] wherein said polypeptide binds BLYS and/or BLYS-like polypeptides; or

(G) X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃, (SEQ ID NO:7)

X₁₂ is Ala, Asn, Gln, Gly, Leu, Lys, Phe, Pro, Thr, Trp, or Tyr (preferably Thr or Trp);

X₁₃ is Ala, Arg, Gln, His, Lys, Met, Phe, Pro, Thr, Trp, or Tyr (preferably Met or Phe);

X₁₄ is Arg, Gln, Glu, Gly, His, Leu, Met, Phe, Pro, Ser, Thr, Tyr, or Val (preferably Val);

X₁₆ is Arg, Asp, Gly, His, Lys, Met, Phe, Pro, Ser, or Trp (preferably Met);

X₁₇ is Arg, Asn, Asp, Gly, His, Phe, Pro, Ser, Trp or Tyr, (preferably Arg, His, or Tyr); and

X₁₈ is Ala, Arg, Asn, Asp, His, Leu, Phe, or Trp (preferably His or Asn),

wherein said polypeptide binds BLYS and/or BLYS-like polypeptides.

[0093] Additional BLYS binding polypeptides that may be used in the methods of the present invention include linear polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from F and G (SEQ ID NOS:6 and 7):

wherein

X₁ is Asp, Gln, Glu, Gly, His, Lys, Met, or Trp (preferably Glu, Lys);

X₂ is Arg, Gln, His, Ile, Leu, or Pro (preferably His or Pro);

X₃ is Asp, Gly, Ile, Lys, Thr, Tyr or Val (preferably Tyr);

X₄ is Asn, Asp, Gln, Glu, Met, Pro, Ser, or Tyr (preferably Asp or Gln);

X₅ is Asn, Asp, His, Ile, Leu, Met, Pro, Thr or Val (preferably Asn or Thr);

X₆ is Asp, Glu, His, Leu, Lys, Pro, or Val (preferably Asp or Pro);

X₇ is Arg, Asn, Gln, His, Ile, Leu, Met, Pro, or Thr (preferably Ile or Pro);

X₈ is Gln, Gly, His, Leu, Met, Ser, or Thr (preferably Leu or Thr);

X₉ is Asn, Gln, Gly, His, Leu, Lys, Ser, or Thr (preferably Lys);

(F) X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂, (SEQ ID NO:6)

wherein

X₁ is Ala, Arg, Gly, His, Leu, Lys, Met, Phe, Trp, Tyr, or Val (preferably Gly, Tyr, or Val);

X₂ is Ala, Arg, Gln, His, Ile, Leu, Phe, Thr, Trp, or Tyr (preferably His or Tyr);

X₃ is Ala, Asp, Lys, Phe, Thr, Trp or Tyr (preferably Asp or Tyr);

X₄ is Arg, Asp, Gln, Lys, Met, Phe, Pro, Ser, Tyr, or Val (preferably Asp or Gln);

X₁₀ is Ala, Gly, Ile, Leu, Lys, Met, or Phe (preferably Gly or Met);

X₁₁ is Ala, Glu, His, Ile, Leu, Met, Ser, Thr, Trp, Tyr, or Val (preferably Ala or Thr);

X₁₂ is Arg, Gln, Glu, Gly, His, Ile, Lys, Tyr, or Val (preferably Arg or His); and

X₁₃ is Arg, Asn, Glu, His, Ile, Ser, Thr, Trp, or Val (preferably His),

wherein said polypeptide binds BLYS and/or BLYS-like polypeptides.

[0095] Additional BLYS binding polypeptides that may be used in the methods of the present invention include BLYS binding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from H-L (SEQ ID NOs:8-12):

(H) Cys-X₂-Phe-X₄-Trp-Glu-Cys, (SEQ ID NO:8)

wherein

X₂ is Phe, Trp, or Tyr (preferably Tyr); and

wherein

X₂ is Asp, Gln, His, Ile, Leu, Lys, Met, Phe, or Thr;

X₃ is His, Ile, Leu, Met, Phe, Pro, Trp, or Tyr;

X₄ is Asp, His, Leu, or Ser (preferably Asp);

X₅ is Ala, Arg, Asp, Glu, Leu, Phe, Pro, or Thr (preferably Glu or Pro);

X₆ is Ala, Arg, Asn, or Leu (preferably Leu);

X₇ is Ile, Leu, Met, Pro, Ser, or Thr (preferably Thr);

X₈ is Ala, Arg, Asn, Gly, His, Lys, Ser, or Tyr;

[0099] X₉ is Ala, Arg, Asn, Gln, Leu, Met, Ser, Trp, Tyr, or Val; or

(L) Cys-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys, (SEQ ID NO:12)

[0096] X₄ is Pro or Tyr (preferably Pro); or

(I) Cys-X₂-X₃-X₄-X₅-X₆-X₇-Cys, (SEQ ID NO:9)

wherein

X₂ is Asp, Ile, Leu, or Tyr (preferably Asp or Leu);

X₃ is Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Pro, Tyr, or Val (preferably Glu or Leu);

X₄ is His, Leu, Lys, or Phe (preferably His or Leu);

X₅ is Leu, Pro, or Thr (preferably Thr or Pro);

X₆ is Arg, Asn, Gly, His, Ile, Lys, Met, or Trp (preferably Lys); and

[0097] X₇ is Ala, Asn, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Trp, Tyr, or Val; or

(J) Cys-X₂-X₃-X₄-X₅-X₆-X₇-X₈-Cys, (SEQ ID NO:10)

wherein

X₂ is Asn, Asp, Pro, Ser, or Thr (preferably Asp);

X₃ is Arg, Asp, Ile, Leu, Met, Pro, or Val (preferably Ile);

X₄ is Ala, Ile, Leu, Pro, Thr, or Val (preferably Val or Leu);

X₅ is Asn, His, Ile, Leu, Lys, Phe, or Thr (preferably Thr);

X₆ is Asn, Glu, Gly, His, Leu, Lys, Met, Pro, or Thr (preferably Leu);

X₇ is Arg, Asn, Asp, Gln, Glu, Gly, Ile, Lys, Met, Pro, Ser, or Trp;

[0098] X₈ is Arg, Glu, Gly, Lys, Phe, Ser, Trp, or Tyr (preferably Ser); or

(K) Cys-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-Cys, (SEQ ID NO:11)

wherein

X₂ is Arg, Asn, Gln, Glu, His, Leu, Phe, Pro, Trp, Tyr, or Val (preferably Trp, Tyr, or Val);

X₃ is Arg, Asp, Gln, Gly, Ile, Lys, Phe, Thr, Trp or Tyr (preferably Asp);

X₄ is Ala, Arg, Asp, Glu, Gly, Leu, Ser, or Tyr (preferably Asp);

X₅ is Asp, Gln, Glu, Leu, Met, Phe, Pro, Ser, or Tyr (preferably Leu);

X₆ is Asp, Leu, Pro, Thr, or Val (preferably Leu or Thr);

X₇ is Arg, Gln, His, Ile, Leu, Lys, Met, Phe, Thr, Trp or Tyr (preferably Lys or Thr);

X₈ is Ala, Arg, Asn, Gln, Glu, His, Leu, Lys, Met, or Thr (preferably Arg or Leu);

X₉ is Ala, Asn, Gln, Gly, Leu, Lys, Phe, Pro, Thr, Trp, or Tyr (preferably Thr or Trp);

X₁₀ is Ala, Arg, Gln, His, Lys, Met, Phe, Pro, Thr, Trp, or Tyr (preferably Met or Phe);

X₁₁ is Arg, Gln, Glu, Gly, His, Leu, Met, Phe, Pro, Ser, Thr, Tyr, or Val (preferably Val);

wherein said polypeptides bind BLYS and/or BLYS-like polypeptides.

[0100] Additional BLYS binding polypeptides that may be used in the methods of the present invention include linear polypeptides comprise the following amino acid sequence M (SEQ ID NO:447):

(M) Ala-X₂-X₃-X₄-Asp-X₆-Leu-Thr-X₉-Leu-X₁₁-X₁₂-X₁₃-X₁₄,

(SEQ ID NO:447)

wherein

X₂ is Asn, Ser, Tyr, Asp, Phe, Ile, Gln, His, Pro, Lys, Leu, Met, Thr, Val, Glu, Ala, Gly, Cys, or Trp (i.e., any amino acid except Arg; preferably Asn);

X₃ is Trp, Glu, Lys, Cys, Leu, Ala, Arg, Gly, or Ser (preferably Trp);

X₄ is Tyr, Phe, Glu, Cys, Asn (preferably Tyr);

X₆ is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala (preferably Pro or Ser);

X₉ is Lys, Asn, Gln, Gly, or Arg (preferably Lys);

X₁₁ is Trp, Ser, Thr, Arg, Cys, Tyr, or Lys (preferably Trp);

X₁₂ is Leu, Phe, Val, Ile, or His (preferably Leu);

X₁₃ is Pro, Leu, His, Ser, Arg, Asn, Gln, Thr, Val, Ala, Cys, Ile, Phe, or Tyr (i.e., not Asp, Glu, Gly, Lys, Met, or Trp; preferably Pro); and

X₁₄ is Asp, Glu, Asn, Val, His, Gln, Arg, Gly, Ser, Tyr, Ala, Cys, Lys, Ile, Thr or Leu (i.e., not Phe, Met, Pro, or Trp; preferably Asp, Val or Glu).

[0101] Preferred BLYS binding polypeptides that may be used in the methods of the present invention include linear polypeptides comprising a core sequence of the formula N:

(N) X₁-X₂-Asp-X₄-Leu-Thr-X₇-Leu-X₉-X₁₀,

(SEQ ID NO:448)

wherein

X₁ is Trp, Glu, Lys, Cys, Leu, Ala, Arg, Gly, or Ser (preferably Trp);

X₂ is Tyr, Phe, Glu, Cys, Asn (preferably Tyr);

X₄ is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala (preferably Pro or Ser);

X₇ is Lys, Asn, Gln, Gly, or Arg (preferably Lys);

X₉ is Trp, Ser, Thr, Arg, Cys, Tyr, or Lys (preferably Trp); and

X₁₀ is Leu, Phe, Val, Ile, or His (preferably Leu).

[0102] Especially preferred BLYS binding polypeptides that may be used in the methods of the present invention include linear polypeptides comprising the core peptide Trp-Tyr-Asp-Pro-Leu-Thr-Lys-Leu-Trp-Leu (SEQ ID NO:436).

[0103] In performing certain methods according to the present invention, it is preferred that the BLYS binding polypeptides, or phage displaying such binding polypeptides, irreversibly bind the BLYS protein in its native, soluble trimeric form.

[0104] In performing certain methods according to the present invention, it is preferred that the BLYS binding polypeptides of the present invention, or phage displaying

such binding polypeptides, reversibly bind the BLYS protein in its native, soluble trimeric form.

[0105] In performing certain methods according to the invention, it may be advantageous for a BLYS binding polypeptide to bind BLYS target protein with high affinity. In specific embodiments, BLYS binding polypeptides used in this invention will bind BLYS target proteins with a dissociation constant or K_D of less than or equal to 5×10⁻² M, 10⁻² M, 5×10⁻³ M, 10⁻³ M, 5×10⁻⁴ M, 10⁻⁴ M, 5×10⁻⁵ M, or 10⁻⁵ M. More preferably, BLYS binding polypeptides used in the invention will bind BLYS target proteins with a dissociation constant or K_D less than or equal to 5×10⁻⁶ M, 10⁻⁶ M, 5×10⁻⁷ M, 10⁻⁷ M, 5×10⁻⁸ M, or 10⁻⁸ M. Even more preferably, BLYS binding polypeptides used in the methods of the invention bind BLYS target proteins with a dissociation constant or K_D less than or equal to 5×10⁻⁹ M, 10⁻⁹ M, 5×10⁻¹⁰ M, 10⁻¹⁰ M, 5×10⁻¹¹ M, 10⁻¹¹ M, 5×10⁻¹² M, 10⁻¹² M, 5×10⁻¹³ M, 10⁻¹³ M, 5×10⁻¹⁴ M, 10⁻¹⁴ M, 5×10⁻¹⁵ M, or 10⁻¹⁵ M.

[0106] In certain preferred embodiments, BLYS binding polypeptides reversibly bind BLYS and/or BLYS-like polypeptides and release bound BLYS protein in an active form, preferably in the native soluble trimeric form, under specific release conditions. In specific embodiments, BLYS binding polypeptides bind BLYS target proteins with off-

rates or k_{off} greater than or equal to 10⁻¹⁰ s⁻¹, 5×10⁻⁹ s⁻¹, 10⁻⁹ s⁻¹, 5×10⁻⁸ s⁻¹, 10⁻⁸ s⁻¹, 5×10⁻⁷ s⁻¹, 10⁻⁷ s⁻¹, 5×10⁻⁶ s⁻¹, 10⁻⁶ s⁻¹, 5×10⁻⁵ s⁻¹, 10⁻⁵ s⁻¹, 5×10⁻⁴ s⁻¹, 10⁻⁴ s⁻¹, 5×10⁻³ s⁻¹, 10⁻³ s⁻¹, 5×10⁻² s⁻¹, 10⁻² s⁻¹, 5×10⁻¹, or 10⁻¹ s⁻¹.

[0107] Binding experiments to determine K_D and off-rates can be performed in a number of conditions including, but not limited to, [pH 6.0, 0.01% Tween 20], [pH 6.0, 0.1% gelatin], [pH5.0, 0.01% Tween 20], [pH9.0, 0.1% Tween 20], [pH6.0, 15% ethylene glycol, 0.01% Tween 20], [pH5.0, 15% ethylene glycol, 0.01% Tween 20], and [pH9.0, 15% ethylene glycol, 0.01% Tween 20] The buffers in which to make these solutions can readily be determined by one of skill in the art, and depend largely on the desired pH of the final solution. Low pH solutions (<pH 5.5) can be made, for example, in citrate buffer, glycine-HCl buffer, or in succinic acid buffer. High pH solutions can be made, for example, in Tris-HCl, phosphate buffers, or sodium bicarbonate buffers. A number of conditions may be used to determine K_D and off-rates for the purpose of determining, for example, optimal pH and/or salt concentrations.

[0108] In certain embodiments, BLYS binding polypeptides reversibly bind BLYS and/or BLYS-like polypeptides, preferably in the native soluble, trimeric form.

[0109] In preferred embodiments, BLYS binding polypeptides reversibly bind only the native soluble, trimeric form of BLYS.

[0110] In certain embodiments, BLYS binding polypeptides irreversibly bind BLYS and/or BLYS-like polypeptides, preferably in the native soluble, trimeric form.

[0111] In preferred embodiments, BLYS binding polypeptides irreversibly bind only the native soluble, trimeric form of BLYS.

[0112] In some screening or assay procedures, it is possible and more convenient to use recombinant bacteriophage that display a particular BLYS binding polypeptide instead of using isolated BLYS binding polypeptide. Such procedures include phage-based ELISA protocols and immobilization of phage displaying a binding polypeptide to chromatographic materials. Such screening assays and procedures are routine in the art and may be readily adapted for procedures using recombinant bacteriophage such as disclosed herein.

[0113] Specific methods of the present invention contemplate the use of BLYS binding polypeptides that competitively inhibit the binding of a BLYS binding molecule. Competitive inhibition can be determined by any suitable method known in the art, for example, using the competitive binding assays described herein. In preferred embodiments, the polypeptide competitively inhibits the binding of a BLYS binding molecule to BLYS by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%. In a more preferred embodiment, the BLYS binding polypeptide competitively inhibits the binding of a BLYS binding molecule to the native soluble trimeric form of BLYS, by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[0114] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) useful in the practice of the methods of the present invention may have one or more of the same biological characteristics as one or more of the BLYS binding polypeptides specifically described herein. By "biological characteristics" is meant, the *in vitro* or *in vivo* activities or properties of the BLYS binding polypeptides, such as, for example, the ability to bind to BLYS (e.g., the soluble form of BLYS, the membrane-bound form of BLYS, the soluble form and membrane-bound form of BLYS), and/or an antigenic and/or epitope region of BLYS), the ability to substantially block BLYS/BLYS receptor (e.g., TACI and BCMA) binding, the ability to substantially increase BLYS/BLYS receptor (e.g., TACI and BCMA) binding, the ability to block BLYS mediated biological activity (e.g., stimulation of B cell proliferation and immunoglobulin production), or, the ability to enhance or stimulate BLYS mediated biological activity (e.g., stimulation of B cell proliferation and immunoglobulin production). Optionally, the BLYS binding polypeptides useful according to the invention will bind to the same epitope as at least one of the BLYS binding polypeptides specifically referred to herein. Such epitope binding can be routinely determined using assays known in the art.

[0115] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) useful in the practice of the methods of the present invention may be polypeptides that neutralize BLYS or a fragment thereof. By a BLYS binding polypeptide that "neutralizes BLYS or a

fragment thereof" is meant a BLYS binding polypeptide that inhibits (i.e., is effective to reduce or abolish) or abolishes the ability of BLYS: to bind to its receptor (e.g., TACI and BCMA), to stimulate B cell activation, to stimulate B cell proliferation, to stimulate immunoglobulin secretion by B cells, to increase B cell lifespan, and/or to stimulate the BLYS receptor signalling cascade.

[0116] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) useful in the practice of the methods of the present invention may also be effective to inhibit or abolish BLYS-mediated B cell proliferation as determined by any method known in the art such as, for example, the assays described in the Examples, *infra*, said BLYS binding polypeptides comprising, or alternatively consisting of, a polypeptide having an amino acid sequence of any one of SEQ ID NOs:1-12, 20-172, and 186-444, preferably of SEQ ID NOs:163-172 and 436-444, or a fragment or variant thereof.

[0117] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) useful in the practice of the methods of the present invention may also be effective to enhance the activity of BLYS or a fragment thereof, said BLYS binding polypeptides comprising, or alternatively consisting of, a polypeptide having an amino acid sequence of any one of SEQ ID NOs:1-12, 20-172, and 186-444, preferably of SEQ ID NOs:163-172 or 436-444, or a fragment or variant thereof. By a BLYS binding polypeptide that "enhances the activity of BLYS or a fragment thereof" is meant a BLYS binding polypeptide that increases the ability of BLYS: to bind to its receptor (e.g., TACI and BCMA), to stimulate B cell proliferation, to stimulate immunoglobulin secretion by B cells, to activate B cells, to increase B cell lifespan and/or to stimulate a BLYS receptor signalling cascade (e.g., to activate calcium-modulator and cyclophilin ligand ("CAML"), calcineurin, nuclear factor of activated T cells transcription factor ("NF-AT"), nuclear factor-kappa B ("NF-kappa B"), activator protein-1 (AP-1), SRF, extracellular-signal regulated kinase 1 (ERK-1), polo like kinases (PLK), ELF-1, high mobility group I (HMG-I), and/or high mobility group Y (HMG-Y)). Nucleic acid molecules encoding these BLYS binding polypeptides are also encompassed by the invention.

[0118] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) useful in the practice of the methods of the present invention may also be effective to stimulate BLYS mediated B cell proliferation as determined by any method known in the art, such as, for example, the assays described in the Examples, *infra*, said BLYS binding polypeptides comprising, or alternatively consisting of, a polypeptide having an amino acid sequence of any one of SEQ ID NOs:1-12, 20-172, and 186-444, preferably of SEQ ID NOs:163-172 or 436-444, or a fragment or variant thereof. Nucleic acid molecules encoding these BLYS binding polypeptides are also encompassed by the invention.

[0119] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) useful in the practice of the methods of the present invention may include

polypeptides effective to specifically bind to the soluble form of BLYS, polypeptides that specifically bind to the membrane-bound form of BLYS, and polypeptides that specifically bind to both the soluble form and membrane-bound form of BLYS.

[0120] The methods of the present invention may also be carried out using mixtures of BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) that specifically bind to BLYS, wherein the mixture contains at least one, two, three, four, five or more different BLYS binding polypeptides. In particular, the invention provides for the use of mixtures of different BLYS binding polypeptides that specifically bind to the soluble form of BLYS, the membrane-bound form of BLYS, and/or both the membrane-bound form and soluble form of BLYS. In specific embodiments, the methods of the invention utilize mixtures of at least 2, preferably at least 4, at least 6, at least 8, at least 10, at least 12, at least 15, at least 20, or at least 25 different BLYS binding polypeptides that specifically bind to BLYS, wherein at least 1, at least 2, at least 4, at least 6, or at least 10, BLYS binding polypeptides of the mixture are BLYS binding polypeptides.

[0121] The methods of the present invention may also be carried out using panels of BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) that specifically bind to BLYS, wherein the panel has at least one, two, three, four, five or more different BLYS binding polypeptides. In particular, the invention provides for the use of panels of different BLYS binding polypeptides that specifically bind to the soluble form of BLYS, the membrane-bound form of BLYS, and/or both the membrane-bound form and soluble form of BLYS. In specific embodiments, the invention provides for the use of panels of BLYS binding polypeptides that have different affinities for BLYS, different specificities for BLYS, or different dissociation rates. The invention provides for the use of panels of at least 10, preferably at least 25, at least 50, at least 75, or at least 100 BLYS binding polypeptides. Panels of BLYS binding polypeptides can be used, for example, in 96 well plates for assays such as ELISAs.

[0122] The methods of the present invention may also be carried out using compositions comprising one or more BLYS binding polypeptides (including molecules comprising, or alternatively consisting of BLYS binding polypeptide fragments or variants). In one embodiment, a composition used in a method of the present invention comprises, one, two, three, four, five, or more BLYS binding polypeptides that comprise or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the BLYS binding polypeptides contained in SEQ ID NOs:1-12, 20-172, and 186-444 as disclosed in Tables 1-8 and 13, or a variant thereof.

[0123] As discussed in more detail below, a composition useful in the methods of the invention may be used either alone or in combination with other compositions. The BLYS binding polypeptides (including molecules comprising, or alternatively consisting of BLYS binding polypeptide fragments or variants of the present invention) may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including

covalently and non-covalently conjugations) to polypeptides or other compositions. For example, BLYS binding polypeptides of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, polypeptide linkers, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 0 396 387.

Production and Modification of BLYS Binding Polypeptides

[0124] BLYS binding polypeptides useful in practicing the methods of the present invention may be produced by chemical synthesis, semi-synthetic methods, and recombinant DNA methodologies known in the art.

[0125] In certain embodiments, BLYS binding polypeptides of the present invention are produced by chemical or semi-synthetic methodologies known in the art (see, Kelley et al. in *Genetic Engineering Principles and Methods*, Setlow, J. K., ed. (Plenum Press, NY., 1990), vol. 12, pp. 1-19; Stewart et al., *Solid-Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, 1989). One advantage of these methodologies is that they allow for the incorporation of non-natural amino acid residues into the sequence of the BLYS binding polypeptide.

[0126] In preferred embodiments, BLYS binding polypeptides are chemically synthesized (see, e.g., Merrifield, *J. Am. Chem. Soc.*, 85: 2149 (1963); Houghten, *Proc. Natl. Acad. Sci. USA*, 82: 5132 (1985)). For example, polypeptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (see, e.g., Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Co., N.Y., 1983), pp. 50-60). BLYS binding polypeptides can also be synthesized by use of a peptide synthesizer. The composition of the synthetic polypeptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Co., N.Y., 1983), pp. 34-49). Furthermore, if desired, BLYS binding polypeptides may contain non-classical amino acids or chemical amino acid analogs, which can routinely be introduced during chemical synthesis as a substitution or addition into the BLYS binding polypeptides. Non-classical amino acids include, but are not limited to, the D-isomers of the common amino acids, 2,4-diaminobutyric acid, alpha-aminoisobutyric acid, 4-aminobutyric acid (4Abu), 2-aminobutyric acid (Abu), 6-aminohexanoic acid (epsilon-Ahx), 2-aminoisobutyric acid (Aib), 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, beta-alanine (bAla), fluoro-amino acids, designer amino acids such as beta-methyl amino acids, Calpha-methyl amino acids, Nalpha-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0127] Solid phase peptide synthesis begins at the carboxy (C) terminus of the putative polypeptide by coupling a protected amino acid to a suitable resin, which reacts with the carboxyl group of the C-terminal amino acid to form a bond that is readily cleaved later, for example, a halomethyl resin such as chloromethyl resin, bromomethyl resin, hydroxymethyl resin, aminomethyl resin, benzhydrylamine

resin, or t-alkyloxycarbonyl-hydrazide resin. After removal of the α -amino protecting group with, for example, trifluoroacetic acid (TFA) in methylene chloride and neutralization with, for example TEA, the next cycle in the synthesis is ready to proceed. The remaining α -amino and, if necessary, side-chain-protected amino acids are then coupled sequentially in the desired order by condensation to obtain an intermediate compound connected to the resin. Alternatively, some amino acids may be coupled to one another forming an oligopeptide prior to addition to the growing solid phase polypeptide chain.

[0128] The condensation between two amino acids, or an amino acid and a peptide, or a peptide and a peptide can be carried out according to condensation methods known in the art, including but not limited to, the azide method, mixed acid anhydride method, DCC (dicyclohexylcarbodiimide) method, active ester method (p-nitrophenyl ester method, BOP [benzotriazole-1-yl-oxy-tris (dimethylamino) phosphonium hexafluorophosphate] method, N-hydroxysuccinic acid imido ester method), and Woodward reagent K method.

[0129] Common to chemical synthesis of peptides is the protection or capping (blocking) of the reactive side chain groups of the various amino acid residues with suitable protecting or capping groups at that site until the group is ultimately removed after the polypeptide chain has been completely assembled. Also common is the protection or capping of the α -amino group on an amino acid or a fragment while that entity reacts at the carboxyl group followed by the selective removal of the α -amino-protecting group to allow subsequent reaction to take place at that location. Accordingly, during synthesis, intermediate compounds are produced which includes each of the amino acid residues located in the desired sequence in the peptide chain with various of these residues having side-chain protecting or capping groups. These protecting or capping groups on amino acid side chains are then removed substantially at the same time so as to produce the desired resultant product following purification.

[0130] The typical protective, capping, or blocking groups for α - and ϵ -amino side chain groups found in amino acids are exemplified by benzyloxycarbonyl (Z), isonicotinoyloxycarbonyl (iNOC), O-chlorobenzyloxycarbonyl [Z(NO₂)], p-methoxybenzyloxycarbonyl [Z(OMe)], t-butoxycarbonyl (Boc), t-amyoioxycarbonyl (Aoc), isobornyloxycarbonyl, adamantyloxycarbonyl, 2-(4-biphenyl)-2-propyloxycarbonyl (Bpoc), 9-fluorenylmethoxycarbonyl (Fmoc), methylsulfonyl ethoxycarbonyl (Msc), trifluoroacetyl, phthalyl, formyl, 2-nitrophenylsulphenyl (NPS), diphenylphosphinothioyl (Ppt), dimethylphosphinothioyl (Mpt), and the like.

[0131] Protective, capping, or blocking groups for the carboxyl group of amino acids include, for example, benzyl ester (OBzl), cyclohexyl ester (Chx), 4-nitrobenzyl ester (ONb), t-butyl ester (Obut), 4-pyridylmethyl ester (OPic), and the like. It is usually also desirable that side chain groups of specific amino acids such as arginine, cysteine, and serine, are protected by a suitable protective group as occasion demands. For example, the guanidino group in arginine may be protected with nitro, p-toluenesulfonyl, benzyloxycarbonyl, adamantyloxycarbonyl, p-methoxybenzenesulfonyl, 4-methoxy-2,6-dimethylbenzenesulfonyl (Mds), 1,3,5-trimethylphenylsulfonyl (Mts), and the like. The thiol group in cysteine may be protected with p-meth-

oxybenzyl, triphenylmethyl, acetylaminoethyl ethylcarbamoyl, 4-methylbenzyl, 2,4,6-trimethylbenzyl (Tmb), etc., and the hydroxyl group in the serine can be protected with benzyl, t-butyl, acetyl, tetrahydropyranyl, etc.

[0132] After the desired amino acid sequence has been completed, the intermediate polypeptide is removed from the resin support by treatment with a reagent, such as liquid HF and one or more thio-containing scavengers, which cleaves the peptide molecule from the resin and all the remaining side-chain protecting groups. Following HF cleavage, the protein sequence is washed with ether, transferred to a large volume of dilute acetic acid, and stirred at pH adjusted to about 8.0 with ammonium hydroxide. Upon pH adjustment, the polypeptide takes its desired conformational arrangement.

[0133] By way of example but not by way of limitation, polypeptides can be chemically synthesized and purified as follows: Peptides can be synthesized by employing the N-alpha-9-fluorenylmethyloxycarbonyl or Fmoc solid phase peptide synthesis chemistry using a Rainin Symphony Multiplex Peptide Synthesizer. The standard cycle used for coupling of an amino acid to the peptide-resin growing chain generally includes: (1) washing the peptide-resin three times for 30 seconds with N,N-dimethylformamide (DMF); (2) removing the Fmoc protective group on the amino terminus by deprotection to with 20% piperidine in DMF by two washes for 15 minutes each, during which process mixing is effected by bubbling nitrogen through the reaction vessel for one second every 10 seconds to prevent peptide-resin settling; (3) washing the peptide-resin three times for 30 seconds with DMF; (4) coupling the amino acid to the peptide resin by addition of equal volumes of a 250 mM solution of the Fmoc derivative of the appropriate amino acid and an activator mix consisting of 400 mM N-methylmorpholine and 250 mM (2-(1H-benzotriazol-1-yl)-1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in DMF; (5) allowing the solution to mix for 45 minutes; and (6) washing the peptide-resin three times for 30 seconds of DMF. This cycle can be repeated as necessary with the appropriate amino acids in sequence to produce the desired peptide. Exceptions to this cycle program are amino acid couplings predicted to be difficult by nature of their hydrophobicity or predicted inclusion within a helical formation during synthesis. For these situations, the above cycle can be modified by repeating step 4 a second time immediately upon completion of the first 45 minute coupling step to "double couple" the amino acid of interest. Additionally, in the first coupling step in peptide synthesis, the resin can be allowed to swell for more efficient coupling by increasing the time of mixing in the initial DMF washes to three 15 minute washes rather than three 30 second washes.

[0134] After peptide synthesis, the peptide can be cleaved from the resin as follows: (1) washing the peptide-resin three times for 30 seconds with DMF; (2) removing the Fmoc protective group on the amino terminus by washing two times for 15 minutes with 20% piperidine in DMF; (3) washing the peptide-resin three times for 30 seconds with DMF; and (4) mixing a cleavage cocktail consisting of 95% trifluoroacetic acid (TFA), 2.4% water, 2.4% phenol, and 0.2% triisopropylsilane with the peptide-resin for two hours, then filtering the peptide in the cleavage cocktail away from the resin, and precipitating the peptide out of solution by addition of two volumes of ethyl ether. Specifically, to isolate the

peptide, the ether-peptide solution can be allowed to sit at -20°C . for 20 minutes, then centrifuged at $6,000\times\text{G}$ for 5 minutes to pellet the peptide, and the peptide can be washed three times with ethyl ether to remove residual cleavage cocktail ingredients. The final peptide product can be purified by reversed phase high pressure liquid chromatography (RP-HPLC) with the primary solvent consisting of 0.1% TFA and the eluting buffer consisting of 80% acetonitrile and 0.1% TFA. The purified peptide can then be lyophilized to a powder.

[0135] In other specific embodiments, branched versions of the BLYS binding polypeptides described herein are provided, e.g., by substituting one or more amino acids within the BLYS binding polypeptide sequence with an amino acid or amino acid analog with a free side chain capable of forming a peptide bond with one or more amino acids (and thus capable of forming a "branch").

[0136] Branched peptides may be prepared by any method known in the art for covalently linking any naturally occurring or synthetic amino acid to any naturally occurring or synthetic amino acid in a peptide chain which has a side chain group able to react with the amino or carboxyl group on the amino acids so as to become covalently attached to the peptide chain. In particular, amino acids with a free amino side chain group, such as, but not limited to, diamminobutyric acid, lysine, arginine, ornithine, diamminopropionic acid and citrulline, can be incorporated into a peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free amino side group, from that residue. Alternatively, amino acids with a free carboxyl side chain group, such as, but not limited to, glutamic acid, aspartic acid and homocitrulline, can be incorporated into the peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free carboxyl side group, from that residue. The amino acid forming the branch can be linked to a side chain group of an amino acid in the peptide chain by any type of covalent bond, including, but not limited to, peptide bonds, ester bonds and disulfide bonds. In a specific embodiment, amino acids, such as those described above, that are capable of forming a branch point, are substituted for BLYS binding polypeptide residues within a peptide including a BLYS binding polypeptide sequence.

[0137] Branched peptides can be prepared by any method known in the art. For example, but not by way of limitation, branched peptides can be prepared as follows: (1) the amino acid to be branched from the main peptide chain can be purchased as an N-alpha-tert-butyloxycarbonyl (Boc) protected amino acid pentafluorophenyl (Opfp) ester and the residue within the main chain to which this branched amino acid will be attached can be an N-Fmoc-alpha-gammadiaminobutyric acid; (2) the coupling of the Boc protected amino acid to diamminobutyric acid can be achieved by adding 5 grams of each precursor to a flask containing 150 ml DMF, along with 2.25 ml pyridine and 50 mg dimethylaminopyridine and allowing the solution to mix for 24 hours; (3) the peptide can then be extracted from the 150 ml coupling reaction by mixing the reaction with 400 ml dichloromethane (DCM) and 200 ml 0.12N HCl in a 1 liter separatory funnel, and allowing the phases to separate, saving the bottom aqueous layer and re-extracting the top layer two more times with 200 ml 0.12N HCl; (4) the solution containing the peptide can be dehydrated by adding

2-5 grams magnesium sulfate, filtering out the magnesium sulfate, and evaporating the remaining solution to a volume of about 2-5 ml; (5) the dipeptide can then be precipitated by addition of ethyl acetate and then 2 volumes of hexanes and then collected by filtration and washed two times with cold hexanes; and (6) the resulting filtrate can be lyophilized to achieve a light powder form of the desired dipeptide. Branched peptides prepared by this method will have a substitution of diamminobutyric acid at the amino acid position which is branched. Branched peptides containing an amino acid or amino acid analog substitution other than diamminobutyric acid can be prepared analogously to the procedure described above, using the N-Fmoc coupled form of the amino acid or amino acid analog.

[0138] In a preferred embodiment, the BLYS binding polypeptide is a cyclic peptide. Cyclization can be, for example, but not by way of limitation, via a disulfide bond between two cysteine residues or via an amide linkage. For example, but not by way of limitation, disulfide bridge formation can be achieved by (1) dissolving the purified peptide at a concentration of between 0.1-0.5 mg/ml in 0.01 M ammonium acetate, pH 7.5; (2) adding to the dissolved peptide 0.01 M potassium ferricyanide dropwise until the solution appears pale yellow in color and allowing this solution to mix for 24 hours; (3) concentrating the cyclized peptide to 5-10 ml of solution, repurifying the peptide by reverse phase-high pressure liquid chromatography (RP-HPLC) and finally lyophilizing the peptide. In a specific embodiment, in which the peptide does not contain two appropriately situated cysteine residues, cysteine residues can be introduced at the amino-terminus and/or carboxy-terminus and/or internally such that the peptide to be cyclized contains two cysteine residues spaced such that the residues can form a disulfide bridge. Alternatively, a cyclic peptide can be obtained by generating an amide linkage using, for example but not limited to, the following protocol: An allyl protected amino acid, such as aspartate, glutamate, asparagine or glutamine, can be incorporated into the peptide as the first amino acid, and then the remaining amino acids are coupled on. The allyl protective group can be removed by a two hour mixing of the peptide-resin with a solution of tetrakis(triphenylphosphine) palladium (0) in a solution of chloroform containing 5% acetic acid and 2.5% N-methylmorpholine. The peptide resin can be washed three times with 0.5% N,N-diisopropylethylamine (DIEA) and 0.5% sodium diethyldithiocarbamate in DMF. The amino terminal Fmoc group on the peptide chain can be removed by two incubations for 15 minutes each in 20% piperidine in DMF, and washed three times with DMF for 30 seconds each. The activator mix, N-methylmorpholine and HBTU in DMF, can be brought onto the column and allowed to couple the free amino terminal end to the carboxyl group generated by removal of the allyl group to cyclize the peptide. The peptide can be cleaved from the resin as described in the general description of chemical peptide synthesis above and the peptide purified by reverse phase-high pressure liquid chromatography (RP-HPLC). In a specific embodiment, in which the peptide to be cyclized does not contain an allyl protected amino acid, an allyl protected amino acid can be introduced into the sequence of the peptide, at the amino-terminus, carboxy-terminus or internally, such that the peptide can be cyclized.

[0139] In addition, according to certain embodiments, it is preferable that the BLYS binding polypeptides are produced

having or retaining an amino terminal (N-terminal) and/or a carboxy terminal (C-terminal) capping group, which may protect the N-terminal or C-terminal amino acid from undesirable chemical reactions during use or which may permit further conjugations or manipulations of the binding polypeptide, for example, in conjugating the binding polypeptide to a chromatographic support resin or matrix or to another peptide to tether the binding polypeptide to a resin or support. Such N-terminal and C-terminal groups may also be used to label or tag the binding polypeptide to detect bound complexes or to locate the binding polypeptide (whether bound or unbound to a BLYS target protein) for example, at some point in a separation procedure. Accordingly, a BLYS binding polypeptide synthesized in its final form for use in a detection or separation procedure may contain an N-terminal and/or a C-terminal capping group. A particularly preferred N-terminal capping group, which may be present or retained in binding polypeptides, is an acetyl group (Ac). A particularly preferred C-terminal capping group, which may be present or retained in binding polypeptides, is an amide group. In a further preferred embodiment, the BLYS binding polypeptides have an acetyl group as an N-terminal capping group and an amide group as a C-terminal capping group.

[0140] The BLYS binding polypeptides may also be prepared commercially by companies providing polypeptide synthesis as a service (e.g., BACHEM Bioscience, Inc., King of Prussia, Pa.; Quality Controlled Biochemicals, Inc., Hopkinton, Mass.).

[0141] The nucleic acid sequence encoding a BLYS binding polypeptide can be produced and isolated using well-known techniques in the art. In one example, nucleic acids encoding the BLYS binding polypeptides are chemically synthesized based on knowledge of the amino acid sequence of the BLYS binding polypeptide (preferably the sequence is codon optimized to the host system in which the polypeptide will be expressed). In another example, nucleic acids encoding a BLYS binding polypeptide are obtained by screening an expression library (e.g., a phage display library) to identify phage expressing BLYS binding polypeptides, and isolating BLYS binding polypeptide encoding nucleic acid sequences from the identified library member (e.g., via polymerase chain reaction methodology using primers flanking the polypeptide encoding sequences).

[0142] Thus, BLYS binding polypeptides can also be obtained by recombinant expression techniques. (See, e.g., Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual, 2d Ed.*, Glover, D. M. (ed.), (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989); *DNA Cloning: A Practical Approach* (MRL Press, Ltd., Oxford, U.K., 1985), Vols. I, II.

[0143] To produce a recombinant BLYS binding polypeptide, a nucleic acid sequence encoding the BLYS binding polypeptide is operatively linked to a promoter such that the BLYS binding polypeptide is produced from said sequence. For example, a vector can be introduced into a cell, within which cell the vector or a portion thereof is expressed, producing the BLYS binding polypeptides. In a preferred embodiment, the nucleic acid is DNA if the source of RNA polymerase is DNA-directed RNA polymerase, but the nucleic acid may also be RNA if the source of polymerase is RNA-directed RNA polymerase or if reverse transcriptase

is present in the cell or provided to produce DNA from the RNA. Such a vector can remain episomal or, become chromosomally integrated, as long as it can be transcribed to produce the desired RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be bacteriophage, plasmid, viral, retroviral, or others known in the art, used for replication and expression in bacterial, fungal, plant, insect or mammalian cells. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells. Introduction of the vector construct into the host cell can be effected by techniques known in the art which include, but are not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are well known in the art and are described, for example, in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986).

[0144] The present invention also contemplates the use of BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) that are recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a heterologous polypeptide (or portion thereof, preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids of the heterologous polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. For example, BLYS binding polypeptides may be used to target heterologous polypeptides to particular cell types (e.g., cells of monocytic lineage and B-cells), either in vitro or in vivo, by fusing or conjugating the heterologous polypeptides to BLYS binding polypeptides that are specific for particular cell surface antigens (e.g., membrane-bound BLYS on cells of monocytic lineage) or which bind antigens (i.e., BLYS) that bind particular cell surface receptors (e.g., TACI and/or BCMA located on B cells). BLYS binding polypeptides fused or conjugated to heterologous polypeptides may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/2 1232; EP 439 095; Naramura et al., *Immunol. Lett.*, 39:91-99 (1994); U.S. Pat. No. 5,474,981; Gillies et al., *Proc. Nat'l Acad. Sci. USA*, 89:1428-1432 (1992); Fell et al., *J. Immunol.*, 146:2446-2452 (1991), which are incorporated by reference in their entireties.

[0145] The present invention further contemplates the use of compositions comprising, or alternatively consisting of, heterologous polypeptides fused or conjugated to BLYS binding polypeptide fragments.

[0146] Fusion proteins useful in the methods of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof), such methods can be used to generate BLYS binding polypeptides with altered activity (e.g., BLYS binding polypeptides with higher affinities and lower dissociation rates). See, generally, U.S. Pat. Nos.

5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opinion Biotechnol.*, 8:724-33 (1997); Harayama, *Trends Biotechnol.*, 16(2):76-82 (1998); Hansson, et al., *J. Mol. Biol.*, 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques*, 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, polynucleotides encoding BLYS binding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more portions of a polynucleotide encoding a BLYS binding polypeptide which portions specifically bind to BLYS may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[0147] Polypeptides of the present invention include products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

[0148] The BLYS binding polypeptides that are used in the methods of the present invention may be modified during or after synthesis or translation, e.g., by glycosylation, acetylation, benzoylation, phosphorylation, amidation, pegylation, formylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, ubiquitination, etc. (See, for instance, Creighton, *Proteins: Structures and Molecular Properties*, 2d Ed. (W.H. Freeman and Co., N.Y., 1992); *Postranslational Covalent Modification of Proteins*, Johnson, ed. (Academic Press, New York, 1983), pp. 1-12; Seifter et al., *Meth. Enzymol.*, 182:626-646 (1990); Rattan et al., *Ann. NY Acad. Sci.*, 663:48-62 (1992).) In specific embodiments, the peptides are acetylated at the N-terminus and/or amidated at the C-terminus.

[0149] BLYS binding polypeptides containing two or more residues that have the potential to interact, such as for example, two cysteine residues in a polypeptide, may be treated under oxidizing conditions or other conditions that promote interaction of these residues (e.g. disulfide bridge formation).

[0150] Further BLYS binding polypeptide modifications contemplated herein include, for example, any of numerous chemical modifications carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc. Additional post-translational/post-synthesis modifications that may be employed include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or

O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression.

[0151] Chemically modified derivatives of BLYS binding polypeptides may be used which may provide additional advantages such as increased affinity, decreased off-rate, solubility, stability and in vivo or in vitro circulating time of the polypeptide, or decreased immunogenicity (see, U.S. Pat. No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0152] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any, on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0153] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Pat. No. 5,643,575; Morpurgo et al., *Appl. Biochem. Biotechnol.*, 56:59-72 (1996); Vorobjev et al., *Nucleosides Nucleotides*, 18:2745-2750 (1999); and Caliceti et al., *Bioconj. Chem.*, 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0154] The polyethylene glycol molecules (or other chemical moieties) should be attached to the BLYS binding polypeptide with consideration of effects on functional domains of the polypeptide. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., *Exp. Hematol.*, 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include, for example, lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulf-

hydriyl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. In a preferred embodiment, the polyethylene glycol molecule is attached at an amino group, such as attachment at the N-terminus or to a lysine side chain amino group.

[0155] As suggested above, polyethylene glycol may be attached to polypeptides via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a polypeptide via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the polypeptide or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the polypeptide.

[0156] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to polypeptide molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated polypeptide. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated polypeptide molecules. Selective N-terminal modification of proteins may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminus) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0157] As indicated above, pegylation of the polypeptides may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.*, 9:249-304 (1992); Francis et al., *Intern. J. of Hematol.*, 68:1-18 (1998); U.S. Pat. No. 4,002,531; U.S. Pat. No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0158] One system for attaching polyethylene glycol directly to amino acid residues of polypeptides without an intervening linker employs tresylated MPEG, which is produced by the modification of monomethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{ClSO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the polypeptide. Thus, the invention includes polypeptide-polyethylene glycol conjugates produced by reacting polypeptides with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

[0159] Polyethylene glycol can also be attached to polypeptides using a number of different intervening linkers. For example, U.S. Pat. No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to

polypeptides. Polypeptide-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the polypeptide by a linker can also be produced by reaction of polypeptides with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichlorophenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number of additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to polypeptides are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated BLYS binding polypeptide products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0160] The number of polyethylene glycol moieties attached to each polypeptide (i.e., the degree of substitution) may also vary. For example, the pegylated polypeptides may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution may range within ranges such as 1-3,2-4, 3-5,4-6, 5-7,6-8, 7-9,8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per polypeptide molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.*, 9:249-304 (1992).

BLYS Binding Polypeptide Multimers, Conjugates and Fusions

[0161] The methods of the present invention may also be carried out using multivalent BLYS binding polypeptides. BLYS binding polypeptides may be monomeric, dimeric, trimeric, or higher-order multimers. In a preferred embodiment multivalent BLYS binding polypeptides are homotrimeric. In another preferred embodiment a homotrimeric BLYS binding polypeptide binds a single homotrimeric BLYS.

[0162] In another preferred embodiment, monomeric or multimeric BLYS binding polypeptides are conjugated with another polypeptide or other chemical compound. For example, BLYS binding polypeptide(s) may be conjugated to a radioactive or other toxic compound so as to target and destroy cells expressing BLYS.

[0163] The present invention also encompasses the use of heteromeric multimers comprised of one or more BLYS binding polypeptides and one or more non-BLYS binding polypeptides or other chemical moieties. Such heteromeric multimers may be monomeric, dimeric, trimeric, tetrameric, pentameric, or higher-order multimers. Heteromeric BLYS binding multimers may be used to target, bind, inhibit, and/or activate responses in cells expressing BLYS and receptors for the heterologous, non-BLYS binding polypeptide or other chemical moiety. Such activated responses may include, for example, apoptosis or other biologically and chemically mediated forms of cell destruction. Heteromeric BLYS binding multimers may also be used to target BLYS expressing cells so as to introduce a desired molecule or compound to the cells. For example, a heteromeric BLYS binding multimer may be conjugated with a radioactive or otherwise toxic compound so as to kill BLYS expressing cells. As an alternative example, a heteromeric BLYS binding and Adenovirus-binding multimer could be used to specifically target and introduce adenovirus-mediated gene therapeutics into BLYS expressing cells.

[0164] BLYS binding polypeptide multimers may be fused or conjugated as homopolymers and heteropolymers using methods known in the art. In a preferred embodiment BLYS binding polypeptides are linked as homomultimers wherein the linker or linkers provide sufficient length and flexibility such that each BLYS binding polypeptide may simultaneously bind an individual BLYS molecule. In another preferred embodiment BLYS binding polypeptides are linked as heteromultimers wherein the linker or linkers provide sufficient length and flexibility such that each BLYS binding polypeptide may simultaneously bind individual BLYS molecules and the heterologous polypeptide or chemical moiety may simultaneously bind to its target. Numerous examples of suitable linker molecules are known in the art. (See, for example, Todorovska et al., *J. Immunol. Methods*, 248(1-2):47-66 (2001); Mehvar, *J. Control Release*, 69(1):1-25 (2000); Francis et al., *Int. J. Hematol.*, 68(1):1-18 (1998).) In specific embodiments, the linker is a member selected from the group consisting of: (a) a peptide linker; (b) a glutamate linker; and (c) a polyethylene glycol linker. The length of linkers to be used according to the methods of the invention may routinely be determined using techniques known in the art. In specific embodiments, the linker is 5-60 angstroms in length. In other embodiments, the linker is 10-50, 10-40, 10-30, or 10-20 angstroms in length. In further embodiments, the linker is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 angstroms in length. In this context "about" includes the recited length, and/or lengths that are larger or smaller by several (5, 4, 3, 2, or 1) angstroms. In other embodiments, the linker is at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 angstroms in length.

[0165] In a preferred embodiment, BLYS binding polypeptides may be fused with human serum albumin (HA). See, e.g., U.S. application Ser. No. 09/833,245, filed Apr. 12, 2001, which is hereby incorporated by reference herein. In one embodiment, the albumin fusion protein comprises HA as the N-terminal portion, and a BLYS binding polypeptide as the C-terminal portion. In another embodiment the albumin fusion protein comprise HA as the C-terminal portion, and a BLYS binding polypeptide as the N-terminal portion.

[0166] In other embodiments, the albumin fusion protein has a BLYS binding polypeptide fused to both the N-terminus and the C-terminus of albumin. In one preferred embodiment, the BLYS binding polypeptides fused at the N- and C-termini are the same BLYS binding polypeptides. In another preferred embodiment, the BLYS binding polypeptides fused at the N- and C-termini are different BLYS binding polypeptides. In another preferred embodiment, a BLYS binding polypeptide is fused at either the N- or C-terminus of HA and a different (non-BLYS binding) polypeptide is fused at either the C- or N-terminus, respectively.

[0167] In addition to albumin fusion proteins in which the BLYS binding polypeptide(s) is (are) fused to the N-terminus and/or C-terminus of HA, BLYS binding polypeptide/albumin fusion proteins may also be produced by inserting the BLYS binding polypeptide into an internal region or regions of HA. For instance, within the protein sequence of the HA molecule a number of loops or turns exist between the end and beginning of α -helices, which are stabilized by disulfide bonds (see FIGS. 9-11 in U.S. application Ser. No. 09/833,245). The loops, as determined from the crystal

structure of HA (FIG. 13 of U.S. application Ser. No. 09/833,245) (PDB identifiers 1AO6, 1BJ5, 1BKE, 1BM0, 1E7E to 1E7I and 1UOR) for the most part extend away from the body of the molecule. These loops are useful for the insertion, or internal fusion, of therapeutically active peptides (particularly those requiring a secondary structure to be functional) or therapeutic proteins, to essentially generate an albumin molecule with specific biological activity.

[0168] Loops in human albumin structure into which binding polypeptides may be inserted to generate albumin fusion proteins include: Val54-Asn61, Thr76-Asp89, Ala92-Glu100, Gln170-Ala176, His 247-Glu252, Glu 266-Glu277, Glu 280-His288, Ala362-Glu368, Lys439-Pro447, Val462-Lys475, Thr478-Pro486, and Lys560-Thr566. In more preferred embodiments, polypeptides are inserted into the Val54-Asn61, Gln170-Ala176, and/or Lys560-Thr566 loops of mature human serum albumin (SEQ ID NO:445).

[0169] In specific embodiments, BLYS binding polypeptides are attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, ^{111}In , ^{177}Lu , ^{90}Y , ^{166}Ho , and ^{153}Sm , to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators attached to BLYS binding polypeptides is ^{111}In . In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to BLYS binding polypeptides is ^{90}Y . In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the BLYS binding polypeptides via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art—see, for example, DeNardo et al., *Clin. Cancer Res.*, 4(10):2483-90 (1998); Peterson et al., *Bioconjug. Chem.*, 10(4):553-7 (1999); and Zimmerman et al., *Nucl. Med. Biol.*, 26(8):943-50 (1999), which are hereby incorporated by reference in their entirety. In addition, U.S. Pat. Nos. 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entireties. Though U.S. Pat. Nos. 5,652,361 and 5,756,065 focus on conjugating chelating agents to antibodies, one skilled in the art would be readily able to adapt the method disclosed therein in order to conjugate chelating agents to other polypeptides.

[0170] The BLYS binding polypeptides can be recovered and purified by known methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

[0171] The BLYS binding polypeptides may also be modified with a detectable label, including, but not limited to, an enzyme, prosthetic group, fluorescent material, luminescent material, bioluminescent material, radioactive material, positron emitting metal, nonradioactive paramagnetic metal ion, and affinity label for detection and isolation of BLYS target. The detectable substance may be coupled or conjugated either directly to the polypeptides or indirectly, through an intermediate (such as, for example, a linker

known in the art) using techniques known in the art. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, glucose oxidase or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include biotin, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi , or other radioisotopes such as, for example, iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium ($^{99\text{m}}\text{Tc}$, $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , $^{0.47}\text{Sc}$, ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru , ^{68}Ge , ^{57}Co , ^{65}Zn , ^{85}Sr , ^{32}P , ^{153}Gd , ^{169}Yb , ^{51}Cr , ^{54}Mn , ^{75}Se , ^{113}Sn , and ^{117}Tm .

[0172] In specific embodiments, BLYS binding polypeptides are attached macrocyclic chelators useful for conjugating radiometal ions, including but not limited to ^{177}Lu , ^{90}Y , ^{166}Ho , and ^{153}Sm , to polypeptides. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the BLYS binding polypeptide via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art—see, for example, DeNardo et al., *Clin. Cancer Res.*, 4(10):2483-90 (1998); Peterson et al., *Bioconjug. Chem.*, 10(4):553-7 (1999); and Zimmerman et al., *Nucl. Med. Biol.*, 26(8):943-50 (1999), which are hereby incorporated by reference in their entirety.

[0173] In a specific embodiment, BLYS binding polypeptides are labeled with biotin.

[0174] The present invention further encompasses the use of BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof), conjugated to a diagnostic or therapeutic agent. The BLYS binding polypeptides can be used diagnostically to, for example, monitor or prognose the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the BLYS binding polypeptide to a detectable substance. Examples of detectable substances include, but are not limited to, various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and non-radioactive paramagnetic metal ions such as, for example, those described herein. The detectable substance may be coupled or conjugated either directly to the BLYS binding polypeptide or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to BLYS binding polypeptides for use as diagnostics according to the present invention.

[0175] Further, a BLYS binding polypeptide (including a molecule comprising, or alternatively consisting of, BLYS

binding polypeptide fragments or variants thereof), may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi , or other radioisotopes such as, for example, ^{103}Pd , ^{133}Xe , ^{131}I , ^{68}Ge , ^{57}Co , ^{65}Zn , ^{85}Sr , ^{32}P , ^{35}S , ^{90}Y , ^{153}Sm , ^{153}Gd , ^{169}Yb , ^{51}Cr , ^{54}Mn , ^{75}Se , ^{113}Sn , $^{90}\text{Yttrium}$, ^{117}Tm , $^{86}\text{Rhenium}$, $^{166}\text{Holmium}$, and $^{188}\text{Rhenium}$. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include, but are not limited to, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, thymidine kinase, endonuclease, RNase, and puromycin and fragments, variants or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thiopa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0176] Techniques known in the art may be applied to label BLYS binding polypeptides. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see, e.g., U.S. Pat. Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

[0177] The BLYS binding polypeptides which are conjugates can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, but are not limited to, for example, a toxin such as abrin, ricin A, alpha toxin, pseudomonas exotoxin, or diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (see, International Publication No. WO 97/33899), AIM II (see, International Publication No. WO 97/34911), fas ligand (Takahashi et al., *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (see, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), or other growth factors.

[0178] A BLYS binding polypeptide (including a molecule comprising, or alternatively consisting of, a BLYS binding

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

Characterization of BLYS Binding Polypeptides

[0179] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) may be characterized in a variety of ways. In particular, BLYS binding polypeptides and related molecules may be assayed for the ability to specifically bind to BLYS or a fragment of BLYS (e.g., to the soluble form or the membrane-bound form of BLYS) using techniques described herein or routinely modifying techniques known in the art. BLYS or BLYS fragments that may be specifically bound by the compositions useful according to the invention include, but are not limited to, human BLYS (SEQ ID NOs:173 and/or 174) or BLYS expressed on human monocytes; murine BLYS (SEQ ID NOs:175 and/or 176) or BLYS expressed on murine monocytes; rat BLYS (either the soluble forms as given in SEQ ID NOs:177, 178, 179 and/or 180 or in a membrane associated form, e.g., on the surface of rat monocytes); or monkey BLYS (e.g., the monkey BLYS polypeptides of SEQ ID NOS:181 and/or 182, the soluble form of monkey BLYS, or BLYS expressed on monkey monocytes) or fragments thereof. Preferably compositions useful according to the invention bind human BLYS (SEQ ID NOs:173 and/or 174) or fragments thereof. Assays for the ability of the BLYS binding polypeptides to specifically bind BLYS or a fragment of BLYS may be performed in solution (e.g., Houghten, *Bio/Techniques*, 13:412-421(1992)), on beads (e.g., Lam, *Nature*, 354:82-84 (1991)), on chips (e.g., Fodor, *Nature*, 364:555-556 (1993)), on bacteria (e.g., U.S. Pat. No. 5,223,409), on spores (e.g., U.S. Pat. Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (e.g., Cull et al., *Proc. Natl. Acad. Sci. USA*, 89:1865-1869 (1992)) or on phage (e.g., Scott and Smith, *Science*, 249:386-390 (1990); Devlin, *Science*, 249:404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci. USA*, 87:6378-6382 (1990); and Felici, *J. Mol. Biol.*, 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). BLYS binding polypeptides that have been identified to specifically bind to BLYS or a fragment of BLYS can then be assayed for their specificity and affinity for BLYS or a fragment of BLYS using or routinely modifying techniques described herein or otherwise known in the art.

[0180] The BLYS binding polypeptides may be assayed for specific binding to BLYS and cross-reactivity with other BLYS-like polypeptides by any method known in the art. In particular, the ability of a BLYS binding polypeptide to specifically bind to the soluble form or membrane-bound form of BLYS and the specificity of the BLYS binding polypeptide, fragment, or variant for BLYS polypeptide from a particular species (e.g., murine, monkey or human, preferably human) may be determined using or routinely modifying techniques described herein or otherwise known in art.

[0181] Assays which can be used to analyze specific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" assays, "immunoprecipitation" assays, precipitin reactions, gel dif-

fusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, radiometric assays, and fluorescent assays, to name but a few. Such assays are routine and well known in the art (see, e.g., *Current Protocols in Molecular Biology*, Vol. 1, Ausubel et al, eds. (John Wiley & Sons, Inc., New York 1994), which is incorporated by reference herein in its entirety) and could easily be adapted to make use of a BLYS binding polypeptide (possibly in conjunction with an anti-BLYS binding polypeptide antibody) in place of an anti-BLYS antibody. Exemplary immunoassays that could be modified to use a BLYS binding polypeptide are described briefly below (but are not intended by way of limitation).

[0182] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%-20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), incubating the membrane with BLYS binding polypeptide (the BLYS binding polypeptide of interest) diluted in blocking buffer, washing the membrane in washing buffer, incubating the membrane with a secondary antibody (which recognizes the BLYS binding polypeptide) conjugated to an enzyme (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ³²P or ¹²⁵I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. Alternatively, the BLYS binding polypeptide may be directly conjugated to a detection molecule (e.g., an enzyme or radiolabel), thereby omitting the need for a secondary anti-BLYS binding polypeptide antibody. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., *Current Protocols in Molecular Biology*, Vol. 1, Ausubel et al, eds. (John Wiley & Sons, Inc., New York 1994) at 10.8.1.

[0183] ELISAs comprise preparing antigen (e.g., BLYS target), coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the BLYS binding polypeptide of interest conjugated to a detectable compound such as an enzyme (e.g., horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound BLYS binding polypeptides or non-specifically bound BLYS binding polypeptides, and detecting the presence of the BLYS binding polypeptides specifically bound to the antigen coating the well. In ELISAs the BLYS binding polypeptide employed in the assay does not have to be conjugated to a detectable compound; instead, an antibody that recognizes the BLYS binding polypeptide and that is conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the BLYS binding polypeptide may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzyme (e.g., horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., *Current Protocols in*

Molecular Biology, Vol. 1, Ausubel et al, eds. (John Wiley & Sons, Inc., New York 1994) at 11.2.1.

[0184] Immunoprecipitation protocols generally use antibody molecules to immunoprecipitate a protein of interest. A BLYS precipitation protocol could easily be modified to use a BLYS binding polypeptide in place of an anti-BLYS antibody. Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasyolol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1 to 4 hours) at 40 degrees C., adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40 degrees C., washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. If one wanted to substitute a BLYS binding polypeptide for the anti-BLYS antibody one could readily do so, and then isolate the BLYS-BLYS binding polypeptide complexes with an antibody that recognizes the BLYS binding polypeptide. Then the triple complex of BLYS, BLYS binding polypeptide, and anti-BLYS binding polypeptide antibody could be isolated using protein A and/or Protein G as described above. Such a protocol may be desirable if, for example, the anti-BLYS binding polypeptide antibody has a higher affinity for the BLYS binding polypeptide than the anti-BLYS antibody may have for BLYS.

[0185] The effectiveness of incorporating a BLYS binding polypeptide in an immunoprecipitation protocol to precipitate BLYS can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the BLYS binding polypeptide to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., *Current Protocols in Molecular Biology*, Vol. 1, Ausubel et al, eds. (John Wiley & Sons, Inc., New York 1994) at 10.16.1.

[0186] The binding affinity of a BLYS binding polypeptide (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) to an antigen and the off-rate of an BLYS binding polypeptide-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a modified radioimmunoassay comprising the incubation of labeled antigen (e.g., ³H- or ¹²⁵I-labeled BLYS target) with the BLYS binding polypeptide of interest in the presence of increasing amounts of unlabeled antigen, followed by detection of the BLYS binding polypeptide bound to the labeled antigen. The affinity of the BLYS binding polypeptide of the present invention for BLYS and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with an anti-BLYS antibody or BLYS binding polypeptide can also be determined using radioimmunoassays. In this case, BLYS is incubated with a BLYS binding polypeptide of the present invention conjugated to a labeled compound (e.g., with ³H or ¹²⁵I) in the presence of increasing amounts of an unlabeled BLYS binding polypeptide or anti-BLYS antibody.

[0187] In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of BLYS

binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) to BLYS, or fragments of BLYS. BIAcore kinetic analysis comprises analyzing the binding and dissociation of BLYS from chips with immobilized BLYS binding polypeptides on their surface (see Example 6, infra).

[0188] The BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) can also be assayed for their ability to inhibit, increase, or not significantly alter, the binding of BLYS to a BLYS receptor (e.g., TACI and BCMA) using techniques known to those skilled in the art. For example, cells expressing a receptor for BLYS (e.g., IM9, REH, ARH-77 cells, Namalwa, and RPMI-8226 B cell tumor lines as well as peripheral CD20+ B cells) can be contacted with BLYS in the presence or absence of a BLYS binding polypeptide, and the ability of the BLYS binding polypeptide to inhibit, increase, or not significantly alter, BLYS binding to the cells can be measured. Alternatively, the BLYS binding polypeptide may be preincubated with the BLYS prior to exposure of the BLYS to cells expressing the BLYS receptor. BLYS binding to cells can be measured by, for example, flow cytometry or a scintillation assay. BLYS or the BLYS binding polypeptide can be labeled with a detectable compound such as a radioactive label (e.g., ³²P, ³⁵S, and ¹²⁵I) or a fluorescent label (e.g., fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine) to enable detection of an interaction between BLYS and a BLYS receptor and/or BLYS and a BLYS binding polypeptide.

[0189] The ability of BLYS binding polypeptides to inhibit, increase, or not significantly alter, BLYS binding to a BLYS receptor can also be determined in cell-free assays. For example, native or recombinant BLYS (e.g., having the amino acid sequence of amino acids 134-285 of SEQ ID NO:173) or a fragment thereof can be contacted with a BLYS binding polypeptide and the ability of the BLYS binding polypeptide to inhibit, increase, or not significantly alter, BLYS from binding to a BLYS receptor can be determined. Preferably, the BLYS binding polypeptide or BLYS receptor is immobilized on a solid support and BLYS or a BLYS fragment is labeled with a detectable compound. Alternatively, BLYS or a BLYS fragment is immobilized on a solid support and the BLYS binding polypeptide is labeled with a detectable compound. BLYS may be partially or completely purified (e.g., partially or completely free of other polypeptides) or part of a cell lysate. Further, the BLYS polypeptide may be a fusion protein comprising BLYS or a biologically active portion thereof and a domain such as an Immunoglobulin Fc or glutathionine-S-transferase. Additionally, the BLYS binding polypeptide and/or BLYS receptor may be a fusion protein comprising a BLYS binding portion of the polypeptide or receptor and a domain such as an Immunoglobulin Fc or glutathionine-S-transferase. For example, amino acid residues 1-154 of TACI (GenBank accession number AAC51790), or 1-48 of BCMA (GenBank accession number NP_001183) may be fused to the Fc region of an IgG molecule and used in a cell free assay to determine the ability of BLYS binding polypeptides to inhibit, increase, or not significantly alter, BLYS binding to a BLYS receptor. Alternatively, BLYS can be biotinylated using techniques well known to those skilled in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, Ill.).

[0190] The BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof), can also be assayed for their ability to inhibit, stimulate, or not significantly alter, BLYS-induced B-cell proliferation using techniques known to those of skill in the art. For example, B-cell proliferation can be assayed by ³H-thymidine incorporation assays and trypan blue cell counts (see, e.g., Moore et al., *Science*, 285: 260-263 (1999)). Further, the BLYS binding polypeptides, or fragments or variants thereof, can be assayed for their ability to inhibit, stimulate, or not significantly alter, BLYS-induced activation of cellular signaling molecules and transcription factors such as calcium-modulator and cyclophilin ligand (CAML), calcineurin, nuclear factor of activated T cells transcription factor (NF-AT), nuclear factor-kappa B (NF-kappa B), SRF, activator protein-1 (AP-1), extracellular-signal regulated kinase 1 (ERK-1), polo like kinases (PLK), ELF-1, high mobility group I (HMG-I), and/or high mobility group Y (HMG-Y) using techniques known to those of skill in the art (see, e.g., von Bulow and Bram, *Science*, 278:138-141(1997)). For example, NF-AT activity can be determined by electromobility gel shift assays, by detecting the expression of a protein known to be regulated by NF-AT (e.g., IL-2 expression), by detecting the induction of a reporter gene (e.g., an NF-AT regulatory element operably linked to a nucleic acid encoding a detectable marker such as luciferase, beta-galactosidase or chloramphenicol acetyltransferase (CAT)), or by detecting a cellular response (e.g., cellular differentiation, or cell proliferation).

[0191] The BLYS binding polypeptides, or fragments or variants thereof can also be assayed for their ability to neutralize, enhance, or not significantly alter, BLYS activity. For example, BLYS binding polypeptides or fragments or variants thereof, may be routinely tested for their ability to inhibit BLYS from binding to cells expressing the receptor for BLYS.

Uses of the Binding Polypeptides and Recombinant Bacteriophage

[0192] The BLYS binding polypeptides described herein are especially useful to detect, isolate, or remove BLYS target proteins in solutions. Such solutions may be simple dispersions or solutions of BLYS and/or BLYS-like polypeptide in water or aqueous buffer or more complex solutions, such as, a blood and other biological fluids, tissue homogenates cell extracts, or biopsy samples, and cell culture media containing BLYS or BLYS-like polypeptides. Biological fluids include, but are not limited to sera, plasma, lymph, blood, blood fractions urine, synovial fluid, spinal fluid, saliva, and mucous.

[0193] In one embodiment, the present invention provides a method for detecting a BLYS protein and/or a BLYS-like polypeptide in a solution comprising contacting the solution with a BLYS binding polypeptide and detecting binding of BLYS or BLYS-like polypeptide to the BLYS binding polypeptide. The BLYS binding polypeptide may be either free or immobilized. Preferably, the BLYS binding polypeptide is a polypeptide immobilized on a solid surface or chromatographic material or the well of a plastic microtiter assay dish.

[0194] Another embodiment of the present invention is a method for isolating BLYS protein and/or BLYS-like polypeptide from a solution containing it, comprising:

[0195] (a) contacting the solution with a BLYS binding polypeptide under conditions that permit binding of BLYS and/or BLYS-like polypeptides to BLYS binding polypeptide, and

[0196] (b) recovering the BLYS and/or BLYS-like polypeptides.

[0197] A further embodiment of the present invention is a method for isolating BLYS protein and/or BLYS-like polypeptide from a solution containing it, comprising:

[0198] (a) contacting the solution with a BLYS binding polypeptide under conditions that permit binding of BLYS and/or BLYS-like polypeptides to BLYS binding polypeptide, and

[0199] (b) separating the complex(es) formed by the BLYS binding polypeptide and BLYS and/or BLYS-like polypeptides from other components of the solution.

[0200] Preferably such method also includes the further steps of:

[0201] (c) dissociating the BLYS binding polypeptide from the BLYS and/or BLYS-like polypeptides, and

[0202] (d) recovering the dissociated, BLYS and/or BLYS-like polypeptide.

[0203] The invention also provides for the use of kits containing a binding polypeptide for use in methods of detecting or isolating BLYS and/or BLYS-like polypeptides.

[0204] According to the invention, detection or isolation of BLYS target proteins comprises contacting a solution containing a BLYS target protein with a BLYS binding polypeptide. Depending on the particular application, the BLYS binding polypeptide may be free in solution or immobilized on a solid support or chromatographic material. Sufficient time is allowed to permit binding between the BLYS target protein and the binding polypeptides, and non-binding components in the solution or mixture are removed or washed away. The formation of a binding complex between the binding polypeptide and the BLYS target protein can then be detected, for example, by detecting the signal from a label on the binding polypeptide, which is one component of the binding complex. A label may be any label that generates a signal that can be detected by standard methods, such as a fluorescent label, a radioactive compound, or an enzyme that reacts with a substrate to generate a detectable signal. Suitable such labels are discussed above. A phage binding polypeptide according to the invention, that is, a recombinant phage displaying a BLYS binding polypeptide on its surface, may form a complex with BLYS and/or BLYS-like polypeptides that is detectable as a precipitate or sediment in a reaction tube, which can be detected visually after settling or centrifugation. Alternatively, a sandwich-type assay may be used, wherein a BLYS binding polypeptide is immobilized on a solid support such as a plastic tube or well, or a chromatographic support matrix such as agarose beads, then the solution suspected of containing the BLYS target is contacted with the immobilized binding polypeptide and non-binding materials or components are removed or washed away.

[0205] The binding polypeptides according to this invention are particularly useful for detection and/or isolation of BLYS and/or BLYS-like polypeptides by affinity chromatog-

raphy methods. Any conventional method of chromatography may be employed. Preferably, a BLYS binding polypeptide will be immobilized on a solid support suitable, for example, for packing a chromatography column. The immobilized BLYS binding polypeptide affinity ligand can then be loaded or contacted with a feed stream under conditions favorable to formation of binding polypeptide/BLYS (or BLYS-like polypeptide) complexes. Non-binding materials can be washed away. Examples of suitable wash conditions can readily be determined by one of skill in the art and include but are not limited to [PBS/0.01% Tween 20, pH7.2] and [1M NaCl/10 mM Tris, pH7.5]. Tris wash buffers may be preferable since phosphates can precipitate in 50% ethylene glycol. In general, non-limiting terms, wash buffers are pH7.0, optionally containing 0.0 to 1.5 M NaCl, more preferably 1 M NaCl. Additionally, wash buffers may optionally contain a mild detergent, such as, for example, Tween 20, Tween 80, or NP-80. BLYS or BLYS-like polypeptide can be eluted from the BLYS binding polypeptide by introducing solution conditions that favor dissociation of the binding complex. Suitable elution solutions can readily be determined by one of skill in the art and include but are not limited to [50% ethylene glycol/100 mM NaOAc]. By way of non-limiting example, useful elution buffers, for the purposes of the present invention contain 40-60% ethylene glycol, preferably 50% ethylene glycol; and 50-100 mM NaOAc with a pH in the range of pH 4-pH7, more preferably, pH 4-pH 6 and most preferably pH 4.5-pH 5.5. Preferably, a fast flow affinity chromatographic technique is used to bind the molecules and from which purified BLYS or BLYS-like polypeptides are eluted.

[0206] Alternatively, batch chromatography can be carried out by mixing a solution containing the BLYS target and the BLYS binding polypeptide, then isolating complexes of the BLYS target and the binding polypeptides. For this type of separation, many methods are known. For example, the binding polypeptide may be immobilized on a solid support such as beads, then separated from the feed stream along with the BLYS target by filtration. In another example, the BLYS binding polypeptide may be modified with its own affinity tag, such as a polyHis tail or streptavidin binding region, which can be used to isolate the binding polypeptide after complexes have formed using an immobilized metal affinity chromatographic resin or streptavidin-coated substrate. Once separated, the BLYS target can be released from the binding polypeptide under elution conditions and recovered in a purified form.

[0207] Methods of producing BLYS or a BLYS-like polypeptides usually yield BLYS or BLYS-like polypeptides in a feed stream that additionally contains impurities (with respect to the BLYS target). One purpose of the present invention is to produce BLYS binding polypeptides and preparations (such as affinity chromatography media or surfaces) comprising BLYS binding polypeptides that allow rapid and highly specific purification of BLYS target proteins from a feed stream. BLYS binding polypeptides obtained herein may easily be tailored to isolate BLYS target protein from a particular feed stream, using or routinely modifying conditions and techniques known in the art. If an alternate production method for BLYS is used, producing a different feed stream, a different set of BLYS binding polypeptides and/or conditions may be necessary to achieve the same level of purification. The new set of BLYS binding polypep-

tides and/or conditions can be readily obtained following or modifying procedures outlined herein, or otherwise known in the art.

Use of BLYS Binding Polypeptides for Epitope Mapping

[0208] The present invention provides BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof), that can be used to identify epitopes of BLYS. In particular, the BLYS binding polypeptides of the present invention can be used to identify epitopes of human BLYS (SEQ ID NOS:173 and/or 174) or BLYS expressed on human monocytes; murine BLYS (SEQ ID NOS:175 and/or 176) or BLYS expressed on murine monocytes; rat BLYS (either the soluble forms as given in SEQ ID NOS:177, 178, 179 and/or 180 or in a membrane associated form, e.g., on the surface of rat monocytes); or monkey BLYS (e.g., the monkey BLYS polypeptides of SEQ ID NOS:181 and/or 182, the soluble form of monkey BLYS, or BLYS expressed on monkey monocytes) using techniques described herein or otherwise known in the art. Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, *Proc. Natl. Acad. Sci. USA*, 82:5131-5135 (1985), further described in U.S. Pat. No. 4,631,211.)

Diagnostic Uses of BLYS Binding Polypeptides

[0209] Labeled and non-labelled BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) which specifically bind to BLYS can be used for diagnostic purposes to detect, diagnose, prognose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of BLYS or BLYS receptor. The invention provides for the detection of aberrant expression of BLYS comprising: (a) assaying the expression of BLYS in a biological sample from an individual using one or more BLYS binding polypeptides that specifically binds to BLYS; and (b) comparing the level of BLYS with a standard level of BLYS, e.g., in normal biological samples, whereby an increase or decrease in the assayed level of BLYS compared to the standard level of BLYS is indicative of aberrant expression.

[0210] By "biological sample" is intended any fluids and/or cells obtained from an individual, body fluid, body tissue, body cell, cell line, tissue culture, or other source which may contain BLYS protein or mRNA. Body fluids include, but are not limited to, sera, plasma, urine, synovial fluid, spinal fluid, saliva, and mucous. Tissues samples may be taken from virtually any tissue in the body. Tissue samples may also be obtained from autopsy material. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0211] The invention also provides for the detection of aberrant expression of BLYS receptor comprising (a) assaying the expression of BLYS receptor in a biological sample from an individual using one or more BLYS binding polypeptides or fragments or variants thereof that specifically binds only to soluble BLYS, but does not inhibit BLYS/BLYS receptor binding. Such a BLYS binding polypeptide, by way of an example that is not to be construed as limiting, would be one that is able to capture a biotinylated BLYS from solution, but that would not prevent

BLYS from binding to its receptor expressed, for example on IM-9 cells, and (b) comparing the level of BLYS receptor with a standard level of BLYS receptor, e.g., in normal tissue or cell samples, whereby an increase or decrease in the assayed level of BLYS receptor compared to the standard level of BLYS receptor is indicative of aberrant expression.

[0212] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) which specifically bind to BLYS can be used for diagnostic purposes to detect, diagnose, prognose, or monitor immune system diseases and disorders, including but not limited to autoimmune diseases and disorders and/or immunodeficiencies, and/or diseases, disorders, or conditions associated therewith. The invention provides for the detection of aberrant expression of BLYS comprising: (a) assaying the expression of BLYS in a biological sample from an individual using one or more BLYS binding polypeptides that specifically binds to BLYS; and (b) comparing the level of BLYS with a standard level of BLYS, e.g., in normal biological samples, whereby an increase or decrease in the assayed level of BLYS compared to the standard level of BLYS is indicative of an autoimmune disorder or disease and/or an immunodeficiency. In specific embodiments, an increase in the assayed level of BLYS is indicative of an autoimmune disorder or disease. In other specific embodiments, a decrease in the assayed level of BLYS is indicative of an immunodeficiency.

[0213] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) which specifically bind to BLYS but do not inhibit BLYS/BLYS receptor binding can be used for diagnostic purposes to detect, diagnose, prognose, or monitor immune system diseases and disorders, including but not limited to autoimmune diseases and disorders and/or immunodeficiencies, and/or diseases, disorders, or conditions associated therewith. The invention provides for the detection of aberrant expression of BLYS receptor comprising: (a) assaying the expression of BLYS receptor in a biological sample from an individual using one or more BLYS binding polypeptides that specifically binds to BLYS; and (b) comparing the level of BLYS receptor with a standard level of BLYS receptor, e.g., in normal biological samples, whereby an increase or decrease in the assayed level of BLYS receptor compared to the standard level of BLYS receptor is indicative of an autoimmune disorder or disease and/or an immunodeficiency. In specific embodiments, an increase in the assayed level of BLYS receptor is indicative of an autoimmune disorder or disease. In other specific embodiments, a decrease in the assayed level of BLYS receptor is indicative of an immunodeficiency.

[0214] Autoimmune disorders, diseases, or conditions that may be detected, diagnosed, prognosed, or monitored using the BLYS binding polypeptides include, but are not limited to, autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmune neutropenia, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, gluten-sensitive enteropathy, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g., IgA nephropathy), multiple sclerosis, neuritis, uveitis ophthalmia, polyendocrinopathies, purpura (e.g., Henoch-Schoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, autoimmune pulmonary inflammation,

myocarditis, IgA glomerulonephritis, dense deposit disease, rheumatic heart disease, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis), systemic lupus erythematosus, discoid lupus, Goodpasture's syndrome, Pemphigus, receptor autoimmunities such as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rheumatoid arthritis, scleroderma with anti-collagen BLYS binding polypeptides, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerular nephritis such as primary glomerular nephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiomyopathy, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulomatous, degenerative, and atrophic disorders.

[0215] In specific embodiments, the present invention encompasses methods and compositions for detecting, diagnosing, prognosing, and/or monitoring diseases or disorders associated with hypergammaglobulinemia (e.g., AIDS, autoimmune diseases, and some immunodeficiencies). In other specific embodiments, the present invention encompasses methods and compositions for detecting, diagnosing, prognosing, and/or monitoring diseases or disorders associated with hypogammaglobulinemia (e.g., an immunodeficiency).

[0216] Immunodeficiencies that may be detected, diagnosed, prognosed, or monitored using the BLYS binding polypeptides include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia/aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0217] Elevated levels of soluble BLYS have been observed in the serum of patients with Systemic Lupus

Erythematosus (SLE). In comparing the sera of 150 SLE patients with that of 38 control individuals, it was found that most of the SLE patients had more than 5 ng/ml of serum BLYS, more than 30% of SLE patients had levels greater than 10 ng/ml, and approximately 10% of SLE patients had serum BLYS levels greater than 20 ng/ml. In contrast, the majority of normal controls had BLYS levels less than 5 ng/ml, and less than 10% had levels higher than 10 ng/ml. The elevated levels of BLYS protein in sera is present in the soluble form and has biologic activity as assayed by the ability to stimulate anti-IgM treated B cells in vitro. SLE patients with more than 15 ng/ml serum BLYS were also found to have elevated levels of anti-dsDNA antibodies compared to both normal controls and SLE patients with less than 5 ng/ml of serum BLYS (unpublished data).

[0218] In addition the serum of two subgroups of patients which were positive for anti-nuclear antibodies (ANA+) but did not meet the formal requirements of the American College of Rheumatology (ACR) for classification of SLE were analyzed for BLYS levels. The first subgroup of sera was ANA+ sera that came from patients who did not present with the clinical impression of SLE. This group had only slightly elevated levels of BLYS (~9 ng/ml BLYS). The second subgroup, however, which was ANA+ sera from patients who presented with the clinical impression of SLE, had significantly increased BLYS levels (~15 ng/ml). These results suggest that an elevated level of BLYS precedes the formal fulfillment of the ACR criteria. The ACR criteria are described in Tan et al., *Arthritis and Rheumatism*, 25:1271-1277 (1982).

[0219] Thus, in specific embodiments, BLYS binding polypeptides which specifically bind to BLYS can be used for diagnostic purposes to detect, diagnose, prognose, or monitor Systemic Lupus Erythematosus or conditions associated therewith. The invention provides for the detection of aberrant expression of BLYS comprising: (a) assaying the expression of BLYS in a biological sample (e.g., serum, synovial fluid) of an individual using one or more BLYS binding polypeptides that specifically binds to BLYS; and (b) comparing the level of BLYS with a standard level of BLYS, e.g., in normal biological samples, whereby an increase in the assayed level of BLYS compared to the standard level of BLYS is indicative of SLE.

[0220] In additional embodiments, BLYS binding polypeptides which specifically bind to BLYS can be used for diagnostic purposes to detect, diagnose, prognose, or monitor Rheumatoid Arthritis. The invention provides for the detection of aberrant expression of BLYS comprising: (a) assaying the expression of BLYS in a biological sample (e.g., serum, synovial fluid) of an individual using one or more BLYS binding polypeptides that specifically binds to BLYS; and (b) comparing the level of BLYS with a standard level of BLYS, e.g., in normal biological samples, whereby an increase in the assayed level of BLYS compared to the standard level of BLYS is indicative of Rheumatoid Arthritis.

[0221] In specific embodiments, the present invention encompasses methods and compositions for detecting, diagnosing and/or prognosing diseases or disorders of cells of hematopoietic origin. Cells of hematopoietic origin include, but are not limited to, lymphocytes (e.g., B cells and T cells), monocytes, macrophages, dendritic cells, polymorphonuclear leukocytes (e.g., basophils, eosinophils, neutro-

phils), mast cells, platelets, erythrocytes and progenitor cells of these lineages. Cells of hematopoietic origin include, but are not limited to, healthy and diseased cell as found present in an animal, preferably a mammal and most preferably a human, or as isolated from an animal, transformed cells, cell lines derived from the above listed cell types, and cell cultures derived from the above listed cell types. Cells of hematopoietic origin may be found or isolated in, for example, resting, activated or anergic states.

[0222] In specific embodiments, the present invention encompasses methods and compositions for detecting, diagnosing, prognosing and or monitoring growth, progression, and/or metastases of malignancies and proliferative diseases or disorders associated with increased cell survival, or the inhibition of apoptosis. For a review of such disorders, see Fishman et al., *Medicine*, 2d Ed. (J. B. Lippincott Co., Philadelphia 1985). An extensive list of examples of proliferative diseases and disorders is presented below in the section of this application entitled "Therapeutic Uses of BLYS Binding Polypeptides." Proliferative diseases and disorders is also extended to include premalignant conditions (e.g., benign tumors, hyperproliferative disorders, and benign proliferative disorders—see below) as well as proliferative disorders of B cells, monocytes, macrophages, and T cells. Other abnormal growth conditions that may be treated, diagnosed, prognosed or monitored include, but are not limited to, hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, *Basic Pathology*, 2d Ed. (W.B. Saunders Co., Philadelphia 1976), pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

[0223] In preferred embodiments, the present invention encompasses methods and compositions for detecting, diagnosing, prognosing and or monitoring growth, progression, and/or metastases of malignancies and proliferative diseases or disorders of monocytic cells.

[0224] In specific embodiments, the present invention encompasses methods and compositions for detecting, diagnosing, prognosing and or monitoring growth, progression, and/or metastases of malignancies and proliferative diseases or disorders of B cells.

[0225] The invention provides a diagnostic assay for diagnosing or prognosing a disease or disorder, comprising: (a) assaying for the level of BLYS in a biological sample of an individual using one or more BLYS binding polypeptides

that specifically bind to BLYS; and (b) comparing the level of BLYS with a standard BLYS level, e.g., in a biological sample from a patient without the disease or disorder, whereby an increase or decrease in the assayed BLYS level compared to the standard level of BLYS is indicative of a particular disease or disorder. With respect to cancer, the presence of a relatively high amount of BLYS in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0226] In specific embodiments, the presence of a relatively high amount of membrane-bound BLYS in a biological sample is indicative of monocytic cell related leukemias or lymphomas, such as, for example acute myelogenous leukemia, and/or the severity thereof.

[0227] In other specific embodiments, the presence of a relatively high amount of BLYS receptor in a biological sample (as determined using BLYS binding polypeptides that bind to soluble BLYS, but do not inhibit BLYS/BLYS receptor binding) is indicative of B cell related leukemias or lymphomas (e.g., chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin's lymphoma, and Hodgkin's disease), and/or the severity thereof.

[0228] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) can be used to assay protein levels in a biological sample using classical immunohistological methods as described herein or as known to those of skill in the art (e.g., see Jalkanen et al., *J. Cell. Biol.*, 101:976-985 (1985); Jalkanen et al., *J. Cell. Biol.*, 105:3087-3096 (1987)). Other methods that can be used for detecting protein gene expression that might utilize BLYS binding polypeptides or fragments or variants thereof include, but are not limited to, the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, alkaline phosphatase, and horseradish peroxidase; radioisotopes, such as iodine (^{121}I , ^{123}I , ^{125}I , ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{111}In , ^{112}In , ^{113}In , $^{115\text{m}}\text{In}$), technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , and ^{97}Ru ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0229] Certain embodiments of the invention are directed to the detection and diagnosis of a disease or disorder associated with aberrant expression of BLYS or BLYS receptor in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: (a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled BLYS binding polypeptide (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) that specifically binds to BLYS; (b) waiting for a time interval following the administering for permitting the labeled BLYS binding

polypeptide to preferentially concentrate at sites in the subject where BLYS is expressed (and for unbound labeled molecule to be cleared to background level); (c) determining background level; and (d) detecting the labeled BLYS binding polypeptide in the subject, such that detection of labeled BLYS binding polypeptide or fragment thereof above the background level and above or below the level observed in a person without the disease or disorder indicates that the subject has a particular disease or disorder associated with aberrant expression of BLYS or BLYS receptor. Background level can be determined by various methods, including comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0230] It will be understood by those skilled in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}Tc . The labeled BLYS binding polypeptide will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments," Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S. W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

[0231] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0232] In an embodiment for monitoring of the disease or disorder, the method is carried out by repeating the method for diagnosing the disease or disorder, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc. and comparing the results of the successive tests.

[0233] Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0234] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (see, e.g., Thurston et al., U.S. Pat. No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Immunophenotyping Using BLYS Binding Polypeptides

[0235] The BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) may be utilized for immunophenotyping of cell lines and biological samples by their BLYS expression or BLYS receptor expression. Various techniques can be employed utilizing BLYS binding polypeptides, fragments, or variants to screen for cellular populations (i.e., immune cells, particularly monocytic cells or B-cells) expressing BLYS or BLYS receptor. Such techniques include magnetic separation using BLYS binding polypeptide-coated magnetic beads, "panning" with BLYS binding polypeptide attached to a solid matrix (i.e., plate), and flow cytometry (see, e.g., U.S. Pat. No. 5,985,660; and Morrison et al., *Cell*, 96:737-49 (1999)).

[0236] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e., minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

[0237] In one embodiment, BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) are used to identify cells of monocytic or B cell origin.

Therapeutic Uses of BLYS Binding Polypeptides

[0238] The present invention is further directed to BLYS binding polypeptide-based therapies which involve administering BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, BLYS binding polypeptides and nucleic acids encoding BLYS binding polypeptides and antibodies that bind BLYS binding polypeptides as described herein. The BLYS binding polypeptides can be used to treat, ameliorate or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of BLYS or BLYS receptor, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant BLYS expression and/or activity or aberrant BLYS receptor expression and/or activity includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. BLYS binding polypeptides may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0239] BLYS binding polypeptides of the present invention (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) that function as agonists or antagonists of BLYS, preferably of BLYS-induced signal transduction, can be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, lack of BLYS function, aberrant BLYS receptor expres-

sion, or lack of BLYS receptor function. For example, BLYS binding polypeptides which disrupt the interaction between BLYS and one or more of its receptors may be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, excessive BLYS function, aberrant BLYS receptor expression, or excessive BLYS receptor function. BLYS binding polypeptides which do not prevent BLYS from binding its receptor but inhibit or downregulate BLYS-induced signal transduction can be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, excessive BLYS function, aberrant BLYS receptor expression, or excessive BLYS receptor function. In particular, BLYS binding polypeptides of the present invention which prevent BLYS-induced signal transduction by specifically recognizing the unbound BLYS, receptor-bound BLYS, or both unbound and receptor-bound BLYS can be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, excessive BLYS function, aberrant BLYS receptor expression, or excessive BLYS receptor function.

[0240] The ability of a BLYS binding polypeptide to inhibit or downregulate BLYS-induced signal transduction may be determined by techniques described herein or otherwise known in the art. For example, BLYS-induced receptor activation and the activation of signaling molecules can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or a signaling molecule by immunoprecipitation followed by western blot analysis (for example, as described herein).

[0241] In a specific embodiment, a BLYS binding polypeptide of the present invention (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) that inhibits or reduces BLYS activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to BLYS activity in the absence of the BLYS binding polypeptide, is administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, excessive BLYS function, aberrant BLYS receptor expression, or excessive BLYS receptor function. In another embodiment, a combination of BLYS binding polypeptides, a combination of BLYS binding polypeptide fragments, a combination of BLYS binding polypeptide variants, or a combination of BLYS binding polypeptides, BLYS binding polypeptide fragments, and/or variants that inhibit or reduce BLYS activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to BLYS activity in absence of said BLYS binding polypeptides, BLYS binding polypeptide fragments, and/or BLYS binding polypeptide variants are administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, excessive BLYS function, aberrant BLYS receptor expression, or excessive BLYS receptor function.

[0242] Further, BLYS binding polypeptides of the present invention (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) which activate BLYS-induced signal transduc-

tion can be administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, lack of BLYS function, aberrant BLYS receptor expression, or lack of BLYS receptor function. These BLYS binding polypeptides may potentiate or activate either all or a subset of the biological activities of BLYS-mediated receptor activation, for example, by inducing multimerization of BLYS and/or multimerization of the receptor. The BLYS binding polypeptides may be administered with or without being pre-complexed with BLYS. In a specific embodiment, a BLYS binding polypeptide of the present invention that increases BLYS activity by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% or more relative to BLYS activity in absence of the BLYS binding polypeptide is administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, lack of BLYS function, aberrant BLYS receptor expression, or lack of BLYS receptor function. In another embodiment, a combination of BLYS binding polypeptides, a combination of BLYS binding polypeptide fragments, a combination of BLYS binding polypeptide variants, or a combination of BLYS binding polypeptides, BLYS binding polypeptide fragments and/or BLYS binding polypeptide variants that increase BLYS activity by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% or more relative to BLYS activity in absence of the said BLYS binding polypeptides or BLYS binding polypeptide fragments and/or BLYS binding polypeptide variants is administered to an animal to treat, prevent or ameliorate a disease or disorder associated with aberrant BLYS expression, lack of BLYS function, aberrant BLYS receptor expression, or lack of BLYS receptor function.

[0243] In a specific embodiment, the present invention provides a method of treating, preventing or ameliorating a disease or disorder associated with aberrant BLYS or BLYS receptor expression or activity, comprising administering to an animal in which such treatment, prevention or amelioration is desired, a BLYS binding polypeptide in an amount effective to treat, prevent or ameliorate the disease or disorder. Diseases and disorders which may be treated, prevented or ameliorated by this method include, but are not limited to, immune system diseases and disorders (e.g., autoimmune diseases and disorders, immunodeficiencies, lupus, rheumatoid arthritis, multiple sclerosis, hypogammaglobulinemia and hypergammaglobulinemia), graft vs. host disease, proliferative diseases and disorders (e.g., cancer) and infectious diseases and disorders.

[0244] In a specific embodiment, the present invention provides a method of treating, preventing or ameliorating a disease or disorder of cells of hematopoietic origin, comprising administering to an animal in which such treatment, prevention, or amelioration is desired, a BLYS binding polypeptide in an amount effective to treat, prevent or ameliorate the disease or disorder. Cells of hematopoietic origin include, but are not limited to, lymphocytes (e.g., B cells and T cells), monocytes, macrophages, dendritic cells,

polymorphonuclear leukocytes (e.g., basophils, eosinophils, neutrophils), mast cells, platelets, erythrocytes and progenitor cells of these lineages.

[0245] One or more BLYS binding polypeptides of the present invention (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) that specifically bind to BLYS may be used locally or systemically in the body as a therapeutic. The BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) may also be advantageously utilized in combination with monoclonal or chimeric antibodies, lymphokines and/or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the BLYS binding polypeptides.

[0246] The BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy, anti-tumor agents, anti-angiogenesis and anti-inflammatory agents).

[0247] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) that specifically bind to BLYS, or polynucleotides encoding BLYS binding polypeptides that specifically bind to BLYS, for both immunoassays directed to and therapy of disorders related to BLYS polynucleotides or polypeptides, including fragments thereof. Such BLYS binding polypeptides will preferably have an affinity for BLYS and/or BLYS fragments. Preferred binding affinities include those with a dissociation constant or K_D of less than or equal to 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, or 10^{-5} M. More preferably, BLYS binding polypeptides bind BLYS target proteins with a dissociation constant or K_D less than or equal to 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, or 10^{-8} M. Even more preferably, BLYS binding polypeptides bind BLYS target proteins with a dissociation constant or K_D less than or equal to 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0248] In a preferred embodiment, BLYS binding polypeptides neutralize BLYS activity. In another preferred embodiment, BLYS binding polypeptides inhibit B cell proliferation.

[0249] In a preferred embodiment, BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) inhibit or reduce binding of the soluble form of BLYS to a BLYS receptor. In another preferred embodiment BLYS binding polypeptides inhibit or reduce B cell proliferation induced by the soluble form of BLYS. In another preferred embodiment BLYS binding polypeptides inhibit or reduce immunoglobulin production induced by the soluble form of BLYS.

[0250] In a preferred embodiment, BLYS binding polypeptides (including molecules comprising, or alternatively con-

sisting of, BLyS binding polypeptide fragments or variants thereof) inhibit or reduce binding of membrane-bound BLyS to a BLyS receptor. In another preferred embodiment, BLyS binding polypeptides inhibit or reduce B cell proliferation induced by the membrane-bound form of BLyS. In another preferred embodiment, BLyS binding polypeptides inhibit or reduce immunoglobulin production induced by the membrane bound form of BLyS.

[0251] In a preferred embodiment, BLyS binding polypeptides (including molecules comprising, or alternatively consisting of, BLyS binding polypeptide fragments or variants thereof) inhibit or reduce binding of both the soluble and membrane-bound forms of BLyS to a BLyS receptor. In another preferred embodiment, BLyS binding polypeptides inhibit or reduce B cell proliferation induced by either or both forms of BLyS. In another preferred embodiment, BLyS binding polypeptides inhibit or reduce immunoglobulin production induced by either or both forms of BLyS.

[0252] In one embodiment, the invention provides a method of delivering radiolabelled BLyS binding polypeptide and/or BLyS binding polypeptide conjugates to targeted cells, such as, for example, monocytic cells expressing the membrane-bound form of BLyS, or B cells expressing a BLyS receptor.

[0253] In one embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g., IgM, IgG, and/or IgA production), comprising, or alternatively consisting of, contacting an effective amount of BLyS binding polypeptide with BLyS, wherein the effective amount of BLyS binding polypeptide inhibits or reduces BLyS mediated immunoglobulin production. In another embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g., IgM, IgG, and/or IgA production), comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a BLyS binding polypeptide in an amount effective to inhibit or reduce immunoglobulin production.

[0254] In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g., IgM, IgG, and/or IgA production), comprising, or alternatively consisting of, contacting an effective amount of BLyS binding polypeptide with BLyS, wherein the effective amount of the BLyS binding polypeptide stimulates BLyS mediated immunoglobulin production. In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g., IgM, IgG, and/or IgA production) comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a BLyS binding polypeptide in an amount effective to stimulate immunoglobulin production. Determination of immunoglobulin levels are most often performed by comparing the level of immunoglobulin in a sample to a standard containing a known amount of immunoglobulin using ELISA assays. Determination of immunoglobulin levels in a given sample, can readily be determined using ELISA or other method known in the art.

[0255] Receptors belonging to the TNF receptor (TNFR) super family (e.g., TACI and BCMA) can be classified into two types based on the presence or absence of a conserved cytoplasmic domain responsible for apoptosis called a

“death domain.” TNF receptors without death domains, such as TNF-R2 HVEM/ATAR, RANK, CD27, CD30, CD40, and OX40 interact with TNF receptor associated factors (TRAF 1-6) and mediate anti-apoptotic survival and/or proliferative responses via activation of the transcription factor NF-kappaB (reviewed in Wajant et al., *Cytokine and Growth Factor Reviews*, 10(1):15-26, 1999). TACI and BCMA do not contain death domains.

[0256] Investigation of BLyS induced signaling in human tonsillar B cells co-stimulated with *Staph. aureus* Cowan consistently revealed that mRNA for ERK-1 and PLK were upregulated by BLyS+SAC treatment (see Example 12). Polo like kinases (PLK) belong to a sub family of serine/threonine kinases related to *Saccharomyces cerevisiae* cell cycle protein CDC5 (29). The expression of PLK is induced during G2 and S phase of the cell cycle. PLK is reported to play a role in cell proliferation (Lee et al., *Proc. Natl. Acad. Sci.*, 95:9301-9306, 1998). The role of extracellular-signal related kinases (ERK1/2) in cell survival and proliferative effects of growth factors and other agonists has been extensively studied. The induced expression of PLK and ERK-1 is consistent with the survival and proliferative effects of BLyS on B cells.

[0257] Additionally, in some samples of human tonsillar B cells stimulated with BLyS and SAC, mRNA for CD25 (IL-2Ralpha) was upregulated. Nuclear extracts from Human tonsillar B cells treated with BLyS and from IM-9 cells treated with BLyS were able to shift probes from the CD25 promoter region containing sites for NF-kappaB, SRF, ELF-1 and HMG1/Y in an electromobility shift assay. ELF-1 for example, is a transcription factor that is part of the ETS family of proteins and whose expression appears to be restricted to T and B cells. Binding sites for ELF-1 have been described in the promoters of a number of proteins that are important in the regulation of the immune response.

[0258] Thus BLyS induced signaling has been shown to be consistent with the activation of cellular activation and cellular proliferation pathways as well as with cellular signaling pathways that regulate B cell lifespan. Further, BLyS treatment of B cells induces cellular proliferation immunoglobulin secretion, a characteristic of activated B cells (Moore et al., *Science*, 285:260-263, 1999). BLyS binding polypeptides complexed with BLyS may inhibit, stimulate, or not significantly alter these BLyS mediated activities.

[0259] In one embodiment, the invention provides methods and compositions for inhibiting or reducing B cell proliferation, comprising, or alternatively consisting of, contacting an effective amount of BLyS binding polypeptide with BLyS, wherein the effective amount of BLyS binding polypeptide inhibits or reduces BLyS mediated B cell proliferation. In another embodiment, the invention provides methods and compositions for inhibiting or reducing B cell proliferation comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a BLyS binding polypeptide in an amount effective to inhibit or reduce B cell proliferation.

[0260] In one embodiment, the invention provides methods and compositions for stimulating B cell proliferation, comprising, or alternatively consisting of, contacting an effective amount of BLyS binding polypeptide with BLyS, wherein the effective amount of BLyS binding polypeptide stimulates BLyS mediated B cell proliferation.

[0261] In one embodiment, the invention provides methods and compositions for stimulating B cell proliferation, comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a BLYS binding polypeptide in an amount effective to stimulate B cell proliferation.

[0262] B cell proliferation is most commonly assayed in the art by measuring tritiated thymidine incorporation (see Examples 7 and 8). This and other assays are commonly known in the art and may be routinely adapted for the use of determining the effect of BLYS binding polypeptides on B cell proliferation.

[0263] In one embodiment, the invention provides methods and compositions for inhibiting or reducing activation of B cells, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide inhibits or reduces BLYS mediated B cell activation.

[0264] In one embodiment, the invention provides methods and compositions for inhibiting or reducing activation of B cells, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a BLYS binding polypeptide in an amount effective to inhibit or reduce B cell activation.

[0265] In one embodiment, the invention provides methods and compositions for increasing activation of B cells, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide increases BLYS mediated activation of B cells.

[0266] In one embodiment, the invention provides methods and compositions for increasing activation of B cells, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a BLYS binding polypeptide in an amount effective to increase B cell activation.

[0267] B cell activation can be measured in a variety of ways, such as FACS analysis of activation markers expressed on B cells. B cell activation markers include, but are not limited to, CD26, CD 28, CD 30, CD 38, CD 39, CD 69, CD70, CD71, CD 77, CD 83, CD126, CDw130, and B220. Additionally, B cell activation may be measured by analysis of the activation of signaling molecules involved in B cell activation. By way of non-limiting example, such analysis may take the form of analyzing mRNA levels of signaling molecules by Northern analysis or real time PCR (Example 12). One can also measure, for example, the phosphorylation of signaling molecules using anti-phosphotyrosine antibodies in a Western blot. B cell activation may also be measured by measuring the calcium levels in B cells. These and other methods of determining B cell activation are commonly known in the art and may be routinely adapted for the use of determining the effect of BLYS binding polypeptides on B cell activation.

[0268] In one embodiment, the invention provides methods and compositions for decreasing lifespan of B cells, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide inhibits or reduces BLYS regulated lifespan of B cells.

[0269] In one embodiment, the invention provides methods and compositions for decreasing lifespan of B cells, comprising, or alternatively consisting of, administering to an animal in which such decrease is desired, a BLYS binding polypeptide in an amount effective to decrease B cell lifespan.

[0270] In one embodiment, the invention provides methods and compositions for increasing lifespan of B cells, comprising, or alternatively consisting of, contacting an effective amount of BLYS binding polypeptide with BLYS, wherein the effective amount of BLYS binding polypeptide increases BLYS regulated lifespan of B cells.

[0271] In one embodiment, the invention provides methods and compositions for increasing lifespan of B cells, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a BLYS binding polypeptide in an amount effective to increase lifespan of B cells.

[0272] B cell life span in vivo may be measured by 5-bromo-2'-deoxyuridine (BrdU) labeling experiments which are well known to one skilled in the art. BrdU is a thymidine analogue that gets incorporated into the DNA of dividing cells. Cells containing BrdU in their DNA can be detected using, for example fluorescently labeled anti-BrdU antibody and flow cytometry. Briefly, an animal is injected with BrdU in an amount sufficient to label developing B cells. Then, a sample of B cells is withdrawn from the animal, for example, from peripheral blood, and analyzed for the percentage of cells that contain BrdU. Such an analysis performed at several time points can be used to calculate the half life of B cells. Alternatively, B cell survival may be measured in vitro. For example B cells may be cultured under conditions where proliferation does not occur, (for example the media should contain no reagents that crosslink the immunoglobulin receptor, such as anti-IgM antibodies) for a period of time (usually 2-4 days). At the end of this time, the percent of surviving cells is determined, using for instance, the vital dye Trypan Blue, or by staining cells with propidium iodide or any other agent designed to specifically stain apoptotic cells and analyzing the percentage of cells stained using flow cytometry. One could perform this experiment under several conditions, such as B cells treated with BLYS, B cells treated with BLYS/BLYS binding polypeptide complexes, and untreated B cells in order to determine the effects of BLYS and BLYS binding polypeptides on B cells survival. These and other methods for determining B cell lifespan are commonly known in the art and could routinely be adapted to determining the effect of BLYS binding polypeptides on BLYS regulated B cell lifespan.

[0273] In one embodiment, the invention provides a method for the specific delivery of BLYS binding polypeptides and BLYS binding polypeptide conjugates to cells by administering molecules that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) in the targeted cell.

[0274] In another embodiment, the invention provides for a method of killing cells of hematopoietic origin, comprising, or alternatively consisting of, contacting BlyS binding polypeptides with BlyS to form a complex; and contacting the complex with cells of hematopoietic origin. In specific embodiments, the method of killing cells of hematopoietic origin, comprises, or alternatively consists of, administering to an animal in which such killing is desired, a BlyS binding polypeptide in an amount effective to kill cells of hematopoietic origin. Cells of hematopoietic origin include, but are not limited to, lymphocytes (e.g., B cells and T cells), monocytes, macrophages, dendritic cells, polymorphonuclear leukocytes (e.g., basophils, eosinophils, neutrophils), mast cells, platelets, erythrocytes and progenitor cells of these lineages. Cells of hematopoietic origin include, but are not limited to, healthy and diseased cell as found present in an animal, preferably a mammal and most preferably a human, or as isolated from an animal, transformed cells, cell lines derived from the above listed cell types, and cell cultures derived from the above listed cell types. Cells of hematopoietic origin may be found or isolated in, for example, resting, activated or anergic states.

[0275] In another embodiment, the invention provides a method for the specific destruction (i.e., killing) of cells (e.g., the destruction of tumor cells) by administering BlyS binding polypeptides or BlyS binding polypeptide conjugates (e.g., radiolabeled BlyS binding polypeptides and/or BlyS binding polypeptides conjugated with radioisotopes, toxins, or cytotoxic prodrugs). In a specific embodiment, the invention provides a method for the specific destruction of cells of monocytic lineage (e.g., monocytic cell related leukemias or lymphomas, such as, for example acute myelogenous leukemia) by administering BlyS binding polypeptides or BlyS binding polypeptide conjugates (e.g., BlyS binding polypeptides conjugated with radioisotopes, toxins, or cytotoxic prodrugs) that specifically bind the membrane-bound form of BlyS. In another specific embodiment, the invention provides a method for the specific destruction of cells of B cell lineage (e.g., B cell related leukemias or lymphomas (e.g., chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin's lymphoma, and Hodgkin's disease) by administering BlyS binding polypeptides or BlyS binding polypeptide conjugates (e.g., BlyS binding polypeptides conjugated with radioisotopes, toxins, or cytotoxic prodrugs) that bind soluble BlyS, but do not inhibit BlyS binding to a BlyS receptor on B cells.

[0276] In another embodiment of the invention, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent or ameliorate diseases and disorders of the immune system. In a specific embodiment, the invention provides a method of treating, preventing, or ameliorating an immune system disease or disorder, comprising, or alternatively consisting of, administering to an animal in which such treatment, prevention, or amelioration is desired, a BlyS binding polypeptide in an amount effective to treat, prevent, or ameliorate the immune system disease or disorder. Diseases and disorders of the immune system include, but are not limited to, autoimmune diseases and disorders (e.g., arthritis, graft rejection, Hashimoto's thyroiditis, insulin-dependent diabetes, lupus, rheumatoid arthritis, idiopathic thrombocytopenic purpura, systemic lupus erythematosus and multiple sclerosis, and other autoimmune diseases or disorders named or described herein), hypogammaglobulinemia, hypergammaglobulinemia, elective IgA deficiency,

ataxia-telangiectasia, immunodeficiencies (e.g., common variable immunodeficiency (CVID), X-linked agammaglobulinemia, severe combined immunodeficiency (SCID), and Wiskott-Aldrich syndrome), graft vs. host disease, idiopathic hyper-eosinophilic syndrome, monocytic leukemoid reaction, monocytic leukocytosis, monocytic leukopenia, monocytopenia, monocytosis, graft or transplant rejection, as well as infectious diseases (e.g., AIDS and hepatitis).

[0277] As discussed herein, BlyS binding polypeptides and BlyS binding polypeptide compositions, may be used to treat, prevent, ameliorate, diagnose or prognose various immune system-related disorders and/or conditions associated with these disorders, in mammals, preferably humans. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of BlyS binding polypeptides and BlyS binding polypeptide compositions that can inhibit an immune response, particularly the proliferation of B cells and/or the production of immunoglobulins, may be an effective therapy in treating and/or preventing autoimmune disorders. Thus, in preferred embodiments, BlyS binding polypeptides and BlyS binding polypeptide compositions are used to treat, prevent, ameliorate, diagnose and/or prognose an autoimmune disorder, or condition(s) associated with such disorder.

[0278] Autoimmune disorders and conditions associated with these disorders that may be treated, prevented, ameliorated, diagnosed and/or prognosed according to the invention with the therapeutic and pharmaceutical compositions described herein include, but are not limited to, autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmune neutropenia, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, gluten-sensitive enteropathy, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g., IgA nephropathy), multiple sclerosis, neuritis, uveitis ophthalmia, polyendocrinopathies, purpura (e.g., Henoch-Schoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, myocarditis, IgA glomerulonephritis, dense deposit disease, rheumatic heart disease, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

[0279] Additional autoimmune disorders and conditions associated with these disorders that may be treated, prevented, ameliorated, diagnosed and/or prognosed according to the present invention with the therapeutic and pharmaceutical compositions described herein include, but are not limited to, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis) (often characterized, e.g., by cell-mediated and humoral thyroid cytotoxicity), systemic lupus erythematosus (often characterized, e.g., by circulating and locally generated immune complexes), discoid lupus, Goodpasture's syndrome (often characterized, e.g., by anti-basement membrane antibodies), Pemphigus (often characterized, e.g., by epidermal acantholytic antibodies), Receptor autoimmunities such as, for example, (a) Graves' Disease (often characterized, e.g., by TSH receptor antibodies), (b) Myasthenia Gravis (often characterized, e.g., by acetylcholine receptor antibodies), and (c) insulin resistance (often characterized, e.g., by insulin receptor antibodies), autoim-

mune hemolytic anemia (often characterized, e.g., by phagocytosis of antibody-sensitized RBCs), autoimmune thrombocytopenic purpura (often characterized, e.g., by phagocytosis of antibody-sensitized platelets).H

[0280] Additional autoimmune disorders and conditions associated with these disorders that may be treated, prevented, ameliorated, diagnosed and/or prognosed according to the present invention with the therapeutic and pharmaceutical compositions described herein include, but are not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis/dermatomyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes) such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies), chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomyopathy (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), inflammatory myopathies, and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

[0281] In a preferred embodiment, therapeutic and pharmaceutical compositions are used to treat, prevent, ameliorate, diagnose or prognose, a member of the group: autoimmune hemolytic anemia, as primary glomerulonephritis, IgA glomerulonephritis, Goodpasture's syndrome, idiopathic thrombocytopenia, Multiple Sclerosis, Myasthenia Gravis, Pemphigus, polymyositis/dermatomyositis, relapsing polychondritis, rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus, Uveitis, vasculitis, and primary biliary cirrhosis.

[0282] In another specific preferred embodiment, therapeutic and pharmaceutical compositions are used to treat, prevent, ameliorate, diagnose or prognose, rheumatoid arthritis and/or medical conditions associated therewith.

[0283] In a specific preferred embodiment, therapeutic and pharmaceutical compositions are used to treat, prevent, ameliorate, diagnose or prognose, lupus and/or medical conditions associated therewith. Lupus-associated conditions that may be treated, prevented, ameliorated, prognosed and/or diagnosed with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, hematologic disorders (e.g., hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia), immunologic disorders (e.g., anti-DNA antibodies, and anti-Sm antibodies), rashes, photosensitivity, oral ulcers, arthritis, fever, fatigue, weight loss, serositis (e.g., pleuritis (pleurisy)), renal disorders (e.g., nephritis), neurological disorders (e.g., seizures, peripheral neuropathy, CNS related disorders), gastrointestinal disorders, Raynaud phenomenon, and pericarditis. In a preferred embodiment, therapeutic and pharmaceutical compositions are used to treat, prevent, ameliorate, diagnose, or prognose, renal disorders associated with systemic lupus erythematosus. In a most preferred embodiment, therapeutic and pharmaceutical compositions are used to treat, prevent, ameliorate, diagnose, or prognose, nephritis associated with systemic lupus erythematosus. In another most preferred embodiment, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent or ameliorate lupus or glomerular nephritis.

[0284] In another embodiment, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent or ameliorate an IgE-mediated allergic reaction or histamine-mediated allergic reaction. Examples of allergic reactions include, but are not limited to, asthma, rhinitis, eczema, chronic urticaria, and atopic dermatitis. In another embodiment, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent, or ameliorate anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility. In another embodiment, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent or ameliorate or modulate inflammation or an inflammatory disorder. Examples of chronic and acute inflammatory disorders that may be treated prevented or ameliorated with the therapeutic and pharmaceutical compositions include, but are not limited to, chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, Crohn's disease, inflammatory bowel disease, chronic and acute inflammatory pulmonary diseases, bacterial infection, psoriasis, septicemia, cerebral malaria, arthritis, gastroenteritis, and glomerular nephritis.

[0285] In another embodiment, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent or ameliorate ischemia and arteriosclerosis. Examples of such disorders include, but are not limited to, reperfusion damage (e.g., in the heart and/or brain) and cardiac hypertrophy.

[0286] Therapeutic or pharmaceutical compositions may also be administered to modulate blood clotting and to treat or prevent blood clotting disorders, such as, for example, antibody-mediated thrombosis (i.e., antiphospholipid antibody syndrome (APS)). For example, therapeutic or phar-

maceutical compositions as described herein may be used to inhibit the proliferation and differentiation of cells involved in producing anticardiolipin antibodies. These compositions can be used to treat, prevent, ameliorate, diagnose, and/or prognose thrombotic related events including, but not limited to, stroke (and recurrent stroke), heart attack, deep vein thrombosis, pulmonary embolism, myocardial infarction, coronary artery disease (e.g., antibody-mediated coronary artery disease), thrombosis, graft reocclusion following cardiovascular surgery (e.g., coronary arterial bypass grafts, recurrent fetal loss, and recurrent cardiovascular thromboembolic events.

[0287] Therapeutic or pharmaceutical compositions containing BLYS binding polypeptides may also be administered to treat, prevent, or ameliorate organ rejection or graft-versus-host disease (GVHD) and/or conditions associated therewith. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Administration of BLYS binding polypeptides that inhibit an immune response may be an effective therapy in preventing organ rejection or GVHD. In specific embodiments the present invention provides a method of inhibiting or reducing graft rejection, comprising administering to an animal in which such inhibition or reduction is desired, a BLYS binding polypeptide in an amount effective to inhibit or reduce graft rejection.

[0288] In another embodiment, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent or ameliorate a disease or disorder diseases associated with increased apoptosis including, but not limited to, AIDS, neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration), myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. In another embodiment, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent or ameliorate bone marrow failure, for example, aplastic anemia and myelodysplastic syndrome.

[0289] In other embodiment, therapeutic or pharmaceutical compositions as described herein are used to treat or prevent a proliferative disorder (e.g., cancer). In preferred embodiments, therapeutic or pharmaceutical compositions as described herein are used to treat or prevent proliferative disorders of monocytic cells. In other preferred embodiments, therapeutic or pharmaceutical compositions as described herein are used to treat or prevent a proliferative disorders of B cells (e.g., leukemia).

[0290] In another embodiment, therapeutic or pharmaceutical compositions as described herein are administered to an animal to treat, prevent or ameliorate growth, progression, and/or metastases of malignancies and proliferative diseases and disorders associated with increased cell survival, or the inhibition of apoptosis. In a specific embodiment, the present invention provides a method of treating a proliferative disease or disorder, comprising administering to an animal in which such treatment is desired, a BLYS binding polypeptide in an amount effective to treat the proliferative

disease or disorder. For a review of such disorders, see Fishman et al., *Medicine*, 2d Ed. (J. B. Lippincott Co., Philadelphia 1985). Examples of such disorders, include, but are not limited to, leukemia (e.g., acute leukemia such as acute lymphocytic leukemia and acute myelocytic leukemia, myeloblastic leukemia, promyelocytic leukemia, myelomonocytic leukemia, monocytic leukemia, erythroleukemia, chronic leukemia, chronic myelocytic (granulocytic) leukemia, and chronic lymphocytic leukemia), Polycythemia vera, lymphomas (e.g. Hodgkin's lymphoma, non-Hodgkin's lymphoma) Hodgkin's disease, non-Hodgkin's disease, multiple myeloma, Waldenstrom's macroglobulinemia, neoplasms, tumors (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, osteosarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovium, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, nasopharyngeal carcinoma, bronchogenic carcinoma, esophageal carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, uterine cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma) heavy chain disease, metastases, or any disease or disorder characterized by uncontrolled cell growth. This method of treating a proliferative diseases or disorders can also be used to treat premalignant conditions (e.g., benign tumors, hyperproliferative disorders, and benign proliferative disorders—see below) as well as proliferative disorders of B cells, monocytes, macrophages, and T cells.

[0291] In another embodiment of the present invention, therapeutic or pharmaceutical compositions as described herein can also be administered to treat a subset of proliferative disorders, namely, premalignant conditions (e.g., benign tumors, hyperproliferative disorders, benign proliferative disorders) and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed above. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, *Basic Pathology*, 2d Ed. (W.B. Saunders Co., Philadelphia 1976), pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it

is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

[0292] Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a therapeutic or pharmaceutical composition as described herein. Characteristics of a transformed phenotype include, but are not limited to, morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, and disappearance of the 250,000 dalton cell surface protein.

[0293] In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a therapeutic or pharmaceutical composition as described herein: a chromosomal translocation associated with a malignancy (e.g., the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (e.g., familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; see Robbins and Angell, *supra*, pp. 112-113), etc.)

[0294] In a specific embodiment, therapeutic or pharmaceutical compositions as described herein are used to treat or prevent a disorder characterized by hypergammaglobulinemia (e.g., AIDS, autoimmune diseases, and some immunodeficiencies).

[0295] In a specific embodiment, therapeutic or pharmaceutical compositions as described herein are used to treat or prevent a disorder characterized by deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, therapeutic or pharmaceutical compositions as described herein may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other

primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii.

[0296] Therapeutic or pharmaceutical compositions as described herein thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases or disorders, or conditions associated therewith: primary immunodeficiencies, immune-mediated thrombocytopenia, Kawasaki syndrome, bone marrow transplant (e.g., recent bone marrow transplant in adults or children), chronic B-cell lymphocytic leukemia, HIV infection (e.g., adult or pediatric HIV infection), chronic inflammatory demyelinating polyneuropathy, and post-transfusion purpura.

[0297] Additionally, therapeutic or pharmaceutical compositions as described herein may be used to diagnose, prognose, treat or prevent one or more of the following diseases, disorders, or conditions associated therewith, Guillain-Barre syndrome, anemia (e.g., anemia associated with parvovirus B19, patients with stable multiple myeloma who are at high risk for infection (e.g., recurrent infection), autoimmune hemolytic anemia (e.g., warm-type autoimmune hemolytic anemia), thrombocytopenia (e.g., neonatal thrombocytopenia), and immune-mediated neutropenia), transplantation (e.g., cytomegalovirus (CMV)-negative recipients of CMV-positive organs), hypogammaglobulinemia (e.g., hypogamma-globulinemic neonates with risk factor for infection or morbidity), epilepsy (e.g., intractable epilepsy), systemic vasculitic syndromes, myasthenia gravis (e.g., decompensation in myasthenia gravis), dermatomyositis, and polymyositis.

[0298] Additional preferred embodiments of the invention include, but are not limited to, the use of therapeutic or pharmaceutical compositions as described herein in the following applications:

[0299] Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response. In a specific nonexclusive embodiment, therapeutic or pharmaceutical compositions as described herein are administered to boost the immune system to produce increased quantities of IgG. In another specific nonexclusive embodiment, BlyS binding polypeptides of the are administered to boost the immune system to produce increased quantities of IgA. In another specific non-limiting embodiment, BlyS binding polypeptides are administered to boost the immune system to produce increased quantities of IgM.

[0300] Administration to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT applications WO 98/24893, WO 96/34096, WO 96/33735, and WO 91/10741).

[0301] Additional preferred embodiments of the invention include, but are not limited to, the use of therapeutic or pharmaceutical compositions as described herein in the following applications:

[0302] A vaccine adjuvant that enhances immune responsiveness to specific antigen. In a specific embodiment, the vaccine is a BLYS binding polypeptide described herein. In another specific embodiment, the vaccine adjuvant is a polynucleotide described herein (e.g., a BLYS binding polypeptide polynucleotide genetic vaccine adjuvant). As discussed herein, therapeutic or pharmaceutical compositions as described herein may be administered using techniques known in the art, including but not limited to, liposomal delivery, recombinant vector delivery, injection of naked DNA, and gene gun delivery.

[0303] An adjuvant to enhance tumor-specific immune responses.

[0304] An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions as described herein as an adjuvant, include, but are not limited to, virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever. In another specific embodiment, the compositions as described herein are used as an adjuvant to enhance an immune response to the HIV gp120 antigen.

[0305] An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions as described herein as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions as described herein are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, Group B *Streptococcus*, *Shigella* spp., Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, *Borrelia burgdorferi*, and *Plasmodium* (malaria).

[0306] An adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions as described herein as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions are used as an adjuvant to enhance an immune response to a parasite. In

another specific embodiment, the compositions are used as an adjuvant to enhance an immune response to *Plasmodium* (malaria).

[0307] As a stimulator of B cell responsiveness to pathogens.

[0308] As an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

[0309] As an agent to induce higher affinity antibodies.

[0310] As an agent to increase serum immunoglobulin concentrations.

[0311] As an agent to accelerate recovery of immunocompromised individuals.

[0312] As an agent to boost immunoresponsiveness among aged populations.

[0313] As an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions as described herein may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions are administered after transplantation, prior to the beginning of recovery of T cell populations. In another specific embodiment, compositions are first administered after transplantation, after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

[0314] As an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy. B cell immunodeficiencies that may be ameliorated or treated by administering the BLYS binding polypeptides and/or compositions as described herein include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non-X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia/aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0315] In a specific embodiment, BLyS binding polypeptides and/or compositions are administered to treat or ameliorate selective IgA deficiency.

[0316] In another specific embodiment, BLyS binding polypeptides and/or compositions are administered to treat or ameliorate ataxia-telangiectasia.

[0317] In another specific embodiment BLyS binding polypeptides and/or compositions are administered to treat or ameliorate common variable immunodeficiency.

[0318] In another specific embodiment, BLyS binding polypeptides and/or compositions are administered to treat or ameliorate X-linked agammaglobulinemia.

[0319] In another specific embodiment, BLyS binding polypeptides and/or compositions are administered to treat or ameliorate severe combined immunodeficiency (SCID).

[0320] In another specific embodiment, BLyS binding polypeptides and/or compositions are administered to treat or ameliorate Wiskott-Aldrich syndrome.

[0321] In another specific embodiment, BLyS binding polypeptides and/or compositions are administered to treat or ameliorate X-linked Ig deficiency with hyper IgM.

[0322] As an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering BLyS binding polypeptides and/or compositions include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

[0323] As an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering BLyS binding polypeptides and/or compositions include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery.

[0324] As a regulator of antigen presentation by monocytes, dendritic cells, T cells and/or B cells. In one embodiment, BLyS binding polypeptides or polynucleotides enhance antigen presentation or antagonize antigen presentation in vitro or in vivo. Moreover, in related embodiments, this enhancement or antagonization of antigen presentation may be useful in anti-tumor treatment or to modulate the immune system.

[0325] As a mediator of mucosal immune responses. The expression of BLyS on monocytes, the expression of BLyS receptor on B cells, and the responsiveness of B cells to BLyS suggests that it may be involved in exchange of signals between B cells and monocytes or their differentiated progeny. This activity is in many ways analogous to the CD40-CD 154 signaling between B cells and T cells. BLyS binding polypeptides and compositions may therefore be good regulators of T cell independent immune responses to environmental pathogens. In particular, the unconventional B cell populations (CD5+) that are associated with mucosal sites and responsible for much of the innate immunity in humans may respond to BLyS binding polypeptides or

compositions as described herein thereby enhancing or inhibiting individual's immune status.

[0326] As an agent to direct an individual's immune system towards development of a humoral response (i.e., TH2) as opposed to a TH1 cellular response.

[0327] As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly, their susceptibility profile would likely change.

[0328] As a monocyte cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic monocyte cell populations.

[0329] As a macrophage cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic macrophage cell populations.

[0330] As a B cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic B cell populations.

[0331] As a means of detecting monocyte cells by virtue of its specificity. This application may require labeling the protein with biotin or other agents (e.g., as described herein) to afford a means of detection.

[0332] As a means of detecting macrophage cells by virtue of its specificity. This application may require labeling the protein with biotin or other agents (e.g., as described herein) to afford a means of detection.

[0333] As a means of detecting B-lineage cells by virtue of its specificity. This application may require labeling the protein with biotin or other agents (e.g., as described herein) to afford a means of detection.

[0334] As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

[0335] As part of a monocyte selection device the function of which is to isolate monocytes from a heterogeneous mixture of cell types. BLyS binding polypeptides could be coupled to a solid support to which monocytes would then specifically bind. Unbound cells would be washed out and the bound cells subsequently eluted. A non-limiting use of this selection would be to allow purging of tumor cells from, for example, bone marrow or peripheral blood prior to transplant.

[0336] As part of a B cell selection device the function of which is to isolate B cells from a heterogeneous mixture of cell types. BLyS binding polypeptides (that do not inhibit BLyS/BLyS Receptor interaction) binding soluble BLyS could be coupled to a solid support to which B cells would then specifically bind. Unbound cells would be washed out and the bound cells subsequently eluted. A non-limiting use

of this selection would be to allow purging of tumor cells from, for example, bone marrow or peripheral blood prior to transplant.

[0337] As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

[0338] As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.

[0339] As an antigen for the generation of antibodies to inhibit or enhance BLYS mediated responses.

[0340] As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

[0341] As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recovery.

[0342] As a means of regulating secreted cytokines that are elicited by BLYS and/or BLYS receptor.

[0343] BLYS binding polypeptides or polynucleotides may be used to modulate IgE concentrations in vitro or in vivo.

[0344] Additionally, BLYS binding polypeptides or polynucleotides may be used to treat, prevent, and/or diagnose IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.

[0345] In a specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate selective IgA deficiency.

[0346] In another specific embodiment BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate ataxia-telangiectasia.

[0347] In another specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate common variable immunodeficiency.

[0348] In another specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate X-linked agammaglobulinemia.

[0349] In another specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate severe combined immunodeficiency (SCID).

[0350] In another specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate Wiskott-Aldrich syndrome.

[0351] In another specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate X-linked Ig deficiency with hyper IgM. In a specific embodiment BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate X-linked Ig deficiency with hyper IgM.

[0352] In another specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat,

prevent, and/or diagnose chronic myelogenous leukemia, acute myelogenous leukemia, leukemia, hystiocytic leukemia, monocytic leukemia (e.g., acute monocytic leukemia), leukemic reticulosis, Shilling Type monocytic leukemia, and/or other leukemias derived from monocytes and/or monocytic cells and/or tissues.

[0353] In another specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate monocytic leukemoid reaction, as seen, for example, with tuberculosis.

[0354] In another specific embodiment, BLYS binding polypeptides or polynucleotides are administered to treat, prevent, diagnose, and/or ameliorate monocytic leukocytosis, monocytic leukopenia, monocytopenia, and/or monocytosis.

[0355] In a specific embodiment, BLYS binding polypeptides or polynucleotides are used to treat, prevent, detect, and/or diagnose monocyte disorders and/or diseases, and/or conditions associated therewith.

[0356] In a specific embodiment, BLYS binding polypeptides or polynucleotides are used to treat, prevent, detect, and/or diagnose primary B lymphocyte disorders and/or diseases, and/or conditions associated therewith. In one embodiment, such primary B lymphocyte disorders, diseases, and/or conditions are characterized by a complete or partial loss of humoral immunity. Primary B lymphocyte disorders, diseases, and/or conditions associated therewith that are characterized by a complete or partial loss of humoral immunity and that may be prevented, treated, detected and/or diagnosed with compositions as described herein include, but are not limited to, X-Linked Agammaglobulinemia (XLA), severe combined immunodeficiency disease (SCID), and selective IgA deficiency.

[0357] In a preferred embodiment BLYS binding polypeptides or polynucleotides are used to treat, prevent, and/or diagnose diseases or disorders affecting or conditions associated with any one or more of the various mucous membranes of the body. Such diseases or disorders include, but are not limited to, for example, mucositis, mucoclasia, mucocolitis, mucocutaneous leishmaniasis (such as, for example, American leishmaniasis, leishmaniasis americana, nasopharyngeal leishmaniasis, and New World leishmaniasis), mucocutaneous lymph node syndrome (for example, Kawasaki disease), mucoenteritis, mucoepidermoid carcinoma, mucoepidermoid tumor, mucoepithelial dysplasia, mucoid adenocarcinoma, mucoid degeneration, myxoid degeneration; myxomatous degeneration; myxomatosis, mucoid medial degeneration (for example, cystic medial necrosis), mucopolipidosis (including, for example, mucopolipidosis I, mucopolipidosis II, mucopolipidosis III, and mucopolipidosis IV), mucolysis disorders, mucomembranous enteritis, mucoenteritis, mucopolysaccharidosis (such as, for example, type I mucopolysaccharidosis (i.e., Hurler's syndrome), type IS mucopolysaccharidosis (i.e., Scheie's syndrome or type V mucopolysaccharidosis), type II mucopolysaccharidosis (i.e., Hunter's syndrome), type III mucopolysaccharidosis (i.e., Sanfilippo's syndrome), type IV mucopolysaccharidosis (i.e., Morquio's syndrome), type VI mucopolysaccharidosis (i.e., Maroteaux-Lamy syndrome), type VII mucopolysaccharidosis (i.e. mucopolysaccharidosis due to beta-glucuronidase deficiency), and mucosulfatidosis), mucopolysacchariduria, mucopurulent

conjunctivitis, mucopus, mucormycosis (i.e., zygomycosis), mucosal disease (i.e., bovine virus diarrhea), mucous colitis (such as, for example, mucocolitis and myxomembranous colitis), and mucoviscidosis (such as, for example, cystic fibrosis, cystic fibrosis of the pancreas, Clarke-Hadfield syndrome, fibrocystic disease of the pancreas, mucoviscidosis, and viscidosis). In a highly preferred embodiment, BLYS binding polypeptides or polynucleotides are used to treat, prevent, and/or diagnose mucositis, especially as associated with chemotherapy.

[0358] In a preferred embodiment, BLYS binding polypeptides or polynucleotides are used to treat, prevent, and/or diagnose diseases or disorders affecting or conditions associated with sinusitis.

[0359] An additional condition, disease or symptom that can be treated, prevented, and/or diagnosed by BLYS binding polypeptides or polynucleotides is osteomyelitis.

[0360] An additional condition, disease or symptom that can be treated, prevented, and/or diagnosed by BLYS binding polypeptides or polynucleotides is endocarditis.

[0361] All of the above described applications as they may apply to veterinary medicine.

[0362] BLYS binding polypeptides or polynucleotides may be used to treat, prevent, and/or diagnose diseases and disorders of the pulmonary system (e.g., sinopulmonary and bronchial infections) and conditions associated with such diseases and disorders and other respiratory diseases and disorders. In specific embodiments, such diseases and disorders include, but are not limited to, bronchial adenoma, bronchial asthma, pneumonia (such as, e.g., bronchial pneumonia, bronchopneumonia, and tuberculous bronchopneumonia), chronic obstructive pulmonary disease (COPD), bronchial polyps, bronchiectasia (such as, e.g., bronchiectasia sicca, cylindrical bronchiectasis, and saccular bronchiectasis), bronchiolar adenocarcinoma, bronchiolar carcinoma, bronchiolitis (such as, e.g., exudative bronchiolitis, bronchiolitis fibrosa obliterans, and proliferative bronchiolitis), bronchiolo-alveolar carcinoma, bronchitic asthma, bronchitis (such as, e.g., asthmatic bronchitis, Castellani's bronchitis, chronic bronchitis, croupous bronchitis, fibrinous bronchitis, hemorrhagic bronchitis, infectious avian bronchitis, obliterative bronchitis, plastic bronchitis, pseudomembranous bronchitis, putrid bronchitis, and verminous bronchitis), bronchocentric granulomatosis, broncho-oedema, bronchoesophageal fistula, bronchogenic carcinoma, bronchogenic cyst, broncholithiasis, bronchomalacia, bronchomycosis (such as, e.g., bronchopulmonary aspergillosis), bronchopulmonary spirochetosis, hemorrhagic bronchitis, bronchorrhea, bronchospasm, bronchostaxis, bronchostenosis, Biot's respiration, bronchial respiration, Kussmaul respiration, Kussmaul-Kien respiration, respiratory acidosis, respiratory alkalosis, respiratory distress syndrome of the newborn, respiratory insufficiency, respiratory scleroma, respiratory syncytial virus, and the like.

[0363] In a specific embodiment, BLYS binding polypeptides or polynucleotides are used to treat, prevent, and/or diagnose chronic obstructive pulmonary disease (COPD).

[0364] In another embodiment, BLYS binding polypeptides or polynucleotides are used to treat, prevent, and/or diagnose fibroses and conditions associated with fibroses,

including, but not limited to, cystic fibrosis (including such fibroses as cystic fibrosis of the pancreas, Clarke-Hadfield syndrome, fibrocystic disease of the pancreas, mucoviscidosis, and viscidosis), endomyocardial fibrosis, idiopathic retroperitoneal fibrosis, leptomeningeal fibrosis, mediastinal fibrosis, nodular subepidermal fibrosis, pericentral fibrosis, perimuscular fibrosis, pipestem fibrosis, replacement fibrosis, subadventitial fibrosis, and Symmers' clay pipestem fibrosis.

[0365] In another embodiment, therapeutic or pharmaceutical compositions are administered to an animal to treat, prevent or ameliorate infectious diseases. Infectious diseases include diseases associated with yeast, fungal, viral and bacterial infections. Viruses causing viral infections which can be treated or prevented in accordance with this invention include, but are not limited to, retroviruses (e.g., human T-cell lymphotropic virus (HTLV) types I and II and human immunodeficiency virus (HIV)), herpes viruses (e.g., herpes simplex virus (HSV) types I and II, Epstein-Barr virus, HHV6-HHV8, and cytomegalovirus), arenaviruses (e.g., lassa fever virus), paramyxoviruses (e.g., morbillivirus virus, human respiratory syncytial virus, mumps, and pneumovirus), adenoviruses, bunyaviruses (e.g., hantavirus), coronaviruses, filoviruses (e.g., Ebola virus), flaviviruses (e.g., hepatitis C virus (HCV), yellow fever virus, and Japanese encephalitis virus), hepadnaviruses (e.g., hepatitis B viruses (HBV)), orthomyoviruses (e.g., influenza viruses A, B and C), papovaviruses (e.g., papillomaviruses), picornaviruses (e.g., rhinoviruses, enteroviruses and hepatitis A viruses), poxviruses, reoviruses (e.g., rotaviruses), togaviruses (e.g., rubella virus), rhabdoviruses (e.g., rabies virus). Microbial pathogens causing bacterial infections include, but are not limited to, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoea*, *Neisseria meningitidis*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter (Vibrio) fetus*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhimurium*, *Treponema pallidum*, *Treponema pertenu*, *Treponema carateneum*, *Borrelia vincentii*, *Borrelia burgdorferi*, *Leptospira icterohemorrhagiae*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Pneumocystis carinii*, *Francisella tularensis*, *Brucella abortus*, *Brucella suis*, *Brucella melitensis*, *Mycoplasma* spp., *Rickettsia prowazeki*, *Rickettsia tsutsugumushi*, *Chlamydia* spp., and *Helicobacter pylori*.

Gene Therapy

[0366] In a specific embodiment, nucleic acids comprising sequences encoding BLYS binding polypeptides or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of BLYS and/or its receptor, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0367] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0368] For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy*, 12:488-505 (1993); Wu and Wu, *Biotherapy*, 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.*, 32:573-596 (1993); Mulligan, *Science*, 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.*, 62:191-217 (1993); May, TIBTECH, 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in *Current Protocols in Molecular Biology*, Ausubel et al., eds. (John Wiley & Sons, NY 1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual* (Stockton Press, NY 1990).

[0369] In a preferred aspect, a composition useful in the methods of the invention comprises, or alternatively consists of, nucleic acids encoding a BLYS binding polypeptide, said nucleic acids being part of an expression vector that expresses the BLYS binding polypeptide or fragment thereof or chimeric protein including it in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the BLYS binding polypeptide coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the BLYS binding polypeptide coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the BLYS binding polypeptide encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); Zijlstra et al., *Nature*, 342:435-438 (1989)).

[0370] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

[0371] In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Pat. No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, micro-particles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.*, 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic

acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT publications WO 92/06180; WO 92/22635; WO 92/20316; WO 93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); Zijlstra et al., *Nature*, 342:435-438 (1989)).

[0372] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding a BLYS binding polypeptide or fragments or variants thereof are used. For example, a retroviral vector can be used (see Miller et al., *Meth. Enzymol.*, 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the BLYS binding polypeptide to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. Additional details concerning retroviral vectors can be found in Boesen et al., *Biotherapy*, 6:29 1-302 (1994), which describes the use of a retroviral vector to deliver the *mdr 1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.*, 93:644-651(1994); Klein et al., *Blood*, 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy*, 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.*, 3:110-114 (1993).

[0373] Other viral vectors that can be used in gene therapy are adenoviruses. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia, where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. See, Kozarsky and Wilson, *Current Opinion in Genetics and Development*, 3:499-503 (1993), presenting a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy*, 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science*, 252:431-434 (1991); Rosenfeld et al., *Cell*, 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.*, 91:225-234 (1993); PCT publication WO 94/12649; and Wang et al., *Gene Therapy*, 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[0374] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.*, 204:289-300 (1993); U.S. Pat. No. 5,436,146).

[0375] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0376] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting

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

[0377] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0378] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0379] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0380] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding a BLYS binding polypeptide or fragment thereof are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells that can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see, e.g., PCT publication WO 94/08598; Stemple and Anderson, *Cell*, 71:973-985 (1992); Rheinwald, *Meth. Cell Bio.*, 21A:229 (1980); and Pittelkow and Scott, *Mayo Clinic Proc.*, 61:771 (1986)).

[0381] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Demonstration of Therapeutic or Prophylactic Utility of a Composition

[0382] The compounds are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays which can be used to determine whether administration of a specific BLYS binding polypeptide or composition of the

present invention is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to, or otherwise administered, a BLYS binding polypeptide or composition of the present invention, and the effect of such a BLYS binding polypeptide or composition of the present invention upon the tissue sample is observed. In various specific embodiments, in vitro assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a BLYS binding polypeptide or composition of the present invention has a desired effect upon such cell types. Preferably, the BLYS binding polypeptides or compositions are also tested in in vitro assays and animal model systems prior to administration to humans.

[0383] BLYS binding polypeptides or compositions of the present invention for use in therapy can be tested for their toxicity in suitable animal model systems, including but not limited to rats, mice, chicken, cows, monkeys, and rabbits. For in vivo testing of a BLYS binding polypeptide or composition's toxicity any animal model system known in the art may be used.

[0384] Efficacy in treating or preventing viral infection may be demonstrated by detecting the ability of a BLYS binding polypeptide or composition to inhibit the replication of the virus, to inhibit transmission or prevent the virus from establishing itself in its host, or to prevent, ameliorate or alleviate the symptoms of disease a progression. The treatment is considered therapeutic if there is, for example, a reduction in viral load, amelioration of one or more symptoms, or a decrease in mortality and/or morbidity following administration of a BLYS binding polypeptide or composition.

[0385] BLYS binding polypeptides or compositions can be tested for the ability to induce the expression of cytokines such as IFN- γ , by contacting cells, preferably human cells, with a BLYS binding polypeptide or composition or a control BLYS binding polypeptide or control composition and determining the ability of the BLYS binding polypeptide or composition to induce one or more cytokines. Techniques known to those skilled in the art can be used to measure the level of expression of cytokines. For example, the level of expression of cytokines can be measured by analyzing the level of RNA of cytokines by, for example, RT-PCR and Northern blot analysis, and by analyzing the level of cytokines by, for example, immunoprecipitation followed by western blot analysis and ELISA. In a preferred embodiment, a compound is tested for its ability to induce the expression of IFN- γ .

[0386] BLYS binding polypeptides or compositions can be tested for their ability to modulate the biological activity of immune cells by contacting immune cells, preferably human immune cells (e.g., T cells, B cells, and Natural Killer cells), with a BLYS binding polypeptide or composition or a control compound and determining the ability of the BLYS binding polypeptide or composition to modulate (i.e., increase or decrease) the biological activity of immune cells. The ability of a BLYS binding polypeptide or composition to modulate the biological activity of immune cells can be assessed by detecting the expression of antigens, detecting the proliferation of immune cells (i.e., B cell proliferation), detecting the activation of signaling molecules, detecting the effector function of immune cells, or detecting the differentiation of

immune cells. Techniques known to those of skill in the art can be used for measuring these activities. For example, cellular proliferation can be assayed by ^3H -thymidine incorporation assays and trypan blue cell counts. Antigen expression can be assayed, for example, by immunoassays including, but not limited to, competitive and non-competitive assay systems using techniques such as western blots, immunohistochemistry radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and FACS analysis. The activation of signaling molecules can be assayed, for example, by kinase assays and electrophoretic shift assays (EMSAs). In a preferred embodiment, the ability of a BLYS binding polypeptide or composition to induce B cell proliferation is measured. In another preferred embodiment, the ability of a BLYS binding polypeptide or composition to modulate immunoglobulin expression is measured.

[0387] BLYS binding polypeptides or compositions can be tested for their ability to reduce tumor formation in *in vitro*, *ex vivo* and *in vivo* assays. BLYS binding polypeptides or compositions can also be tested for their ability to inhibit viral replication or reduce viral load in *in vitro* and *in vivo* assays. BLYS binding polypeptides or compositions can also be tested for their ability to reduce bacterial numbers in *in vitro* and *in vivo* assays known to those of skill in the art. BLYS binding polypeptides or compositions can also be tested for their ability to alleviate one or more symptoms associated with cancer, an immune disorder (e.g., an inflammatory disease), a neurological disorder or an infectious disease. BLYS binding polypeptides or compositions can also be tested for their ability to decrease the time course of the infectious disease. Further, BLYS binding polypeptides or compositions can be tested for their ability to increase the survival period of animals suffering from disease or disorder, including cancer, an immune disorder or an infectious disease. Techniques known to those of skill in the art can be used to analyze the function of the BLYS binding polypeptides or compositions *in vivo*.

Therapeutic/Prophylactic Compositions and Administration

[0388] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of BLYS binding polypeptide (or fragment or variant thereof) or pharmaceutical composition, preferably a BLYS binding polypeptide. In a preferred aspect, a BLYS binding polypeptide or fragment or variant thereof is substantially purified (i.e., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to, animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably a human.

[0389] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0390] Various delivery systems are known and can be used to administer BLYS binding polypeptide or fragment or variant thereof, e.g., encapsulation in liposomes, micropar-

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

[0391] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including a BLYS binding polypeptide, care must be taken to use materials to which the protein does not absorb.

[0392] In another embodiment, the composition can be delivered in a vesicle, in particular a liposome (see, Langer, *Science*, 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler, eds. (Liss, New York 1989), pp. 353-365; Lopez-Berestein, *ibid.*, pp. 317-327; see, generally, *ibid.*).

[0393] In yet another embodiment, the composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.*, 14:201 (1987); Buchwald et al., *Surgery*, 88:507 (1980); Saudek et al., *N. Engl. J. Med.*, 321:574 (1989)). In another embodiment, polymeric materials can be used (see, *Medical Applications of Controlled Release*, Langer and Wise, eds. (CRC Press, Boca Raton, Fla. 1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball, eds. (Wiley, New York 1984); Ranger and Peppas, *Macromol. Sci. Rev. Macromol. Chem.*, 23:61 (1983); see also Levy et al., *Science*, 228:190 (1985); During et al., *Ann. Neurol.*, 25:35 1 (1989); Howard et al., *J. Neurosurg.*, 7 1:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (*Science*, 249:1527-1533 (1990)).

[0394] In a specific embodiment where the composition to be used in the method of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA*, 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0395] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a BLYS binding polypeptide or a fragment thereof, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations, and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed. (Mack Publishing Co., 1990). Such compositions will contain a therapeutically effective amount of the BLYS binding polypeptide or fragment thereof, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0396] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as

lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0397] The compositions for use in the methods of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylaminoethanol, histidine, procaine, etc.

[0398] The amount of the composition which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0399] For BLYS binding polypeptides, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Further, the dosage and frequency of administration of therapeutic or pharmaceutical compositions may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the BLYS binding polypeptides by modifications such as, for example, lipidation.

[0400] The BLYS binding polypeptides and BLYS binding polypeptide compositions may be administered alone or in combination with other molecules including BLYS. In further embodiments of the invention, the BLYS binding polypeptides are administered in complex with BLYS. Preferably the BLYS binding polypeptide is radiolabelled or in complex with a radioisotope, toxin, or prodrug. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0401] The BLYS binding polypeptides and BLYS binding polypeptide compositions may be administered alone or in

combination with other adjuvants. Adjuvants that may be administered with the BLyS binding polypeptides and BLyS binding polypeptide compositions include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with alum. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with QS-21. Further adjuvants that may be administered with the BLyS binding polypeptides and BLyS binding polypeptide compositions include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Viroosomal adjuvant technology. Vaccines that may be administered with the BLyS binding polypeptides and BLyS binding polypeptide compositions include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis, and/or PNEUMOVAX-23™.

[0402] In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated therewith. In one embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose any Gram positive bacterial infection and/or any disease, disorder, and/or condition associated therewith. In another embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the genus *Enterococcus* and/or the genus *Streptococcus*. In another embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the Group B streptococci. In another embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with *Streptococcus pneumoniae*.

[0403] The BLyS binding polypeptides and BLyS binding polypeptide compositions may be administered alone or in combination with other therapeutic agents, including but not limited to, chemotherapeutic agents, antibiotics, antivirals, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents and cytokines. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as

through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0404] In one embodiment, the BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with other members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the BLyS binding polypeptides and BLyS binding polypeptide compositions include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (PCT publication WO 96/14328), TRAIL, AIM-II (PCT publication WO 97/34911), APRIL (*J. Exp. Med.*, 188(6):1185-1190 (1998)), endokine-alpha (PCT publication WO 98/07880), Neutrokine-alpha (PCT publication WO 98/18921), OPG, OX40, and nerve growth factor (NGF), and soluble forms of fas, CD30, CD27, CD40 and 4-1BB, TR2 (PCT publication WO 96/34095), DR3 (PCT publication WO 97/33904), DR4 (PCT publication WO 98/32856), TR5 (PCT publication WO 98/30693), TR6 (PCT publication WO 98/30694), TR7 (PCT publication WO 98/41629), TRANK, TR9 (PCT publication WO 98/56892), 312C2 (PCT publication WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

[0405] In a preferred embodiment, the BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), biologically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0406] In an additional embodiment, the BLyS binding polypeptides and BLyS binding polypeptide compositions are administered alone or in combination with an anti-angiogenic agent(s). Anti-angiogenic agents that may be administered with the BLyS binding polypeptides and BLyS binding polypeptide compositions include, but are not limited to, Angiostatin (Entremed, Rockville, Md.), Troponin-1 (Boston Life Sciences, Boston, Mass.), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEG1, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

[0407] Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

[0408] Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

[0409] Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

[0410] A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., *Cancer Res.*, 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP-PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha, alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., *J. Bio. Chem.*, 267:17321-17326, 1992); Chymostatin (Tomkinson et al., *Biochem J.*, 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Epone-mycin; Camptothecin; Fumagillin (Ingber et al., *Nature*, 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, *J. Clin. Invest.*, 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., *J. Biol. Chem.*, 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., *Agents Actions*, 36:312-316, 1992); and metalloproteinase inhibitors such as BB94.

[0411] Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, N.J.); Angiostatic steroid; AGM-1470 (Brem and Folkman, *J. Pediatr. Surg.*, 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (Storgard et al., *J. Clin. Invest.*, 103:47-54 (1999)); carboxyaminoimidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, Md.); Conbretastatin A-4 (CA4P) (OXIGENE, Boston, Mass.); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, Pa.); TNP-470, (Tap Pharmaceuticals, Deerfield, Ill.); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

[0412] Anti-angiogenic agents that may be administered in combination with the compounds may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, AG-3340 (Agouron, La Jolla, Calif.), BAY-12-9566 (Bayer, West Haven, Conn.), BMS-275291 (Bristol Myers Squibb, Princeton, N.J.), CGS-27032A (Novartis, East Hanover, N.J.), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, EMD-121974 (Merck KcgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, Calif./Medimmune, Gaithersburg, Md.). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, Angiozyme (Ribozyme, Boulder, Colo.), Anti-VEGF BLYS binding polypeptide (Genentech, S. San Francisco, Calif.), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, Calif.), SU-5416 (Sugen/Pharmacia Upjohn, Bridgewater, N.J.), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, IM-862 (Cytran, Kirkland, Wash.), Interferon-alpha, IL-12 (Roche, Nutley, N.J.), and Pentosan polysulfate (Georgetown University, Washington, D.C.).

[0413] In particular embodiments, the use of BLYS binding polypeptides and BLYS binding polypeptide compositions in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

[0414] In a particular embodiment, the use of BLYS binding polypeptides and BLYS binding polypeptide compositions in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of BLYS binding polypeptides and BLYS binding polypeptide compositions in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

[0415] In another embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with an anticoagulant. Anticoagulants that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, heparin, warfarin, and aspirin. In a specific embodiment, BLYS binding polypeptides and

BLyS binding polypeptide compositions are administered in combination with heparin and/or warfarin. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with warfarin. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with warfarin and aspirin. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with heparin. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with heparin and aspirin.

[0416] In another embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with an agent that suppresses the production of anticardiolipin antibodies. In specific embodiments, the polypeptides are administered in combination with an agent that blocks and/or reduces the ability of anticardiolipin antibodies to bind phospholipid-binding plasma protein beta 2-glycoprotein I (b2GPI).

[0417] In certain embodiments, BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with anti-retroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the BLyS binding polypeptides and BLyS binding polypeptide compositions, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the BLyS binding polypeptides and BLyS binding polypeptide compositions, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the BLyS binding polypeptides and BLyS binding polypeptide compositions, include, but are not limited to, CRIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with BLyS binding polypeptides and BLyS binding polypeptide compositions to treat, prevent, and/or diagnose AIDS and/or to treat, prevent, and/or diagnose HIV infection.

[0418] In other embodiments, BLyS binding polypeptides and BLyS binding polypeptide compositions may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the BLyS binding polypeptides and BLyS binding polypeptide compositions, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONA-

ZOLE™, ACYCLOVIR™, FAMCICLOVIR™, PYRIMETHAMNE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic cytomegalovirus infection. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat, prevent, and/or diagnose an opportunistic fungal infection. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with ACYCLOVIR™ and/or FAMCICLOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, BLyS binding polypeptides and BLyS binding polypeptide compositions are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat, prevent, and/or diagnose an opportunistic bacterial infection.

[0419] In a further embodiment, the BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with an antiviral agent. Antiviral agents that may be administered with the BLyS binding polypeptides and BLyS binding polypeptide compositions include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0420] In a further embodiment, the BLyS binding polypeptides and BLyS binding polypeptide compositions are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the BLyS binding polypeptides and BLyS binding polypeptide compositions include, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin,

streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

[0421] Conventional nonspecific immunosuppressive agents, that may be administered in combination with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, steroids, cyclosporine, cyclosporine analogs cyclophosphamide, cyclophosphamide IV, methylprednisolone, prednisolone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

[0422] In specific embodiments, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with immunosuppressants. Immunosuppressant preparations that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0423] In a preferred embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with steroid therapy. Steroids that may be administered in combination with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, oral corticosteroids, prednisone, and methylprednisolone (e.g., IV methylprednisolone). In a specific embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with prednisone. In a further specific embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with prednisone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions and prednisone are those described herein, and include, but are not limited to, azathioprine, cyclophosphamide, and cyclophosphamide IV. In another specific embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with methylprednisolone. In a further specific embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with methylprednisolone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions and methylprednisolone are those described herein, and include, but are not limited to, azathioprine, cyclophosphamide, and cyclophosphamide IV.

[0424] In a preferred embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with an antimalarial. Antimalarials that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, hydroxychloroquine, chloroquine, and/or quinacrine.

[0425] In a preferred embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with an NSAID.

[0426] In a nonexclusive embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with one, two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecasemin (Chiron), T-614 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000 (Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-1Ra gene therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIII-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-663 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

[0427] In a preferred embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL™ (Etanercept), anti-TNF antibody, LJP 394 (La Jolla Pharmaceutical Company, San Diego, Calif.) and prednisolone.

[0428] In a more preferred embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with an antimalarial, methotrexate, anti-TNF antibody, REMICADE™, ENBREL™ and/or sulfasalazine. In one embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with methotrexate. In another embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with anti-TNF antibody. In another embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with methotrexate and anti-TNF antibody. In another embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with sulfasalazine. In another specific embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with methotrexate, anti-TNF antibody, and sulfasalazine. In another embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with ENBREL™. In another embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with ENBREL™ and methotrexate. In another embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with ENBREL™, methotrexate and sulfasalazine. In another embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with ENBREL™, methotrexate and sulfasalazine. In other embodiments, one

or more antimalarials is combined with one of the above-recited combinations. In a specific embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL™, methotrexate and sulfasalazine. In another specific embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with an antimalarial (e.g., hydroxychloroquine), sulfasalazine, anti-TNF antibody, and methotrexate.

[0429] In an additional embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but not limited to, GAMMAR™, IVEEGAM™, SANDOglobulin™, GAMMAGARD S/D™, and GAMMUNE™. In a specific embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0430] CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), biologically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0431] In an additional embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0432] In another embodiment, compositions are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mus-

tard) and thiotepa); steroids and combinations (e.g., betamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

[0433] In a specific embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with Rituximab. In a further embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered with Rituximab and CHOP, or Rituximab and any combination of the components of CHOP.

[0434] In an additional embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with cytokines. Cytokines that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, GM-CSF, G-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, anti-CD40, CD40L, IFN-alpha (IFN- α), IFN-beta (IFN- β), IFN-gamma (IFN- γ), TNF-alpha (TNF- α), and TNF-beta (TNF- β). In preferred embodiments, BLYS binding polypeptides and BLYS binding polypeptide compositions are administered with BLYS (e.g., amino acids 134-285 of SEQ ID NO:173). In another embodiment, BLYS binding polypeptides and BLYS binding polypeptide compositions may be administered with any interleukin, including, but not limited to, IL-1 alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, and IL-22. In preferred embodiments, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with IL-4 and IL-10.

[0435] In one embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with one or more chemokines. In specific embodiments, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with an α (CxC) chemokine selected from the group consisting of gamma-interferon inducible protein-10 (γ IP-10), interleukin-8 (IL-8), platelet factor-4 (PF4), neutrophil activating protein (NAP-2), GRO- α , GRO- β , GRO- γ , neutrophil-activating peptide (ENA-78), granulocyte chemoattractant protein-2 (GCP-2), and stromal cell-derived factor-1 (SDF-1, or pre-B cell stimulatory factor (PBSF)); and/or a β (CC) chemokine selected from the group consisting of: RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory protein-1 alpha (MIP-1 α), macrophage inflammatory protein-1 beta (MIP-1 β), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-2 (MCP-2), monocyte chemotactic protein-3 (MCP-3), monocyte chemotactic protein-4 (MCP-4) macrophage inflammatory protein-1 gamma (MIP-1 γ), macrophage inflammatory protein-3 alpha (MIP-3 α), macrophage inflammatory protein-3 beta (MIP-3 β), macrophage inflammatory protein-4 (MIP-4/DC-CK-1/PARC), eotaxin, Exodus, and 1-309; and/or the γ (C) chemokine, lymphotactin.

[0436] In another embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions

are administered with chemokine beta-8, chemokine beta-1, and/or macrophage inflammatory protein-4. In a preferred embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered with chemokine beta-8.

[0437] In an additional embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with an IL-4 antagonist. IL-4 antagonists that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to: soluble IL-4 receptor polypeptides, multimeric forms of soluble IL-4 receptor polypeptides; anti-IL-4 receptor antibodies that bind the IL-4 receptor without transducing the biological signal elicited by IL-4, anti-IL4 antibodies that block binding of IL-4 to one or more IL-4 receptors, and muteins of IL-4 that bind IL-4 receptors but do not transduce the biological signal elicited by IL-4. Preferably, the antibodies employed according to this method are monoclonal antibodies (including BLYS binding polypeptide fragments, such as, for example, those described herein).

[0438] The invention also encompasses combining the polynucleotides and/or polypeptides (and/or agonists or antagonists thereof) with other proposed or conventional hematopoietic therapies. Thus, for example, the polynucleotides and/or polypeptides (and/or agonists or antagonists thereof) can be combined with compounds that singly exhibit erythropoietic stimulatory effects, such as erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, and triiodothyronine. Also encompassed are combinations of the BLYS binding polypeptides and BLYS binding polypeptide compositions with compounds generally used to treat aplastic anemia, such as, for example, methenolene, stanozolol, and nandrolone; to treat iron-deficiency anemia, such as, for example, iron preparations; to treat malignant anemia, such as, for example, vitamin B₁₂ and/or folic acid; and to treat hemolytic anemia, such as, for example, adrenocortical steroids, e.g., corticoids. See, e.g., Resegotti et al., *Panminerva Medica*, 23:243-248 (1981); Kurtz, *FEBS Letters*, 14a:105-108 (1982); McGonigle et al., *Kidney Int.*, 25:437-444 (1984); and Pavlovic-Kantera, *Expt. Hematol.*, 8(supp. 8):283-291 (1980), the contents of each of which are hereby incorporated by reference in their entireties.

[0439] Compounds that enhance the effects of or synergize with erythropoietin are also useful as adjuvants herein, and include but are not limited to, adrenergic agonists, thyroid hormones, androgens, hepatic erythropoietic factors, erythropoietins, and erythropoietins. See, for example, Dunn, *Current Concepts in Erythropoiesis* (John Wiley and Sons, Chichester, England 1983); Kalmani, *Kidney Int.*, 22:383-391 (1982); Shahidi, *New Eng. J. Med.*, 289:72-80 (1973); Urabe et al., *J. Exp. Med.*, 149:1314-1325 (1979); Billat et al., *Expt. Hematol.*, 10:133-140 (1982); Naughton et al., *Acta Haemat.*, 69:171-179 (1983); Cognote et al., in abstract 364, *Proceedings 7th Intl. Cong. of Endocrinology* (Quebec City, Quebec, Jul. 1-7, 1984); and Rothman et al., *J. Surg. Oncol.*, 20:105-108 (1982). Methods for stimulating hematopoiesis comprise administering a hematopoietically effective amount (i.e., an amount which effects the formation of blood cells) of a pharmaceutical composition containing polynucleotides and/or polypeptides (and/or agonists or antagonists thereof) to a patient. The polynucleotides and/or

polypeptides and/or agonists or antagonists thereof are administered to the patient by any suitable technique, including but not limited to parenteral, sublingual, topical, intrapulmonary and intranasal, and those techniques further discussed herein. The pharmaceutical composition optionally contains one or more members of the group consisting of erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, triiodothyronine, methenolene, stanozolol, and nandrolone, iron preparations, vitamin B₁₂, folic acid and/or adrenocortical steroids.

[0440] In an additional embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, LEUKINE™(sargramostim) and NEUPOGEN™ (filgrastim).

[0441] In an additional embodiment, the BLYS binding polypeptides and BLYS binding polypeptide compositions are administered in combination with fibroblast growth factors. Fibroblast growth factors that may be administered with the BLYS binding polypeptides and BLYS binding polypeptide compositions include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0442] Additionally, the BLYS binding polypeptides and BLYS binding polypeptide compositions may be administered alone or in combination with other therapeutic regimens, including but not limited to, radiation therapy. Such combinatorial therapy may be administered sequentially and/or concomitantly.

Kits for Detecting and/or Quantitating BLYS or BLYS-Like Polypeptides

[0443] The present invention is also directed to an assay kit which can be useful in screening for the presence of BLYS and/or quantitating BLYS concentrations in a fluid, such as, for example, a biological fluid (e.g., blood, serum, synovial fluid).

[0444] In a particular embodiment of the present invention, an assay kit is contemplated which comprises in one or more containers one or more BLYS binding polypeptides and optionally, a detection means for determining the presence of a BLYS-BLYS binding polypeptide interaction or the absence thereof. The kit further optionally contains BLYS protein that may be used, for example as a control. The BLYS binding polypeptide may be free or expressed on the surface of a phage.

[0445] In a specific embodiment, either the BLYS binding polypeptide or the BLYS protein is labeled. As further discussed herein, a wide range of labels can be used according to the present invention, including but not limited to conjugating the recognition unit to biotin by conventional means. Alternatively, the label may comprise, e.g., a fluorogen, an enzyme, an epitope, a chromogen, or a radionuclide. Preferably, the biotin is conjugated by covalent attachment to either the BLYS binding polypeptide or the BLYS protein. Preferably, the BLYS binding polypeptide is immobilized on a solid support. The detection means employed to

detect the label will depend on the nature of the label and can be any known in the art, e.g., film to detect a radionuclide, an enzyme substrate that gives rise to a detectable signal to detect the presence of an enzyme, antibody to detect the presence of an epitope, etc.

[0446] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In one preferred embodiment the kit comprises a vial containing BLYS binding polypeptides conjugated to a toxin or a label (as described herein). Such conjugated binding polypeptide may be used to kill a particular population of cells or to quantitate a particular population of cells. In a preferred embodiment, such conjugated BLYS binding polypeptides are used to kill monocyte cells expressing the membrane-bound form of BLYS. In another preferred embodiment, such conjugated BLYS binding polypeptides are used to quantitate monocyte cells expressing the membrane-bound form of BLYS. In another preferred embodiment, such conjugated BLYS binding polypeptides are used to kill B cells expressing BLYS receptor on their surface. In another preferred embodiment, such conjugated BLYS binding polypeptides are used to quantitate B cells expressing BLYS receptor on their surface.

[0447] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises a BLYS binding polypeptide, preferably a purified BLYS binding polypeptide, in one or more containers. In an alternative embodiment, a kit comprises a BLYS binding polypeptide fragment that specifically binds to BLYS. In a specific embodiment, the kits of the present invention contain a substantially isolated BLYS polypeptide as a control. Preferably, the kits of the present invention further comprise a control binding polypeptide which does not react with BLYS. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of a BLYS binding polypeptide to BLYS (e.g., the BLYS binding polypeptide may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the BLYS binding polypeptide may be conjugated to a detectable substrate). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized BLYS. The BLYS provided in the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above-described kit includes a solid support to which BLYS is attached. Such a kit may also include a non-attached reporter-labeled anti-BLYS binding polypeptide antibody. In this embodiment, binding of the BLYS binding polypeptide to BLYS can be detected by binding of the said reporter-labeled antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0448] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing BLYS or BLYS-like polypeptides. The diagnostic kit includes a substantially isolated BLYS binding polypeptide specifically reactive with BLYS target, and means for detect-

ing the binding of BLYS target to the BLYS binding polypeptide. In one embodiment, the BLYS binding polypeptide is attached to a solid support.

[0449] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound BLYS binding polypeptide according to the present invention. After BLYS binds to a specific BLYS binding polypeptide, the unbound serum components are removed by washing, reporter-labeled anti-BLYS binding polypeptide antibody is added, unbound anti-BLYS binding polypeptide antibody is removed by washing, and a reagent is reacted with reporter-labeled anti-BLYS binding polypeptide antibody to bind reporter to the reagent in proportion to the amount of bound BLYS binding polypeptide on the solid support. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or calorimetric substrate.

[0450] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated BLYS binding polypeptides.

[0451] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant BLYS, and a reporter-labeled anti-BLYS binding polypeptide antibody for detecting surface-bound anti-BLYS binding polypeptide.

Methods of Screening for BLYS Binding Molecules

[0452] The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind BLYS, and the BLYS binding molecules identified thereby. This method comprises the steps of:

[0453] (a) contacting BLYS or BLYS-like polypeptide with a plurality of molecules; and

[0454] (b) identifying molecule(s) that binds the BLYS or BLYS-like polypeptide.

[0455] The step of contacting the BLYS protein or BLYS-like protein with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing BLYS target on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized BLYS target. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized BLYS protein or BLYS-like polypeptide. The molecules having a selective affinity for the BLYS or BLYS-like polypeptide can then be purified by affinity selection. The nature of the solid support, process for attachment of the BLYS or BLYS-like polypeptide to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

[0456] Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising

a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" using a BLYS target protein, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the BLYS target protein and the individual clone. Prior to contacting the BLYS target protein with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for BLYS or BLYS-like polypeptide. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the BLYS protein or BLYS-like protein can be determined directly by conventional means, or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary amino acid sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

[0457] In certain situations, it may be desirable to wash away any BLYS or BLYS-like polypeptide, or alternatively, unbound polypeptides, from a mixture of BLYS or BLYS-like polypeptide and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction. One or more such a wash steps may be particularly desirable when the BLYS or BLYS-like polypeptide or the plurality of polypeptides is bound to a solid support.

[0458] The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or non-peptide libraries which can be screened for molecules that specifically bind to BLYS. Peptide libraries may be designed such that the polypeptides encoded by the libraries are automatically fused to a polypeptide linker moiety, for example. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., *Science*, 251:767-773 (1991); Houghten et al., *Nature*, 354:84-86 (1991); Lam et al., *Nature*, 354:82-84 (1991); Medynski, *BioTechnology*, 12:709-710 (1994); Gallop et al., *J. Medicinal Chemistry*, 37(9):1233-1251 (1994); Ohlmeyer et al., *Proc. Natl. Acad. Sci. USA*, 90:10922-10926 (1993); Erb et al., *Proc. Natl. Acad. Sci. USA*, 91:11422-11426 (1994); Houghten et al., *Biotechniques*, 13:412 (1992); Jayawickreme et al., *Proc. Natl. Acad. Sci. USA*, 91:1614-1618 (1994); Salmon et al., *Proc. Natl. Acad. Sci. USA*, 90:11708-11712 (1993); PCT publication WO 93/20242; and Brenner and Lerner, *Proc. Natl. Acad. Sci. USA*, 89:5381-5383 (1992).

[0459] Examples of phage display libraries are described in Scott and Smith, *Science*, 249:386-390 (1990); Devlin et al., *Science*, 249:404-406 (1990); Christian et al., *J. Mol. Biol.*, 227:711-718 (1992); Lenstra, *J. Immunol. Meth.*, 152:149-157 (1992); Kay et al., *Gene*, 128:59-65 (1993); and PCT publication WO 94/18318.

[0460] In vitro translation-based libraries include but are not limited to those described in PCT publication WO 91/05058 and Mattheakis et al., *Proc. Natl. Acad. Sci. USA*, 91:9022-9026 (1994).

[0461] By way of examples of non-peptide libraries, a benzodiazepine library (see, e.g., Bunin et al., *Proc. Natl. Acad. Sci. USA*, 91:4708-4712 (1994)) can be adapted for use. Peptoid libraries (Simon et al., *Proc. Natl. Acad. Sci. USA*, 89:9367-9371 (1992)) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (*Proc. Natl. Acad. Sci. USA*, 91:11138-11142 (1994)).

[0462] The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke, *BioTechnology*, 13:351-360 (1995) list benzodiazepines, hydantoin, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

[0463] Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety of functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

[0464] Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

[0465] Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, *Adv. Exp. Med. Biol.*, 251:215-218 (1989); Scott and Smith, *Science*, 249:386-390 (1990); Fowlkes et al., *BioTechniques*, 13:422-427 (1992); Oldenburg et al., *Proc. Natl. Acad. Sci. USA*, 89:5393-5397 (1992); Yu et al., *Cell*, 76:933-945 (1994); Staudt et al., *Science*, 241:577-580 (1988); Bock et al., *Nature*, 355:564-566 (1992); Tuerk et al., *Proc. Natl. Acad. Sci. USA*, 89:6988-6992 (1992); Ellington et al., *Nature*, 355:850-852 (1992); U.S. Pat. No. 5,096,815; U.S. Pat. No. 5,223,409; and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, *Science*, 263:671-673 (1993); and PCT publication WO 94/18318.

[0466] In a specific embodiment, screening to identify a molecule that binds BLYS can be carried out by contacting the library members with BLYS or BLYS-like polypeptide immobilized on a solid phase and harvesting those library members that bind to the BLYS or BLYS-like polypeptide. Examples of such screening methods, termed “panning” techniques are described by way of example in Parmley and Smith, *Gene*, 73:305-318 (1998); Fowlkes et al., *BioTechniques*, 13:422-427 (1992); PCT publication WO 94/18318; and in references cited therein.

[0467] In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, *Nature*, 340:245-246 (1989); Chien et al., *Proc. Natl. Acad. Sci. USA*, 88:9578-9582 (1991)) can be used to identify molecules that specifically bind to BLYS or BLYS-like polypeptides.

[0468] Where the BLYS binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term “biased” is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

[0469] Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid, that certain amino acid positions in a peptide remain fixed (e.g., as cysteine), or that positions 4, 8, and 9, for example, of a decapeptide library be limited to permit several but less than all of the twenty naturally-occurring amino acids. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

[0470] As mentioned above, in the case of a BLYS binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a BLYS binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

[0471] The selected BLYS binding polypeptide can be obtained by chemical synthesis or recombinant expression.

[0472] The specific BLYS binding polypeptides disclosed herein were isolated using phage display technology, to identify BLYS binding polypeptides exhibiting particular preselected binding properties. These BLYS binding polypeptides were isolated initially by screening nine phage display libraries, that is, populations of recombinant bacteriophage transformed to express an exogenous recombinant polypeptide on their surface. In order to isolate new polypeptide binding moieties for a particular target, such as BLYS, screening of peptide libraries, for example using phage display techniques, is especially advantageous, in that very large numbers (e.g., 5×10^9) of potential binders can be tested and successful binders isolated in a short period of time.

[0473] In order to prepare a phage library of potential binding polypeptides to screen for members of the library that are BLYS binding polypeptides, a candidate binding domain is selected to serve as a structural template for the polypeptides to be displayed in the library. The phage library is made up of polypeptide analogues of this template or “parental binding domain.” The parental binding domain is a polypeptide molecule that may be a naturally occurring or synthetic protein or polypeptide, or polypeptide region or domain of a protein. The parental binding domain may be selected based on knowledge of a known interaction between the parental binding domain and a target protein, but this is not critical. In fact, it is not essential that the parental binding domain have any affinity for a target at all because its purpose is to provide a structure from which a multiplicity of polypeptide analogues (a “library”) can be generated, which multiplicity of polypeptide analogues will include one or more binding polypeptides that exhibit the desired binding and release properties with respect to BLYS target proteins (and any other properties selected).

[0474] Knowledge of the exact polypeptide that will serve as the parental binding domain, or knowledge of a class of proteins or domains to which the parental binding domain belongs can be useful in determining the conditions under which BLYS binding polypeptides optimally bind BLYS target proteins as well as the conditions under which BLYS binding polypeptides optimally release BLYS target proteins. Similarly, the binding and/or release conditions may be selected with regard to known interactions between a binding domain and the BLYS target protein, for example, to favor the interaction under the binding and/or release conditions, or they may be selected without regard to such known interactions. Likewise, the parental binding domain can be selected taking into account a desired binding and/or release condition or not. It is understood that if the binding domain analogues of a library are unstable under a proposed or desired binding or release condition, no useful binding polypeptides may be obtained.

[0475] In selecting the parental binding domain, the most important consideration is how the analogue domains will be presented to the BLYS target protein, that is, in what conformations the BLYS target and the polypeptide analogues will contact one another. In preferred embodiments, for example, the polypeptide analogues will be generated by insertion of synthetic DNA encoding the polypeptide analogue into a replicable genetic package, resulting in display of the domain on the surface of a microorganism, such as M13 phage, using techniques as described in Kay et al., *Phage Display of Peptides and Proteins: A Laboratory Manual* (Academic Press, Inc.; San Diego 1996) and U.S. Pat. No. 5,223,409 (Ladner et al.), incorporated herein by reference. For formation of phage display libraries, it is preferred to use structured polypeptides as the parental binding domain or template, as opposed to unstructured, linear peptides. Mutation of surface residues in a protein domain or polypeptide molecule will usually have little effect on the overall structure or general properties (such as size, stability, and temperature of denaturation) of the protein; while at the same time mutation of surface residues may profoundly affect the binding properties of the molecule. The more tightly a polypeptide segment is constrained, the less likely it is to bind to any particular target. If it does bind, however, the binding is likely to be tighter and more specific. Thus, it is preferred to select a parental

binding domain wherein the parental polypeptide has structure and, thereby in turn, select a structure for the polypeptide analogues of the library, which is constrained within a framework having some degree of rigidity.

[0476] Preferably the protein domain that is used as the template or parental domain for generating the library of domain analogues will be a peptide molecule that is a relatively small protein or polypeptide. Small polypeptides offer several advantages over large proteins: First, the mass per binding site is reduced. Highly stable protein domains having low molecular weights, for example, Kunitz domains (~7 kilodaltons, kDa), Kazal domains (~7 kDa), *Cucurbita maxima* trypsin inhibitor (CMTI) domains (~3.5 kDa), and endothelin (~2 kDa), can show much higher binding per gram than do antibodies (150 kDa) or single chain scFv antibodies (30 kDa). Second, the possibility of non-specific binding is reduced because there is less molecular surface available for nonspecific binding. Third, small polypeptides can be engineered to have unique tethering sites in a way that is impracticable for larger proteins or antibodies. For example, small proteins and polypeptides can be engineered to have lysines only at sites suitable for tethering to a chromatography matrix. This is not feasible for antibodies. Fourth, a constrained polypeptide structure is more likely to retain its functionality when transferred (with the structural domain intact) from one framework to another. For instance, the binding domain structure is likely to be transferable from the framework used for presentation in a library, such as displayed on a phage, to an isolated protein removed from the presentation framework or immobilized on a chromatographic substrate.

[0477] In specific embodiments, the BLYS binding polypeptides are immobilized. BLYS binding polypeptide molecules according to the invention may be immobilized, for example, on chromatographic support materials to form efficient BLYS separation or affinity chromatographic media. Immobilized BLYS binding polypeptides have uses that include, but are not limited to, detecting, isolating or removing BLYS target proteins from solutions. One strategy for generating BLYS binding polypeptide molecules that can be immobilized, for example, on matrices, resins, or supports, involves selecting appropriate binding domain templates such that BLYS binding polypeptide molecules are generated that have one or more amino acids that may be used to covalently link the BLYS binding polypeptide to a chromatographic resin or substrate to form an affinity resin. Similarly, the N-terminal amino group or the C-terminal carboxyl group of a peptide molecule may be modified by adding a capping group to render it inert or a functional group, which permits linkage to a support medium. For example, the C-terminal carboxyl group of a protein domain may be converted to an amide or a hydrazide (—NH—NH_2) group for reaction with an aldehyde-functional substrate or other amine-reactive substrate. This technique is preferred. Another preferred modification of BLYS binding polypeptides useful for linking a BLYS binding polypeptide molecule to a chromatography material is a polypeptide linker comprising, or alternatively consisting of, the amino acid sequence Pro-Gly-Pro-Glu-Gly-Gly-Gly-Lys (SEQ ID NO:13).

[0478] In one non-limiting example of a screening procedure to obtain BLYS binding polypeptides encompassed by the invention, the phage in a phage display library are

contacted with and allowed to bind a BLYS target protein that is immobilized on a solid support. Those phage that display non-binding polypeptides are separated from those that bind the BLYS target protein. Any of various techniques known in the art may be applied to dissociate the bound phage from the immobilized BLYS protein, and to collect and/or amplify the phage and/or their nucleic acid contents. Using these techniques it is possible to identify a BLYS binding phage that is about 1 in 20 million in the population. Libraries, displaying 10-20 million or more potential binding peptide molecules each, are rapidly screened to find high-affinity BLYS binding polypeptides.

[0479] In each round of screening, the diversity of a population falls until only efficient binding polypeptides remain, that is, the process converges. Typically, a phage display library will contain several closely related binding polypeptides (10 to 50 different binding polypeptides out of 10 million). Indications of convergence include increased binding (measured by phage titers) and recovery of closely related sequences. After a first set of binding polypeptide molecules is identified, the sequence information can be used to design other libraries biased for members having additional desired properties, for example, discrimination between different forms of BLYS (e.g., the membrane form and the soluble form of BLYS) and fragments thereof, or discrimination between BLYS and closely related impurities in a feed stream.

[0480] Such techniques make it possible not only to screen a large number of potential binding polypeptides, but make it practical to repeat the binding and elution cycles and to build secondary, biased libraries for screening polypeptide analogue-displaying phage that meet specific criteria. Using these techniques, a polypeptide analogue biased library may be screened to reveal members that bind tightly, that is, have high affinity for BLYS target protein, under the screening conditions.

[0481] In the present invention target BLYS protein molecules were biotinylated and then bound to streptavidin-coated magnetic particles. Nine phage display libraries of different design were screened for the ability to bind the immobilized BLYS. Each library was characterized by M13 phage displaying variegated peptides of different lengths and overall structure: A library designated TN6/6 (2×10^8 variants) displayed a variegated 12-mer with two internal invariant cyteines to form a hexamer loop structure. A library designated TN7/4 (2.3×10^9 variants) presented a variegated 13-mer having two internal invariant cyteines to form a heptamer loop structure. A library designated TN8/9 (5×10^9 variants) displayed a variegated 14-mer with two internal invariant cyteines to form an octamer loop structure. A library designated TN9/4 (3.2×10^9 variants) presented a variegated 16-mer having two internal invariant cyteines to form a nonamer loop structure. A library designated TN10/9 (2.5×10^9 variants) displayed a variegated 16-mer with two internal invariant cyteines to form a decamer loop structure. A library designated TN12/1 (1.4×10^9 variants) presented a variegated 18-mer having two internal invariant cyteines to form a dodecamer loop structure. A library designated as Substrate Phage Library #2, having a diversity of about 2×10^8 amino acid sequences, was designed to include a linear peptide-variegated region in the display polypeptide consisting of 13 consecutive amino acids, and the display polypeptide design allowed any amino acid residue except

cysteine to occur at each position. Finally, two commercially available linear phage display libraries were also screened, designated PhD 7 and PhD 12, respectively (New England Biolabs). The PhD 7 library displayed a linear random-sequence 7-mer; the PhD 12 library displayed a random-sequence 12-mer.

[0482] BLYS binding phage were isolated and collected from all of the libraries except PhD 7.

[0483] After analysis of the sequences isolated from the library screenings, several families of BLYS binding peptides were defined (see, consensus sequences A-G and H-L, above). The amino acid sequences of the BLYS-binding "hits" from the first rounds of screening are set forth in Tables 1-8 (infra).

[0484] In order to obtain BLYS binding polypeptides having an even higher affinity for BLYS targets, a specialized library was prepared, i.e., a BLYS affinity maturation library, based on variegation of high affinity exemplars of the PhD 12 library (see Example 6). This library was designed to provide a population enriched with polypeptides likely to show high affinity for BLYS. The selections from this library were performed to eliminate, by prolonged competition with soluble eluants of soluble BLYS or other BLYS binding polypeptides, all but the polypeptides having the highest affinity for BLYS. A large family of high affinity BLYS binding polypeptides was isolated from four rounds of screening the affinity maturation library, and their amino acid sequences appear in Table 13 (infra).

[0485] As it within the scope of the present invention to screen phage libraries that bind one or more of the various forms of BLYS, the following outlines some assays that may be used in screening for BLYS binding polypeptides that bind the soluble form of BLYS, the membrane-bound form of BLYS, or both the soluble and the membrane-bound forms of BLYS. Assays to determine the specificity of binding polypeptides for different forms of a protein are commonly known in the art and may be readily adapted for determining the specificity of BLYS binding polypeptides for different forms of BLYS.

[0486] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) may be screened in a variety of assays to identify those BLYS binding polypeptides that specifically bind to the soluble form of BLYS. BLYS binding polypeptides may be assayed in neutralization assays described herein (see Examples 7 and 8) or otherwise known in the art. For example, BLYS binding polypeptides may be tested for their ability to inhibit soluble BLYS from binding a BLYS receptor. The BLYS receptor used in these assays may be an isolated BLYS receptor (e.g., BLYS receptor conjugated to agarose beads) or may be present on the cell surface of cell lines that express BLYS receptors which include, but are not limited to, peripheral CD20+ B cells, IM-9, REH, ARH-77, Namalwa, and RPMI-8226 B cell tumor lines.

[0487] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants thereof) may be screened in a variety of assays commonly known in the art to identify those BLYS binding polypeptides that specifically bind to the membrane-bound form of BLYS. For example, BLYS bind-

ing polypeptides may be assayed for binding BLYS protein present on cell membranes of cells that express BLYS. Cell lines that express BLYS that might be useful for testing BLYS binding polypeptide binding to membrane-bound form of BLYS include, K-562, HL-60, THP-1, and U937 cells.

[0488] Additionally, BLYS binding polypeptides may be screened against cells engineered to express an "uncleavable" form of BLYS in order to determine their specificity for the membrane-bound form of BLYS. Mutations in BLYS which may achieve this result include, but are not limited to, the mutation or deletion of amino acid residues Lys-132 and/or Arg-133 of the BLYS sequence shown in SEQ ID NO:173. A typical mutagenesis might include mutation of one or both of residues Lys-132 or Arg-133 to alanine residues. Cells expressing such an "uncleavable" form of BLYS provide a profound reagent to use in assaying the ability of BLYS binding polypeptides to bind the membrane-bound form of BLYS.

[0489] BLYS binding polypeptides (including molecules comprising, or alternatively consisting of, BLYS binding polypeptide fragments or variants) may be screened in a variety of assays to identify those BLYS binding polypeptides or BLYS binding polypeptide fragments or variants that specifically bind to the soluble form and membrane-bound form of BLYS. This can readily be determined by performing assays to distinguish binding to the soluble form and assays to distinguish binding to the membrane-bound form (such as the assays described herein or otherwise known in the art), and identifying the BLYS binding polypeptides that bind both forms.

[0490] Additionally, BLYS binding polypeptides may be screened for the ability to inhibit, stimulate or not significantly alter BLYS activity, e.g., the ability of BLYS: to bind to its receptor (e.g., TAC1 and BCMA), to stimulate B cell proliferation, to stimulate immunoglobulin secretion by B cells, to activate B cells, to increase B cell lifespan and/or to stimulate a BLYS receptor signaling cascade (e.g., to activate calcium-modulator and cyclophilin ligand ("CAML"), calcineurin, nuclear factor of activated T cells transcription factor ("NF-AT"), nuclear factor-kappa B ("NF-kappa B"; NF- κ B), activator protein-1 (AP-1), SRF, extracellular-signal regulated kinase 1 (ERK-1), polo like kinases (PLK), ELF-1, high mobility group I (HMG-I), and/or high mobility group Y (HMG-Y)). Assays that may be used to screen for the effects on BLYS activity are described herein (see, for example, Examples 7, 8, and 12) and are commonly known in the art.

Anti-BLYS Binding Polypeptide Antibodies

[0491] Further polypeptides useful herein relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a BLYS binding polypeptide (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-id) antibodies (including, e.g., anti-id antibodies to antibodies), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen

binding site that immunospecifically binds an antigen. The immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Immunoglobulins may have both a heavy and light chain. In specific embodiments, the immunoglobulin molecules are IgG1. In other specific embodiments, the immunoglobulin molecules are IgG4. An array of IgG, IgE, IgM, IgD, IgA, and IgY heavy chains may be paired with a light chain of the kappa or lambda forms.

[0492] Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 to Kucherlapati et al.

[0493] The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715, WO 92/08802, WO 91/00360, WO 92/05793; Tutt et al., *J. Immunol.*, 147:60-69 (1991); U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., *J. Immunol.*, 148:1547-1553 (1992).

[0494] Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a BLYS binding polypeptide of the present invention which they recognize or specifically bind. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind BLYS binding polypeptides of the present invention, and allows for the exclusion of the same.

[0495] In further preferred, nonexclusive embodiments, the antibodies (e.g., anti-idiotypic antibodies) inhibit one or more biological activities of BLYS through specific binding to BLYS. In more preferred embodiments, the antibody inhibits BLYS-mediated B cell proliferation.

[0496] Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other BLYS binding

polypeptide are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a BLYS binding polypeptide of the present invention are also included in the present invention. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a BLYS binding polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides, the complement of which hybridize to a polynucleotides of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a BLYS binding polypeptide. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10⁻⁵ M, 10⁻⁵ M, 5×10⁻⁶ M, 10⁻⁶ M, 5×10⁻⁷ M, 10⁻⁷ M, 5×10⁻⁸ M, 10⁻⁸ M, 5×10⁻⁹ M, 10⁻⁹ M, 5×10⁻¹⁰ M, 10⁻¹⁰ M, 5×10⁻¹¹ M, 10⁻¹¹ M, 5×10⁻¹² M, 10⁻¹² M, 5×10⁻¹³ M, 10⁻¹³ M, 5×10⁻¹⁴ M, 10⁻¹⁴ M, 5×10⁻¹⁵ M, or 10⁻¹⁵ M.

[0497] The invention also provides antibodies that competitively inhibit binding of an antibody to a BLYS binding polypeptide as determined by any method known in the art for determining competitive binding. In preferred embodiments, the antibody competitively inhibits binding to the BLYS binding polypeptide by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[0498] Antibodies of the present invention (e.g., anti-idiotypic antibodies) may act as agonists or antagonists of BLYS or alternatively may not significantly alter BLYS mediated activity. For example, the present invention includes antibodies (e.g., anti-idiotypic antibodies) which disrupt BLYS/BLYS receptor (e.g., TACI and BCMA) interactions either partially or fully. In another example, antibodies of the invention enhance BLYS/BLYS receptor interactions either partially or fully. Such activity may be the result of, for example, the antibody binding to a BLYS binding polypeptide, or alternatively as a result of direct binding of the antibody (e.g., an anti-idiotypic antibody to BLYS).

[0499] Preferably, antibodies of the present invention bind a BLYS binding polypeptide disclosed herein, a portion thereof, or an antibody that binds a BLYS binding polypeptide disclosed herein, or a portion thereof. The invention features both BLYS binding polypeptide-specific antibodies and antibodies that are specific to BLYS binding polypeptide/BLYS complexes. The invention features antibodies that enhance BLYS/BLYS binding polypeptide binding and/or BLYS/BLYS receptor binding. The invention also features antibodies that do not inhibit or reduce BLYS/BLYS binding polypeptide binding and/or BLYS/BLYS receptor binding. The invention also features BLYS binding polypeptide specific antibodies that inhibit binding of the BLYS binding polypeptide to BLYS or BLYS binding to BLYS receptor. In specific embodiments, antibodies are provided that inhibit BLYS activity or BLYS receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least

70%, at least 60%, or at least 50% of the activity in absence of the antibody. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra).

[0500] The antibodies of the present invention may be used, for purposes including, but not limited to, purify, detect, and target the BLyS binding polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of BLyS in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1988).

[0501] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugated) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 396 387.

[0502] The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0503] The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *corynebacterium parvum*. Such adjuvants are also well known in the art.

[0504] According to certain embodiments of the invention, multivalent BLyS binding polypeptides are administered to the host animal. Multivalent BLyS binding polypeptide complexes may be prepared using techniques and materials known in the art such as, for example, by cross-linking the polypeptide to a carrier protein (e.g., bovine serum albumin (BSA), human albumin, keyhole limpet hemocyanin (KLH), or succinylated KLH) by use of conventional cross-linking reagents.

[0505] In specific embodiments multivalent BLyS binding polypeptides are administered in the form of multiple antigen peptides (MAP) (Tam, *J. Imm. Meth.*, 124:53-61 (1989); Tam, *Proc. Natl. Acad. Sci. USA*, 85:5409-5413 (1988)). In this form, the multivalent BLyS binding polypeptide is synthesized on a branching lysyl matrix using solid-phase peptide synthesis methods. Recognition units in the form of MAP may be prepared by methods known in the art (Tam, 1989, supra; Tam, 1988, supra), or, for example, by a stepwise solid-phase procedure on MAP resins (Applied Biosystems), utilizing methodology established by the manufacturer. MAP peptides may be synthesized comprising (BLyS binding polypeptide)₂ Lys₁, (BLyS binding polypeptide)₄ Lys₃, (BLyS binding polypeptide)₈ Lys₆ or more levels of branching.

[0506] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1988); Hammerling et al., in *Monoclonal Antibodies and T-Cell Hybridomas* (Elsevier, N.Y. 1981), pp. 563-681 (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0507] A "monoclonal antibody" may comprise, or alternatively consist of, two proteins, i.e., a heavy and a light chain.

[0508] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 9). In a non-limiting example, mice can be immunized with a polypeptide or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the American Type Culture Collection (ATCC), to form hybridoma cells. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0509] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen according to the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a BLYS binding polypeptide.

[0510] Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH 1 domain of the heavy chain.

[0511] For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles that carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods*, 182:41-50 (1995); Ames et al., *J. Immunol. Methods*, 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.*, 24:952-958 (1994); Persic et al., *Gene*, 187 9-18 (1997); Burton et al., *Advances in Immunology*, 57:191-280 (1994); PCT international application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0512] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques*, 12(6):864-869 (1992); and Sawai et al., *AJRI*, 34:26-34 (1995); and Better et al., *Science*, 240:1041-1043 (1988) (said references incorporated herein by reference in their entireties).

[0513] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology*, 203:46-88 (1991); Shu et al., *Proc. Natl. Acad. Sci. USA*, 90:7995-7999 (1993); and Skerra et al., *Science*, 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science*, 229:1202 (1985); Oi et al., *BioTechniques*, 4:214 (1986); Gillies et al., *J. Immunol. Methods*, 125:191-202 (1989); U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. A humanized antibody is an antibody molecule made using one or more complementarity determining regions (CDRs) from a non-human species antibody that binds the desired antigen and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature*, 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239 400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592 106; EP 519 596; Padlan, *Molecular Immunology*, 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering*, 7(6):805-814 (1994); Roguskau. et al., *Proc. Natl. Acad. Sci. USA*, 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

[0514] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0515] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered

non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a binding polypeptide. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.*, 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, each of which is incorporated by reference herein in its entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.) and GenPharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0516] Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach, a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (See, Jespers et al., *Bio/technology*, 12:899-903 (1988).)

[0517] Further, antibodies to the BLYS binding polypeptides can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" BLYS binding polypeptides, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.*, 7(5):437-444 (1989) and Nissinoff, *J. Immunol.*, 147(8):2429-2438 (1991).) For example, antibodies which bind to and competitively inhibit the binding of BLYS binding polypeptide to BLYS can be used to generate anti-idiotypes that "mimic" the BLYS/BLYS binding polypeptide binding domain and, as a consequence, bind to and neutralize or enhance BLYS binding to BLYS receptor (e.g., TACI and BCMA). Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to bind BLYS and/or neutralize or enhance BLYS mediated activity. In a specific embodiment, anti-idiotypic antibodies can be used to bind BLYS, and thereby block its biological activity. In another specific embodiment, anti-idiotypic antibodies can be used to bind BLYS, and thereby enhance its biological activity (e.g., via multimerization of BLYS).

Polynucleotides Encoding Antibodies

[0518] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of

the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to BLYS or a BLYS binding polypeptide.

[0519] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques*, 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0520] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method known in the art.

[0521] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., *Molecular Cloning: A Laboratory Manual, 2d Ed.* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 1990) and *Current Protocols in Molecular Biology*, Ausubel et al., eds. (John Wiley & Sons, NY 1993), which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0522] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.*, 278: 457-479 (1998) for a listing of human

framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds BLYS or a BLYS binding polypeptide. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0523] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:851-855 (1984); Neuberger et al., *Nature*, 312:604-608 (1984); Takeda et al., *Nature*, 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

[0524] Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, *Science*, 242:423-42 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879-5883 (1988); and Ward et al., *Nature*, 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., *Science*, 242:1038-1041 (1988)).

Methods of Producing Antibodies

[0525] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0526] Recombinant expression of an antibody, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody or a single chain antibody), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody or portion thereof (preferably containing the heavy or light chain variable domain) has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody-encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides

replicable vectors comprising a nucleotide sequence encoding an antibody molecule, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT publication WO 86/05807; PCT publication WO 89/01036; and U.S. Pat. No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0527] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody. Thus, the invention includes host cells containing a polynucleotide encoding an antibody, or a heavy or light chain thereof, or a single chain antibody, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0528] A variety of host-expression vector systems may be utilized to express the antibody molecules. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene*, 45:101 (1986); Cockett et al., *Bio/Technology*, 8:2 (1990)).

[0529] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but

are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.*, 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.*, 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.*, 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0530] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0531] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. See, e.g., Logan & Shenk, *Proc. Natl. Acad. Sci. USA*, 81:355-359 (1984). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, Bittner et al., *Methods in Enzymol.*, 153:51-544 (1987)).

[0532] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, NSO, 293, 3T3, WI38, and in

particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[0533] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0534] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., *Cell*, 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA*, 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., *Cell*, 22:817 (1980)) genes can be employed in tk-, hgprrt- or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., *Proc. Natl. Acad. Sci. USA*, 77:357 (1980)); O'Hare et al., *Proc. Natl. Acad. Sci. USA*, 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418; Wu and Wu, *Biotherapy*, 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.*, 32:573-596 (1993); Mulligan, *Science*, 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.*, 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215; and hygromycin, which confers resistance to hygromycin (Santerre et al., *Gene*, 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in *Current Protocols in Molecular Biology*, Ausubel et al., eds. (John Wiley & Sons, NY 1993); Krieglger, *Gene Transfer and Expression, A Laboratory Manual* (Stockton Press, NY 1990); and *Current Protocols in Human Genetics*, Dracopoli et al., eds. (John Wiley & Sons, NY 1994), Chapters 12 and 13; Colberre-Garapin et al., *J. Mol. Biol.*, 150:1 (1981), which are incorporated by reference herein in their entireties.

[0535] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor

present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., *Mol. Cell. Biol.*, 3:257 (1983)).

[0536] The host cell may be co-transfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature*, 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA*, 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0537] Once an antibody molecule has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

[0538] The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than BLYS binding polypeptides of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.*, 39:91-99 (1994); U.S. Pat. No. 5,474,981; Gillies et al., *Proc. Natl. Acad. Sci. USA*, 89:1428-1432 (1992); Fell et al., *J. Immunol.*, 146:2446-2452 (1991), which are incorporated by reference in their entireties.

[0539] The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or

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

[0540] As discussed, supra, the polypeptides corresponding to a BLYS binding polypeptide may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the BLYS binding polypeptides may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394 827; Traunecker et al., *Nature*, 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (see, EP-A-232 262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., *J. Molecular Recognition*, 8:52-58 (1995); Johanson et al., *J. Biol. Chem.*, 270:9459-9471 (1995)).

[0541] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA*, 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell*, 37:767 (1984)) and the "flag" tag.

[0542] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically

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

[0543] Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0544] The conjugates can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, PCT publication WO 97/33899), AIM II (See, PCT publication WO 97/34911), Fas Ligand (Takahashi et al., *Int. Immunol.*, 6:1567-1574

(1994)), VEGF (See, PCT publication WO 99/23105), CD40 Ligand, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0545] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Amon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al., eds. (Alan R. Liss, Inc. 1985), pp. 243-56; Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery (2nd Ed.)*, Robinson et al., eds. (Marcel Dekker, Inc. 1987), pp. 623-53; Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al., eds., pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al., eds. (Academic Press 1985), pp. 303-16; and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982).

[0546] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the BLYS binding polypeptide. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0547] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980, which is incorporated herein by reference in its entirety.

[0548] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Assays for Antibody Binding

[0549] The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., *Current Protocols in Molecular Biology*, Ausubel et al., eds. (John Wiley & Sons, NY 1993), which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0550] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycho-

late, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C., adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C., washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., *Current Protocols in Molecular Biology*, Ausubel et al., eds. (John Wiley & Sons, NY 1993) at 10.16.1.

[0551] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%-20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ³²P or ¹²⁵I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., *Current Protocols in Molecular Biology*, Ausubel et al., eds. (John Wiley & Sons, NY 1993) at 10.8.1.

[0552] ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., *Current Protocols in Molecular Biology*, Ausubel et al., eds. (John Wiley & Sons, NY 1993) at 11.2.1.

[0553] The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be

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

Therapeutic Uses of Antibodies

[0554] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the diseases, disorders, or conditions disclosed herein. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant BLYS expression and/or activity, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein.

[0555] The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of BLYS or BLYS receptor includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. The antibodies of the invention may also be used to target and kill cells expressing BLYS on their surface and/or cells having BLYS bound to their surface. This targeting may be the result of binding of the antibody to BLYS binding polypeptides that have been coadministered, or alternatively, the result of direct binding of the antibody to BLYS. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0556] Non-limiting examples of the ways in which the antibodies of the present invention may be used therapeutically includes binding BLYS binding polypeptides of the present invention that have been coadministered in order to bind or neutralize BLYS, or by direct cytotoxicity of the antibody, e.g., as mediated by complement (CDC) or by effector cells (ADCC). BLYS binding polypeptides and anti-BLYS binding polypeptide antibodies may be administered either locally or systemically. Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[0557] The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

[0558] The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy, anti-tumor agents, antibiotics, and immunoglobulin). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0559] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_D less than 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Demonstration of Therapeutic or Prophylactic Activity of Antibodies

[0560] The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic and/or Prophylactic Administration and Composition

[0561] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a BlyS binding compound or pharmaceutical composition, preferably an antibody. In a preferred embodiment, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0562] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0563] Various delivery systems are known and can be used to administer a compound, e.g., encapsulation in lipo-

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

[0564] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, care must be taken to use materials to which the protein does not absorb.

[0565] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science*, 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler, eds. (Liss, New York 1989), pp. 353-365; Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

[0566] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.*, 14:201 (1987); Buchwald et al., *Surgery*, 88:507 (1980); Saudek et al., *N. Engl. J. Med.*, 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise, eds. (CRC Press, Boca Raton, Fla. 1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball, eds. (Wiley, New York 1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.*, 23:61 (1983); see also Levy et al., *Science*, 228:190 (1985); During et al., *Ann. Neurol.*, 25:351 (1989); Howard et al., *J. Neurosurg.*, 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, Langer and Wise, eds. (CRC Press, Boca Raton, Fla. 1974), vol. 2, pp. 115-138 (1984)).

[0567] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0568] In a specific embodiment where the compound is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA*, 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0569] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed. (Mack Publishing Co., 1990). Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0570] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed

together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0571] The compounds for use in the methods of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0572] The amount of the compound used which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0573] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[0574] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

[0575] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a BLYS binding polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of BLYS. The invention provides for the detection of aberrant expression of BLYS, comprising (a) contacting cells or body fluid with

a BLYS binding polypeptide; (b) assaying the expression of BLYS in cells or body fluid of an individual using one or more antibodies specific to the BLYS binding polypeptide and (c) comparing the level of BLYS expression with a standard BLYS expression level, whereby an increase or decrease in the assayed BLYS expression level compared to the standard expression level is indicative of aberrant expression.

[0576] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) contacting cells or body fluid with a BLYS binding polypeptide; (b) assaying the expression of BLYS in cells or body fluid of an individual using one or more antibodies specific to the BLYS binding polypeptide of interest and (c) comparing the level of BLYS expression with a standard BLYS expression level, whereby an increase or decrease in the assayed BLYS expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of BLYS in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0577] Antibodies can be used to assay BLYS protein levels in a biological sample using or routinely modifying classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen et al., *J. Cell. Biol.*, 101:976-985 (1985); Jalkanen et al., *J. Cell. Biol.*, 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium ($^{99\text{m}}\text{Tc}$, $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0578] Techniques known in the art may be applied to label antibodies. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see, e.g., U.S. Pat. Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

[0579] One embodiment of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of BLYS in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: (a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to BLYS (e.g., a BLYS binding polypeptide) or which specifically binds to a molecule that specifically binds to BLYS (e.g., an anti-BLYS binding polypeptide antibody);

(b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); (c) determining background level; and (d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system. As described herein, specific embodiments of the invention are directed to the use of the antibodies to quantitate or qualitate concentrations of cells of B cell lineage or cells of monocytic lineage.

[0580] It will be understood by those skilled in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific polypeptide. In vivo tumor imaging is described in S. W. Burchiel et al., "Immunopharmacokinetics of Radio-labeled Antibodies and Their Fragments." (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S. W. Burchiel and B. A. Rhodes, eds. (Masson Publishing Inc. 1982).

[0581] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0582] In a further embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disorder, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc. and comparing the results.

[0583] Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include but are not limited to computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0584] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Pat. No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodi-

ment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Antibody Kits

[0585] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention comprise two or more antibodies (monoclonal and/or polyclonal) that recognize the same and/or different sequences or regions of a polypeptide according to the invention. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0586] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0587] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0588] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0589] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound

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

[0590] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated protein(s).

[0591] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

[0592] In another specific embodiment, any of the antibodies listed above are conjugated to a toxin or a label (as described supra). Such conjugated antibodies are used to kill a particular population of cells or to quantitate a particular population of cells. In a preferred embodiment, such conjugated antibodies are used to kill B cells expressing BLYS receptor on their surface. In another preferred embodiment, such conjugated antibodies are used to quantitate B cells expressing BLYS receptor on their surface.

[0593] In another specific embodiment, any of the antibodies listed above are conjugated to a toxin or a label (as described supra). Such conjugated antibodies are used to kill a particular population of cells or to quantitate a particular population of cells. In a preferred embodiment, such conjugated antibodies are used to kill monocyte cells expressing the membrane-bound form of BLYS. In another preferred embodiment, such conjugated antibodies are used to quantitate monocyte cells expressing the membrane-bound form of BLYS.

[0594] The antibodies of the invention also have uses as therapeutics and/or prophylactics which include, but are not limited to, in activating monocytes or blocking monocyte activation and/or killing monocyte lineages that express the membrane bound form of BLYS on their cell surfaces (e.g., to treat, prevent, and/or diagnose myeloid leukemias, monocyte based leukemias and lymphomas, monocytosis, monocytopenia, rheumatoid arthritis, and other diseases or conditions associated with activated monocytes). In a specific embodiment, the antibodies fix complement. In other specific embodiments, as further described herein, the antibodies (or fragments thereof) are associated with heterologous polypeptides or nucleic acids (e.g. toxins, such as, compounds that bind and activate endogenous cytotoxic effector systems, and radioisotopes; and cytotoxic prodrugs).

[0595] As discussed above, antibodies to the BLYS binding polypeptides can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" the BLYS binding polypeptide, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.*, 7(5):437-444 (1989), and Nissinoff, J. *Immunol.*, 147(8):2429-2438 (1991)). For example, antibodies which bind to BLYS binding polypeptides and competitively inhibit BLYS/BLYS binding polypeptide binding can be used to generate anti-idiotypes that "mimic" the BLYS binding polypeptide/BLYS binding domain and, as a consequence, bind to and, for example, neutralize BLYS. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize BLYS. For example, such anti-idiotypic antibodies can be used to bind BLYS and thereby block BLYS mediated B cell activation, proliferation, survival and/or differentiation.

EXAMPLES

[0596] Isolation of BLYS binding polypeptides and their use in accordance with this invention will be further illustrated below. The specific parameters included in the following examples are intended to illustrate the practice of the invention, and they are not presented to in any way limit the scope of the invention.

Example 1

Screening of Phage Display Libraries

[0597] Streptavidin-coated magnetic beads (Dynal M-280) were chosen for presentation of the target during screening because of their superior binding capacity compared to that of a 96 well plate. The binding capacity of the beads for biotinylated antibodies was 5-10 $\mu\text{g}/\text{mg}$ of beads as stated by the manufacturer. For this study and the screening to follow, 5 μg of biotinylated recombinant BLYS (obtained from Human Genome Sciences, Inc.) was allowed for each mg of beads. This amount of biotinylated BLYS represents a 10-fold excess of target, for saturation of the beads. Unbound BLYS was washed away. Bound biotinylated BLYS was confirmed with detection using Mab 16C9 (murine anti-BLYS, Human Genome Sciences) primary antibody and a goat anti-mouse HRP conjugate as the secondary antibody. An irrelevant monoclonal antibody (anti-TNF α) was used to probe a second set of beads to control for nonspecific binding. The color reagent TMB was used and the assay read at OD 630 nm.

[0598] Nine phage display libraries, TN6/6, TN7/4, TN8/9, TN9/4, TN10/9, TN12/1, and Substrate Phage #2 (Dyax Corp., Cambridge, Mass. (US)), and PhD7 and PhD12 (New England Biolabs), were screened for BLYS binders. The makeup of these libraries was as follows:

[0599] The TN6/6 phage display library was composed of recombinant M13 phage displaying variegated peptides with the potential to form loop structures based on a polypeptide template having the structure Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa (SEQ ID NO:14) and providing 2.0×10^8 peptide diversity.

[0600] The TN7/4 phage display library was composed of recombinant M13 phage displaying variegated peptides with the potential to form loop structures based on a polypeptide template having the structure Xaa-Xaa-Xaa-Cys-Xaa-Xaa-

Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa (SEQ ID NO:15) and providing 2.3×10^9 peptide diversity.

[0601] The TN8/9 phage display library was composed of recombinant M13 phage displaying variegated peptides with the potential to form loop structures based on a polypeptide template having the structure Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa (SEQ ID NO:16) and providing about 5×10^9 peptide diversity.

[0602] The TN9/4 phage display library was composed of recombinant M13 phage displaying variegated peptides with the potential to form loop structures based on a polypeptide template having the structure Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa (SEQ ID NO:17) and providing about 3.2×10^9 peptide diversity.

[0603] The TN10/9 phage display library was composed of recombinant M13 phage displaying variegated peptides with the potential to form loop structures based on a polypeptide template having the structure Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa (SEQ ID NO:18) and providing 2.5×10^9 peptide diversity.

[0604] The TN12/1 phage display library was composed of recombinant M13 phage displaying variegated peptides with the potential to form loop structures based on a polypeptide template having the structure Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa (SEQ ID NO:19) and providing 1.4×10^9 peptide diversity.

[0605] Substrate Phage Library #2 was composed of recombinant M13 phage displaying a polypeptide insert of approximately 80 amino acids, having two streptavidin binding domains, a linear variegated segment of thirteen amino acids where all amino acids except Cys were permitted at each position, and a Factor Xa cleavage site, linked together with peptide linkers. This library provided a diversity of 2×10^8 display polypeptides.

[0606] Libraries PhD7 and PhD 12 were composed of recombinant M13 phage displaying randomized linear seven- and twelve-amino acid peptides, respectively.

[0607] Screening was performed as described in PCT/US01/[____], entitled "Binding Polypeptides for B Lymphocyte Stimulator Protein (BLYS)", filed concurrently herewith.

[0608] At the conclusion of screening individual phage isolates were randomly selected and tested by ELISA for binding to BLYS. The same isolates were submitted for DNA sequence analysis to identify the nucleotide and deduced amino acid sequence of the displayed peptide. Isolates were also tested for their ability to bind to recombinant BLYS in feed streams of CHO supernatant and Sf9 supernatant (supplied by Human Genome Sciences, Inc.).

[0609] Each isolate was tested for binding to BLYS by standard ELISA techniques where bound phage were detected with a monoclonal anti-phage antibody/HRP conjugate.

[0610] Amino acid sequences of the displayed peptides were derived from sequencing the phage isolate DNA inserts. Sequence data from the phage isolates were grouped by library and sorted according to the degree of similarity.

The BLYS binding phage isolate peptides are shown in Tables 1-8 below. These peptides represent the translation of the DNA sequences across the varied regions of the genes encoding the phage display fusion/peptide.

TABLE 1

<u>TN6/6 Library BLYS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-B06	HLRCWSTNCRYD	20
453-01-A04	VMDCLINRCDTV	21

[0611]

TABLE 2

<u>TN7/4 Library BLYS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-B04	KSKCFPPWECQQA	22
453-01-D11	AMKCYFPWECANG	23
453-01-A05	NVACYFPWECCHHP	24
453-01-D01	NAPCYFPWECFSI	25
453-01-D03	SVNCWFPWECVGN	26
453-01-A08	KEPCYFYWECAVS	27

[0612]

TABLE 3

<u>TN8/9 Library BLYS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-D04	DTNCDLLTKMCGPQ	28
453-01-C06	GTPCDLLTKLCLLW	29
453-01-D10	MSECDLLTKICLMG	30
453-01-B07	VVPCDLLTKHCFEA	31
453-01-B09	VVPCDLLTKHCFEA	32
453-01-C02	WSACDLLTKQCVQV	33
453-01-A06	-DGCDELTKICGMK	34
453-01-B03	KSWCDELTKVCFDP	35
453-01-B11	KWMCDELTKQCQYV	36
453-01-A02	MKYCDELTKICVWG	37
453-01-B05	YFQCDELTKMCWQK	38
453-01-A11	AMHCDKLTKHCKFH	39
453-01-A03	VPYCDKLTKICQW-	40
453-01-A07	EVFCDVLTkVCFHD	41
453-01-C09	KPKCDVLTkMCDWL	42

TABLE 3-continued

<u>TN8/9 Library BLYS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-B02	TQHCDVLTkQCFTI	43
453-01-C01	GHFCDRLTkYCFEP	44
453-01-A09	HIQCDRLTKSCLSV	45
453-01-D05	IKACDILTKVCWPP	46
453-01-A01	QFDCDPLTKYCGEF	47
453-01-C07	KMYCDHLTKYCWPE	48
453-01-C11	MQSCDILTKYCFKR	49
453-01-D12	GPWCDILTKGFLAQ	50
453-01-C04	SVRCDLLTKGCPVW	51
453-01-B10	PADCPLTKNICFWK	52
453-01-D02	TNVCDPLTKVCFMN	53
453-01-C05	EHWCDDLTKLFCRL	54
453-01-D08	GYWCDVLTNNCWKI	55
453-01-C10	LYNCDYLTKLFCFEP	56
453-01-C08	HVDCLLHPKACYKY	57
453-01-D07	VQDCLLHPKACQMQ	58
453-01-D09	KFDCLLKPMPFCSNH	59
453-01-C12	FADCLIHPKSCKPL	60
453-01-D06	HGNCYFPWECESK	61
453-01-B01	MIIVLLLLRFAISR	62
453-01-A12	SLLVIFLLIGAGSL	63

[0613]

TABLE 4

<u>TN9/4 Library BLYS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-G06	FHPCDMLTKGIWCQPN	64
453-01-H01	SKRCDLLTKMWCETE	65
453-01-F02	TKFCDRLTKMPKCVWK	66
453-01-E03	NTFCFDPLTKGRVNP	67
453-01-E11	DWTCDPLFKHRECIFE	68
453-01-H09	PQPCDLLFEKKSIIK	69
453-01-H02	RWHCDMLINPSCLPD	70
453-01-E04	KIQCDIVNLSSCVYP	71
453-01-G11	LNACDIVHPNYCSGM	72
453-01-F01	AKACSIVNLESCCYL	73

TABLE 4-continued

<u>TN9/4 Library BlyS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-H06	RQACSIITPWGCPPI	74
453-01-F10	ADNCTVATLDFCYWT	75
453-01-E05	KPECNITKQFCFGE	76

[0614]

TABLE 5

<u>TN10 Library BlyS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-H07	-NNCQWDELTSMDPF	77
453-01-F05	SRLCHMDELTHVCVHF	78
453-01-F09	SRPCQIDELTKACFYN	79
453-01-G09	DRVCKLDFLTYNCLNH	80
453-01-F04	HSNCIMDLLTNRCFYD	81
453-01-H03	PFNCFHDPLTGLCLHS	82
453-01-F03	YDSCYDRLTKQCYPS	83
453-01-F07	FHDCMYDALLGYCLPY	84
453-01-G08	NRSCDPLTRPKSCGL	85
453-01-G04	LSNCDWDDLIRQCLHD	86
453-01-E01	FWDCLFHPNSRYCVLS	87
453-01-E10	SRDCLLSPAMAWCGLD	88

[0615]

TABLE 6

<u>TN12/1 Library BlyS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-H05	GGNCYTDSLTKLHFCMGD	89
453-01-H04	--MCPRDPLTKAKLCNWH	90
453-01-G03	PNQCQDDLTKQWYSCHYH	91
453-01-F11	FDMCFDALTKQNFYCRFH	92
453-01-F06	RNMCVDRLTKLQHGCEGA	93
453-01-G07	DPECLTSFDRLTKMCWPW	94
453-01-H11	DDECHYDYLTHYMRCDYR	95
453-01-G05	FGGCNIDLLTNTMMCHR	96
453-01-G10	HGFCYWDELTMQWHCNHH	97
453-01-H12	GAMCVDLLTYTFRPCMYA	98

TABLE 6-continued

<u>TN12/1 Library BlyS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-01-E07	SNKCWDELTHAWAECGRF	99
453-01-E09	RPVCYKGYDILTTQCMFW	100
453-01-G01	PSRCWFLLFNKFVCKRN	101
453-01-H08	RSGCVYDMLLMTMYCPSN	102
453-01-H10	SNRCEGDQLMRPPSCRHL	103
453-01-F08	YRMCWDDLRLRGFVCDPH	104
453-01-E06	HDGCYDELLYRWTRCEHR	105
453-01-E08	WAWCFDELVQRYFTCFDH	106
453-01-E02	LPECRQYFPWEKQVCSYW	107

[0616]

TABLE 7

<u>PhD 12 Library BlyS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-02-B05	VHYDSLTKMWTR	108
453-02-D09	FTDPLTKMSLHS	109
453-02-C12	GVDVLTCLYFVP	110
453-02-A05	YYDRLTKLYSSM	111
453-02-B06	L?KDPLTKLYIS	112
453-02-A04	GVDVLTCL?FVP	113
453-02-B03	RLYDPLTKLVLS	114
453-02-B01	MFDPLTKIAFPA	115
453-02-D04	FYDSLTKTNLRD	116
453-02-B02	GIYDKLTRAWLP	117
453-02-B08	KYDPLTRAR?PL	118
453-02-D06	YIDQLTRLSLPS	119
453-02-A09	HqTFDILTRLHF	120
453-02-B04	WQFDVLTTRSWTP	121
453-02-A02	GAAYDHLTRTWL	122
453-02-D05	YFDQLTHLSIKK	123
453-02-A06	AWDPLTMLVLPW	124
453-02-D03	ALWMDPLTGLAF	125
453-02-B12	WQFDVLT?SWTP	126
453-02-A01	WTDPLTHMEIYH	127
453-02-C04	WTDSLTGLWFPD	128
453-02-C05	YTDPLTGIV?PF	129

TABLE 7-continued

<u>PhD 12 Library BLYS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-02-D08	YWDKLTMLHLGV	130
453-02-D02	YYDFLTRTVLPS	131
453-02-A03	RLDPLSKNDFPR	132
453-02-A11	LRYPDLLKS?IY	133
453-02-D07	LRYPDLLKSYIY	134
453-02-A07	YFDQFTHLSIKK	135
453-02-C08	YFDQ?THLSIKK	136

[0617]

TABLE 8

<u>Substrate Phage Library BLYS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-02-E04	EHYYTDPLTGARI	137
453-02-F01	EHY?TDPLTGARI	138
453-02-E09	EHYSTDPLTGARI	139
453-02-E07	EHYYTDPL?G?RI	140
453-02-G05	EHYYTDPL?G?R?	141
453-02-G09	EHYYTDPL?GAR?	142
453-02-E06	EH?YTDPLNGAR?	143
453-02-E05	EHYYNDPLNGAR?	144
453-02-F04	?H?YNDPLNGAR?	145
453-02-G07	KPYYPDITKMTHH	146
453-02-F06	KPYYPDITKMSHH	147
453-02-E08	KPYYPDIPISKMTHH	148
453-02-G08	KP??DPISKMTHH	149
453-02-E01	QIGYDELTKAWVT	150
453-02-G02	QLGYDELTKAWVT	151
453-02-H06	KIDEL?MQNIIIW	152
453-02-F08	DHTDPLIQGLTKR	153
453-02-H01	WHDPLKMHMFHHE	154
453-02-F03	KHIDMETGLILQN	155
453-02-G03	MQVDPETGLKYEYH	156
453-02-E03	?LDQHVN??YQS	157
453-02-F10	E??T??LTGAR?	158
453-02-F02	GPYNI?RL?GEF?	159

TABLE 8-continued

<u>Substrate Phage Library BLYS-binding Sequences</u>		
Phage Isolate	Amino Acid Sequence	SEQ ID NO:
453-02-E02	HIKMLHQGSFVGV	160
453-02-H08	HPTNT??HQ?VYS	161
453-02-H05	HRGQV??LNGMv?	162

? = amino acid unknown (all tables)
 lower case = amino acid identity probable but not completely characterized

Example 2

Immobilization of BLYS Binding Polypeptides on Sepharose-4FF Beads

[0618] On the basis of the above results, six display phage sequences were chosen for further study:

[0619] TN7-01-A08 (SEQ ID NO:27), TN8-01-B07 (SEQ ID NO:31), TN10-01-F05 (SEQ ID NO:78), TN12-01-H05 (SEQ ID NO:89), PhD-02-C04 (SEQ ID NO:128), and PhD-02-C12 (SEQ ID NO:110). In order to develop a suitable BLYS affinity ligand, the identified display peptides were synthesized to order by a commercial vendor, with slight modifications:

[0620] Two amino acids of leader were added to each binding peptide at the N-terminus, in order to avoid leaving a free amine at the first amino acid of the sequence corresponding to the variegated region of the phage display template; the N-terminus was acetylated to prevent immobilization of the peptide to the chromatographic matrix through that position; a C-terminal linker was added (i.e., -PGPEGGGK; SEQ ID NO:13); and any internal lysines in the peptide were blocked with the group: ivDde (i.e., 1-(4, 4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methyl butyl-L-lysine). This group was intact on the finished synthesized peptides and was removed after immobilization or fluorescence labeling. As an alternative modification, peptides with internal lysines were also synthesized with C-terminal hydrazide functional groups, which could be immobilized onto activated aldehyde chromatographic media.

[0621] The peptides were immobilized onto NHS-activated SEPHAROSE-4 Fast Flow agarose media (Pharmacia) at ligand densities targeted to 2 $\mu\text{mol/ml}$. Actual ligand densities of peptides on the media ranged from 0.76 $\mu\text{mol/ml}$ to 1.98 $\mu\text{mol/ml}$, as determined by amino acid analysis of immobilized peptide. All but one peptide was immobilized in aqueous conditions of 100 mM KH_2PO_4 /150 mM NaCl/0.05% Tween 20, pH 7.5. For solubility reasons, the peptide DX217 (see, Table 9, below) was immobilized in 30% dimethyl formamide(DMF)/100 mM KH_2PO_4 /150 mM NaCl/0.05% Tween 20, pH 7.5. Immobilization reactions were allowed to proceed for 2 hours at ambient temperature, followed by brief washing with pH 7.5 buffer. The Fast Flow SEPHAROSE media was then allowed to tumble at ambient temperature overnight to hydrolyze remaining NHS esters after which the media was washed to remove any unbound peptide. A solution of 2% hydrazine/DMF was used to de-block ligands containing ivDde-lysine. Media was then further washed with aqueous buffers and stored at 4° C. until

packed into columns. Table 9 shows the sequences of the synthesized peptides and their measured densities on the agarose media.

peptides (DX212, DX214, DX216, DX217, DX219, and DX221) bound BLYS in solution with approximately the same affinity ($K_D=0.5-2 \mu\text{M}$). Cross-competition studies

TABLE 9

<u>BLYS Binding Peptides Synthesized as Affinity Ligands</u>			
Peptide Name	Isolate source	Sequence (potential disulfide loop underlined)	SEQ ID NO:
DX212	01-A08	Ac-AGKEPCYFYWECAVSGPGPEGGGK	163
DX214	01-B07	Ac-AGVPFCDLLTKHCFEAGPGPEGGGK	164
DX216	01-F-5	Ac-GSSRLCHMDELTHVCVHFAPPGPEGGGK	165
DX217	01-H05	Ac-GDGGNCYTDSLTKLHFCMGDEPGPEGGGK	166
DX219	02-C12	Ac-GYDVLTKLYFVPGGGPEGGGK	167
DX221	02-C04	Ac-WTDSLTLGLWFPDGGPGPEGGGK	168

Ac denotes N-terminal acetylation

BLYS-Ligand Affinity Determination (Overview of Procedure)

[0622] Dissociation constants between the synthetic peptides and BLYS (free in solution) were measured by fluorescence anisotropy (FA). In these experiments, the concentration of the fluorescein-labeled peptide is held constant and the BLYS protein concentration was varied. The observed change in anisotropy is fit to the following equation via nonlinear regression to obtain the apparent K_D .

$$\text{Peptide} + \text{BLYS} \xrightleftharpoons{K_D} \text{Peptide} \cdot \text{BLYS}$$

$$r_{\text{obs}} = r_{\text{free}} + (r_{\text{bound}} - r_{\text{free}}) \frac{(K_D + \text{BLYS} + P) - \sqrt{(K_D + \text{BLYS} + P)^2 - 4 \cdot \text{BLYS} \cdot P}}{2 \cdot P}$$

where:

r_{obs} =observed anisotropy, r_{free} =anisotropy of free peptide, r_{bound} =anisotropy of bound peptide, K_D =dissociation constant, BLYS=total BLYS concentration, and P=total fluorescein labeled peptide concentration.

[0623] Binding reactions containing 50 nM fluorescein-labeled peptide and a varied concentration of BLYS in a volume between 10 and 20 μL per well were performed in 384 well microplates. Reactions were assayed using a Tecan Polarion fluorescence polarization plate reader. Cross-competition studies between peptides were performed using 50 nM fluorescein-labeled peptide and 1-2 μM BLYS in the presence and absence of 100 μM unlabeled peptide. The influence of pH on the observed K_D was investigated at pH 6.0 using the primary binding buffer [15 mM sodium citrate, 120 mM NaCl, 0.01% Tween 20] and at pH 9.0 using 200 mM sodium bicarbonate, 125 mM sodium chloride. Other buffers in which dissociation constants of BLYS Binding polypeptides were determined include: [pH 6.0, 0.01% Tween], [pH 6.0, 0.1% gelatin], [pH5.0, 0.01% Tween], [pH9.0, 0.1% Tween], [pH6.0, 15% ethylene glycol, 0.01% Tween], [pH5.0, 15% ethylene glycol, 0.01% Tween], and [pH9.0, 15% ethylene glycol, 0.01% Tween]. All six of the

demonstrated that all peptides compete with each other for BLYS binding, which suggests that they all bind to the same site on BLYS.

Example 3

Design of modified BLYS Binding Peptides

[0624] Once a promising BLYS binding polypeptide has been isolated, improvements to that polypeptide can be

made by changing, adding or removing individual or multiple amino acid residues from the polypeptide. Amino acid substitutions can be conservative or non conservative. Conservative amino acids exchanges include, for example, the exchange of aromatic residues (e.g., phenylalanine, tryptophan, and tyrosine) for one another, the exchange of hydrophobic residues (e.g. leucine, isoleucine, and valine) for one another, the exchange of polar residues (e.g., glutamine and asparagine) for one another, the exchange of acidic residues (e.g., arginine, lysine, and histidine) for one another, and the exchange of small residues (e.g., alanine, serine, threonine, methionine, and glycine) for one another, the exchange of aromatic residues for one another. Additionally, nonclassical amino acids, chemical amino acid analogs, or chemically modified classical amino acids can be introduced as a substitution or addition to a BLYS binding polypeptide of the invention. Non-classical amino acids include, but are not limited to, the D-isomers of the common amino acids, 2,4-diaminobutyric acid (Dbu), 4-aminobutyric acid (bAbu), 2-aminobutyric acid (Abu), 6-amino hexanoic acid (epsilon-Ahx), 2-aminoisobutyric acid (Aib), 3-aminoisobutyric acid (bAib), 3-aminopropanoic acid (bAla), ornithine (Orn), norleucine (Nle), norvaline (Nva), 3-hy-

droxyproline (3Hyp), 4-hydroxyproline (4Hyp), sarcosine (MeGly), citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, Ca-methyl amino acids, N α -methyl amino acids, and amino acid analogs in general. By way of example, four modified peptides based on the DX212 sequence have been designed:

1. Ac-AGK(Ac)EPCYFYWECAVSGPGPEGGGK (SEQ ID NO:169)—internal lysine side chain acetylated;
2. Ac-AGREPCYFYWECAVSGPGPEGGGK (SEQ ID NO:170)—arginine substitution;
3. Ac-AGQEPYFYWECAVSGPGPEGGGK (SEQ ID NO:171)—glutamine substitution;
4. Ac-AGNIePCYFYWECAVSGPGPEGGGK (SEQ ID NO:172)—norleucine substitution. Ac denotes N-terminal acetylation.

Example 4

Biacore Analysis of the Affinity of BLyS Binding Polypeptides

[0625] Binding of BLyS binding polypeptides to BLyS, for example, can be analyzed by BIAcore analysis. Either BLyS (or another antigen for which one wants to know the affinity of a BLyS binding polypeptide) or BLyS binding polypeptide can be covalently immobilized to a BIAcore sensor chip (CM5 chip) via amine groups using N-ethyl-N'-(dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide chemistry. Various dilutions of BLyS binding polypeptides or BLyS (or other antigen for which one wants to know the affinity of a BLyS binding polypeptide), respectively are flowed over the derivatized CM5 chip in flow cells at 15 microliters/min. for a total volume of 50 microliters. The amount of bound protein is determined during washing of the flow cell with HBS buffer (10 mM HEPES, pH7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005% surfactant P20). Binding specificity for the protein of interest is determined by competition with soluble competitor in the presence of the protein of interest.

[0626] The flow cell surface can be regenerated by displacing bound protein by washing with 20 microliters of 10 mM glycine-HCl, pH2.3. For kinetic analysis, the flow cells are tested at different flow rates and different polypeptide densities on the CM5 chip. The on-rates and off-rates can be determined using the kinetic evaluation program in BIAevaluation 3 software.

Example 5

BLyS Binding Polypeptide Neutralization of Murine Splenocyte Proliferation

[0627] To determine if an BLyS binding polypeptide inhibits BLyS mediated B cell proliferation, a splenocyte proliferation assay can be performed. Briefly, murine splenocytes are isolated by flushing spleen with complete medium using a 25 g needle and 10 ml of complete medium (RPMI 1640 with 10% FBS containing 100 U/ml penicillin, 100 μ g/ml streptomycin, 4 mM glutamine, 5×10^{-5} M β -mercaptoethanol). The cells are passed through a 100 micron nylon filter to remove cell clumps. The cell suspension is

then separated by gradient centrifugation at 400 \times g for 25 minutes at room temperature (one 15 ml conical tube/spleen; 3 ml Ficol, 10 ml cell suspension/spleen; Ficol 1083 from Sigma). The recovered cells are washed 3 times in complete medium and counted. Recovered cells are then diluted to a concentration of 3×10^6 /ml in complete medium containing a 3 \times concentration of SAC (3 \times =1:33,333 dilution of stock *Staph. aureus* Cowan strain; Calbiochem).

[0628] For each BLyS binding polypeptide, 50 microliters of dilutions at 30 μ g/ml, 3.0 μ g/ml, and 0.3 μ g/ml concentrations are aliquotted into individual wells of a 96 well plate in triplicate. Suitable positive controls, such as, for example monoclonal antibody 15C10, can also be used. Medium containing no BLyS binding polypeptide is used as negative control. BLyS protein is diluted in complete medium to concentrations of 300 ng/ml, 90 ng/ml and 30 ng/ml. 50 microliters of each of the BLyS dilutions were then added to the BLyS binding polypeptide dilution series in the plates. The plate containing the BLyS binding polypeptide and BLyS dilutions are then incubated for 30 minutes at 37 $^\circ$ C., 5% CO₂, after which 50 microliters of the splenocyte cell suspension containing SAC is added to all wells. The plates are then incubated for 72 hours (37 $^\circ$ C., 5% CO₂).

[0629] After 72 hours, each well is supplemented with 50 μ l of complete medium containing 0.5 μ Ci of ³H-thymidine (6.7 Ci/mM; Amersham) and cells are incubated for an additional 20-24 hours at (37 $^\circ$ C., 5% CO₂). Following incubation cells are harvested using a Tomtec Cell Harvester and filters counted in a TopCount Scintillation counter (Packard).

Example 6

In Vitro Screening of BLyS Antagonists

[0630] The bioassay for assessing the effects of putative BLyS antagonists is performed in triplicate in 96 well format by mixing equal volumes of BLyS, responder cells, and putative antagonist each of which is prepared as a 3 \times stock reagent.

[0631] B-lymphocytes are purified from human tonsil by MACS (anti-CD3 depletion), washed, and resuspended in complete medium (CM) (RPMI 1640 with 10% FBS containing 100 U/ml penicillin, 100 μ g/ml streptomycin, 4 mM glutamine, 5×10^{-5} M beta-mercaptoethanol) at a concentration of 3×10^6 cells/mL. *Staphylococcus aureus*, Cowan I (SAC, CalBiochem) is added to cells at 3 \times concentration (3 \times =1:33,333 dilution of stock).

[0632] Meanwhile, eight serial dilutions (3-fold) of potential antagonists are prepared in CM such that the diluted antagonists are at 3 \times the final concentrations to be tested in the assay. BLyS binding polypeptides are routinely tested starting at a final concentration of 10 μ g/mL and going down to about 1.5 ng/mL.

[0633] Human rBLyS was prepared in CM to 3 \times concentration (3 \times =300 ng/mL, 30 ng/mL, and 3 ng/mL) in CM. Potential inhibitors are routinely tested at several concentrations of BLyS to avoid false negatives due to unexpectedly low affinity or antagonist concentration.

[0634] Fifty microliters of diluted antagonist and 50 μ l of diluted BLyS are added to the putative antagonist dilution series. Cells are then incubated for 72 hours (37 $^\circ$ C., 5%

CO₂) in a fully humidified chamber. After 72 hrs., the cells are supplemented with 0.5 μ Ci/well 3H-thymidine (e.g., 6.7 Ci/mmol) and incubated for an additional 24 hours. Plates are harvested using a Tomtec Cell Harvester and filters counted in a TopCount Scintillation counter (Packard).

Example 7

Protein Fusions of BLyS Binding Polypeptides

[0635] BLyS binding polypeptides of the invention are optionally fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of BLyS binding polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See, EP A 394 827; Traunecker et al., *Nature*, 331:84-86 (1988)). Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to BLyS binding polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made using techniques known in the art or by using or routinely modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

[0636] Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below (SEQ ID NO:447). These primers also preferably contain convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

[0637] For example, if the pC4 (Accession No. 209646) expression vector is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and BLyS binding polynucleotide is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

[0638] If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

[0639] Human IgG Fc Region:

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GGGATCCGGAGCCCAAACTTCTGACAAAACCTCACACATGCCACCCTGCCAGCACCTGAATTCGAGGGTGCACCG (SEQ ID NO:449)
TCAGTCTTCCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGT
GGACGTAAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAA
AGCCGCGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAAGGTCCTCACCGTCTGCACCCAGGACTGGCTGAAT
GGCAAGGAGTACAAGTGAAGGTCTCCAACAAGCCCTCCCAACCCCATCGAGAAAACCATCTCCAAGCCAAAGG
GCAGCCCGAGAACCAAGTGTACACCCCTGCCCCATCCCGGGATGAGTGCACCAAGAACCAAGGTCAGCCCTGACCT
GCCTGGTCAAAGGCTTCTATCCAAGGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACCAACTACAAG
ACCACGCTCCCGTGGTGGACTCCGACGGCTCCTTCTTCCTTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
GCAGGGGAACGTCTTCTCATGCTCCGTGATGATGAGGCTCTGCACAACCACTACACGAGAGAGCCTCTCCCTGT
CTCCGGTAAATGAGTCCGACGGCCGACTCTAGAGGAT
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Example 8

Isolation of scFV Molecules Recognizing BLyS Binding Polypeptides

[0640] Naturally occurring V-genes isolated from human PBLs are constructed into a large library of antibody fragments which contain reactivities against polypeptides of the present invention to which the donor may or may not have been exposed (see, e.g., U.S. Pat. No. 5,885,793, incorporated herein by reference in its entirety).

Rescue of the Library

[0641] A library of scFvs is constructed from the RNA of human PBLs as described in WO 92/01047. To rescue phage displaying antibody fragments, approximately 10⁹ *E. coli* harbouring the phagemid are used to inoculate 50 ml of 2 \times TY containing 1% glucose and 100 μ g/ml of ampicillin (2 \times TY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2 \times TY-AMP-GLU, 2 \times 10⁸ TU of Δ gene 3 helper phage (M13 Δ gene III, see WO 92/01047) are added and the culture incubated at 37° C. for 45 minutes without shaking and then at 37° C. for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 minutes and the pellet resuspended in 2 liters of 2 \times TY containing 100 μ g/ml ampicillin and 50 μ g/ml kanamycin and grown overnight. Phage are prepared as described in WO92/01047.

[0642] M13 Δ gene III is prepared as follows: M13 Δ gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 Δ gene III particles are made by growing the helper phage in cells harboring a pUC 19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C. without shaking and then for a further hour at 37° C. with shaking. Cells are pelleted (IEC-Centra 8, 4000 revs/min. for 10 min.), resuspended in 300 ml 2 \times TY broth containing 100 μ g ampicillin/ml and 25 μ g kanamycin/ml (2 \times TY-AMP-KAN) and grown overnight, shaking at 37° C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 μ m filter (Minisart NML; Sartorius) to give a final concentration of approximately 1013 transducing units/ml (ampicillin-resistant clones).

Panning of the Library

[0643] Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 mg/ml or 10 mg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-

PBS for 2 hours at 37° C. and then washed 3 times in PBS. Approximately 1013 TU of phage are applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log *E. coli* TG1 by incubating eluted phage with bacteria for 30 minutes at 37° C. The *E. coli* are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with Δ gene III helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders

[0644] Eluted phage from the 3rd and 4th rounds of selection are used to infect *E. coli* HB 2151 and soluble scFv is produced (Marks et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate, pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., WO 92/01047) and then by sequencing.

[0645] Additionally, scFvs may be converted to complete Ig molecules using techniques which are commonly known in the art.

Example 9

Production of an Anti-BLyS Binding Polypeptide Antibody

[0646] a) Hybridoma Technology

[0647] The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing BLyS binding polypeptides are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of BLyS binding polypeptide is prepared and purified to render it substantially free of natural contaminants which is then conjugated to a carrier molecule such as keyhole limpet hemocyanin (KLH), succinylated KLH, or chicken gamma globulin (CGG). Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

[0648] In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or BLyS protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Kohler et al., *Nature*, 256:495 (1975); Kohler et al., *Eur. J. Immunol.*, 6:511 (1976); Kohler et al., *Eur. J. Immunol.*, 6:292 (1976); Hammerling et al., in *Monoclonal Antibodies and T-Cell Hybridomas* (Elsevier, N.Y. 1981), pp. 563-681.) In general, such procedures involve immunizing an animal (preferably a mouse) with BLyS binding polypeptide or, more preferably, with a secreted BLyS binding polypeptide-expressing

cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56° C.), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

[0649] The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2/0), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology*, 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the BLyS binding polypeptide.

[0650] Alternatively, additional antibodies capable of binding to BLyS binding polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the BLyS binding polypeptide-specific antibody can be blocked by BLyS binding polypeptide. Such antibodies comprise anti-idiotypic antibodies to the BLyS binding protein-specific antibody and can be used to immunize an animal to induce formation of further BLyS binding polypeptide-specific antibodies.

[0651] It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). Alternatively, secreted BLyS binding protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

[0652] For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, *Science*, 229:1202 (1985); Oi et al., *BioTechniques*, 4:214 (1986); Cabilly et al., U.S. Pat. No. 4,816,567; Taniguchi et al., EP 171 496; Morrison et al., EP 173 494; Neuberger et al., WO 86/01533; Robinson et al., WO 87/02671; Boulianne et al., *Nature*, 312:643 (1984); Neuberger et al., *Nature*, 314:268 (1985).)

Example 10

BLyS-Induced Signalling in B Cells

[0653] Total RNA was prepared from tonsillar B cells unstimulated or stimulated with SAC or SAC plus BLyS

(100 ng/mL) for 12 hours. Messenger RNA levels of ERK-1 and PLK was determined by real time quantitative PCR using ABI 7700 Taqman sequence detector. Amplification primers and probes were designed to span the region from nucleotides 252-332 of the human PLK sequence and nucleotides 373 to 446 of the human ERK-1 mRNA (GenBank accession numbers X75932 and X60188, respectively). For quantitation of RNA, the comparative delta CT method was used (Perkin-Elmer user Bulletin #2 and #4, 1997) using an 18S ribosomal RNA probe as endogenous reference. Expression levels were characterized relative to observed levels in unstimulated B-cells.

Example 11

Affinity Maturation of BLYS Binding Polypeptides

[0654] In order to identify high affinity BLYS-binding polypeptides, a BLYS Affinity Maturation Library (BAML) was designed around a 14-mer linear peptide template sequence having fixed amino acid residues at 5 of the 14 positions. 3 of the 5 fixed residues corresponded to a highly conserved DxLT tetrapeptide amino acid motif (SEQ ID NO:446) isolated from both the constrained and linear peptide libraries. The design of the 14-mer allowed for some amino acid variation at each of the remaining 9 positions, however, preference was given for a particular amino acid at each of these positions. Analysis of binding affinity of the newly isolated peptides for BLYS was evaluated by direct and indirect phage ELISA and fluorescence anisotropy.

[0655] BAML was designed on a 14-mer linear (non-constrained) template peptide sequence having fixed residues at positions 1 (Ala), 5 (Asp), 7 (Leu), 8 (Thr), and 10 (Leu). The amino acid sequence of positions 3-14 in the BAML template most closely resembles a binding polypeptide isolated from the PhD 12 linear polypeptide library (see Table 7, supra). Residues at position 1 (fixed Ala) and position 2 (variable) were included to extend the length and presentation of the BLYS-binding sequence. Positions 5-8 correspond to the DxLT motif found in peptide isolates from both the constrained and linear peptide libraries (see Tables 1-8, supra). Since hydrophobic amino acids (L, M, I, A, and G) were found at position 10 in 85% of the original isolates, a Leu residue, occurring in 42% of the isolates, was fixed at that position in the BAML template peptide.

TABLE 10

BAML template sequence (14-mer)														SEQ ID
amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	NO:
A	n	w	y	D	s	L	T	k	L	w	l	p	d	184

[0656] Referring to Table 10, the upper case letters indicate the fixed residues at positions 1, 5, 7, 8, and 10 of the template. Lower case letters designate preferred amino acids at those positions, however the design of the variegated DNA template encoding the 14-mer allows for some sequence variation at these positions.

[0657] Table 11 shows the design of the variegated DNA template used to generate the BAML peptides.

TABLE 11

BAML DNA template sequence (14-mer)													
codon position													
1	2	3	4	5	6	7	8	9	10	11	12	13	14
codons*													
GCT	eez	zjj	zez	GAT	zqz	CTT	ACT	eej	CTC	zjj	qzz	qqz	jez

*The sequence of codons is SEQ ID NO:185.

[0658] Referring to Table 11, the nucleotide coding sequences for the fixed amino acids in the BAML 14-mer template are shown in upper case letters. The letters “e”, “j”, “q”, and “z” in the variegated DNA template each represent a particular mixture of nucleoside bases present in the input dNTPs for each position:

[0659] j=79% guanine, 7% cytosine, 7% adenine, 7% thymine

[0660] q=7% guanine, 79% cytosine, 7% adenine, 7% thymine

[0661] e=7% guanine, 7% cytosine, 79% adenine, 7% thymine

[0662] z=7% guanine, 7% cytosine, 7% adenine, 79% thymine.

The codons of the DNA template were designed to skew the encoded variable amino acid toward the preferred amino acid at each position shown in SEQ ID NO:184 (Table 10, lower case). Later sequencing of phage isolates showed that, at any particular position, preferred residues occurred at a frequency of from 44% to 70%.

[0663] Synthetic DNA sequences fitting the DNA template were amplified by large scale PCR. The amplified DNAs were restriction digested for insertion into a M13 phage expression vector (MANP vector, Dyax Corp., Cambridge, Mass.), and vectors bearing the inserts were used to transform M13 phage by electroporation, to produce the BAML.

Recombinant phage were collected and purified by PEG precipitation and titered. A total of 3.2x10¹³ PFU were amplified in BAML from 1.6x10⁹ transformants.

Screening BAML

[0664] As outlined in Table 12 below, a two-step competition method, starting with the original BAML library, was used over 4 rounds of screening to isolate the highest affinity BLYS-binding polypeptides from the BAML. Prior to screening, the amplified BAML was contacted with Seradyn streptavidin-coated magnetic beads (MG-SA, Seradyn, Indianapolis, Ind.), to remove bead- and streptavidin-binding phage.

[0665] For screening BAML, phage were incubated in solution with biotinylated BLYS (b-BLYS) in 200 µl PBS, pH 7.4, Tween-20 (0.1%), to form phage/b-BLYS binding complexes. For the first competition step, unlabeled BLYS (1-2 µM) was added to the phage/b-BLYS binding complex mixture in solution and incubated for 1-20 hrs. (See Table 12.) The phage/b-BLYS complexes remaining in solution

after incubation with unlabeled BLyS were captured by brief (10 min. on rotator) incubation with MG-SA streptavidin beads (50 μ l). After capture of the phage/b-BLyS complexes on streptavidin beads, the unbound fraction was removed and beads were washed 15-20 times with 1 ml PBS-Tween 20 prior to the second competition step. The phage/unlabeled BLyS complexes from the round 1 competition step only, were collected and used as a fraction of the input phage for the second round of screening along with the bead-captured phage/b-BLyS complexes, however, in each subsequent round of screening only the bead-associated phage were collected after the first competition step for further screening, and the phage/unlabeled BLyS complexes were discarded.

[0666] For the second competition step, the competitor peptide was a polypeptide (DX221; SEQ ID NO:168) based on a BLyS-binding polypeptide isolated from the PhD 12 library in the initial screenings described above. The phage/b-BLyS complexes bound to streptavidin, collected after the first competition incubation step, were serially diluted with 50 μ M DX221 BLyS-binding peptide ($K_D=3 \mu$ M) in 300 μ l PBS-Tween-20 (0.1%). A series of short incubations (3-4 per round, for 1 hour) of the phage/b-BLyS complexes with DX221 followed by a final incubation of from overnight (O/N, for rounds 1, 2, and 4) to 3 days (for round 3). (See Table 12.) The second competition step in round 4 included an incubation with 67 nM BLyS for 1 hour prior to incubation with DX221. The streptavidin bead-associated phage/b-BLyS binding complexes remaining after the DX221 competition step in round 4 were collected for further analysis.

dilutions of b-BLyS (0-0.1 μ g/ml in PBS) were immobilized in the streptavidin-coated wells (1 hr, 37° C.). After washing, 1-25 μ l of overnight culture of *E. coli* infected with the individual phage plaques were added to the appropriate wells and incubated for 1 hour, followed by 10 washes with PBS-Tween-20. Anti-M13 antibody conjugated to horseradish peroxidase (1:10,000 in PBS-Tween-20) was added to the wells (30 min., room temperature), the color reagent TMB was used and the plates read at OD 630 nm.

[0669] Individual phage isolates binding to immobilized BLyS were sequenced and the sequences analyzed. The unique sequences of the BAML BLyS-binding 14-mer display peptides are shown in Table 13.

[0670] Analysis of the peptides reveals a significant sequence "collapse" around one motif: W₃YDPLTKLWL_{1,2} (SEQ ID NO:436) (subscripts indicate amino acid position in the 14-mer display peptide sequence). This most numerous core motif includes the four fixed residues from the original BAML template, i.e., Asp (D) at position 5, Leu (L) at position 7, Thr (T) at position 8, and Leu (L) at position 10. In addition, 5 of the 6 preferred residues from the original BAML template sequence were included in this motif (see Table 10).

[0671] 73% (143 of 197) of the round 4 isolates included this core motif (SEQ ID NO:436). Single residue substitutions within the 10-mer core motif centered on positions 4 (Y→F) and 12 (L→F, I, or V), with the substitutions at position 12 being alternative hydrophobic residues for Leu.

[0672] For the three remaining variable positions (i.e., 2, 13, and 14), selection was not as stringent, although some

TABLE 12

BLyS affinity maturation library (BAML) screening conditions						
Screening Round	Input phage ¹	b-BLyS ²	First Competition Incubation Time (hrs)	Competitor (BLyS)	Second Competition Incubation Time (hrs)	Peptide Elutions
1	1.5 × 10 ¹¹	100 nM	2	2 μ M	1	50 μ M DX221, 4 × 1 hr, then O/N
2	2 × 10 ¹⁰	100 nM	1	1 μ M	20	50 μ M DX221, 3 × 1 hr, then O/N
3	6.5 × 10 ¹⁰	100 pM	16	1 μ M	3	50 μ M DX221, 4 × 1 hr, then 3 days
4	6.0 × 10 ¹⁰	10 pM	16	1 μ M	2	67 nM BLyS, 1 hr; 50 μ M DX221 + 67 nM BLyS 3 × 1 hr, O/N, then add ¹ 4 hrs

¹Input phage for round 1 was original BAML; for round 2 was amplified output phage from overnight (final) peptide elution and bead-associated phage from round 1; for round 3 was amplified bead-associated output phage from round 2; and for round 4 was amplified bead-associated output phage from round 3. All amplified phage samples were pre-cleared on streptavidin beads before incubation with biotin-BLyS in solution.

²b-BLyS = biotinylated BLyS

ELISA Analysis

[0667] Approximately four hundred BAML isolates from rounds 2, 3 and 4 of the above screening were analyzed by direct and indirect phage ELISA assays.

[0668] For indirect phage ELISA, Immulon-2HB plates (Dyex Technologies, Inc., Chantilly, Va.) were coated with 100 μ l of 1 μ g/ml Immunopure streptavidin (Pierce, Rockford, Ill.) diluted in PBS. 100 μ l of a series of 10-fold

preferences were apparent, being either built into the library or persisting through rounds of selection. For example, in round 4 isolates, 51% included Asn at position 2; 77% included Pro at position 13; and 32% included Asp at position 14. The presence of Val (27%) or Glu (19%) at position 14 was among the most highly selected in the round 4 isolates, in comparison to their theoretical proportion (4% each) at position 14 in BAML.

[0673] The sequences in Table 13 are grouped according to their degree of difference from the core sequence (SEQ ID NO:436).

TABLE 13

Sequences of BAML Phage Isolates (from Rounds 2, 3, 4)														
14-mer amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEQ ID NO:
An	w	y	D	s	L	T	k	L	w	l	p	d	consensus;	184
AN	W	Y	D	P	L	T	K	L	W	L	P	D		186
AN	W	Y	D	P	L	T	K	L	W	L	P	E		187
AN	W	Y	D	P	L	T	K	L	W	L	P	G		188
AN	W	Y	D	P	L	T	K	L	W	L	P	V		189
AN	W	Y	D	P	L	T	K	L	W	L	P	D		190
AN	W	Y	D	P	L	T	K	L	W	L	P	D		191
AN	W	Y	D	P	L	T	K	L	W	L	P	T		192
AN	W	Y	D	P	L	T	K	L	W	L	P	A		193
AN	W	Y	D	P	L	T	K	L	W	L	P	N		194
AN	W	Y	D	P	L	T	K	L	W	L	V	D		195
AN	W	Y	D	P	L	T	K	L	W	L	H	D		196
AN	W	Y	D	P	L	T	K	L	W	L	T	D		197
AN	W	Y	D	P	L	T	K	L	W	L	P	H		198
AN	W	Y	D	P	L	T	K	L	W	L	T	V		199
AN	W	Y	D	P	L	T	K	L	W	L	L	D		200
AN	W	Y	D	P	L	T	K	L	W	L	L	E		201
AN	W	Y	D	P	L	T	K	L	W	L	H	E		202
AN	W	Y	D	P	L	T	K	L	W	L	P	R		203
AN	W	Y	D	P	L	T	K	L	W	L	A	D		204
AN	W	Y	D	P	L	T	K	L	W	L	P	Y		205
AN	W	Y	D	P	L	T	K	L	W	L	P	I		206
AN	W	Y	D	P	L	T	K	L	W	L	I	D		207
AN	W	Y	D	P	L	T	K	L	W	L	R	D		208
A	Y	W	Y	D	P	L	T	K	L	W	L	P	D	209
A	Y	W	Y	D	P	L	T	K	L	W	L	L	E	210
A	Y	W	Y	D	P	L	T	K	L	W	L	R	V	211
A	Y	W	Y	D	P	L	T	K	L	W	L	P	E	212
A	Y	W	Y	D	P	L	T	K	L	W	L	P	V	213
A	Y	W	Y	D	P	L	T	K	L	W	L	H	Q	214
A	Y	W	Y	D	P	L	T	K	L	W	L	P	A	215
A	Y	W	Y	D	P	L	T	K	L	W	L	R	V	216
A	Y	W	Y	D	P	L	T	K	L	W	L	P	G	217

TABLE 13-continued

Sequences of BAML Phage Isolates (from Rounds 2, 3, 4)														
14-mer amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEQ ID NO:
A	Y	W	Y	D	P	L	T	K	L	W	L	R	Y	218
A	Y	W	Y	D	P	L	T	K	L	W	L	P	Y	219
A	Y	W	Y	D	P	L	T	K	L	W	L	L	Y	220
A	Y	W	Y	D	P	L	T	K	L	W	L	R	D	221
A	Y	W	Y	D	P	L	T	K	L	W	L	P	V	222
A	Y	W	Y	D	P	L	T	K	L	W	L	L	G	223
A	Y	W	Y	D	P	L	T	K	L	W	L	T	H	224
A	Y	W	Y	D	P	L	T	K	L	W	L	P	T	225
A	Y	W	Y	D	P	L	T	K	L	W	L	L	V	226
A	Y	W	Y	D	P	L	T	K	L	W	L	Y	Y	227
A	Y	W	Y	D	P	L	T	K	L	W	L	S	D	228
A	S	W	Y	D	P	L	T	K	L	W	L	P	A	229
A	S	W	Y	D	P	L	T	K	L	W	L	H	D	230
A	S	W	Y	D	P	L	T	K	L	W	L	P	G	231
A	S	W	Y	D	P	L	T	K	L	W	L	P	Q	232
A	S	W	Y	D	P	L	T	K	L	W	L	P	Y	233
A	S	W	Y	D	P	L	T	K	L	W	L	P	H	234
A	S	W	Y	D	P	L	T	K	L	W	L	P	V	235
A	S	W	Y	D	P	L	T	K	L	W	L	P	I	236
A	S	W	Y	D	P	L	T	K	L	W	L	P	E	237
A	F	W	Y	D	P	L	T	K	L	W	L	R	V	238
A	F	W	Y	D	P	L	T	K	L	W	L	P	E	239
A	F	W	Y	D	P	L	T	K	L	W	L	L	E	240
A	F	W	Y	D	P	L	T	K	L	W	L	P	V	241
A	I	W	Y	D	P	L	T	K	L	W	L	P	E	242
A	I	W	Y	D	P	L	T	K	L	W	L	P	D	243
A	I	W	Y	D	P	L	T	K	L	W	L	H	D	244
A	I	W	Y	D	P	L	T	K	L	W	L	T	D	245
A	I	W	Y	D	P	L	T	K	L	W	L	P	F	246
A	I	W	Y	D	P	L	T	K	L	W	L	L	D	247
A	I	W	Y	D	P	L	T	K	L	W	L	P	R	248
A	I	W	Y	D	P	L	T	K	L	W	L	P	A	249
A	I	W	Y	D	P	L	T	K	L	W	L	T	A	250
A	I	W	Y	D	P	L	T	K	L	W	L	A	V	251

TABLE 13-continued

Sequences of BAML Phage Isolates (from Rounds 2, 3, 4)														
14-mer amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEQ ID NO:
A	I	W	Y	D	P	L	T	K	L	W	L	P	G	252
A	I	W	Y	D	P	L	T	K	L	W	L	R	V	253
A	I	W	Y	D	P	L	T	K	L	W	L	P	H	254
A	I	W	Y	D	P	L	T	K	L	W	L	R	E	255
A	I	W	Y	D	P	L	T	K	L	W	L	S	D	256
A	T	W	Y	D	P	L	T	K	L	W	L	S	D	257
A	T	W	Y	D	P	L	T	K	L	W	L	P	A	258
A	T	W	Y	D	P	L	T	K	L	W	L	A	D	259
A	T	W	Y	D	P	L	T	K	L	W	L	T	S	260
A	T	W	Y	D	P	L	T	K	L	W	L	P	G	261
A	T	W	Y	D	P	L	T	K	L	W	L	P	Y	262
A	T	W	Y	D	P	L	T	K	L	W	L	S	G	263
A	T	W	Y	D	P	L	T	K	L	W	L	P	V	264
A	D	W	Y	D	P	L	T	K	L	W	L	P	V	265
A	D	W	Y	D	P	L	T	K	L	W	L	P	K	266
A	D	W	Y	D	P	L	T	K	L	W	L	P	D	267
A	D	W	Y	D	P	L	T	K	L	W	L	P	E	268
A	D	W	Y	D	P	L	T	K	L	W	L	H	Q	269
A	E	W	Y	D	P	L	T	K	L	W	L	R	Q	270
A	E	W	Y	D	P	L	T	K	L	W	L	P	D	271
A	E	W	Y	D	P	L	T	K	L	W	L	P	Y	272
A	L	W	Y	D	P	L	T	K	L	W	L	P	A	273
A	L	W	Y	D	P	L	T	K	L	W	L	P	D	274
A	L	W	Y	D	P	L	T	K	L	W	L	R	G	275
A	L	W	Y	D	P	L	T	K	L	W	L	L	G	276
A	M	W	Y	D	P	L	T	K	L	W	L	P	A	277
A	M	W	Y	D	P	L	T	K	L	W	L	Q	V	278
A	M	W	Y	D	P	L	T	K	L	W	L	L	G	279
A	A	W	Y	D	P	L	T	K	L	W	L	P	D	280
A	A	W	Y	D	P	L	T	K	L	W	L	A	D	281
A	A	W	Y	D	P	L	T	K	L	W	L	L	D	282
A	H	W	Y	D	P	L	T	K	L	W	L	T	D	283
A	H	W	Y	D	P	L	T	K	L	W	L	P	V	284
A	H	W	Y	D	P	L	T	K	L	W	L	H	D	285
A	H	W	Y	D	P	L	T	K	L	W	L	P	D	286

TABLE 13-continued

Sequences of BAML Phage Isolates (from Rounds 2, 3, 4)														
14-mer amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEQ ID NO:
A	P	W	Y	D	P	L	T	K	L	W	L	H	D	287
A	P	W	Y	D	P	L	T	K	L	W	L	P	V	288
A	Q	W	Y	D	P	L	T	K	L	W	L	P	Y	289
A	Q	W	Y	D	P	L	T	K	L	W	L	P	R	290
A	Q	W	Y	D	P	L	T	K	L	W	L	P	D	291
A	K	W	Y	D	P	L	T	K	L	W	L	P	V	292
A	K	W	Y	D	P	L	T	K	L	W	L	P	V	293
A	K	W	Y	D	P	L	T	K	L	W	L	N	G	294
A	K	W	Y	D	P	L	T	K	L	W	L	P	A	295
A	W	W	Y	D	P	L	T	K	L	W	L	T	D	296
A	V	W	Y	D	P	L	T	K	L	W	L	P	A	297
A	Y	E	Y	D	P	L	T	K	L	W	L	L	Y	298
A	T	K	Y	D	P	L	T	K	L	W	L	P	D	299
A	T	L	Y	D	P	L	T	K	L	W	L	P	G	300
A	I	R	Y	D	P	L	T	K	L	W	L	P	Y	301
A	E	R	Y	D	P	L	T	K	L	W	L	P	H	302
A	D	R	Y	D	P	L	T	K	L	W	L	P	Q	303
A	N	S	Y	D	P	L	T	K	L	W	L	P	E	304
A	I	L	Y	D	P	L	T	K	L	W	L	P	D	305
A	N	W	F	D	P	L	T	K	L	W	L	P	Q	306
A	N	W	F	D	P	L	T	K	L	W	L	P	V	307
A	N	W	F	D	P	L	T	K	L	W	L	T	D	308
A	N	W	F	D	P	L	T	K	L	W	L	P	D	309
A	N	W	F	D	P	L	T	K	L	W	L	P	G	310
A	N	W	F	D	P	L	T	K	L	W	L	P	E	311
A	N	W	F	D	P	L	T	K	L	W	L	P	A	312
A	N	W	F	D	P	L	T	K	L	W	L	P	N	313
A	N	W	F	D	P	L	T	K	L	W	L	S	E	314
A	N	W	F	D	P	L	T	K	L	W	L	H	D	315
A	N	W	F	D	P	L	T	K	L	W	L	V	D	316
A	Y	W	F	D	P	L	T	K	L	W	L	P	D	317
A	Y	W	F	D	P	L	T	K	L	W	L	P	V	318
A	Y	W	F	D	P	L	T	K	L	W	L	P	A	319
A	Q	W	F	D	P	L	T	K	L	W	L	P	D	320

TABLE 13-continued

Sequences of BAML Phage Isolates (from Rounds 2, 3, 4)														
14-mer amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEQ ID NO:
AHW	FDP	LTK	L	W	L	P	D							321
ATW	FDP	LTK	L	W	L	P	V							322
AYW	YDS	LTK	L	W	L	P	V							323
AYW	YDS	LTK	L	W	L	H	D							324
ANW	YDS	LTK	L	W	I	P	D							325
ANW	YDS	LTK	L	W	L	P	V							326
ANW	YDS	LTK	L	W	L	P	D							327
ANW	YDS	LTK	L	W	L	A	D							328
ANW	YDS	LTK	L	W	L	P	A							329
ANW	YDS	LTK	L	W	L	Y	E							330
AGW	YDS	LTK	L	W	L	P	D							331
AVW	YDS	LTK	L	W	L	T	D							332
ANW	YDA	LTK	L	W	L	P	V							333
AYW	YDT	LTK	L	W	L	P	N							334
AFW	YDP	LTN	L	W	L	L	E							335
AYW	YDP	LTG	L	W	L	L	V							336
AYW	YDP	LTG	L	W	L	L	Y							337
AYW	YDP	LTG	L	W	L	R	V							338
AYW	YDP	LTE	L	W	L	R	L							339
AMW	YDP	LTK	L	S	L	P	D							340
AYW	YDP	LTK	L	S	L	L	V							341
AIW	YDP	LTK	L	S	L	T	V							342
AIW	YDP	LTK	L	S	L	L	V							343
ADW	YDP	LTK	L	S	L	L	L							344
AYW	YDP	LTK	L	R	L	L	E							345
ADW	YDP	LTK	L	R	L	L	V							346
ADW	YDP	LTK	L	R	L	I	V							347
AIW	YDP	LTK	L	Y	L	P	D							348
AIW	YDP	LTK	L	G	L	L	V							349
ANW	YDP	LTK	L	T	L	L	V							350
ANW	YDP	LTK	L	L	L	P	N							351
ASW	YDP	LTK	L	W	F	P	D							352
ANW	YDP	LTK	L	W	F	P	D							353
ANW	YDP	LTK	L	W	F	S	D							354
ASW	YDP	LTK	L	W	F	P	V							355

TABLE 13-continued

Sequences of BAML Phage Isolates (from Rounds 2, 3, 4)														
14-mer amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEQ ID NO:
ADW	YDP	LTK	L	W	F	P	V							356
ASW	YDP	LTK	L	W	F	P	K							357
AKW	YDP	LTK	L	W	F	P	D							358
ASW	YDP	LTK	L	W	F	L	E							359
ANW	YDP	LTK	L	W	F	P	A							360
ATW	YDP	LTK	L	W	F	P	D							361
AIW	YDP	LTK	L	W	F	P	E							362
AIW	YDP	LTK	L	W	F	P	D							363
AIW	YDP	LTK	L	W	F	P	G							364
AYW	YDP	LTK	L	W	F	P	H							365
ANW	YDP	LTK	L	W	F	P	V							366
AYW	YDP	LTK	L	W	F	P	D							367
AGW	YDP	LTK	L	W	F	P	D							368
AIW	YDP	LTK	L	W	F	P	T							369
AKW	YDP	LTK	L	W	F	P	A							370
AYW	YDP	LTK	L	W	F	F	D							371
ANW	YDP	LTK	L	W	F	A	D							372
ANW	YDP	LTK	L	W	F	P	Y							373
ADW	YDP	LTK	L	W	F	R	D							374
ANW	YDP	LTK	L	W	V	P	D							375
ADW	YDP	LTK	L	W	V	P	A							376
ANW	YDP	LTK	L	W	V	P	N							377
ANW	YDP	LTK	L	W	V	P	E							378
ANW	YDP	LTK	L	W	V	P	Q							379
AEW	YDP	LTK	L	W	V	P	K							380
AQW	YDP	LTK	L	W	V	P	V							381
ANW	YDP	LTK	L	W	V	P	Y							382
ALW	YDP	LTK	L	W	V	P	Y							383
ANW	YDP	LTK	L	W	V	P	G							384
ASW	YDP	LTK	L	W	I	P	Y							385
ADW	YDP	LTK	L	W	I	P	G							386
ANW	YDP	LTK	L	W	I	P	Y							387
AKW	YDP	LTK	L	W	I	P	Y							388
AIW	YDP	LTK	L	W	I	P	N							389

TABLE 13-continued

Sequences of BAML Phage Isolates (from Rounds 2, 3, 4)														
14-mer amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEQ ID NO:
A	T	W	Y	D	P	L	T	K	L	W	I	P	Q	390
A	S	W	Y	D	P	L	T	N	L	W	V	P	D	391
A	Y	E	Y	D	P	L	T	N	L	W	L	L	Y	392
A	Y	W	Y	D	P	L	T	N	L	S	L	L	V	393
A	Y	W	Y	D	P	L	T	K	L	S	I	L	E	394
A	N	W	Y	D	S	L	T	K	L	W	I	P	Y	395
A	H	W	F	D	P	L	T	Q	L	K	I	R	V	396
A	Y	W	C	D	P	L	T	K	L	C	I	L	E	397
A	N	S	Y	D	P	L	T	K	L	W	F	P	Y	398
A	N	L	Y	D	P	L	T	K	L	W	V	P	Y	399
A	N	W	Y	D	A	L	T	K	L	W	L	H	D	400
A	N	W	Y	D	S	L	T	K	L	W	F	P	D	401
A	T	S	Y	D	S	L	T	K	L	W	L	P	A	402
A	C	W	Y	D	S	L	T	K	L	C	H	R	E	403
A	I	G	N	D	P	L	T	K	L	W	I	P	Y	404
A	N	W	Q	D	C	L	T	K	L	C	L	A	G	405
A	Y	W	F	D	P	L	T	N	L	W	L	L	E	406
A	Y	W	Y	D	P	L	T	N	L	S	L	L	V	407
A	N	C	F	D	S	L	T	R	L	W	L	C	D	408
A	C	A	Y	D	A	L	T	K	L	C	L	P	A	409
A	N	W	Y	D	P	L	T	N	L	S	L	L	L	410
A	Y	W	Y	D	P	L	T	Q	L	S	L	L	V	411
A	Y	R	Y	D	A	L	T	G	L	W	L	L	Y	412
A	Y	W	N	D	P	L	T	K	L	K	L	R	L	413
A	Y	W	Y	D	P	L	T	Q	L	S	L	L	V	414
A	Y	R	Y	D	A	L	T	G	L	W	L	L	Y	415
A	Y	R	Y	D	S	L	T	N	L	W	L	L	Y	416
A	Y	W	Y	D	P	L	T	K	L	S	I	L	E	417
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A	F	W	F	D	P	L	T	G	L	W	L	L	E	419
A	H	W	Y	D	P	L	T	K	L	S	I	R	V	420
A	P	W	Y	D	S	L	T	K	L	W	F	P	S	421
A	N	C	Y	D	T	L	T	K	L	W	L	T	C	422
A	N	W	Y	D	S	L	T	K	L	S	L	P	D	423
A	Y	A	Y	D	F	L	T	Q	L	S	L	P	D	424

TABLE 13-continued

Sequences of BAML Phage Isolates (from Rounds 2, 3, 4)														
14-mer amino acid position														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEQ ID NO:
A	F	R	Y	D	S	L	T	G	L	W	L	R	Y	425
A	N	C	Y	D	S	L	T	K	L	W	L	P	C	426
A	N	G	Y	D	L	L	T	N	L	S	V	S	D	427
A	N	W	Y	D	P	L	T	R	L	W	I	P	V	428
A	L	K	F	D	Y	L	T	K	L	W	L	P	D	429
A	Y	R	Y	D	S	L	T	K	L	W	L	P	G	430
A	Y	C	Y	D	S	L	T	K	L	W	I	P	D	431
A	S	W	E	D	S	L	T	K	L	W	L	S	K	432
A	Y	W	Y	D	S	L	T	G	L	S	L	L	V	433
A	Y	W	Y	D	P	L	T	Y	L	R	L	R	V	434
A	K	C	Y	D	S	L	T	N	L	W	L	C	D	435

[0674] Nearly all of the ELISA signals of the BAML isolates were higher than those isolated in the initial screen (see Example 1). For comparison, peptide 453-01-B07 (SEQ ID NO:31) ($K_D=700$ nM) was used as a reference (positive control). Negative control MAEX (M13 phage with no insert) did not bind b-BLyS at any concentration tested.

[0675] For direct phage ELISA, the signal measured is a reflection of the ability of a set number of phage to bind to various concentrations of b-BLyS. Peptides tested by the direct phage ELISA assay were chosen based on high affinity for BLyS as determined in the indirect phage ELISA assay. For this assay, Immulon-2HB plates were coated with 0 or 100 ng anti-Fd antibody (Sigma, St. Louis, Mo.). After washing (PBS-Tween-20), phage dilutions were added to saturate the available antibody and incubated for 1 hour, washed, then incubated with 100 μ l of 10-fold dilutions of b-BLyS (0-1 μ g/ml) for 1 hour at room temperature. Streptavidin-HRP (1:1000 in PBS-tween-20; Endogen, Woburn, Mass.) was added to the wells and incubated for 1 hour, developed using TMB and reading at OD 630 nm.

Determination of BAML Peptide K_D by Fluorescence Anisotropy.

[0676] Several peptides containing the 10-mer core structural motif or single-position variants of that motif identified by sequence analysis were synthesized with a short Gly-Gly-Lys linker sequence and the C-terminal lysine was labeled with fluorescein. These peptides, shown in Table 14, below, were synthesized by solid phase synthesis for determination of dissociation constant with respect to BLyS. The DX815 and DX876 polypeptides were derived from DX814 (SEQ ID NO:186) by deletion of two N-terminal amino acids or the two amino acids N-terminal and C-terminal to the core peptide at (positions 3-12). DX816, DX817, DX819, and DX822 correspond to other BAML isolates (SEQ ID NOs:189, 309, 353, 327, respectively). DX818

corresponds to isolate SEQ ID NO:340, except that Asn has been substituted for Met at position 2. The K_D of several BlyS binding BAML peptides was determined by fluorescence anisotropy, performed as previously described. The sequence of DX822 without the -GGK linker (see SEQ ID NO:327) matches the BAML template sequence (see Table 10). The BAML consensus sequence found in DX822 resulted in a more than 10-fold improvement in binding affinity for BlyS, as compared to one of the highest affinity binders isolated in the initial screen (453-01-B07, SEQ ID NO:31).

TABLE 14

Dissociation Constants of Synthetic BlyS-binding Polypeptides		SEQ ID NO:	K_d (nM)
Peptide Sequence			
DX814	Ac-ANWYDPLTKLWLPDGGK-fitc	437	26 ± 7
DX815	Ac-WYDPLTKLWLPDGGK-fitc	438	31 ± 13
DX876	Ac-WYDPLTKLWLGKK-fitc	439	171 ± 90
DX816	Ac-ANWYDPLTKLWLPVGGK-fitc	440	44 ± 15
DX817	Ac-ANWFDPLTKLWLPDGGK-fitc	441	32 ± 26
DX818	Ac-ANWYDPLTKLSLPDGGK-fitc	442	342 ± 108
DX819	Ac-ANWYDPLTKLWFPDGGK-fitc	443	69 ± 38
DX822	Ac-ANWYDSLTKLWLPDGGK-fitc	444	79 ± 54

[0677] Analysis of the BAML isolates revealed a lack of sequence conservation at position 2 (varied in the BAML template, see Table 10). To examine whether the N-terminal residues at positions 1 and 2 in the BAML sequence were necessary for binding to BlyS, a truncated version of DX814 comprising only residues 3-14 (DX815; see Table 14) was synthesized and analyzed by fluorescence anisotropy. The K_D for DX815 was indistinguishable from that of

DX814, suggesting that residues 1-2 are not required for high affinity binding to BlyS. Further truncation of DX814 to the minimal core (residues 1-10, DX876) increased the K_D to 171 nM, indicating a contribution from Pro at position 13 and/or Asp at position 14 of the 14-mer to high affinity BlyS binding. Substitution of Val in DX816 at that position had little effect on the K_D (see Table 14). In comparing the BlyS-binding polypeptide DX221 (Ac-WTDSLTLGLWFPDGGPGPEGGGK; $K_D=3 \mu\text{M}$; SEQ ID NO:168) with the BAML peptide closest in sequence (DX819, Ac-ANWYDPLTKLWFPDGGK; $K_D=69 \text{ nM}$; SEQ ID NO:443), differences are seen at three positions 4 (T→Y), 6 (S→P), and 9 (G→K), indicating the contribution of these residues in binding affinity.

[0678] The synthesized BAML peptides exhibited K_D values in the low nanomolar range, two orders of magnitude lower than primary isolate-derived peptides (see Example 1). Phenylalanine substitutions ($F_4 \rightarrow Y_4$; $F_{12} \rightarrow L_{12}$; Table 14) were the most common minor variations to the core sequence and these changes failed to significantly affect the dissociation constants of the synthesized peptides. A change at position 11 ($W_{11} \rightarrow S_{11}$; DX818), however, resulted in an approximately 10-fold decrease in affinity compared to DX814.

[0679] Following the foregoing description, the characteristics important for using various affinity binding polypeptides for targeting of BlyS or BlyS-like polypeptides (BlyS target protein) in vitro or in vivo can be appreciated. Additional binding polypeptide uses of the invention and alternative methods adapted to a particular use will be evident from studying the foregoing description. For instance, any spacer or linker sequences associated with BlyS binding polypeptides discussed above may be removed or substituted to yield additional BlyS binding polypeptides for use in the methods of this invention. All such embodiments and obvious alternatives are intended to be within the scope of this invention, as defined by the claims that follow.

[0680] Publications referred to above are hereby incorporated by reference.

SEQUENCE LISTING

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<223> OTHER INFORMATION: X3 is Ala, Asn, Lys, or Pro (preferably Lys)

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<223> OTHER INFORMATION: X is Leu, Pro, or Thr
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<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 is Ala, Asp, Lys, Phe, Thr, Trp or Tyr
    (preferably Asp or Tyr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is Arg, Asp, Gln, Lys, Met, Phe, Pro, Ser,
    Tyr, or Val (preferably Asp or Gln)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(5)

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<223> OTHER INFORMATION: X5 is Asp, Leu, Lys, Phe, Pro, Ser, or Val
(preferably Leu or Ser)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X6 is His, Ile, Leu, Pro, Ser, or Thr
(preferably Leu or Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: X7 is Arg, Gly, His, Leu, Lys, Met, or Thr
(preferably Lys or Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: X8 is Ala, Arg, Asn, Ile, Leu, Lys, Met, or Thr
(preferably Leu or Lys)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X9 is Ala, Asn, Arg, Asp, Glu, Gly, His, Leu,
Met, Ser, Trp, Tyr, or Val (preferably Met or Ser)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: X10 is Ile, Leu, Phe, Ser, Thr, Trp, Tyr, or
Val (preferably Thr or Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: X11 is Ala, Arg, Gly, His, Ile, Leu, Lys, Pro,
Ser, Thr, Trp, Tyr, or Val (preferably Pro or Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: X12 is Arg, Asp, His, Leu, Lys, Met, Phe, Pro,
Ser, Trp, Tyr, or Val (preferably Arg or Pro),

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<400> SEQUENCE: 6

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1           5           10

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<210> SEQ ID NO 7
<211> LENGTH: 13
<212> TYPE: PRP
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: X1 is Asp, Gln, Glu, Gly, His, Lys, Met, or Trp
(preferably Glu, Lys)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 is Arg, Gln, His, Ile, Leu, or Pro
(preferably His or Pro)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 is Asp, Gly, Ile, Lys, Thr, Tyr or Val
(preferably Tyr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is Asn, Asp, Gln, Glu, Met, Pro, Ser, or Tyr
(preferably Asp or Gln)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: X5 is Asn, Asp, His, Ile, Leu, Met, Pro, Thr or
Val (preferably Asn or Thr)
<220> FEATURE:

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<221> NAME/KEY: SITE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X6 is Asp, Glu, His, Leu, Lys, Pro, or Val
      (preferably Asp or Pro)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: X7 is Arg, Asn, Gln, His, Ile, Leu, Met, Pro,
      or Thr (preferably Ile or Pro)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: X8 is Gln, Gly, His, Leu, Met, Ser, or Thr
      (preferably Leu or Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X9 is Asn, Gln, Gly, His, Leu, Lys, Ser, or Thr
      (preferably Lys)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: X10 is Ala, Gly, Ile, Leu, Lys, Met, or Phe
      (preferably Gly or Met)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: X11 is Ala, Glu, His, Ile, Leu, Met, Ser, Thr,
      Trp, Tyr, or Val (preferably Ala or Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: X12 is Arg, Gln, Glu, Gly, His, Ile, Lys, Tyr,
      or Val (preferably Arg or His)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X13 is Arg, Asn, Glu, His, Ile, Ser, Thr, Trp,
      or Val (preferably His),

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<400> SEQUENCE: 7

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1           5           10

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<210> SEQ ID NO 8
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 is Phe, Trp, or Tyr (preferably Tyr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is Pro or Tyr (preferably Pro)

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<400> SEQUENCE: 8

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Cys Xaa Pro Xaa Thr Gly Cys
1           5

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<210> SEQ ID NO 9
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (2)..(2)

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<223> OTHER INFORMATION: X2 is Asp, Ile, Leu, or Tyr (preferably Asp or Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 is Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Pro, Tyr, or Val (preferably Glu or Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is His, Leu, Lys, or Phe (preferably His or Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: X5 is Leu, Pro, or Thr (preferably Thr or Pro)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X6 is Arg, Asn, Gly, His, Ile, Lys, Met, or Trp (preferably Lys)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: X7 is Ala, Asn, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Trp, Tyr, or Val

<400> SEQUENCE: 9

Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys
1 5

<210> SEQ ID NO 10
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 is Asn, Asp, Pro, Ser, or Thr (preferably Asp)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 is Arg, Asp, Ile, Leu, Met, Pro, or Val (preferably Ile)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is Ala, Ile, Leu, Pro, Thr, or Val (preferably Val or Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: X5 is Asn, His, Ile, Leu, Lys, Phe, or Thr (preferably Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X6 is Asn, Glu, Gly, His, Leu, Lys, Met, Pro, or Thr (preferably Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: X7 is Arg, Asn, Asp, Gln, Glu, Gly, Ile, Lys, Met, Pro, Ser, or Trp
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: X8 is Arg, Glu, Gly, Lys, Phe, Ser, Trp, or Tyr (preferably Ser)

<400> SEQUENCE: 10

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Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
1 5

<210> SEQ ID NO 11
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 is Asp, Gln, His, Ile, Leu, Lys, Met, Phe,
or Thr
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 is His, Ile, Leu, Met, Phe, Pro, Trp, or Tyr
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is Asp, His, Leu, or Ser (preferably Asp)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: X5 is Ala, Arg, Asp, Glu, Leu, Phe, Pro, or Thr
(preferably Glu or Pro)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X6 is Ala, Arg, Asn, or Leu (preferably Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: X7 is Ile, Leu, Met, Pro, Ser, or Thr
(preferably Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: X8 is Ala, Arg, Asn, Gly, His, Lys, Ser, or Tyr
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X9 is Ala, Arg, Asn, Gln, Leu, Met, Ser, Trp,
Tyr, or Val
<400> SEQUENCE: 11

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
1 5 10

<210> SEQ ID NO 12
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 is Arg, Asn, Gln, Glu, His, Leu, Phe, Pro,
Trp, Tyr, or Val (preferably Trp, Tyr, or Val)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 is Arg, Asp, Gln, Gly, Ile, Lys, Phe, Thr,
Trp or Tyr (preferably Asp)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is Ala, Arg, Asp, Glu, Gly, Leu, Ser, or Tyr
(preferably Asp)
<220> FEATURE:

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<221> NAME/KEY: SITE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: X5 is Asp, Gln, Glu, Leu, Met, Phe, Pro, Ser,
or Tyr (preferably Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X6 is Asp, Leu, Pro, Thr, or Val (preferably
Leu or Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: X7 is Arg, Gln, His, Ile, Leu, Lys, Met, Phe,
Thr, Trp or Tyr (preferably Lys or Thr)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: X8 is Ala, Arg, Asn, Gln, Glu, His, Leu, Lys,
Met, or Thr (preferably Arg or Leu)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X9 is Ala, Asn, Gln, Gly, Leu, Lys, Phe, Pro,
Thr, Trp, or Tyr (preferably Thr or Trp)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: X10 is Ala, Arg, Gln, His, Lys, Met, Phe, Pro,
Thr, Trp, or Tyr (preferably Met or Phe)
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: X11 is Arg, Gln, Glu, Gly, His, Leu, Met, Phe,
Pro, Ser, Thr, Tyr, or Val (preferably Val)

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<400> SEQUENCE: 12

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Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
1           5           10

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<210> SEQ ID NO 13
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: c-terminal linker

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<400> SEQUENCE: 13

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Pro Gly Pro Glu Gly Gly Gly Lys
1           5

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<210> SEQ ID NO 14
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: phage display library template
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(8)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (10)..(12)
<223> OTHER INFORMATION: X is any amino acid except Cys

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<400> SEQUENCE: 14

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Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa

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1	5	10
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<210> SEQ ID NO 15
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: phage display library template
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(9)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (11)..(13)
<223> OTHER INFORMATION: X is any amino acid except Cys

<400> SEQUENCE: 15

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
1 5 10

<210> SEQ ID NO 16
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: phage display library template
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(14)
<223> OTHER INFORMATION: X is any amino acid except Cys

<400> SEQUENCE: 16

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
1 5 10

<210> SEQ ID NO 17
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: phage display library template
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(11)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(15)
<223> OTHER INFORMATION: X is any amino acid except Cys

<400> SEQUENCE: 17

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
1 5 10 15

<210> SEQ ID NO 18
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: phage display library template

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<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(12)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (14)..(16)
<223> OTHER INFORMATION: X is any amino acid except Cys

<400> SEQUENCE: 18

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
1           5           10           15

<210> SEQ ID NO 19
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: phage display library template
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(14)
<223> OTHER INFORMATION: X is any amino acid except Cys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (16)..(18)
<223> OTHER INFORMATION: X is any amino acid except Cys

<400> SEQUENCE: 19

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa
1           5           10           15

Xaa Xaa

<210> SEQ ID NO 20
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 20

His Leu Arg Cys Trp Ser Thr Asn Cys Arg Tyr Asp
1           5           10

<210> SEQ ID NO 21
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 21

Val Met Asp Cys Leu Ile Asn Arg Cys Asp Thr Val
1           5           10

<210> SEQ ID NO 22
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 22

Lys Ser Lys Cys Phe Phe Pro Trp Glu Cys Gln Gln Ala
1 5 10

<210> SEQ ID NO 23

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 23

Ala Met Lys Cys Tyr Phe Pro Trp Glu Cys Ala Asn Gly
1 5 10

<210> SEQ ID NO 24

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 24

Glu Asn Val Ala Cys Tyr Phe Pro Trp Glu Cys His His Pro
1 5 10

<210> SEQ ID NO 25

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 25

Asn Ala Pro Cys Tyr Phe Pro Trp Glu Cys Phe Ser Ile
1 5 10

<210> SEQ ID NO 26

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 26

Ser Val Asn Cys Trp Phe Pro Trp Glu Cys Val Gly Asn
1 5 10

<210> SEQ ID NO 27

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 27

Lys Glu Pro Cys Tyr Phe Tyr Trp Glu Cys Ala Val Ser
1 5 10

<210> SEQ ID NO 28

<211> LENGTH: 14

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 28

Asp Thr Asn Cys Asp Leu Leu Thr Lys Met Cys Gly Pro Gln
1 5 10

<210> SEQ ID NO 29
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 29

Gly Thr Pro Cys Asp Leu Leu Thr Lys Leu Cys Leu Leu Trp
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 30

Met Ser Glu Cys Asp Leu Leu Thr Lys Ile Cys Leu Met Gly
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 31

Val Pro Phe Cys Asp Leu Leu Thr Lys His Cys Phe Glu Ala
1 5 10

<210> SEQ ID NO 32
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 32

Val Pro Phe Cys Asp Leu Leu Thr Lys His Cys Phe Glu Ala
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 33

Trp Ser Ala Cys Asp Leu Leu Thr Lys Gln Cys Val Gln Val
1 5 10

-continued

<210> SEQ ID NO 34
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 34

Asp Gly Cys Asp Glu Leu Thr Lys Ile Cys Gly Met Lys
1 5 10

<210> SEQ ID NO 35
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 35

Lys Ser Trp Cys Asp Glu Leu Thr Lys Val Cys Phe Asp Pro
1 5 10

<210> SEQ ID NO 36
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 36

Lys Trp Met Cys Asp Glu Leu Thr Lys Gln Cys Gln Tyr Val
1 5 10

<210> SEQ ID NO 37
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 37

Met Lys Tyr Cys Asp Glu Leu Thr Lys Ile Cys Val Gly Trp
1 5 10

<210> SEQ ID NO 38
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 38

Tyr Phe Gln Cys Asp Glu Leu Thr Lys Met Cys Trp Gln Lys
1 5 10

<210> SEQ ID NO 39
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 39

Ala Met His Cys Asp Lys Leu Thr Lys His Cys Lys Phe His
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<210> SEQ ID NO 40
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<400> SEQUENCE: 40

Val Pro Tyr Cys Asp Lys Leu Thr Lys Ile Cys Gln Trp
1 5 10

<210> SEQ ID NO 41
<211> LENGTH: 14
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<400> SEQUENCE: 41

Glu Val Phe Cys Asp Val Leu Thr Lys Val Cys Phe His Asp
1 5 10

<210> SEQ ID NO 42
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<400> SEQUENCE: 42

Lys Pro Lys Cys Asp Val Leu Thr Lys Met Cys Asp Trp Leu
1 5 10

<210> SEQ ID NO 43
<211> LENGTH: 14
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<400> SEQUENCE: 43

Thr Gln His Cys Asp Val Leu Thr Lys Gln Cys Phe Thr Ile
1 5 10

<210> SEQ ID NO 44
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<400> SEQUENCE: 44

Gly His Phe Cys Asp Arg Leu Thr Lys Tyr Cys Phe Glu Pro
1 5 10

<210> SEQ ID NO 45
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<400> SEQUENCE: 45

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His Ile Gln Cys Asp Arg Leu Thr Lys Ser Cys Leu Ser Val
1 5 10

<210> SEQ ID NO 46
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<400> SEQUENCE: 46

Ile Lys Ala Cys Asp Ile Leu Thr Lys Val Cys Trp Pro Pro
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<210> SEQ ID NO 47
<211> LENGTH: 14
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<400> SEQUENCE: 47

Gln Phe Asp Cys Asp Pro Leu Thr Lys Tyr Cys Gly Glu Phe
1 5 10

<210> SEQ ID NO 48
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<400> SEQUENCE: 48

Lys Met Tyr Cys Asp His Leu Thr Gly Tyr Cys Trp Pro Glu
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<210> SEQ ID NO 49
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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 49

Met Gln Ser Cys Asp Ile Leu Thr Gly Tyr Cys Phe Lys Arg
1 5 10

<210> SEQ ID NO 50
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<400> SEQUENCE: 50

Gly Pro Trp Cys Asp Ile Leu Thr Gly Phe Cys Leu Ala Gln
1 5 10

<210> SEQ ID NO 51
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Ser Val Arg Cys Asp Leu Leu Thr Gly Trp Cys Pro Val Trp
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<400> SEQUENCE: 52

Pro Ala Asp Cys Asp Pro Leu Thr Asn Ile Cys Phe Trp Lys
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<210> SEQ ID NO 53

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<400> SEQUENCE: 53

Thr Asn Val Cys Asp Pro Leu Thr Asn Val Cys Phe Met Asn
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<210> SEQ ID NO 54

<211> LENGTH: 14

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<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 54

Glu His Trp Cys Asp Asp Leu Thr His Leu Cys Phe Arg Leu
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<210> SEQ ID NO 55

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 55

Gly Tyr Trp Cys Asp Val Leu Thr Asn Asn Cys Trp Lys Ile
1 5 10

<210> SEQ ID NO 56

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 56

Leu Tyr Asn Cys Asp Tyr Leu Thr Arg Leu Cys Phe Glu Pro
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<210> SEQ ID NO 57

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 57

His Val Asp Cys Leu Leu His Pro Lys Ala Cys Tyr Lys Tyr
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<210> SEQ ID NO 58

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 58

Val Gln Asp Cys Leu Leu His Pro Lys Ala Cys Gln Met Gln
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<210> SEQ ID NO 59

<211> LENGTH: 14

<212> TYPE: PRT

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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 59

Lys Phe Asp Cys Leu Leu Lys Pro Met Phe Cys Ser Asn His
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<210> SEQ ID NO 60

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 60

Phe Ala Asp Cys Leu Ile His Pro Lys Ser Cys Lys Pro Leu
1 5 10

<210> SEQ ID NO 61

<211> LENGTH: 14

<212> TYPE: PRT

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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 61

His Gly Asn Cys Tyr Pro Phe Pro Trp Glu Cys Glu Ser Lys
1 5 10

<210> SEQ ID NO 62

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 62

Met Ile Ile Val Leu Leu Leu Leu Arg Phe Ala Ile Ser Arg
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<210> SEQ ID NO 63

<211> LENGTH: 14

<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 63

Ser Leu Leu Val Ile Phe Leu Leu Ile Gly Ala Gly Ser Leu
1 5 10

<210> SEQ ID NO 64
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 64

Phe His Pro Cys Asp Met Leu Thr Gly Ile Trp Cys Gln Pro Asn
1 5 10 15

<210> SEQ ID NO 65
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 65

Ser Lys Arg Cys Asp Leu Leu Thr Lys Met Trp Cys Glu Thr Glu
1 5 10 15

<210> SEQ ID NO 66
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 66

Thr Lys Phe Cys Asp Arg Leu Thr Met Pro Lys Cys Val Trp Lys
1 5 10 15

<210> SEQ ID NO 67
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 67

Asn Thr Phe Cys Pro Asp Pro Leu Thr Gly Arg Cys Val Asn Pro
1 5 10 15

<210> SEQ ID NO 68
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 68

Asp Trp Thr Cys Asp Pro Leu Phe His Arg Glu Cys Ile Phe Glu
1 5 10 15

<210> SEQ ID NO 69

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<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 69

Pro Gln Pro Cys Asp Leu Leu Phe Glu Lys Lys Cys Ser Ile Lys
1 5 10 15

<210> SEQ ID NO 70
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 70

Arg Trp His Cys Asp Met Leu Ile Asn Pro Ser Cys Leu Pro Asp
1 5 10 15

<210> SEQ ID NO 71
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 71

Lys Ile Gln Cys Asp Ile Val Asn Leu Ser Ser Cys Val Tyr Pro
1 5 10 15

<210> SEQ ID NO 72
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 72

Leu Asn Ala Cys Asp Ile Val His Pro Asn Tyr Cys Ser Gly Met
1 5 10 15

<210> SEQ ID NO 73
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 73

Ala Lys Ala Cys Ser Ile Val Asn Leu Glu Ser Cys Glu Tyr Leu
1 5 10 15

<210> SEQ ID NO 74
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 74

Arg Gln Ala Cys Ser Ile Ile Thr Pro Trp Gly Cys Pro Ile Pro
1 5 10 15

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<210> SEQ ID NO 75
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 75

Ala Asp Asn Cys Thr Val Ala Thr Leu Asp Phe Cys Tyr Trp Thr
1 5 10 15

<210> SEQ ID NO 76
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 76

Lys Pro Glu Cys Asn Ile Thr Lys Pro Gln Phe Cys Phe Gly Glu
1 5 10 15

<210> SEQ ID NO 77
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 77

Asn Asn Cys Gln Trp Asp Glu Leu Thr Ser Met Cys Asp Pro Phe
1 5 10 15

<210> SEQ ID NO 78
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 78

Ser Arg Leu Cys His Met Asp Glu Leu Thr His Val Cys Val His Phe
1 5 10 15

<210> SEQ ID NO 79
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 79

Ser Arg Pro Cys Gln Ile Asp Glu Leu Thr Lys Ala Cys Phe Tyr Asn
1 5 10 15

<210> SEQ ID NO 80
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 80

Asp Arg Val Cys Lys Leu Asp Phe Leu Thr Tyr Asn Cys Leu Asn His

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<210> SEQ ID NO 81
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 81

His Ser Asn Cys Ile Met Asp Leu Leu Thr Asn Arg Cys Phe Tyr Asp
1 5 10 15

<210> SEQ ID NO 82
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 82

Pro Phe Asn Cys Phe His Asp Pro Leu Thr Gly Leu Cys Leu His Ser
1 5 10 15

<210> SEQ ID NO 83
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 83

Tyr Asp Ser Cys Thr Tyr Asp Arg Leu Thr Lys Gln Cys Tyr Pro Ser
1 5 10 15

<210> SEQ ID NO 84
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 84

Phe His Asp Cys Met Tyr Asp Ala Leu Leu Gly Tyr Cys Leu Pro Tyr
1 5 10 15

<210> SEQ ID NO 85
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 85

Asn Arg Ser Cys Asp Pro Leu Thr Arg Pro Lys Ser Cys Gly Leu
1 5 10 15

<210> SEQ ID NO 86
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 86

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Leu Ser Asn Cys Asp Trp Asp Asp Leu Ile Arg Gln Cys Leu His Asp
1 5 10 15

<210> SEQ ID NO 87
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 87

Phe Trp Asp Cys Leu Phe His Pro Asn Ser Arg Tyr Cys Val Leu Ser
1 5 10 15

<210> SEQ ID NO 88
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 88

Ser Arg Asp Cys Leu Leu Ser Pro Ala Met Ala Trp Cys Gly Leu Asp
1 5 10 15

<210> SEQ ID NO 89
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 89

Gly Gly Asn Cys Tyr Thr Asp Ser Leu Thr Lys Leu His Phe Cys Met
1 5 10 15

Gly Asp

<210> SEQ ID NO 90
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 90

Met Cys Pro Arg Asp Pro Leu Thr Lys Ala Lys Leu Cys Asn Trp His
1 5 10 15

<210> SEQ ID NO 91
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 91

Pro Asn Gln Cys Gln Asp Asp Leu Thr Lys Gln Trp Tyr Ser Cys His
1 5 10 15

Tyr His

<210> SEQ ID NO 92
<211> LENGTH: 18

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 92

Phe Asp Met Cys Phe Asp Ala Leu Thr Lys Gln Asn Phe Tyr Cys Arg
1 5 10 15

Phe His

<210> SEQ ID NO 93
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 93

Arg Asn Met Cys Val Asp Arg Leu Thr Lys Leu Gln His Gly Cys Glu
1 5 10 15

Gly Ala

<210> SEQ ID NO 94
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 94

Asp Pro Glu Cys Leu Thr Ser Phe Asp Arg Leu Thr Lys Met Cys Trp
1 5 10 15

Pro Trp

<210> SEQ ID NO 95
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 95

Asp Asp Glu Cys His Tyr Asp Tyr Leu Thr His Tyr Met Arg Cys Asp
1 5 10 15

Tyr Arg

<210> SEQ ID NO 96
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 96

Phe Gly Gly Cys Asn Ile Asp Leu Leu Thr Asn Thr Met Met Cys His
1 5 10 15

Arg Asn

<210> SEQ ID NO 97
<211> LENGTH: 18
<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 97
His Gly Pro Cys Tyr Trp Asp Glu Leu Thr Met Gln Trp His Cys Asn
1 5 10 15

His His

<210> SEQ ID NO 98
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 98
Gly Ala Met Cys Val Asp Leu Leu Thr Tyr Thr Phe Arg Pro Cys Met
1 5 10 15

Tyr Ala

<210> SEQ ID NO 99
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 99
Ser Asn Lys Cys Trp Asp Glu Leu Thr His Ala Trp Ala Glu Cys Gly
1 5 10 15

Arg Phe

<210> SEQ ID NO 100
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 100
Arg Pro Val Cys Tyr Lys Gly Tyr Asp Ile Leu Thr Thr Gln Cys Met
1 5 10 15

Pro Trp

<210> SEQ ID NO 101
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 101
Pro Ser Arg Cys Trp Phe Asp Leu Leu Phe Asn Lys Phe Val Cys Lys
1 5 10 15

Arg Asn

<210> SEQ ID NO 102
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 102

Arg Ser Gly Cys Val Tyr Asp Met Leu Leu Met Thr Met Tyr Cys Pro
1 5 10 15

Ser Asn

<210> SEQ ID NO 103

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 103

Ser Asn Arg Cys Glu Gly Asp Gln Leu Met Arg Pro Pro Ser Cys Arg
1 5 10 15

His Leu

<210> SEQ ID NO 104

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 104

Tyr Arg Met Cys Trp Trp Asp Asp Leu Leu Arg Gly Phe Val Cys Asp
1 5 10 15

Phe His

<210> SEQ ID NO 105

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 105

His Asp Gly Cys Tyr Asp Glu Leu Leu Tyr Arg Trp Thr Arg Cys Glu
1 5 10 15

His Arg

<210> SEQ ID NO 106

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 106

Trp Ala Trp Cys Phe Asp Glu Leu Val Gln Arg Tyr Phe Thr Cys Phe
1 5 10 15

Asp His

<210> SEQ ID NO 107

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 107

Leu Pro Glu Cys Arg Gln Tyr Phe Pro Trp Glu Lys Gln Val Cys Ser
1 5 10 15

Tyr Trp

<210> SEQ ID NO 108

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 108

Val His Tyr Asp Ser Leu Thr Lys Met Trp Thr Arg
1 5 10

<210> SEQ ID NO 109

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 109

Phe Thr Asp Pro Leu Thr Lys Met Ser Leu His Ser
1 5 10

<210> SEQ ID NO 110

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 110

Gly Tyr Asp Val Leu Thr Lys Leu Tyr Phe Val Pro
1 5 10

<210> SEQ ID NO 111

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 111

Tyr Tyr Asp Arg Leu Thr Lys Leu Tyr Ser Ser Met
1 5 10

<210> SEQ ID NO 112

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<220> FEATURE:

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<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 112

Leu Xaa Lys Asp Pro Leu Thr Lys Leu Tyr Ile Ser

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<210> SEQ ID NO 113
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 113

Gly Tyr Asp Val Leu Thr Lys Leu Xaa Phe Val Pro
1 5 10

<210> SEQ ID NO 114
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 114

Arg Leu Tyr Asp Pro Leu Thr Lys Leu Val Leu Ser
1 5 10

<210> SEQ ID NO 115
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 115

Met Phe Asp Pro Leu Thr Lys Ile Ala Phe Pro Ala
1 5 10

<210> SEQ ID NO 116
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 116

Phe Tyr Asp Ser Leu Thr Lys Thr Asn Leu Arg Asp
1 5 10

<210> SEQ ID NO 117
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 117

Gly Ile Tyr Asp Lys Leu Thr Arg Ala Trp Leu Pro
1 5 10

<210> SEQ ID NO 118
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
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<221> NAME/KEY: SITE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 118

Lys Tyr Asp Pro Leu Thr Arg Ala Arg Xaa Pro Leu
1 5 10

<210> SEQ ID NO 119
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 119

Tyr Ile Asp Gln Leu Thr Arg Leu Ser Leu Pro Ser
1 5 10

<210> SEQ ID NO 120
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 120

His Gln Thr Phe Asp Ile Leu Thr Arg Leu His Phe
1 5 10

<210> SEQ ID NO 121
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 121

Trp Gln Phe Asp Val Leu Thr Arg Ser Trp Thr Pro
1 5 10

<210> SEQ ID NO 122
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 122

Gly Ala Ala Tyr Asp His Leu Thr Arg Thr Trp Leu
1 5 10

<210> SEQ ID NO 123
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 123

Tyr Phe Asp Gln Leu Thr His Leu Ser Ile Lys Lys
1 5 10

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<210> SEQ ID NO 124
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 124

Ala Trp Asp Pro Leu Thr Met Leu Val Leu Pro Trp
1 5 10

<210> SEQ ID NO 125
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 125

Ala Leu Trp Met Asp Pro Leu Thr Gly Leu Ala Phe
1 5 10

<210> SEQ ID NO 126
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 126

Trp Gln Phe Asp Val Leu Thr Xaa Ser Trp Thr Pro
1 5 10

<210> SEQ ID NO 127
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 127

Trp Thr Asp Pro Leu Thr His Met Glu Ile Tyr His
1 5 10

<210> SEQ ID NO 128
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 128

Trp Thr Asp Ser Leu Thr Gly Leu Trp Phe Pro Asp
1 5 10

<210> SEQ ID NO 129
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 129

Tyr Thr Asp Pro Leu Thr Gly Ile Val Xaa Pro Phe
1 5 10

<210> SEQ ID NO 130
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 130

Tyr Trp Asp Lys Leu Thr Met Leu His Leu Gly Val
1 5 10

<210> SEQ ID NO 131
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 131

Tyr Tyr Asp Phe Leu Thr Arg Thr Val Leu Pro Ser
1 5 10

<210> SEQ ID NO 132
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 132

Arg Leu Asp Pro Leu Ser Lys Asn Asp Phe Pro Arg
1 5 10

<210> SEQ ID NO 133
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 133

Leu Arg Tyr Asp Pro Leu Leu Lys Ser Xaa Ile Tyr
1 5 10

<210> SEQ ID NO 134
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 134

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Leu Arg Tyr Asp Pro Leu Leu Lys Ser Tyr Ile Tyr
1 5 10

<210> SEQ ID NO 135
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 135

Tyr Phe Asp Gln Phe Thr His Leu Ser Ile Lys Lys
1 5 10

<210> SEQ ID NO 136
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 136

Tyr Phe Asp Gln Xaa Thr His Leu Ser Ile Lys Lys
1 5 10

<210> SEQ ID NO 137
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 137

Glu His Tyr Tyr Thr Asp Pro Leu Thr Gly Ala Arg Ile
1 5 10

<210> SEQ ID NO 138
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 138

Glu His Tyr Xaa Thr Asp Pro Leu Thr Gly Ala Arg Ile
1 5 10

<210> SEQ ID NO 139
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 139

Glu His Tyr Ser Thr Asp Pro Leu Thr Gly Ala Arg Ile
1 5 10

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<210> SEQ ID NO 140
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 140

Glu His Tyr Tyr Thr Asp Pro Leu Xaa Gly Xaa Arg Ile
1 5 10

<210> SEQ ID NO 141
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 141

Glu His Tyr Tyr Thr Asp Pro Leu Xaa Gly Xaa Arg Xaa
1 5 10

<210> SEQ ID NO 142
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 142

Glu His Tyr Tyr Thr Asp Pro Leu Xaa Gly Ala Arg Xaa
1 5 10

<210> SEQ ID NO 143
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:

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<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 143

Glu His Xaa Tyr Thr Asp Pro Leu Asn Gly Ala Arg Xaa
1 5 10

<210> SEQ ID NO 144
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 144

Glu His Tyr Tyr Asn Asp Pro Leu Asn Gly Ala Arg Xaa
1 5 10

<210> SEQ ID NO 145
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 145

Xaa His Xaa Tyr Asn Asp Pro Leu Asn Gly Ala Arg Xaa
1 5 10

<210> SEQ ID NO 146
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 146

Lys Pro Tyr Tyr Asp Pro Ile Thr Lys Met Thr His His
1 5 10

<210> SEQ ID NO 147
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

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<400> SEQUENCE: 147

Lys Pro Tyr Tyr Asp Pro Ile Thr Lys Met Ser His His
1 5 10

<210> SEQ ID NO 148

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 148

Lys Pro Tyr Tyr Asp Pro Ile Ser Lys Met Thr His His
1 5 10

<210> SEQ ID NO 149

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLys binding polypeptide

<220> FEATURE:

<221> NAME/KEY: SITE

<222> LOCATION: (3)..(4)

<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 149

Lys Pro Xaa Xaa Asp Pro Ile Ser Lys Met Thr His His
1 5 10

<210> SEQ ID NO 150

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 150

Gln Ile Gly Tyr Asp Glu Leu Thr Lys Ala Trp Val Thr
1 5 10

<210> SEQ ID NO 151

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 151

Gln Leu Gly Tyr Asp Glu Leu Thr Lys Ala Trp Val Thr
1 5 10

<210> SEQ ID NO 152

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLys binding polypeptide

<220> FEATURE:

<221> NAME/KEY: SITE

<222> LOCATION: (6)..(6)

<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 152

Lys Ile Asp Glu Leu Xaa Met Gln Asn Ile Ile Ile Trp

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1 5 10

<210> SEQ ID NO 153
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 153

Asp His Thr Asp Pro Leu Ile Gln Gly Leu Thr Lys Arg
1 5 10

<210> SEQ ID NO 154
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 154

Trp His Asp Pro Leu Lys His Met His Phe His His Glu
1 5 10

<210> SEQ ID NO 155
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 155

Lys His Ile Asp Met Glu Thr Gly Leu Ile Leu Gln Asn
1 5 10

<210> SEQ ID NO 156
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 156

Met Gln Val Asp Pro Glu Thr Gly Leu Lys Tyr Glu His
1 5 10

<210> SEQ ID NO 157
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (8)..(10)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 157

Xaa Leu Asp Gln His Val Asn Xaa Xaa Xaa Tyr Gln Ser
1 5 10

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<210> SEQ ID NO 158
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (2)..(4)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 158

Glu Xaa Xaa Xaa Thr Xaa Xaa Leu Thr Gly Ala Arg Xaa
1 5 10

<210> SEQ ID NO 159
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 159

Gly Pro Tyr Asn Ile Xaa Arg Leu Xaa Gly Glu Arg Xaa
1 5 10

<210> SEQ ID NO 160
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 160

His Ile Lys Met Leu His Gln Gly Ser Phe Val Gly Val
1 5 10

<210> SEQ ID NO 161
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: X is unknown
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (10)..(10)

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<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 161

His Pro Thr Asn Thr Xaa Xaa His Gln Xaa Val Tyr Ser
 1 5 10

<210> SEQ ID NO 162

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<220> FEATURE:

<221> NAME/KEY: SITE

<222> LOCATION: (6)..(7)

<223> OTHER INFORMATION: X is unknown

<220> FEATURE:

<221> NAME/KEY: SITE

<222> LOCATION: (12)..(12)

<223> OTHER INFORMATION: X is unknown

<220> FEATURE:

<221> NAME/KEY: SITE

<222> LOCATION: (13)..(13)

<223> OTHER INFORMATION: X is unknown

<400> SEQUENCE: 162

His Arg Gly Gln Val Xaa Xaa Leu Asn Gly Met Val Xaa
 1 5 10

<210> SEQ ID NO 163

<211> LENGTH: 24

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 163

Ala Gly Lys Glu Pro Cys Tyr Phe Tyr Trp Glu Cys Ala Val Ser Gly
 1 5 10 15

Pro Gly Pro Glu Gly Gly Gly Lys
 20

<210> SEQ ID NO 164

<211> LENGTH: 25

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 164

Ala Gly Val Pro Phe Cys Asp Leu Leu Thr Lys His Cys Phe Glu Ala
 1 5 10 15

Gly Pro Gly Pro Glu Gly Gly Gly Lys
 20 25

<210> SEQ ID NO 165

<211> LENGTH: 28

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 165

Gly Ser Ser Arg Leu Cys His Met Asp Glu Leu Thr His Val Cys Val
 1 5 10 15

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His Phe Ala Pro Pro Gly Pro Glu Gly Gly Gly Lys
 20 25

<210> SEQ ID NO 166
 <211> LENGTH: 29
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 166

Gly Asp Gly Gly Asn Cys Tyr Thr Asp Ser Leu Thr Lys Leu His Phe
 1 5 10 15

Cys Met Gly Asp Glu Pro Gly Pro Glu Gly Gly Gly Lys
 20 25

<210> SEQ ID NO 167
 <211> LENGTH: 22
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 167

Gly Tyr Asp Val Leu Thr Lys Leu Tyr Phe Val Pro Gly Gly Pro Gly
 1 5 10 15

Pro Glu Gly Gly Gly Lys
 20

<210> SEQ ID NO 168
 <211> LENGTH: 22
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 168

Trp Thr Asp Ser Leu Thr Gly Leu Trp Phe Pro Asp Gly Gly Pro Gly
 1 5 10 15

Pro Glu Gly Gly Gly Lys
 20

<210> SEQ ID NO 169
 <211> LENGTH: 24
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: modified BLYS binding polypeptide

<400> SEQUENCE: 169

Ala Gly Lys Glu Pro Cys Tyr Phe Tyr Trp Glu Cys Ala Val Ser Gly
 1 5 10 15

Pro Gly Pro Glu Gly Gly Gly Lys
 20

<210> SEQ ID NO 170
 <211> LENGTH: 24
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: modified BLYS binding polypeptide

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<400> SEQUENCE: 170

Ala Gly Arg Glu Pro Cys Tyr Phe Tyr Trp Glu Cys Ala Val Ser Gly
 1 5 10 15

Pro Gly Pro Glu Gly Gly Lys
 20

<210> SEQ ID NO 171

<211> LENGTH: 24

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: modified BLYS binding polypeptide

<400> SEQUENCE: 171

Ala Gly Gln Glu Pro Cys Tyr Phe Tyr Trp Glu Cys Ala Val Ser Gly
 1 5 10 15

Pro Gly Pro Glu Gly Gly Lys
 20

<210> SEQ ID NO 172

<211> LENGTH: 25

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: modified BLYS binding polypeptide

<220> FEATURE:

<221> NAME/KEY: SITE

<222> LOCATION: (4)..(4)

<223> OTHER INFORMATION: X is norleucine

<400> SEQUENCE: 172

Ala Gly Asn Xaa Glu Pro Cys Tyr Phe Tyr Trp Glu Cys Ala Val Ser
 1 5 10 15

Gly Pro Gly Pro Glu Gly Gly Lys
 20 25

<210> SEQ ID NO 173

<211> LENGTH: 285

<212> TYPE: PRT

<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 173

Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
 1 5 10 15

Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
 20 25 30

Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
 35 40 45

Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
 50 55 60

Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
 65 70 75 80

Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
 85 90 95

Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
 100 105 110

Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
 115 120 125

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Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Val Thr Gln
 130 135 140

Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys
 145 150 155 160

Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser
 165 170 175

Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr
 180 185 190

Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
 195 200 205

Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu
 210 215 220

Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
 225 230 235 240

Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly
 245 250 255

Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu
 260 265 270

Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
 275 280 285

<210> SEQ ID NO 174

<211> LENGTH: 266

<212> TYPE: PRT

<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 174

Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
 1 5 10 15

Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
 20 25 30

Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
 35 40 45

Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
 50 55 60

Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
 65 70 75 80

Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
 85 90 95

Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
 100 105 110

Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
 115 120 125

Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Gly Ser Tyr
 130 135 140

Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu
 145 150 155 160

Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile
 165 170 175

Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His Leu
 180 185 190

Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu Val
 195 200 205

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Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn Asn
 210                               215                               220

Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu
225                               230                               235                               240

Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp
                               245                               250                               255

Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
                               260                               265

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<210> SEQ ID NO 175
<211> LENGTH: 309
<212> TYPE: PRT
<213> ORGANISM: mouse

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<400> SEQUENCE: 175

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Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys
 1                               5                               10                               15

Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro
                               20                               25                               30

Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
                               35                               40                               45

Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala
 50                               55                               60

Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu
65                               70                               75                               80

Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala
                               85                               90                               95

Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
                               100                              105                              110

Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe
                               115                              120                              125

Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro
                               130                              135                              140

Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly
145                              150                              155                              160

Met Asn Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp
                               165                              170                              175

Ser Asp Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp
                               180                              185                              190

Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
                               195                              200                              205

Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu
                               210                              215                              220

Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
225                              230                              235                              240

Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
                               245                              250                              255

Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala
                               260                              265                              270

Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
                               275                              280                              285

Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly

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290 295 300

Ala Leu Lys Leu Leu
305

<210> SEQ ID NO 176
<211> LENGTH: 290
<212> TYPE: PRT
<213> ORGANISM: mouse

<400> SEQUENCE: 176

Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys
1 5 10 15

Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro
20 25 30

Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
35 40 45

Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala
50 55 60

Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu
65 70 75 80

Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala
85 90 95

Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
100 105 110

Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe
115 120 125

Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro
130 135 140

Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly
145 150 155 160

Met Asn Leu Arg Asn Arg Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser
165 170 175

Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val Val
180 185 190

Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp
195 200 205

Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys Val His Val
210 215 220

Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn
225 230 235 240

Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala
245 250 255

Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn
260 265 270

Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys
275 280 285

Leu Leu
290

<210> SEQ ID NO 177
<211> LENGTH: 239
<212> TYPE: PRT
<213> ORGANISM: rat

-continued

<400> SEQUENCE: 177

Ala Val Gln Ala Asp Leu Met Ser Leu Arg Met Glu Leu Gln Ser Tyr
 1 5 10 15
 Arg Ser Ser Ala Thr Pro Ala Ala Pro Gly Ala Pro Gly Leu Ser Ala
 20 25 30
 Gly Val Lys Leu Pro Thr Pro Ala Ala Pro Gly Pro His Asn Ser Ser
 35 40 45
 Arg Gly Gln Arg Asn Arg Arg Ala Phe Gln Gly Pro Glu Glu Thr Glu
 50 55 60
 Gln Asp Val Asp Leu Ser Ala Thr Pro Ala Pro Ser Leu Pro Gly Asn
 65 70 75 80
 Cys His Ala Ser His His Asp Glu Asn Gly Leu Asn Leu Arg Thr Ile
 85 90 95
 Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Asn Thr Pro Thr Ile
 100 105 110
 Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg
 115 120 125
 Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val Val Arg Gln Thr
 130 135 140
 Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro Ile Phe
 145 150 155 160
 Ala Met Gly His Val Ile Gln Arg Lys Lys Ile His Val Phe Gly Asp
 165 170 175
 Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Lys
 180 185 190
 Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu
 195 200 205
 Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile
 210 215 220
 Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu Leu
 225 230 235

<210> SEQ ID NO 178

<211> LENGTH: 220

<212> TYPE: PRT

<213> ORGANISM: rat

<400> SEQUENCE: 178

Ala Val Gln Ala Asp Leu Met Ser Leu Arg Met Glu Leu Gln Ser Tyr
 1 5 10 15
 Arg Ser Ser Ala Thr Pro Ala Ala Pro Gly Ala Pro Gly Leu Ser Ala
 20 25 30
 Gly Val Lys Leu Pro Thr Pro Ala Ala Pro Gly Pro His Asn Ser Ser
 35 40 45
 Arg Gly Gln Arg Asn Arg Arg Ala Phe Gln Gly Pro Glu Glu Thr Glu
 50 55 60
 Gln Asp Val Asp Leu Ser Ala Thr Pro Val Pro Ser Leu Pro Gly Asn
 65 70 75 80
 Cys His Ala Ser His His Asp Glu Asn Gly Leu Asn Leu Arg Thr Arg
 85 90 95
 Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala
 100 105 110

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Leu Glu Glu Lys Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe
    115                                120                                125

Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly
    130                                135                                140

His Val Ile Gln Arg Lys Lys Ile His Val Phe Gly Asp Glu Leu Ser
    145                                150                                155                                160

Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro
    165                                170                                175

Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp
    180                                185                                190

Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn
    195                                200                                205

Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu Leu
    210                                215                                220

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<210> SEQ ID NO 179

<211> LENGTH: 207

<212> TYPE: PRT

<213> ORGANISM: rat

<400> SEQUENCE: 179

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Ala Val Gln Ala Asp Leu Met Ser Leu Arg Met Glu Leu Gln Ser Tyr
  1                                5                                10                                15

Arg Ser Ser Ala Thr Pro Ala Ala Pro Gly Ala Pro Gly Leu Ser Ala
    20                                25                                30

Gly Val Lys Leu Pro Thr Pro Ala Ala Pro Gly Pro His Asn Ser Ser
    35                                40                                45

Arg Gly Gln Arg Asn Arg Arg Ala Phe Gln Gly Pro Glu Glu Thr Val
    50                                55                                60

Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Asn Thr Pro Thr Ile
    65                                70                                75                                80

Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg
    85                                90                                95

Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val Val Arg Gln Thr
    100                                105                                110

Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro Ile Phe
    115                                120                                125

Ala Met Gly His Val Ile Gln Arg Lys Lys Ile His Val Phe Gly Asp
    130                                135                                140

Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Lys
    145                                150                                155                                160

Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu
    165                                170                                175

Glu Gly Asp Glu Val Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile
    180                                185                                190

Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu Leu
    195                                200                                205

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<210> SEQ ID NO 180

<211> LENGTH: 188

<212> TYPE: PRT

<213> ORGANISM: rat

<400> SEQUENCE: 180

-continued

Ala Val Gln Ala Asp Leu Met Ser Leu Arg Met Glu Leu Gln Ser Tyr
 1 5 10 15
 Arg Ser Ser Ala Thr Pro Ala Ala Pro Gly Ala Pro Gly Leu Ser Ala
 20 25 30
 Gly Val Lys Leu Pro Thr Pro Ala Ala Pro Gly Pro His Asn Ser Ser
 35 40 45
 Arg Gly Gln Arg Asn Arg Arg Ala Phe Gln Gly Pro Glu Glu Thr Gly
 50 55 60
 Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala
 65 70 75 80
 Leu Glu Glu Lys Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe
 85 90 95
 Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly
 100 105 110
 His Val Ile Gln Arg Lys Lys Ile His Val Phe Gly Asp Glu Leu Ser
 115 120 125
 Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro
 130 135 140
 Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp
 145 150 155 160
 Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn
 165 170 175
 Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu Leu
 180 185

<210> SEQ ID NO 181
 <211> LENGTH: 243
 <212> TYPE: PRT
 <213> ORGANISM: monkey

<400> SEQUENCE: 181

Lys Asp Arg Lys Leu Leu Ala Ala Ala Leu Leu Leu Ala Leu Leu Ser
 1 5 10 15
 Cys Cys Leu Met Val Val Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly
 20 25 30
 Asp Leu Ala Ser Leu Arg Ala Glu Leu Gln Gly His His Ala Glu Lys
 35 40 45
 Leu Pro Ala Arg Ala Arg Ala Pro Lys Ala Gly Leu Gly Glu Ala Pro
 50 55 60
 Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu
 65 70 75 80
 Gly Asn Ser Ser Gln Ser Ser Arg Asn Lys Arg Ala Ile Gln Gly Ala
 85 90 95
 Glu Glu Thr Val Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu
 100 105 110
 Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu
 115 120 125
 Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu
 130 135 140
 Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr
 145 150 155 160
 Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val His
 165 170 175

-continued

Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln
 180 185 190

Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile
 195 200 205

Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu
 210 215 220

Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu
 225 230 235 240

Lys Leu Leu

<210> SEQ ID NO 182
 <211> LENGTH: 219
 <212> TYPE: PRT
 <213> ORGANISM: monkey

<400> SEQUENCE: 182

Tyr Gln Val Ala Ala Val Gln Gly Asp Leu Ala Ser Leu Arg Ala Glu
 1 5 10 15

Leu Gln Ser His His Ala Glu Lys Leu Pro Ala Arg Ala Arg Ala Pro
 20 25 30

Lys Ala Gly Leu Gly Glu Ala Pro Ala Val Thr Ala Gly Leu Lys Ile
 35 40 45

Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Ser Ser Arg
 50 55 60

Asn Lys Arg Ala Ile Gln Gly Ala Glu Glu Thr Val Ile Gln Asp Cys
 65 70 75 80

Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser
 85 90 95

Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu
 100 105 110

Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe
 115 120 125

Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His
 130 135 140

Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu
 145 150 155 160

Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn
 165 170 175

Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu
 180 185 190

Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly
 195 200 205

Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
 210 215

<210> SEQ ID NO 183
 <211> LENGTH: 8
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: epitope tag

<400> SEQUENCE: 183

Asp Tyr Lys Asp Asp Asp Lys

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1 5

<210> SEQ ID NO 184
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: concensus BLYS binding polypeptide

<400> SEQUENCE: 184

Ala Asn Trp Tyr Asp Ser Leu Thr Lys Leu Trp Leu Pro Asp
1 5 10

<210> SEQ ID NO 185
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: coding sequence for BLYS affinity maturation
library template
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(12)
<223> OTHER INFORMATION: N=A or G or C or T
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (16)..(18)
<223> OTHER INFORMATION: N=A or G or C or T
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (25)..(27)
<223> OTHER INFORMATION: N=A or G or C or T
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (31)..(42)
<223> OTHER INFORMATION: N=A or G or C or T

<400> SEQUENCE: 185

gctnnnnnnn nngatnnnct tactnnnctc nnnnnnnnnn nn 42

<210> SEQ ID NO 186
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 186

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp
1 5 10

<210> SEQ ID NO 187
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 187

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Glu
1 5 10

<210> SEQ ID NO 188
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 188

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Gly
1 5 10

<210> SEQ ID NO 189

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 189

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Val
1 5 10

<210> SEQ ID NO 190

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 190

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Ser Asp
1 5 10

<210> SEQ ID NO 191

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 191

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Asn Asp
1 5 10

<210> SEQ ID NO 192

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 192

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Thr
1 5 10

<210> SEQ ID NO 193

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 193

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Ala
1 5 10

<210> SEQ ID NO 194

<211> LENGTH: 14

<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 194

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asn
1 5 10

<210> SEQ ID NO 195
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 195

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Val Asp
1 5 10

<210> SEQ ID NO 196
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 196

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu His Asp
1 5 10

<210> SEQ ID NO 197
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 197

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Thr Asp
1 5 10

<210> SEQ ID NO 198
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 198

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro His
1 5 10

<210> SEQ ID NO 199
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 199

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Thr Val
1 5 10

<210> SEQ ID NO 200

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<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 200

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Asp
1 5 10

<210> SEQ ID NO 201
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 201

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Glu
1 5 10

<210> SEQ ID NO 202
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 202

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu His Glu
1 5 10

<210> SEQ ID NO 203
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 203

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Arg
1 5 10

<210> SEQ ID NO 204
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 204

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Ala Asp
1 5 10

<210> SEQ ID NO 205
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 205

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Tyr
1 5 10

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<210> SEQ ID NO 206
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 206

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Ile
1 5 10

<210> SEQ ID NO 207
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 207

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Ile Asp
1 5 10

<210> SEQ ID NO 208
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 208

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Asp
1 5 10

<210> SEQ ID NO 209
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 209

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp
1 5 10

<210> SEQ ID NO 210
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 210

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Glu
1 5 10

<210> SEQ ID NO 211
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 211

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Val

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1 5 10

<210> SEQ ID NO 212
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 212

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Glu
1 5 10

<210> SEQ ID NO 213
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 213

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Val
1 5 10

<210> SEQ ID NO 214
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 214

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu His Gln
1 5 10

<210> SEQ ID NO 215
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 215

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Ala
1 5 10

<210> SEQ ID NO 216
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 216

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Val
1 5 10

<210> SEQ ID NO 217
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 217

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Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Gly
1 5 10

<210> SEQ ID NO 218
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 218

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Tyr
1 5 10

<210> SEQ ID NO 219
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 219

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Tyr
1 5 10

<210> SEQ ID NO 220
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 220

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Tyr
1 5 10

<210> SEQ ID NO 221
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 221

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Asp
1 5 10

<210> SEQ ID NO 222
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 222

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Val
1 5 10

<210> SEQ ID NO 223
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

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<400> SEQUENCE: 223

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Gly
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<211> LENGTH: 14

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<400> SEQUENCE: 224

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<210> SEQ ID NO 225

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<210> SEQ ID NO 226

<211> LENGTH: 14

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<223> OTHER INFORMATION: BLYS binding polypeptide

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<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<211> LENGTH: 14

<212> TYPE: PRT

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Ala Ser Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu His Asp
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Ala Ser Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Gly
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Ala Ser Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Gln
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<210> SEQ ID NO 235
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<212> TYPE: PRT
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<400> SEQUENCE: 237

Ala Ser Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Glu
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<400> SEQUENCE: 238

Ala Phe Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Val
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<400> SEQUENCE: 239

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1 5 10

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<400> SEQUENCE: 240

Ala Phe Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Glu
1 5 10

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<210> SEQ ID NO 241
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<400> SEQUENCE: 241

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1 5 10

<210> SEQ ID NO 242
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<400> SEQUENCE: 242

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Glu
1 5 10

<210> SEQ ID NO 243
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<400> SEQUENCE: 243

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1 5 10

<210> SEQ ID NO 244
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<400> SEQUENCE: 244

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1 5 10

<210> SEQ ID NO 245
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<400> SEQUENCE: 245

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1 5 10

<210> SEQ ID NO 246
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<400> SEQUENCE: 246

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Phe
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<212> TYPE: PRT
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1 5 10

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Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Arg
1 5 10

<210> SEQ ID NO 249
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<400> SEQUENCE: 249

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Ala
1 5 10

<210> SEQ ID NO 250
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1 5 10

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<220> FEATURE:
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<400> SEQUENCE: 251

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<210> SEQ ID NO 252
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 252

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Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Gly
1 5 10

<210> SEQ ID NO 253
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<212> TYPE: PRT
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<220> FEATURE:
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<400> SEQUENCE: 253

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Val
1 5 10

<210> SEQ ID NO 254
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<400> SEQUENCE: 254

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro His
1 5 10

<210> SEQ ID NO 255
<211> LENGTH: 14
<212> TYPE: PRT
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<220> FEATURE:
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<400> SEQUENCE: 255

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Glu
1 5 10

<210> SEQ ID NO 256
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 256

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Ser Asp
1 5 10

<210> SEQ ID NO 257
<211> LENGTH: 14
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<400> SEQUENCE: 257

Ala Thr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Ala
1 5 10

<210> SEQ ID NO 258
<211> LENGTH: 14
<212> TYPE: PRT
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Ala Thr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Ala Asp
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<210> SEQ ID NO 259

<211> LENGTH: 14

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<210> SEQ ID NO 260

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<210> SEQ ID NO 261

<211> LENGTH: 14

<212> TYPE: PRT

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<210> SEQ ID NO 262

<211> LENGTH: 14

<212> TYPE: PRT

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<400> SEQUENCE: 262

Ala Thr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Ser Gly
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<210> SEQ ID NO 263

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 263

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<210> SEQ ID NO 264

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: BLYS binding polypeptide

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<210> SEQ ID NO 265

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<212> TYPE: PRT

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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 265

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<210> SEQ ID NO 266

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<212> TYPE: PRT

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<400> SEQUENCE: 266

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<210> SEQ ID NO 267

<211> LENGTH: 14

<212> TYPE: PRT

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<400> SEQUENCE: 267

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<210> SEQ ID NO 268

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 268

Ala Asp Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Glu
1 5 10

<210> SEQ ID NO 269

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 269

Ala Asp Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu His Gln
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<210> SEQ ID NO 270

<211> LENGTH: 14

<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 270

Ala Glu Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Asp
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 271

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<210> SEQ ID NO 272
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<400> SEQUENCE: 272

Ala Glu Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Tyr
1 5 10

<210> SEQ ID NO 273
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<400> SEQUENCE: 273

Ala Leu Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Ala
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<210> SEQ ID NO 274
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<212> TYPE: PRT
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<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 274

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1 5 10

<210> SEQ ID NO 275
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 275

Ala Leu Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Arg Gly
1 5 10

<210> SEQ ID NO 276

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<211> LENGTH: 14
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 276

Ala Leu Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Gly
1 5 10

<210> SEQ ID NO 277
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 277

Ala Met Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Ala
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<210> SEQ ID NO 278
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 278

Ala Met Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Gln Val
1 5 10

<210> SEQ ID NO 279
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 279

Ala Met Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Gly
1 5 10

<210> SEQ ID NO 280
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 280

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<210> SEQ ID NO 281
<211> LENGTH: 14
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<400> SEQUENCE: 281

Ala Ala Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Ala Asp
1 5 10

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<210> SEQ ID NO 282
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<212> TYPE: PRT
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<400> SEQUENCE: 282

Ala Ala Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Asp
1 5 10

<210> SEQ ID NO 283
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<400> SEQUENCE: 283

Ala His Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Thr Asp
1 5 10

<210> SEQ ID NO 284
<211> LENGTH: 14
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<400> SEQUENCE: 284

Ala His Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Val
1 5 10

<210> SEQ ID NO 285
<211> LENGTH: 14
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<400> SEQUENCE: 285

Ala His Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu His Asp
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<210> SEQ ID NO 286
<211> LENGTH: 14
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<400> SEQUENCE: 286

Ala His Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp
1 5 10

<210> SEQ ID NO 287
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 287

Ala Pro Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu His Asp

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<210> SEQ ID NO 288
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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 288

Ala Pro Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Val
1 5 10

<210> SEQ ID NO 289
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 289

Ala Gln Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Glu
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<210> SEQ ID NO 290
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 290

Ala Gln Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Tyr
1 5 10

<210> SEQ ID NO 291
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<212> TYPE: PRT
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<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 291

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<210> SEQ ID NO 292
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<400> SEQUENCE: 292

Ala Lys Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp
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<210> SEQ ID NO 293
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<212> TYPE: PRT
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<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 293

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Ala Lys Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Val
1 5 10

<210> SEQ ID NO 294
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<212> TYPE: PRT
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Ala Lys Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Val
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<210> SEQ ID NO 295
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<400> SEQUENCE: 295

Ala Lys Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Asn Gly
1 5 10

<210> SEQ ID NO 296
<211> LENGTH: 14
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<400> SEQUENCE: 296

Ala Trp Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Ala
1 5 10

<210> SEQ ID NO 297
<211> LENGTH: 14
<212> TYPE: PRT
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Ala Val Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Thr Asp
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<210> SEQ ID NO 298
<211> LENGTH: 14
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<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 298

Ala Tyr Glu Tyr Asp Pro Leu Thr Lys Leu Trp Leu Leu Tyr
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<211> LENGTH: 14
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<400> SEQUENCE: 299

Ala Thr Lys Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp
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<210> SEQ ID NO 300

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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Ala Thr Leu Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Gly
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<210> SEQ ID NO 301

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: BLYS binding polypeptide

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Ala Ile Arg Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Tyr
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<210> SEQ ID NO 302

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 302

Ala Glu Arg Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro His
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<210> SEQ ID NO 303

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 303

Ala Asp Arg Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Gln
1 5 10

<210> SEQ ID NO 304

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 304

Ala Asn Ser Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Glu
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<210> SEQ ID NO 305

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
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Ala Ile Leu Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp
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<210> SEQ ID NO 306
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Ala Asn Trp Phe Asp Pro Leu Thr Lys Leu Trp Leu Val Asp
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Ala Asn Trp Tyr Asp Ser Leu Thr Lys Leu Trp Leu Tyr Glu
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Ala Gly Trp Tyr Asp Ser Leu Thr Lys Leu Trp Leu Pro Asp
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Ala Val Trp Tyr Asp Ser Leu Thr Lys Leu Trp Leu Thr Asp
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Ala Asn Trp Tyr Asp Ala Leu Thr Lys Leu Trp Leu Pro Val
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<210> SEQ ID NO 335

<211> LENGTH: 14

<212> TYPE: PRT

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<212> TYPE: PRT

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<210> SEQ ID NO 337

<211> LENGTH: 14

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<210> SEQ ID NO 338

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<400> SEQUENCE: 339

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<223> OTHER INFORMATION: BLYS binding polypeptide

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<210> SEQ ID NO 342

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<211> LENGTH: 14

<212> TYPE: PRT

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<211> LENGTH: 14

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<210> SEQ ID NO 346

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<400> SEQUENCE: 348

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<400> SEQUENCE: 349

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1 5 10

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<400> SEQUENCE: 355

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<210> SEQ ID NO 356
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Ala Ser Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Pro Lys
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<210> SEQ ID NO 358
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<400> SEQUENCE: 359

Ala Ser Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Leu Glu
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<400> SEQUENCE: 361

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<400> SEQUENCE: 363

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Pro Asp

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<400> SEQUENCE: 364

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<210> SEQ ID NO 365
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<400> SEQUENCE: 365

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Pro His
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<210> SEQ ID NO 366
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<400> SEQUENCE: 366

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<210> SEQ ID NO 367
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<400> SEQUENCE: 368

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Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Pro Thr
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Ala Lys Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Pro Ala
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Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Phe Asp
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<400> SEQUENCE: 372

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Ala Asp
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<400> SEQUENCE: 374

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Ala Asp Trp Tyr Asp Pro Leu Thr Lys Leu Trp Val Pro Ala
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Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Val Pro Glu
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Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Val Pro Gln
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<220> FEATURE:
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1 5 10

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Ala Leu Trp Tyr Asp Pro Leu Thr Lys Leu Trp Val Pro Tyr
1 5 10

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Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Val Pro Gly
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Ala Asp Trp Tyr Asp Pro Leu Thr Lys Leu Trp Ile Pro Gly
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<210> SEQ ID NO 387
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<212> TYPE: PRT
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1 5 10

<210> SEQ ID NO 388
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 388

Ala Lys Trp Tyr Asp Pro Leu Thr Lys Leu Trp Ile Pro Tyr
1 5 10

<210> SEQ ID NO 389
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 389

Ala Ile Trp Tyr Asp Pro Leu Thr Lys Leu Trp Ile Pro Asn
1 5 10

<210> SEQ ID NO 390
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 390

Ala Thr Trp Tyr Asp Pro Leu Thr Lys Leu Trp Ile Pro Gln
1 5 10

<210> SEQ ID NO 391
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 391

Ala Ser Trp Tyr Asp Pro Leu Thr Asn Leu Trp Val Pro Asp
1 5 10

<210> SEQ ID NO 392
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 392

Ala Tyr Glu Tyr Asp Pro Leu Thr Asn Leu Trp Leu Leu Tyr
1 5 10

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<210> SEQ ID NO 393
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 393

Ala Tyr Trp Tyr Asp Pro Leu Thr Asn Leu Ser Leu Leu Val
1 5 10

<210> SEQ ID NO 394
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 394

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Ser Ile Leu Glu
1 5 10

<210> SEQ ID NO 395
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 395

Ala Asn Trp Tyr Asp Ser Leu Thr Lys Leu Trp Ile Pro Tyr
1 5 10

<210> SEQ ID NO 396
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 396

Ala His Trp Phe Asp Pro Leu Thr Gln Leu Lys Ile Arg Val
1 5 10

<210> SEQ ID NO 397
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 397

Ala Tyr Trp Cys Asp Pro Leu Thr Lys Leu Cys Ile Leu Glu
1 5 10

<210> SEQ ID NO 398
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 398

Ala Asn Ser Tyr Asp Pro Leu Thr Lys Leu Trp Phe Pro Tyr
1 5 10

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<210> SEQ ID NO 399
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 399

Ala Asn Leu Tyr Asp Pro Leu Thr Lys Leu Trp Val Pro Tyr
1 5 10

<210> SEQ ID NO 400
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 400

Ala Asn Trp Tyr Asp Ala Leu Thr Lys Leu Trp Leu His Asp
1 5 10

<210> SEQ ID NO 401
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 401

Ala Asn Trp Tyr Asp Ser Leu Thr Lys Leu Trp Phe Pro Asp
1 5 10

<210> SEQ ID NO 402
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 402

Ala Thr Ser Tyr Asp Ser Leu Thr Lys Leu Trp Leu Pro Ala
1 5 10

<210> SEQ ID NO 403
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 403

Ala Cys Trp Tyr Asp Ser Leu Thr Lys Leu Cys His Arg Glu
1 5 10

<210> SEQ ID NO 404
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 404

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Ala Ile Gly Asn Asp Pro Leu Thr Lys Leu Trp Ile Pro Tyr
1 5 10

<210> SEQ ID NO 405
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 405

Ala Asn Trp Gln Asp Cys Leu Thr Lys Leu Cys Leu Ala Gly
1 5 10

<210> SEQ ID NO 406
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 406

Ala Tyr Trp Phe Asp Pro Leu Thr Asn Leu Trp Leu Leu Glu
1 5 10

<210> SEQ ID NO 407
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 407

Ala Tyr Trp Tyr Asp Pro Leu Thr Asn Leu Ser Leu Leu Val
1 5 10

<210> SEQ ID NO 408
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 408

Ala Asn Cys Phe Asp Ser Leu Thr Arg Leu Trp Leu Cys Asp
1 5 10

<210> SEQ ID NO 409
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 409

Ala Cys Ala Tyr Asp Ala Leu Thr Lys Leu Cys Leu Pro Ala
1 5 10

<210> SEQ ID NO 410
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

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<400> SEQUENCE: 410

Ala Asn Trp Tyr Asp Pro Leu Thr Asn Leu Ser Leu Leu Leu
1 5 10

<210> SEQ ID NO 411

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 411

Ala Tyr Trp Tyr Asp Pro Leu Thr Gln Leu Ser Leu Leu Val
1 5 10

<210> SEQ ID NO 412

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 412

Ala Tyr Arg Tyr Asp Ala Leu Thr Gly Leu Trp Leu Leu Tyr
1 5 10

<210> SEQ ID NO 413

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 413

Ala Tyr Trp Asn Asp Pro Leu Thr Lys Leu Lys Leu Arg Leu
1 5 10

<210> SEQ ID NO 414

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 414

Ala Tyr Trp Tyr Asp Pro Leu Thr Gln Leu Ser Leu Leu Val
1 5 10

<210> SEQ ID NO 415

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 415

Ala Tyr Arg Tyr Asp Ala Leu Thr Gly Leu Trp Leu Leu Tyr
1 5 10

<210> SEQ ID NO 416

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 416

Ala Tyr Arg Tyr Asp Ser Leu Thr Asn Leu Trp Leu Leu Tyr
1 5 10

<210> SEQ ID NO 417

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 417

Ala Tyr Trp Tyr Asp Pro Leu Thr Lys Leu Ser Ile Leu Glu
1 5 10

<210> SEQ ID NO 418

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 418

Ala Ser Cys Tyr Asp Pro Leu Thr Lys Leu Cys Phe Pro Val
1 5 10

<210> SEQ ID NO 419

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 419

Ala Phe Trp Phe Asp Pro Leu Thr Gly Leu Trp Leu Leu Glu
1 5 10

<210> SEQ ID NO 420

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 420

Ala His Trp Tyr Asp Pro Leu Thr Lys Leu Ser Ile Arg Val
1 5 10

<210> SEQ ID NO 421

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 421

Ala Pro Trp Tyr Asp Ser Leu Thr Lys Leu Trp Phe Pro Ser
1 5 10

<210> SEQ ID NO 422

<211> LENGTH: 14

<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 422

Ala Asn Cys Tyr Asp Thr Leu Thr Lys Leu Trp Leu Thr Cys
1 5 10

<210> SEQ ID NO 423
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 423

Ala Asn Trp Tyr Asp Ser Leu Thr Lys Leu Ser Leu Pro Asp
1 5 10

<210> SEQ ID NO 424
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 424

Ala Tyr Ala Tyr Asp Phe Leu Thr Gln Leu Ser Leu Pro Asp
1 5 10

<210> SEQ ID NO 425
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 425

Ala Phe Arg Tyr Asp Ser Leu Thr Gly Leu Trp Leu Arg Tyr
1 5 10

<210> SEQ ID NO 426
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 426

Ala Asn Cys Tyr Asp Ser Leu Thr Lys Leu Trp Leu Pro Cys
1 5 10

<210> SEQ ID NO 427
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLys binding polypeptide

<400> SEQUENCE: 427

Ala Asn Gly Tyr Asp Leu Leu Thr Asn Leu Ser Val Ser Asp
1 5 10

<210> SEQ ID NO 428

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<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 428

Ala Asn Trp Tyr Asp Pro Leu Thr Arg Leu Trp Ile Pro Val
1 5 10

<210> SEQ ID NO 429
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 429

Ala Leu Lys Phe Asp Tyr Leu Thr Lys Leu Trp Leu Pro Asp
1 5 10

<210> SEQ ID NO 430
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 430

Ala Tyr Arg Tyr Asp Ser Leu Thr Lys Leu Trp Leu Pro Gly
1 5 10

<210> SEQ ID NO 431
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 431

Ala Tyr Cys Tyr Asp Ser Leu Thr Lys Leu Trp Ile Pro Asp
1 5 10

<210> SEQ ID NO 432
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 432

Ala Ser Trp Glu Asp Ser Leu Thr Lys Leu Trp Leu Ser Lys
1 5 10

<210> SEQ ID NO 433
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 433

Ala Tyr Trp Tyr Asp Ser Leu Thr Gly Leu Ser Leu Leu Val
1 5 10

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<210> SEQ ID NO 434
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 434

Ala Tyr Trp Tyr Asp Pro Leu Thr Tyr Leu Arg Leu Arg Val
1 5 10

<210> SEQ ID NO 435
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 435

Ala Lys Cys Tyr Asp Ser Leu Thr Asn Leu Trp Leu Cys Asp
1 5 10

<210> SEQ ID NO 436
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: core peptide of high affinity BLYS binders

<400> SEQUENCE: 436

Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu
1 5 10

<210> SEQ ID NO 437
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 437

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp Gly Gly
1 5 10 15

Lys

<210> SEQ ID NO 438
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 438

Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp Gly Gly Lys
1 5 10 15

<210> SEQ ID NO 439
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 439

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Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Gly Gly Lys
1 5 10

<210> SEQ ID NO 440
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 440

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Val Gly Gly
1 5 10 15

Lys

<210> SEQ ID NO 441
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 441

Ala Asn Trp Phe Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp Gly Gly
1 5 10 15

Lys

<210> SEQ ID NO 442
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 442

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Ser Leu Pro Asp Gly Gly
1 5 10 15

Lys

<210> SEQ ID NO 443
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 443

Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Trp Phe Pro Asp Gly Gly
1 5 10 15

Lys

<210> SEQ ID NO 444
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide

<400> SEQUENCE: 444

Ala Asn Trp Tyr Asp Ser Leu Thr Lys Leu Trp Leu Pro Asp Gly Gly
1 5 10 15

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Lys

<210> SEQ ID NO 445

<211> LENGTH: 585

<212> TYPE: PRT

<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 445

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Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1          5          10          15
Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
20          25          30
Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
35          40          45
Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
50          55          60
Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65          70          75          80
Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
85          90          95
Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100         105         110
Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
115         120         125
Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
130         135         140
Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145         150         155         160
Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
165         170         175
Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
180         185         190
Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
195         200         205
Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
210         215         220
Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225         230         235         240
Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
245         250         255
Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
260         265         270
Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
275         280         285
Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
290         295         300
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305         310         315         320
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
325         330         335
Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
340         345         350

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Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
 355 360 365
 Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
 370 375 380
 Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
 385 390 395 400
 Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
 405 410 415
 Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
 420 425 430
 Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
 435 440 445
 Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
 450 455 460
 Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
 465 470 475 480
 Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
 485 490 495
 Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
 500 505 510
 Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
 515 520 525
 Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
 530 535 540
 Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
 545 550 555 560
 Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
 565 570 575
 Ala Ala Ser Gln Ala Ala Leu Gly Leu
 580 585

<210> SEQ ID NO 446
 <211> LENGTH: 4
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: recurring structural motif of BLys binding
 polypeptides
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (2)..(2)
 <223> OTHER INFORMATION: X is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala
 <400> SEQUENCE: 446

Asp Xaa Leu Thr
 1

<210> SEQ ID NO 447
 <211> LENGTH: 14
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: BLys binding polypeptide
 <220> FEATURE:
 <221> NAME/KEY: SITE
 <222> LOCATION: (2)..(2)
 <223> OTHER INFORMATION: X2 is any amino acid except Arg
 <220> FEATURE:

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<221> NAME/KEY: SITE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X3 is Trp, Glu, Lys, Cys, Leu, Ala, Arg, Gly,
or Ser
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is Tyr, Phe, Glu, Cys, Asn
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X6 is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X9 is Lys, Asn, Gln, Gly, or Arg
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: X11 is Trp, Ser, Thr, Arg, Cys, Tyr, or Lys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: X12 is Leu, Phe, Val, Ile, or His
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: X13 is Pro, Leu, His, Ser, Arg, Asn, Gln, Thr,
Val, Ala, Cys, Ile, Phe, or Tyr
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: X14 is Asp, Glu, Asn, Val, His, Gln, Arg, Gly,
Ser, Tyr, Ala, Cys, Lys, Ile, Thr or Leu.

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<400> SEQUENCE: 447

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Ala Xaa Xaa Xaa Asp Xaa Leu Thr Xaa Leu Xaa Xaa Xaa Xaa
1           5           10

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<210> SEQ ID NO 448
<211> LENGTH: 10
<212> TYPE: PRP
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLYS binding polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: X1 is Trp, Glu, Lys, Cys, Leu, Ala, Arg, Gly,
or Ser
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: X2 is Tyr, Phe, Glu, Cys, Asn
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X4 is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: X7 is Lys, Asn, Gln, Gly, or Arg
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: X9 is Trp, Ser, Thr, Arg, Cys, Tyr, or Lys
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: X10 is Leu, Phe, Val, Ile, or His.

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<400> SEQUENCE: 448

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Xaa Xaa Asp Xaa Leu Thr Xaa Leu Xaa Xaa

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His Phe Ala Pro
20

<210> SEQ ID NO 453
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 453

Gly Asp Gly Gly Asn Cys Tyr Thr Asp Ser Leu Thr Lys Leu His Phe
1 5 10 15

Cys Met Gly Asp Glu
20

<210> SEQ ID NO 454
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 454

Gly Tyr Asp Val Leu Thr Lys Leu Tyr Phe Val Pro Gly Gly
1 5 10

<210> SEQ ID NO 455
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 455

Trp Thr Asp Ser Leu Thr Gly Leu Trp Phe Pro Asp Gly Gly
1 5 10

<210> SEQ ID NO 456
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 456

Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu Pro Asp
1 5 10

<210> SEQ ID NO 457
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLyS binding polypeptide

<400> SEQUENCE: 457

Trp Tyr Asp Pro Leu Thr Lys Leu Trp Leu
1 5 10

-continued

<210> SEQ ID NO 458
 <211> LENGTH: 14
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: BLYS binding polypeptide

 <400> SEQUENCE: 458

 Ala Asn Trp Tyr Asp Pro Leu Thr Lys Leu Ser Leu Pro Asp
 1 5 10

What is claimed is:

1. A method of treating, preventing or ameliorating a disease or disorder associated with aberrant B Lymphocyte Stimulator (BLYS) or BLYS receptor expression or activity, comprising administering to an animal in which such treatment, prevention or amelioration is desired, a BLYS binding polypeptide in an amount effective to treat, prevent or ameliorate the disease or disorder.

2. The method of claim 1, wherein the disease or disorder is an immune system disease or disorder.

3. The method of claim 2, wherein the immune system disease or disorder is an autoimmune disease or disorder.

wherein

X₁ is Ala, Asn, Lys, or Ser;

X₂ is Ala, Glu, Met, Ser, or Val;

X₃ is Ala, Asn, Lys, or Pro (preferably Lys);

X₅ is Phe, Trp, or Tyr (preferably Tyr);

X₇ is Pro or Tyr (preferably Pro);

X₁₁ is Ala, Gln, His, Phe, or Val;

X₁₂ is Asn, Gln, Gly, His, Ser, or Val; and

X₁₃ is Ala, Asn, Gly, Ile, Pro, or Ser;

(3) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-Cys-X₁₂-X₁₃-X₁₄, (SEQ ID NO:2)

4. The method of claim 2, wherein the immune system disease or disorder is an immunodeficiency.

5. The method of claim 3, wherein the autoimmune disease or disorder is lupus.

6. The method of claim 1, wherein the disease or disorder is glomerular nephritis.

7. The method of claim 2, wherein the immune system disease or disorder is rheumatoid arthritis, multiple sclerosis, hypogammaglobulinemia or hypergammaglobulinemia.

8. The method of claim 2 wherein the immune system disease or disorder is graft vs. host disease.

9. The method of claim 2, wherein the immune system disease or disorder is a proliferative disease or disorder.

10. The method of claim 9, wherein the proliferative disorder is cancer.

11. The method of claim 1, wherein the disease or disorder is an infectious disease or disorder.

12-68. (canceled)

69. The method according to claim 1, wherein the BLYS binding polypeptide comprises an amino acid sequence selected from the group consisting of:

(1) Asp-Xaa-Leu-Thr, (SEQ ID NO:446)

wherein Xaa is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala (preferably Pro or Ser);

wherein

X₁ is Ala, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, or is absent;

X₂ is Ala, Asn, Asp, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val;

X₃ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val (preferably Asp);

X₅ is Asp, Ile, Leu, or Tyr (preferably Asp or Leu);

X₆ is Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Pro, Tyr, or Val (preferably Glu or Leu);

X₇ is His, Leu, Lys, or Phe (preferably His or Leu);

X₈ is Leu, Pro, or Thr (preferably Thr or Pro);

X₉ is Arg, Asn, Gly, His, Ile, Lys, Met, or Trp (preferably Lys);

X₁₀ is Ala, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Trp, Tyr, or Val;

X₁₂ is Asp, Gln, Glu, Gly, Ile, Leu, Lys, Phe, Ser, Trp, Tyr, or Val;

X₁₃ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; and

(2) X₁-X₂-X₃-Cys-X₅-Phe-X₇-Trp-Glu-Cys-X₁₁-X₁₂-X₁₃, (SEQ ID NO:1)

X₁₄ is Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Trp, Tyr, Val, or is absent;

(4) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-Cys-X₁₃-X₁₄-X₁₅,

(SEQ ID NO:3)

wherein

X₁ is Ala, Arg, Asn, Asp, Leu, Lys, Phe, Pro, Ser, or Thr;

X₂ is Asn, Asp, Gln, His, Ile, Lys, Pro, Thr, or Trp;

X₃ is Ala, Arg, Asn, Gln, Glu, His, Phe, Pro, or Thr (preferably Ala);

X₅ is Asn, Asp, Pro, Ser, or Thr (preferably Asp);

X₁₂ is Ala, Arg, Asn, Gln, Leu, Met, Ser, Trp, Tyr, or Val;

X₁₄ is Asp, Gly, Leu, Phe, Tyr, or Val (preferably Leu);

X₁₅ is Asn, His, Leu, Pro, or Tyr (preferably His, Leu or Pro); and

X₁₆ is Asn, Asp, His, Phe, Ser, or Tyr, (preferably Asp or Ser);

(6) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃-X₁₄-Cys-X₁₆-X₁₇-X₁₈,

(SEQ ID NO:5)

X₆ is Arg, Asp, Ile, Leu, Met, Pro, or Val (preferably Ile);

X₇ is Ala, Ile, Leu, Pro, Thr, or Val (preferably Val or Leu);

X₈ is Asn, His, Ile, Leu, Lys, Phe, or Thr (preferably Thr);

X₉ is Asn, Glu, Gly, His, Leu, Lys, Met, Pro, or Thr (preferably Leu);

X₁₀ is Arg, Asn, Asp, Gln, Glu, Gly, Ile, Lys, Met, Pro, Ser, or Trp;

X₁₁ is Arg, Glu, Gly, Lys, Phe, Ser, Trp, or Tyr (preferably Ser);

X₁₃ is Gln, Glu, Ile, Leu, Phe, Pro, Ser, Tyr, or Val (preferably Val);

X₁₄ is Asn, Gly, Ile, Phe, Pro, Thr, Trp, or Tyr; and

X₁₅ is Asn, Asp, Glu, Leu, Lys, Met, Pro, or Thr (preferably Glu or Pro);

wherein

X₁ is Arg, Asp, Gly, His, Leu, Phe, Pro, Ser, Trp, Tyr, or is absent (preferably Arg);

X₂ is Ala, Arg, Asn, Asp, Gly, Pro, Ser, or is absent (preferably Asn, Asp, Gly, or Pro);

X₃ is Arg, Asn, Gln, Glu, Gly, Lys, Met, Pro, Trp or Val (preferably Gly or Met);

X₅ is Arg, Asn, Gln, Glu, His, Leu, Phe, Pro, Trp, Tyr, or Val preferably Trp, Tyr, or Val);

X₆ is Arg, Asp, Gln, Gly, Ile, Lys, Phe, Thr, Trp or Tyr (preferably Asp);

X₇ is Ala, Arg, Asp, Glu, Gly, Leu, Ser, or Tyr (preferably Asp);

X₈ is Asp, Gln, Glu, Leu, Met, Phe, Pro, Ser, or Tyr (preferably Leu);

X₉ is Asp, Leu, Pro, Thr, or Val (preferably Leu or Thr);

(5) X₁-X₂-X₃-Cys-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-Cys-X₁₄-X₁₅-X₁₆,

(SEQ ID NO:4)

wherein

X₁ is Asn, Asp, His, Leu, Phe, Pro, Ser, Tyr, or is absent (preferably Ser);

X₂ is Arg, Asn, Asp, His, Phe, Ser, or Trp (preferably Arg);

X₃ is Asn, Asp, Leu, Pro, Ser, or Val (preferably Asn or Asp);

X₅ is Asp, Gln, His, Ile, Leu, Lys, Met, Phe, or Thr;

X₆ is His, Ile, Leu, Met, Phe, Pro, Trp, or Tyr;

X₇ is Asp, His, Leu, or Ser (preferably Asp);

X₈ is Ala, Arg, Asp, Glu, Leu, Phe, Pro, or Thr (preferably Glu or Pro);

X₉ is Ala, Arg, Asn, or Leu (preferably Leu);

X₁₀ is Ile, Leu, Met, Pro, Ser, or Thr (preferably Thr);

X₁₁ is Ala, Arg, Asn, Gly, His, Lys, Ser, or Tyr;

X₁₀ is Arg, Gln, His, Ile, Leu, Lys, Met, Phe, Thr, Trp or Tyr (preferably Lys or Thr);

X₁₁ is Ala, Arg, Asn, Gln, Glu, His, Leu, Lys, Met, or Thr (preferably Arg or Leu);

X₁₂ is Ala, Asn, Gln, Gly, Leu, Lys, Phe, Pro, Thr, Trp, or Tyr (preferably Thr or Trp);

X₁₃ is Ala, Arg, Gln, His, Lys, Met, Phe, Pro, Thr, Trp, or Tyr (preferably Met or Phe);

X₁₄ is Arg, Gln, Glu, Gly, His, Leu, Met, Phe, Pro, Ser, Thr, Tyr, or Val (preferably Val);

X₁₆ is Arg, Asp, Gly, His, Lys, Met, Phe, Pro, Ser, or Trp (preferably Met);

X₁₇ is Arg, Asn, Asp, Gly, His, Phe, Pro, Ser, Trp or Tyr, (preferably Arg, His, or Tyr); and

X₁₈ is Ala, Arg, Asn, Asp, His, Leu, Phe, or Trp (preferably His or Asn);

(7) X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂, (SEQ ID NO:6)

wherein

X₁ is Ala, Arg, Gly, His, Leu, Lys, Met, Phe, Trp, Tyr, or Val (preferably Gly, Tyr, or Val);

X₂ is Ala, Arg, Gln, His, Ile, Leu, Phe, Thr, Trp, or Tyr (preferably His or Tyr);

X₃ is Ala, Asp, Lys, Phe, Thr, Trp or Tyr (preferably Asp or Tyr);

X₄ is Arg, Asp, Gln, Lys, Met, Phe, Pro, Ser, Tyr, or Val (preferably Asp or Gln);

X₅ is Asp, Leu, Lys, Phe, Pro, Ser, or Val (preferably Leu or Ser);

X₆ is His, Ile, Leu, Pro, Ser, or Thr (preferably Leu or Thr);

X₇ is Arg, Gly, His, Leu, Lys, Met, or Thr (preferably Lys or Thr);

X₈ is Ala, Arg, Asn, Ile, Leu, Lys, Met, or Thr (preferably Leu or Lys);

X₉ is Ala, Asn, Arg, Asp, Glu, Gly, His, Leu, Met, Ser, Trp, Tyr, or Val (preferably Met or Ser);

X₁₀ is Ile, Leu, Phe, Ser, Thr, Trp, Tyr, or Val (preferably Thr or Leu);

X₁₁ is Ala, Arg, Gly, His, Ile, Leu, Lys, Pro, Ser, Thr, Trp, Tyr, or Val (preferably Pro or Thr); and

X₁₂ is Arg, Asp, His, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val (preferably Arg or Pro);

X₁₁ is Ala, Glu, His, Ile, Leu, Met, Ser, Thr, Trp, Tyr, or Val (preferably Ala or Thr);

X₁₂ is Arg, Gln, Glu, Gly, His, Ile, Lys, Tyr, or Val (preferably Arg or His); and

X₁₃ is Arg, Asn, Glu, His, Ile, Ser, Thr, Trp, or Val (preferably His);

(9) Cys-X₂-Phe-X₄-Trp-Glu-Cys, (SEQ ID NO:8)

wherein

X₂ is Phe, Trp, or Tyr (preferably Tyr); and

X₄ is Pro or Tyr (preferably Pro);

(10) Cys-X₂-X₃-X₄-X₅-X₆-X₇-Cys, (SEQ ID NO:9)

wherein

X₂ is Asp, Ile, Leu, or Tyr (preferably Asp or Leu);

X₃ is Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Pro, Tyr, or Val (preferably Glu or Leu);

X₄ is His, Leu, Lys, or Phe (preferably His or Leu);

X₅ is Leu, Pro, or Thr (preferably Thr or Pro);

X₆ is Arg, Asn, Gly, His, Ile, Lys, Met, or Trp (preferably Lys); and

(8) X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃, (SEQ ID NO:7)

wherein

X₁ is Asp, Gln, Glu, Gly, His, Lys, Met, or Trp (preferably Glu or Lys);

X₂ is Arg, Gln, His, Ile, Leu, or Pro (preferably His or Pro);

X₃ is Asp, Gly, Ile, Lys, Thr, Tyr or Val (preferably Tyr);

X₄ is Asn, Asp, Gln, Glu, Met, Pro, Ser, or Tyr (preferably Asp or Gln);

X₅ is Asn, Asp, His, Ile, Leu, Met, Pro, Thr or Val (preferably Asn or Thr);

X₆ is Asp, Glu, His, Leu, Lys, Pro, or Val (preferably Asp or Pro);

X₇ is Arg, Asn, Gln, His, Ile, Leu, Met, Pro, or Thr (preferably Ile or Pro);

X₈ is Gln, Gly, His, Leu, Met, Ser, or Thr (preferably Leu or Thr);

X₉ is Asn, Gln, Gly, His, Leu, Lys, Ser, or Thr (preferably Lys);

X₁₀ is Ala, Gly, Ile, Leu, Lys, Met, or Phe (preferably Gly or Met);

X₇ is Ala, Asn, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Trp, Tyr, or Val;

(11) Cys-X₂-X₃-X₄-X₅-X₆-X₇-X₈-Cys, (SEQ ID NO:10)

wherein

X₂ is Asn, Asp, Pro, Ser, or Thr (preferably Asp);

X₃ is Arg, Asp, Ile, Leu, Met, Pro, or Val (preferably Ile);

X₄ is Ala, Ile, Leu, Pro, Thr, or Val (preferably Val or Leu);

X₅ is Asn, His, Ile, Leu, Lys, Phe, or Thr (preferably Thr);

X₆ is Asn, Glu, Gly, His, Leu, Lys, Met, Pro, or Thr (preferably Leu);

X₇ is Arg, Asn, Asp, Gln, Glu, Gly, Ile, Lys, Met, Pro, Ser, or Trp;

X₈ is Arg, Glu, Gly, Lys, Phe, Ser, Trp, or Tyr (preferably Ser);

(12) Cys-X2-X3-X4-X5-X6-X7-X8-X9-Cys, (SEQ ID NO:11)

wherein

X₂ is Asp, Gln, His, Ile, Leu, Lys, Met, Phe, or Thr;
 X₃ is His, Ile, Leu, Met, Phe, Pro, Trp, or Tyr;
 X₄ is Asp, His, Leu, or Ser (preferably Asp);
 X₅ is Ala, Arg, Asp, Glu, Leu, Phe, Pro, or Thr (preferably Glu or Pro);
 X₆ is Ala, Arg, Asn, or Leu (preferably Leu);
 X₇ is Ile, Leu, Met, Pro, Ser, or Thr (preferably Thr);
 X₈ is Ala, Arg, Asn, Gly, His, Lys, Ser, or Tyr;
 X₉ is Ala, Arg, Asn, Gln, Leu, Met, Ser, Trp, Tyr, or Val;

(13) Cys-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-Cys, (SEQ ID NO:12)

wherein

X₂ is Arg, Asn, Gln, Glu, His, Leu, Phe, Pro, Trp, Tyr, or Val (preferably Trp, Tyr, or Val);
 X₃ is Arg, Asp, Gln, Gly, Ile, Lys, Phe, Thr, Trp or Tyr (preferably Asp);
 X₄ is Ala, Arg, Asp, Glu, Gly, Leu, Ser, or Tyr (preferably Asp);
 X₅ is Asp, Gln, Glu, Leu, Met, Phe, Pro, Ser, or Tyr (preferably Leu);
 X₆ is Asp, Leu, Pro, Thr, or Val (preferably Leu or Thr);
 X₇ is Arg, Gln, His, Ile, Leu, Lys, Met, Phe, Thr, Trp or Tyr (preferably Lys or Thr);
 X₈ is Ala, Arg, Asn, Gln, Glu, His, Leu, Lys, Met, or Thr (preferably Arg or Leu);
 X₉ is Ala, Asn, Gln, Gly, Leu, Lys, Phe, Pro, Thr, Trp, or Tyr (preferably Thr or Trp);
 X₁₀ is Ala, Arg, Gln, His, Lys, Met, Phe, Pro, Thr, Trp, or Tyr (preferably Met or Phe);
 X₁₁ is Arg, Gln, Glu, Gly, His, Leu, Met, Phe, Pro, Ser, Thr, Tyr, or Val (preferably Val);

(14) Ala-X2-X3-X4-Asp-X6-Leu-Thr-X9-Leu-X11-X12-X13-X14, (SEQ ID NO:447)

wherein

X₂ is Asn, Ser, Tyr, Asp, Phe, Ile, Gln, His, Pro, Lys, Leu, Met, Thr, Val, Glu, Ala, Gly, Cys, or Trp (i.e., any amino acid except Arg; preferably Asn);

X₃ is Trp, Glu, Lys, Cys, Leu, Ala, Arg, Gly, or Ser (preferably Trp);

X₄ is Tyr, Phe, Glu, Cys, Asn (preferably Tyr);

X₆ is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala (preferably Pro or Ser);

X₉ is Lys, Asn, Gln, Gly, or Arg (preferably Lys);

X₁₁ is Trp, Ser, Thr, Arg, Cys, Tyr, or Lys (preferably Trp);

X₁₂ is Leu, Phe, Val, Ile, or His (preferably Leu);

X₁₃ is Pro, Leu, His, Ser, Arg, Asn, Gln, Thr, Val, Ala, Cys, Ile, Phe, or Tyr (i.e., not Asp, Glu, Gly, Lys, Met, or Trp; preferably Pro); and

X₁₄ is Asp, Glu, Asn, Val, His, Gln, Arg, Gly, Ser, Tyr, Ala, Cys, Lys, Ile, Thr or Leu (i.e., not Phe, Met, Pro, or Trp; preferably Asp); and,

(15) X1-X2-Asp-X4-Leu-Thr-X7-Leu-X9-X10, (SEQ ID NO:448)

wherein

X₁ is Trp, Glu, Lys, Cys, Leu, Ala, Arg, Gly, or Ser (preferably Trp);

X₂ is Tyr, Phe, Glu, Cys, Asn (preferably Tyr);

X₄ is Pro, Ser, Thr, Phe, Leu, Tyr, Cys, or Ala preferably Pro or Ser);

X₇ is Lys, Asn, Gln, Gly, or Arg (preferably Lys);

X₉ is Trp, Ser, Thr, Arg, Cys, Tyr, or Lys (preferably Trp); and

X₁₀ is Leu, Phe, Val, Ile, or His (preferably Leu).

70. The method according to claim 69, wherein the BLYS binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 20-168 and 186-435, as depicted in Tables 1-8 and 13.

71. The method according to claim 69, wherein the BLYS binding polypeptide comprises an amino acid sequence selected from the group consisting of: Ala-Gly-Lys-Glu-Pro-

Cys-Tyr-Phe-Tyr-Trp-Glu-Cys-Ala-Val-Ser-Gly (SEQ ID NO: 450); Ala-Gly-Val-Pro-Phe-Cys-Asp-Leu-Leu-Thr-Lys-His-Cys-Phe-Glu-Ala-Gly (SEQ ID NO: 451); Gly-Ser-Ser-Arg-Leu-Cys-His-Met-Asp-Glu-Leu-Thr-His-Val-Cys-Val-His-Phe-Ala-Pro (SEQ ID NO: 452); Gly-Asp-Gly-Gly-

Asn-Cys-Tyr-Thr-Asp-Ser-Leu-Thr-Lys-Leu-His-Phe-Cys-Met-Gly-Asp-Glu (SEQ ID NO: 453); Gly-Tyr-Asp-Val-Leu-Thr-Lys-Leu-Tyr-Phe-Val-Pro-Gly-Gly (SEQ ID NO: 454); Trp-Thr-Asp-Ser-Leu-Thr-Gly-Leu-Trp-Phe-Pro-Asp-Gly-Gly (SEQ ID NO: 455); Ala-Asn-Trp-Tyr-Asp-Pro-Leu-Thr-Lys-Leu-Trp-Leu-Pro-Asp (SEQ ID NO:186); Trp-Tyr-Asp-Pro-Leu-Thr-Lys-Leu-Trp-Leu-Pro-Asp (SEQ ID NO: 456); Trp-Tyr-Asp-Pro-Leu-Thr-Lys-Leu-Trp-Leu (SEQ ID NO: 457); Ala-Asn-Trp-Tyr-Asp-Pro-Leu-Thr-

Lys-Leu-Trp-Leu-Pro-Val (SEQ ID NO:189); Ala-Asn-Trp-Phe-Asp-Pro-Leu-Thr-Lys-Leu-Trp-Leu-Pro-Asp (SEQ ID NO: 309); Ala-Asn-Trp-Tyr-Asp-Pro-Leu-Thr-Lys-Leu-Ser-Leu-Pro-Asp (SEQ ID NO: 458); Ala-Asn-Trp-Tyr-Asp-Pro-Leu-Thr-Lys-Leu-Trp-Phe-Pro-Asp (SEQ ID NO: 353); Ala-Asn-Trp-Tyr-Asp-Ser-Leu-Thr-Lys-Leu-Trp-Leu-Pro-Asp (SEQ ID NO: 327).

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