ANTI-TYRP1 ANTIBODIES

CROSS-REFERENCE

This application claims the benefit of U.S. Provisional Application No. 61/069199 filed March 12, 2008.

FIELD OF THE INVENTION

The present invention is directed to human and chimeric antibodies, including fragments or portions thereof, that are specific to human tyrosinase-related protein-1 (TYRP1). The antibodies are used for treating growth of cancer cells and can be used alone or in combination with an anti-neoplastic agent or treatment.

BACKGROUND OF THE INVENTION

Human tyrosinase-related protein-1 (TYRP1), also known as gp75, (WO 91/14775) (SEQ ID NO: 28) is a melanosomal membrane glycoprotein involved in melanin biosynthesis. It is found predominantly within the melanosomes of melanocytes and is also found expressed on the cell surface of melanocytes and human melanomas.

The TYRP1 antigen is highly immunogenic. Antibodies and T-cells to TYRP1 have been identified in melanoma patients. It appears that both cellular and humoral responses are effective in eliminating melanoma *in vivo*. Adoptive transfer of melanoma reactive T-cells also results in tumor regression. Antibody response induced by TYRP1 vaccine also could inhibit melanoma growth and metastasis in animals.

Although a variety treatment options for melanoma have been studied including, small molecule inhibitors, chemotherapeutics, immunotherapies including vaccines (for example United States Patent 6,168,946), gene therapy/immunostimulants, and anti-angiogenics, at the present time, there are no effective therapies for patients with melanoma. Development of new treatments for this unmet medical need is highly warranted.

Animal studies have resulted in the discovery of the antibody TA99. TA99 (IgG2a), a murine monoclonal antibody (MAb) specific for human and murine TYRP1, localizes to subcutaneous human melanoma *in vivo*. *See* Welt et al., *Proc. Natl. Acad.*

Sci. USA 84: 4200-4204 (1987) and United States Patent 4,798,790. TA99 treatment inhibited tumor growth and metastasis in mice. *See* Takechi et al., *Clin Cancer Res. 2*:1837-42 (1996).

Mice treated with TA99 often lose hair color (depigmentation), suggesting destruction of melanocyte in the skin. The Fc receptor-mediated effector activation appears to play a critical role in the elimination of cells targeted by TA99. The anti-tumor effect of TA99 was dramatically reduced in FcR knockout mice. *See* Clynes et al., *Proc. Natl. Acad. Sci. USA 95*:652-656 (1998). However, the murine nature of TA99 means it would be precluded from use as a therapeutic in humans due to potential issues of immunogenicity and further its ability to activate downstream immune effector functions would be limited.

Thus there is a need to provide alternative anti-TYRP1 antibodies which are effective in the treatment of melanoma. The present invention provides alternative anti-TYRP1 antibodies that are effective in the treatment of melanoma.

Additionally, there is a need to provide alternative anti-TYRP1 antibodies which have improved binding affinity for TYRP1 compared with those antibodies known in the art. The present invention provides alternative anti-TYRP1 antibodies which have improved binding affinity for TYRP1 compared with those antibodies known in the art.

Further, there is a need to provide alternative anti-TYRP1 antibodies which have reduced immunogenicity in humans and improved ability to activate downstream immune effector functions such as antibody dependent cellular cytotoxicity (ADCC). The present invention provides chimeric and human anti-TYRP1 antibodies which have reduced immunogenicity in humans and an improved ability to activate downstream immune effector functions such as antibody dependent cellular cytotoxicity (ADCC) compared with those antibodies known in the art.

There also remains a need to provide alternative anti-TYRP1 antibodies which have improved stability via a reduction in protein misfolding and incorrect processing. Preferred antibodies of the present invention have improved stability via reduced protein misfolding and incorrect processing.

SUMMARY

In a first aspect, there is provided a monoclonal antibody or fragment thereof that specifically binds human TYRP1 (SEQ ID NO. 28) comprising a VH comprising the amino acid sequence:
QVQLVQSGSELKKPGASVKISCKASGYTFTSYAMNWVRQAPGQGLESMGWINT NTGNPTYAQGFTGRFVFSMDTSVSTAYLQISSLKAEDTAIYYCAPRYSSSWYLDY WGQGTLVTVSS (SEQ ID NO:14), a CDRL1 having the sequence RASQSVSSYLA (SEQ ID NO:4), a CDRL2 having the sequence DASNRAT (SEQ ID NO:5), and a CDRL3 having the sequence QQRSNWLMYT (SEQ ID NO:6).

In a second aspect, there is provided an isolated polynucleic acid comprising a nucleotide sequence encoding the antibody or fragment as defined above.

In a third aspect, there is provided an expression vector comprising the polynucleic acid as defined above operably linked to expression control elements such that the encoded antibody or fragment may be expressed.

In a fourth aspect, there is provided a recombinant cell comprising the expression vector as defined above, which recombinant cell is capable of producing an antibody or fragment as defined above.

In a fifth aspect, there is provided an antibody or fragment produced by culturing the recombinant cell as defined above so that the antibody or fragment is produced and recovering the antibody or fragment from the culture.

In a sixth aspect, there is provided a pharmaceutical composition comprising an antibody or fragment as defined above together with a pharmaceutically acceptable carrier, diluent or excipient.

In a seventh aspect, there is provided an antibody or fragment as defined above for use as a medicament.

In an eighth aspect, there is provided an antibody or fragment as defined above for use in the treatment of cancer.

In a ninth aspect, there is provided the use of an antibody or fragment as defined above in the manufacture of a medicament for treatment of cancer.

The present disclosure is directed to human and chimeric monoclonal antibodies, and fragments thereof, that bind to the melanoma antigen TYRP1 (SEQ ID NO:28).

One embodiment of the present disclosure is a monoclonal antibody that specifically binds human TYRP1 with a dissociation constant, K_D, at ambient laboratory temperature (20 °C – 25 °C), in the range from 0.1 x 10^{-9} M to 1.6 x 10^{-9} M, wherein said K_D values are determined by surface plasmon resonance. In other embodiments of the present disclosure the monoclonal antibody is chimeric or human. Fragments of such antibodies, which fragments retain the ability to specifically bind human TYRP1, are part of the disclosure even though dissociation constants for such fragments are not in the specified range.

In other embodiments of the present disclosure the monoclonal antibody specifically binds human TYRP1 with a K_D of about 0.1 x 10^{-9} M to about 1.2 x 10^{-9} M, about 0.1 x 10^{-9} M to about 0.8 x 10^{-9} M, about 0.1 x 10^{-9} M to about 0.4 x 10^{-9} M, about 0.2 x 10^{-9} M to about 1.2 x 10^{-9} M, about 0.2 x 10^{-9} M to about 0.8 x 10^{-9} M, about 0.2 x 10^{-9} M to about 0.4 x 10^{-9} M, about 0.2 x 10^{-9} M to about 0.3 x 10^{-9} M, or about 0.28 x 10^{-9} M.

One embodiment of the present disclosure is an antibody or fragment thereof that binds TYRP1 comprising a CDRH1 having the sequence GYTFTSYAMN (SEQ ID NO:1), a CDRH2 having the sequence WINTNTGNPTYAQGFTG (SEQ ID NO:2), a CDRH3 having the sequence RYSSSWYLDY (SEQ ID NO:3), a CDRL1 having the sequence RASQSVSSYLA (SEQ ID NO:4), a CDRL2 having the sequence DASNRAT (SEQ ID NO:5), and a CDRL3 having the sequence QQRSNWLMYT (SEQ ID NO:6), wherein said antibody further comprises an amino acid substitution within one of said CDR sequences. In another embodiment, the aforementioned CDRs do not have an amino acid substitution in one of the CDR sequences. In yet another embodiment, the antibody having the aforementioned CDRs specifically binds human TYRP1 with a dissociation constant K_D in the range from 0.1 x 10^{-9} M to 1.6 x 10^{-9} M.

In another embodiment of the present disclosure the antibody or fragment thereof that specifically binds TYRP1, comprising a CDRH1 having the sequence

GFNIKDYFLH (SEQ ID NO:7), a CDRH2 having the sequence WINPDNGNTVYDPKFQG (SEQ ID NO:8), a CDRH3 having the sequence DYTYEKAALDY (SEQ ID NO:9), a CDRL1 having the sequence RASGNIYNYLA (SEQ ID NO:10), a CDRL2 having the sequence DAKTLAD (SEQ ID NO:11), and a CDRL3 having the sequence QHFWSLPFT (SEQ ID NO:12), further comprises an amino acid substitution within one of said CDR sequences. In another embodiment, the aforementioned CDRs (SEQ ID NOs:7-12) do not have any amino acid substitutions.

Another embodiment of the present disclosure is an antibody or fragment thereof that binds TYRP1, and comprises

a VL having the sequence:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDA SNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWLMYTFGQG TKLEIK (SEQ ID NO: 16)

and a VH sequence of

QVQLVQSGSELKKPGASVKISCKASGYTFTSYAMNWVRQAPGQGLECMG WINTNTGNPTYAQGFTGRFVFSMDTSVSTAYLQISSLKAEDTAIYYCAPRY SSSWYLDYWGQGTLVTVSS (SEQ ID NO: 13)

or

QVQLVQSGSELKKPGASVKISCKASGYTFTSYAMNWVRQAPGQGLESMG WINTNTGNPTYAQGFTGRFVFSMDTSVSTAYLQISSLKAEDTAIYYCAPRY SSSWYLDYWGQGTLVTVSS (SEQ ID NO: 14).

Another embodiment of the present disclosure is a monoclonal antibody comprising a heavy chain of SEQ ID NO: 29 and a light chain of SEQ ID NO: 32; or a heavy chain of SEQ ID NO: 30 and a light chain of SEQ ID NO: 32. In one embodiment, an antibody comprises two heavy chains of SEQ ID NO: 29 and two light chains of SEQ ID NO: 32, or comprises two heavy chains of SEQ ID NO: 30 and two light chains of SEQ ID NO: 32. TYRP1 –binding fragments of such antibodies are part of the disclosure.

The present disclosure is also directed to isolated DNAs encoding such antibodies and portions thereof. Other embodiments of the present disclosure include: an isolated polynucleic acid comprising a nucleotide sequence encoding the antibody, or a fragment thereof; an expression vector comprising said nucleotide sequence linked to an expression

sequence or a recombinant host cell comprising said expression vector or a recombinant host cell or a progeny thereof, wherein said cell expresses the antibody, or fragment thereof. Yet another embodiment of the present disclosure is a method of producing or purifying an antibody, or fragment thereof, comprising culturing said cells under conditions permitting expression of the antibody or fragment thereof.

Additionally, the present disclosure is directed to methods of inhibiting growth of a cancer cell, and methods of treating melanoma, all in mammals, by administering an effective amount of antibody. One embodiment of the present disclosure is using the previously described antibodies or fragments thereof as a medicament. In yet another embodiment, the previously described antibodies or fragments thereof are to be used in the treatment of cancer, including but not limited to malignant melanoma.

In another embodiment, the disclosure provides the use of an antibody of the disclosure for the manufacture of a medicament for the treatment of cancer. In a preferred embodiment the cancer is malignant melanoma.

The antibodies may be used alone or in combination with an anti-neoplastic agent or treatment. One embodiment of the present disclosure is using the previously described antibodies in combination with an additional anti-cancer agent or treatment. In yet another embodiment, the anti-cancer agent is dacarbazine.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for human and chimeric antibodies, and fragments thereof, specific to the TYRP1 antigen, as well as isolated or purified polynucleotide sequences encoding the antibodies. Antibodies of the present invention can be used to treat neoplastic diseases, including solid and non-solid tumors, and for treatment of hyperproliferative disorders. The term antibody includes fragments that bind TYRP-1, unless otherwise noted. Binding parameters herein are for full-length antibodies, not fragments, which will necessarily have different binding parameters because of their different size.

Naturally occurring antibodies typically have two identical heavy chains and two identical light chains with each light chain covalently linked to a heavy chain by an inter-chain disulfide bond. Multiple disulfide bonds further link the two heavy chains to one

another. As used herein, the term "antibody" includes immunoglobulin molecules comprising 4 polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Individual chains can fold into domains having similar sizes (110-125 amino acids) and structures, but different functions.

The light chain can comprise one variable domain (abbreviated herein as VL) and/or one constant domain (abbreviated herein as CL). The light chains of antibodies (immunoglobulins) are either kappa (K) light chains or lambda (λ) light chains. The expression VL, as used herein, is intended to include both the variable regions from kappa-type light chains (VK) and from lambda-type light chains (Vλ). The light chain constant region is comprised of one domain, CL.

The heavy chain can also comprise one variable domain (abbreviated herein as VH) and/or, depending on the class or isotype of antibody, three or four constant domains (CH1, CH2, CH3 and CH4) (abbreviated herein collectively as CH). In humans, the isotypes are IgA, IgD, IgE, IgG, and IgM, with IgA and IgG further subdivided into subclasses or subtypes (IgA_{1-2} and IgG_{1-4}). The present invention includes antibodies of any of the aforementioned classes or subclasses (isotypes). Human IgG_1 is the preferred isotype for the antibodies of the present invention.

Generally, the variable domains show considerable amino acid sequence variability from one antibody to the next, particularly at the location of the antigen-binding site. Three regions, called hypervariable or complementarity-determining regions (CDRs), are found in each of VL and VH, which are supported by less variable regions called framework regions (FR). Amino acids are assigned to a particular CDR region or domain in accordance with Kabat convention (Kabat, *et al., Ann. NY Acad. Sci.* 190:382-93 (1971); Kabat, *et al., Sequences of Proteins of Immunological Interest,* Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

The portion of an antibody consisting of VL and VH domains is designated Fv (Fragment variable) and constitutes the antigen-binding site. Single chain Fv (scFv) is an antibody fragment containing a VL domain and a VH domain on one polypeptide chain,

wherein the N terminus of one domain and the C terminus of the other domain are joined by a flexible linker (see, e.g., U.S. Pat. No. 4,946,778 (Ladner et al.); WO 88/09344, (Huston et al.); WO 92/01047 (McCafferty et al.) describes the display of scFv fragments on the surface of soluble recombinant genetic display packages, such as bacteriophage.

The peptide linkers used to produce the single chain antibodies can be flexible peptides selected to assure that the proper three-dimensional folding of the VL and VH domains occurs. The linker is generally 10 to 50 amino acid residues. Preferably, the linker is 10 to 30 amino acid residues. More preferably the linker is 12 to 30 amino acid residues. The most preferable linker is 15 to 25 amino acid residues. An example of such linker peptides includes $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$.

An "isolated antibody" is an antibody that (1) has been partially, substantially, or fully purified from a mixture of components; (2) has been identified and separated and/or recovered from a component of its natural environment; (3) is monoclonal; (4) is free of other proteins from the same species; (5) is expressed by a cell from a different species; or (6) does not occur in nature. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Examples of isolated antibodies include an antibody that has been affinity purified, an antibody that has been made by a hybridoma or other cell line *in vitro*, and a human antibody derived from a transgenic mouse.

The term "monoclonal antibody," as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are substantially identical except for possible naturally occurring mutations or minor post-translational variations that may be present. Monoclonal antibodies are highly specific, being directed against a single antigenic site (also known as determinant or epitope). Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants, each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies,

and is not to be construed as requiring production of the antibody by any particular method.

The term "antibodies," as used herein, also includes "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species (*e.g.*, mouse or rat) or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity. *See, e.g.*, Morrison *et al.*, Proc. Natl. Acad. Sci. USA *81*:6851-6855 (1984)). Thus, the present invention includes, for example, chimeric antibodies comprising a chimeric heavy chain and/or a chimeric light chain. The chimeric heavy chain may comprise any of the heavy chain variable (VH) regions described herein or mutants or variants thereof fused to a heavy chain constant region of a non-human antibody. The chimeric light chain may comprise any of the light chain variable (VL) regions described herein or mutants or variants thereof fused to a light chain constant region of a non-human antibody.

The term "human antibody," as used herein, includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences (as described in Kabat, *et al.*, (1991) *Sequences of Proteins of Immunological Interest*, 5th Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs. The human antibody can have at least one position replaced with an amino acid residue, *e.g.*, an activity enhancing amino acid residue which is not encoded by the human germline immunoglobulin sequence. However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The phrase "recombinant human antibody" includes human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal that is transgenic for human immunoglobulin genes, or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences (*See*, Kabat, *et al.*, *supra.*).

Fc (Fragment crystallization) is the designation for the portion or fragment of an antibody that comprises paired heavy chain constant domains. In an IgG antibody, for example, the Fc comprises CH2 and CH3 domains. The Fc of an IgA or an IgM antibody further comprises a CH4 domain. The Fc is associated with Fc receptor binding, activation of complement-mediated cytotoxicity and ADCC. For antibodies such as IgA and IgM, which are complexes of multiple IgG like proteins, complex formation requires Fc constant domains.

Thus, antibodies of the invention include, but are not limited to, naturally occurring antibodies, antibodies, human antibodies, humanized antibodies, recombinant human antibodies, monoclonal antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof; each containing at least one CDR. Functional fragments include antigen binding fragments that bind to a TYRP1 antigen. For example, antibody fragments capable of binding to TYRP1 or a portion thereof, and which are embraced by the present invention include bivalent fragments such as $(Fab')_2$ with inter-chain disulfide bonds intact, monovalent fragments such as Fab (Fragment, antigen binding) which refers to the fragments of the antibody consisting of VL CL VL CH1 domains and do not retain the heavy chain hinge region (*e.g.*, by papain digestion), fabs which retain the heavy chain hinge region, facb (*e.g.*, by plasmin digestion), $F(ab')_2$, Fab' which lack disulfide bonds, pFc' (*e.g.*, by pepsin or plasmin digestion), Fd (*e.g.*, by pepsin digestion, partial reduction and re-aggregation) and Fv or scFv (*e.g.*, by molecular biology techniques). Antibody

fragments are also intended to include, *e.g.*, domain deleted antibodies, linear antibodies, single chain antibodies, scFv, single domain antibodies, multivalent single chain antibodies, multi-specific antibodies formed from antibody fragments including diabodies, triabodies, and the like that bind specifically with antigens.

The hinge region separates the Fab and Fc portions of the antibody, providing for mobility of Fabs relative to each other and relative to Fc, as well as including multiple disulfide bonds for covalent linkage of the two heavy chains.

The antibodies, or fragments thereof, of the present invention are specific for TYRP1. Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Antibodies, or fragments thereof, of the present invention, for example, can be mono-specific, bi-specific, or multi-specific. Bi-specific antibodies (BsAbs) are antibodies that have two different antigen-binding specificities or sites. Multi-specific antibodies have more than two different antigen-binding specificities or sites. Where an antibody has more than one specificity, the recognized epitopes can be associated with a single antigen or with more than one antigen. Thus, the present invention provides bi-specific antibodies, or fragments thereof, that bind to two different antigens, with at least one specificity for TYRP1.

The present invention provides isolated antibodies or fragments thereof specific for TYRP1. The TYRP1 protein is mammalian, and is preferably human. In an especially preferred embodiment, an antibody of the invention is capable of binding to both human TYRP1 and murine TYRP1 [Shibahara et al., Nucleic Acids Res. 14(6) 2413-2427 (1986)] and is hence useful in both both pre-clinical and clinical *in vivo* studies. The antibodies of the invention are capable of one or more of the following activities: 1) displaying high affinity binding towards TYRP1 exclusively; and 2) inhibiting tumor growth *in vitro* and *in vivo*.

Specificity of the present antibodies, or fragments thereof, for TYRP1 can be determined based on affinity and/or avidity. Affinity, represented by the equilibrium constant for the dissociation of an antigen with an antibody (K_D), measures the binding strength between an antigenic determinant and an antibody-binding site.

Antibodies of the present invention, or fragments thereof, also include those for which binding characteristics have been modified or improved by direct mutation,

methods of affinity maturation, phage display, or chain shuffling. Affinity and specificity can be modified or improved by mutating CDRs and screening for antigen binding sites having the desired characteristics (see, e.g., Yang et al., J. Mol. Biol., (1995) 254: 392-403). CDRs are mutated in a variety of ways. One way is to randomize individual residues or combinations of residues so that in a population of otherwise identical antigen binding sites, all twenty amino acids are found at particular positions. Alternatively, mutations are induced over a range of CDR residues by error prone PCR methods (see, e.g., Hawkins et al., J. Mol. Biol., (1992) 226: 889-896). For example, phage display vectors containing heavy and light chain variable region genes can be propagated in mutator strains of *E. coli* (see, e.g., Low et al., J. Mol. Biol., (1996) 250: 359-368). These methods of mutagenesis are illustrative of the many methods known to one of skill in the art.

A convenient way for generating substitutional variants is affinity maturation using phage display. Briefly, several CDR region sites are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity, specificity, IC50, EC50, K_D) as herein disclosed. In order to identify candidate CDR region sites for modification, alanine scanning mutagenesis can be performed to identify CDR region residues contributing significantly to antigen binding. Alternatively, or in addition, random mutagenesis may be performed on one or more CDR sequences at one or more residue positions, either while the CDR is operably linked to the variable region or while the CDR is independent of other variable region sequence and then the altered CDR returned to a variable region using recombinant DNA technology. Once such variant antibodies are generated and expressed, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

As described herein, in addition to the antibodies specifically described herein, other "substantially homologous" modified antibodies can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled

in the art. For example, the framework regions can vary from the native sequences at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis.

The present invention includes TYRP1-binding polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the described full-length anti-TYRP1 antibodies. Substantially the same amino acid sequence is defined herein as a sequence with at least 70%, preferably at least about 80%, and more preferably at least about 90%, 95%, 96%, 97%, 98%, or 99% homology to another amino acid sequence, as determined by the FASTA search method in accordance with Pearson and Lipman (*Proc. Natl. Acad. Sci. USA* (1988) 85:2444-8).

The antibodies of the present invention, or fragments thereof, include human antibodies having one, two, three, four, five, and/or six complementarity determining regions (CDRs) selected from the group consisting of the amino acid sequences of the CDRs as set forth in Table 1.

In another embodiment, the present antibodies, or fragments thereof, can have a heavy chain variable region of 20D7 or 20D7S and/or a light chain variable region of 20D7 or 20D7S, outlined below. 20D7 and 20D7S are particularly preferred antibodies of the present invention. These antibodies have human VH and VL framework regions (FRs) as well as human CDRs.

The present invention includes nucleic acid sequences that encode an anti-TYRP1 antibody heavy chain, comprising any one of the VH regions or a portion thereof, or any one of the VH CDRs, including any variants thereof, as disclosed herein. The invention also includes nucleic acid molecules that encode an anti-TYRP1 antibody light chain comprising any one of the VL regions or a portion thereof or any one of the VL CDRs, including any variants thereof as disclosed herein.

Each domain of the antibodies of this invention can be a complete antibody with the heavy or light chain variable domain, or it can be a functional equivalent or a mutant or derivative of a naturally-occurring domain, or a synthetic domain constructed, for example, *in vitro* using a technique such as one described in WO 93/11236 (Griffiths et al.). For instance, it is possible to join together domains corresponding to antibody variable domains, which are missing at least one amino acid. Also included is an antibody with one or more amino acid substitution, mutation or deletion within one of the CDR sequences. The important characterizing feature is the ability of each domain to associate with a complementary domain to form an antigen-binding site. Accordingly, the terms variable heavy and light chain fragment should not be construed to exclude variants, including variants to the CDRs, that do not have a material effect on specificity.

The antibodies of the present invention may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein, *Nature 256*: 495-497 (1975) and Campbell, Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas, Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA method described by Huse et al., *Science 246*: 1275-1281 (1989).

Antibody fragments can be produced by cleaving a whole antibody, or by expressing DNA that encodes the fragment. Fragments of antibodies may be prepared by methods described by Lamoyi et al., *J. Immunol. Methods* 56: 235-243 (1983) and by Parham, *J. Immunol.* 131: 2895-2902 (1983). Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Such fragments may also contain single-chain fragment variable region antibodies, i.e. scFv, diabodies, or other antibody fragments. Methods of producing such functional equivalents are disclosed in PCT Application WO 93/21319, European Patent Application No. 239,400; PCT Application WO 89/09622; European Patent Application 338,745; and European Patent Application EP 332,424.

Preferred host cells for transformation of vectors and expression of the antibodies of the present invention are mammalian cells, e.g., NSO cells (non-secreting (0) mouse myeloma cells), 293 and CHO cells and other cell lines of lymphoid origin such as

lymphoma, myeloma, or hybridoma cells. Other eukaryotic hosts, such as yeasts, can be alternatively used.

The transformed host cells are cultured by methods known in the art in a liquid medium containing assimilable sources of carbon (carbohydrates such as glucose or lactose), nitrogen (amino acids, peptides, proteins or their degradation products such as peptones, ammonium salts or the like), and inorganic salts (sulfates, phosphates and/or carbonates of sodium, potassium, magnesium and calcium). The medium furthermore contains, for example, growth-promoting substances, such as trace elements, for example iron, zinc, manganese and the like.

Where it is desired to express a gene construct in yeast, a suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7. Stinchcomb et al. *Nature*, 282: 39 (1979); Kingsman et al., *Gene*, 7: 141 (1979). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics*, 85: 12 (1977). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

The antibodies of the invention may be isolated or purified by any method known in the art, including precipitation by ammonium sulfate or sodium sulfate followed by dialysis against saline, ion exchange chromatography, affinity or immuno-affinity chromatography as well as gel filtration or zone electrophoresis. A preferred method of purification for the antibodies of the current invention is Protein-A affinity chromatography.

DNA encoding human antibodies can be prepared by recombining DNA encoding human constant regions and variable regions, other than the CDRs, derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived from a human.

Suitable sources of DNAs that encode fragments of antibodies include any cell, such as hybridomas and spleen cells, which express the full-length antibody. The fragments may be used by themselves as antibody equivalents, or may be recombined into

equivalents, as described above. The DNA deletions and recombinations described in this section may be carried out by well-known methods. Another source of DNA is a phage display library of antibodies, as is known in the art. The exemplified antibodies of the current invention were made via hybridoma technology from immunized mice.

Additionally, the present invention provides expression vectors containing the polynucleotide sequences previously described operably linked to an expression sequence, a promoter and an enhancer sequence. A variety of expression vectors for the efficient synthesis of antibody polypeptide in prokaryotic systems, such as bacteria, and eukaryotic systems, including but not limited to yeast and mammalian cell culture systems, have been developed. The vectors of the present invention can comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences.

Any suitable expression vector can be used. For example, prokaryotic cloning vectors include plasmids from *E. coli*, such as *colE1*, *pCR1*, *pBR322*, *pMB9*, *pUC*, *pKSM*, and *RP4*. Prokaryotic vectors also include derivatives of phage DNA such as *M13* and other filamentous single-stranded DNA phages. An example of a vector useful in yeast is the 2μ plasmid. Suitable vectors for expression in mammalian cells include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and shuttle vectors derived from combination of functional mammalian vectors, such as those described above, and functional plasmids and phage DNA.

Additional eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, *J. Mol. Appl. Genet. 1*: 327-341 (1982); Subramani et al., *Mol. Cell. Biol. 1*: 854-864 (1981); Kaufmann and Sharp, *J. Mol. Biol. 159*: 601-664 (1982); Scahill et al., *Proc. Natl. Acad. Sci. USA 80*: 4654-4659 (1983); and Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA 77*: 4216-4220 (1980).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the *lac* system, the *trp* system, the *tac* system, the *trc* system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the

promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

The present invention also provides recombinant host cells containing the expression vectors previously described. Antibodies of the present invention can be expressed in cell lines other than in hybridomas. Nucleic acids, which comprise a sequence encoding a polypeptide according to the invention, can be used for transformation of a suitable mammalian host cell.

Cell lines of particular preference are selected based on high level of expression, constitutive expression of protein of interest and minimal contamination from host proteins. Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines, such as but not limited to, NSO cells (non-secreting (0) mouse myeloma cells), mouse myeloma cells, Chinese Hamster Ovary (CHO) cells, Baby Hamster Kidney (BHK) cells and many others. Suitable additional eukaryotic cells include yeast and other fungi. Useful prokaryotic hosts include, for example, *E. coli*, such as *E. coli* SG-936, *E. coli* HB 101, *E. coli* W3110, *E. coli* X1776, *E. coli* X2282, *E. coli* DHI, and *E. coli* MRCl, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis*, and *Streptomyces*.

These present recombinant host cells can be used to produce an antibody, or fragment thereof, by culturing the cells under conditions permitting expression of the antibody or fragment thereof and purifying the antibody or fragment thereof from the host cell or medium surrounding the host cell. Targeting of the expressed antibody or fragment for secretion in the recombinant host cells can be facilitated by inserting a signal or secretory leader peptide-encoding sequence (see, Shokri et al., (2003) Appl Microbiol Biotechnol. 60(6):654-64, Nielsen et al., Prot. Eng. (1997) 10:1-6 and von Heinje et al., (1986) Nucl. Acids Res. 14:4683-4690) at the 5' end of the antibody-encoding gene of interest. These secretory leader peptide elements can be derived from either prokaryotic or eukaryotic sequences. Accordingly suitably, secretory leader peptides are used, being

amino acids joined to the N-terminal end of a polypeptide to direct movement of the polypeptide out of the host cell cytosol and secretion into the medium.

The antibodies of this invention can be fused to additional amino acid residues. Such amino acid residues can be a peptide tag, perhaps to facilitate isolation. Other amino acid residues for homing of the antibodies to specific organs or tissues are also contemplated.

Another embodiment for the preparation of antibodies in the present invention is the expression of the nucleic acid encoding the antibody according to the invention in a transgenic animal that has a substantial portion of the human antibody producing genome inserted and is rendered deficient in the production of endogenous antibodies. Transgenic animals include, but are not limited to mice, goat, and rabbit. One further embodiment of the invention includes expression of the antibody-coding gene in, for example, the mammary gland of the animal for secretion of the polypeptide during lactation.

A method of treating tumor growth in a mammal by administering to the mammal an effective amount of an antibody is also provided by the present invention. Suitable tumors to be treated according to the present invention preferably express TYRP1. While not intended to be bound to any particular mechanism, the present methods provide for treatment of the growth of cancer cells including malignant melanoma. "Treatment" or "treat", in the context of the present invention refers to therapeutic treatment including inhibiting, slowing, lessening or reversing the progress of the underlying condition or undesired physiological change associated with a disease or disorder, ameliorating clinical symptoms of a condition or preventing the appearance of clinical symptoms of the condition. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of the extent of a disease or disorder, stabilization of a disease or disorder (*i.e.*, where the disease or disorder does not worsen), delay or slowing of the progression of a disease or disorder, amelioration or palliation of the disease or disorder, and remission (whether partial or total) of the disease or disorder, whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the disease. In one embodiment, the present invention can be used as a medicament.

The term "melanoma" includes, but is not limited to, melanomas, metastatic melanomas, melanomas derived from either melanocytes or melanocyte related nevus cells, melanocarcinomas, melanoepitheliomas, melanosarcomas, melanoma *in situ*, superficial spreading melanoma, modular melanoma, lentigo malignant melanoma, acral lentiginous melanoma, invasive melanoma and familial atypical mole and melanoma (FAM-M) syndrome. In one embodiment of the invention, melanoma is a specific form of cancer. In another embodiment the melanoma can be malignant. One embodiment of the present invention would be to utilize, as described below, the presently described anti-TYRP1 antibodies to treat first line melanoma. In another embodiment, the presently described anti-TYRP1 antibodies would be first line treatment for metastatic melanoma, that is, they would be used in a first round of treatment of newly diagnosed metastatic melanoma.

In the methods of the present invention, a therapeutically effective amount of an antibody of the invention is administered to a mammal in need thereof. Effective doses of the compositions of the present invention, for treatment of disorders as described herein vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. The term administering as used herein means delivering the antibodies of the present invention to a mammal by any method that can achieve the result sought. They can be administered, for example, intravenously or intramuscularly. Although human antibodies of the invention are particularly useful for administration to humans, they can be administered to other mammals as well. The term mammal as used herein is intended to include, but is not limited to, humans, laboratory animals, domestic pets and farm animals. Therapeutically effective amount means an amount of antibody of the present invention that, when administered to a mammal, is effective in producing the desired therapeutic effect, such as inhibiting tumor growth. Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" of an anti-TYRP1 antibody of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time

necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

The identification of such disease is well within the ability and knowledge of one skilled in the art. For example, human individuals who are suffering from malignant melanoma or who are at risk of developing clinically significant symptoms are suitable for administration of the present anti-TYRP1 antibodies.

The present anti-TYRP1 antibodies are administered for therapeutic treatments to a patient suffering from malignant melanoma in an amount sufficient to inhibit, or reduce the progression of the tumor or pathologic condition. Progression includes, e.g., the growth, invasiveness, metastases and/or recurrence of the tumor or pathologic condition. An amount adequate to accomplish this is defined as a therapeutically effective dose. Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's own immune system. Dosing schedules will also vary with the disease state and status of the patient, and will typically range from a single bolus dosage or continuous infusion to multiple administrations per day (e.g., every 4-6 hours), or as indicated by the treating physician and the patient's condition. An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the invention is 0.1-50 mg/kg, more preferably 3-35 mg/kg, and more preferably 5-20 mg/kg. Dosing amounts and frequencies will be determined by the physicians treating the patient and may include doses from less than 1 mg/kg to over 100 mg/kg given daily, three times per week, weekly, once every two weeks, or less often. Dose per administration may be in the range of 1-100, 2-75, or 5-60 mg/kg. It should be noted, however, that the present invention is not limited to any particular dose.

In an embodiment of the invention, anti-TYRP1 antibodies can be administered in combination with one or more other anti-neoplastic agents. Any suitable anti-neoplastic agent can be used, such as a chemotherapeutic agent, radiation or combinations thereof.

The anti-neoplastic agent can be an alkylating agent or an anti-metabolite. Examples of alkylating agents include, but are not limited to, cisplatin, cyclophosphamide, melphalan, and dacarbazine (DTIC). In vivo studies indicate that administering 20D7 in combination with dacarbazine (DTIC) demonstrate stronger anti-tumor activity compared to mono-therapy on a human 624mel xenograft. In one embodiment of the present invention, the presently described anti-TYRP1 antibodies are given in combination with dacarbazine. Examples of anti-metabolites include, but are not limited to, doxorubicin, daunorubicin, paclitaxel, irinotecan (CPT-11), and topotecan. When the anti-neoplastic agent is radiation, the source of the radiation can be either external (external beam radiation therapy – EBRT) or internal (brachytherapy – BT) to the patient being treated. The dose of anti-neoplastic agent administered depends on numerous factors, including, for example, the type of agent, the type and severity of the tumor being treated and the route of administration of the agent. It should be emphasized, however, that the present invention is not limited to any particular dose.

In the present invention, any suitable method or route can be used to administer anti- TYRP1 antibodies of the invention, and optionally, to co-administer anti-neoplastic agents and/or antagonists of other receptors. The anti-neoplastic agent regimens utilized according to the invention, include any regimen believed to be optimally suitable for the treatment of the patient's neoplastic condition. Different malignancies can require use of specific anti-tumor antibodies and specific anti-neoplastic agents, which will be determined on a patient to patient basis. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. Parenteral routes are preferred. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

In another aspect of the invention, an anti-TYRP1 antibody of the invention can be chemically or biosynthetically linked to one or more anti-neoplastic or anti-angiogenic agents.

The invention further contemplates anti-TYRP1 antibodies linked to target or reporter moieties, including by way of example only anti-neoplastic agents, other antibodies or reporters, such as radiolabled isotopes, in a diagnostic system where a detectable signal-producing agent is conjugated to the antibody.

It is understood that the anti-TYRP1 antibodies of the invention, where used in a mammal for the purpose of prophylaxis or treatment, will be administered in the form of a composition additionally comprising a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. Pharmaceutically acceptable carriers can further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the binding proteins. The compositions of the injection can, as is well known in the art, be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the mammal.

Moreover, included within the scope of the present invention is use of the present antibodies *in vivo* and *in vitro* for investigative or diagnostic methods, which are well known in the art. The diagnostic methods include kits, which contain antibodies of the present invention.

In one embodiment, the invention is a human monoclonal antibody, or fragment thereof, specific for TYRP1. In another embodiment, the invention is a chimeric monoclonal antibody, or fragment thereof, specific for TYRP1. In another embodiment the CDR regions of the antibody are identical to the CDR regions of CTA99. In a different embodiment the CDR regions of the antibody are identical to the CDR regions of 20D7 or 20D7S.

In one embodiment, the antibody binds to TYRP1 with a dissociation rate constant (K_d or k_{off}) between 1.7 x 10^{-4} 1/s (sec^{-1}, 1/seconds) and 5 x 10^{-4} 1/s, as measured by surface plasmon resonance, described herein, at ambient laboratory temperature (20 °C – 25 °C). In another embodiment, the antibody binds to TYRP1 with a K_d or k_{off} between 1.7 x 10^{-4} 1/s and 3.5 x 10^{-4} 1/s. In a further embodiment, the antibody binds to TYRP1 with a dissociation rate constant, as measured by surface plasmon resonance, that is within 10% of the dissociation rate constant determined for 20D7, 20D7S, or CTA99 under the same conditions.

One embodiment of the present invention comprises a monoclonal antibody, or fragment thereof, specific for TYRP1 comprising one or more complementarity determining regions (CDRs) selected from the group consisting of the CDRs in Tables 1 and 2. In another embodiment, the invention is a monoclonal antibody, or fragment thereof, specific for TYRP1 having a light chain CDR1 region with the sequence: RASQSVSSYLA (SEQ ID NO:4). In another embodiment, the invention is a monoclonal antibody, or fragment thereof, specific for TYRP1 having a heavy chain CDR3 with the sequence: RYSSSWYLDY (SEQ ID NO:3). In a different embodiment, the invention is a monoclonal antibody, or fragment thereof, comprising (i) a light chain variable region selected from the group consisting of 20D7, 20D7S and CTA99 and (ii) a heavy chain variable region selected from the group consisting of 20D7, 20D7S and CTA99. In another embodiment, the invention is a monoclonal antibody, or fragment thereof, specific for TYRP1 comprising (i) a light chain variable region of CTA99 (ii) a heavy chain variable region of CTA99, and (iii) human immunoglobulin G_1 ($hIgG_1$) constant regions.

Another embodiment of this invention is a method of treating cancer in a mammal comprising administering to the mammal an effective amount of an antibody, or fragment thereof, of any of the embodiments already described. The invention also provides a method to treat a malignant melanoma. Another treatment method provided by this invention combines using the antibodies or fragments thereof of this invention along with administering an additional anti-cancer agent or treatment. In one treatment method, the anti-cancer agent is dacarbazine (DTIC).

It is to be understood and expected that variations in the principles of invention herein disclosed can be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

All references mentioned herein are incorporated in their entirety.

EXAMPLES

The examples that follow further illustrate the invention, but should not be construed to limit the scope in any way. They should in no way be construed, however, as limiting the broad scope of the invention. Detailed description of conventional

methods, such as those employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, the introduction of plasmids into host cells, and the expression and determination thereof of genes and gene products can be obtained from numerous publications, including Sambrook, J. et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press.

Animals and Cell Lines

Maintain SKmel28, SKmel23, 624mel, 1102mel and A375 in RPMI 1640 (Invitrogen Life Technologies) with 10% heat-inactivated fetal bovine serum (HyClone Laboratories, Logan, UT) and routinely test for Mycoplasma contamination. SKmel23 and SKmel28 were provided by Dr. Alan Houghton (Memorial Sloan-Kettering Cancer Center, New York, NY). 624mel and 1102mel were obtained from Dr. Steve Rosenberg (National Cancer Institute, Bethesda, MD). A375 was purchased from American Type Culture Collection (Manassas, VA). Six to eight week old female Nu/Nu mice were purchased from Taconic Farms (Germantown, NY).

Expression and Purification of Human and Chimeric Anti-TYRP1 Antibodies

For each antibody, engineer a suitable heavy chain nucleotide sequence, for example SEQ ID NOs 21, 22, or 23 (for 20D7, 20D7S and CTA99 respectively) into a suitable expression plasmid, for example pGSHC, and engineer a suitable light chain nucleotide sequence, for example SEQ ID No. 26 or 27 (for 20D7/20D7S and CTA99 respectively) into a suitable expression plasmid, such as pGSLC, by a suitable method such as PCR cloning. To establish a stable cell line, co-transfect in a suitable host cell line, such as NSO cells, with linearized heavy and light chain plasmids by electroporation and culture in suitable media such as glutamine free Dulbecco's Modified Eagle Medium with dialyzed fetal calf serum and glutamine synthetase supplement. Screen clones for antibody expression by an enzyme-linked immunosorbent assay (ELISA) and select the highest producer for culture in spinner flasks. Purify antibodies by a suitable method such as protein-A affinity chromatography.

One embodiment of the present invention is the recombinant human monoclonal antibody 20D7, a full length IgG1κ targeting cell surface expressed tyrosinase-related protein-1 (TYRP1 or TRP1). The antibody is comprised of a human gamma-1 heavy chain (HC) (subgroup I) and a human kappa light chain (subgroup III). 20D7 was shown to selectively bind to human TYRP1 with high affinity and mediated potent anti-tumor activity in xenograft models by a mechanism involving activation of immune effector function.

One embodiment of the present invention is the recombinant human monoclonal antibody 20D7S, a full length IgG1κ targeting cell surface expressed tyrosinase-related protein-1 (TYRP1 or TRP1). The antibody is comprised of a human gamma-1 heavy chain (HC) (subgroup I) and a human kappa light chain (subgroup III). 20D7S was created in an effort to generate an even more stable molecule; a free cysteine residue within the heavy chain variable region (C47) was converted to a serine residue by site-directed mutagenesis. This unpaired or free cysteine has the potential to mis-pair with other cysteines that participate in intra- and inter-chain disulfide bridging of heavy and light chain polypeptides. Mis-pairing could potentially result in improper folding and processing, increasing the heterogeneity of the product and potentially its stability. The SDS PAGE gel analysis described herein confirms the presence of free light chain and free heavy chain in the preparation of 20D7, but the presence of free light chains and free heavy chains are reduced or eliminated in the preparation of 20D7S.

Another embodiment of the present invention is CTA99, a chimeric antibody with a human constant region IgG1. Murine antibody TA99 (United States Patent 4,798,790) contains two light chains; one TA99 light chain is specific to TYRP1 and the other is from parental mouse myeloma cells. There is decreased activity because the contaminated light chain is unable to bind to TYRP1. CTA99 was constructed to remediate this flaw and thereby improve activity. In one embodiment of the present invention, CTA99 was designed with two identical heavy chains and two identical light chains. Studies described herein illustrate CTA99's increased activity and binding affinity, as well as effector functions more suitable for humans.

Tables 1 and 2 provide the amino acid sequences and SEQ ID NOs of the various CDRs of the present invention. Table 3 provides the SEQ ID NOs of the various

sequences related to the present invention. Polynucleic acid sequences that encode the amino acid sequences disclosed below are also included within the scope of the present invention.

Table 1: Amino Acid Sequence of 20D7 and 20D7S Antibody Heavy and Light Chain Variable Region CDRs.

	Heavy Chain	SEQ ID NO.	Light Chain	SEQ ID NO.
CDR1	GYTFTSYAMN	1	RASQSVSSYLA	4
CDR2	WINTNTGNPTYAQGFTG	2	DASNRAT	5
CDR3	RYSSSWYLDY	3	QQRSNWLMYT	6

Table 2: Amino Acid Sequence of CTA99 Antibody Heavy and Light Chain Variable Region CDRs.

	Heavy Chain	SEQ ID NO.	Light Chain	SEQ ID NO.
CDR1	GFNIKDYFLH	7	RASGNIYNYLA	10
CDR2	WINPDNGNTVYDPKFQG	8	DAKTLAD	11
CDR3	DYTYEKAALDY	9	QHFWSLPFT	12

Table 3: Amino Acid Sequence SEQ. ID. NOs of 20D7, 20D7S, and CTA99 Antibodies

	Heavy Chain			Light Chain		
Antibody	With signal	Variable region	Without signal	With signal	Variable region	Without signal
20D7	18	13	29	24	16	32
20D7S	19	14	30	24	16	32
CTA99	20	15	31	25	17	33

Antibodies used in experiments comprised full-length heavy and light chains without signals, as given in Table 3.

Table 4: Summary of Data from 20D7, 20D7S, and CTA99 Antibodies

Antibody	Competition ELISA (EC_{50}) (M)	FACS (mel)	Affinity K_D (M)	Human FcR binding (M)	Human ADCC (%)	Human CDC (%)
TA99	4.0×10^{-10}	+++	1.7×10^{-9}	ND	ND	ND

CTA99	1.1×10^{-10}	+++	1.1×10^{-9}	ND	48	94
20D7	1.1×10^{-10}	+++	0.28×10^{-9}	1.1×10^{-10}	42	100
20D7S	0.98×10^{-10}	+++	0.28×10^{-9}	0.97×10^{-10}	56	ND

ND = not determined.

Competition Enzyme-Linked Immunosorbent Assay (ELISA)

Coat falcon flexible 96-well flat-bottomed plates with recombinant human TYRP (.5ug/mL x 50 µL) at 4° Celsius overnight. The next day, block the plate with 5% FBS in PBS containing 0.1% Tween-20 for 2 h at room temperature. Add various amounts of antibodies in 100 µL samples. Wash the plate 3x with PBS/Tween and add 100 µL of horseradish peroxidase-conjugated goat anti-human antibody (Biosource, Camarillo, CA) diluted at 1:5000 in 100 µL to the plate and incubate for one hour at room temperature (20 – 25 °C). Wash the plate 3x and add 50 µL /well of 3, 3', 5, 5'-tetramethylbenzidine (TMB; KPL, Gaithersburg, MD) substrate to the plate. Read the plates at 450 nm using a microplate reader (such as Molecular Devices).

Table 5: Enzyme-Linked Immunosorbent Assay (ELISA)

Antibody	Binding to TYRP1 (EC_{50}) (M)
TA99	4.0×10^{-10}
CTA99	1.1×10^{-10}
20D7	1.1×10^{-10}
20D7S	0.98×10^{-10}

The half minimal effect concentration (EC_{50}) is measured in Molar (M). Antibodies, including human 20D7, human 20D7S and CTA99, exhibit specific binding to human TYRP1 in an ELISA assay.

Flow Cytometry

Treat 624mel cells for 1 hour on ice with either 5 µg/mL human IgG, or anti-TYRP1 MAbs in 1%BSA/PBS. Wash cells 3x in 1% BSA/PBS and incubate for 1 hour with fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG. Wash cells and

analyze by a suitable flow cytometer, such as Epics XL Flow Cytometer (Coulter). Flow cytometry analysis shows that 20D7S antibodies as exemplified herein exhibit binding to native TYRP1 expressing in human cell lines SKmel28 and SKmel23 as compared to control human IgG_1. Similarly, flow cytometry analysis shows that for CTA99 and 20D7 antibodies exemplified herein exhibit binding to native TYRP1 expressing human cell line 624mel as compared to control human IgG_1.

Surface Plasmon Resonance / Biacore Analysis

Measure the binding kinetics of the antibodies to recombinant human TYRP1 at ambient laboratory temperature (20 °C – 25 °C) using the surface plasmon resonance, for example a Biacore biosensor (Pharmacia). Immobilize TYRP1 protein onto a CM5 research grade sensor chip and inject the antibodies at concentrations ranging from 0.5 nM to 100 nM. Acquire sensorgrams for each concentration and evaluate using the BIA Evaluations 3.2 program to determine the rate constants k_{on} and k_{off}. K_d, also referred to as k_{off}, is the rate constant of the dissociation reaction. K_a, also referred to as k_{on}, is the rate constant of the association reaction. K_D is a measure of binding affinity; calculate K_D from the ratio of the rate constants k_{off} / k_{on} measured in Molar (M). K_a, the K_d, and K_D for the antibodies exemplified herein, TA99, CTA99, 20D7, and 20D7S, are summarized below in Table 6.

Table 6: Binding Kinetics of Antibodies to Recombinant Human TYRP1

Antibody	K_a (1/Ms) k_{on}	K_d (1/s) k_{off}	K_D (M)
TA99	9.5×10^4	1.6×10^{-4}	1.7×10^{-9}
CTA99	2.8×10^4	3.0×10^{-4}	1.1×10^{-9}
20D7	6.8×10^5	1.9×10^{-4}	0.28×10^{-9}
20D7S	6.4×10^5	1.8×10^{-4}	0.28×10^{-9}

Biacore analysis of the binding of 20D7 and 20D7S to human TYRP1 demonstrate substantial specific binding affinities; accordingly 20D7 and 20D7S are valid candidates for therapeutic antibodies.

Complement Dependent Cytotoxicity (CDC) Assay

Wash human melanoma cell line 624mel 3 times and bring to a concentration of 10^6 viable cells/mL in a suitable media, such as AIM V Media (Invitrogen Life Technologies). Plate one hundred microliters of cells in 96-well, round-bottom Falcon plates and incubate with MAb 20D7 or hIgG (Jackson Immunoresearch, West Grove, PA) starting at 3.7 μg/mL diluted down 1:3, for 1 hr at 37 °C. Add low-Tox-M Rabbit complement (Cedarlane Labs, Westbury, NY) diluted 1:5 in AIM V at 50 μl/well and incubate for 1hr at 37°C. Count cells for viability with Trypan Blue (Invitrogen Life Technologies).

Table 7 shows the percentage of cytotoxicity at various antibody concentrations in the CDC assay. The data demonstrate that 20D7 and CTA99 antibodies induce CDC against TYRP1 (+) human 624mel cells *in vitro*. 20D7 triggered dose-dependent complement-mediated cell lysis of 624mel cells, reaching complete cell lysis in this assay with an antibody concentration of 3.7 μg/mL. Accordingly, there is a strong immune effector response for CDC in 20D7 and CTA99. % Specific Lysis = Test % Cytotoxicity – Negative Control % Cytotoxicity.

Table 7: CDC Data Demonstrating Complement Activation by 20D7 and CTA99

Antibody Concentration (μg/mL)	CTA99	20D7
3.7	95%	100%
1.23	54%	84%
0.41	53%	38%
0.14	17%	35%
0.05	9.5%	24%

Antibody Dependent Cellular Cytotoxicity (ADCC) against Human Melanoma *In Vitro*

Collect and wash 624mel cells with a suitable media, such as AIM V media (Invitrogen Life Technologies) and plate the cells at a density of 10,000 cells /well in a 100 μL volume, in a 96-well Falcon U bottom plate. Add antibodies at 5 μg/mL in a 50 μL volume and incubate at 37 °C for 0.5 hr with target cells. Effector cells were added in

a volume of 50 µL at various E:T (effector:target) ratios. Plates were further incubated for 4 hrs at 37°C. After incubation, plates were spun down at 800 g, and 100 µL of supernatants were gently transferred to 96 well flat bottom plates. Lactate dehydrogenase assay reagent was added as specified by the manufacturer (Roche) and plates were read at 490 nm. Controls in assay: Target Spontaneous and Target Maximum (by adding 50 µL of 4% triton).

Lysis is dependent on the effector to target concentration with lysis of 50% of target cells occurring at an E:T ratio of 100:1. At a fixed concentration (5 µg/mL) 20D7 and CTA99 activate the lysing of 624mel cells. Table 8 shows the percentage of cell cytotoxicity in the presence of various effector cell to tumor cell ratios (E:T Ratio) in the ADCC assay. 20D7S, 20D7 and CTA99 antibodies induce ADCC against TYRP1 (+) human 624mel cells *in vitro*. There is a strong immune effector response for ADCC in 20D7S, 20D7 and CTA99.

% Cytotoxicity = (Experimental – target spontaneous) /
(Target Maximum – target spontaneous) X 100

Table 8: ADCC against Human Melanoma *In Vitro*

	% Cytotoxicity		
E:T Ratio	20D7	20D7S	CTA99
100:1	43%	56%	49%
50:1	33%	47%	39%
25:1	31%	43%	17%
12.5:1	17%	23%	12%

20D7S and 20D7 Stability Assays

Load 20D7 and 20D7S into SDS-PAGE gel (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Run gels using BioRad apparatus until the bromophenol blue dye is just off. Visualize the separated proteins using Coomassie Dye.

In the SDS PAGE analysis, more stable molecules are seen as a single band with few if any free light and or heavy chains; the presence of free light chains and free heavy chains are evidence of less stable molecules. The SDS PAGE gel of 20D7 showed the presence of obvious free light and heavy chains. The SDS PAGE gel of 20D7S showed

very few free light and heavy chains. Accordingly, 20D7S is a more stable IgG1 molecule than 20D7.

CTA99 and 20D7 Effectively Treat Human Melanoma Xenografts.

For the following subcutaneous studies, tumor volumes are calculated by the formula [π/6(W1xW2xW2)], where W1 represents the largest tumor diameter and W2 represents the smallest tumor diameter. %T/C = 100 x (Treatment Volume/Initial Volume) / (Control Volume/Initial Volume). Statistical analysis is done using traditional p-value techniques. For the subcutaneous studies the p value is calculated based on the tumor volume of animals receiving the anti-TYPR antibody verses the tumor volume of the control animals. For the following metastatic studies, tumor inhibition is measured by counting lung surface nodules. % inhibition = 100 x (control nodules# – treatment nodules#) / (control nodules#). Statistical analysis is done using traditional p-value techniques. For the metastatic studies the p-value is calculated based on the nodules observed in the animals receiving the anti-TYPR antibody verses the nodules observed in the control animals.

In vivo single agent activity of anti-TYRP1 antibody on xenograft models of human melanoma

Harvest, wash and resuspend 624mel cultured cells in a 50/50 solution of Matrigel and RPMI 1640 media (10% FBS heat inactivated). For the subcutaneous tumor model, inject 2 x 10^6 cells subcutaneously into the left flank of nude mice. When tumors reach 200 mm^3, treat mice with anti-TYRP1 antibodies or control human IgG; administer antibodies 1 mg/mouse three times per week. Measure tumors with calipers twice a week and calculate % T/C. For the *in vivo* single agent activity of anti-TYRP1 antibody on xenograft models of human melanoma, growth of SKmel28 xenografts was inhibited by 20D7 treatment compared to human IgG control (T/C = 51%; P = 0.01 at day 43). Established 624mel tumors were also shown to be inhibited significantly by 20D7 treatment. Tumor growth was inhibited and reached statistical significance at day 16 post initiation of antibody treatment (T/C = 44%; P = 0.01). Additional human melanoma xenografts were evaluated for anti-tumor activity of 20D7. Cell lines A375 and 1102mel

were shown to be significantly inhibited by single agent 20D7 11 days post antibody treatment (T/C = 42%; *P* = 0.01 and T/C = 43%; *P* = 0.004 for A375 and 1102mel respectively). Lysates of skins from different species are incubated with TA99 (5 μg/ml) for 2 hours at room temperature. The lysates are then precipitated with protein A and subjected to SDS-PAGE under reducing and non-reducing conditions using four 12% gradient gels. After electrophoresis, the gels are transferred to PVDF membrane (Invitrogen Life Technologies). The membrane is probed with 20D7S (5 μg/ml) followed by an HRP labeled anti-human IgG (Zymed, South San Francisco, CA). The blot is developed using a chemiluminescent substrate (KPL, Gaithersburg, MD). The data show that 20D7 readily cross-reacts with mouse TYRP1. However, no overt toxicity was apparent in any animals treated with 20D7. Body weight and overall appearance were not significantly different in 20D7 treated animals relative to human IgG control treated mice.

In vivo single agent dose response activity of anti-TYRP1 antibody on xenograft models of human melanoma

Mix Skmel28 cells in a 50/50 solution of Matrigel and RPMI 1640 media (10% FBS heat inactivated). Inject 2 x 10^6 cells subcutaneously into the left flank of nude mice. When tumors reach 200 mm^3, treat mice with anti-TYRP1 antibodies (6 mg/kg, 20 mg/kg, or 60 mg/kg) or control human IgG three times per week. Measure tumors with calipers twice a week and calculate % T/C. The dose-response study of 20D7 on SKmel28 xenografts indicated a dose-dependent anti-tumor response. Even at 6 mg/kg dose, tumors were significantly inhibited by 20D7 (T/C = 69%; $P < 0.0001$). The anti-tumor effects at each dose were statistically significant: 6 mg/kg and 20 mg/kg (T/C = 50%; $P = 0.04$), 6 mg/kg and 60 mg/kg (T/C = 19%; $P < 0.003$).

In vivo single agent activity of anti-TYRP1 antibody in two metastatic melanoma models

B16BL6 is an aggressive and spontaneously arising murine melanoma. It forms lung metastases in nude mice after intravenous administration. Harvest, wash and resuspend cultured SKmel23 and B16BL6 cells melanoma cells in RPMI 1640 media (10% FBS heat inactivated).

Model 1: Inject 1 x 10^5 B16BL6 cells intravenously. On the second day after the tumor injection, administer to mice anti-TYRP1 antibodies or control human IgG, according to three different dose concentrations (200 μg/mouse, 500 μg/mouse, and 1 mg/mouse). Sacrifice mice on day 20, remove lungs, count lung surface nodules and calculate percent inhibition. Heavy metastases are detected over the surface of the lungs in human IgG treated mice; significantly fewer metastases are noted in 20D7 treated animals. All three concentrations of 20D7 reduce the level of lung metastases (Inhibition = 65%, 74%, and 95%, respectively).

Model 2: Inject 1 x 10^5 human SKmel23 cells intravenously. On the second day after the tumor injection, administer to mice anti-TYRP1 antibodies or control human IgG, according to two different dose concentrations (200 μg/mouse and 500 μg/mouse). Sacrifice mice on day 20, remove lungs, count lung surface nodules and calculate percent inhibition. Metastatic nodules are significantly reduced by treatment with 20D7 or CTA99 at 200 μg/dose and at 500 μg/dose. 20D7 reduced metastasis by 58% and 73% respectively. CTA99 reduced metastasis by 63% and 75% respectively. These results demonstrate that in two separate models, melanoma metastasis is inhibited by 20D7 and CTA99.

In vivo comparative studies of 20D7 and 20D7S inhibition of tumor growth on subcutaneous xenograft and metastatic models of human melanoma

For the subcutaneous tumor model, harvest, wash and resuspend 624mel cultured cells in a 50/50 solution of Matrigel and RPMI 1640 media (10% FBS heat inactivated), then inject 2 x 10^6 624mel cells subcutaneously into the left flank of nude mice. When tumors reach 200 mm^3, treat mice with 20D7 or 20D7S, 40 mg/kg twice per week. Measure tumors with calipers twice a week and calculate % T/C. In the 624mel subcutaneous xenograft model, 20D7 inhibited tumor growth T/C =21% and for 20D7S T/C =25%. Both 20D7 and 20D7S inhibited tumor growth in the xenograft model.

For the metastatic model, harvest, wash and resuspend 888mel cultured cells in RPMI 1640 media (10% FBS heat inactivated), inject 888mel cells intravenously. On the second day after the tumor injection, administer to mice anti-TYRP1 antibodies or control

human IgG. Sacrifice mice on day 20, remove lungs, count lung surface nodules and calculate percent inhibition. In the metastatic model of 888mel in nude mice, both 20D7 and 20D7S significantly inhibited lung surface metastasis: 20D7 inhibition = 77%, p=0.0005; 20D7S inhibition = 80%, p=0.0005. Both 20D7 and 20D7S reduced metastasis of melanoma in the metastatic model.

20D7 and Dacarbazine (DTIC) combination treatment demonstrated stronger anti-tumor activity compared to mono-therapy on human xenograft

For the subcutaneous model, harvest, wash and resuspend cultured 624mel cells, in a 50/50 solution of Matrigel and RPMI 1640 media (10% FBS heat inactivated). Inject 2 x 10^6 624mel cells into the left flank of nude mice. When tumors reach 200 mm^3, treat mice with anti-TYRP1 antibodies, DTIC, or a combination of anti-TYRP1 antibodies and DTIC. Administer 40 mg/kg antibodies once per week. Administer DTIC 5 mg/kg once per week. Measure tumors with calipers twice a week and calculate % T/C. For metastatic model, harvest, wash and resuspend cultured SKmel23, 888mel and B16 melanoma cells, in RPMI 1640 media (10% FBS heat inactivated). Inject SKmel23, 888mel and B16 melanoma cells intravenously. On the second day after the tumor injection, administer to mice anti-TYRP1 antibodies or control human IgG. Sacrifice mice on day 20, remove lungs, count lung surface nodules and calculate percent inhibition.

As demonstrated in the subcutaneous model, 20D7 and DTIC combination treatment inhibited tumor growth significantly better than 20D7 ($p<0.001$) or DTIC ($p<0.001$) alone.

Table 9: Summary of anti-tumor Activities of 20D7 *In Vivo*

Model	Tumor	Measurement	20D7	20D7+DTIC
Metastatic	SKmel23	% inhibition	80%	99%*
Metastatic	888 mel	% inhibition	77%	96%*
Metastatic	B16	% inhibition	95%	ND
Subcutaneous	624mel	% T/C	50% P=0.004	19% P<0.0001

Table 9 summarizes *in vivo* anti-tumor activities of 20D7S compared to monotherapy 20D7 or DTIC in 4 models. The anti-tumor activities of 20D7 *in vivo* are shown by percent inhibition in metastatic models (the * denotes statistical significance; ND denotes not determined. The anti-tumor activities of 20D7 *in vivo* are shown by percent T/C in subcutaneous models. Treatment with 20D7 and Dacarbazine (DTIC) in combination demonstrated stronger anti-tumor activity as compared to mono-therapy.

WE CLAIM:

1. A monoclonal antibody or fragment thereof that specifically binds human TYRP1 (SEQ ID NO. 28) comprising a VH comprising the amino acid sequence: QVQLVQSGSELKKPGASVKISCKASGYTFTSYAMNWVRQAPGQGLESMGWINT NTGNPTYAQGFTGRFVFSMDTSVSTAYLQISSLKAEDTAIYYCAPRYSSSWYLDY WGQGTLVTVSS (SEQ ID NO:14), a CDRL1 having the sequence RASQSVSSYLA (SEQ ID NO:4), a CDRL2 having the sequence DASNRAT (SEQ ID NO:5), and a CDRL3 having the sequence QQRSNWLMYT (SEQ ID NO:6).

2. The antibody or fragment thereof of claim 1, comprising a VL comprising the amino acid sequence: EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQ APRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWLMYT FGQGTKLEIK (SEQ ID NO:16) and a VH comprising the amino acid sequence: QVQLVQSGSELKKPGASVKISCKASGYTFTSYAMNWVRQAPGQGLESMGWINT NTGNPTYAQGFTGRFVFSMDTSVSTAYLQISSLKAEDTAIYYCAPRYSSSWYLDY WGQGTLVTVSS (SEQ ID NO:14).

3. The antibody or fragment thereof of any preceding claim comprising a heavy chain of SEQ ID NO: 30 and a light chain of SEQ ID NO: 32.

4. An antibody or fragment thereof as claimed in any one of claims 1-3 comprising two heavy chains of SEQ ID NO: 30 and two light chains of SEQ ID NO: 32.

5. An isolated polynucleic acid comprising a nucleotide sequence encoding the antibody or fragment according to any one of claims 1-4.

6. An expression vector comprising the polynucleic acid of claim 5 operably linked to expression control elements such that the encoded antibody or fragment may be expressed.

7. A recombinant cell comprising the expression vector of claim 6, which recombinant cell is capable of producing an antibody or fragment according to any one of claims 1-4.

8. An antibody or fragment produced by culturing the recombinant cell according to claim 7 so that the antibody or fragment is produced and recovering the antibody or fragment from the culture.

9. A pharmaceutical composition comprising an antibody or fragment as claimed in any one of claims 1-4 or 8 together with a pharmaceutically acceptable carrier, diluent or excipient.

10. An antibody or fragment as claimed in any one of claims 1-4 or 8 for use as a medicament.

11. An antibody or fragment as claimed in any one of claims 1-4 or 8 for use in the treatment of cancer.

12. An antibody or fragment as claimed in claim 11, wherein the cancer is malignant melanoma.

13. Use of an antibody or fragment as claimed in any one of claims 1-4 or 8 in the manufacture of a medicament for treatment of cancer.

14. The use of claim 13, wherein the cancer is malignant melanoma.

SEQUENCE LISTING

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tgtgacaaaa ctcacacatg cccaccgtgc ccagcacctg aactcctggg gggaccgtca 780

gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc 840

acatgcgtgg tggtggacgt gagccacgaa gaccctgagg tcaagttcaa ctggtatgtg 900

gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg 960

taccgtgtgg tcagcgtcct caccgtcctg caccaagact ggctgaatgg caaggagtac 1020

aagtgcaagg tctccaacaa agccctccca gccccatcg agaaaaccat ctccaaagcc 1080

aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga ggagatgacc 1140

aagaaccaag tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg 1200

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac 1260

tccgacggct ccttcttcct ctattccaag ctcaccgtgg acaagagcag gtggcagcag 1320

gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag 1380

agcctctccc tgtctccggg caaatga 1407

<210> 22
<211> 1407
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 22
atgggatggt catgtatcat ccttttttcta gtagcaactg caactggagt acattcacag 60

gtgcagctgg tccaatctgg gtctgagttg aagaagcctg gggcctcagt gaagatttcc 120

tgcaaggctt ctggatacac cttcactagc tatgctatga attgggtgcg acaggcccct 180

ggacaagggc ttgagtctat gggatggatc aacaccaaca ctgggaaccc aacgtatgcc 240

cagggcttca caggacggtt tgtcttctcc atggacacct ctgtcagcac ggcatatctg 300

cagatcagca gcctaaaggc tgaggacact gccatatatt actgtgcgcc ccgatatagc 360

agcagctggt accttgatta ctggggccag ggaaccctgg tcaccgtgtc ctcagctagc 420

accaagggcc catcggtctt ccccctggca ccctcctcca agagcacctc tgggggcaca 480

gcggccctgg gctgcctggt caaggactac ttccccgaac cggtgacggt gtcgtggaac 540

tcaggcgccc tgaccagcgg cgtgcacacc ttcccggctg tcctacagtc ctcaggactc 600

tactccctca gcagcgtggt gaccgtgccc tccagcagct tgggcaccca gacctacatc 660

tgcaacgtga atcacaagcc cagcaacacc aaggtggaca agagagttga gcccaaatct 720

tgtgacaaaa ctcacacatg cccaccgtgc ccagcacctg aactcctggg gggaccgtca 780

gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc 840

acatgcgtgg tggtggacgt gagccacgaa gaccctgagg tcaagttcaa ctggtatgtg 900

gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg 960

taccgtgtgg tcagcgtcct caccgtcctg caccaagact ggctgaatgg caaggagtac 1020

aagtgcaagg tctccaacaa agccctccca gcccccatcg agaaaaccat ctccaaagcc 1080

aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga ggagatgacc 1140

aagaaccaag tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg 1200

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac 1260

tccgacggct ccttcttcct ctattccaag ctcaccgtgg acaagagcag gtggcagcag 1320

gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag 1380

agcctctccc tgtctccggg caaatga 1407

<210> 23
<211> 1413
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 23
atgggatggt catgtatcat ccttttttcta gtagcaactg caactggagt acattcagag 60

gttcagctgc agcagtctgg ggctgagctt gtgaggccag gggccttggt caagttgtcc 120

tgcaaaactt ctggcttcaa cattaaagac tacttttac actgggtgag acagaggcct 180

gaccagggcc tggagtggat tggatggatt aatcctgata atggtaatac tgtttatgac 240

ccgaagtttc agggcacggc cagtttaaca gcagacacat cctccaacac agtctacttg 300

cagctcagcg gcctgacatc tgaggacact gccgtctatt tctgtactcg gagggactat 360

acttatgaaa aggctgctct ggactactgg ggtcagggag cctcagtcat cgtctcctca 420

gctagcacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg 480

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 540

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 600

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 660

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagag agttgagccc 720

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 780

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccccct 840

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 900

tatgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 960

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aagactggct gaatggcaag 1020

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 1080

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggaggag 1140

atgaccaaga accaagtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1200

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1260

ctggactccg acggctcctt cttcctctat tccaagctca ccgtggacaa gagcaggtgg 1320

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1380

cagaagagcc tctccctgtc tccgggcaaa tga 1413

<210> 24
<211> 234
<212> PRT
<213> Homo sapiens

<400> 24

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu
20 25 30

Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val
35 40 45

Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
50 55 60

Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg
65 70 75 80

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
85 90 95

Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn
100 105 110

Trp Leu Met Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
115 120 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130 135 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145 150 155 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165 170 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr

180 185 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195 200 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210 215 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

<210> 25
<211> 233
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 25

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Ala Ile Gln Met Ser Gln Ser Pro Ala Ser Leu Ser Ala
20 25 30

Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile
35 40 45

Tyr Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro His
50 55 60

Leu Leu Val Tyr Asp Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg
65 70 75 80

Phe Ser Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Ser
85 90 95

Leu Gln Thr Glu Asp Ser Gly Asn Tyr Tyr Cys Gln His Phe Trp Ser
100 105 110

Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg Thr
115 120 125

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
130 135 140

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
145 150 155 160

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
165 170 175

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
180 185 190

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
195 200 205

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
210 215 220

Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

<210> 26
<211> 705
<212> DNA
<213> Homo sapiens

<400> 26
atgggatggt catgtatcat ccttttttcta gtagcaactg caactggagt acattcagaa 60

attgtgttga cacagtctcc agccaccctg tctttgtctc caggggaaag agccaccctc 120

tcctgcaggg ccagtcagag tgttagcagc tacttagcct ggtaccaaca gaaacctggc 180

caggctccca ggctcctcat ctatgatgca tccaacaggg ccactggcat cccagccagg 240

ttcagtggca gtgggtctgg gacagacttc actctcacca tcagcagcct agagcctgaa 300

gattttgcag tttattactg tcagcagcgt agcaactggc tcatgtacac ttttggccag 360

gggaccaagc tggagatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 420

tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 480

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 540

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 600

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 660

ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag 705

<210> 27
<211> 702
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 27
atgggatggt catgtatcat ccttttttcta gtagcaactg caactggagt acattcagcc 60

atccagatgt ctcagtctcc agcctcccta tctgcatctg tgggagaaac tgtcaccatc 120

acatgtcgag caagtggaaa tatttacaat tatttagcat ggtatcagca gaaacaggga 180

aaatctcctc acctcctggt ctatgatgca aaaaccttag cagatggtgt gccatcaagg 240

ttcagtggca gtggctcagg gacacaatat tctctcaaga ttagcagcct gcagactgaa 300

gattctggga attattactg tcaacatttt tggagtcttc cattcacgtt cggctcgggg 360

acaaagttgg aaataaaacg aactgtggct gcaccatctg tcttcatctt cccgccatct 420

gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc tgctgaataa cttctatccc 480

agagaggcca aagtacagtg gaaggtggat aacgccctcc aatcgggtaa ctcccaggag 540

agtgtcacag agcaggacag caaggacagc acctacagcc tcagcagcac cctgacgctg 600

agcaaagcag actacgagaa acacaaagtc tacgcctgcg aagtcaccca tcagggcctg 660

agctcgcccg tcacaaagag cttcaacagg ggagagtgtt ag 702

<210> 28
<211> 537
<212> PRT
<213> Homo sapiens

<400> 28

Met Ser Ala Pro Lys Leu Leu Ser Leu Gly Cys Ile Phe Phe Pro Leu
1 5 10 15

Leu Leu Phe Gln Gln Ala Arg Ala Gln Phe Pro Arg Gln Cys Ala Thr
20 25 30

Val Glu Ala Leu Arg Ser Gly Met Cys Cys Pro Asp Leu Ser Pro Val
35 40 45

Ser Gly Pro Gly Thr Asp Arg Cys Gly Ser Ser Ser Gly Arg Gly Arg
50 55 60

Cys Glu Ala Val Thr Ala Asp Ser Arg Pro His Ser Pro Gln Tyr Pro
65 70 75 80

His Asp Gly Arg Asp Asp Arg Glu Val Trp Pro Leu Arg Phe Phe Asn
85 90 95

Arg Thr Cys His Cys Asn Gly Asn Phe Ser Gly His Asn Cys Gly Thr
100 105 110

Cys Arg Pro Gly Trp Arg Gly Ala Ala Cys Asp Gln Arg Val Leu Ile
115 120 125

Val Arg Arg Asn Leu Leu Asp Leu Ser Lys Glu Glu Lys Asn His Phe
130 135 140

Val Arg Ala Leu Asp Met Ala Lys Arg Thr Thr His Pro Leu Phe Val
145 150 155 160

Ile Ala Thr Arg Arg Ser Glu Glu Ile Leu Gly Pro Asp Gly Asn Thr
165 170 175

Pro Gln Phe Glu Asn Ile Ser Ile Tyr Asn Tyr Phe Val Trp Thr His
180 185 190

```
Tyr Tyr Ser Val Lys Lys Thr Phe Leu Gly Val Gly Gln Glu Ser Phe
        195                 200                 205

Gly Glu Val Asp Phe Ser His Glu Gly Pro Ala Phe Leu Thr Trp His
    210                 215                 220

Arg Tyr His Leu Leu Arg Leu Glu Lys Asp Met Gln Glu Met Leu Gln
225                 230                 235                 240

Glu Pro Ser Phe Ser Leu Pro Tyr Trp Asn Phe Ala Thr Gly Lys Asn
                245                 250                 255

Val Cys Asp Ile Cys Thr Asp Asp Leu Met Gly Ser Arg Ser Asn Phe
            260                 265                 270

Asp Ser Thr Leu Ile Ser Pro Asn Ser Val Phe Ser Gln Trp Arg Val
        275                 280                 285

Val Cys Asp Ser Leu Glu Asp Tyr Asp Thr Leu Gly Thr Leu Cys Asn
    290                 295                 300

Ser Thr Glu Asp Gly Pro Ile Arg Arg Asn Pro Ala Gly Asn Val Ala
305                 310                 315                 320

Arg Pro Met Val Gln Arg Leu Pro Glu Pro Gln Asp Val Ala Gln Cys
                325                 330                 335

Leu Glu Val Gly Leu Phe Asp Thr Pro Pro Phe Tyr Ser Asn Ser Thr
            340                 345                 350

Asn Ser Phe Arg Asn Thr Val Glu Gly Tyr Ser Asp Pro Thr Gly Lys
        355                 360                 365

Tyr Asp Pro Ala Val Arg Ser Leu His Asn Leu Ala His Leu Phe Leu
    370                 375                 380

Asn Gly Thr Gly Gly Gln Thr His Leu Ser Pro Asn Asp Pro Ile Phe
385                 390                 395                 400

Val Leu Leu His Thr Phe Thr Asp Ala Val Phe Asp Glu Trp Leu Arg
                405                 410                 415

Arg Tyr Asn Ala Asp Ile Ser Thr Phe Pro Leu Glu Asn Ala Pro Ile
            420                 425                 430

Gly His Asn Arg Gln Tyr Asn Met Val Pro Phe Trp Pro Pro Val Thr
        435                 440                 445

Asn Thr Glu Met Phe Val Thr Ala Pro Asp Asn Leu Gly Tyr Thr Tyr
    450                 455                 460
```

```
Glu Ile Gln Trp Pro Ser Arg Glu Phe Ser Val Pro Glu Ile Ile Ala
465                 470                 475                 480

Ile Ala Val Val Gly Ala Leu Leu Leu Val Ala Leu Ile Phe Gly Thr
                485                 490                 495

Ala Ser Tyr Leu Ile Arg Ala Arg Arg Ser Met Asp Glu Ala Asn Gln
            500                 505                 510

Pro Leu Leu Thr Asp Gln Tyr Gln Cys Tyr Ala Glu Glu Tyr Glu Lys
        515                 520                 525

Leu Gln Asn Pro Asn Gln Ser Val Val
    530                 535
```

<210> 29
<211> 449
<212> PRT
<213> Homo sapiens

<400> 29

```
Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Cys Met
        35                  40                  45

Gly Trp Ile Asn Thr Asn Thr Gly Asn Pro Thr Tyr Ala Gln Gly Phe
    50                  55                  60

Thr Gly Arg Phe Val Phe Ser Met Asp Thr Ser Val Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Pro Arg Tyr Ser Ser Ser Trp Tyr Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160
```

```
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430
```

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

<210> 30
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 30

Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Ser Met
35 40 45

Gly Trp Ile Asn Thr Asn Thr Gly Asn Pro Thr Tyr Ala Gln Gly Phe
50 55 60

Thr Gly Arg Phe Val Phe Ser Met Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Pro Arg Tyr Ser Ser Ser Trp Tyr Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

```
Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
```

<210> 31
<211> 452

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 31

```
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala
1               5                  10                  15

Leu Val Lys Leu Ser Cys Lys Thr Ser Gly Phe Asn Ile Lys Asp Tyr
            20                  25                  30

Phe Leu His Trp Val Arg Gln Arg Pro Asp Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Trp Ile Asn Pro Asp Asn Gly Asn Thr Val Tyr Asp Pro Lys Phe
    50                  55                  60

Gln Gly Thr Ala Ser Leu Thr Ala Asp Thr Ser Ser Asn Thr Val Tyr
65                  70                  75                  80

Leu Gln Leu Ser Gly Leu Thr Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Thr Arg Arg Asp Tyr Thr Tyr Glu Lys Ala Ala Leu Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Ala Ser Val Ile Val Phe Ser Ala Ala Ser Thr Lys Gly Pro
        115                 120                 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
    130                 135                 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145                 150                 155                 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
                165                 170                 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
            180                 185                 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
        195                 200                 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
    210                 215                 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225                 230                 235                 240
```

```
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            260                 265                 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
        275                 280                 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
    290                 295                 300

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
305                 310                 315                 320

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
                325                 330                 335

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            340                 345                 350

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
        355                 360                 365

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    370                 375                 380

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
385                 390                 395                 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
                405                 410                 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
            420                 425                 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
        435                 440                 445

Ser Pro Gly Lys
    450
```

<210> 32
<211> 215
<212> PRT
<213> Homo sapiens

<400> 32

```
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
```

20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Leu Met
85 90 95

Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 33
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

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Ala Ile Gln Met Ser Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile Tyr Asn Tyr
20 25 30

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Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro His Leu Leu Val
        35                  40                  45

Tyr Asp Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Ser Leu Gln Thr
65                  70                  75                  80

Glu Asp Ser Gly Asn Tyr Tyr Cys Gln His Phe Trp Ser Leu Pro Phe
                85                  90                  95

Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
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