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(57) **Abrégé/Abstract:**
The present invention provides T cell receptors (TCRs) having the property of binding to SLLMWITQC-HLA-A*0201, the SLLMWITQC peptide being derived from the NY-ESO-1 protein which is expressed by a range of tumour cells. The TCRs have a K_D for the said that peptide-HLA complex of less than or equal to 1μM and/or have an off-rate (k_{off}) of $1x10^{-3}$ S^{-1} or slower.

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(54) Title: HIGH AFFINITY NY-ESO T CELL RECEPTOR

(57) Abstract: The present invention provides T cell receptors (TCRs) having the property of binding to SLLMWITQC-HLA-A*0201, the SLLMWITQC peptide being derived from the NY-ESO-1 protein which is expressed by a range of tumour cells. The TCRs have a K_D for the said that peptide-HLA complex of less than or equal to 1μM and/or have an off-rate (k_{off}) of $1x10^{-3}$ S^{-1} or slower.

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High Affinity NY-ESO T cell receptor

The present invention relates to T cell receptors (TCRs) having the property of binding to SLLMWITQC-HLA-A*0201 and comprising at least one TCR α chain variable domain and/or at least one TCR β chain variable domain CHARACTERISED IN THAT said TCR has a K_D for the said SLLMWITQC-HLA-A*0201 complex of less than or equal to 1μM and/or has an off-rate (k_{off}) for the SLLMWITQC-HLA-A*0201 complex of $1x10^{-3}$ S^{-1}or slower.

Background to the Invention

The SLLMWITQC peptide is derived from the NY-ESO-1 protein that is expressed by a range of tumours (Chen *et al.*, (1997) *PNAS USA* **94** 1914-1918). The Class I HLA molecules of these cancerous cells present peptides from this protein, including SLLMWITQC. Therefore, the SLLMWITQC-HLA-A2 complex provides a cancer marker that TCRs can target, for example for the purpose of delivering cytotoxic or immuno-stimulatory agents to the cancer cells. However, for that purpose it would be desirable if the TCR had a higher affinity and/or a slower off-rate for the peptide-HLA complex than native TCRs specific for that complex.

Brief Description of the Invention

This invention makes available for the first time TCRs having high affinity (K_D) of the interaction less than or equal to 1μM, and/or a slower off-rate (k_{off}) of $1x10^{-3}$ S^{-1} or slower, for the SLLMWITQC-HLA-A*0201 complex. Such TCRs are useful, either alone or associated with a therapeutic agent, for targeting cancer cells presenting that complex

Detailed Description of the Invention

The present invention provides a T-cell receptor (TCR) having the property of binding to SLLMWITQC-HLA-A*0201 and comprising at least one TCR α chain variable domain and/or at least one TCR β chain variable domain CHARACTERISED IN THAT said TCR has a K_D for the said SLLMWITQC-HLA-A*0201 complex of less than or equal to 1μM and/or has an off-rate (k_{off}) for the SLLMWITQC-HLA-A*0201 complex of $1x10^{-3}$ S^{-1}or slower. The K_D and/or (k_{off}) measurement can be made by any of the known methods. A preferred method is the Surface Plasmon Resonance (Biacore) method of Example 5.

For comparison, the interaction of a disulfide-linked soluble variant of the native 1G4 TCR (see SEQ ID NO: 9 for TCR α chain and SEQ ID NO: 10 for TCR β chain) and the SLLMWITQC-HLA-A*0201 complex has a K_D of approximately 10μM, an off-rate (k_{off}) of 1.28 x 10^{-1} S^{-1} and a half-life of 0.17 minutes as measured by the Biacore-base method of Example 5.

The native 1G4 TCR specific for the SLLMWITQC-HLA-A*0201 complex has the following Valpha chain and Vbeta chain gene usage:

Alpha chain - TRAV21
Beta chain: - TRBV 6.5

The native 1G4 TCR can be used as a template into which various mutations that impart high affinity and/or a slow off-rate for the interaction between TCRs of the invention and the SLLMWITQC-HLA-A*0201 complex can be introduced. Thus the invention includes TCRs which are mutated relative to the native 1G4 TCR α chain variable domain (see Figure 1a and SEQ ID No: 1) and/or β chain variable domain (see Figure 1b and SEQ ID NO: 2) in at least one complementarity determining region (CDR) and/or variable domain framework region thereof. It is also contemplated that other hypervariable regions in the variable domains of the TCRs of the invention, such as the hypervariable 4 (HV4) regions, may be mutated so as to produce a high affinity mutant.

Native TCRs exist in heterodimeric $\alpha\beta$ or $\gamma\delta$ forms. However, recombinant TCRs consisting of a single TCR α or TCR β chain have previously been shown to bind to peptide MHC molecules.

In one embodiment the TCR of the invention comprise both an α chain variable domain and an TCR β chain variable domain.

As will be obvious to those skilled in the art the mutation(s) in the TCR α chain sequence and/or TCR β chain sequence may be one or more of substitution(s), deletion(s) or insertion(s). These mutations can be carried out using any appropriate method including, but not limited to, those based on polymerase chain reaction (PCR), restriction enzyme-based cloning, or ligation independent cloning (LIC) procedures. These methods are detailed in many of the standard molecular biology texts. For further details regarding polymerase chain reaction (PCR) mutagenesis and restriction enzyme-based cloning see (Sambrook & Russell, (2001) Molecular Cloning – A Laboratory Manual (3rd Ed.) CSHL Press) Further information on LIC procedures can be found in (Rashtchian, (1995) *Curr Opin Biotechnol* **6** (1): 30-6)

It should be noted that any αβ TCR that comprises similar Valpha and Vbeta gene usage and therefore amino acid sequence to that of the 1G4 TCR could make a convenient template TCR. It would then be possible to introduce into the DNA encoding one or both of the variable domains of the template αβ TCR the changes required to produce the mutated high affinity TCRs of the invention. As will be obvious to those skilled in the art, the necessary mutations could be introduced by a number of methods, for example site-directed mutagenesis.

The TCRs of the invention include those in which one or more of the TCR alpha chain variable domain amino acids corresponding to those listed below are mutated relative to the amino acid occurring at these positions in the sequence provided for the native 1G4 TCR alpha chain variable domain in Figure 1a and SEQ ID No: 1.

Unless stated to the contrary, the TCR amino acid sequences herein are generally provided including an N-terminal methionine (Met or M) residue. As will be known to those skilled in the art this residue may be removed during the production of recombinant proteins. Furthermore, unless stated to the contrary, the soluble TCR and

TCR variable domain sequences have been truncated at the N-terminus thereof. (Resulting in the lose of the N-terminal "K" and "NA" in the TCR alpha and beta chain sequences respectively.). As will be obvious to those skilled in the art these "missing" N-terminal TCR residues may be re-introduced into the TCRs of the present invention. As will also be obvious to those skilled in the art, it may be possible to truncate the sequences provided at the C-terminus and/or N-terminus thereof, by 1, 2, 3, 4, 5 or more residues, without substantially affecting the pMHC binding characteristics of the TCR, all such trivial variants are encompassed by the present invention.

As used herein the term "variable domain" is understood to encompass all amino acids of a given TCR which are not included within the constant domain as encoded by the TRAC gene for TCR α chains and either the TRBC1 or TRBC2 for TCR β chains. (T cell receptor Factsbook, (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8)

As is known to those skilled in the art, part of the diversity of the TCR repertoire is due to variations which occur in the amino acid encoded by the codon at the boundary between the variable domain, as defined herein, and the constant domain. For example, the codon that is present at this boundary in the wild-type IG4 TCR sequence results in the presence of the Tyrosine (Y) residue at the C-terminal of the variable domain sequences herein. This Tyrosine replaces the N-terminal Asparagine (N) residue encoded by the TRAC gene shown in Figure 8A.

Embodiments of the invention include mutated TCRs which comprise mutation of one or more of alpha chain variable domain amino acids corresponding to: 20V, 51Q, 52S, 53S, 94P, 95T, 96S, 97G, 98G, 99S, 100Y, 101I and 103T, for example the amino acids:

20A

51P/S/T or M

52P/F or G

53W/H or T

94H or A

95L/M/A/Q/Y/E/I/F/V/N/G/S/D or R

96L/T/Y/I/Q/V/E/X/A/W/R/G/H/D or K

97D/N/V/S/T or A

98P/H/S/T/W or A

99T/Y/D/H/V/N/E/G/Q/K/A/I or R

100F/M or D

101P/T/ or M

103A

The numbering used above is the same as that shown in Figure 1a and SEQ ID No: 1

Embodiments of the invention also include TCRs which comprise mutation of one or more of the TCR beta chain variable domain amino acids corresponding to those listed below, are relative to the amino acid occurring at these positions in the sequence provided for the native 1G4 TCR alpha chain variable domain of the nativè 1G4 TCR beta chain in Figure 1b and SEQ ID No: 2. The amino acids referred to which may be mutated are:18M, 50G, 51A, 52G, 53I, 55D, 56Q, 70T, 94Y, 95V and 97N, for example:

18V

50S or A

51V or I

52Q

53T or M

55R

56R

70I

94N or F

95L

97G or D

The numbering used above is the same as that shown in Figure 1b and SEQ ID No: 2

Further preferred embodiments of the invention are provided by TCRs comprising one of the mutated alpha chain variable domain amino acid sequences shown in Fig 6 (SEQ ID Nos: 11 to 83). Phenotypically silent variants of such TCRs also form part of this invention.

Additional preferred embodiments of the invention are provided by TCRs comprising one of the mutated beta chain variable domain amino acid sequences shown in Figures 7 or 13. (SEQ ID Nos: 84 to 99 or 117 to 121). Phenotypically silent variants of such TCRs also form part of this invention.

Native TCRs exist in heterodimeric αβ or γδ forms. However, recombinant TCRs consisting of αα or ββ homodimers have previously been shown to bind to peptide MHC molecules. Therefore, one embodiment of the invention is provided by TCR αα or TCR ββ homodimers.

Further preferred embodiments are provided by TCRs of the invention comprising the alpha chain variable domain amino acid sequence and the beta chain variable domain amino acid sequence combinations listed below, phenotypically silent variants of such TCRs also form part of this invention:

Alpha chain variable domain sequence, SEQ ID NO:	Beta chain variable domain sequence, SEQ ID NO:
1	84
1	85
1	86
1	87
1	88
11	84

12	84
12	85
12	90
11	85
11	86
11	92
11	93
13	86
14	84
14	85
15	84
15	85
16	84
16	85
17	86
18	86
19	84
20	86
21	84
21	85
22	84
23	86
24	84
25	84
26	84
27	84
28	84
29	84
30	84
31	84

32	84
33	84
20	86
34	86
35	89
36	89
37	89
38	89
39	89
16	89
17	89
31	89
40	89
1	90
1	91
41	90
42	2
42	85
42	92
1	92
1	93
43	92
44	92
45	92
46	92
47	92
48	84
49	94
50	84
50	94

51	94
51	95
1	94
1	85
51	84
52	84
52	94
52	95
53	84
49	95
49	94
54	92
55	92
56	92
57	92
58	92
59	92
60	92
61	92
62	92
63	92
64	92
65	92
66	92
67	92
68	92
69	92
70	92
71	92
72	92

73	92
74	92
75	92
76	92
77	92
78	92
79	92
80	92
81	92
82	92
83	92
11	96
11	97
11	98
11	99
1	89
50	117
49	117
50	118
49	119
50	119
58	93
49	118
1	119
1	117
55	120
56	120
50	121
50	120
49	121

49	120
48	118
53	95

Preferred embodiments provide a TCR of the invention comprising:

the alpha chain variable domain shown in the SEQ ID NO: 49 and the beta chain variable domain shown in the SEQ ID NO: 94, or phenotypically silent variants thereof.

In another preferred embodiment TCRs of the invention comprising the variable domain combinations detailed above further comprise the alpha chain constant region amino acid sequence shown in Figure 8a (SEQ ID NO: 100) and one of the beta chain amino acid constant region sequences shown in Figures 8b and 8c (SEQ ID NOs: 101 and 102) or phenotypically silent variants thereof.

As used herein the term "phenotypically silent variants" is understood to refer to those TCRs which have a K_D for the said SLLMWITQC-HLA-A*0201 complex of less than or equal to 1μM and/or have an off-rate (k_{off}) of $1x10^{-3}$ S^{-1} or slower. For example, as is known to those skilled in the art, it may be possible to produce TCRs that incoporporate minor changes in the constant and/or variable domains thereof compared to those detailed above without altering the affinity and/or off-rate for the interaction with the SLLMWITQC-HLA-A*0201 complex. Such trivial variants are included in the scope of this invention. Those TCRs in which one or more conservative substitutions have been made also form part of this invention.

In one broad aspect, the TCRs of the invention are in the form of either single chain TCRs (scTCRs) or dimeric TCRs (dTCRs) as described in WO 04/033685 and WO 03/020763.

A suitable scTCR form comprises a first segment constituted by an amino acid sequence corresponding to a TCR α chain variable domain, a second segment constituted by an amino acid sequence corresponding to a TCR β chain variable domain sequence fused to the N terminus of an amino acid sequence corresponding to a TCR β chain constant domain extracellular sequence, and a linker sequence linking the C terminus of the first segment to the N terminus of the second segment.

Alternatively the first segment may be constituted by an amino acid sequence corresponding to a TCR β chain variable domain, the second segment may be constituted by an amino acid sequence corresponding to a TCR α chain variable domain sequence fused to the N terminus of an amino acid sequence corresponding to a TCR α chain constant domain extracellular sequence

The above scTCRs may further comprise a disulfide bond between the first and second chains, said disulfide bond being one which has no equivalent in native αβT cell receptors, and wherein the length of the linker sequence and the position of the disulfide bond being such that the variable domain sequences of the first and second segments are mutually orientated substantially as in native αβ T cell receptors.

More specifically the first segment may be constituted by an amino acid sequence corresponding to a TCR α chain variable domain sequence fused to the N terminus of an amino acid sequence corresponding to a TCR α chain constant domain extracellular sequence, the second segment may be constituted by an amino acid sequence corresponding to a TCR β chain variable domain fused to the N terminus of an amino acid sequence corresponding to TCR β chain constant domain extracellular sequence, and a disulfide bond may be provided between the first and second chains, said disulfide bond being one which has no equivalent in native αβ T cell receptors.

In the above scTCR forms, the linker sequence may link the C terminus of the first segment to the N terminus of the second segment, and may have the formula -PGGG-$(SGGGG)_n$-P- wherein n is 5 or 6 and P is proline, G is glycine and S is serine.

-PGGG-SGGGGSGGGGSGGGGSGGGGSGGGG-P (SEQ ID NO: 103)
-PGGG-SGGGGSGGGGSGGGGSGGGGSGGGGSGGGG-P (SEQ ID NO: 104)

A suitable dTCR form of the TCRs of the present invention comprises a first polypeptide wherein a sequence corresponding to a TCR α chain variable domain sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant domain extracellular sequence, and a second polypeptide wherein a sequence corresponding to a TCR β chain variable domain sequence fused to the N terminus a sequence corresponding to a TCR β chain constant domain extracellular sequence, the first and second polypeptides being linked by a disulfide bond which has no equivalent in native αβ T cell receptors.

The first polypeptide may comprise a TCR α chain variable domain sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant domain extracellular sequence, and a second polypeptide wherein a sequence corresponding to a TCR β chain variable domain sequence is fused to the N terminus a sequence corresponding to a TCR β chain constant domain extracellular sequence, the first and second polypeptides being linked by a disulfide bond between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof. ("TRAC" etc. nomenclature herein as per T cell receptor Factsbook, (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8)

The dTCR or scTCR form of the TCRs of the invention may have amino acid sequences corresponding to human αβ TCR extracellular constant and variable domain sequences, and a disulfide bond may link amino acid residues of the said constant domain sequences, which disulfide bond has no equivalent in native TCRs.

The disulfide bond is between cysteine residues corresponding to amino acid residues whose β carbon atoms are less than 0.6 nm apart in native TCRs, for example between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof. Other sites where cysteines can be introduced to form the disulfide bond are the following residues in exon 1 of TRAC*01 for the TCR α chain and TRBC1*01 or TRBC2*01 for the TCR β chain:

TCR α chain	TCR β chain	Native β carbon separation (nm)
Thr 45	Ser 77	0.533
Tyr 10	Ser 17	0.359
Thr 45	Asp 59	0.560
Ser 15	Glu 15	0.59

In addition to the non-native disulfide bond referred to above, the dTCR or scTCR form of the TCRs of the invention may include a disulfide bond between residues corresponding to those linked by a disulfide bond in native TCRs.

The dTCR or scTCR form of the TCRs of the invention preferably does not contain a sequence corresponding to transmembrane or cytoplasmic sequences of native TCRs.

Preferred embodiments of the invention provide a soluble TCR consisting of:

the alpha chain amino acid sequence of SEQ ID NO: 122 and beta chain amino acid sequence SEQ ID NO: 123:

the alpha chain amino acid sequence of SEQ ID NO: 122 and beta chain amino acid sequence SEQ ID NO: 124;

SEQ ID NOs: 122, 123 and 124 have been provided in a form which includes the N-terminal methionine (M) and the N-terminal "K" and "NA" in the TCR alpha and beta chain sequences respectively.

PEGylated TCR Monomers

In one particular embodiment a TCR of the invention is associated with at least one polyalkylene glycol chain(s). This association may be cause in a number of ways known to those skilled in the art. In a preferred embodiment the polyalkylene chain(s) is/are covalently linked to the TCR. In a further embodiment the polyethylene glycol chains of the present aspect of the invention comprise at least two polyethylene repeating units.

Multivalent TCR Complexes

One aspect of the invention provides a multivalent TCR complex comprising at least two TCRs of the invention. In one embodiment of this aspect, at least two TCR molecules are linked via linker moieties to form multivalent complexes. Preferably the complexes are water soluble, so the linker moiety should be selected accordingly. Furthermore, it is preferable that the linker moiety should be capable of attachment to defined positions on the TCR molecules, so that the structural diversity of the complexes formed is minimised. One embodiment of the present aspect is provided by a TCR complex of the invention wherein the polymer chain or peptidic linker sequence extends between amino acid residues of each TCR which are not located in a variable region sequence of the TCR.

Since the complexes of the invention may be for use in medicine, the linker moieties should be chosen with due regard to their pharmaceutical suitability, for example their immunogenicity.

Examples of linker moieties which fulfil the above desirable criteria are known in the art, for example the art of linking antibody fragments.

There are two classes of linker that are preferred for use in the production of multivalent TCR molecules of the present invention. A TCR complex of the invention in which the TCRs are linked by a polyalkylene glycol chain provides one embodiment of the present aspect.

The first are hydrophilic polymers such as polyalkylene glycols. The most commonly used of this class are based on polyethylene glycol or PEG, the structure of which is shown below.

$$HOCH_2CH_2O\ (CH_2CH_2O)_n\text{-}CH_2CH_2OH$$

Wherein n is greater than two. However, others are based on other suitable, optionally substituted, polyalkylene glycols include polypropylene glycol, and copolymers of ethylene glycol and propylene glycol.

Such polymers may be used to treat or conjugate therapeutic agents, particularly polypeptide or protein therapeutics, to achieve beneficial changes to the PK profile of the therapeutic, for example reduced renal clearance, improved plasma half-life, reduced immunogenicity, and improved solubility. Such improvements in the PK profile of the PEG-therapeutic conjugate are believe to result from the PEG molecule or molecules forming a 'shell' around the therapeutic which sterically hinders the reaction with the immune system and reduces proteolytic degradation. (Casey *et al*, (2000) Tumor Targetting **4** 235-244) The size of the hydrophilic polymer used my in particular be selected on the basis of the intended therapeutic use of the TCR complex. Thus for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use low molecular weight polymers in the order of 5 KDa. There are numerous review papers and books that detail the use of PEG and similar molecules in pharmaceutical formulations. For example, see Harris (1992) Polyethylene Glycol Chemistry - Biotechnical and Biomedical Applications, Plenum, New York, NY. or Harris &

Zalipsky (1997) Chemistry and Biological Applications of Polyethylene Glycol ACS Books, Washington, D.C.

The polymer used can have a linear or branched conformation. Branched PEG molecules, or derivatives thereof, can be induced by the addition of branching moieties including glycerol and glycerol oligomers, pentaerythritol, sorbitol and lysine.

Usually, the polymer will have a chemically reactive group or groups in its structure, for example at one or both termini, and/or on branches from the backbone, to enable the polymer to link to target sites in the TCR. This chemically reactive group or groups may be attached directly to the hydrophilic polymer, or there may be a spacer group/moiety between the hydrophilic polymer and the reactive chemistry as shown below:

Reactive chemistry-Hydrophilic polymer-Reactive chemistry

Reactive chemistry-Spacer-Hydrophilic polymer-Spacer-Reactive chemistry

The spacer used in the formation of constructs of the type outlined above may be any organic moiety that is a non-reactive, chemically stable, chain, Such spacers include, by are not limited to the following:

$-(CH_2)_n-$ wherein $n = 2$ to 5

$-(CH_2)_3NHCO(CH_2)_2$

A TCR complex of the invention in which a divalent alkylene spacer radical is located between the polyalkylene glycol chain and its point of attachment to a TCR of the complex provides a further embodiment of the present aspect.

A TCR complex of the invention in which the polyalkylene glycol chain comprises at least two polyethylene glycol repeating units provides a further embodiment of the present aspect.

There are a number of commercial suppliers of hydrophilic polymers linked, directly or via a spacer, to reactive chemistries that may be of use in the present invention. These suppliers include Nektar Therapeutics (CA, USA), NOF Corporation (Japan), Sunbio (South Korea) and Enzon Pharmaceuticals (NJ, USA).

Commercially available hydrophilic polymers linked, directly or via a spacer, to reactive chemistries that may be of use in the present invention include, but are not limited to, the following:

PEG linker Description	Source of PEG	Catalogue Number
TCR Monomer attachment		
5K linear (Maleimide)	Nektar	2D2MOHO1
20K linear (Maleimide)	Nektar	2D2MOPO1
20K linear (Maleimide)	NOF Corporation	SUNBRIGHT ME-200MA
20K branched (Maleimide)	NOF Corporation	SUNBRIGHT GL2-200MA
30K linear (Maleimide)	NOF Corporation	SUNBRIGHT ME-300MA
40K branched PEG (Maleimide)	Nektar	2D3XOTO1
5K-NP linear (for Lys attachment)	NOF Corporation	SUNBRIGHT MENP-50H
10K-NP linear (for Lys attachment)	NOF Corporation	SUNBRIGHT MENP-10T
20K-NP linear (for Lys attachment)	NOF Corporation	SUNBRIGHT MENP-20T
TCR dimer linkers		
3.4K linear (Maleimide)	Nektar	2D2DOFO2
5K forked (Maleimide)	Nektar	2D2DOHOF
10K linear (with orthopyridyl ds-linkers in place of Maleimide)	Sunbio	
20K forked (Maleimide)	Nektar	2D2DOPOF
20K linear (Maleimide)	NOF Corporation	
40K forked (Maleimide)	Nektar	2D3XOTOF

Higher order TCR multimers		
15K, 3 arms, Mal_3 (for trimer)	Nektar	OJOONO3
20K, 4 arms, Mal_4 (for tetramer)	Nektar	OJOOPO4
40 K, 8 arms, Mal_8 (for octamer)	Nektar	OJOOTO8

A wide variety of coupling chemistries can be used to couple polymer molecules to protein and peptide therapeutics. The choice of the most appropriate coupling chemistry is largely dependant on the desired coupling site. For example, the following coupling chemistries have been used attached to one or more of the termini of PEG molecules (Source: Nektar Molecular Engineering Catalogue 2003):

N-maleimide
Vinyl sulfone
Benzotriazole carbonate
Succinimidyl proprionate
Succinimidyl butanoate
Thio-ester
Acetaldehydes
Acrylates
Biotin
Primary amines

As stated above non-PEG based polymers also provide suitable linkers for multimerising the TCRs of the present invention. For example, moieties containing maleimide termini linked by aliphatic chains such as BMH and BMOE (Pierce, products Nos. 22330 and 22323) can be used.

Peptidic linkers are the other class of TCR linkers. These linkers are comprised of chains of amino acids, and function to produce simple linkers or multimerisation domains onto which TCR molecules can be attached. The biotin / streptavidin system has previously been used to produce TCR tetramers (see WO/99/60119) for in-vitro binding studies. However, stepavidin is a microbially-derived polypeptide and as such not ideally suited to use in a therapeutic.

A TCR complex of the invention in which the TCRs are linked by a peptidic linker derived from a human multimerisation domain provides a further embodiment of the present aspect.

There are a number of human proteins that contain a multimerisation domain that could be used in the production of multivalent TCR complexes. For example the tetramerisation domain of p53 which has been utilised to produce tetramers of scFv antibody fragments which exhibited increased serum persistence and significantly reduced off-rate compared to the monomeric scFV fragment. (Willuda *et al.* (2001) J. Biol. Chem. 276 (17) 14385-14392) Haemoglobin also has a tetramerisation domain that could potentially be used for this kind of application.

A multivalent TCR complex of the invention comprising at least two TCRs provides a final embodiment of this aspect, wherein at least one of said TCRs is associated with a therapeutic agent.

In one aspect a TCR (or multivalent complex thereof) of the present invention may alternatively or additionally comprise a reactive cysteine at the C-terminal or N-terminal of the alpha or beta chains thereof.

Diagnostic and therapeutic Use

In one aspect the TCR of the invention may be associated with a therapeutic agent or detectable moiety. For example, said therapeutic agent or detectable moiety may be covalently linked to the TCR.

In one embodiment of the invention said therapeutic agent or detectable moiety is covalently linked to the C-terminus of one or both TCR chains.

In one aspect the scTCR or one or both of the dTCR chains of TCRs of the present invention may be labelled with an detectable moiety, for example a label that is suitable for diagnostic purposes. Such labelled TCRs are useful in a method for detecting a SLLMWITQC-HLA-A*0201 complex which method comprises contacting the TCR ligand with a TCR (or a multimeric high affinity TCR complex) which is specific for the TCR ligand; and detecting binding to the TCR ligand. In tetrameric TCR complexes formed for example, using biotinylated heterodimers, fluorescent streptavidin can be used to provide a detectable label. Such a fluorescently-labelled TCR tetramer is suitable for use in FACS analysis, for example to detect antigen presenting cells carrying theSLLMWITQC-HLA-A*0201 complex for which these high affinity TCRs are specific.

Another manner in which the soluble TCRs of the present invention may be detected is by the use of TCR-specific antibodies, in particular monoclonal antibodies. There are many commercially available anti-TCR antibodies, such as αFl and βFl, which recognise the constant domains of the α and β chains, respectively.

In a further aspect a TCR (or multivalent complex thereof) of the present invention may alternatively or additionally be associated with (e.g. covalently or otherwise linked to) a therapeutic agent which may be, for example, a toxic moiety for use in cell killing, or an immune effector molecule such as an interleukin or a cytokine. A multivalent TCR complex of the invention may have enhanced binding capability for a TCR ligand compared to a non-multimeric wild-type or T cell receptor heterodimer of the invention. Thus, the multivalent TCR complexes according to the invention are particularly useful for tracking or targeting cells presenting particular antigens *in vitro* or *in vivo*, and are also useful as intermediates for the production of further multivalent TCR complexes having such uses. These TCRs or multivalent TCR complexes may therefore be provided in a pharmaceutically acceptable formulation for use *in vivo*.

The invention also provides a method for delivering a therapeutic agent to a target cell, which method comprises contacting potential target cells with a TCR or multivalent TCR complex in accordance with the invention under conditions to allow attachment of the TCR or multivalent TCR complex to the target cell, said TCR or multivalent TCR complex being specific for theSLLMWITQC-HLA-A*0201 complex and having the therapeutic agent associated therewith.

In particular, the soluble TCR or multivalent TCR complex of the present invention can be used to deliver therapeutic agents to the location of cells presenting a particular antigen. This would be useful in many situations and, in particular, against tumours. A therapeutic agent could be delivered such that it would exercise its effect locally but not only on the cell it binds to. Thus, one particular strategy envisages anti-tumour molecules linked to TCRs or multivalent TCR complexes according to the invention specific for tumour antigens.

Many therapeutic agents could be employed for this use, for instance radioactive compounds, enzymes (perforin for example) or chemotherapeutic agents (cis-platin for example). To ensure that toxic effects are exercised in the desired location the toxin could be inside a liposome linked to streptavidin so that the compound is released slowly. This will prevent damaging effects during the transport in the body and ensure that the toxin has maximum effect after binding of the TCR to the relevant antigen presenting cells.

Other suitable therapeutic agents include:

- small molecule cytotoxic agents, i.e. compounds with the ability to kill mammalian cells having a molecular weight of less than 700 daltons. Such compounds could also contain toxic metals capable of having a cytotoxic effect. Furthermore, it is to be understood that these small molecule cytotoxic agents also include pro-drugs, i.e. compounds that decay or are converted under physiological conditions to release cytotoxic agents. Examples of such agents include cis-platin, maytansine derivatives, rachelmycin, calicheamicin, docetaxel, etoposide, gemcitabine, ifosfamide, irinotecan, melphalan, mitoxantrone, sorfimer sodiumphotofrin II, temozolmide, topotecan, trimetreate glucuronate, auristatin E vincristine and doxorubicin;

- peptide cytotoxins, i.e. proteins or fragments thereof with the ability to kill mammalian cells. Including but not limited to, ricin, diphtheria toxin, pseudomonas bacterial exotoxin A, DNAase and RNAase;
- radio-nuclides, i.e. unstable isotopes of elements which decay with the concurrent emission of one or more of α or β particles, or γ rays. including but not limited to, iodine 131, rhenium 186, indium 111, yttrium 90, bismuth 210 and 213, actinium 225 and astatine 213; chelating agents may be used to facilitate the association of these radio-nuclides to the high affinity TCRs, or multimers thereof;
- prodrugs, including but not limited to, antibody directed enzyme pro-drugs;
- immuno-stimulants, i.e. moieties which stimulate immune response. Including but not limited to, cytokines such as IL-2 and IFN, Superantigens and mutants thereof, TCR-HLA fusions and chemokines such as IL-8, platelet factor 4, melanoma growth stimulatory protein, etc, antibodies or fragments thereof, complement activators, xenogeneic protein domains, allogeneic protein domains, viral/bacterial protein domains, viral/bacterial peptides and anti-T cell determinant antibodies (e.g. anti-CD3 or anti-CD28).

Functional antibody fragments and variants

Antibody fragments and variants/analogues which are suitable for use in the compositions and methods described herein include, but are not limited to, the following.

Antibody Fragments

As is known to those skilled in the art, it is possible to produce fragments of a given antibody which retain substantially the same binding characteristics as those of the parent antibody. The following provides details of such fragments:

Minibodies – These constructs consist of antibodies with a truncated Fc portion. As such they retain the complete binding domains of the antibody from which are derived.

Fab fragments – These comprise a single immunoglobulin light chain covalently-linked to part of an immunoglobulin heavy chain. As such, Fab fragments comprise a single antigen combining site. Fab fragments are defined by the portion of an IgG that can be liberated by treatment with papain. Such fragments are commonly produced via recombinant DNA techniques. (Reeves *et al.*, (2000) *Lecture Notes on Immunology* (4th Edition) Published by Blackwell Science)

$F(ab')_2$ fragments – These comprise both antigen combining sites and the hinge region from a single antibody. $F(ab')_2$ fragments are defined by the portion of an IgG that can be liberated by treatment with pepsin. Such fragments are commonly produced via recombinant DNA techniques. (Reeves *et al.*, (2000) *Lecture Notes on Immunology* (4tth Edition) Published by Blackwell Science)

Fv fragments – These comprise an immunoglobulin variable heavy domain linked to an immunoglobulin variable light domain. A number of Fv designs have been produced. These include dsFvs, in which the association between the two domains is enhanced by an introduced disulfide bond. Alternatively, scFVs can be formed using a peptide linker to bind the two domains together as a single polypeptide. Fvs constructs containing a variable domain of a heavy or light immunoglobulin chain associated to the variable and constant domain of the corresponding immunoglobulin heavy or light chain have also been produced. FV have also been multimerised to form diabodies and triabodies (Maynard et al., (2000) *Annu Rev Biomed Eng* **2** 339-376)

Nanobodies™ – These constructs, marketed by Ablynx (Belgium), comprise synthetic single immunoglobulin variable heavy domain derived from a camelid (e.g. camel or llama) antibody.

Domain Antibodies - These constructs, marketed by Domantis (Belgium), comprise an affinity matured single immunoglobulin variable heavy domain or immunoglobulin variable light domain.

Antibody variants and analogues

The defining functional characteristic of antibodies in the context of the present invention is their ability to bind specifically to a target ligand. As is known to those skilled in the art it is possible to engineer such binding characteristics into a range of other proteins. Examples of antibody variants and analogues suitable for use in the compositions and methods of the present invention include, but are not limited to, the following.

Protein scaffold-based binding polypeptides – This family of binding constructs comprise mutated analogues of proteins which contain native binding loops. Examples include Affibodies, marketed by Affibody (Sweden), which are based on a three-helix motif derived from one of the IgG binding domains of *Staphylococcus aureus* Protein A. Another example is provided by Evibodies, marketed by EvoGenix (Australia) which are based on the extracellular domains of CTLA-4into which domains similar to antibody binding loops are grafted. A final example, Cytokine Traps marketed by Regeneron Pharmaceuticals (US), graft cytokine receptor domains into antibody scaffolds. (Nygren *et al.*, (2000) *Current Opinion in Structural biology* 7 463-469) provides a review of the uses of scaffolds for engineering novel binding sites in proteins. This review mentions the following proteins as sources of scaffolds: CP1 zinc finger, Tendamistat, Z domain (a protein A analogue), PST1, Coiled coils, LACI-D1 and cytochrome b_{562}. Other protein scaffold studies have reported the use of Fibronectin, Green fluorescent protein (GFP) and ankyrin repeats.

As is known to those skilled in the art antibodies or fragments, variants or analogues thereof can be produced which bind to various parts of a given protein ligand. For example, anti-CD3 antibodies can be raised to any of the polypeptide chains from which this complex is formed (i.e.γ, δ, ε, ζ, and η CD3 chains) Antibodies which bind to the ε CD3 chain are the preferred anti-CD3 antibodies for use in the compositions and methods of the present invention.

Soluble TCRs or multivalent TCR complexes of the invention may be linked to an enzyme capable of converting a prodrug to a drug. This allows the prodrug to be converted to the drug only at the site where it is required (i.e. targeted by the sTCR).

It is expected that the high affinity SLLMWITQC (SEQ ID NO: 125)-HLA-A*0201 specific TCRs disclosed herein may be used in methods for the diagnosis and treatment of cancer.

For cancer treatment, the localisation in the vicinity of tumours or metastasis would enhance the effect of toxins or immunostimulants. For vaccine delivery, the vaccine antigen could be localised in the vicinity of antigen presenting cells, thus enhancing the efficacy of the antigen. The method can also be applied for imaging purposes.

One embodiment is provided by an isolated cell presenting a TCR of the invention. For example, said cell may be a T cell.

Further embodiments of the invention are provided by a pharmaceutical composition comprising:

a TCR or a multivalent TCR complex of the invention (optionally associated with a therapeutic agent), or a plurality of cells presenting at least one TCR of the invention, together with a pharmaceutically acceptable carrier;

The invention also provides a method of treatment of cancer comprising administering to a subject suffering such cancer disease an effective amount of a TCR or a multivalent TCR complex of the invention (optionally associated with a therapeutic agent), or a plurality of cells presenting at least one TCR of the invention. In a related embodiment the invention provides for the use of a TCR or a multivalent TCR complex of the invention (optionally associated with a therapeutic agent), or a plurality of cells presenting at least one TCR of the invention, in the preparation of a composition for the treatment of cancer.

Therapeutic or imaging TCRs in accordance with the invention will usually be supplied as part of a sterile, pharmaceutical composition which will normally include a pharmaceutically acceptable carrier. This pharmaceutical composition may be in any suitable form, (depending upon the desired method of administering it to a patient). It may be provided in unit dosage form, will generally be provided in a sealed container and may be provided as part of a kit. Such a kit would normally (although not necessarily) include instructions for use. It may include a plurality of said unit dosage forms.

The pharmaceutical composition may be adapted for administration by any appropriate route, for example parenteral, transdermal or via inhalation, preferably a parenteral (including subcutaneous, intramuscular, or, most preferably intravenous) route. Such compositions may be prepared by any method known in the art of pharmacy, for example by admixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

Dosages of the substances of the present invention can vary between wide limits, depending upon the disease or disorder to be treated, the age and condition of the individual to be treated, etc. and a physician will ultimately determine appropriate dosages to be used.

Additional Aspects

A scTCR or dTCR (which preferably is constituted by constant and variable sequences corresponding to human sequences) of the present invention may be provided in substantially pure form, or as a purified or isolated preparation. For example, it may be provided in a form which is substantially free of other proteins.

The invention also provides a method of producing a high affinity TCR having the property of binding to SLLMWITQC-HLA-A*0201.CHARACTERISED IN THAT the TCR (i) comprises at least one TCR α chain variable domain and/or at least one TCR β chain variable domain and (ii) has a K_D for the said SLLMWITQC-HLA-A*0201 complex of less than or equal to 1μM and/or an off-rate (k_{off}) for the SLLMWITQC-HLA-A*0201 complex of $1x10^{-3}$ S^{-1} or slower, wherein the method comprises:

(a) the production of a TCR comprising the α and β chain variable domains of the 1G4 TCR wherein one or both of the α and β chain variable domains comprise a mutation(s) in one or more of the amino acids identified in claims 7 and 8;

(b) contacting said mutated TCR with SLLMWITQC-HLA-A*0201 under conditions suitable to allow the binding of the TCR to SLLMWITQC-HLA-A*0201;

and measuring the K_D and/or k_{off} of the interaction.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*.

Examples

The invention is further described in the following examples, which do not limit the scope of the invention in any way.

Reference is made in the following to the accompanying drawings in which:

Figure 1a and 1b details the alpha chain variable domain amino acid and beta chain variable domain amino acid sequences of the native 1G4 TCR respectively.

Figures 2a and 2b show respectively the DNA sequence of soluble versions of the native 1G4 TCR α and β chains.

Figures 3a and 3b show respectively the 1G4 TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 2a and 2b.

Figures 4a and 4b show respectively the DNA sequence of soluble versions of the 1G4 TCR α and β chains mutated to include additional cysteine residues to form a non-native disulphide bond. The mutated codon is indicated by shading.

Figures 5a and 5b show respectively the 1G4 TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 4a and 4b. The introduced cysteine is indicated by shading.

Figure 6 details the alpha chain variable domain amino acid sequences of the high affinity 1G4 TCR variants.

Figure 7 details the beta chain variable domain amino acid sequences of the high affinity 1G4 TCR variants.

Figure 8a details the amino acid sequence of a soluble form of TRAC.

Figure 8b details the amino acid sequence of a soluble form of TRBC1.

Figure 8c details the amino acid sequence of a soluble form of TRBC2.

Figure 9 details the DNA sequence of the pEX954 plasmid.

Figure 10 details the DNA sequence of the pEX821 plasmid.

Figure 11 details the DNA sequence of the pEX202 plasmid.

Figure 12 details the DNA sequence of the pEX205 plasmid.

Figure 13 details further beta chain variable domain amino acid sequences of the high affinity 1G4 TCR variants.

Figure 14a details the alpha chain amino acid sequences of a preferred soluble high affinity 1G4 TCR variant.

Figure 14b details the beta chain amino acid sequences of a preferred (c58c61) soluble high affinity 1G4 TCR variant utilising the TRBC1 constant domain.

Figure 14c details the beta chain amino acid sequences of a preferred (c58c61) soluble high affinity 1G4 TCR variant utilising the TRBC2 constant domain.

Figure 14d details the beta chain amino acid sequences of a preferred (c58c61) soluble high affinity 1G4 TCR using the TRBC2 encoded constant region fused via a peptide linker to wild-type human IL-2.

Figure 15a shows FACs staining of T2 cell pulsed with a range of NY-ESO-analogue SLLMWITQV peptide concentrations using the high affinity c58c61 1G4 TCR-IL-2 fusion proteins.

Figure 15b shows FACs staining of T2 cell pulsed with a range of NY-ESO-derived SLLMWITQC peptide concentrations using the high affinity c58c61 1G4 TCR-IL-2 fusion proteins.

Figure 16 shows FACs staining of SK-MEL-37, ScaBER, J82, HcT119 and Colo 205 cancer cells transfected with an SLLMWITQC peptide producing ubiquitin minigene (± proteosome inhibitors) using the high affinity c58c61 1G4 TCR-IL-2 fusion proteins.

Figure 17 shows ELISPOT data demonstrating the ability of soluble high affinity c58c61 1G4 TCR to inhibit CTL activation against the MEL-624 cancer cell.

Figure 18 shows ELISPOT data demonstrating the ability of soluble high affinity c58c61 1G4 TCR to inhibit CTL activation against the SK-MEL-37 cancer cell.

Figure 19 shows inhibition of T cell activation against peptide pulsed T2 cells by the soluble c58c61 high affinity 1G4 TCR as measured by IFNγ production.

Figure 20 shows lack of inhibition of T cell activation against peptide pulsed T2 cells by the soluble wild-type 1G4 TCR as measured by IFNγ production.

Figure 21 shows tumor growth inhibition caused by soluble c58c61 high affinity 1G4 TCR-IL-2 immunoconjugates.

Figure 22 shows the number of SLLMWITQC-HLA-A*0201 antigens on the surface of Mel 526, Mel 624 and SK-Mel-37 cancer cells as determined by fluorescent microscopy. The visualisation of cell-bound biotinylated soluble c58c61 high affinity 1G4 TCRs was facilitated by conjugation with streptavidin-R phycoerythrin (PE).

Example 1 – Production of a soluble disulfide-linked TCR comprising the native 1G4 TCR variable domain

RNA isolation

Total RNA was isolated from 10000 clonal T cells by re-suspension in 100μl tri-reagent (Sigma) and processing of the lysate according to the manufacturer's instructions. After the final precipitation the RNA was re-dissolved in 12.5 μl RNAse free water.

cDNA Production

To the above sample of RNA, 2.5μl of 10 mM oligo-dT^{15} (Promega) was added and the sample incubated at 60°C for 2 minutes then placed on ice. Reverse transcription was carried out using OmniscriptRT™kit (Qiagen) by addition of 2μl RT buffer (10 ×), 2μl 5 mM dNTP, 1 μl Omniscript reverse transcriptase. The sample was mixed and incubated for 1 hour at 37°C. cDNA was then stored at -80°C.

The above cDNA was used as template. A panel of forward primers covering all possible alpha and beta variable chains was used to screen for, and amplify by PCR, alpha and beta chains genes. Primer sequences used for TCR chain gene amplification were designed from the NCBI website (http://www.ncbi.nlm.nih.gov/Entrez/) using accession numbers obtained from the T cell receptor Factsbook, (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8. Alpha -chain forward primers were designed to contain a *ClaI* restriction site and the universal alpha chain reverse primer a *SalI* restriction site. Beta-chain forward primers were designed to contain a *AseI* restriction site and universal beta reverse primer an *AgeI* restriction site.

Recipient vectors for the TCR gene fragments were based on a pGMT7 parent plasmid, which contains the T7 promoter for high level expression in E.coli strain BL21-DE3(pLysS) (Pan *et al., Biotechniques* (2000) **29** (6): 1234-8)

Alpha chain purified PCR products were digested with *ClaI* and *SalII* and ligated into pEX954 (see figure 9) cut with *ClaI* and *XhoI.*

Beta chain purified PCR products were digested with *AseI* and *AgeI* and ligated into pEX821 (See Figure 10) cut with *NdeI/AgeI.*

Ligation

The cut PCR product and cut vector were ligated using a rapid DNA ligation kit (Roche) following the manufacturers instructions.

Ligated plasmids were transformed into competent E.coli strain XL1-blue cells and plated out on LB/agar plates containing 100mg/ml ampicillin. Following incubation overnight at 37°C, single colonies were picked and grown in 10 ml LB containing 100mg/ml ampicillin overnight at 37°C with shaking. Cloned plasmids were purified using a Miniprep™ kit (Qiagen) and the insert was sequenced using an automated DNA sequencer (Lark Technologies).

Figures 4a and 4b show respectively the DNA sequence of soluble versions of the 1G4 TCR α and β chains mutated to include additional cysteine residues to form a non-native disulphide bond.

Figures 5a and 5b show respectively the NY-ESO TCR α and β chain extracellular amino acid sequences produced from the DNA sequences of Figures 4a and 4b

Example 2- Production of high affinity variants of the soluble disulfide linked 1G4 TCR

The soluble disulfide-linked native 1G4 TCR produced as described in Example 1 can be used a template from which to produce the TCRs of the invention which have an increased affinity for the SLLMWITQC (SEQ ID NO: 125) -HLA-A*0201 complex.

The amino sequences of the mutated TCR alpha and beta chain variable domains which demonstrate high affinity for the SLLMWITQC-HLA-A*0201 complex are listed in Figures 6 and 7 respectively. (SEQ ID Nos: 11-83 and 84-99 respectively) As is known to those skilled in the art the necessary codon changes required to produce these mutated chains can be introduced into the DNA encoding these chains by site-directed mutagenesis. (QuickChange™ Site-Directed Mutagenesis Kit from Stratagene)

Briefly, this is achieved by using primers that incorporate the desired codon change(s) and the plasmids containing the relevant 1G4 TCR chain as a template for the mutagenesis:

Mutagenesis was carried out using the following conditions : 50ng plasmid template, 1μl of 10mM dNTP, 5 μl of 10x Pfu DNA polymerase buffer as supplied by the manufacturer, 25 pmol of fwd primer, 25 pmol of rev primer, 1μl pfu DNA polymerase in total volume 50 μl. After an initial denaturation step of 2 mins at 95C, the reaction was subjected to 25 cycles of denaturation (95C, 10 secs), annealing (55C 10 secs), and elongation (72C, 8 mins). The resulting product was digested with DpnI restriction enzyme to remove the template plasmid and transformed into E. coli strain XL1-blue. Mutagenesis was verified by sequencing.

Example 3 - Production of soluble "zippered" high affinity TCRs

Alpha chain – c-jun leucine zipper

The construct was made by PCR stitching.

For the 5'-end of the gene the plasmid coding for the high affinity TCR alpha chains and containing the code for the introduced inter-chain di-sulfide bridge was used as template. PCR with the following two primer pairs generated the desired variable domain.

5'-TRAV21 fwd tctctcattaatgaaacaggaggtgacgcagattcct
(SEQ ID NO: 105)

C-alpha rev CGGCAGGGTCAGGGTTCTGG
(SEQ ID NO: 106)

For the 3'-end of the gene the plasmid pEX202 (see Figure 11), coding for a wild type affinity TCR alpha chain fused to human c-jun leucine zipper domain and not containing the code for the introduced inter-chain di-sulfide bridge, was used as template. PCR with the following primer pair generated the desired constant domain.

C-alpha fwd CCAGAACCCTGACCCTGCCG
(SEQ ID NO: 107)
3'-alpha rev aagcttcccgggggaactttctgggctggg
(SEQ ID NO: 108)

The two products were mixed and diluted 1000 fold and 1 µl was used as template in a 50 µl PCR with 5'-TRAV21 fwd and 3'-alpha rev primers.

The resulting PCR product was digested using restriction enzymes AseI and XmaI and ligated into pEX202 cut with NdeI and XmaI .

PCRs were carried out using the following conditions : 50 pg plasmid template, 1 µl of 10 mM dNTP, 5 µl of 10x Pfu DNA polymerase buffer as supplied by the manufacturer, 25 pmol of fwd primer, 25 pmol of rev primer, 1µl Pfu DNA polymerase in total volume 50 µl. After an initial denaturation step of 2 mins at 95C, the reaction was subjected to 30 cycles of denaturation (95C, 10 secs), annealing (55C 10 secs), and elongation (72C, 2 mins).

Beta chain – c-fos leucine zipper

The construct was made by PCR stitching.

For the 5'-end of the gene plasmids coding for the high affinity TCR beta chains and containing the introduced inter-chain di-sulfide bridge were used as template. PCR with the following two primers generated the desired variable domain gene fragment.

TRBV6-5 fwd tctctcattaatgaatgctggtgtcactcagacccc
(SEQ ID NO: 109)
C-beta rev CTTCTGATGGCTCAAACACAGC
(SEQ ID NO: 110)

For the 3'-end of the gene the plasmid pEX205 (see Figure 12), coding for a wild type affinity TCR beta chain fused to the human c-fos leucine zipper domain and not containing the code for the introduced inter-chain di-sulfide bridge, was used as template. PCR with the following two primers generated the desired constant domain gene fragment.

C-beta fwd GCTGTGTTTGAGCCATCAGAAG
(SEQ ID NO: 111)
TRBC rev aagcttcccggggtctgctctaccccaggc
(SEQ ID NO: 112)

The two products were mixed and diluted 1000 fold and 1 μl was used as template in a 50 μl PCR with TRBV6-5 fwd and TRBC rev primers. PCRs were carried out as described above.

The resulting PCR product was digested using restriction enzymes AseI and XmaI and ligated into pEX205 cut with NdeI and XmaI.

Example 4 – Expression, refolding and purification of soluble TCR

The expression plasmids containing the mutated α-chain and β-chain respectively as prepared in Examples 1, 2 or 3 were transformed separately into *E.coli* strain BL21pLysS, and single ampicillin-resistant colonies were grown at 37°C in TYP (ampicillin 100μg/ml) medium to OD_{600} of 0.4 before inducing protein expression with 0.5mM IPTG. Cells were harvested three hours post-induction by centrifugation for 30 minutes at 4000rpm in a Beckman J-6B. Cell pellets were re-suspended in a buffer containing 50mM Tris-HCI, 25% (w/v) sucrose, 1mM NaEDTA, 0.1% (w/v) NaAzide, 10mM DTT, pH 8.0. After an overnight freeze-thaw step, re-suspended cells were sonicated in 1 minute bursts for a total of around 10 minutes in a Milsonix™ XL2020 sonicator using a standard 12mm diameter probe. Inclusion body pellets were recovered by centrifugation for 30 minutes at 13000rpm in a Beckman J2-21 centrifuge. Three detergent washes were then carried out to remove cell debris and membrane components. Each time the inclusion body pellet was homogenised in a Triton™buffer (50mM Tris-HCI, 0.5% Triton-X100, 200mM NaCI, 10mM NaEDTA, 0.1% (w/v) NaAzide, 2mM DTT, pH 8.0) before being pelleted by centrifugation for 15 minutes at 13000rpm in a Beckman J2-21. Detergent and salt was then removed by a similar wash in the following buffer: 50mM Tris-HCl, 1mM NaEDTA, 0.1% (w/v) NaAzide, 2mM DTT, pH 8.0. Finally, the inclusion bodies were divided into 30 mg aliquots and frozen at -70°C. Inclusion body protein yield was quantitated by solubilising with 6M guanidine-HCl and measurement with a Bradford dye-binding assay (PerBio).

Approximately 30mg of TCR β chain and 60mg of TCR α chain solubilised inclusion bodies were thawed from frozen stocks, samples were then mixed and the mixture diluted into 15ml of a guanidine solution (6 M Guanidine-hydrochloride, 10mM Sodium Acetate, 10mM EDTA), to ensure complete chain de-naturation. The guanidine solution containing fully reduced and denatured TCR chains was then injected into 1 litre of the following refolding buffer: 100mM Tris pH 8.5, 400mM L-Arginine, 2mM EDTA, 5mM reduced Glutathione, 0.5mM oxidised Glutathione, 5M urea, 0.2mM PMSF. The redox couple (2-mercaptoethylamine and cystamine (to final concentrations of 6.6mM and 3.7mM, respectively) were added approximately 5 minutes before addition of the denatured TCR chains. The solution was left for 5 hrs ± 15minutes. The refolded TCR was dialysed in Spectrapor 1 membrane (Spectrum; Product No. 132670) against 10 L 10 mM Tris pH 8.1 at 5°C ± 3°C for 18-20 hours. After this time, the dialysis buffer was changed to fresh 10 mM Tris pH 8.1 (10 L) and dialysis was continued at 5°C ± 3°C for another 20-22 hours.

sTCR was separated from degradation products and impurities by loading the dialysed refold onto a POROS 50HQ anion exchange column and eluting bound protein with a gradient of 0-500mM NaCI over 50 column volumes using an Akta purifier (Pharmacia). Peak fractions were stored at 4°C and analysed by Coomassie-stained SDS-PAGE before being pooled and concentrated. Finally, the sTCR was purified and characterised using a Superdex 200HR gel filtration column pre-equilibrated in HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.5 mM EDTA, 0.05% nonidet p40). The peak eluting at a relative molecular weight of approximately 50 kDa was pooled and concentrated prior to characterisation by BIAcore surface plasmon resonance analysis.

Example 5 – Biacore surface plasmon resonance characterisation of sTCR binding to specific pMHC

A surface plasmon resonance biosensor (Biacore 3000™) was used to analyse the binding of a sTCR to its peptide-MHC ligand. This was facilitated by producing single pMHC complexes (described below) which were immobilised to a streptavidin-coated binding surface in a semi-oriented fashion, allowing efficient testing of the binding of a soluble T-cell receptor to up to four different pMHC (immobilised on separate flow cells) simultaneously. Manual injection of HLA complex allows the precise level of immobilised class I molecules to be manipulated easily.

Biotinylated class I HLA-A*0201 molecules were refolded *in vitro* from bacterially-expressed inclusion bodies containing the constituent subunit proteins and synthetic peptide, followed by purification and *in vitro* enzymatic biotinylation (O'Callaghan *et al.* (1999) *Anal. Biochem.* **266**: 9-15). HLA-A*0201-heavy chain was expressed with a C-terminal biotinylation tag which replaces the transmembrane and cytoplasmic domains of the protein in an appropriate construct. Inclusion body expression levels of ~75 mg/litre bacterial culture were obtained. The MHC light-chain or β2-microglobulin was also expressed as inclusion bodies in *E.coli* from an appropriate construct, at a level of ~500 mg/litre bacterial culture.

E. coli cells were lysed and inclusion bodies are purified to approximately 80% purity. Protein from inclusion bodies was denatured in 6 M guanidine-HCl, 50 mM Tris pH 8.1, 100 mM NaCl, 10 mM DTT, 10 mM EDTA, and was refolded at a concentration of 30 mg/litre heavy chain, 30 mg/litre β2m into 0.4 M L-Arginine-HCl, 100 mM Tris pH 8.1, 3.7 mM cystamine, mM cysteamine, 4 mg/ml of the SLLMWITQC peptide required to be loaded by the HLA-A*0201 molecule, by addition of a single pulse of denatured protein into refold buffer at < 5°C. Refolding was allowed to reach completion at 4°C for at least 1 hour.

Buffer was exchanged by dialysis in 10 volumes of 10 mM Tris pH 8.1. Two changes of buffer were necessary to reduce the ionic strength of the solution sufficiently. The protein solution was then filtered through a 1.5μm cellulose acetate filter and loaded onto a POROS 50HQ anion exchange column (8 ml bed volume). Protein was eluted with a linear 0-500 mM NaCl gradient. HLA-A*0201-peptide complex eluted at approximately 250 mM NaCl, and peak fractions were collected, a cocktail of protease inhibitors (Calbiochem) was added and the fractions were chilled on ice.

Biotinylation tagged pMHC molecules were buffer exchanged into 10 mM Tris pH 8.1, 5 mM NaCl using a Pharmacia fast desalting column equilibrated in the same buffer. Immediately upon elution, the protein-containing fractions were chilled on ice and protease inhibitor cocktail (Calbiochem) was added. Biotinylation reagents were then added: 1 mM biotin, 5 mM ATP (buffered to pH 8), 7.5 mM MgCl2, and 5 μg/ml BirA enzyme (purified according to O'Callaghan *et al.* (1999) *Anal. Biochem.* **266**: 9-15). The mixture was then allowed to incubate at room temperature overnight.

The biotinylated pHLA-A*0201 molecules were purified using gel filtration chromatography. A Pharmacia Superdex 75 HR 10/30 column was pre-equilibrated with filtered PBS and 1 ml of the biotinylation reaction mixture was loaded and the column was developed with PBS at 0.5 ml/min. Biotinylated pHLA-A*0201 molecules eluted as a single peak at approximately 15 ml. Fractions containing protein were pooled, chilled on ice, and protease inhibitor cocktail was added. Protein concentration was determined using a Coomassie-binding assay (PerBio) and aliquots of biotinylated pHLA-A*0201 molecules were stored frozen at –20°C. Streptavidin was immobilised by standard amine coupling methods.

Such immobilised complexes are capable of binding both T-cell receptors and the coreceptor CD8αα, both of which may be injected in the soluble phase. Specific binding of TCR is obtained even at low concentrations (at least 40μg/ml), implying the TCR is relatively stable. The pMHC binding properties of sTCR are observed to be qualitatively and quantitatively similar if sTCR is used either in the soluble or immobilised phase. This is an important control for partial activity of soluble species and also suggests that biotinylated pMHC complexes are biologically as active as non-biotinylated complexes.

The interactions between 1G4 sTCR containing a novel inter-chain bond and its ligand/ MHC complex or an irrelevant HLA-peptide combination, the production of which is described above, were analysed on a Biacore 3000™ surface plasmon resonance (SPR) biosensor. SPR measures changes in refractive index expressed in response units (RU) near a sensor surface within a small flow cell, a principle that can be used to detect receptor ligand interactions and to analyse their affinity and kinetic parameters. The probe flow cells were prepared by immobilising the individual HLA-peptide complexes in separate flow cells via binding between the biotin cross linked onto β2m and streptavidin which have been chemically cross linked to the activated surface of the flow cells. The assay was then performed by passing sTCR over the surfaces of the different flow cells at a constant flow rate, measuring the SPR response in doing so.

To measure Equilibrium binding constant

Serial dilutions of WT 1G4 sTCR were prepared and injected at constant flow rate of 5 μl min-1 over two different flow cells; one coated with ~1000 RU of specific SLLMWITQC-HLA-A*0201 complex, the second coated with ~1000 RU of non-specific HLA-A2 -peptide complex. Response was normalised for each concentration using the measurement from the control cell. Normalised data response was plotted versus concentration of TCR sample and fitted to a hyperbola in order to calculate the equilibrium binding constant, K_D. (Price & Dwek, Principles and Problems in Physical Chemistry for Biochemists (2nd Edition) 1979, Clarendon Press, Oxford).

To measure Kinetic Parameters

For high affinity TCRs K_D was determined by experimentally measuring the dissociation rate constant, kd, and the association rate constant, ka. The equilibrium constant K_D was calculated as kd/ka.
TCR was injected over two different cells one coated with ~300 RU of specific HLA-A2-nyeso peptide complex, the second coated with ~300 RU of non-specific HLA-A2 -peptide complex. Flow rate was set at 50 μl/min. Typically 250 μl of TCR at ~3 μM concentration was injected. Buffer was then flowed over until the response had returned to baseline. Kinetic parameters were calculated using Biaevaluation software. The dissociation phase was also fitted to a single exponential decay equation enabling calculation of half-life.

Results

The interaction between a soluble disulfide-linked native 1G4 TCR (consisting of the α and β TCR chains detailed in SEQ ID NOs 9 and 10 respectively) and the SLLMWITQC-HLA-A*0201 complex was analysed using the above methods and demonstrated a K_D of 15 μM and a k_{off} of 1.28×10^{-1} S^{-1}.

The TCRs specified in the following table have a K_D of less than or equal to 1 μM and/or a k_{off} of 1 x 10^{-3} S^{-1} or slower.

Alpha chain variable domain sequence, SEQ ID NO:	Beta chain variable domain sequence, SEQ ID NO:
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1	85
1	86
1	87
1	88
11	84
12	84
12	85
12	90
11	85
11	86
11	92
11	93
13	86
14	84
14	85
15	84
15	85
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16	85
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33	84
20	86
34	86
35	89
36	89
37	89
38	89
39	89
16	89
17	89
31	89
40	89
1	90
1	91
41	90
42	2

42	85
42	92
1	92
1	93
43	92
44	92
45	92
46	92
47	92
48	84
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50	84
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51	95
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82	92
83	92
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11	97
11	98
11	99
1	89
50	117

49	117
50	118
49	119
50	119
58	93
49	118
1	119
1	117
55	120
56	120
50	121
50	120
49	121
49	120
48	118
53	95

Example 6 – In-vitro cell staining using a high affinity c58c61 NY-ESO TCR- IL-2 fusion protein.

T2 lymphoblastoid cells were pulsed with the NY-ESO-derived SLLMWITQC, NY-ESO-analogue SLLMWITQV peptide, or an irrelevant peptide at a range of concentrations (10^{-5} – 10^{-10}M) for 180 minutes at 37°C. The NY-ESO-analogue SLLMWITQV peptide (V-variant peptide) was used as this peptide is known to have a higher affinity for the binding cleft of the HLA-A*0201 complex than the native NY-ESO-derived SLLMWITQC peptide. After pulsing, cells were washed in serum-free RPMI and 5 x 10^5 cells were incubated with high affinity c58c61 NY-ESO TCR- IL-2 fusion protein for 10min at room temperature, followed by secondary anti-IL-2 mAb conjugated with PE (Serotec) for 15min at room temperature. After washing, bound TCR-IL-2 was quantified by flow cytometry using a FACSVantage SE (Becton

Dickinson).Controls, also using peptide-pulsed T2 cells were included where TCR-IL-2 was omitted.

Figure 14a details the amino acid sequence of the alpha chain of the c58c61 NY-ESO TCR. (SEQ ID NO: 122).

Figure 14c (SEQ ID NO: 124) details the amino acid sequence of the beta chain of the c58c61 NY-ESO TCR using the TRBC2 encoded constant region.

Figure 14d (SEQ ID NO: 125) details the amino acid sequence of the beta chain of the c58c61 NY-ESO TCR using the TRBC2 encoded constant region fused via a peptide linker to wild-type human IL-2.

The alpha and beta chain variable domain mutations contained within the soluble c58c61 1G4 TCR – IL-2 fusion protein correspond to those detailed in SEQ ID NO: 49 and SEQ ID NO: 94 respectively. Note that SEQ ID NOs: 121 -125 have been provided in a form which includes the N-terminal methionine (M) and the "K" and "NA" residues omitted in the majority of the other TCR alpha chain and beta chain amino acid sequences.

In similar experiments SK-MEL-37, ScaBER, J82, HcT119 and Colo 205 cancer cells transfected with a NY-ESO-derived SLLMWITQC peptide expressing ubiquitin minigene construct were used. The cancer cells were transfected using substantially the methods described in (Rimoldi *et al.*, (2000) *J. Immunol.* **165** 7253-7261). Cells were labelled as described above.

Results

Figure 15a shows FACs staining of T2 cell pulsed with a range of NY-ESO-analogue SLLMWITQV peptide concentrations using the high affinity c58c61 1G4 TCR-IL-2 fusion proteins.

Figure 15a shows FACs staining of T2 cell pulsed with a range of NY-ESO-analogue SLLMWITQV peptide concentrations using the high affinity c58c61 1G4 TCR-IL-2 fusion proteins.

Figure 15b shows FACs staining of T2 cell pulsed with a range of NY-ESO-derived SLLMWITQC peptide concentrations using the high affinity c58c61 1G4 TCR-IL-2 fusion proteins.

Figure 16 shows FACs staining of SK-MEL-37, ScaBER, J82, HcT119 and Colo 205 cancer cells transfected with an SLLMWITQC peptide producing ubiquitin minigene (± proteosome inhibitors) using the high affinity c58c61 1G4 TCR-IL-2 fusion proteins.

Example 9 – CTL activation ELISPOT assay

The following assay was carried out to demonstrate that the soluble high affinity c58c61 NY-ESO TCR was capable of inhibiting activation of an SLLMWITQC-HLA-A*0201 specific CTL clone (1G4). IFN-γ production was used as the read-out for CTL activation.

Reagents

R10 Assay media: 10% FCS (heat-inactivated, Gibco, cat# 10108-165), 88% RPMI 1640 (Gibco, cat# 42401-018), 1% glutamine (Gibco, cat# 25030-024) and 1% penicillin/streptomycin (Gibco, cat# 15070-063).

Peptide: (obtained from various sources) initially dissolved in DMSO (Sigma, cat# D2650) at 4mg/ml and frozen.

Wash buffer: 0.01M PBS/0.05% Tween™20 (1 sachet of Phosphate buffered saline with Tween 20, pH7.4 from Sigma, Cat. # P-3563 dissolved in 1litre distilled water gives final composition 0.01M PBS, 0.138M NaCl, 0.0027M KCl, 0.05% Tween 20).
PBS (Gibco, cat#10010-015).

The EliSpot™kit contains all other reagents required i.e. capture and detection antibodies, skimmed milk powder, BSA, streptavidin-alkaline phosphatase, BCIP/NBT solution (Human IFN-g PVDF Eli-spot 20 x 96 wells with plates (IDS cat# DC-856.051.020, DC-856.000.000). The following method is based on the instructions supplied with each kit but contains some alterations.

MEL-624 and SK-MEL-37 melanoma cell lines were treated with trypsin for 5 minutes at 37 °C. The cells are then washed and re-suspended in R10 media.

50000 target cells were then plated out per well in 50µl of R10 media in a 96 well ELISPOT plate (Diaclone).

The following was then added to the above target cell cultures:

1 x 10^{-7} M high affinity c58c61 TCR, or an irrelevant TCR, in 50µl of R10 media.

600 SLLMWITQC-HLA-A*0201 specific T cells (clone 1G4) in 50µl of R10 media.

These cultures were then incubated for 24 hours at 37°C, 5%CO_2. The ELISPOT plates were processed according to the manufacturers instructions.

Results

The soluble high affinity c58c61 1G4 TCR strongly inhibited the activation of 1G4 T cell clones against the melanoma cells, as measured by IFN-γ production. Whereas the irrelevant high affinity TCR had no inhibitory effect. (See Figure 17 for MEL-624 cancer cell line results and Figure 18 for SK-MEL-37 cancer cell line results)

Example 10 – CTL activation ELISA assay

The following assay was carried out to demonstrate that the soluble high affinity c58c61 1G4 TCR was capable of inhibiting activation of an SLLMWITQC-HLA-A*0201 specific CTL clone (1G4). IFN-γ production was used as the read-out for CTL activation.

Reagents

R10 Assay media: 10% FCS (heat-inactivated, Gibco, cat# 10108-165), 88% RPMI 1640 (Gibco, cat# 42401-018), 1% glutamine (Gibco, cat# 25030-024) and 1% penicillin/streptomycin (Gibco, cat# 15070-063).

Peptide: (obtained from various sources) initially dissolved in DMSO (Sigma, cat# D2650) at 4mg/ml and frozen.

Wash buffer: 0.01M PBS/0.05% Tween 20 (1 sachet of Phosphate buffered saline with Tween 20, pH7.4 from Sigma, Cat. # P-3563 dissolved in 1litre distilled water gives final composition 0.01M PBS, 0.138M NaCl, 0.0027M KCl, 0.05% Tween 20).
PBS (Gibco, cat#10010-015).

The ELISA kit contains all other reagents except BSA (Sigma). required i.e. capture and detection antibodies, skimmed milk powder, streptavidin-HRP, TMB solution (Human IFN-g Eli-pair 20 x 96 wells with plates. The following method is based substantially on the instructions supplied with each kit.

Method

ELISA plates were prepared according to the manufacturers instructions. (Diaclone kit, Immunodiagnostic systems, UK

T2 cell line target cells were washed and re-suspended in R10 media with or without varying concentrations (100nM – 10pM) of SLLMWITQC peptide, then incubated for 1 hour at 37°C, 5%CO_2.

10,000 target cells per well were then plated out into a 96 well ELISA plate.

To these plates the following was added to the relevant well:

1 x 10^{-6} M to 3 x 10^{-12} M of the high affinity c58c61 1G4 TCR or wild-type 1G4 TCR in 50 µl of R10 media.

5000 1G4 effector cells in 50 µl of R10 media.

The plates were then incubated for 48 hours at 37°C, 5% CO_2. The ELISA was then processed according to manufacturer's instructions.

Results

The soluble high affinity c58c61 1G4 TCR strongly inhibited the activation of 1G4 T cell clones against the peptide-pulsed target cells, as measured by IFN-γ production. Whereas the wild-type 1G4 TCR had no inhibitory effect. (See Figure 19 for the high affinity c58c61 1G4 TCR and Figure 20 for the wild-type 1G4 TCR)

Example 11 –In-vivo tumour targeting using a high affinity c58c61 IG4 TCR-IL-2 fusion protein

This work was carried out to investigate the ability of a high affinity c58c61 1G4 TCR-IL-2 fusion protein described in Example 6, to inhibit growth of human tumor cells engrafted in nude mice.

Fifty female nude mice (HARLAN, France) were used in this trial.

All animals were injected subcutaneously with the human melanoma tumour-forming cell line (SK-MEL-37) which had been stably transfected with a NY-ESO peptide/ubiquitin minigene construct in ensure enhanced expression of the appropriate class I-peptide target at the cell surface. Tumors were allowed to grow in the animals for 5 days to allow tumour development prior to commencement of treatment.

The rats then received the following i.v. bolus dosage of c58c61 high affinity NY-ESO TCR/IL-2 fusion protein:

Doses ranged between 0.02 and 1.0 mg/kg high affinity 1G4 TCR/IL-2 fusion proteins in PBS, administered at 5, 6, 7, 8, 11, 13, 17, 20, 24, 28, and 30 day post-tumor engraftment. In all experiments, a control treatment group was included where PBS alone was substituted for the TCR/IL-2 immunoconjugate.

Tumor size was then measured using callipers and tumor volume determined according to the following formula (W^2 x L)/2, where W=the smallest diameter of the tumor, and L= is the longest diameter.

Results

The therapeutic effect of the TCR/IL-2 immunoconjugates in terms of tumor growth inhibition is shown in Figure 21.

Conclusions

The TCR/IL-2 immunoconjugate exhibited a clear dose-dependent anti-tumor effect as shown by the tumour growth curves depicted in Figure 21.

Example 12 - Quantification of cell surface TCR ligands by fluorescence microscopy using high affinity c58c61 1G4 TCR

The number of SLLMWITQC-HLA-A*0201 antigens on cancer cells (Mel 526, Mel 624 and SK-Mel-37 cell lines) was determined (on the assumption that one fluorescence signal relates to a single labelled TCR bound to its cognate pMHC ligand on the surface of the target cell) by single molecule fluorescence microscopy using the high-affinity c58c61 1G4 TCR. This was facilitated by using biotinylated TCR to target the antigen-expressing cancer cells and subsequent labelling of cell-bound TCR by streptavidin-R phycoerythrin (PE) conjugates. Individual PE molecules were then imaged by 3-dimensional fluorescence microscopy.

Staining of adherent cells. The cancer cells were plated into chamber well slides and allowed to adhere overnight in incubator. (37°C, 5% CO_2) Media was removed and replaced with fresh R10. Media was removed, and cells washed twice with 500 µl of PBS supplemented with 400 µM $MgCl_2$ (PBS/Mg). Cells were incubated in 200 µl of TCR solution (5 µg ml^{-1} high affinity c58c61 1G4 TCR, or 5 µg ml^{-1} of an "irrelevant" HLA-A2-tax peptide-specific high affinity TCR, in PBS/Mg containing 0.5% BSA albumin) for 30 min at 4°C. TCR solution was removed, and cells were washed three times with 500 µl of PBS/Mg. Cells were incubated in 200 µl of streptavidin-PE solution (5 µg ml^{-1} streptavidin-PE in PBS/Mg containing 0.5% BSA) at room temperature in the dark for 20 min. Streptavidin-PE solution was removed and cells were washed five times with 500 µl of PBS/Mg. Wash media was removed, and cells kept in 400 µl of imaging media before imaging by fluorescence microscopy.

Fluorescence microscopy. Fluorescent microscopy was carried out using an Axiovert 200M (Zeiss) microscope with a 63x Oil objective (Zeiss). A Lambda LS light source containing a 300W Xenon Arc lamp (Sutter) was used for illumination, and light intensity was reduced to optimal levels by placing a 0.3 and a 0.6 neutral density filter into the light path. Excitation and emission spectra were separated using a TRITC/DiI filter set (Chroma). Cells were imaged in three dimensions by *z*-stack acquisition (21 planes, 1 µm apart). Image acquisition and analysis was performed using Metamorph software (Universal Imaging) as described (Irvine *et al.,* Nature (419), p845-9, and Purbhoo *et al.,* Nature Immunology (5), p524-30.).

Results

As demonstrated by Figure 22 the above method was used successfully to image high affinity 1G4 TCR bound to SLLMWITQC-HLA-A*0201 antigens on the surface of Mel 526, Mel 624 and SK-Mel-37 cancer cells.

SEQUENCE LISTING

<110> Avidex Ltd
Jakobsen, Bent K
Boulter, Jonathan M
Li, Yi
Molloy, Peter E
Dunn, Steven M

<120> High Affinity NY-ESO T cell receptors

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<141> 2005-05-18

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Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
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Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
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            20   25   30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
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65   70   75   80

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35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp
115 120 125

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Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp
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20 25 30

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35 40 45

Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro
115 120 125

Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu
130 135 140

Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
145 150 155 160

Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Pro Leu Lys
165 170 175

Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu Ser Ser Arg Leu
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<223> DNA encoding a soluble version of 1G4 TCR alpha chain containing a non native cysteine codon

<400> 7
atgcaggagg tgacacagat tcctgcagct ctgagtgtcc cagaaggaga aaacttggtt 60

ctcaactgca gtttcactga tagcgctatt tacaacctcc agtggtttag gcaggaccct 120 gggaaaggtc tcacatctct gttgcttatt cagtcaagtc agagagagca aacaagtgga 180

agacttaatg cctcgctgga taaatcatca ggacgtagta ctttatacat tgcagcttct 240

cagcctggtg actcagccac ctacctctgt gctgtgaggc ccacatcagg aggaagctac 300

atacctacat ttggaagagg aaccagcctt attgttcatc cgtatatcca gaaccctgac 360

cctgccgtgt accagctgag agactctaaa tccagtgaca agtctgtctg cctattcacc 420

gattttgatt ctcaaacaaa tgtgtcacaa agtaaggatt ctgatgtgta tatcacagac 480

aaatgtgtgc tagacatgag gtctatggac ttcaagagca acagtgctgt ggcctggagc 540

aacaaatctg actttgcatg tgcaaacgcc ttcaacaaca gcattattcc agaagacacc 600

ttcttcccca gcccagaaag ttcctaa 627

<210> 8

<211> 729

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA encoding a soluble version of the 1G4 TCR beta chain containi ng a non native cysteine codon

<400> 8

atgggtgtca ctcagacccc aaaattccag gtcctgaaga caggacagag catgacactg 60

cagtgtgccc aggatatgaa ccatgaatac atgtcctggt atcgacaaga cccaggcatg 120

gggctgaggc tgattcatta ctcagttggt gctggtatca ctgaccaagg agaagtcccc 180

aatggctaca atgtctccag atcaaccaca gaggatttcc cgctcaggct gctgtcggct 240

gctccctccc agacatctgt gtacttctgt gccagcagtt acgtcgggaa caccggggag 300

ctgtttttgg gagaaggctc taggctgacc gtactggagg acctgaaaaa cgtgttccca 360

cccgaggtcg ctgtgtttga gccatcagaa gcagagatct cccacaccca aaaggccaca 420

ctggtgtgcc tggccacagg cttctacccc gaccacgtgg agctgagctg gtgggtgaat 480

gggaaggagg tgcacagtgg ggtctgcaca gacccgcagc ccctcaagga gcagcccgcc 540

ctcaatgact ccagatacgc tctgagcagc cgcctgaggg tctcggccac cttctggcag 600

gacccccgca accacttccg ctgtcaagtc cagttctacg ggctctcgga gaatgacgag 660

tggacccagg atagggccaa acccgtcacc cagatcgtca gcgccgaggc ctggggtaga 720

gcagactaa 729

<210> 9

<211> 208

<212> PRT

<213> Artificial Sequence

<220>

<223> Soluble version of the 1G4 TCR alpha chain containing a non-nativ
e cysteine residue

<400> 9

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp
115 120 125

Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser
130 135 140

Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp
145 150 155 160

Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala
165 170 175

Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn
180 185 190

Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser

195 200 205

<210> 10

<211> 242

<212> PRT

<213> Artificial Sequence

<220>

<223> Soluble version of the 1G4 TCR beta chain containing a non native cysteine residue

<400> 10

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro
115 120 125

Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu
130 135 140

Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
145 150 155 160

Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys
165 170 175

Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Ala Leu Ser Ser Arg Leu
180 185 190

Arg Val Ser Ala Thr Phe Trp Gln Asp Pro Arg Asn His Phe Arg Cys
195 200 205

Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp
210 215 220

Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg
225 230 235 240

Ala Asp

<210> 11

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 11

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Leu
85 90 95

Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 12

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 12

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg His Thr Ser
85 90 95

Asn Gly Tyr Phe Pro Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 13

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 13

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Met Thr
85 90 95

Gly Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 14

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 14

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Tyr
85 90 95

Gly Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 15

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 15

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Met Ile
85 90 95

Gly Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 16

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L
Q W F R Q D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S
L D K S S G R S T L Y I A A S Q P G D S A T Y L C A V R P M I G G
T Y I P T F G R G T S L I V H P Y

<400> 16

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Thr
85 90 95

Gly Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 17

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 17

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Thr
                85   90   95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 18

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 18

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala Thr
85 90 95

Gly Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 19

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 19

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

Ser Leu Asp Lys Ser Ser Gly Arg Ser Thr Leu Tyr Ile Ala Ala Ser

65 70 75 80

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Gln Thr
85 90 95

Val Pro Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 20

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 20

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Met Ser
85 90 95

Gly Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 21

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 21

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Tyr Gln
85 90 95

Ser Gly His Tyr Met Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 22

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 22

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Asp Tyr Thr Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 23

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 23

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala

50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Met Leu
85 90 95

Gly Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 24

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 24

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Gln
85 90 95

Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 25

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 25

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Met Thr
85 90 95

Asp Ser Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 26

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 26

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Val
85 90 95

Asp Pro Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 27

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 27

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu

35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

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

Asp Ala Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 28

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 28

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

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

Asp Ser Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 29

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 29

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Val
85 90 95

Gly Gly Val Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 30

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 30

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
                85   90   95

Gly Gly Asn Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 31

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 31

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn

20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Thr
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 32

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 32

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ile Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 33

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 33

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Met Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 34

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 34

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Met Thr
                85   90   95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 35

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 35

Met Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val Pro Glu Gly

1 5 10 15

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ile Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 36

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 36

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 37

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 37

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ala
85 90 95

Gly Gly Ser Tyr Ile Pro Ala Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 38

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 38

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 39

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 39

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala Thr
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 40

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 40

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Gln
85 90 95

Tyr Thr Gln Val Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val His
100 105 110

Pro Tyr

<210> 41

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 41

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

Glu Asn Leu Ala Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Pro Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 42

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 42

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Pro Phe Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 43

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 43

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 44

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 44

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Thr Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 45

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 45

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 46

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 46

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Met Gly His Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 47

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 47

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Met Gly Thr Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser
85 90 95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 48

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 48

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Pro Phe Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Leu
85 90 95

Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 49

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 49

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Thr Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Leu
85 90 95

Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 50

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 50

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Leu
85 90 95

Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 51

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 51

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Met Gly His Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Leu
                85   90   95

Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 52

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 52

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Met Gly Thr Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Leu
                85   90   95

Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 53

<211> 115

<212> PRT

<213> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 53

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Met Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

```
Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Leu
                85                  90                  95

Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100                 105                 110

His Pro Tyr
        115
```

<210> 54

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 54

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20                  25                  30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35                  40                  45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50                  55                  60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Arg
                85                  90                  95

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100                 105                 110

His Pro Tyr
        115
```

<210> 55

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 55

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Asn Asp
85 90 95

Gly Ser Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile
100 105 110

Val His Pro Tyr
115

<210> 56

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 56

Met Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val Pro Glu Gly

1 5 10 15

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala Trp
85 90 95

Gly Gly Asn Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 57

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 57

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

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

Gly Gly Glu Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 58

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 58

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

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

Gly Gly Gly Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 59

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 59

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Val Ser
85 90 95

Gly Gly Asp Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 60

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 60

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Asp
85 90 95

Asp Gly Gly Arg Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile
100 105 110

Val His Pro Tyr
115

<210> 61

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 61

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Asn Thr
85 90 95

Gly Gly Gln Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 62

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 62

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ile Ala
85 90 95

Gly Gly Lys Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
        115

<210> 63

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 63

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20                  25                  30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35                  40                  45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50                  55                  60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Gly Thr
                85                  90                  95

Gly Gly Asp Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100                 105                 110

His Pro Tyr
        115
```

<210> 64

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 64

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala Ala
85 90 95

Gly Gly Ser Asp Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 65

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 65

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Ala
85 90 95

Gly Gly Ala Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 66

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 66

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala Arg
85 90 95

Gly Gly Asp Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 67

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 67

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Gly
85 90 95

Gly Gly Ile Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 68

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 68

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Met Gly
85 90 95

Gly Gly Arg Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 69

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 69

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ser Val
85 90 95

Gly Gly Asn Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 70

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 70

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

```
Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala Thr
                85                  90                  95

Gly Gly Asn Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100                 105                 110

His Pro Tyr
        115
```

<210> 71

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 71

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20                  25                  30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35                  40                  45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50                  55                  60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Thr
                85                  90                  95

Gly Gly Ala Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100                 105                 110

His Pro Tyr
        115
```

<210> 72

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 72

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

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

Ser Gly Asn Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 73

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 73

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ile Ser
                85   90   95

Gly Gly Asp Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 74

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 74

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala His
85 90 95

Asn Gly Asn Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 75

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 75

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Asp Asn
85 90 95

Thr Trp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile
100 105 110

Val His Pro Tyr
115

<210> 76

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 76

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

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

Gly Gly Asp Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 77

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s

equence

<400> 77

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ala Ala
85 90 95

Ser Gly Asn Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 78

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 78

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ile Ser
                85   90   95

Gly Gly Glu Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
        115

<210> 79

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 79

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20   25   30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35   40   45

Leu Ile Ser Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50   55   60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ile Thr
                85   90   95

Gly Gly Gly Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100   105   110

His Pro Tyr
115

<210> 80

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA sequence

<400> 80

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20                  25                  30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35                  40                  45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50                  55                  60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Lys
                85                  90                  95

Gly Gly Ala Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100                 105                 110

His Pro Tyr
        115
```

<210> 81

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 81

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20                  25                  30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
        35                  40                  45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
    50                  55                  60

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

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

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
            100                 105                 110

His Pro Tyr
        115
```

<210> 82

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s
equence

<400> 82

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
            20                  25                  30
```

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Ser Thr
85 90 95

Gly Gly Asn Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val
100 105 110

His Pro Tyr
115

<210> 83

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR alpha chain variable domain AA s equence

<400> 83

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

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45

Leu Ile Gln Gly Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60

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

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Val Asp
85 90 95

Asp Gly Gly Lys Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile
100 105 110

Val His Pro Tyr
115

<210> 84

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 84

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Ala Gly Thr Thr Asp Arg Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

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

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 85

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 85

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1   5   10   15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
            20   25   30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
        35   40   45

Val Gly Ala Gly Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
    50   55   60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65   70   75   80

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

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
            100   105   110

<210> 86

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 86

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1   5   10   15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
            20   25   30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
        35   40   45

Val Gly Ala Gly Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Asn Val Gly
85 90 95

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 87

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 87

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Ala Gly Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Gly Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 88

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 88

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 89

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 89

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Ala Gly Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

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

Asp Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 90

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 90

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Val Gly Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

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

Asp Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 91

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 91

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Val Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Val Gly Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

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

Asp Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 92

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 92

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Ser Val Gly Met Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 93

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 93

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Ala Ile Gln Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

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Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 94

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se
quence

<400> 94

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Ala Ile Gln Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

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

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 95

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 95

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
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Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Ser Val Gly Met Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

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

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 96

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 96

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser

55-75

35 40 45

Val Gly Ala Gly Thr Thr Asp Arg Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Val Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 97

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 97

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Ala Gly Thr Thr Asp Arg Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asp Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 98

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA sequence

<400> 98

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Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
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Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
            20                  25                  30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
        35                  40                  45

Val Gly Ala Gly Thr Thr Asp Arg Gly Glu Val Pro Asn Gly Tyr Asn
    50                  55                  60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65                  70                  75                  80

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

Asp Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
            100                 105                 110
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<210> 99

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA sequence

<400> 99

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Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1               5                   10                  15
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Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Gly Ala Gly Thr Thr Asp Arg Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Phe Val Gly
85 90 95

Asp Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 100

<211> 47

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of a soluble form of TRAC

<400> 100

Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
1 5 10 15

Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
20 25 30

Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys
35 40 45

<210> 101

<211> 56

<212> PRT

<213> Artificial Sequence

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<223> Amino acid sequence of a soluble form of TRBC1

<400> 101

Glu Asp Leu Asn Lys Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro
1 5 10 15

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

Ala Thr Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
35 40 45

Gly Lys Glu Val His Ser Gly Val
50 55

<210> 102

<211> 56

<212> PRT

<213> Artificial Sequence

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<223> Amino acid sequence of a soluble form of TRBC2

<400> 102

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1 5 10 15

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

Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
35 40 45

Gly Lys Glu Val His Ser Gly Val
50 55

<210> 103

<211> 30

<212> PRT

<213> Artificial Sequence

<220>

<223> Short scTCR linker

<400> 103

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Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Pro
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<210> 104

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> Long scTCR linker

<400> 104

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Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
            20   25   30

Gly Gly Pro
        35

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<211> 37

<212> DNA

<213> Artificial Sequence

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<223> Primer

<400> 105
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<210> 106

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> Primer

<400> 106
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<210> 107

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 107
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<210> 108

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 108
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<210> 109

<211> 36

<212> DNA

<213> Artificial Sequence

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<223> Primer

<400> 109
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<210> 110

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 110
cttctgatgg ctcaaacaca gc 22

<210> 111

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> Primer

<400> 111
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<210> 112

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 112
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<210> 113

<211> 3342

<212> DNA

<213> Artificial Sequence

<220>

<223> pEX954 Vector

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<211> 3836

<212> DNA

<213> Artificial Sequence

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<211> 3857
<212> DNA
<213> Artificial Sequence

<220>
<223> pEX202 Vector

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<211> 4019

<212> DNA

<213> Artificial Sequence

<220>

<223> pEX205

<400> 116

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<210> 117

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se
quence

<400> 117

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Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Ala Ile Gln Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asp Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 118

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 118

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Ala Ile Gln Thr Thr Asp Arg Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

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

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 119

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se
quence

<400> 119

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Ala Ile Gln Thr Thr Asp Gln Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 120

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 120

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser
35 40 45

Val Ser Val Gly Met Thr Asp Arg Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asp Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 121

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> High affinity variant of 1G4 TCR beta chain variable domain AA se quence

<400> 121

Met Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr Gly Gln
1 5 10 15

Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr Met Ser
20 25 30

Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His Tyr Ser

35 40 45

Val Ala Ile Gln Thr Thr Asp Arg Gly Glu Val Pro Asn Gly Tyr Asn
50 55 60

Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu Ser Ala
65 70 75 80

Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr Val Gly
85 90 95

Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr Val Leu
100 105 110

<210> 122

<211> 162

<212> PRT

<213> Artificial Sequence

<220>

<223> C58c61 alpha with truncated TRAC

<400> 122

Met Lys Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val Pro Glu
1 5 10 15

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

Asn Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu
35 40 45

Leu Leu Ile Thr Pro Trp Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn
50 55 60

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

Ser Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu
85 90 95

Leu Asp Gly Thr Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile
100 105 110

Val His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg

115 120 125

Asp Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp
130 135 140

Ser Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr
145 150 155 160

Asp Lys

<210> 123

<211> 170

<212> PRT

<213> Artificial Sequence

<220>

<223> C58c61 beta with truncated TRBC1

<400> 123

Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
1 5 10 15

Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
20 25 30

Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
35 40 45

Tyr Ser Val Ala Ile Gln Thr Thr Asp Gln Gly Glu Val Pro Asn Gly
50 55 60

Tyr Asn Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu
65 70 75 80

Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr
85 90 95

Leu Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr
100 105 110

Val Leu Glu Asp Leu Asn Lys Val Phe Pro Pro Glu Val Ala Val Phe
115 120 125

Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val

55-96
130 135 140

Cys Leu Ala Thr Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp
145 150 155 160

Val Asn Gly Lys Glu Val His Ser Gly Val
165 170

<210> 124

<211> 170

<212> PRT

<213> Artificial Sequence

<220>

<223> C58c61 beta with truncated TRBC2

<400> 124

Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
1 5 10 15

Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
20 25 30

Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
35 40 45

Tyr Ser Val Ala Ile Gln Thr Thr Asp Gln Gly Glu Val Pro Asn Gly
50 55 60

Tyr Asn Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu
65 70 75 80

Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr
85 90 95

Leu Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr
100 105 110

Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe
115 120 125

Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val
130 135 140

Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp

145 150 155 160

Val Asn Gly Lys Glu Val His Ser Gly Val
165 170

<210> 125

<211> 305

<212> PRT

<213> Artificial Sequence

<220>

<223> C58c61 beta with truncated TRBC2 fused to wt human IL-2

<400> 125

Met Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu Lys Thr
1 5 10 15

Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His Glu Tyr
20 25 30

Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu Ile His
35 40 45

Tyr Ser Val Ala Ile Gln Thr Thr Asp Gln Gly Glu Val Pro Asn Gly
50 55 60

Tyr Asn Val Ser Arg Ser Thr Ile Glu Asp Phe Pro Leu Arg Leu Leu
65 70 75 80

Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Ser Tyr
85 90 95

Leu Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg Leu Thr
100 105 110

Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe
115 120 125

Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val
130 135 140

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

Val Asn Gly Lys Glu Val His Ser Gly Val Pro Gly Ala Pro Thr Ser

55-98

165 170 175

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
180 185 190

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
195 200 205

Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu
210 215 220

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
225 230 235 240

Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
245 250 255

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
260 265 270

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
275 280 285

Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu
290 295 300

Thr
305

What is claimed is:

1. A T-cell receptor (TCR) having the property of binding to SLLMWITQC- HLA-A*0201 and comprising one or both of

(i) at least one TCR α chain variable domain comprising one or more amino acid substitutions relative to the native IG4 TCR α chain variable domain shown in SEQ ID NO: 1 selected from 20A, 51P, 51S, 51T, 51M, 52P, 52F, 52G, 53W, 53H, 53T, 94H, 94A, 95L, 95M, 95A, 95Q, 95Y, 95E, 95I, 95F, 95V, 95N, 95G, 95S, 95R, 95D, 96L, 96T, 96Y, 96I, 96Q, 96V, 96E, 96X, 96A, 96W, 96R, 96G, 96H, 96K, 96D, 97D, 97N, 97V, 97S, 97A, 97T, 98P, 98H, 98S, 98T, 98W, 98A, 99T, 99Y, 99D, 99H, 99V, 99N, 99E, 99G, 99Q, 99K, 99A, 99I, 99R, 100F, 100M, 100D, 101P, 101T, 101M or 103A using the numbering shown in SEQ ID NO: 1; and

(ii) at least one TCR β chain variable domain comprising one or more amino acid substitutions relative to the native IG4 TCR β chain variable domain shown in SEQ ID NO: 2 selected from 18V, 50S, 50A, 51V, 51I, 52Q, 53T, 53M, 55R, 56R, 70I, 94N, 94F, 95L, 97G or 97D using the numbering shown in SEQ ID NO: 2;

said TCR having a K_D for the said SLLMWITQC-HLA-A*0201 complex of less than or equal to 1μM and/or has an off-rate (k_{off}) for the SLLMWITQC-HLA-A*0201 complex of $1x10^{-3}$ S^{-1} or slower.

2. A TCR as claimed in claim 1 comprising both an α chain variable domain and an TCR β chain variable domain.

3. A TCR as claimed in claim 1 which is an αα or ββ homodimer.

4. A T-cell receptor (TCR) as claimed in any one of claims 1 to 3 wherein the said K_D and/or k_{off} is/are as measured by Surface Plasmon Resonance.

5. A TCR as claimed in any one of claims 1 to 4 comprising one of the alpha chain variable domain amino acid sequences shown in (SEQ ID Nos: 11 to 83).

6. A TCR as claimed in any one of claims 1 to 5 comprising any one of the beta chain variable domain amino acid sequences shown in (SEQ ID Nos: 84 to 99, or 117 to 121).

7. A TCR as claimed in claim 2 comprising the alpha and beta chain variable domain pairings shown in the following table:

Alpha chain variable domain sequence, SEQ ID NO:	Beta chain variable domain sequence, SEQ ID NO:
1	84
1	85
1	86
1	87
1	88
11	84
12	84
12	85
12	90
11	85
11	86
11	92
11	93
13	86
14	84
14	85
15	84
15	85
16	84
16	85
17	86
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21	85
22	84
23	86

24	84
25	84
26	84
27	84
28	84
29	84
30	84
31	84
32	84
33	84
20	86
34	86
35	89
36	89
37	89
38	89
39	89
16	89
17	89
31	89
40	89
1	90
1	91
41	90
42	2
42	85
42	92
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1	93
43	92

44	92
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46	92
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81	92
82	92
83	92
11	96
11	97
11	98
11	99
1	89
50	117
49	117
50	118
49	119
50	119
58	93

49	118
1	119
1	117
55	120
56	120
50	121
50	120
49	121
49	120
48	118
53	95

8. A TCR as claimed in claim 2 comprising the alpha chain variable domain shown in the SEQ ID NO: 49 and the beta chain variable domain shown in the SEQ ID NO: 94 .

9. A TCR as claimed in any one of claims 1 to 8 further comprising the alpha chain constant region amino acid sequence shown in SEQ ID NO: 100, and/or the beta chain amino acid constant region sequence according to SEQ ID NO: 101 or the beta chain amino acid constant region sequence according to SEQ ID NO: 102.

10. A TCR as claimed in any one of claims 1 to 9 which is a dimeric T cell receptor (dTCR) or a single chain T cell receptor (scTCR).

11. A TCR as claimed in any one of claims 4 to 10 which is an scTCR comprising a first segment constituted by an amino acid sequence corresponding to a TCR α chain variable domain

a second segment constituted by an amino acid sequence corresponding to a TCR β chain variable domain sequence fused to the N terminus of an amino acid sequence corresponding to a TCR β chain constant domain extracellular sequence, and

a linker sequence linking the C terminus of the first segment to the N terminus of the second segment, the linker sequence having the sequence –PGGG-(SGGGG)$_n$-P- wherein n is 5 or 6, P is proline, G is glycine and S is serine.

12. A TCR as claimed in any one of claims 4 to 10 which is an scTCR comprising a first segment constituted by an amino acid sequence corresponding to a TCR β chain variable domain

a second segment constituted by an amino acid sequence corresponding to a TCR α chain variable domain sequence fused to the N terminus of an amino acid sequence corresponding to a TCR α chain constant domain extracellular sequence, and

a linker sequence linking the C terminus of the first segment to the N terminus of the second segment, the linker sequence having the sequence –PGGG-(SGGGG)$_n$-P- wherein n is 5 or 6, P is proline, G is glycine and S is serine.

13. A TCR as claimed in claim 11 or 12 further comprising a disulfide bond between the first and second chains, said disulfide bond being one which has no equivalent in native αβT cell receptors, and wherein the length of the linker sequence and the position of the disulfide bond being such that the variable domain sequences of the first and second segments are mutually orientated as in native αβ T cell receptors.

14. An scTCR as claimed in any one of claims 11 to 13 wherein in the binding part the linker sequence links the C terminus of the first segment to the N terminus of the second segment.

15. A scTCR as claimed in any one of claims 11 to 14 wherein in the binding part the linker sequence has the formula -PGGG-(SGGGG)$_5$-P- (SEQ ID NO: 103) or -PGGG-(SGGGG)$_6$-P- (SEQ ID NO: 104) wherein P is proline, G is glycine and S is serine.

16. A TCR as claimed in any one of claims 1, 2 or 4 to 10 which is a dTCR comprising a first polypeptide wherein a sequence corresponding to a TCR α chain variable domain sequence is fused to the N terminus of a sequence corresponding to a TCR α chain constant domain extracellular sequence, and

a second polypeptide wherein a sequence corresponding to a TCR βchain variable domain sequence fused to the N terminus a sequence corresponding to a TCR β chain constant domain extracellular sequence,

the first and second polypeptides being linked by a disulfide bond which has no equivalent in native αβ T cell receptors, wherein the said disulfide bond is between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof.

17. A TCR as claimed in any one of claims 10 to 16 wherein the dTCR or scTCR binding part comprises a disulfide bond between residues corresponding to those linked by a disulfide bond in native TCRs.

18. A TCR as claimed in any one of claims 8 to 15 wherein the dTCR or scTCR binding part does not contain a sequence corresponding to transmembrane or cytoplasmic sequences of native TCRs.

19. A soluble TCR consisting of the alpha chain amino acid sequence of SEQ ID NO: 122 and beta chain amino acid sequence SEQ ID NO: 123.

20. A soluble TCR consisting of the alpha chain amino acid sequence of SEQ ID NO: 122 and beta chain amino acid sequence SEQ ID NO: 124.

21. A TCR as claimed in any one of claims 1 to 20 wherein the TCR is covalently or otherwise linked to at least one polyalkylene glycol chain(s).

22. A TCR as claimed in claim 21 wherein the polyalkylene glycol chain(s) is/are covalently linked to the TCR.

23. A TCR as claimed in claim 21 or claim 22 wherein the polyalkylene glycol chain(s) comprise(s) at least two polyethylene glycol repeating units.

24. A TCR as claimed in any one of claims 1 to 23 further comprising a reactive cysteine at the C terminal or N-terminal of the alpha or beta chains thereof.

25. A TCR as claimed in any one of claims 1 to 24 covalently or otherwise linked to a therapeutic agent or detectable moiety.

26. A TCR as claimed in claim 25 wherein the TCR is covalently linked to a therapeutic agent or detectable moiety.

27. A TCR as claimed in claim 25 wherein the therapeutic agent or detectable moiety is covalently linked to the C terminus of one or both TCR chains.

28. A TCR as claimed in any one of claims 25 to 27 covalently or otherwise linked to a therapeutic agent which is an immune effector molecule.

29. A TCR as claimed in claim 28 wherein the immune effector molecule is a cytokine.

30. A TCR as claimed in claim 28 wherein the immune effector molecule is IL-2.

31. A TCR as claimed in any one of claims 25 to 27 wherein the therapeutic agent is a cytotoxic agent.

32. A TCR as claimed in any one of claims 25 to 27 wherein the therapeutic agent is a radionuclide.

33. A multivalent TCR complex comprising at least two TCRs as claimed in any one of claims 1 to 32.

34. A multivalent TCR complex comprising at least two TCRs as claimed in any one of claims 1 to 33 linked by a linker which is polyethylene glycol, polypropylene glycol, or a copolymer of ethylene glycol and propylene glycol or by a multimerisation domain sequence.

35. A TCR complex as claimed in claim 34 wherein the linker multimerisation domain sequence extends between amino acid residues of each TCR which are not located in a variable region sequence of the TCR.

36. A TCR complex as claimed in claim 34 or 35 wherein a divalent alkylene spacer radical is located between the polyalkylene glycol chain and its point of attachment to a TCR of the complex.

37. A TCR complex as claimed in claim 34 or claim 35 wherein the polyalkylene glycol chain comprises at least two polyethylene glycol repeating units.

38. A multivalent TCR complex comprising at least two TCRs as claimed in any one of claims 1 to 24 wherein at least one of said TCRs is associated with a therapeutic agent as claimed in any one of claims 25 to 32.

39. An isolated cell presenting a TCR as defined in any one of claims 1 to 17.

40. A pharmaceutical composition comprising a TCR or a multivalent TCR complex as claimed in any one of claims 1 to 38, or a plurality of cells as claimed in claim 39, together with a pharmaceutically acceptable carrier.

41. Use of a TCR or a multivalent TCR complex as claimed in any one of claims 1 to 38 or a plurality of cells as claimed in claim 39 for treatment of cancer.

42. Use of a TCR or a multivalent TCR complex as claimed in any one of claims 1 to 38, or a plurality of cells as claimed in claim 39 in the manufacture of a medicament for treatment of cancer.

43. A method of producing a high affinity TCR having the property of binding to SLLMWITQC-HLA-A*0201 and comprising one or both of

(i) at least one TCR α chain variable domain comprising one or more amino acid substitutions relative to the native IG4 TCR α chain variable domain shown in SEQ ID NO: 1, which are 20A, 51P, 51S, 51T, 51M, 52P, 52F, 52G, 53W, 53H, 53T, 94H, 94A, 95L, 95M, 95A, 95Q, 95Y, 95E, 95I, 95F, 95V, 95N, 95G, 95S, 95R, 95D, 96L, 96T, 96Y, 96I, 96Q, 96V, 96E, 96X, 96A, 96W, 96R, 96G, 96H, 96K, 96D, 97D, 97N, 97V, 97S, 97A, 97T, 98P, 98H, 98S, 98T, 98W, 98A, 99T, 99Y, 99D, 99H, 99V, 99N, 99E, 99G, 99Q, 99K, 99A, 99I, 99R, 100F, 100M, 100D, 101P, 101T, 101M or 103A using the numbering shown in SEQ ID NO: 1; and

(ii) at least one TCR β chain variable domain comprising one or more amino acid substitutions relative to the native IG4 TCR β chain variable domain shown in SEQ ID NO: 2, which are 18V, 50S, 50A, 51V, 51I, 52Q, 53T, 53M, 55R, 56R, 70I, 94N, 94F, 95L, 97G or 97D using the numbering shown in SEQ ID NO: 2; the TCR having a K_D for the said SLLMWITQC-HLA-A*0201 complex of less than or equal to 1μM and/or an off-rate (k_{off}) for the SLLMWITQC-HLA-A*0201 complex of $1x10^{-3}$ S^{-1} or slower, said method comprising:

(a) the production of a TCR comprising one or both of (i) and (ii);

(b) contacting said TCR with SLLMWITQC-HLA-A*0201 under conditions suitable to allow the binding of the TCR to SLLMWITQC-HLA-A*0201;

and measuring the K_D and/or k_{off} of the interaction.

44. A T cell receptor (TCR) comprising the α chain variable domain sequence of SEQ ID NO: 1 and the β chain variable domain sequence of SEQ ID NO: 2 except that amino acids 95T and 96S of SEQ ID NO: 1 are replaced by 95L and 96Y.

45. A TCR as claimed in claim 44, which is a TCR comprising a first polypeptide having a sequence corresponding to the TCR α chain variable domain sequence fused to the N terminus of a sequence corresponding to a TCR α chain constant domain extracellular sequence, and

a second polypeptide having a sequence corresponding to the TCR β chain variable domain sequence fused to the N terminus a sequence corresponding to a TCR β chain constant domain extracellular sequence,

the first and second polypeptide being linked by a disulfide bond which has no equivalent in native αβ T cell receptors.

46. A TCR as claimed in claim 45, wherein the said disulfide bond is between cysteine residues substituted for Thr 48 of exon 1 TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof.

47. A nucleic acid encoding a TCR α chain variable domain sequence of the TCR as claimed in any one of claims 44 to 46.

48. An isolated cell presenting a TCR as defined in any one of claims 44 to 46.

49. An isolated cell as claimed in claim 48, which is a T cell.

50. A pharmaceutical composition comprising a TCR as claimed in any one of claims 44 to 46, or a plurality of isolated cells as claimed in claim 48 or claim 49 together with a pharmaceutically acceptable carrier.

51. Use of a TCR as claimed in any one of claims 44 to 46 or a plurality of isolated cells as claimed in claim 48 or claim 49 for the treatment of cancer.

52. Use of a TCR as claimed in any one of claims 44 to 46, or a plurality of isolated cells as claimed in claim 48 or claim 49 in the manufacture of a medicament for the treatment of cancer.

53. Use of a pharmaceutical composition as claimed in claim 50 for treatment of cancer.

Figure 1a

```
                  10                  20
                  *                   *
M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A

30                  40                  50
*                   *                   *
I Y N L Q W F R Q D P G K G L T S L L L I Q S S Q R E Q T

  60                  70                  80
  *                   *                   *
S G R L N A S L D K S S G R S T L Y I A A S Q P G D S A T

    90                  100                 110
    *                   *                   *
Y L C A V R P T S G G S Y I P T F G R G T S L I V H P Y
```

(SEQ ID No: 1)

Figure 1b

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                10                  20
                 *                   *
M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E

30                  40                  50
*                    *                   *
Y M S W Y R Q D P G M G L R L I H Y S V G A G I T D Q G E

 60                  70                  80
  *                   *                   *
V P N G Y N V S R S T T E D F P L R L L S A A P S Q T S V

   90                  100                 110
    *                   *                   *
Y F C A S S Y V G N T G E L F F G E G S R L T V L
```

(SEQ ID No: 2)

Figure 2a

atgcaggaggtgacacagattcctgcagctctgagtgtcccagaaggagaaaacttggttctcaactgcagtttcactgatag
cgctatttacaacctccagtggtttaggcaggaccctgggaaaggtctcacatctctgttgcttattcagtcaagtcagagaga
gcaaacaagtggaagacttaatgcctcgctggataaatcatcaggacgtagtactttatacattgcagcttctcagcctggtga
ctcagccacctacctctgtgctgtgaggcccacatcaggaggaagctacatacctacatttggaagaggaaccagccttatt
gttcatccgtatatccagaaccctgaccctgccgtgtaccagctgagagactctaaatccagtgacaagtctgtctgcctattc
accgattttgattctcaaacaaatgtgtcacaaagtaaggattctgatgtgtatatcacagacaaaactgtgctagacatgaggt
ctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttgcatgtgcaaacgccttcaacaacagcatt
attccagaagacaccttcttccccagcccagaaagttcctaa

(SEQ ID No: 3)

Figure 2b

atgggtgtcactcagaccccaaaattccaggtcctgaagacaggacagagcatgacactgcagtgtgcccaggatatgaac
catgaatacatgtcctggtatcgacaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatcactga
ccaaggagaagtccccaatggctacaatgtctccagatcaaccacagaggatttcccgctcaggctgctgtcggctgctccc
tcccagacatctgtgtacttctgtgccagcagttacgtcgggaacaccggggagctgttttttggagaaggctctaggctgac
cgtactggaggacctgaaaaacgtgttcccacccgaggtcgctgtgtttgagccatcagaagcagagatctcccacaccca
aaaggccacactggtgtgcctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatgggaaggag
gtgcacagtggggtcagcacagacccgcagcccctcaaggagcagcccgccctcaatgactccagatacgctctgagca
gccgcctgagggtctcggccaccttctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcgg
agaatgacgagtggacccaggatagggccaaacccgtcacccagatcgtcagcgccgaggcctggggtagagcagact
aa
(SEQ ID No: 4)

Figure 3a

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W
F R Q D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S
G R S T L Y I A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G
R G T S L I V H P Y I Q N P D P A V Y Q L R D S K S S D K S V C L F T
D F D S Q T N V S Q S K D S D V Y I T D K T V L D M R S M D F K S N S
A V A W S N K S D F A C A N A F N N S I I P E D T F F P S P E S S

(SEQ ID No: 5)

Figure 3b

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R
Q D P G M G L R L I H Y S V G A G I T D Q G E V P N G Y N V S R S T T
E D F P L R L L S A A P S Q T S V Y F C A S S Y V G N T G E L F F G E
G S R L T V L E D L K N V F P P E V A V F E P S E A E I S H T Q K A T
L V C L A T G F Y P D H V E L S W W V N G K E V H S G V S T D P Q P L
K E Q P A L N D S R Y A L S S R L R V S A T F W Q D P R N H F R C Q V
Q F Y G L S E N D E W T Q D R A K P V T Q I V S A E A W G R A D

(SEQ ID No: 6)

Figure 4a

atgcaggaggtgacacagattcctgcagctctgagtgtcccagaaggagaaaacttggttctcaactgcagtttcactgatag
cgctatttacaacctccagtggtttaggcaggaccctgggaaaggtctcacatctctgttgcttattcagtcaagtcagagaga
gcaaacaagtggaagacttaatgcctcgctggataaatcatcaggacgtagtactttatacattgcagcttctcagcctggtga
ctcagccacctacctctgtgctgtgaggcccacatcaggaggaagctacatacctacatttggaagaggaaccagccttatt
gttcatccgtatatccagaaccctgaccctgccgtgtaccagctgagagactctaaatccagtgacaagtctgtctgcctattc
accgattttgattctcaaacaaatgtgtcacaaagtaaggattctgatgtgtatatcacagacaaatgtgtgctagacatgaggt
ctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttgcatgtgcaaacgccttcaacaacagcatt
attccagaagacaccttcttccccagcccagaaagttcctaa
(SEQ ID No: 7)

Figure 4b

atgggtgtcactcagaccccaaaattccaggtcctgaagacaggacagagcatgacactgcagtgtgcccaggatatgaac
catgaatacatgtcctggtatcgacaagacccaggcatggggctgaggctgattcattactcagttggtgctggtatcactga
ccaaggagaagtccccaatggctacaatgtctccagatcaaccacagaggatttcccgctcaggctgctgtcggctgctccc
tcccagacatctgtgtacttctgtgccagcagttacgtcgggaacaccggggagctgttttttggagaaggctctaggctgac
cgtactggaggacctgaaaaacgtgttcccacccgaggtcgctgtgtttgagccatcagaagcagagatctcccacaccca
aaaggccacactggtgtgcctggccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatgggaaggag
gtgcacagtggggtctgcacagacccgcagcccctcaaggagcagcccgccctcaatgactccagatacgctctgagca
gccgcctgagggtctcggccaccttctggcaggacccccgcaaccacttccgctgtcaagtccagttctacgggctctcgg
agaatgacgagtggacccaggatagggccaaacccgtcacccagatcgtcagcgccgaggcctggggtagagcagact
aa
(SEQ ID No: 8)

Figure 5a

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W
F R Q D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S
G R S T L Y I A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G
R G T S L I V H P Y I Q N P D P A V Y Q L R D S K S S D K S V C L F T
D F D S Q T N V S Q S K D S D V Y I T D K C V L D M R S M D F K S N S
A V A W S N K S D F A C A N A F N N S I I P E D T F F P S P E S S
(SEQ ID No: 9)

Figure 5b

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R
Q D P G M G L R L I H Y S V G A G I T D Q G E V P N G Y N V S R S T T
E D F P L R L L S A A P S Q T S V Y F C A S S Y V G N T G E L F F G E
G S R L T V L E D L K N V F P P E V A V F E P S E A E I S H T Q K A T
L V C L A T G F Y P D H V E L S W W V N G K E V H S G V C T D P Q P L
K E Q P A L N D S R Y A L S S R L R V S A T F W Q D P R N H F R C Q V
Q F Y G L S E N D E W T Q D R A K P V T Q I V S A E A W G R A D
(SEQ ID No: 10)

Figure 6

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L L D G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 11)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R H T S N G Y F P P T F G R G T S L I V H P Y
(SEQ ID No: 12)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P M T G G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 13)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L Y G G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 14)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P M I G G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 15)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L T G G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 16)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L T G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 17)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A T G G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 18)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P Q T V P T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 19)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P M S G G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 20)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P Y Q S G H Y M P T F G R G T S L I V H P Y
(SEQ ID No: 21)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G D Y T P T F G R G T S L I V H P Y
(SEQ ID No: 22)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P M L G G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 23)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L Q D G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 24)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P M T D S T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 25)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L V D P T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 26)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P E V D A T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 27)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L E D S T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 28)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L V G G V Y I P T F G R G T S L I V H P Y
(SEQ ID No: 29)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G N Y I P T F G R G T S L I V H P Y
(SEQ ID No: 30)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T T G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 31)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P I S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 32)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P M S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 33)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P M T G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 34)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P I S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 35)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 36)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T A G G S Y I P A F G R G T S L I V H P Y
(SEQ ID No: 37)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 38)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A T G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 39)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q S S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T - Q Y T Q V P T F G R G T S L I V H P Y
(SEQ ID No: 40)

M Q E V T Q I P A A L S V P E G E N L A L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q P S Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 41)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I P F W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 42)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 43)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I T P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 44)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 45)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I M G H Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 46)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I M G T Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P T S G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 47)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I P F W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L L D G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 48)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I T P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L L D G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 49)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L L D G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 50)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I M G H Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L L D G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 51)

MQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQ
DPGKGLTSLLLIMGTQREQTSGRLNASLDKSSGRSTLYI
AASQPGDSATYLCAVRPLLDGTYIPTFGRGTSLIVHPY
(SEQ ID No: 52)

MQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQ
DPGKGLTSLLLIMGWQREQTSGRLNASLDKSSGRSTLYI
AASQPGDSATYLCAVRPLLDGTYIPTFGRGTSLIVHPY
(SEQ ID No: 53)

MQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQ
DPGKGLTSLLLISPWQREQTSGRLNASLDKSSGRSTLYI
AASQPGDSATYLCAVRPLRGGSYIPTFGRGTSLIVHPY
(SEQ ID No: 54)

MQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQ
DPGKGLTSLLLISPWQREQTSGRLNASLDKSSGRSTLYI
AASQPGDSATYLCAVRPNDGSGSYIPTFGRGTSLIVHPY
(SEQ ID No: 55)

MQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQ
DPGKGLTSLLLISPWQREQTSGRLNASLDKSSGRSTLYI
AASQPGDSATYLCAVRPAWGGNYIPTFGRGTSLIVHPY
(SEQ ID No: 56)

MQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQ
DPGKGLTSLLLISPWQREQTSGRLNASLDKSSGRSTLYI
AASQPGDSATYLCAVRPAEGGEYIPTFGRGTSLIVHPY
(SEQ ID No: 57)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P F T G G G Y I P T F G R G T S L I V H P Y
(SEQ ID No: 58)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P V S G G D Y I P T F G R G T S L I V H P Y
(SEQ ID No: 59)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L D D G G R Y I P T F G R G T S L I V H P Y
(SEQ ID No: 60)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P N T G G Q Y I P T F G R G T S L I V H P Y
(SEQ ID No: 61)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P I A G G K Y I P T F G R G T S L I V H P Y
(SEQ ID No: 62)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P G T G G D Y I P T F G R G T S L I V H P Y
(SEQ ID No: 63)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A A G G S D I P T F G R G T S L I V H P Y
(SEQ ID No: 64)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L A G G A Y I P T F G R G T S L I V H P Y
(SEQ ID No: 65)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A R G G D Y I P T F G R G T S L I V H P Y
(SEQ ID No: 66)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L G G G I Y I P T F G R G T S L I V H P Y
(SEQ ID No: 67)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P M G G G R Y I P T F G R G T S L I V H P Y
(SEQ ID No: 68)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P S V G G N Y I P T F G R G T S L I V H P Y
(SEQ ID No: 69)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A T G G N Y I P T F G R G T S L I V H P Y
(SEQ ID No: 70)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L T G G A Y I P T F G R G T S L I V H P Y
(SEQ ID No: 71)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P R E S G N Y I P T F G R G T S L I V H P Y
(SEQ ID No: 72)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P I S G G D Y I P T F G R G T S L I V H P Y
(SEQ ID No: 73)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A H N G N Y I P T F G R G T S L I V H P Y
(SEQ ID No: 74)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P D N T W G T Y I P T F G R G T S L I V H P Y
(SEQ ID No: 75)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P V E G G D Y I P T F G R G T S L I V H P Y
(SEQ ID No: 76)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A A S G N Y I P T F G R G T S L I V H P Y
(SEQ ID No: 77)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P I S G G E Y I P T F G R G T S L I V H P Y
(SEQ ID No: 78)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I S P W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P I T G G G Y I P T F G R G T S L I V H P Y
(SEQ ID No: 79)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P L K G G A Y I P T F G R G T S L I V H P Y
(SEQ ID No: 80)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q
D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I
A A S Q P G D S A T Y L C A V R P A E G G S Y I P T F G R G T S L I V H P Y
(SEQ ID No: 81)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I A A S Q P G D S A T Y L C A V R P S T G G N Y I P T F G R G T S L I V H P Y (SEQ ID No: 82)

M Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R Q D P G K G L T S L L L I Q G W Q R E Q T S G R L N A S L D K S S G R S T L Y I A A S Q P G D S A T Y L C A V R P V D D G G K Y I P T F G R G T S L I V H P Y (SEQ ID No: 83)

Figure 7

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D R G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y L G N T G E L F F G E G S R L T V L
(SEQ ID No: 84)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D Q G E V P N G Y N V S R S T I E D F P
L R L L S A A P S Q T S V Y F C A S S Y L G N T G E L F F G E G S R L T V L
(SEQ ID No: 85)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D Q G E V P N G Y N V S R S T T E D F P
L R L L S A A P S Q T S V Y F C A S S N V G N T G E L F F G E G S R L T V L
(SEQ ID No: 86)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D Q G E V P N G Y N V S R S T T E D F P
L R L L S A A P S Q T S V Y F C A S S Y V G G T G E L F F G E G S R L T V L
(SEQ ID No: 87)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G I T D Q G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y V G N T G E L F F G E G S R L T V L
(SEQ ID No: 88)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D Q G E V P N G Y N V S R S T T E D F P
L R L L S A A P S Q T S V Y F C A S S Y L G D T G E L F F G E G S R L T V L
(SEQ ID No: 89)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G V G T T D Q G E V P N G Y N V S R S T T E D F P
L R L L S A A P S Q T S V Y F C A S S Y L G D T G E L F F G E G S R L T V L
(SEQ ID No: 90)

M G V T Q T P K F Q V L K T G Q S V T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G V G T T D Q G E V P N G Y N V S R S T T E D F P
L R L L S A A P S Q T S V Y F C A S S Y L G D T G E L F F G E G S R L T V L
(SEQ ID No: 91)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V S V G M T D Q G E V P N G Y N V S R S T T E D F P
L R L L S A A P S Q T S V Y F C A S S Y V G N T G E L F F G E G S R L T V L
(SEQ ID No: 92)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V A I Q T T D Q G E V P N G Y N V S R S T T E D F P L
R L L S A A P S Q T S V Y F C A S S Y V G N T G E L F F G E G S R L T V L
(SEQ ID No: 93)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V A I Q T T D Q G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y L G N T G E L F F G E G S R L T V L
(SEQ ID No: 94)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V S V G M T D Q G E V P N G Y N V S R S T I E D F P
L R L L S A A P S Q T S V Y F C A S S Y L G N T G E L F F G E G S R L T V L
(SEQ ID No: 95)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D R G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y V G V T G E L F F G E G S R L T V L
(SEQ ID No: 96)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D R G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y V G D T G E L F F G E G S R L T V L
(SEQ ID No: 97)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D R G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y L G D T G E L F F G E G S R L T V L
(SEQ ID No: 98)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V G A G T T D R G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S F V G D T G E L F F G E G S R L T V L
(SEQ ID No: 99)

Figure 8a

N I Q N P D P A V Y Q L R D S K S S D K S V C L F T
D F D S Q T N V S Q S K D S D V Y I T D K
(SEQ ID NO: 100)

Figure 8b

E D L N K V F P P E V A V F E P S E A E I S H T Q K A T
L V C L A T G F F P D H V E L S W W V N G K E V H S G V
(SEQ ID NO: 101)

Figure 8c

E D L K N V F P P E V A V F E P S E A E I S H T Q K A T
L V C L A T G F Y P D H V E L S W W V N G K E V H S G V
(SEQ ID NO: 102)

Figure 9

PEX954

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(SEQ ID NO: 113)

Figure 10

PEX821

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(SEQ ID NO: 114)

Figure 11

pEX202

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(SEQ ID NO: 115)

Figure 12

pEX205

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ataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctt
tttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttc
cgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgtag
caccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacg
atagttaccggataaggcgcagcggtcgggctgaacggggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaac
tgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtc
ggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagc
gtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggcctttttacggttcctggccttttgctggcc
ttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgataccgctcgccgcagccga
acgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcctgatgcggtattttctccttacgcatctgtgcggtatttcaca
ccgcaatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatacactccgctatcgctacgtgactgggtcatggc
tgcgccccgacacccgccaacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgtc
tccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgaggcag (SEQ ID NO: 116)

Figure 13

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V A I Q T T D Q G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y V G D T G E L F F G E G S R L T V L
(SEQ ID No: 117)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V A I Q T T D R G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y L G N T G E L F F G E G S R L T V L
(SEQ ID No: 118)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V A I Q T T D Q G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y V G N T G E L F F G E G S R L T V L
(SEQ ID No: 119)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V S V G M T D R G E V P N G Y N V S R S T T E D F P
L R L L S A A P S Q T S V Y F C A S S Y V G D T G E L F F G E G S R L T V L
(SEQ ID No: 120)

M G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y R Q
D P G M G L R L I H Y S V A I Q T T D R G E V P N G Y N V S R S T I E D F P L
R L L S A A P S Q T S V Y F C A S S Y V G N T G E L F F G E G S R L T V L
(SEQ ID No: 121)

Figure 14a

C58c61 alpha with TRAC

M K Q E V T Q I P A A L S V P E G E N L V L N C S F T D S A I Y N L Q W F R
Q D P G K G L T S L L L I T P W Q R E Q T S G R L N A S L D K S S G R S T L Y
I A A S Q P G D S A T Y L C A V R P L L D G T Y I P T F G R G T S L I V H P Y I
Q N P D P A V Y Q L R D S K S S D K S V C L F T D F D S Q T N V S Q S K D S
D V Y I T D K

(SEQ ID NO: 122)

Figure 14b

C58c61 beta with TRBC1

M N A G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y
R Q D P G M G L R L I H Y S V A I Q T T D Q G E V P N G Y N V S R S T I E D F
P L R L L S A A P S Q T S V Y F C A S S Y L G N T G E L F F G E G S R L T V L
E D L N K V F P P E V A V F E P S E A E I S H T Q K A T L V C L A T G F F P D
H V E L S W W V N G K E V H S G V

(SEQ ID NO: 123)

Figure 14c

C58c61 beta with TRBC2

M N A G V T Q T P K F Q V L K T G Q S M T L Q C A Q D M N H E Y M S W Y
R Q D P G M G L R L I H Y S V A I Q T T D Q G E V P N G Y N V S R S T I E D F
P L R L L S A A P S Q T S V Y F C A S S Y L G N T G E L F F G E G S R L T V L
E D L K N V F P P E V A V F E P S E A E I S H T Q K A T L V C L A T G F Y P D
H V E L S W W V N G K E V H S G V

(SEQ ID NO: 124)

Figure 14d

C58c61 beta with TRBC2 fused to wt human IL-2

MNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWY
RQDPGMGLRLIHYSV<u>AIQT</u>TDQGEVPNGYNVSRST<u>I</u>EDF
PLRLLSAAPSQTSVYFCASSY<u>L</u>GNTGELFFGEGSRLTVL
EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPD
HVELSWWVNGKEVHSGV*PGAPTSSSTKKTQLQLEHLLL*
DLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCL
EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLT
(SEQ ID NO: 125)

Figure 15a

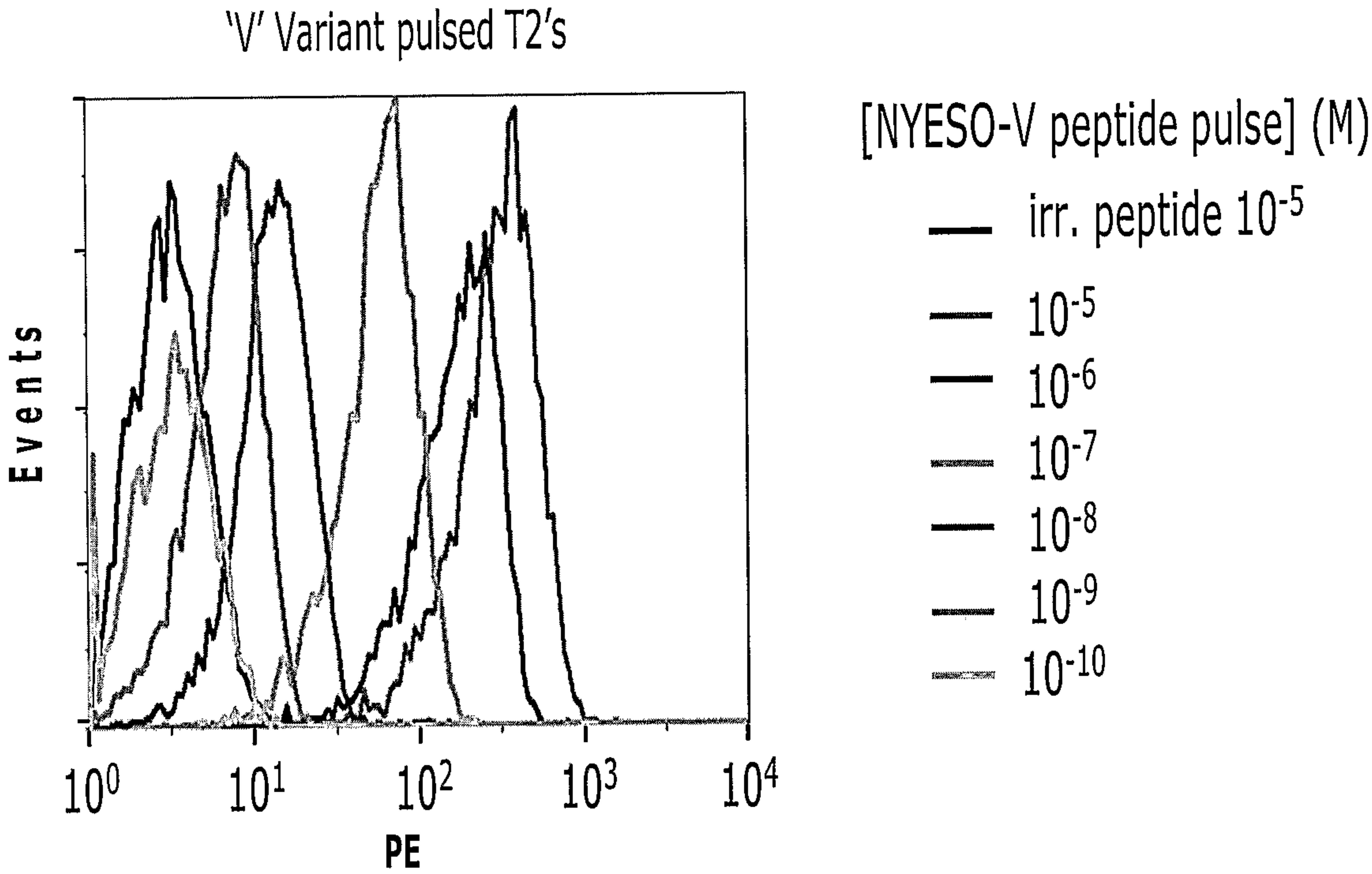


Figure 15b

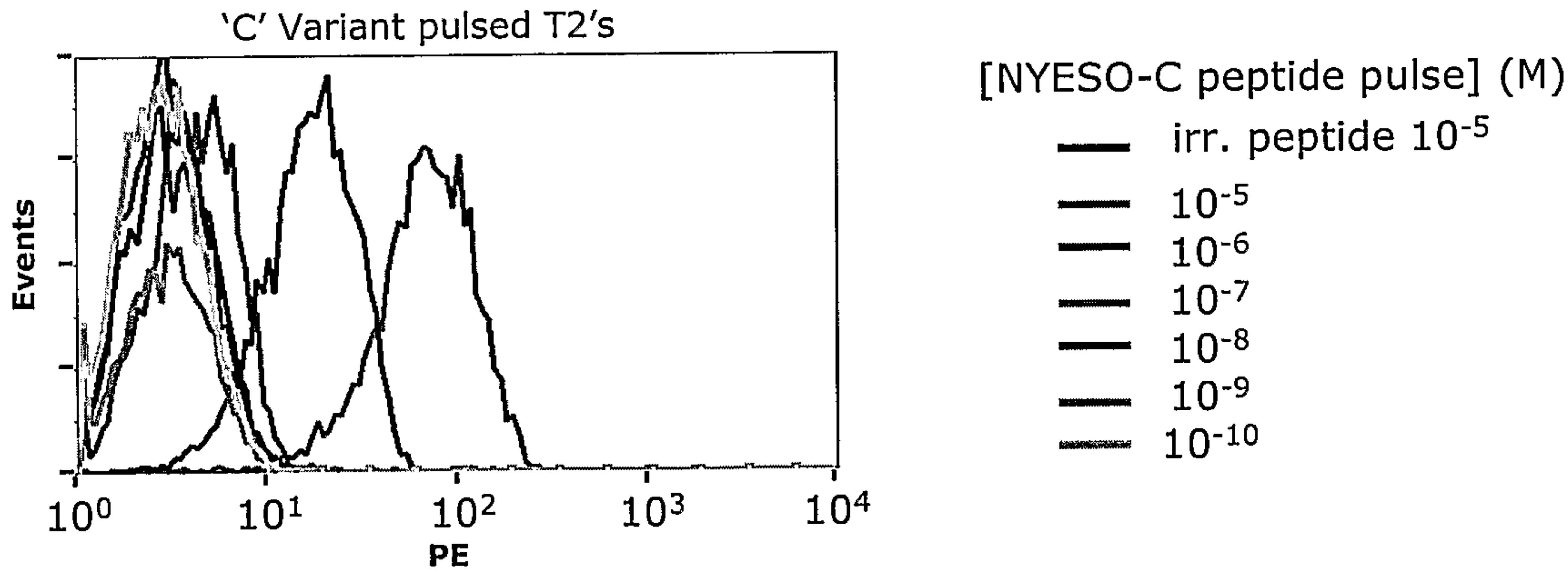


Figure 16

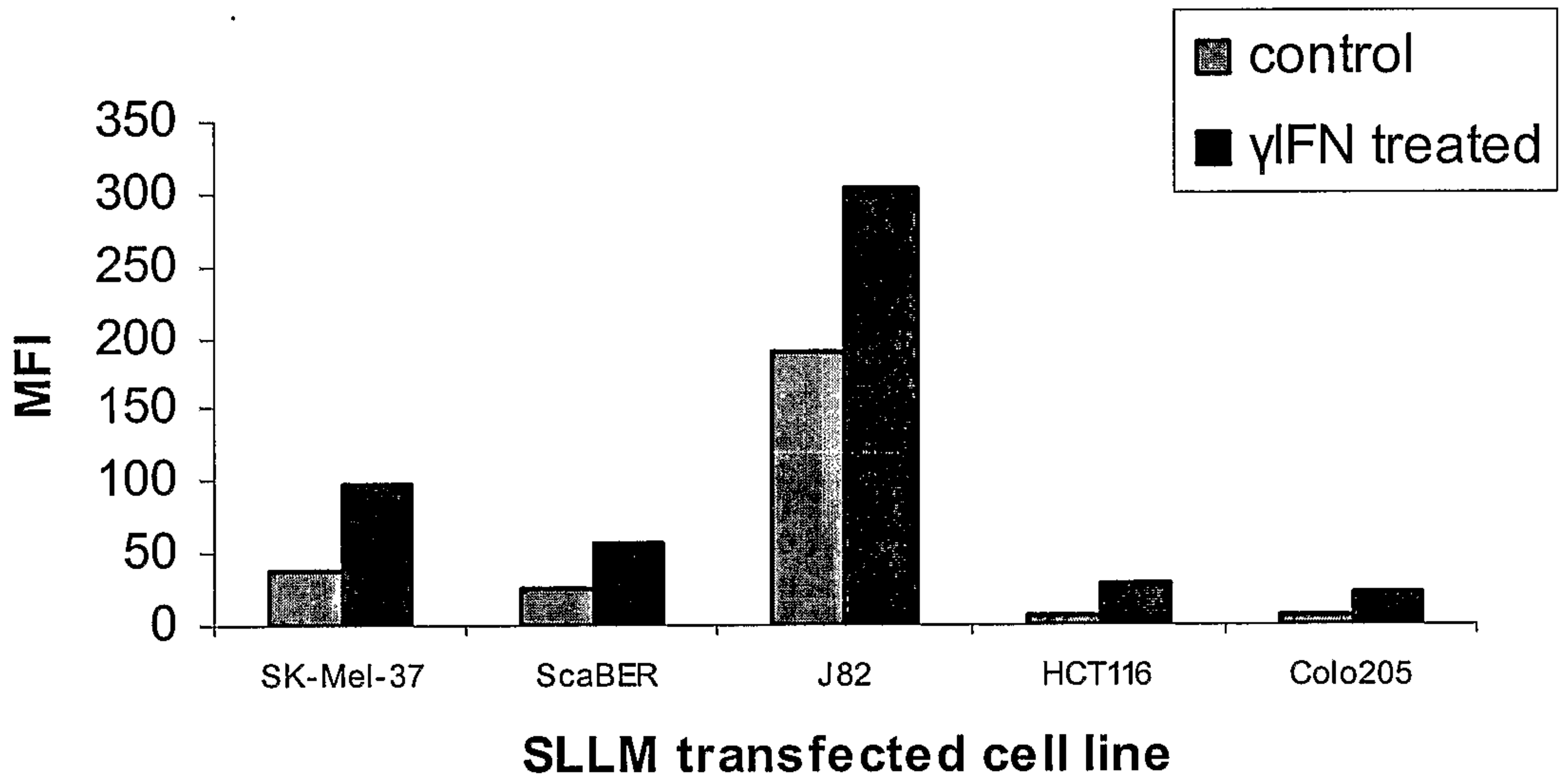

control
γIFN treated
350
300
250
200
150
100
50
0
MFI
SK-Mel-37
ScaBER
J82
HCT116
Colo205
SLLM transfected cell line

Figure 17

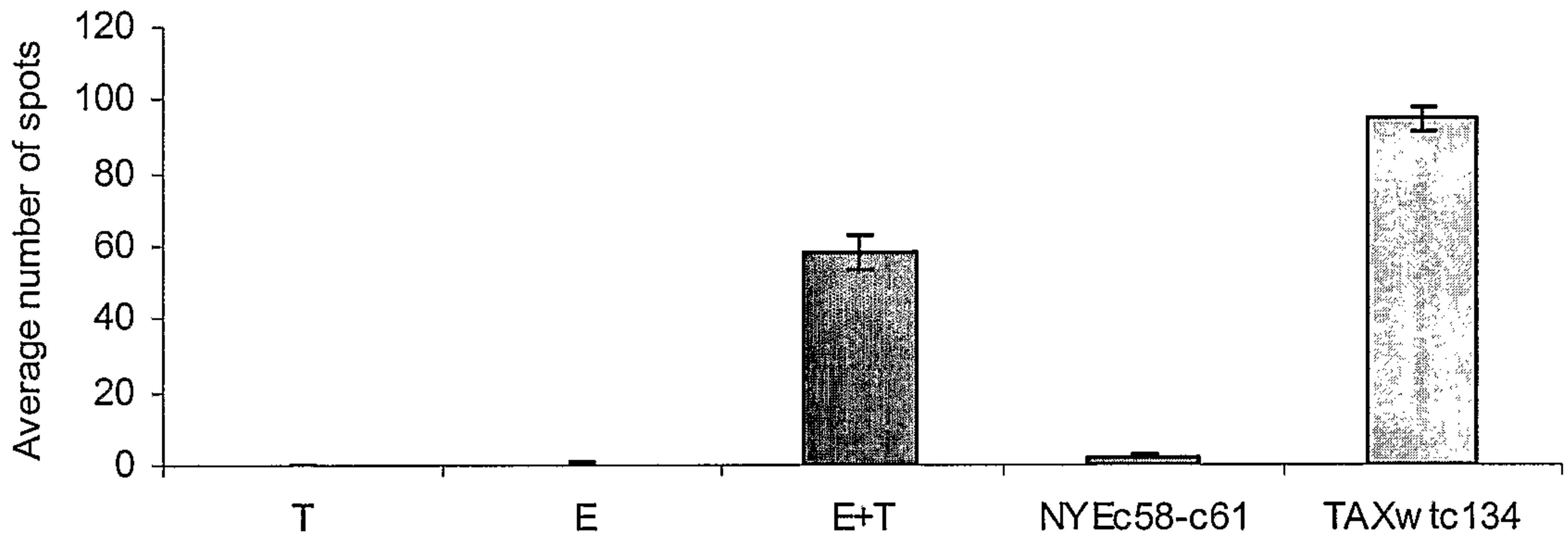

Average number of spots
120
100
80
60
40
20
0
T
E
E+T
NYEc58-c61
TAXwtc134

Figure 18

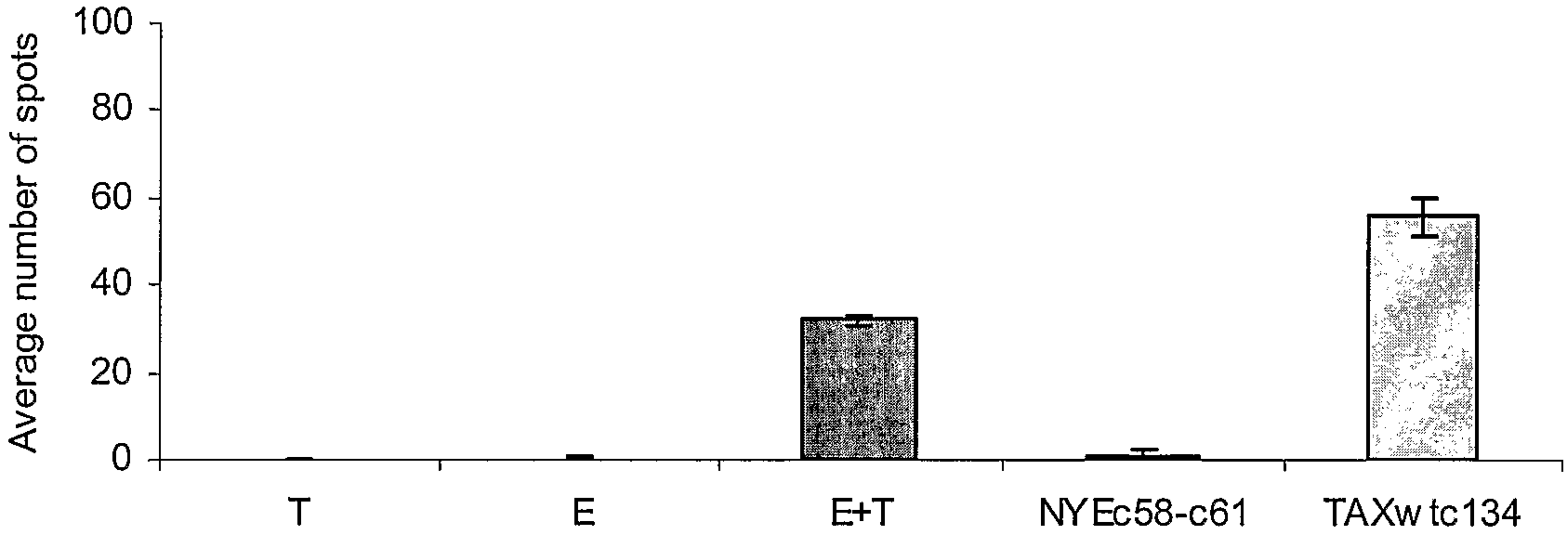

100
80
60
40
20
0
Average number of spots
T
E
E+T
NYEc58-c61
TAXwtc134

Figure 19

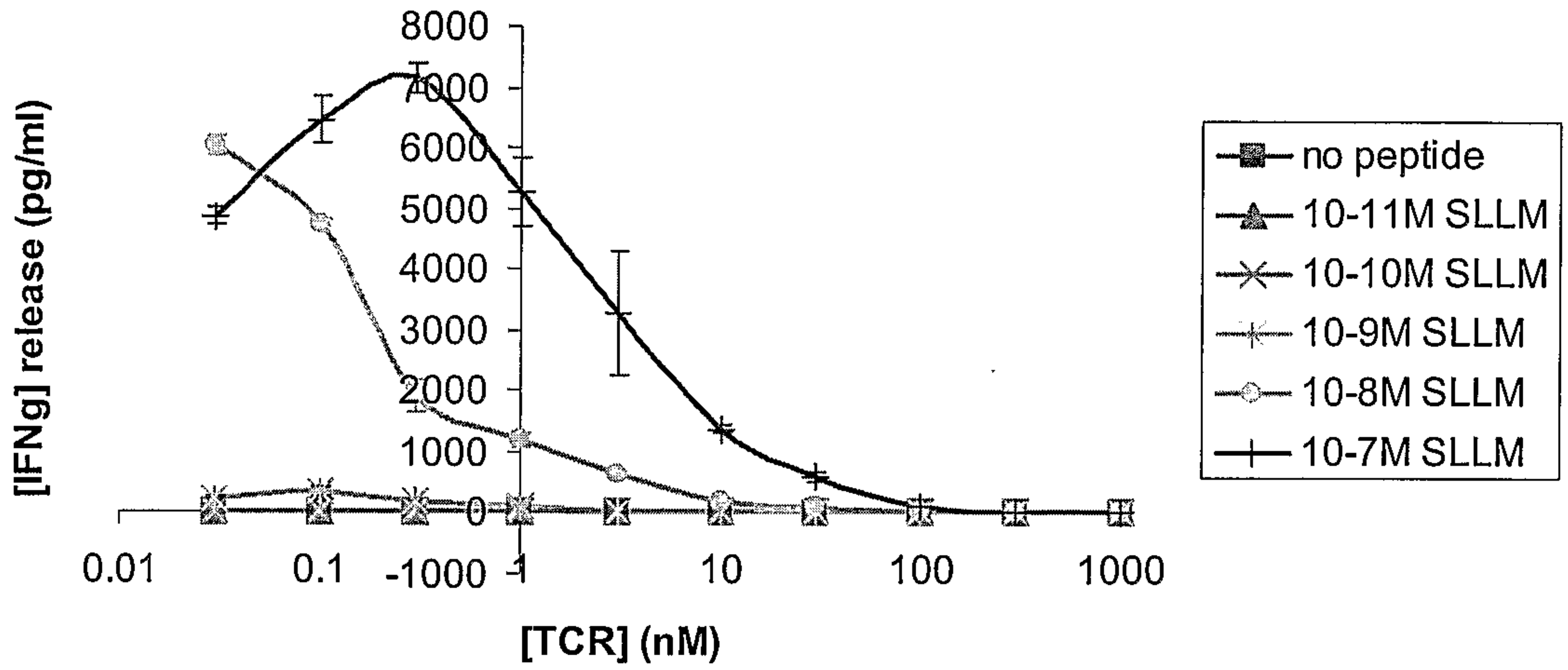

8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
[IFNg] release (pg/ml)
0.01
0.1
1
10
100
1000
[TCR] (nM)
no peptide
10-11M SLLM
10-10M SLLM
10-9M SLLM
10-8M SLLM
10-7M SLLM

Figure 20

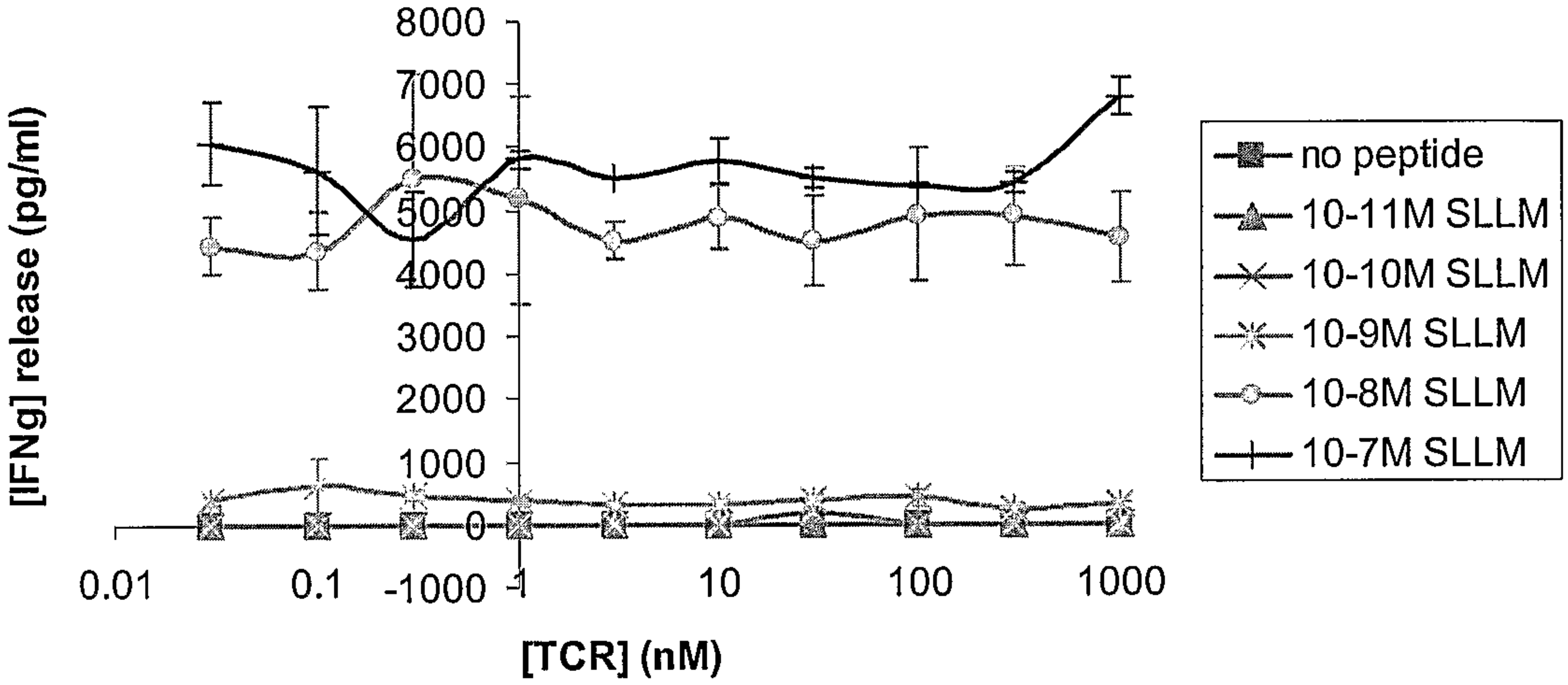

8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
[IFNg] release (pg/ml)
0.01
0.1
1
10
100
1000
[TCR] (nM)
no peptide
10-11M SLLM
10-10M SLLM
10-9M SLLM
10-8M SLLM
10-7M SLLM

Figure 21

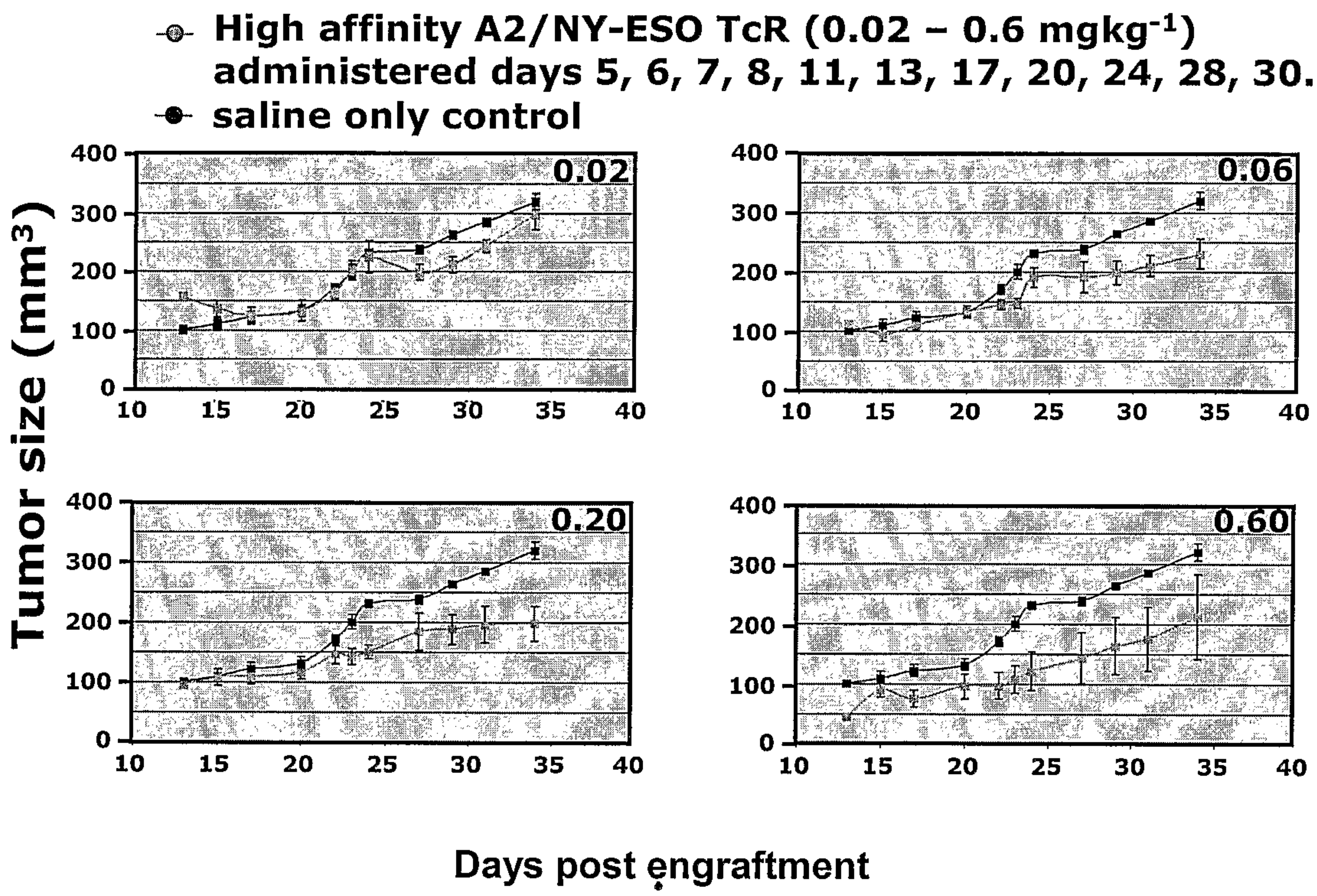
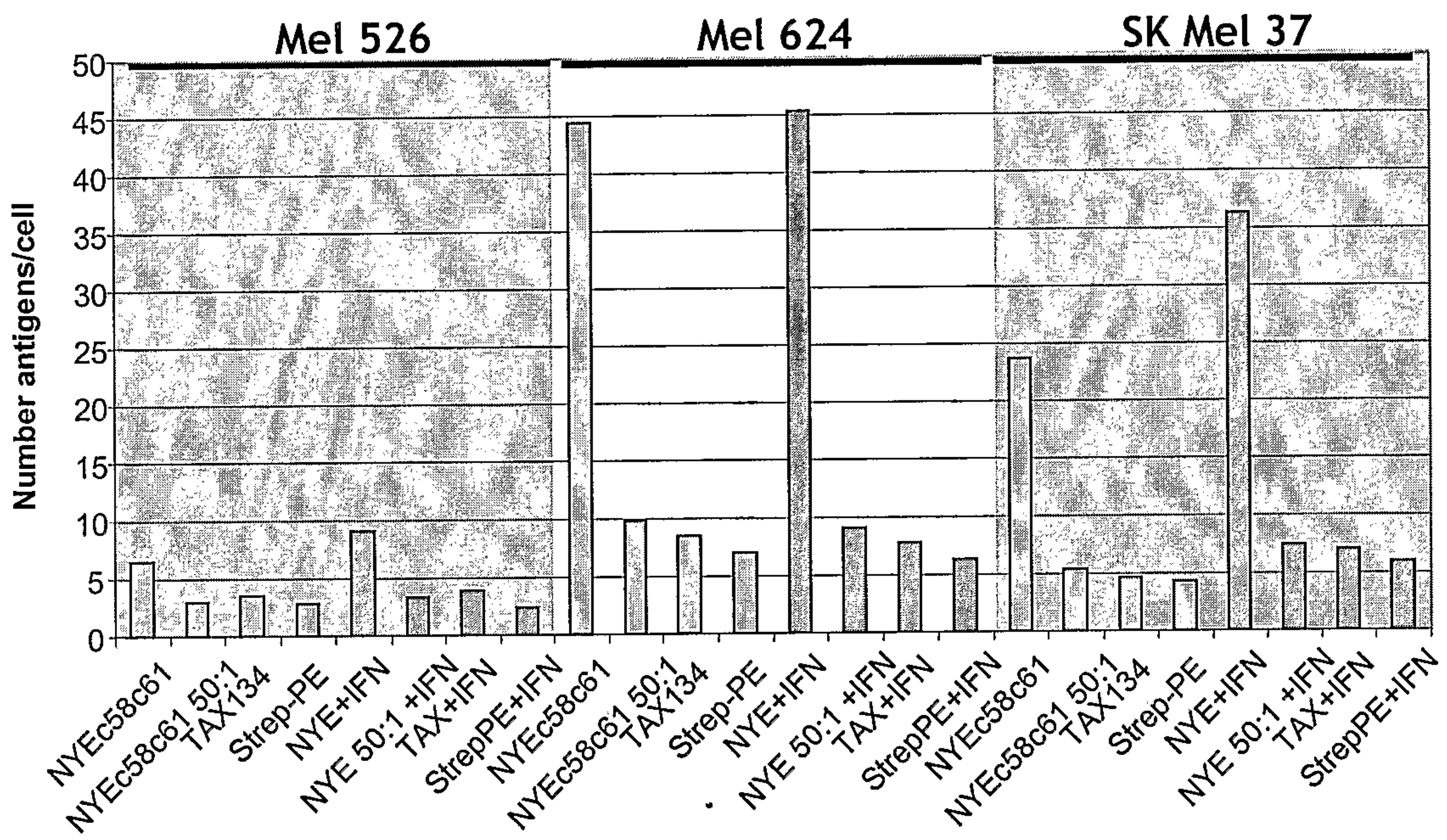

High affinity A2/NY-ESO TcR (0.02 – 0.6 mgkg-1) administered days 5, 6, 7, 8, 11, 13, 17, 20, 24, 28, 30.
saline only control
Tumor size (mm3)
0.02
0.06
0.20
0.60
Days post engraftment

Figure 22


Mel 526
Mel 624
SK Mel 37
Number antigens/cell
0
5
10
15
20
25
30
35
40
45
50
NYEc58c61
NYEc58c61 50:1
TAX134
Strep-PE
NYE+IFN
NYE 50:1 +IFN
TAX+IFN
StrepPE+IFN