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(54) Title: MULTI-SPECIFIC IGG-(FAB)2 CONSTRUCTS CONTAINING T-CELL RECEPTOR CONSTANT DOMAINS

(57) Abstract: The present invention provides IgG-(Fab)2 multispecific compounds containing T-Cell Receptor (TCR) constant do-
mains. In addition, the present invention provides methods of making such IgG-(Fab)2 multispecific compounds.



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Multi-Specific IgG-(Fab)₂ Constructs Containing T-Cell Receptor Constant Domains

The present disclosure relates to IgG-(Fab)₂ multi-specific compounds containing T-Cell receptor constant domains and methods of making such multi-specific compounds.

5 Therapeutic antibodies allow for the modulation of many disease conditions which provides benefits to patients. There are instances in which modulating more than one target could provide even greater benefit to patients. Although administration of two therapeutic antibodies is possible, practical considerations such as non-compatibility of formulations when combining multiple therapeutic antibodies into a single dosage form, and increased injections when administering multiple therapeutic antibodies as separate agents, may limit the possible benefit. A multi-specific compound could help minimize these concerns, and may also provide biological activities distinct from the combined administration of individual agents.

15 One of the most commonly used multi-specific compound platforms is the IgG-scFv. In this platform, an scFv is linked to each end of the heavy or light chain of an antibody. The IgG-scFv platform attempts to combine the activities of two antibodies while maintaining IgG-like pharmacokinetics and immune effector function. In scFvs, the V_H and V_L domains can exhibit attenuated stability and the exposure of hydrophobic surface area due to weaker HC/LC association can lead to aggregation.

20 To make IgG-scFv multispecific compounds viable, the biophysical aspects of the scFv within each particular IgG-scFv must be engineered to overcome expression, folding, affinity, solubility, and stability problems. Engineering scFvs to sufficiently overcome these issues is not straightforward, requiring significant time and resources for every multi-specific compound. As such, the IgG-scFv configuration may not always be a viable option for generating a multi-specific compound.

25 In Fabs, antibody heavy chain (HC) and light chain (LC) association is strong, with limited interdomain dynamics. However, manufacturing an antibody with a Fab linked to each HC or LC of the antibody (IgG-(Fab)₂) compound is challenging because the two LCs of an IgG-(Fab)₂ compound will bind heterogeneously to the two HC Fd (i.e., V_H and C_{H1}) regions within the compound generating unacceptable heterogeneity (sixteen HC/LC/LC pairings are possible).

The compounds of the present invention achieve specific assembly of IgG-(Fab)₂ compounds by replacing the Fab or antibody constant domains (C_H1/C_L) with T-cell receptor (TCR) constant domains. In addition, the present disclosure provides that the IgG-(Fab)₂ of the present invention contain the TCR α constant domain in the HC polypeptide to allow for proper assembly of the IgG-(Fab)₂ such that the specificities of the Fab and the antibody are maintained. The compounds of the present invention allow for the specificities and binding activities of the variable regions of two therapeutic antibodies to be combined in one compound.

While chimeric proteins containing an antibody variable region linked to a TCR constant domain have been disclosed (Kuwana, et al., Biochemical and Biophysical Research Communications, Vol. 149, No. 3, 1987 and Seimiya, et al., Journal of Biochemistry 113, 687-691, 1993), these references do not demonstrate appropriate assembly of a V_H-Cα and a V_L-Cβ polypeptides in the context of an IgG-(Fab)₂ as presently disclosed. Kuwana, et al. and Seimiya, et al. did not provide any disclosure or suggestion that using a TCR α constant domain in the heavy chain of an IgG-Fab, would allow for specific assembly of the polypeptide chains of an IgG-(Fab)₂ compound such that the binding specificities of the Fab and the antibody would be maintained.

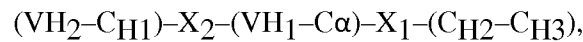
The present disclosure provides a compound comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, in which

- a. the first polypeptide chain has the formula VL₁-Cβ, wherein
 - i. VL₁ is a first antibody light chain variable region, and
 - ii. Cβ is a T-Cell Receptor β constant domain; and
- b. the second polypeptide has the formula VL₂-C_L, wherein
 - i. VL₂ is a second antibody light chain variable region, and
 - ii. C_L is a light chain κ or λ constant domain; and
- c. the third polypeptide chain has a formula selected from the group consisting of

(VH₁-Cα)-X₁-(CH₂-CH₃)-X₂-(VH₂-CH₁),

(VH₂-CH₁)-(H-CH₂-CH₃)-X₂-(VH₁-Cα),

(VH₁-Cα)-X₂-(VH₂-CH₁)-(H-CH₂-CH₃), and



wherein

VH₁ is a first antibody heavy chain variable region, and

C α is a T-Cell Receptor α constant domain and

5 X₁ is a peptide linker or is absent, and

C_{H2}-C_{H3} are heavy chain constant domains 2 and 3, and

H is a hinge region between C_{H1} and C_{H2}, and

X₂ is a peptide linker or is absent, and

VH₂ is a second antibody heavy chain variable region and

10 C_{H1} is a heavy chain constant domain 1; and

in which the first and second polypeptide chains associate with the third polypeptide chain to form a binding site (VL₁-VH₁) for a first binding partner and a binding site for a second binding partner (VL₂-VH₂), respectively. The binding partners may be distinct antigens or proteins, or may be different positions on an antigen or protein.

15

The present disclosure also provides a mammalian cell containing DNA encoding three polypeptides,

- a. wherein a first polypeptide has a formula the first polypeptide chain has the formula VL₁-C β , wherein

20 VL₁ is a first antibody light chain variable region, and

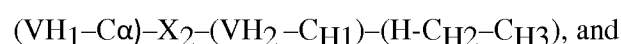
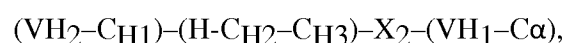
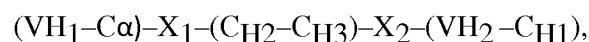
C β is a T-Cell Receptor β constant domain;

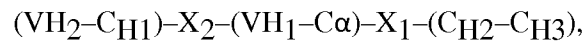
- b. the second polypeptide has the formula VL₂-C_L, wherein

VL₂ is a second antibody light chain variable region, and

CL is a light chain κ or λ constant domain; and

25 c. the third polypeptide has a formula selected from the group consisting of





wherein

VH₁ is a first antibody heavy chain variable region, and

C α is a T-Cell Receptor α constant domain, and

5 X₁ is a peptide linker or is absent, and

C_{H2}-C_{H3} are heavy chain constant domains 2 and 3, and

H is a hinge region between C_{H1} and C_{H2}, and

X₂ is a peptide linker or is absent, and

VH₂ is a second antibody heavy chain variable region and

10 C_{H1} is a heavy chain constant domain 1.

The present disclosure provides a compound comprising an antibody and two Fabs, wherein the V_H containing polypeptides of the Fab are linked to the heavy chains of the antibody and wherein the C_{H1} constant regions of the antibody or Fab fragments are replaced with T-cell receptor α constant domains (C α) and the corresponding light chain constant domains of the antibody or Fabs are replaced with T-cell receptor β constant domains (C β) such that the C α and C β domains allow the corresponding variable regions to associate to form an antigen binding fragment and the C_{H1} and light chain constant regions allow the variable regions associate to form another antigen binding fragment.

The present disclosure provides a compound comprising an antibody and two Fabs, wherein the V_H containing polypeptides of the Fab are linked to the heavy chains of the antibody and wherein the C_{H1} regions of the Fab fragments are replaced with T-cell receptor α constant domains (C α) and the constant domains of the Fab light chains are replaced with T-cell receptor β constant domains (C β) such that the C α and C β domains associate to form part of an antigen binding fragment. The present disclosure also provides a compound comprising an antibody and two Fabs, wherein the V_H containing polypeptides of the Fab are linked to the heavy chains of the antibody and wherein the C_{H1} regions of the antibody are replaced with T-cell receptor α constant domains (C α) and the constant domains of the antibody light chain are replaced with T-cell receptor β constant domains (C β) such that the C α and C β domains associate to form part of an antigen binding fragment.

The present disclosure provides a compound comprising three polypeptide chains, in which

- a) the first polypeptide chain comprises the formula $VH_1-C\alpha$ wherein
 VH_1 is a first antibody heavy chain variable region, and
 $C\alpha$ is a T-cell receptor α constant domain, and
- b) the second polypeptide chain comprises the formula $VH_2-C_{H1}-H-C_{H2}-C_{H3}$ wherein
 VH_2 is a second antibody heavy chain variable region, and
 C_{H1} , C_{H2} , and C_{H3} are heavy chain constant domains 1, 2, and 3, respectively, and
 H is a hinge region, and
- c) the third polypeptide chain comprises the formula $VL_1-C\beta-X_1-VL_2-CL$, wherein
 VL_1 is a first antibody light chain variable region,
 $C\beta$ is a T-Cell Receptor β constant domain,
 X_1 is a linker which can be present or absent,
 VL_2 is a second antibody light chain region and
 CL is either a C_k or C_λ light chain antibody constant domain,
in which the first and second polypeptide chains associate with the third polypeptide chain to form a binding site (VL_1-VH_1) for a first binding partner and a binding site for a second binding partner (VL_2-VH_2).

The present disclosure also provides a compound comprising three polypeptide chains, wherein

- a) a first polypeptide chain has the formula $VH_1-C_{H1}-H-C_{H2}-C_{H3}$, wherein VH_1 is a first antibody heavy chain variable region, and
 C_{H1} , C_{H2} , and C_{H3} are heavy chain constant domains 1, 2, and 3, respectively, and
 H is a hinge region, and
- b) a second polypeptide chain has the formula $VL_1-CL-VL_2-C\beta$, wherein
 VL_1 is a first antibody light chain variable region and
 CL is either C_k or C_λ , and
 VL_2 is a second light chain variable region, and

- $C\beta$ is a T-Cell Receptor β constant domain, and
- c) the third polypeptide has the formula $VH_2-C\alpha$, wherein
- VH_2 is a second antibody heavy chain variable region and
- $C\alpha$ is a T-Cell Receptor α constant domain,

5 in which the first and third polypeptide chains associate with the second polypeptide chain to form a binding site (VL_1-VH_1) for a first binding partner and a binding site for a second binding partner (VL_2-VH_2).

The present disclosure also provides a compound comprising three polypeptide chains, wherein

- 10 a) a first polypeptide chain has the formula VL_1-CL , wherein
- VL_1 is a first antibody light chain variable region and
- CL is either C_k or C_λ , and
- b) a second polypeptide chain has the formula $VL_2-C\beta$, wherein
- VL_2 is a second antibody light chain variable region and
- 15 $C\beta$ is a T-Cell Receptor β constant domain,
- c) a third polypeptide chain has the formula $VH_1-C_{H1}-X_1-VH_2-C\alpha$, wherein
- VH_1 is a first antibody heavy chain variable region, and
- C_{H1} is a heavy chain constant domain 1, and
- X_1 is a linker, and
- 20 VH_2 is a second antibody heavy chain variable region, and
- $C\alpha$ is a T-Cell Receptor α constant domain, and

in which the first and second polypeptide chains associate with the third polypeptide chain to form a binding site (VH_1-VL_1) for a first binding partner and a binding site for a second binding partner (VH_2-VL_2).

25 The present disclosure also provides a compound comprising three polypeptide chains, wherein

- a) a first polypeptide chain has the formula $VL_1-C\beta$, wherein
- VL_1 is a first antibody light chain variable region, and
- $C\beta$ is a T-Cell Receptor β constant domain,
- 30 b) a second polypeptide has the formula VL_2-CL , wherein
- VL_2 is a second antibody light chain variable region, and
- CL is either C_k or C_λ , and

c) a third polypeptide has the formula $VH_1-C\alpha-X_1-VH_2-C_{H1}$, wherein

VH_1 is a first antibody heavy chain variable region, and

$C\alpha$ is a T-Cell Receptor α constant domain, and

X_1 is a linker, and

5 VH_2 is a second antibody heavy chain variable, and

C_{H1} is heavy chain constant domain 1, and

in which the first and second polypeptide chains associate with the third polypeptide chain to form a binding site (VH_1-VL_1) for a first binding partner and a binding site for a second binding partner (VH_2-VL_2).

10 The present invention also provides a process for producing any of the proceeding compounds comprising cultivating the mammalian cells containing DNA encoding a compound of the present invention under conditions such that the compound is expressed and recovering the compound.

Brief Description of the Figures

15 **Fig. 1.** Schematic diagram of Format #1 of a TCR-based IgG-(Fab)₂ compound, comprising three polypeptide chains: $VL_1-C\beta$, VL_2-CL , and $(VH_1-C\alpha)-X_1-(C_{H2}-C_{H3})-X_2-(VH_2-C_{H1})$, in which VL_1 is a first antibody light chain variable region, $C\beta$ is a T-Cell Receptor β constant domain; VL_2 is a second antibody light chain variable region, CL is a light chain κ or λ constant domain; VH_1 is a first antibody heavy chain variable region, $C\alpha$ is a T-Cell Receptor α constant domain, X_1 is a peptide linker or is absent, $C_{H2}-C_{H3}$ are heavy chain constant domains 2 and 3, X_2 is a peptide linker or is absent, VH_2 is a second antibody heavy chain variable region and C_{H1} is a heavy chain constant domain 1.

Fig. 2. Schematic diagram of Format #2 of a TCR-based IgG-(Fab)₂ compound, comprising three polypeptide chains: $VL_1-C\beta$, VL_2-CL , and $(VH_2-C_{H1})-(C_{H2}-C_{H3})-X_2-(VH_1-C\alpha)$. **Fig. 3.** Schematic diagram of Format #3 of a TCR-based IgG-(Fab)₂ compound, comprising three polypeptide chains: $VL_1-C\beta$, VL_2-CL , and $(VH_1-C\alpha)-X_2-(VH_2-C_{H1})-(C_{H2}-C_{H3})$.

Fig. 4. Schematic diagram of Format #4 of a TCR-based IgG-(Fab)₂ compound, comprising three polypeptide chains: VL₁-C β , VL₂-CL, and (VH₂-C_{H1})-X₂-(VH₁-C α)-X₁-(C_{H2}-C_{H3}).

The general structure of an “antibody” is very well-known. For an antibody of the IgG type, there are four amino acid chains (two “heavy” chains and two “light” chains) that are cross-linked via intra- and inter-chain disulfide bonds. When expressed in certain biological systems, antibodies having unmodified human Fc sequences are glycosylated in the Fc region. Antibodies may be glycosylated at other positions as well. The subunit structures and three-dimensional configurations of antibodies are well known. Each heavy chain is comprised of an N-terminal heavy chain variable region (“V_H”) and a heavy chain constant region (“C_H”). The heavy chain constant region is comprised of three domains (C_{H1}, C_{H2}, and C_{H3}) for IgG as well as a hinge region (“hinge”) between the C_{H1} and C_{H2} domains. Each light chain is comprised of a light chain variable region (“V_L”) and a light chain constant region (“C_L”). The C_L may be of the kappa (“κ”) or lambda (“λ”) isotypes.

The variable regions of each light/heavy chain pair to form binding sites. The heavy chain variable region (V_H) and the light chain variable region (V_L) of the compounds of the present invention can be subdivided into regions of hypervariability, termed complementarity determining regions (“CDRs”), interspersed with regions that are more conserved, termed framework regions (“FR”). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Herein, the 3 CDRs of the heavy chain are referred to as “CDRH1, CDRH2, and CDRH3” and the 3 CDRs of the light chain are referred to as “CDRL1, CDRL2 and CDRL3.” The CDRs contain most of the residues which form specific interactions with the antigen. The assignment of amino acids to each domain is in accordance with well-known conventions [e.g., Kabat, “Sequences of Proteins of Immunological Interest,” National Institutes of Health, Bethesda, Md. (1991)].

An antibody may be derived from a single copy or clone (monoclonal antibody (mAb)), including e.g., any eukaryotic, prokaryotic, or phage clone. Preferably, an antibody of the present invention exists in a homogeneous or substantially homogeneous

population of antibody molecules. A full-length antibody comprises full length or substantially full length constant regions, including the Fc region. An “antigen-binding fragment” of such an antibody is any shortened form of a full length antibody that comprises the antigen-binding variable regions and retains antigen-binding capability.

- 5 Such shortened forms include, e.g., a Fab fragment, Fab' fragment or F(ab')₂ fragment that includes the CDRs or the variable regions of the antibodies disclosed.

An antibody of the present invention can be produced using techniques well known in the art, e.g., recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in
10 the art.

An antibody of the present invention is an engineered antibody that has been engineered to comprise framework, hinge, or constant regions derived from fully human frameworks, hinge, or constant regions containing one or more amino acid substitutions, deletions, or additions therein. Further, an antibody of the present invention is
15 substantially non-immunogenic in humans.

A variety of different human framework sequences may be used singly or in combination as a basis for an antibody of the present invention. Preferably, the framework regions of an antibody of the present invention are of human origin or substantially human (at least 95%, 97% or 99% of human origin.) The sequences of
20 framework regions of human origin may be obtained from The Immunoglobulin Factsbook, by Marie-Paule Lefranc, Gerard Lefranc, Academic Press 2001, ISBN 012441351.

T-cell receptors are used to recognize foreign antigenic peptides displayed by various antigen presenting cells of the immune system. Binding of T-cells to antigen-
25 presenting cells occurs via interactions between major histocompatibility complex (MHC) moieties of the antigen-presenting cells, which carry an antigenic peptide, and T-cell receptors (TCRs) on the surface of T-cells. Formation of a cell-bridging MHC-peptide-TCR complex typically results in stimulation and activation of the T-cells, a primary step of an adaptive immune response. TCRs are related to immunoglobulins in
30 that they contain hypervariable V-class Ig-fold domains capable of generating an incredible diversity that enables them to recognize diverse peptidic antigens displayed by MHCs. Like antibody Fabs, they also contain C-class Ig-fold constant domains that help

stabilize the TCR complex. However, structurally, TCR constant domains have very different primary sequences than immunoglobulin Fab constant domains (~20% identity between the TCR α - and β -domains and IgG CH1 and CL constant domains). Additional information regarding T-cell receptors and their constant domains may be found in The T

5 Cell Receptor Factsbook, by Marie-Paule Lefranc, Gerard Lefranc, Academic Press 2001, ISBN 0124413528.

Example 1- Certain Orientations of TCR Constant Regions May Substitute for CH1/CL Regions; Variable Region-Constant Region Linker Length

10 To determine the ability of the TCR α - and β -constant domains ($C\alpha$ and $C\beta$, respectively) to replace the C_{H1} of the heavy chain and light chain constant (CL) domains, varying constructs in a mammalian expression vector for transient expression in HEK293 cells are generated. Both orientations with the $C\alpha$ or $C\beta$ domains replacing the C_{H1} domain or the CL domain are constructed. For simplicity, this chimeric antibody format

15 is called IgG-TCR. An anti-human IL-17 IgG4/ κ (HC of SEQ ID NO:78 and LC of SEQ ID NO:79) antibody is utilized for generating IgG-TCR constructs. The C_{H1} /CL heterodimeric unit of the antibody is replaced using both $C\alpha/C\beta$ and $C\beta/C\alpha$ orientations and varying V-gene/TCR constant domain linker sequences. Each heavy chain ("HC") construct is co-transfected with each light chain ("LC") construct to determine whether

20 any format will allow for assembly, binding, and stability.

The linking regions between IgG V-genes and their constant regions are shorter than the linking regions between TCR V-genes and their respective constant regions. The linker regions between the antibody V-genes and the TCR constant domains, as well as truncated versions of the α - and β -constant connector regions are generated within the

25 IgG-TCR format. The sequences of the constructs with truncations are denoted in **Table 1**.

All gene constructs are synthesized using PCR-based overlapping oligonucleotide synthesis. Heavy chain genes are subcloned into a vector containing the gene sequence coding for a human IgG4 constant region resulting in the fusion products of the antibody

30 V-genes and the TCR $C\alpha$ and $C\beta$ genes with the IgG4PAA hinge-Fc domain. Light chains are also subcloned into a vector. The vector contains a common mouse antibody

LC signal sequence that is translated in-frame as part of the expressed protein and cleaved prior to secretion. The ligation mixture was used to transform *E. coli* strain TOP 10 competent cells (Invitrogen Corporation, Carlsbad, CA). Sequences are confirmed by DNA sequencing. Plasmid DNAs are used to transfect 293F cells for transient production of antibody protein.

Table 1: Substitution of C α and C β for C_{H1} and CL in an anti-human IL-17-IgG4; Orientations and Linker Lengths.

Construct Name	C _{H1} Substitute (SEQ ID NO:)	CL Substitute (SEQ ID NO:)	HC SEQ ID NO:	LC SEQ ID NO:
TCR2	C α (1)	C β (10)	29	23
TCR4	C α (2)	C β (11)	30	24
TCR6	C α (1)	C β (11)	29	24
TCR8	C α (2)	C β (10)	30	23
TCR1	C β (10)	C α (1)	27	25
TCR3	C β (11)	C α (2)	28	26
TCR5	C β (11)	C α (1)	28	25
TCR7	C β (10)	C α (2)	27	26
Anti-IL17/IgG4	-	-	78	79

The sequence given by SEQ ID NO:2 represents a truncation of N-terminal Pro-Asn (PN) from the native human C α sequence (given by SEQ ID NO:1). The sequence given by SEQ ID NO:11 represents a truncation of N-terminal Glu-Asp-Leu-Asn (EDLN) from the native human C β sequence (given by SEQ ID NO:10). The sequences of the variable regions, hinge, CH2, and CH3 are identical for these constructs. The only variation is in the CH₁/CL domains.

A vector harboring the LC/TCR DNA sequence and a vector harboring the HC/TCR DNA sequence are transfected (1:2 plasmid ratio for the HC and LC plasmids) into 293F cells using FreeStyle™ transfection reagents (Life Technologies). Transfected

cells are grown at 37 °C in a 5% CO₂ incubator while shaking at 125 rpm. Secreted protein is harvested by centrifugation at >10,000 rpm for 5 min.

To purify expressed IgG-TCR constructs, supernatants are passed through 2 µm filters. 1 mL of supernatant is incubated with 100 µL resuspended, phosphate buffered saline ("PBS")-washed Protein G magnetic beads (Millipore). Beads are washed two
5 times with PBST (phosphate buffered saline/0.02% Tween 80) according to the manufacturer's protocols. Protein is eluted from the beads by adding 130 µL 0.01 M Acetate, pH 3.0. After harvesting, the eluants are immediately neutralized by adding 20 µL 0.1 M Tris, pH 9.0.

10 Each expressed IgG-TCR is evaluated by SDS-PAGE. Approximately 5 µg protein is loaded in each well of Novex[®] 4-20% Tris Glycine gels or 3-8% Tris-Acetate gels according to manufacturer protocols (Life Technologies). For reduced samples, 10% 0.5 M DTT in H₂O is added to the same prior to loading.

As shown in Table 2, all the proteins expressed well. However, none of the
15 constructs having Cβ in the HC and Cα in the LC (TCR1, TCR3, TCR5, and TCR7) were assembled. Qualitative assembly was determined by SDS-PAGE based on the size of the IgG-TCR protein bands under non-reducing conditions and based on the ability to detect a LC band under reducing conditions (**Table 2**).

Analytical size exclusion chromatography (SEC) with in-line light scattering
20 (SEC/LS) is performed for each sample. 30-80 µL of each sample (~0.2-0.8 mg/mL) are injected onto a Sepax Zenix SEC 200 analytical HPLC (7.8×300 mm) column equilibrated in 10 mM phosphate, 150 mM NaCl, 0.02% NaN₃, pH 6.8, using an Agilent 1100 HPLC system (Agilent Technologies). Static light scattering data for material eluted from the SEC column are collected using a miniDAWN TREOS static light
25 scattering detector coupled to an Optilab T-rEX in-line refractive index meter (Wyatt Technologies). UV data are analyzed using HPCHEM (Agilent). Molecular weights of the complexes are determined by their static light scattering profiles using ASTRA V (Wyatt Technologies). The SEC results (**Table 2**) demonstrate that the constructs aggregate to a similar level as a control IgG4 antibody.

30 The extent of HC/LC assembly is assessed using a kinetic Biacore experiment. Briefly, kinetic surface Plasmon resonance (SPR) experiments are performed using a

Biacore 3000 (GE Healthcare). IgG-TCR proteins are captured onto CM5 sensorchip surfaces with an immobilized goat anti-human IgG-Fc polyclonal antibody (Jackson ImmunoResearch, Cat. #109-005-098). The goat polyclonal antibody immobilization is achieved by injecting the protein (at 50 µg/mL, pH 5) over an NHS/EDC activated
 5 sensorchip surface followed by blocking with ethanolamine (as described by manufacturer). The IgG or IgG-TCRs are captured onto the anti-human Fc sensorchip surface by injecting 10 µL of protein at 0.1 and 0.5 mg/mL using a 2 µL/mL flow rate. Flow rates are increased to 10 µL/min followed by secondary 30 µL injections of IL-17 (SEQ ID NO:80) at 10 and 50 nM. Following a 15 minute dissociation period, the flow
 10 rate is increased to 60 µL/min and sensorchip surfaces are regenerated using two consecutive 10 µL injections of 0.1 M glycine, pH 2.0.

The experiment measures the percent activity by relating the amount of captured IgG or IgG-TCR (based on resonance units) with the secondary response generated using a saturating level of IL-17. The percent activity of each IgG-TCR is calculated based on
 15 a comparison with the WT IgG:

$$\% \text{Activity} = \frac{\left(\frac{RU_{IL-17}}{RU_{IgG_TCR}} \right)}{\left(\frac{RU_{IL-17}}{RU_{IgG}} \right)},$$

where RU_{IgG_TCR} and RU_{IgG} are the resonance units of the captured IgG-TCR or IgG, respectively, and RU_{IL-17} is the saturating level of resonance units generated by the binding of IL-17 to the pre-loaded anti-IL-17 IgG or IgG-TCR. TCR3, which had the
 20 Cα- and Cβ domains replacing the CL and CH1 domains, respectively, demonstrated little activity in the assay indicating a near complete lack of LC/HC assembly (**Table 2**). TCR2, TCR4, TCR6, and TCR8 (with the α- and β-constant regions replacing the CH1 and CL domains, respectively) all had activity. (**Table 2**).

25

Table 2: Biochemical characterization of TCR1-TCR8.

Construct Name	Expression Level (μg/mL)	Apparent LC association by SDS-PAGE	Monomer/Aggregate by SEC/LS	Activity by kinetic Biacore
Anti-17 IgG4	70	+	64/36	100
TCR2	81	+	63/37	35
TCR4	56	+	67/33	54
TCR6	29	+	64/36	35
TCR8	74	+	65/35	48
TCR1	34	-	~25 ^a /~75 ^a	_b
TCR3	60	-	25/75	17
TCR5	44	-	~25 ^a /~75 ^a	_b
TCR7	27	-	~25 ^a /~75 ^a	_b

^a Estimated based on characterization of microscale purified material.

^b Not measured.

- 5 Thus, replacing the C_H1 domain of an immunoglobulin with Cα and replacing the C_L domain of an immunoglobulin with Cβ allows for the native-like heterotetramer formation of an IgG

Example 2- IgG-TCR hinge and LC C-terminus modifications

- Antibody I (HC SEQ ID NO. 34; LC SEQ ID NO. 33, trastuzumab), which binds
 10 to HER2, is used for the following experiments. The variable regions of Antibody I are appended to the N-termini of TCR4 above. The IgG-TCR construct containing the Antibody I Fv is denoted as tTCR-G4(+) and has HC and LC sequences of SEQ ID NO:36 and SEQ ID NO:35, respectively. Next, the IgG4 hinge (SEQ ID NO:5) of tTCR-G4 is replaced with an IgG1 hinge (SEQ ID NO:6) resulting in tTCR-G1(+) which has a
 15 HC of SEQ ID NO:37 and a LC of SEQ ID NO:35.

- To determine whether changes to Cβ in the LC can improve assembly, truncations are made in the C-terminus of Cβ. A new construct, with four residues truncated from the C-terminal sequence of Cβ is created (SEQ ID NO:12). A construct tTCR-G1(-) contains the same tTCR-G1 HC (SEQ ID NO:37) with the modified tTCR-G1 LC (SEQ ID
 20 NO:38) truncated at its C-terminus. Similarly, pTCR-G1(-) containing the Antibody II

(HC SEQ ID NO:42; LC SEQ ID NO:41) variable regions (HCVR SEQ ID NO:40; LCVR SEQ ID NO:39) is constructed.

All gene constructs are synthesized using protocols similar to those described in Example 1. Protein expression and purification are performed as described in Example 1.

- 5 Protein characterization is performed as described in Example 2, except that the kinetic Biacore assay used to measure IL-17 activity is replaced with a solution equilibrium assay that measures HER2 equilibrium affinity and stoichiometry. Briefly, Antibody I is directly immobilized to 12000 RUs onto an NHS/EDC activated CM5 sensor chip surface by injection of a 50 µg/mL solution of Antibody I in 10 mM Acetate, pH 5.0. A linear
- 10 RU response is observed by injecting human (h)HER-2-Fc protein (Cat. #1129-ER-050, R&D systems) at 2 µL/min at multiple concentrations between 1 and 100 nM over the sensorchip surface and monitoring the response after 80 seconds of binding. To evaluate the activity/assembly of the IgG-TCR, 20 nM hHER-2-Fc are mixed with varying concentrations (ranging from 1 nM to 100 nM) of each test article. The Antibody I
- 15 sensorchip surface measures the concentration of unbound or free hHER-2-Fc ($[R]_F$) in solutions containing 20 nM hHER-2-Fc and IgG-TCR. Unbound hHER-2-Fc is equal to the total amount of receptor in solution ($[R]_T$) minus the bound concentration ($[R]_B$). The equilibrium dissociation constant, K_D , and binding stoichiometry, n (used to determine the %assembly), between the IgG-TCRs and hHER-2-Fc are determined using the linear
- 20 relationship between $[R]_F$ and linear slope of RU/time (known as the velocity or V_i) within the first 80 seconds of the experiment:

$$V_i = m \cdot \left[[R]_T - \frac{1}{2} \left\{ \frac{(n[IgG_TCR]_T + [R]_T + K_D) - \sqrt{(n[IgG_TCR]_T + [R]_T + K_D)^2 - 4n[IgG_TCR]_T[R]_T}}{2} \right\} \right]$$

where m = slope of the hHER-2-Fc concentration-dependent standard curve and

- 25 $[IgG_TCR]_T$ = total IgG_TCR concentration.

Table 3:–tTCR-G1(+), tTCR-G1(-), and pTCR-G1(-) construct compositions.

Construct Name	Heavy Chain Compositions	Light Chain Compositions	HC SEQ ID NO:	LC SEQ ID NO:
Antibody I (trastuzumab)	VH = SEQ ID NO:32 CH1 = SEQ ID NO:14 H = SEQ ID NO:6 CH2-CH3 = SEQ ID NO:15	VL = SEQ ID NO:31 CL = SEQ ID NO:21	34	33
Antibody II (pertuzumab)	VH = SEQ ID NO:40 CH1 = SEQ ID NO:14 H = SEQ ID NO:6 CH2-CH3 = SEQ ID NO:15	VL = SEQ ID NO:39 CL = SEQ ID NO:21	42	41
tTCR-G1(+)	VH = SEQ ID NO:32 C α = SEQ ID NO:2 X ₁ = SEQ ID NO:6 CH2-CH3 = SEQ ID NO:15 ^a	VL=SEQ ID NO:31 C β = SEQ ID NO:11	37	35
tTCR-G1(-)	VH = SEQ ID NO:32 C α = SEQ ID NO:2 X ₁ = SEQ ID NO:6 CH2-CH3 = SEQ ID NO:15 ^a	VL=SEQ ID NO:31 C β = SEQ ID NO:12	37	38
pTCR-G1(-)	VH = SEQ ID NO:40 C α = SEQ ID NO:2 X ₁ = SEQ ID NO:6 CH2-CH3 = SEQ ID NO:15 ^a	VL = SEQ ID NO:39 C β = SEQ ID NO:12	46	45

^a The sequence for CH2-CH3 was that of SEQ ID NO:15, except that the terminal Lys (K) was deleted.

5

Table 4: Assembly of IgG-TCR constructs

Construct	Mol. Weight (kDa)	Apparent Affinity Trastuzumab or Pertuzumab Arm (nM)	Fraction Assembly Relative to
Antibody I (trastuzumab)	144	n.d	1
Antibody II (pertuzumab)	142	0.2±0.2	1
tTCR-G1(+)	166	n.d	0.75±0.2
tTCR-G1(-)	177	0.3±0.15	0.96±0.05
pTCR-G1(-)	180	0.13±0.1	1.03±0.2

“n.d ” denotes not determined.

Truncation of the C-terminal four amino acids of C β results in a 5-fold average increase in protein expression as determined according to the procedure in Example 2 (data not shown). The proteins with the LC-C β truncation are more uniformly the expected molecular weight based on in-line light scattering measurements and do not appear to be incompletely assembled. Additionally, truncating four LC-C β -terminal amino acids eliminates apparent proteolysis, mis-assembly, or protein degradation that had been observed as multiple absorbance peaks eluting for non-truncated molecules (data not shown).

A Biacore-based equilibrium binding experiment between tTCR-G1(+), tTCR-G1(-), and pTCR-G1(-) proteins with the hHER-2-Fc protein demonstrates that both the tTCR-G1(-) and pTCR-G1(-) proteins are fully assembled, while the tTCR-G1(+) protein is only ~75% assembled (Table 4). As a control, Antibody I and Antibody II are shown to block hHER-2-Fc binding to surfaces immobilized with the antibodies, but to not block each other with a stoichiometry matching the hHER-2-Fc concentration used in the assay (20 nM) (Table 4). The tTCR-G1 compounds could not inhibit hHER-2-Fc from binding an Antibody II-labeled surface and the pTCR-G1(-) compound could not inhibit hHER-2-Fc binding to an Antibody I-labeled surface, indicating that the specificity for their particular epitopes is intact. Different batches of tTCR-G1(+) appeared to give varying levels of assembly, as measured by the stoichiometry of hHER2-Fc blocking in the assay, perhaps because of differences in plasmid levels that were transfected (data not shown). The tTCR-G1(-) and pTCR-G1(-) proteins consistently block binding of 20 nM hHER2-Fc to surfaces with Antibody I and Antibody II, respectively, at 20 nM concentrations indicating they are 100% assembled. Overall, the presence of an IgG1 hinge (including the interchain cysteine that forms a disulfide with LC) and the LC-C β truncation results in fully assembled IgG-TCRs.

Example 3- Specificity of LC and HC Assembly

To determine the extent to which introduction of TCR-constant domains into a Fab enables specific HC/LC assembly that discriminates from wild-type HC/LC assembly (i.e., HCs and LCs containing CH1 and CL domains, respectively), tTCR-G1(-) and pTCR-G1(-) HC plasmids are transfected with both their cognate C β constant domain

containing LCs and the natural (non-TCR-domain containing) LC plasmids (Table 5). Additionally, the wild-type IgG1 Antibody I (trastuzumab) and Antibody II (pertuzumab) HC plasmids are transfected with their cognate natural LCs and their LCs containing C β constant domains (Table 5).

- 5 An OctetRed (ForteBio) biosensor assay is used to evaluate the specificity of the optimized Antibody I and Antibody II IgG-TCR constructs within this Example. The method includes the use of an anti-human IgG-Fc specific biosensor (ForteBio) to capture the IgG-TCR HC or wild-type IgG HC transfected in the presence of both the wild-type and TCR β -containing LCs (Table 5) at 10 μ g/mL in Octet buffer for 5 minutes. The captured IgGs or IgG-TCRs are then used to capture an anti-kappa CL domain-specific murine mAb (Sigma-Aldrich Cat. #SAB4700607) or an anti-C β constant domain-specific murine mAb (ThermoScientific Cat. #TCR1151) at 10 μ g/mL in Octet buffer for 5 minutes. The % non-specific binding is assessed as the saturated signal of the anti-C β or anti-C κ antibodies compared to the controls.

15

Table 5: Co-transfection of Antibody I and Antibody II IgG-TCR and IgG HCs with both natural LCs or LCs containing TCR- β constant domains.

Construct	SEQ ID NO:			% Non-Specific Assembly
	HC	LC1	LC2	
tC α HC/tC β LC (control)	37	38	-	0
tC α HC/tC β LC/tC κ LC	37	38	33	24 $\pm$ 4
tG1 HC/tC κ LC (control)	34	33	-	0
tG1 HC/tC κ LC/tC β LC	34	33	38	10 $\pm$ 2
pC α HC/pC β LC (control)	46	45	-	0
pC α HC/pC β LC/pC κ LC	46	45	41	11 $\pm$ 2
pG1 HC/pC κ LC (control)	42	41	-	0
pG1 HC/pC κ LC/pC β LC	42	41	45	0 $\pm$ 2

- 20 Wild-type IgG HCs show strong selective binding to their cognate wild-type LC when expressed in the presence of a C β -containing LC with the same variable domains. Additionally, C α -containing IgG HCs show selective binding to their cognate C β -containing LCs when expressed in the presence of a wild-type LC.

Example 4- Construction of IgG-(Fab)₂ compounds

To determine whether C α and C β domains can be used to generate compounds in which a Fab with one specificity is linked to an antibody with another specificity (IgG-(Fab)₂) and whether the HCs and LCs of such an IgG-(Fab)₂ can assemble appropriately to maintain their binding activities, IgG-(Fab)₂ with different C α and C β configurations are constructed. In one configuration, the HC portion of the Fab is linked to the N-terminus of the HC of the antibody and in another configuration, the HC portion of the Fab is linked to the C-terminus of the antibody. For each configuration, the C_{H1} region of the antibody or Fab is replaced with a C α domain, while the corresponding LC constant domain is replaced with C β . Each of the compounds contains a HC containing both a V_H/C_{H1} domain and a V_H/C α domain and two LCs, one containing a V_L/C_L pair and the other containing a V_L/C β -constant domain pair. The combinations of HCs and LCs that compose each compound are listed in **Table 7 and 8** and illustrated in **Figures 1-4**. All constructs fall into one of four formats, as shown below:

Table 6: IgG-(Fab)₂ compositions

	Figure	Format #
TCR-Fab – (CH2-CH3) – IgG-Fab	1	1
IgG-Fab – (CH2-CH3) – TCR-Fab	2	2
TCR-Fab – IgG-Fab – (CH2-CH3)	3	3
IgG-Fab – TCR-Fab – (CH2-CH3)	4	4

All the methods for generation of the IgG-Fab constructs including, gene synthesis, subcloning, and plasmid preparations are described in Example 1.

Table 7: TCR-based IgG-(Fab)₂ Constructs

Construct Name	Polypeptide Components SEQ ID NOs:	Format #
C-tTCRpG1	<u>tVH-Cα-X1-(C_{H2}-C_{H3})-X2-pVH-C_{H1}</u> ^a 32 - 2 - 6 - 15 -13- 40 - 14 <u>tVL-Cβ</u> 31 - 12 <u>pVL-κ</u> 39 - 21	1
C-pG1tTCR	<u>pVH-C_{H1}-(H-C_{H2}-C_{H3})-X2-tVH-Cα</u> ^b 40 - 14 - 6 - 15- 13 - 32 - 2 <u>pVL-κ</u> 39 - 21 <u>tVL-Cβ</u> 31 - 12	2

N-tTCRpG1	$\frac{\text{tVH-C}\alpha\text{-X2-pVH-C}_{\text{H1}}\text{-(H-C}_{\text{H2}}\text{-C}_{\text{H3}})}{32-2-13-40-14-6-15}^{\mathbf{c}}$ $\frac{\text{tVL-C}\beta}{31-12} \quad \frac{\text{pVL-}\kappa}{39-21}$	3
N-tTCRpG1ds	$\frac{\text{tVH-C}\alpha\text{-X2-pVH-C}_{\text{H1}}\text{-(H-C}_{\text{H2}}\text{-C}_{\text{H3}})}{32-2-81-40-14-6-15}^{\mathbf{c}}$ $\frac{\text{tVL-C}\beta}{31-12} \quad \frac{\text{pVL-}\kappa}{39-21}$	3
N-pG1tTCR	$\frac{\text{pVH-CH1-X2-tVH-C}\alpha\text{-X1-(C}_{\text{H2}}\text{-C}_{\text{H3}})}{40-14-13-32-2-6-15}^{\mathbf{c}}$ $\frac{\text{pVL-}\kappa}{39-21} \quad \frac{\text{tVL-C}\beta}{31-12}$	4
N-pG1tTCRds	$\frac{\text{pVH-C}_{\text{H1}}\text{-X2-tVH-C}\alpha\text{-X1-(C}_{\text{H2}}\text{-C}_{\text{H3}})}{40-14-82-32-2-6-15}^{\mathbf{c}}$ $\frac{\text{pVL-}\kappa}{39-21} \quad \frac{\text{tVL-C}\beta}{31-12}$	4
C-pTCRtG1	$\frac{\text{pVH-C}\alpha\text{-X1-(C}_{\text{H2}}\text{-C}_{\text{H3}})\text{-X2-tVH-C}_{\text{H1}}}{40-2-6-15-13-32-14}^{\mathbf{a}}$ $\frac{\text{pVL-C}\beta}{39-12} \quad \frac{\text{tVL-}\kappa}{31-21}$	1
C-tG1pTCR	$\frac{\text{tVH-C}_{\text{H1}}\text{-(H-C}_{\text{H2}}\text{-C}_{\text{H3}})\text{-X2-pVH-C}\alpha}{32-14-6-15-13-40-2}^{\mathbf{b}}$ $\frac{\text{tVL-}\kappa}{31-21} \quad \frac{\text{pVL-C}\beta}{39-12}$	2
N-pTCRtG1	$\frac{\text{pVH-C}\alpha\text{-X2-tVH-C}_{\text{H1}}\text{-(H-C}_{\text{H2}}\text{-C}_{\text{H3}})}{40-2-13-32-14-6-15}^{\mathbf{c}}$ $\frac{\text{pVL-C}\beta}{39-12} \quad \frac{\text{tVL-}\kappa}{31-21}$	3
N-pTCRtG1ds	$\frac{\text{pVH-C}\alpha\text{-X2-tVH-C}_{\text{H1}}\text{-(H-C}_{\text{H2}}\text{-C}_{\text{H3}})}{40-2-81-32-14-6-15}^{\mathbf{c}}$ $\frac{\text{pVL-C}\beta}{39-12} \quad \frac{\text{tVL-}\kappa}{31-21}$	3
N-tG1pTCR	$\frac{\text{tVH-C}_{\text{H1}}\text{-X2-pVH-C}\alpha\text{-X1-(C}_{\text{H2}}\text{-C}_{\text{H3}})}{32-14-13-40-2-6-15}^{\mathbf{c}}$ $\frac{\text{tVL-}\kappa}{31-21} \quad \frac{\text{pVL-C}\beta}{39-12}$	4
N-tG1pTCRds	$\frac{\text{tVH-C}_{\text{H1}}\text{-X2-pVH-C}\alpha\text{-X1-(C}_{\text{H2}}\text{-C}_{\text{H3}})}{32-14-82-40-2-6-15}^{\mathbf{c}}$ $\frac{\text{tVL-}\kappa}{31-21} \quad \frac{\text{pVL-C}\beta}{39-12}$	4

a C-terminal Glu-Pro-Lys-Ser-Cys-Asp-Gly-Gly-Gly (EPKSCDGGG) on HC.

b C-terminal Glu-Ser-Ser-Cys-Asp-Val-Gly-Gly-Gly (ESSCDVGGG) on HC.

c C-terminal is des-Ly (des K).

Table 8: Sequence composition of IgG-(Fab)₂ compounds

Construct Name	SEQ ID NOs:			Format #
	HC	K LC	TCR LC	
C-tTCRpG1	47	41	55	1
C-pG1tTCR	48	41	55	2
N-tTCRpG1	49	41	55	3
N-tTCRpG1ds	73	41	55	3
N-pG1tTCR	50	41	55	4
N-pG1tTCRds	74	41	55	4
C-pTCRtG1	51	33	56	1
C-tG1pTCR	52	33	56	2
N-pTCRtG1	53	33	56	3
N-pTCRtG1ds	71	33	56	3
N-tG1pTCR	54	33	56	4
N-tG1pTCRds	72	33	56	4
Antibody I (trastuzumab)	34	33	-	-
Antibody II (pertuzumab)	42	41	-	-

5

The following nomenclature is used when referring the IgG-Fab compounds of the present invention. “C-“ or “N-“ denotes an additional Fab region (either wild-type Fab or Fab containing TCR-constant domains) appended to the C- or N-terminus of the HC, respectively. “t” refers to Antibody I Fv (trastuzumab Fv), “p” refers to Antibody II (pertuzumab Fv), “G1” denotes a wild-type Fab while “TCR” denotes a Fab containing TCR-constant domains. The order with which the G1 and TCR sequences are listed within each name indicates the order within the primary sequence that the Fab regions occur. For example, C-tTCRpG1 indicates that a Fab of Antibody II (pertuzumab Fab), which has antibody C_{H1}/C_L domains, is linked to the C-terminal end of the HC of Antibody I (trastuzumab) whose C_{H1}/C_L domains are replaced by TCR Cα/Cβ, respectively.

10

15

IgG-(Fab)₂ compound characterization, including protein expression, protein purification, and in vitro biochemical are performed as described in Example 1. The

binding/assembly properties of the IgG-Fab compounds are determined using the SPR-based solution equilibrium methodology described in Example 2.

Table 9: Characterization of the IgG-Fab recognizing two epitopes of HER2

Construct	Mol. Wt (kDa) ^a	Trastuzumab arm apparent affinity (nM)	Fraction Trastuzumab Arm Assembly ^b (IgG_TCR <i>n</i> /Trastuz. IgG1 <i>n</i>)	Pertuzumab arm apparent affinity (nM)	Fraction Pertuzumab Arm Assembly ^b (IgG_TCR <i>n</i> /Pertuz. IgG1 <i>n</i>)
C-tTCRpG1	263	0.09±0.2	0.73±0.2	0.12±0.2 *	1.1±0.2*
C-pG1tTCR	275	0.21±0.3	0.85±0.2	1.9±1.5*	0.92±0.2*
N-tTCRpG1	264	0.19±0.3	0.72±0.2	0.11±0.2*	1.2±0.2*
N-pG1tTCR	274	0.06±0.2	0.88±0.2	0.01±0.2*	1.15±0.2*
C-pTCRtG1	256	0.31±0.5*	0.92±0.2*	3.2±2.8	1.2±0.2
C-tG1pTCR	- ^d	5.0±6.0*	0.73±0.3*	0.35±0.5	1.2±0.2
N-pTCRtG1	256	0.21±0.5*	1.0±0.3*	2.4±2.1	1.2±0.2
N-tG1pTCR	264	0.22±0.5*	1.1±0.2*	0.1±1.0	1.0±0.2
N-tTCRpG1ds	266	0.08±0.1	0.87 ±0.2	0.03±0.1*	1.2±0.2*
N-pG1tTCRds	277	0.04±0.1	1.29±0.2	0.09±0.1*	1.7±0.4*
N-pTCRtG1ds	256	0.03±0.1*	1.34±0.2*	0.08±0.1	1.3±0.2
N-tG1pTCRds	259	0.11±0.1*	1.39±0.25*	0.15±0.1	1.4±0.3

5 ^aDetermined by SEC with in-line static light scattering. mAb data ±5 kDa; BsAb data ±10 kDa.

^bDetermined by equilibrium solution SPR experiments.

^cProtein displayed varying HC/LC compositions based on SEC.

^dProtein displayed varying HC/LC compositions based on SEC.

10 “*” Indicates WT-IgG Fab binding stoichiometry/assembly results
“n.d.” denotes not determined.

The IgG-(Fab)₂ compounds could all block hHER-2-Fc from binding both surfaces (Table 9). The data suggest that four binding sites are intact.

15 **Example 5- Activity of IgG-(Fab)₂ Compounds in cell assays**

Next, we assayed IgG-(Fab)₂ compounds for their ability to inhibit HER-2-driven tumor cell growth. The NCI-N87 (gastric) and BT474 (breast) tumor cell lines both highly over express HER-2 on their cell surfaces and have been shown to be sensitive to treatment using the trastuzumab/pertuzumab combination.

20 For the inhibition assays, HER-2-positive NCI-N87 (ATCC Cat. #CRL-5822) or BT474 (ATCC Cat. #HTB-20) tumor cell lines are cultured according to the guidelines

provided by the ATCC. For flow cytometry, cells (~75% confluent) are lifted from their culture flasks using cell dissociation buffer (Cat. #13151014 Life Technologies), counted, and plated in 96-well round bottom tissue culture plates at 0.5×10^6 cells per well.

Mouse Ig G1-PE (BD), Mouse anti-EGFR-PE (BD), Mouse anti-Her-2/neu-PE (BD), and

- 5 Mouse anti-Her-3/erbB3-PE (BD) all are used at $20 \mu\text{l}/0.5 \times 10^6$ cells. All mAbs are diluted in flow cytometry buffer (Dulbecco PBS w/2% FBS & 0.05% sodium azide & 10% NGS) and incubated for 45 minutes. The cells are centrifuged at 1500 rpm for 5 minutes at 4°C and washed three times with flow cytometry buffer. After final wash the cells are resuspended in flow cytometry buffer containing propidium iodide (PI,
- 10 Molecular Probes) to stain for dead cells. Samples are run on the LSR Fortessa acquiring with Diva software (both from Becton Dickinson) and analyzed with FloJo (version 7.6.3).

For fetal bovine serum (FBS)-mediated tumor cell proliferation experiments, NCI-N87 or BT-474 cell lines are seeded on 96-well plates at 1×10^3 cells per well and

- 15 precultured in RPMI-1640 medium containing 10% FBS overnight. To evaluate the impact of the IgG-(Fab)₂ compounds on cell proliferation, 100, 10, 1, and 0.1 nM solutions of each test compound (or combinations of 100, 10, 1, and 0.1 nM of each test compound) in RPMI-1640 medium containing 10% FBS are added to the cells.
- Alternately, 300, 30, 3, and 0.1 nM solutions of each test compound or combinations of
- 20 test compounds are evaluated in the same buffer solutions. After 5 days of treatment, cell viability is determined with a Cell Titer Glo reagent (Promega). The percentage of growth inhibition is calculated according to the formula $[1 - (\text{signal with mAb (or IgG-(Fab)}_2)) / (\text{signal with FBS only})] \times 100$.

To measure the levels of phosphorylated EGFR, HER-2, and HER-3 on the BT-

- 25 474 cell line the following protocol is used. BT-474(breast cancer) cell lines are seeded in 12-well culture plates at 2.5×10^5 cells per well and grown in RPMI-1640 medium containing 10% FBS overnight. The next day, cells are treated with 100 nM mAbs or IgG-(Fab)₂ for 24, 48 and 72 hours at 37°C in 10% FBS containing medium. Cell lysates are made using cell lysis buffer (MSD Cat. #R60TX-3), and protein concentrations are
- 30 measured using BCA protein assay (Pierce). Phospho-HER-2, Phospho-HER-3 and Phospho-EGFR in cell lysates are measured using a Phospho-HER-2, Phospho-HER-3 and Phospho-EGFR multiplex MSD kit (Meso Scale Discovery). Plates are loaded with

16 µg total protein in duplicate, and incubated for 2 hours at room temperature with shaking. Next, plates are washed and detection antibody is added and incubated for 2 hours at room temperature with shaking. Plates are read on a Sector Imager6000 (Meso Scale Discovery).

5

Table 10: Inhibition of BT474 breast and NCI-N87 gastric cancer cell lines by the IgG-(Fab)₂ compounds.

Construct	Inhibition of FBS-driven BT474 cell proliferation (100 nM mAb, IgG-(Fab) ₂)	Inhibition of FBS-driven N87 cell proliferation (100 nM mAb, IgG-(Fab) ₂)	Inhibition of FBS-driven BT474 cell proliferation (300 nM mAb, IgG-(Fab) ₂)	Inhibition of FBS-driven N87 cell proliferation (300 nM mAb, IgG-(Fab) ₂)
IgG1 control	8±7	9±8	-2±4	-10±4
Trastuzumab IgG1	48±1	34±1	44±11	28±11
Pertuzumab IgG1	41±2	46±7	52±2	46±5
Trastuzumab + Pertuzumab	55±1	62±4	59±3	60±5
tTCR_G1(-)	-5±2	-1±12	n.d.	n.d.
pTCR_G1(-)	-5±3	-6±11	n.d.	n.d.
tTCR_G1 + pTCR_G1	-4±5	35±3	n.d.	n.d.
C-tTCRpG1	-4±2	15±17	n.d.	n.d.
C-pG1tTCR	-13±7	10±16	n.d.	n.d.
N-tTCRpG1	-9±3	11±4	n.d.	n.d.
N-pG1tTCR	25±1	35±4	16±4	50±9
C-pTCRtG1	-10±11	-19±6	-37±2	-2±6
C-tG1pTCR	-10±5	-1±20	n.d.	n.d.
N-pTCRtG1	59±3	77±1	62±1	82±2
N-tG1pTCR	-8±5	-26±4	n.d.	n.d.
N-pG1tTCRds	n.d.	n.d.	33±3	64±0
N-pTCRtG1ds	n.d.	n.d.	64±2	80±1

“n.d.” denotes not determined.

As shown in **Table 10**: Antibody I (trastuzumab) and Antibody II (pertuzumab) individually inhibited FBS-mediated tumor cell growth of both the BT474 and NCI-N87

10

cell lines. In addition, the combination of Antibody I and II resulted in an increase in anti-proliferative activity. The individual IgG-TCR proteins, tTCR-G1(-) and pTCR-G1(-), and the combination of these proteins demonstrated significant decreases in anti-proliferative activity presumably due to the different Fab/hinge dynamics introduced using the subtly different connecting regions within the IgG-TCR format. **Table 10** also shows that the various IgG-(Fab)₂ compounds possess a spectrum of activities on HER-2-mediated tumor cell growth ranging from antagonistic to highly-antagonistic with the N-pTCRtG1 being even more inhibitory in the NCI-N87 cell line than the Antibody I and II combination.

Example 6- IgG-(Fab)₂ combining the specificities of the anti-HER-2 mAb pertuzumab and the anti-EGFR mAbs cetuximab and matuzumab.

To determine whether the IgG-(Fab)₂ of the present invention are able to bind to two different targets, IgG-(Fab)₂ compounds targeting EGFR and HER-2 are generated. Different constructs containing a cetuximab Fab and wild type pertuzumab (with IgG1 C_H1 domain and kappa C_L domain) as well as two additional constructs containing a matuzumab Fab and wild-type pertuzumab (with IgG1 C_H1 domain and kappa C_L domain), each in the IgG-TCR format are generated. Cetuximab is a chimeric mouse/human mAb directed against EGFR (HC of SEQ ID NO:60 and LC of SEQ ID NO:59), while matuzumab is a humanized anti-EGFR mAb (HC of SEQ ID NO:69 and LC of SEQ ID NO:68). The HCs and LCs that comprise the IgG-Fab are provided in **Table 11 and 12** as are the sequences of the antibody controls and an IgG-TCR control, mTCR-G1(-). “c” refers to Antibody III (cetuximab Fv), and “m” refers to Antibody IV (matuzumab Fv) containing Fab. The remainder of the nomenclature is consistent with **Example 4**.

Table 11: Composition of the anti-HER-2/anti-EGFR IgG-Fabs and relevant controls

Construct Name	Polypeptide Components SEQ ID NOs:	Format #
C-cTCRpG1	$\text{cVH-C}\alpha\text{-X1-(C}_{\text{H2}}\text{-C}_{\text{H3}}\text{)-X2-pVH-C}_{\text{H1}}$ ^a 58 - 2 - 6 - 15 13- 40 - 14 $\text{tVL-C}\beta$ $\text{pVL-C}\kappa$ 57 - 12 39 - 21	1
C-mTCRpG1	$\text{mVH-C}\alpha\text{-X1-(C}_{\text{H2}}\text{-C}_{\text{H3}}\text{)-X2-pVH-C}_{\text{H1}}$ ^b 67 14 6- 15- 13 40 - 14 $\text{mVL-C}\beta$ $\text{pVL-C}\kappa$ 66 - 12 39 - 21	1
N-cTCRpG1	$\text{cVH-C}\alpha\text{-X2-pVH-C}_{\text{H1}}\text{-(H-C}_{\text{H2}}\text{-C}_{\text{H3}}\text{)}$ ^c 58 - 2 -81 - 40 - 14 - 6- 15 $\text{cVL-C}\beta$ $\text{pVL-C}\kappa$ 57- 12 39 - 21	3
N-mTCRpG1	$\text{mVH-C}\alpha\text{-X2-pVH-C}_{\text{H1}}\text{-(H-C}_{\text{H2}}\text{-C}_{\text{H3}}\text{)}$ ^c 67 - 2 - 81 - 40 - 14 - 6- 15 $\text{mVL-C}\beta$ $\text{pVL-C}\kappa$ 66 - 12 39 - 21	3

Table 12: HC and LC sequence composition of the anti-HER-2/anti-EGFR IgG-5 **(Fab)₂ and controls**

Construct	HC SEQ ID NO:	LC SEQ ID NO:	IgG-TCR LC SEQ ID NO:
C-cTCRpG1	61	42	62
C-mTCRpG1	63	42	64
N-cTCRpG1	65	42	62
N-mTCRpG1	70	42	64
mTCR_G1(-)	77	-	64
Antibody III (cetuximab)	60	59	-
Antibody IV (matuzumab)	69	68	-

The construction, expression, purification, and biophysical characterization of the anti-HER-2/anti-EGFR IgG-(Fab)₂ compounds is performed as described in the **Example 4**. The four IgG-(Fab)₂ compounds designed to recognize both EGFR and HER-2 are expressed and purified (by protein G magnetic beads only – no secondary purification steps) at the 2 mL and 10 mL scales.

The proteins are characterized by SDS-PAGE and analytical SEC as in **Example 1**.

To determine whether the IgG-(Fab)₂ compounds are able to engage both targets simultaneously, an ELISA assay is run. Specifically, clear 96-well round bottom high binding microtiter plates (Greiner) are coated overnight at 2-8 °C with 50 µL/well 1 µg/mL hEGFR-Fc (Cat. #344-ER-050, R&D systems) in a 50 mM Na₂CO₃ pH 9.4 buffer. The plate is washed 4X times with PBST and blocked with 100 µL/well casein buffer (Pierce) for 1 hr at 37 °C. The plate is then washed 4X times with PBST and test compounds are added at 50 µL/well and 30 µg/mL (20 nM) and serially diluted 1:2 down the plate. The test compounds are incubated on the plate for 1 hr at 37 °C. The plate is washed 4X times with PBST and 50 µL/ well 0.2 µg/mL hHER-2-Histag (Sino Biologics) is added for 1 hr at 37 °C. The plate is then washed 4X times with PBST followed by the addition of a 50 µL/well secondary anti-Histag-HRP antibody (PENTA-His-HRP, Qiagen) diluted 1:1000 in PBST. The secondary antibody is incubated for 1 hr at 37 °C. The plate is then washed 4X times with PBST and 100 µL/well 1-component 3,3',5,5'-Tetramethylbenzidine (TMB) substrate is added (KPL laboratories). After approximately 10 minutes, 100 µL/well 1% H₃PO₄ (in H₂O) is added to quench the reaction. Absorbance (450 nm) of every well in the plate is read using a SpectraMax UV plate reader (Molecular Devices).

Table 13: Characterization of TCR-based IgG-Fabs that recognize EGFR and HER-2.

Construct	%monomer/ %aggregate	Molecular Weight (kDa)	EC ₅₀ (nM, EGFR/HER-2 sandwich ELISA)
C-cTCRpG1	94/6	268±10	1.9±1.5
C-mTCRpG1	86/14	257±10	<1.0
N-cTCRpG1	97/3	259±10	3.3±1.0

N-mTCRpG1	90/10	254±20	2.0±2.0
cetuximab IgG1	99.5/0.5	Not determined	No binding observed
mTCR-G1(-)	Not determined	Not determined	No binding observed

As shown in **Table 13**, the IgG-(Fab)₂ compounds have the correct molecular weight based on SDS-PAGE and analytical SEC and are primarily monodisperse with ~3-10% soluble aggregates. Also, all four IgG-(Fab)₂ compounds are capable of binding both EGFR and HER-2 based on positive signals in the sandwich ELISA and the measured potencies are listed in **Table 13**.

The results of the expression and biophysical characterization indicate the IgG-(Fab)₂ compounds not only express at their expected molecular weights, but display relatively ideal starting biophysical properties. The dual-specificity binding ELISA demonstrates the ability to bind both antigens for these IgG-Fab constructs.

SEQ ID NO:1 TCR α

PNIQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM
MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSP

5

SEQ ID NO:2 TCR α N-term,

IQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM
DFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSP

10 SEQ ID NO:3 TCR α N-terminal truncated IgG4 Fc region

IQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM
DFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVK

SEQ ID NO:4 TCR α N term, C-term truncation + IgG1 hinge seq

15 IQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM
DFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSC

SEQ ID NO:5 IgG4 hinge fragment

ESSCDVK

20

SEQ ID NO:6 IgG1 hinge fragment

EPKSC

SEQ ID NO:7 N-terminal truncated $-(K)$ TCR α

25 IQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM
DFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV

SEQ ID NO:8 N-terminal truncated $-(K)$ TCR α + GGG

30 IQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM
DFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVGGG

SEQ ID NO:9 N-terminal truncated TCR α + Linker

35 IQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM
DFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPGGGGSGGGGSGGGGSGGGG
SGGGG

SEQ ID NO:10 TCR β

EDLNKVFPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHS
 GVSTDPQLKEQPALNDSRYCLSSRLRVSATFWQNP RNHFRCQVQFYGLSENDE
 5 WTQDRAKPVTQIVSAEAWGRADCGFTS

SEQ ID NO:11 N-term truncated TCR β

KVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVST
 DPQLKEQPALNDSRYCLSSRLRVSATFWQNP RNHFRCQVQFYGLSENDEWTQD
 10 RAKPVTQIVSAEAWGRADCGFTS

SEQ ID NO:12 N-term, C-term truncation TCR β

KVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVST
 DPQLKEQPALNDSRYCLSSRLRVSATFWQNP RNHFRCQVQFYGLSENDEWTQD
 15 RAKPVTQIVSAEAWGRADC

SEQ ID NO:13 Linker

GGGGSGGGGSGGGGSGGGGS

20 SEQ ID NO:14 CH1

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
 VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV

SEQ ID NO:15 CH2-CH3

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
 QKSLSLSPGK

30

SEQ ID NO:16 CH1-CH2-CH3

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
 VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
 PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
 35 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
 KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
 GK

SEQ ID NO:17 (CH1-L)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
 VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVGGGGSGGGGSG
 5 GGGSGGGSGGGGS

SEQ ID NO:18 (CH2-CH3-L)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
 10 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
 QKSLSLSPGKGGGGSGGGGSGGGGSGGGGS

SEQ ID NO:19 (CH1CH2CH3-L)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
 VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
 PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGV
 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
 20 KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
 GKGGGGSGGGGSGGGGSGGGGS

SEQ ID NO:20 (A-X1-CH2CH3-X2)

IQNPDAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM
 25 DFKSNSAVAWSNKSDFAFANFNNSIIPEDTFFPSPEPKSCDKTHTCPPCPAPELL
 GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT
 KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
 DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGS
 30 GGGGSGGGGSGGGGS

SEQ ID NO:21 C κ region

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES
 VTEQDSKDSITYSLSSLTLSKADYEEKHKVYACEVTHQGLSPVTKSFNRGEC
 35

SEQ ID NO:22 C λ region

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVET
 TTPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC

SEQ ID NO:23: anti-IL-17 LC V_L/C β

DIVMTQTPLSLSVTPGQPASISCRSSRSLVHSRGNTYLHWYLQKPGQSPQLLIYKV
 SNRFIGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHLPFTFGQGTKLEIK
 5 EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHS
 GVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPРНHRCQVQFYGLSENDE
 WTQDRAKPVTQIVSAEAWGRADCGFTS

SEQ ID NO:24: anti-IL-17 LC V_L/C β _truncated

10 DIVMTQTPLSLSVTPGQPASISCRSSRSLVHSRGNTYLHWYLQKPGQSPQLLIYKV
 SNRFIGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHLPFTFGQGTKLEIK
 KVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVST
 DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPРНHRCQVQFYGLSENDEWTQD
 RAKPVTQIVSAEAWGRADCGFTS

15

SEQ ID NO:25: anti-IL-17 LC V_L/C α

DIVMTQTPLSLSVTPGQPASISCRSSRSLVHSRGNTYLHWYLQKPGQSPQLLIYKV
 SNRFIGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHLPFTFGQGTKLEIKP
NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRS
 20 MDFKSNSA VAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVK

SEQ ID NO:26: anti-IL-17 V_L/C α _truncated

DIVMTQTPLSLSVTPGQPASISCRSSRSLVHSRGNTYLHWYLQKPGQSPQLLIYKV
 SNRFIGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHLPFTFGQGTKLEIKI
 25 QNPDP AVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMD
 FKSNSA VAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVK

SEQ ID NO:27: anti-IL-17 HC V_H/C β /IgG4Fc

QVQLVQSGAEVKKPGSSVKVCKASGYSFTDYHIHWVRQAPGQGLEWMGVINP
 30 MYGTTDYNQRFKGRVTITADESTSTAYMELSSLRSED TAVYYCARYDYFTGTGV
 YWGQGT LTVSSEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVEL
 SWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPРНHRC

QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESKYGPPCPPCPAPE
 AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
 KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG
 QPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
 5 VLDS DGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLG

SEQ ID NO:28 anti-IL-17 HC V_H/C β truncated/IgG4Fc

QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYHHHWVRQAPGQGLEWMGVINP
 MYGTTDYNQRFKGRVTITADESTSTAYMELSSLRSED TAVYYCARYDYFTGTGV
 10 YWGQGT LTVSSKVPPEVA VFEPSEAEISHTQKATLVCLATGFFPDHVELSWW
 VNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRV SATFWQNPRNHFRCQVQF
 YGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESKYGPPCPPCPAPEAAGG
 PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
 REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP
 15 QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD S
 DGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLG

SEQ ID NO:29 anti-IL-17 HC V_H/C α /IgG4Fc

QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYHHHWVRQAPGQGLEWMGVINP
 20 MYGTTDYNQRFKGRVTITADESTSTAYMELSSLRSED TAVYYCARYDYFTGTGV
 YWGQGT LTVSSPNIQNPDAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSD
 VYITDKTVLDMRSMDFKSNSA VAWSNKSDFACANAFNNSIIPEDTFFPSPRESSCD
 VKESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPE
 VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS
 25 NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
 WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHN
 HYTQKSLSLGLG

SEQ ID NO:30 anti-IL-17 HC V_H/C α _truncated/IgG4Fc

QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYHHHWVRQAPGQGLEWMGVINP
 30 MYGTTDYNQRFKGRVTITADESTSTAYMELSSLRSED TAVYYCARYDYFTGTGV
 YWGQGT LTVSSIQNPDAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYI
 TDKTVLDMRSMDFKSNSA VAWSNKSDFACANAFNNSIIPEDTFFPSPRESSCDVKE
 SKY GPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
 35 NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
 LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES

NGQPENNYKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
QKSLSLSLG

SEQ ID NO:31 trastuzumab LCVR

5 DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFLTITSSLPEDFATYYCQQHYTTPPTFGQGTKVEIK

SEQ ID NO:32 trastuzumab HCVR

10 EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT
NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
MDYWGQGTLLTVSS

SEQ ID NO:33 Trastuzumab LC

15 DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFLTITSSLPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:34 trastuzumab HC

20 EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT
NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
MDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
25 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

30 SEQ ID NO:35 anti-HER-2 trastuzumab LC V_I/C β

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFLTITSSLPEDFATYYCQQHYTTPPTFGQGTKVEIKKVFP

EVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPL
KEQPALNDSRYCLSSRLRVSATFWQNP RNHFRCQVQFYGLSENDEWTQDRAKP
VTQIVSAEAWGRADCGFTS

5 SEQ ID NO:36 anti-HER-2 trastuzumab HC V_H/C α /IgG4Fc

EVQLVESGGGLVQPGGSLRLSCAASG FNIKDTYIHWRQAPGKGLEWVARIYPT
NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
MDYWGQGT LVTVSSIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSD
VYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCD
10 VKESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPE
VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHN
HYTQKSLSLSLG

15

SEQ ID NO:37 anti-HER-2 trastuzumab HC V_H/C α /IgG1hinge+Fc

EVQLVESGGGLVQPGGSLRLSCAASG FNIKDTYIHWRQAPGKGLEWVARIYPT
NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
MDYWGQGT LVTVSSIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSD
20 VYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYT
25 QKSLSLSPG

SEQ ID NO:38 anti-HER-2 trastuzumab LC V_L/C β with C-terminal truncation

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGRSGTDFLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKKVFP
30 EVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPL
KEQPALNDSRYCLSSRLRVSATFWQNP RNHFRCQVQFYGLSENDEWTQDRAKP
VTQIVSAEAWGRADC

SEQ ID NO:39 pertuzumab LCVR

DIQMTQSPSSLSASVGDRVITITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRY
TGVPSRFSGSGSGTDFLTISLQPEDFATYYCQQYYIYPYTFGQGGTKVEIK

5 SEQ ID NO:40 pertuzumab HCVR

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
YWGQGTLLTVSS

10 SEQ ID NO:41 pertuzumab LC

DIQMTQSPSSLSASVGDRVITITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRY
TGVPSRFSGSGSGTDFLTISLQPEDFATYYCQQYYIYPYTFGQGGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

15

SEQ ID NO:42 pertuzumab HC

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
YWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
20 GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL

25 HNHYTQKSLSLSPGK

SEQ ID NO:43 IgG_TCR BsAb HC denoted C-tTCRpG1 (C-terminal Fab fusion is wild-type pertuzumab Fab)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT
30 NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
MDYWGQGTLLTVSSIQNPDAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSD
VYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN

WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
 QKSLSLSPGKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAA
 5 SGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKN
 TLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGGQGLTVTVSSASTKGPSVFPLA
 PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
 VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGG

10 SEQ ID NO:44 IgG_TCR BsAb HC denoted C-pG1tTCR (C-terminal Fab fusion is
 IgG_TCR trastuzumab Fab)

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
 15 GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
 EPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP
 EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
 SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
 EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
 20 HNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSL
 RLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSIQNP
 DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKS
 NSAVAWSNKSDFACANAFNNSIIPEDTFFPSPSSCDVGGG

25

SEQ ID NO:45 anti-HER-2 pertuzumab LC V_L/C β with C-terminal truncation

DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRY
 TGVPSRFSGSGSGTDFLTISLQPEDFATYYCQQYYIYPYTFGQGTKVEIKKVFP
 EVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWVNGKEVHSGVSTDPQPL
 30 KEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKP
 VTQIVSAEAWGRADC

SEQ ID NO:46 anti-HER-2 pertuzumab HC V_H/C α /IgG1hinge+Fc

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
 35 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGLTVTVSSIQNPDPNAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYI

TDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSCDKT
 HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
 IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
 5 ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL
 SLSPG

SEQ ID NO:47 C-tTCRpG1 HC

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT
 10 NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
 MDYWGQGTLVTVSSIQNPDAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSD
 VYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSC
 DKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
 15 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYT
 QKSLSLSPGKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAA
 SGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKN
 TLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLA
 20 PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
 VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGG

SEQ ID NO:48 C-pG1tTCR HC

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
 25 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
 GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
 EPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
 EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
 30 SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
 EWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL
 HNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSL
 RLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA
 DTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSIQNP
 35 DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKS
 NSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVGGG

SEQ ID NO:49 N-tTCRpG1 HC

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGTLLTVSSIQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYI
 5 TDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPGGGGSGG
 GSGGGGGSGGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHW
 VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAED
 TAVYYCSRWGGDGFYAMDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTA
 ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT
 10 QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
 LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
 SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

15

SEQ ID NO:50 N-pG1tTCR HC

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
 20 GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
 GGGGSGGGGGSGGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNI
 KDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQ
 MNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVSSIQNPDPVYQLRDS
 KSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNK
 25 DFACANAFNNSIIPEDTFFPSPEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
 MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
 VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

30

SEQ ID NO:51 C-pTCRtG1 HC

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGTLLTVSSIQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYI
 35 TDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSCDKT
 HTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
 VDGEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP

IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
 ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFN
 IKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQ
 5 MNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVSSASTKGPSVFPLAPSS
 KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGG

SEQ ID NO:52 C-tG1pTCR HC

10 EVQLVESGGGLVQPGGSLRLSCAASGFNLIKDTYIHWVRQAPGKGLEWVARIYPT
 NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
 MDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
 KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH
 15 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
 CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCSCVMHE
 ALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGGGGSEVQLVESGGGLVQPGG
 SLRLSCAASGFTFTDYMMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFT
 20 LSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLLTVSSIQNP
 DPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKS
 NSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVGGG

SEQ ID NO:53 N-pTCRtG1 HC

25 EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYMMDWVRQAPGKGLEWVADVNP
 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGTLLTVSSIQNPDPNAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYI
 TDKTVLDMRSMDFKSNNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPGGGGSGG
 GGGGGGGSGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNLIKDTYIHW
 30 VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAED
 TAVYYCSRWGGDGFYAMDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTA
 ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
 QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
 LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
 35 SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVD
 KSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG

SEQ ID NO:54 N-tG1pTCR HC

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWRQAPGKGLEWVARIYPT
NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
5 MDYWGGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAAS
GFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKNT
LYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGGGTLVTVSSIQNPDPAVYQLR
10 DSKSSDKSVCLFTDFDSQTNVSQSKDSVYITDKTVLDMRSMDFKSNSAVAWSN
KSDFACANAFNNSIIPEDTFFPSPEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
15 DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:55 Trastuzumab V_L/TCR-β LC

20 DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFGSGRSGTDFTLTISSLQPEDFATYYCQQHYYTPPTFGQGTKVEIKKVFPP
EVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPL
KEQPALNDSRYCLSSRLRVSATFWQNPRNHFRQCQVQFYGLSENDEWTQDRAKP
VTQIVSAEAWGRADC

SEQ ID NO:56 Pertuzumab V_L/TCR-β LC

25 DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRY
TGVPSRFGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKKVFPP
EVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPL
KEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKP
VTQIVSAEAWGRADC

30

SEO ID NO:57 cetuximab LCVR

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQR TNGSPRLLIKYASESISGIP
SRFSGSGSGTDFTL SINSVESEDIADYYCQONNNWPTTFGAGTKLELK

SEQ ID NO:58 cetuximab HCVR

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
GNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAY
WGQGTLLTVSA

5

SEQ ID NO:59 Cetuximab LC

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNNGSPRLLIKYASESISGIP
SRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDS
10 KDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:60 Cetuximab HC

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
GNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAY
15 WGQGTLLTVSAASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
20 EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL
HNHYTQKSLSLSPG

SEQ ID NO:61 C-cTCRpG1 HC

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
25 GNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAY
WGQGTLLTVSAIQNPDAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYIT
DKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSCDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
30 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL
SPGKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLS CAASGFT
FTDYTMDWVRQAPGKGLEWVADVNPNSSGGSIYNQRFKGRFTLSVDRSKNTLYL
QMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLLTVSSASTKGPSVFPLAPSSK

STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGG

SEQ ID NO:62 Cetuximab VL/TCR- LC

5 DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRRTNGSPRLLIKYASESISGIP
SRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKLELKKVFPPEV
AVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKE
QPALNDSRYCLSSRLRVSATFWQNPRNHFRQCQVQFYGLSENDEWTQDRAKPVT
QIVSAEAWGRADC

10

SEQ ID NO:63 C-mTCRpG1 HC

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSHWMHWVRQAPGQGLEWIGEFN
PSNGRTNYNEKFKSKATMTVDTSTNTAYMELSSLRSEDVAVYYCASRDYDYDG
RYFDYWGGQGLTVTVSSIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKD
15 SDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY
20 TQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLS
ASGFTFTDYMMDWVRQAPGKLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSK
NTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGGQGLTVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGG

25

SEQ ID NO:64 matuzumab VL/TCR- LC

DIQMTQSPSSLSASVGDRVTITCSASSSVTYMYWYQQKPGKAPKLLIYDTSNLA
GVPSRFSGSGSGTDYFTISSLQPEDATYYCQQWSSHIFTFGQGTKVEIKKVPPE
VAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLK
30 EQPALNDSRYCLSSRLRVSATFWQNPRNHFRQCQVQFYGLSENDEWTQDRAKPVT
QIVSAEAWGRADC

SEQ ID NO:65 N-cTCRpG1ds HC

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
 GNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAY
 WGQGTLVTVSAIQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYIT
 5 DKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDGGGG
 SGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFTDYT
 MDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSL
 RAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT
 AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
 10 TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
 TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
 LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
 DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

15

SEQ ID NO:66 matuzumab LCVR

DIQMTQSPSSLSASVGDRVTITCSASSSVTYMYWYQQKPGKAPKLLIYDTSNLA
 GVPSRFSGSGSGTDYFTFISLQPEDATYYCQQWSSSHFTFGQGTKVEIK

20 SEQ ID NO:67 matuzumab HCVR

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSHWMHWVRQAPGQGLEWIGEFN
 PSNGRNTNYNEKFKSKATMTVDTSTNTAYMELSSLRSEDVAVYYCASRDYDYDG
 RYFDYWGQGTLVTVSS

25 SEQ ID NO:68 matuzumab LC

DIQMTQSPSSLSASVGDRVTITCSASSSVTYMYWYQQKPGKAPKLLIYDTSNLA
 GVPSRFSGSGSGTDYFTFISLQPEDATYYCQQWSSSHFTFGQGTKVEIKRTVAAP
 SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
 KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

30

SEQ ID NO:69 matuzumab HC

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSHWMHWVRQAPGQGLEWIGEFN
 PSNGRNTNYNEKFKSKATMTVDTSTNTAYMELSSLRSEDVAVYYCASRDYDYDG

RYFDYWGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
 SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
 DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVS
 HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
 5 KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
 DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMH
 EALHNHYTQKSLSLSPG

SEQ ID NO:70 N-mTCRpG1ds HC

10 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSHWMHWVRQAPGQGLEWIGEFN
 PSNGRTNYNEKFKSKATMTVDTSTNTAYMELSSLRSEDVAVYYCASRDYDYDG
 RYFDYWGGTLVTVSSIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKD
 SDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSC
 DGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGF
 15 TFDYTM DWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLY
 LQMNSLRAEDTAVYYCARNLGP SFYFDYWGGTLVTVSSASTKGPSVFPLAPSS
 KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF
 PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 20 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:71 N-pTCRtG1ds HC

25 EVQLVESGGGLVQPGGSLRLSCAASGFTFDYTM DWVRQAPGKGLEWVADVNP
 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGP SFYFD
 YWGQGLTVTVSSIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYI
 TDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDGGG
 GSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT
 30 YIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSL
 RAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTS
 GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
 SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK
 KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 35 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
 DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
 TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:72 N-tG1pTCRds HC

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT
 NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
 MDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 5 WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
 KKVEPKSCDGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLR
 LSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSV
 DRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLLTVSSIQNPDP
 10 VYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSA
 VAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSCDKTHTCPPCPAPELLGGPSVFLF
 PPKPDKTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

15

SEQ ID NO:73 N-tTCRpG1ds HC

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT
 NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
 MDYWGQGTLLTVSSIQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDS
 20 VYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCD
 GGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFT
 FTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYL
 QMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLLTVSSASTKGPSVFPLAPSSK
 STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
 25 PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDKTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

30

SEQ ID NO:74 N-pG1tTCRds HC

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
 35 GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
 EPKSCDGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSC
 AASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSK

NTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTTLTVSSIQNPDP
 YQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSVDYITDKTVLDMRSMDFKSNSA
 AWSNKSDFACANAFNNSIIPEDTFFPSPEPKSCDKTHTCPPCPAPELLGGPSVFLP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
 5 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
 SKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:75 N-pTCRtG1ds HC

10 EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNP
 NSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFD
 YWGQGTTLTVSSIQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSVDYI
 TDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDGGG
 GSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT
 15 YIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSL
 RAEDTAVYYCSRWGGDGFYAMDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTS
 GGTAAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
 SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK
 KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 20 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
 DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
 TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:76 N-tG1pTCRds HC

25 EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT
 NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYA
 MDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTW
 WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
 KKVEPKSCDGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLR
 30 LSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSV
 DRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTTLTVSSIQNPDP
 VYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSVDYITDKTVLDMRSMDFKSNSA
 VAWSNKSDFACANAFNNSIIPEDTFFPSPEPKSCDKTHTCPPCPAPELLGGPSVFL
 PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 35 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:77 mTCR_G1(-) HC (IgG-TCR)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSHWMHWVRQAPGQGLEWIGEFN
 PSNGRTNYNEKFKSKATMTVDTSTNTAYMELSSLRSEDVAVYYCASRDYDYDG
 5 RYFDYWGQGTLLTVSSIQNPDAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKD
 SDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPEPKS
 CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
 NWWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
 LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
 10 NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
 TQKSLSLSPG

SEQ ID NO:78 IL-17 IgG4 antibody HC

QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYHHHWVRQAPGQGLEWMGVINP
 15 MYGTTDYNQRFKGRVTITADESTSTAYMELSSLRSEDVAVYYCARYDYFTGTGV
 YWGQGTLLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
 GALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTKTYTCNVDHKPSNTKVDKRV
 ESKYGPPCPCPCAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
 FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
 20 GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
 SNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
 TQKSLSLSLG

SEQ ID NO:79 IL-17 kappa LC

DIVMTQTPLSLSVTPGQPASISCRSSRSLVHSRGNTYLHWYLQKPGQSPQLLIYKV
 25 SNRFIGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHLPFTFGQGTKLEIK
 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES
 VTEQDSKDSSTLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

30 SEQ ID NO:80 hIL-17 with C-terminal FlagHis tag

GITIPRNPGPCNSEDKNFPRTVMVNLNIHNRNTNTNPKRSSDYYNRSTSPWNLHR
 NEDPERYPSVIWEAKCRHLGCINADGNVDYHMNSVPIQQEILVLRREPPHPCNSF
 RLEKILVSVGCTCVTPIVHHVAAFIDYKDDDDKHVHHHHHHH

SEQ ID NO:81 Hinge-Linker

ESSCDGGGGSGGGSGGGSGGGGS

SEQ ID NO:82 Hinge-Linker

5 EPKSCDGGGGSGGGSGGGSGGGGS

We claim:

1. A compound comprising a first polypeptide chain, a second polypeptide chain, and a third polypeptide chain, in which
 - 5 a. the first polypeptide chain has the formula $VL_1-C\beta$, wherein
 - i. VL_1 is a first antibody light chain variable region, and
 - ii. $C\beta$ is a T-Cell Receptor β constant domain ($C\beta$); and
 - b. the second polypeptide has the formula VL_2-CL , wherein
 - 10 i. VL_2 is a second antibody light chain variable region, and
 - ii. CL is a light chain κ or λ constant domain; and
 - c. the third polypeptide chain has a formula selected from the group consisting of

$$(VH_1-C\alpha)-X_1-(C_{H2}-C_{H3})-X_2-(VH_2-C_{H1}),$$

$$(VH_2-C_{H1})-(H-C_{H2}-C_{H3})-X_2-(V_{H1}-C\alpha),$$

$$(VH_1-C\alpha)-X_2-(VH_2-C_{H1})-(H-C_{H2}-C_{H3}), \text{ and}$$

$$(VH_2-C_{H1})-X_2-(VH_1-C\alpha)-X_1-(C_{H2}-C_{H3}),$$
 wherein

VH_1 is a first antibody heavy chain variable region,
 $C\alpha$ is a T-Cell Receptor α constant domain ($C\alpha$),
 X_1 is a peptide linker or is absent,
 $C_{H2}-C_{H3}$ are heavy chain constant domains 2 and 3,
 H is a hinge region,
 X_2 is a peptide linker or is absent,
 VH_2 is a second antibody heavy chain variable region and
 C_{H1} is heavy chain constant domain 1; and

in which the first and second polypeptide chains independently associate with the third polypeptide chain to form a first antigen binding site (VH_1-VL_1) and a second antigen binding site (VH_2-VL_2).

2. The compound of claim 1, wherein C α is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:7.
- 5 3. The compound of claim 1 or 2, wherein C β is selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.
4. The compound of any one of claims 1-3, wherein C α is SEQ ID NO:2.
- 10 5. The compound of any one of claims 1-4, wherein C β is SEQ ID NO:11.
6. The compound of any one of claims 1-4, wherein C β is SEQ ID NO:12.
7. The compound of any one of claims 1-6, wherein C_{H2}-C_{H3} is SEQ ID NO:15,
15 C_{H1} is SEQ ID NO:14, X₂ is SEQ ID NO:13, and X₁ is SEQ ID NO:5 or SEQ ID NO:6.
8. The compound of any one of claims 1-7, in which X₁ is SEQ ID NO:6.
- 20 9. The compound of any one of claims 1-8, wherein CL is SEQ ID NO:21.
10. The compound of any one of claims 1-9, comprising two of each of the first, second, third polypeptides, in which a first polypeptide chain and a second polypeptide chain independently associate with one of the third polypeptide chains
25 to form a first antigen binding site and a second antigen binding site, and the other first polypeptide chain and the other second polypeptide chain associate with the other third polypeptide chain to form another first antigen binding site and another second antigen binding site.
- 30 11. A DNA molecule comprising a polynucleotide sequence encoding a polypeptide chain having a formula selected from the group consisting of

$$(VH_1-C\alpha)-X_2-(VH_2-C_{H1})-(C_{H2}-C_{H3}),$$

$(VH_1-C\alpha)-X_1-(CH_2-CH_3)-X_2-(VH_2-CH_1)$,
 $(VH_2-CH_1)-(CH_2-CH_3)-X_2-(VH_1-C\alpha)$, and
 $(VH_2-CH_1)-X_2-(VH_1-C\alpha)-X_1-(CH_2-CH_3)$,

in which

- 5 VH_1 is a first antibody heavy chain variable region,
 $C\alpha$ is a T-Cell Receptor α constant domain ($C\alpha$),
 X_1 is a peptide linker or is absent,
 CH_2-CH_3 are heavy chain constant domains 2 and 3,
 X_2 is a peptide linker or is absent,
 10 VH_2 is a second antibody heavy chain variable region and
 CH_1 is a heavy chain constant domain 1.

12. A mammalian cell comprising DNA encoding three polypeptide chains,

- a. wherein a first polypeptide chain has the formula $VL_1-C\beta$, wherein
 15 VL_1 is a first antibody light chain variable region, and
 $C\beta$ is a T-Cell Receptor β constant domain ($C\beta$);
 b. the second polypeptide chain has the formula VL_2-CL , wherein
 VL_2 is a second antibody light chain variable region, and
 CL is a light chain κ or λ constant domain; and
 20 c. the third polypeptide chain has a formula selected from the group
 consisting of

$(VH_1-C\alpha)-X_1-(CH_2-CH_3)-X_2-(VH_2-CH_1)$,
 $(VH_2-CH_1)-(H-CH_2-CH_3)-X_2-(VH_1-C\alpha)$,
 $(VH_1-C\alpha)-X_2-(VH_2-CH_1)-(H-CH_2-CH_3)$, and
 25 $(VH_2-CH_1)-X_2-(VH_1-C\alpha)-X_1-(CH_2-CH_3)$,

wherein

VH_1 is a first antibody heavy chain variable region,
 $C\alpha$ is a T-Cell Receptor α constant domain ($C\alpha$),
 X_1 is a peptide linker or is absent,

C_{H2}–C_{H3} are heavy chain constant domains 2 and 3,

H is a hinge region between C_{H1} and C_{H2},

X₂ is a peptide linker or is absent,

VH₂ is a second antibody heavy chain variable region and

5 C_{H1} is a heavy chain constant domain 1.

13. A process for producing the compound of any one of Claims 1-10, comprising cultivating the cell of Claim 12 under conditions such that the compound is expressed and recovering the compound.

10 14. A compound produced by the process of Claim 13.

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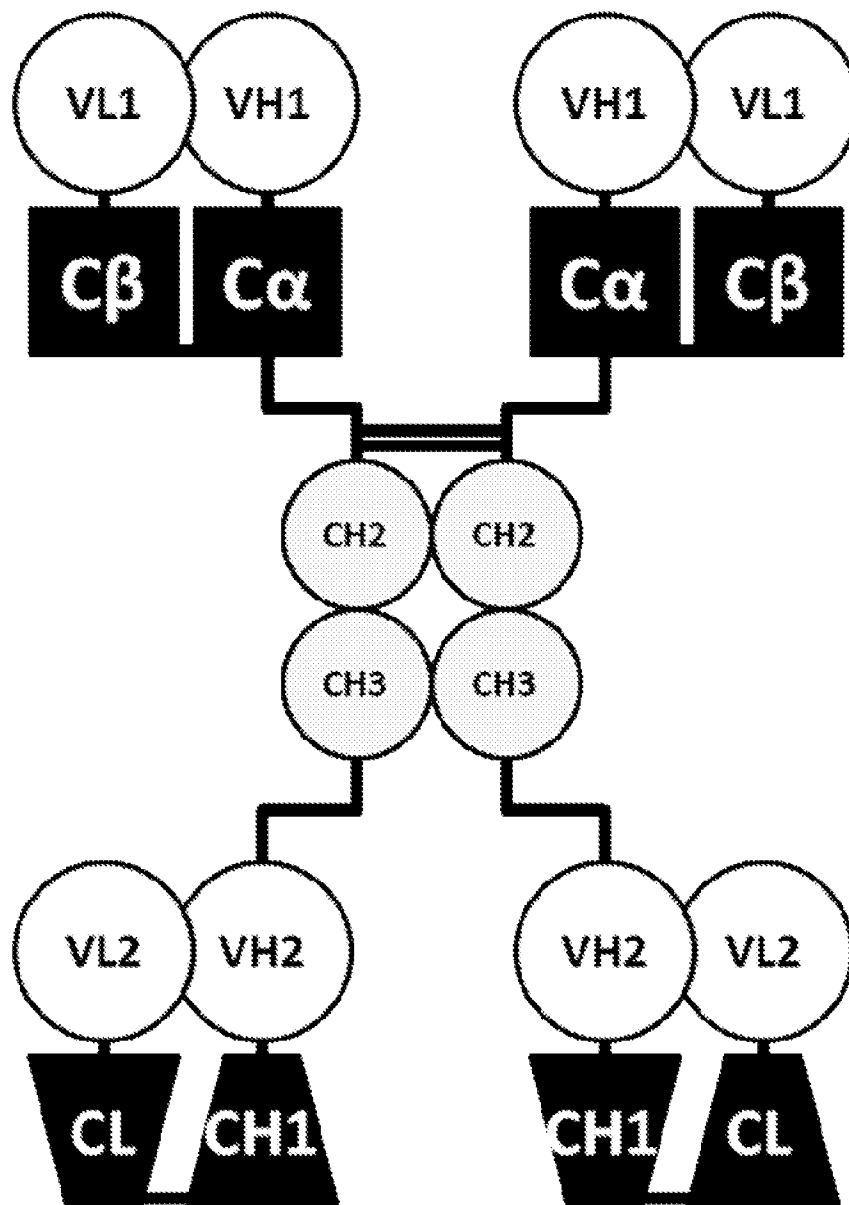
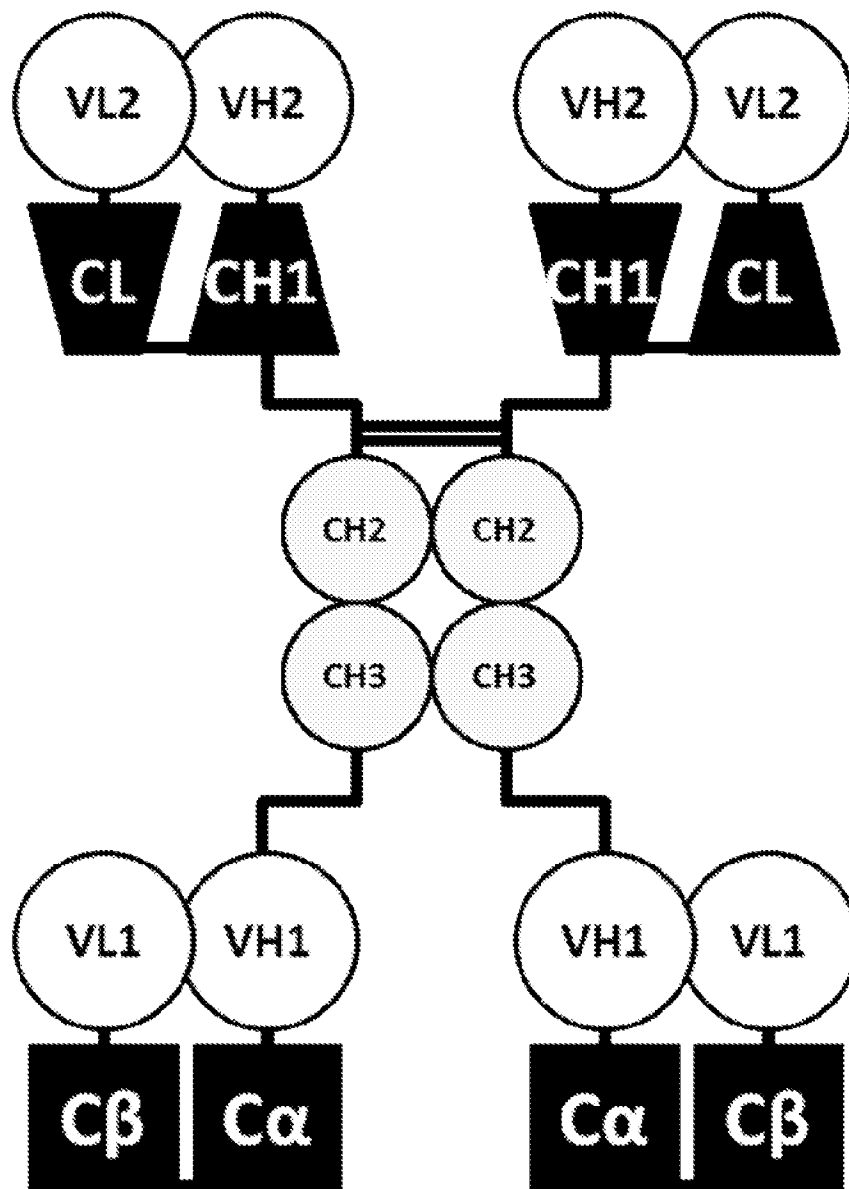


Fig. 1

-2/4 -



-3/4 -

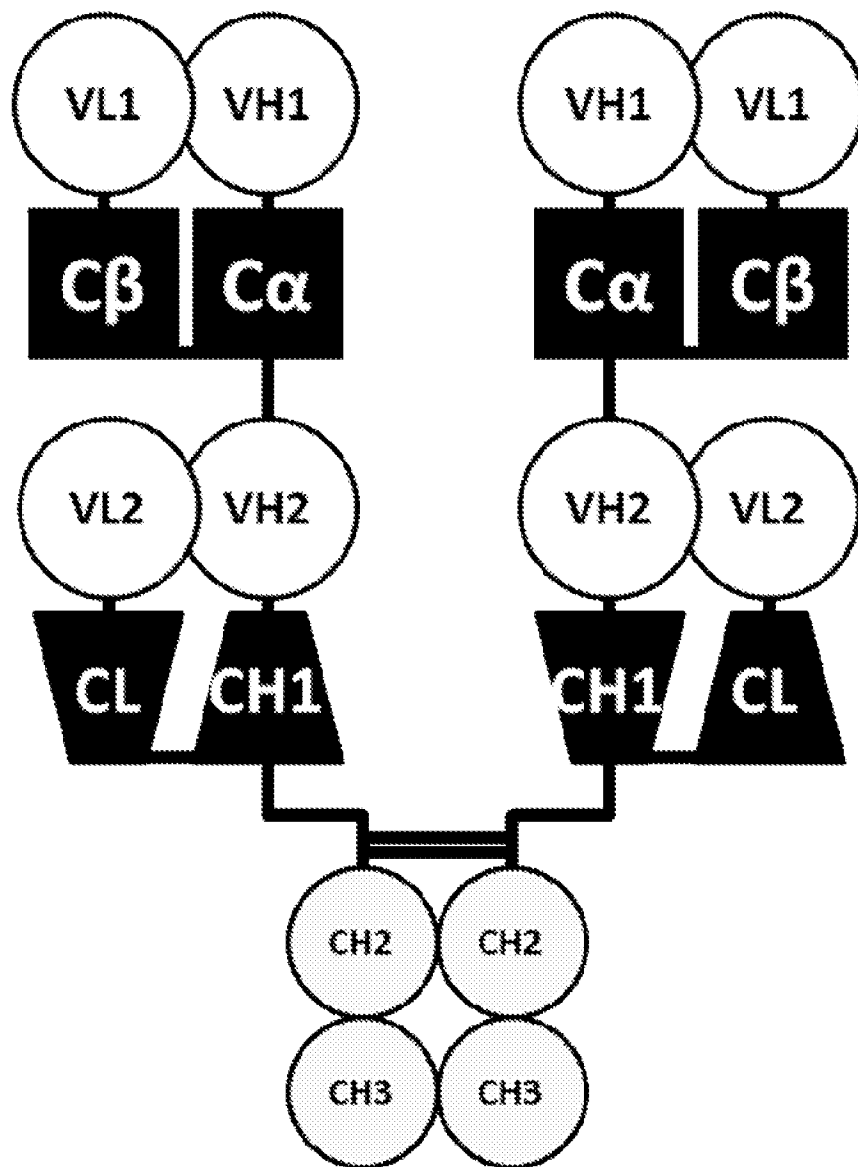
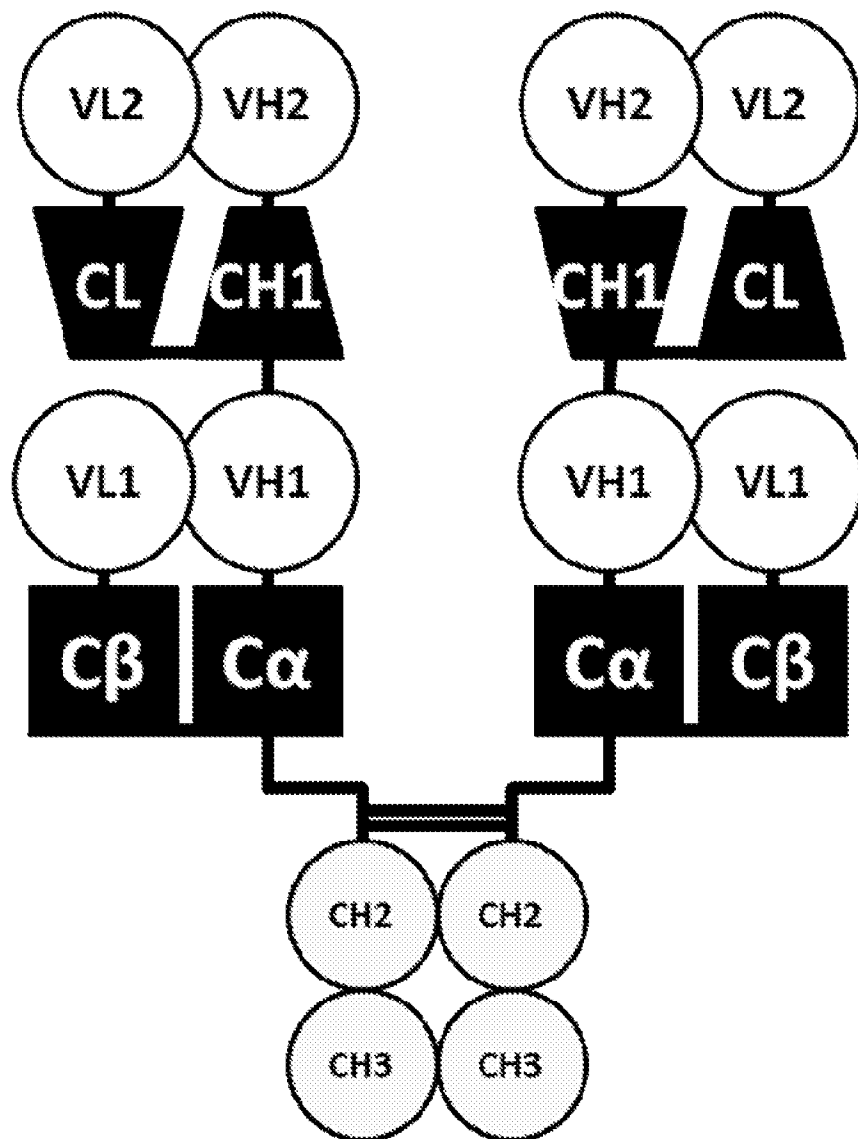


Fig. 3

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/050436

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/24 C07K16/28 C07K16/32 C07K14/725 C07K16/46
C07K19/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SEIMIYA H ET AL: "T cell receptor-extracellular constant regions as hetero-cross-linkers for immunoglobulin variable regions.", JOURNAL OF BIOCHEMISTRY JUN 1993, vol. 113, no. 6, June 1993 (1993-06), pages 687-691, XP008165120, ISSN: 0021-924X page 690, right-hand column, last paragraph - page 691, left-hand column, paragraph 2	1-14
Y	US 6 534 633 B1 (WEIDANZ JON A [US] ET AL) 18 March 2003 (2003-03-18) figure 7a; examples 1-3 ----- -/-	1-14



Further documents are listed in the continuation of Box C.



See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

9 October 2013

Date of mailing of the international search report

17/10/2013

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Le Flao, Katell

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/050436

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KUWANA Y ET AL: "Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions", 31 December 1987 (1987-12-31), BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, PAGE(S) 960 - 968, XP024836482, ISSN: 0006-291X [retrieved on 1987-12-31] abstract	1-14
A	----- ZUO Z ET AL: "An efficient route to the production of an IgG-like bispecific antibody.", PROTEIN ENGINEERING MAY 2000, vol. 13, no. 5, May 2000 (2000-05), pages 361-367, XP002206534, ISSN: 0269-2139 page 361, left-hand column - right-hand column; figures 1,2 page 365, right-hand column, last paragraph - page 366, left-hand column, paragraph 1	1-14
A	----- ATWELL J L ET AL: "DESIGN AND EXPRESSION OF A STABLE BISPECIFIC SCFV DIMER WITH AFFINITY FOR BOTH GLYCOPHORIN AND N9 NEURAMINIDASE", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 33, no. 17/18, 1 January 1997 (1997-01-01), pages 1301-1312, XP009047682, ISSN: 0161-5890, DOI: 10.1016/S0161-5890(96)00097-1 page 1303, left-hand column, last paragraph - right-hand column	1-14
A	----- DÜBEL S ET AL: "Bifunctional and multimeric complexes of streptavidin fused to single chain antibodies (scFv)", JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 178, no. 2, 1 January 1995 (1995-01-01), pages 201-209, XP008135095, ISSN: 0022-1759, DOI: 10.1016/0022-1759(94)00257-W page 202, left-hand column, paragraph 2; figure 1C	1-14
A	----- WO 91/03493 A1 (UNIV SOUTHAMPTON [GB]) 21 March 1991 (1991-03-21) examples 1,2 ----- -/--	1-14

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/050436

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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