Muteins of 4-1BB ligand extracellular domain, fusion proteins comprising the same and uses thereof

Field of the invention

The present invention relates to the field of medicine, in particular to the fields of oncology, immunology, inflammation and immunotherapy of tumors. Specifically, the invention relates to muteins of the 4-1BB ligand (4-1BBL) extracellular domain (ECD), preferably 4-1BBL ECD muteins having an altered affinity, relative to the affinity of wild-type 4-1BBL for 4-1BB. The invention further relates to fusion proteins comprising such 4-1BBL ECD muteins and the use of such 4-1BBL ECD muteins and fusion proteins in medical treatments of e.g. cancer.

Background of the invention

What today we consider a medical milestone in immune oncology was the application of redirecting a patient's immune system, predominantly through manipulating alpha (α) beta (β) T cells to overcome tolerance to consequently attack tumor cells. This can be achieved by multiple means such as (i) antibodies directing such T cell responses called engagers as well as antibodies that overcome various ways of immune suppression by blocking immune checkpoint interactions. (ii) Alternatively, cellular immunotherapy has proven highly effective against certain types of leukemias using chimeric antigen receptors (CAR), genetically introduced into a patient's own T cells which are subsequently grown in commercial manufacturing settings into large numbers and administrated intra venous to an autologous receiver.

Despite initial successes using both technologies, a myriad of problems remain to be overcome. For example, checkpoint inhibitors as well as engagers induce a high occurrence rate of severe toxicities, additionally, predictions on why some but not all patients respond to specific antibodies cannot yet be made. Cellular therapies are also facing struggles on multiple levels, poor manufacturability due to the source of cells coming from very ill patients, lengthy and very costly manufacturing per se and the lack of predictive strategies to know which patients will benefit from the expensive therapy. Finally, cellular immunotherapy is yet limited to a short list of malignant indications.

Advancements in the field to overcome these adversities focus on enhancing existing technologies to modulate αβ T-cells, but increasingly, efforts are expanded to include members of the innate immune system which are capable of orchestrating complete and natural immune responses, involving cells and mechanisms that go beyond the direct mode of action of a therapeutic. Furthermore, innate lymphocytes are often Major Histocompatibility Complex (MHC) un-restricted, allowing for their potential allogeneic use in multiple recipients without causing graft versus host disease (GvHD). Both, antibody and adoptive cell therapies targeting innate cells also show a much lower prevalence for therapy associated toxicities such as cytokine release syndrome (CRS) and neurotoxicity. Candidates for such therapeutics are Natural Killer (NK) cells, induced NK (iNK) cells, macrophages and gamma (γ) delta (δ) T cells.

Strategies based on the recruitment of cytotoxic NK cells are currently being developed. One such strategy employs multifunctional antibodies called natural killer cell engagers (NKCEs) have been developed, which simultaneously target tumor-associated antigens (TAAs), and activate receptors on endogenous NK cells. A number of NKCEs that are currently in development for clinical application is reviewed by Demaria et al. (Eur. J. Immunol. 2021. 51: 1934–1942). NKCEs are designed to strengthen the interaction between the NK cell and targeted tumor cell and to increase NK cell effector functions towards the tumor cell. However, NK cells in tumors are low in number and of poor functionality (have an exhausted phenotype). NKCE's developed thus far only address interaction of NK cells with tumor cells and in most cases do not improve their functionality, and in none of the cases improves the NK cell numbers in the tumor.

Also γδ T cells, predominantly Vδ2 T-cells have been tested as cellular immunotherapies. Despite a great safety profile, induction of growth and/or targeting/redirection in-situ via the Vδ2 TCR with antibodies or drugs has been shown to lead to early exhaustion. Additionally, their canonical role in immunology of predominantly responding to mycobacterial infections makes them poor cellular therapies in the absence of engineered stimuli.

More recently, WO2024/056862 and WO2024/056861 disclose such multifunctional antigen-binding proteins comprising as NK cell-activating cytokine a 4-1BB agonist, such as 4-1BB ligand (4-1BBL). Co-pending applications EP 24208721.1 and EP24208726.0 disclose multispecific antigen binding proteins, which bind to a tumor-associated antigen of interest and/or to a γδ T cell receptor, and which comprise a γδ T cell-activating agonist, such IL-21, and optionally a γδ T cell co-stimulatory agonist such as 4-1BBL. The multifunctional antigen-binding proteins in these applications do address the number, functionality and location of the NK cells and/or γδ T cells. However, the specificity of the 4-BB agonist in these proteins is not yet specific enough for function in the tumor only, potentially causing side effects outside the tumor, e.g. in the periphery.

4-1BB is a member of the TNFR superfamily expressed on dendritic cells, monocytes, macrophages, B cells, T cells and NK cells. Its activation by 4-1BBL on CD8 T cells and NK cells results in increased proliferation, cytokine production, survival, and anti-tumor cytotoxicity. 4-1BB activation can also have a profound impact on myeloid cell activation, the humoral immune system and CD4 T cells. 4-1BB stimulation therefore has been an attractive target for therapy and achieved objective clinical responses in cancer patients. However, the development of 4-1BB- or 4-1BBL-based therapies has been complex due to its broad expression on immune cells in the periphery. In particular, the development of 4-1BB agonists has been hampered by severe and at times fatal hepatotoxicity in patients due to 4-1BB-mediated activation of liver monocyte/macrophages and subsequent T cell infiltration and activation (Segal et al. Clin Cancer Res. 2017;23(8):1929-1936; Bartkowiak et al. Clin Cancer Res. 2018;24(5):1138-1151). Accordingly, care must be taken to avoid broad 4-1BB-activation in leukocytes and the potential toxicity thereof. The therapeutic application of 4-1BBL signaling must therefore be balanced and targeted, such that the effects triggered by 4-1BBL are designed to occur at the right time and place in the body.

There is therefore a need in the art for 4-1BBL treatment modalities that address these issues. It is thus an object of the present invention to provide for such 4-1BBL treatment modalities.

Description of the invention

Definitions

Various terms relating to the methods, compositions, uses and other aspects of the present invention are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art to which the invention pertains, unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definition provided herein. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein.

"A," "an," and "the": these singular form terms include plural referents unless the content clearly dictates otherwise. The indefinite article "a" or "an" thus usually means "at least one". Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like.

"About" and "approximately": these terms, when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods. Additionally, amounts, ratios, and other numerical values are sometimes presented herein in a range format. It is to be understood that such range format is used for convenience and brevity and should be understood flexibly to include numerical values explicitly specified as limits of a range, but also to include all individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly specified. For example, a ratio in the range of about 1 to about 200 should be understood to include the explicitly recited limits of about 1 and about 200, but also to include individual ratios such as about 2, about 3, and about 4, and sub-ranges such as about 10 to about 50, about 20 to about 100, and so forth.

"And/or": The term "and/or" refers to a situation wherein one or more of the stated cases may occur, alone or in combination with at least one of the stated cases, up to with all of the stated cases.

"Comprising": this term is construed as being inclusive and open ended, and not exclusive. Specifically, the term and variations thereof mean the specified features, steps or components are included. These terms are not to be interpreted to exclude the presence of other features, steps or components.

"Exemplary": this term means "serving as an example, instance, or illustration," and should not be construed as excluding other configurations disclosed herein.

As used herein "cancer'' and "cancerous", refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Cancer is also referred to as malignant neoplasm.

As used herein, "in combination with" is intended to refer to all forms of administration that provide a first drug together with a further (second, third) drug. The drugs may be administered

simultaneous, separate or sequential and in any order. Drugs administered in combination have biological activity in the subject to which the drugs are delivered.

As used herein “simultaneous” administration refers to administration of more than one drug at the same time, but not necessarily via the same route of administration or in the form of one combined formulation. For example, one drug may be provided orally whereas the other drug may be provided intravenously during a patient’s visit to a hospital. “Separate” includes the administration of the drugs in separate form and/or at separate moments in time, but again, not necessarily via the same route of administration. “Sequential(ly)” indicates that the administration of a first drug is followed, immediately or in time, by the administration of the second drug.

A used herein "compositions", "products" or "combinations" useful in the methods of the present disclosure include those suitable for various routes of administration, including, but not limited to, intravenous, subcutaneous, intradermal, subdermal, intranodal, intratumoral, intramuscular, intraperitoneal, oral, nasal, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral or mucosal application. The compositions, formulations, and products according to the disclosure invention normally comprise the drugs (alone or in combination) and one or more suitable pharmaceutically acceptable excipients.

As used herein, “an effective amount” is meant the amount of an agent required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active agent(s) used to practice the present invention for therapeutic treatment of a cancer varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an “effective” amount. Thus, in connection with the administration of a drug which, in the context of the current disclosure, is “effective against” a disease or condition indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as an improvement of symptoms, a cure, a reduction in at least one disease sign or symptom, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating the particular type of disease or condition.

“Sequence identity” is herein defined as a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. In the art, “identity” also means the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences. “Similarity” between two amino acid sequences is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. “Identity” and “similarity” can be readily calculated by known methods. The terms “sequence identity” or “sequence similarity” means that two (poly)peptide or two nucleotide sequences, when optimally aligned, preferably over the entire length (of at least the shortest sequence in the comparison) and maximizing the number of matches and minimizes the number of gaps such as by the programs ClustalW (1.83), GAP or BESTFIT using default parameters, share at least a certain percentage of sequence identity as defined

elsewhere herein. GAP uses the Needleman and Wunsch global alignment algorithm to align two sequences over their entire length, maximizing the number of matches and minimizes the number of gaps. Generally, the GAP default parameters are used, with a gap creation penalty = 50 (nucleotides) / 8 (proteins) and gap extension penalty = 3 (nucleotides) / 2 (proteins). For nucleotides the default scoring matrix used is nwsgapdna and for proteins the default scoring matrix is BLOSUM62 (Henikoff & Henikoff, 1992, PNAS 89, 915-919). A preferred multiple alignment program for aligning protein sequences of the invention is ClustalW (1.83) using a BLOSUM matrix and default settings (Gap opening penalty:10; Gap extension penalty: 0.05). Sequence alignments and scores for percentage sequence identity may be determined using computer programs, such as the GCG Wisconsin Package, Version 10.3, available from Accelrys Inc., 9685 Scranton Road, San Diego, CA 92121-3752 USA, or using open source software, such as the program “needle” (using the global Needleman Wunsch algorithm) or “water” (using the local Smith Waterman algorithm) in EmbossWIN version 2.10.0, using the same parameters as for GAP above, or using the default settings (both for ‘needle’ and for ‘water’ and both for protein and for DNA alignments, the default Gap opening penalty is 10.0 and the default gap extension penalty is 0.5; default scoring matrices are BLOSUM62 for proteins and DNAFull for DNA). When sequences have a substantially different overall lengths, local alignments, such as those using the Smith Waterman algorithm, are preferred. Alternatively, percentage similarity or identity may be determined by searching against public databases, using algorithms such as FASTA, BLAST, etc.

Optionally, in determining the degree of amino acid similarity, the skilled person may also take into account so-called “conservative” amino acid substitutions, as will be clear to the skilled person. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. Examples of classes of amino acid residues for conservative substitutions are given in the Tables below.

Acidic Residues	Asp (D) and Glu (E)
Basic Residues	Lys (K), Arg (R), and His (H)
Hydrophilic Uncharged Residues	Ser (S), Thr (T), Asn (N), and Gln (Q)
Aliphatic Uncharged Residues	Gly (G), Ala (A), Val (V), Leu (L), and Ile (I)
Non-polar Uncharged Residues	Cys (C), Met (M), and Pro (P)
Aromatic Residues	Phe (F), Tyr (Y), and Trp (W)

Alternative conservative amino acid residue substitution classes.

1	A	S	T
2	D	E	
3	N	Q	
4	R	K	
5	I	L	M

6	F	Y	W

Alternative physical and functional classifications of amino acid residues.

Alcohol group-containing residues	S and T
Aliphatic residues	I, L, V, and M
Cycloalkenyl-associated residues	F, H, W, and Y
Hydrophobic residues	A, C, F, H, I, L, M, R, T, V, W, and Y
Negatively charged residues	D and E
Polar residues	C, D, E, H, K, N, Q, R, S, and T
Positively charged residues	H, K, and R
Small residues	A, C, D, G, N, P, S, T, and V
Very small residues	A, G, and S
Residues involved in turn formation	A, C, D, E, G, H, K, N, Q, R, S, P and T
Flexible residues	Q, T, K, S, G, P, D, E, and R

The term "agent" refers generally to any entity which is normally not present or not present at the levels being administered to a cell, tissue or subject. An agent can be a compound or a composition. An agent can e.g. be selected from the group consisting of: polynucleotides, polypeptides, small molecules, (multispecific) antigen binding proteins, such as antibodies and functional fragments thereof.

The term "antigen-binding domain" or "antigen-binding region" refers to the portion of an antigen-binding protein that is capable of specifically binding to an antigen or epitope. In one embodiment, the antigen-binding region is an immunoglobulin-derived antigen-binding region, e.g. comprising both an antibody light chain variable region (V_L) and an antibody heavy chain variable region (V_H). Examples of such antigen-binding regions include single-chain Fv (scFv), single-chain antibody, Fv, single-chain Fv2 (scFv2), Fab, and Fab'. In one embodiment, the antigen-binding region is an immunoglobulin-derived antigen-binding region from a single domain antibody consisting only of heavy chains and devoid of light chains as are known e.g. from camelids, wherein the antigen-binding site is present on, and formed by, the single variable domain (also referred to as an "immunoglobulin single variable domain" or "ISVD"). Examples of such ISVDs include the single variable domains of camelid heavy chain antibodies (V_HHs), also referred to as nanobodies, domain antibodies (dAbs), and single domains derived from shark antibodies (IgNAR domains). In other embodiments, an antigen-binding region comprises a non-immunoglobulin-derived domain capable of specifically binding to an antigen or epitope, such as DARPpins; Affilins; anticalins, etc.

The term "antibody" herein is used in the broadest sense and specifically includes full-length monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies), and antibody fragments and derivatives, so long as they exhibit the desired biological and/or immunological activity. Various techniques relevant to the production of antibodies are provided in, e.g., Harlow, et al.. Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold

Spring Harbor, N.Y., (1988). An antibody can be human and/or humanized. "Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody.

The terms "full length antibody", "intact antibody", and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure. "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG-class antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a light chain constant domain (CL), also called a light chain constant region. The heavy chain of an antibody may be assigned to one of five types, called α (IgA), δ (IgD), ε (IgE), γ (IgG), or m (IgM), some of which may be further divided into subtypes, e.g. γ1 (IgG1), γ2 (IgG2), γ3 (IgG3), γ4 (IgG4), α1 (IgA1) and α2 (IgA2). The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

An "antibody fragment" comprises a portion of a full-length antibody, e.g. the antigen-binding or variable regions thereof. Examples of antibody fragments include Fab, Fab', F(ab)2, F(ab')2, F(ab)s, Fv (typically the V_H and V_L domains of a single arm of an antibody), single-chain Fv (scFv), dsFv, Fd fragments (typically the V_H and CH1 domain), and dAb (typically a V_H domain) fragments; V_H, V_L, V_HH, and V-NAR domains; minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g.. Ill et al.. *Protein Eng* 1997;10: 949-57); camel IgG; IgNAR; and multispecific antibody fragments formed from antibody fragments, and one or more isolated CDRs or a functional paratope, where isolated CDRs or antigen-binding residues or polypeptides can be associated or linked together so as to form a functional antibody fragment. For a review of certain antibody fragments, see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, N.Y., pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')2 fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific, see, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Various types of antibody fragments have been described or reviewed in, e.g.. Heiliger and Hudson, Nat Biotechnol 2005; 23, 1126-1136; WO2005/040219, US20050238646 and US20020161201. Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. CHO, *E. coli* or phage), as described herein.

The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow and Lane, "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory Press, N.Y. (1988); Hammerling et al., in: "Monoclonal Antibodies and T-Cell Hybridomas," Elsevier, N.Y. (1981), pp. 563-681 (both of which are incorporated herein by reference in their entireties).

The term "monospecific" antibody as used herein denotes that the antibody-part of a conjugate comprising antigen binding-regions as described herein, has one or more antigen-binding sites each of which bind to the same epitope of the same antigen. The term "bispecific" means that the antibody-part of a conjugate as described herein, has at least two antigen-binding sites that are able to specifically bind to at least two distinct antigenic determinants. Typically, a bispecific antigen binding molecule comprises two antigen-binding sites, each of which is specific for a different antigenic determinant. In certain embodiments the bispecific antigen binding molecule is capable of simultaneously binding two antigenic determinants, particularly two antigenic determinants expressed on two distinct cells.

The term "valent" or "valency" as used within the current application denotes the presence of a specified number of binding sites or number of ligands in an antigen binding molecule or conjugate described herein. As such, the terms "bivalent", "tetravalent", and "hexavalent" denote the presence of two, four, and six binding sites or ligands, respectively, in an antigen binding molecule or conjugate.

An antibody immunologically reactive with a particular antigen can be generated by recombinant methods such as selection of libraries of recombinant antibodies in phage or similar vectors, see, e.g., Huse et al., Science 246:1275-1281 (1989); Ward et al., Nature 341:544-546 (1989); and Vaughan et al., Nature Biotech. 14:309-314 (1996), or by immunizing an animal with the antigen or with DNA encoding the antigen. Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with an antigen of interest or a cell expressing such an antigen. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells. Hybridomas are selected and cloned by limiting dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding the antigen. Ascites fluid, which generally contains high levels of antibodies, can be generated by inoculating mice intraperitoneally with positive hybridoma clones.

Typically, an immunoglobulin has a heavy and light chain. Each heavy and light chain contains a constant region and a variable region, (the regions are also known as "domains"). Light

and heavy chain variable regions contain four "framework" regions interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs". The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, which is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementarity-determining region" or "CDR" (e.g. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light-chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy-chain variable domain; Kabat et al. 1991, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, USA) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light-chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy-chain variable domain; Chothia and Lesk, J. Mol. Biol 1987;196:901-917). Typically, the numbering of amino acid residues in this region is performed by the method described in Kabat et al., supra. Phrases such as “Kabat position”, "variable domain residue numbering as in Kabat" and "according to Kabat" herein refer to this numbering system for heavy chain variable domains or light chain variable domains. Using the Kabat numbering system, the actual linear amino acid sequence of a peptide may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, an FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of CDR H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The term "framework" or "FR" residues as used herein refers to the region of an antibody variable domain exclusive of those regions defined as CDRs. Each antibody variable domain framework can be further subdivided into the contiguous regions separated by the CDRs (FR1, FR2, FR3 and FR4).

The term "constant region" as defined herein refers to an antibody-derived constant region that is encoded by one of the light or heavy chain immunoglobulin constant region genes. By "constant light chain" or "light chain constant region" as used herein is meant the region of an antibody encoded by the kappa (Ck) or lambda (Cλ) light chains. The constant light chain typically comprises a single domain, and as defined herein refers to positions 108-214 of Cκ or Cλ, wherein numbering is according to the EU index (Kabat et al., 1991, *supra*).

The term "constant heavy chain" or "heavy chain constant region" as used herein refers to the region of an antibody encoded by the mu, delta, gamma, alpha, or epsilon genes to define the antibody's isotype as IgM, IgD, IgG, IgA, or IgE, respectively. For full length IgG antibodies, the constant heavy chain, as defined herein, refers to the N-terminus of the CH1 domain to the C-

terminus of the CH3 domain, thus comprising positions 118-447, wherein numbering is according to the EU index.

Papain digestion of intact antibodies produces two identical antigen-binding fragments, called "Fab" fragments containing each the heavy- and light-chain variable domains and also the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. "Fab" fragments can also be recombinantly produced by methods known in the art. As used herein, Thus, the term "Fab fragment" " or "Fab region" refers to an antibody fragment comprising a light chain fragment comprising a VL domain and a constant domain of a light chain (CL), and a VH domain and a first constant domain (CH1) of a heavy chain. Fab may refer to this region in isolation, or this region in the context of a polypeptide, conjugate or antigen-binding region, or any other embodiments as outlined herein. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH are Fab' fragments in which the cysteine residue(s) of the constant domains bear a free thiol group. Pepsin treatment yields an F(ab')2 fragment that has two antigen-combining sites (two Fab fragments) and a part of the Fc region.

The term "single-chain Fv" or "scFv" as used herein refers to antibody fragments comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. Methods for producing scFvs are well known in the art. For a review of methods for producing scFvs see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, N.Y., pp. 269-315 (1994).

"Scaffold antigen-binding proteins" are known in the art, for example, fibronectin and designed ankyrin repeat proteins (DARPins) have been used as alternative scaffolds for antigen-binding domains, see, e.g., Gebauer and Skerra, Engineered protein scaffolds as next-generation antibody therapeutics. Curr Opin Chem Biol 13:245-255 (2009) and Stumpp et al., Darpins: A new generation of protein therapeutics. Drug Discovery Today 13: 695-701 (2008). In one aspect of the invention, a scaffold antigen-binding protein is selected from the group consisting of CTLA-4 (Evibody), Lipocalins (Anticalin), monobodies, centyrins, kunitz domains, knottins, fynomers, lipocalins, a Protein A-derived molecule such as Z-domain of Protein A (Affibody), an A-domain (Avimer/Maxibody), a serum transferrin (*trans*-body); a designed ankyrin repeat protein (DARPin), a variable domain of antibody light chain or heavy chain (single-domain antibody, sdAb), a variable domain of antibody heavy chain (nanobody, aVH), V_{NAR} fragments, a fibronectin (AdNectin), a C-type lectin domain (Tetranectin); a variable domain of a new antigen receptor beta-lactamase (V_{NAR} fragments), a human gamma-crystallin or ubiquitin (Affilin molecules); a Kunitz type domain of human protease inhibitors, microbodies such as the proteins from the knottin family, peptide aptamers and fibronectin (adnectin).

CTLA-4 (Cytotoxic T Lymphocyte-associated Antigen 4) is a CD28-family receptor expressed on mainly $CD4^+$ T-cells. Its extracellular domain has a variable domain- like Ig fold. Loops corresponding to CDRs of antibodies can be substituted with heterologous sequence to confer

different binding properties. CTLA-4 molecules engineered to have different binding specificities are also known as Evibodies (e.g. US7166697B1). Evibodies are around the same size as the isolated variable region of an antibody (e.g. a domain antibody). For further details see Journal of Immunological Methods 248 (1-2), 31-45 (2001).

Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids. They have a rigid beta-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size and are derived from lipocalins. For further details see Biochim Biophys Acta 1482: 337-350 (2000), US7250297B1 and US20070224633.

An affibody is a scaffold derived from Protein A of Staphylococcus aureus which can be engineered to bind to antigen. The domain consists of a three-helical bundle of approximately 58 amino acids. Libraries have been generated by randomization of surface residues. For further details see Protein Eng. Des. Sel. 17, 455-462 (2004) and EP1641818A1.

Avimers are multidomain proteins derived from the A-domain scaffold family. The native domains of approximately 35 amino acids adopt a defined disulfide bonded structure. Diversity is generated by shuffling of the natural variation exhibited by the family of A-domains. For further details see Nature Biotechnology 23(12), 1556 - 1561 (2005) and Expert Opinion on Investigational Drugs 16(6), 909-917 (June 2007).

A transferrin is a monomeric serum transport glycoprotein. Transferrins can be engineered to bind different target antigens by insertion of peptide sequences in a permissive surface loop. Examples of engineered transferrin scaffolds include the Trans-body. For further details see J. Biol. Chem 274, 24066-24073 (1999).

Designed Ankyrin Repeat Proteins (DARPins) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33-residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different target antigens by randomizing residues in the first alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. 332, 489-503 (2003), PNAS 100(4), 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007) and US20040132028A1.

A single-domain antibody is an antibody fragment consisting of a single monomeric variable antibody domain. The first single variable domains were derived from the variable domain of the antibody heavy chain from camelids (nanobodies or V_HH fragments). Furthermore, the term single variable domain antibody includes an autonomous human heavy chain variable domain (aVH) or V_{NAR} fragments derived from sharks.

Fibronectin is a scaffold which can be engineered to bind to antigen. Adnectins consists of a backbone of the natural amino acid sequence of the 10th domain of the 15 repeating units of human fibronectin type III (FN3). Three loops at one end of the β-sandwich can be engineered to enable an Adnectin to specifically recognize a therapeutic target of interest. For further details see Protein Eng. Des. Sel. 18, 435- 444 (2005), US20080139791, WO2005056764 and US6818418B1.

Peptide aptamers are combinatorial recognition molecules that consist of a constant scaffold protein, typically thioredoxin (TrxA) which contains a constrained variable peptide loop inserted at the active site. For further details see Expert Opin. Biol. Ther. 5, 783-797 (2005).

Microbodies are derived from naturally occurring microproteins of 25-50 amino acids in length which contain 3-4 cysteine bridges - examples of microproteins include KalataBI and conotoxin and knottins. The microproteins have a loop which can be engineered to include up to 25 amino acids without affecting the overall fold of the microprotein. For further details of engineered knottin domains, see WO2008098796.

The term "Fv" or "Fv fragment" or "Fv region" as used herein refers to a polypeptide that comprises the V_H and V_L domains of a single antibody.

The term "Fc" or "Fc region", as used herein refers to the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Fc may refer to this region in isolation, or this region in the context of an Fc polypeptide, as described below. By "Fc polypeptide" or “Fc-derived polypeptide” as used herein is meant a polypeptide that comprises all or part of an Fc region. Fc polypeptides herein include but are not limited to antibodies, Fc fusions and Fc fragments. Also, Fc regions according to the invention include variants containing at least one modification that alters (enhances or diminishes) an Fc associated effector function. Also, Fc regions according to the invention include chimeric Fc regions comprising different portions or domains of different Fc regions, e.g., derived from antibodies of different isotype or species. Fc thus refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains Cγ2 (CH2) and Cγ 3 (CH3) and the hinge between Cγ 1 and Cγ 2. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226, P230 or A231 to its carboxyl-terminus, wherein the numbering is according to the EU index. The "CH2 domain" of a human IgG Fc region usually extends from an amino acid residue at about position 231 to an amino acid residue at about position 340. In one embodiment, a carbohydrate chain is attached to the CH2 domain. The CH2 domain herein may be a native sequence CH2 domain or variant CH2 domain. The "CH3 domain" comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (i.e. from an amino acid residue at about position 341 to an amino acid residue at about position 447 of an IgG). The CH3 region herein may be a native sequence CH3 domain or a variant CH3 domain (e.g. a CH3 domain with an introduced "protuberance" ("knob") in one chain thereof and a corresponding introduced "cavity" ("hole") in the other chain thereof; see US Patent No. 5,821,333, expressly incorporated herein by reference). Such variant CH3 domains may be used to promote heterodimerization of two non-identical antibody heavy chains as herein described. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of

Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

The "knob-into-hole" technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis. In a specific embodiment a knob modification comprises the amino acid substitution T366W in one of the two subunits of the Fc region, and the hole modification comprises the amino acid substitutions T366S, L368A and Y407V in the other one of the two subunits of the Fc domain. In a further specific embodiment, the subunit of the Fc region comprising the knob modification additionally comprises the amino acid substitution S354C, and the subunit of the Fc region comprising the hole modification additionally comprises the amino acid substitution Y349C. Introduction of these two cysteine residues results in the formation of a disulfide bridge between the two subunits of the Fc region, thus further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)). The numbering is according to EU index of Kabat et al, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

A "region equivalent to the Fc region of an immunoglobulin" is intended to include naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants having alterations which produce substitutions, additions, or deletions but which do not decrease substantially the ability of the immunoglobulin to mediate effector functions (such as antibody-dependent cellular cytotoxicity). For example, one or more amino acids can be deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of biological function. Such variants can be selected according to general rules known in the art so as to have minimal effect on activity (see, e.g., Bowie, J. U. et al., Science 247:1306-10 (1990)).

The term "effector functions" refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

An "activating Fc receptor" is an Fc receptor that following engagement by an Fc region of an antibody elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Activating Fc receptors include FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32), and

FcaRI (CD89). A particular activating Fc receptor is human FcγRIIIa (see UniProt accession no. P08637, version 141), also referred to as CD16 or CD16A. In humans, CD16 consists of two isoforms, CD16A and CD16B, encoded by two highly homologous genes. CD16A is a transmembrane protein expressed by lymphocytes and some monocytes, whereas CD16B is linked to the plasma membrane via a GPI anchor and primarily expressed by neutrophils. Therefore, when reference is made herein to CD16 in the context of expression on NK cells herein, usually CD16A is meant unless otherwise indicated.

By "variable region" as used herein is meant the region of an antibody that comprises one or more Ig domains substantially encoded by any of the V_L (including Vκ and Vλ) and/or V_H genes that make up the light chain (including κ and λ) and heavy chain immunoglobulin genetic loci respectively. A light or heavy chain variable region (V_L or V_H) comprise four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity.

The term "hypervariable region" or "HVR," as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (HI), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) Hypervariable regions (HVRs) are also referred to as complementarity determining regions (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen-binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia et al., J. Mol. Biol. 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table A as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table A. CDR defintions[1]

CDR	Kabat	Chotia	AbM[2]
V_H CDR1	31-35	26-32	26-35
V_H CDR2	50-65	52-58	50-58
V_H CDR3	95-102	95-102	95-102
V_L CDR1	24-34	26-32	24-34
V_L CDR2	50-56	50-52	50-56
V_L CDR3	89-97	91-96	89-97

[1] Numbering of all CDR definitions in Table A is according to the numbering conventions set forth by Kabat et al. (see below).

[2] "AbM" with a lowercase "b" as used in Table A refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

Kabat *et al.* also defined a numbering system for variable region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable region sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody variable region are according to the Kabat numbering system.

With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDRL2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra.*

As used herein, the term "affinity matured" in the context of antigen binding molecules (e.g., antibodies) refers to an antigen-binding molecule that is derived from a reference antigen-binding molecule, e.g., by mutation, binds to the same antigen, preferably binds to the same epitope, as the reference antibody; and has a higher affinity for the antigen than that of the reference antigen-binding molecule. Affinity maturation generally involves modification of one or more amino acid residues in one or more CDRs of the antigen-binding molecule. Typically, the affinity matured antigen-binding molecule binds to the same epitope as the initial reference antigen-binding molecule.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG1, IgG2, IgG3, IgG4,

IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and m respectively.

A "blocking" antibody or an "antagonist" antibody is one which inhibits or reduces biological activity of the antigen it binds. Preferred blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen. An "agonist antibody", as used herein, is an antibody which mimics at least one of the functional activities of a polypeptide of interest.

The term "specifically binds" refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding region or antigen-binding protein can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity. The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (K_D), is a measure for the binding strength between an antigenic determinant and an antigen-binding site on the antigen-binding protein. Alternatively, the affinity can also be expressed as the affinity constant (K_A), which is $1/K_D$. Affinity can be determined in a manner known per se, depending on the specific combination of antigen-binding protein and antigen of interest. Avidity is herein understood to refer to the strength of binding of a target molecule with multiple binding sites by a larger complex of binding agents, i.e. the strength of binding of multivalent binding. Avidity is related to both the affinity between an antigenic determinant and its antigen-binding site on the antigen-binding protein and the valency, i.e. the number of binding sites present on the antigen-binding protein. Affinity, on the other hand refers to simple monovalent receptor ligand systems.

Typically, an antigen-binding region of a conjugate of the invention thereof will specifically bind its target molecule (antigen) with a dissociation constant (K_D) of about 10^{-6} to 10^{-12} M or less, and preferably 10^{-8} to 10^{-12} M or less, and/or with a binding affinity of at least 10^{-6} M or 10^{-7} M, preferably at least 10^{-8} M, more preferably at least 10^{-9} M, such as at least 10^{-10}, 10^{-11}, 10^{-12} M or less. Any K_D value greater than 10^{-4} M (i.e. less than 100 μM) is generally considered to indicate non-specific binding. Thus, an antigen-binding region that “specifically binds” an antigen, is an antigen-binding domain that binds the antigen with a K_D value of no more than 10^{-4} M, as may be determined as herein described below. Preferably, an antigen-binding region of a conjugate of the invention will specifically bind to the target molecule with an affinity less than 800, 400, 200, 100 50, 10 or 5 nM, more preferably less than 1 nM, such as less than 500, 200, 100, 50, 10 or 5 pM. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention (see e.g. Harlow, et al., *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Coligan et al., eds.. *Current Protocols in Immunology,* Greene Publishing Assoc, and Wiley Interscience, N.Y., (1992, 1993), and Muller, *Meth. Enzymol.* 92:589-601 (1983)). Specific illustrative embodiments are described in the following.

A "K_D" or "K_D value" can be measured by using an ELISA as described in the Examples herein or by using surface plasmon resonance assays using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, NJ) In an exemplary method, carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with N-ethyl-N’-(3-dimethylaminopropyl)-

carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10mM sodium acetate, pH 4.8, into 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of the antibody or Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% Tween 20 (PBST) at 25°C at a flow rate of approximately 25µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2) by simultaneous fitting the association and dissociation sensogram. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen, Y., et al., (1999) J. Mol Biol 293:865-881. If the on-rate exceeds 10^6 M^{-1} S^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-Aminco spectrophotometer (ThermoSpectronic) with a stir red cuvette.

The term "humanized antibody" or "humanized immunoglobulin" refers to an immunoglobulin comprising a human framework, at least one and preferably all complementarity determining regions (CDRs) from a non-human antibody, and in which any constant region present is substantially identical to a human immunoglobulin constant region, i.e., at least about 85%, at least 90%, and at least 95% identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of one or more native human immunoglobulin sequences. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. See, e.g., Queen et al., U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,761; 5,693,762; 6,180,370 (each of which is incorporated by reference in its entirety). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101 and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Mol. Immunol., 28:489 498 (1991); Studnicka et al., Prot. Eng. 7:805 814 (1994); Roguska et al., Proc. Natl. Acad. Sci. 91:969 973 (1994), and chain shuffling (U.S. Pat. No. 5,565,332), all of which are hereby incorporated by reference in their entireties.

One class of antigen-binding regions for use in the invention comprises immunoglobulin single variable domains (ISVDs) with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring single variable domain, but that has been "humanized", i.e. by replacing one or more amino acid residues in the amino acid sequence of said naturally occurring

single variable domain sequence by one or more of the amino acid residues that occur at the corresponding position(s) in a V_H domain from a conventional 4-chain antibody from a human being. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the prior art on humanization including e.g. Jones et al. (Nature 321:522-525, 1986); Riechmann et al., (Nature 332:323-329, 1988); Presta (Curr. Op. Struct. Biol. 2:593-596, 1992), Vaswani and Hamilton (Ann. Allergy, Asthma and Immunol., 1:105-115 1998); Harris (Biochem. Soc. Transactions, 23:1035-1038, 1995); Hurle and Gross (Curr. Op. Biotech., 5:428-433, 1994), and specific prior art relating to humanization of V_HHs such as e.g. Vincke et al. (2009, J. Biol. Chem. 284:3273–3284). Again, it should be noted that such humanized single variable domains of the invention can be obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring single variable domain as a starting material.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

As an alternative to humanization, human antibodies can be generated. By “human antibody” is meant an antibody containing entirely human light and heavy chains as well as constant regions, produced by any of the known standard methods. For example, transgenic animals (e.g., mice) are available that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region PH gene in chimeric and germ-line mutant mice results in the complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ line mutant mice will result in the production of human antibodies after immunization. See, e.g., Jakobovits *et al.*, Proc. Nat. Acad. Sci. USA, 90:255 1 (1993); Jakobovits *et al.*, Nature, 362:255-258 (1993). Alternatively, phage display technology (McCafferty *et al.*, Nature 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd,

and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3:564-57 1 (1993). Human antibodies may also be generated by *in vitro* activated B cells or SCID mice with its immune system reconstituted with human cells. Once a human antibody is obtained, its coding DNA sequences can be isolated, cloned and introduced into an appropriate expression system i.e. a cell line, preferably from a mammal, which subsequently express and liberate it into a culture media from which the antibody can be isolated.

Amino acid substitutions are herein indicated as A_oXA_s, wherein A_o indicates the original amino acid, X indicates the position of that original amino acid in the original amino acid sequence, and A_s indicates the substitute amino acid as present in that position in the modified amino acid sequence. For example, V153Q denotes that the original amino acid valine (V) in position 153 is changed to a glutamine (Q).

Amino acid deletions are herein indicated as A_oX-, wherein A_o indicates the original amino acid, X indicates the position of that original amino acid in the original amino acid sequence and the dash indicates that the original amino acid A_o is no longer present in the modified amino acid sequence. For example N59- indicates that the asparagine (N) in position 59 is deleted in the modified amino acid sequence.

Amino acid deletions are herein indicated as A_oX ins$A_{1\text{-}n}$, wherein A_o indicates the original amino acid, X indicates the position of that original amino acid in the original amino acid sequence and ins$A_{1\text{-}n}$ indicates that amino acids 1 – n replace the original amino acid A_o. For example G84 insGGGGG indicates that the original glycine in position 84 is replaced by a sequence of 5 glycines in the modified amino acid sequence.

As used herein, the phrase “NK cells” refers to a sub-population of lymphocytes that is involved in innate immunity. NK cells can be identified by virtue of certain characteristics and biological properties, such as the expression of specific surface antigens including CD56 and/or NKp46 for human NK cells, the absence of the alpha/beta or gamma/delta TCR complex on the cell surface, the ability to recognize and kill cells that fail to express “self” MHC/HLA antigens by the activation of specific cytolytic machinery, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response. Any of these characteristics and activities can be used to identify NK cells, using methods well known in the art. Any subpopulation of NK cells will also be encompassed by the term NK cells. Within the context herein “active” NK cells designate biologically active NK cells, including NK cells having the capacity of lysing target cells or enhancing the immune function of other cells. NK cells can be obtained by various techniques known in the art, such as isolation from blood samples, cytapheresis, tissue or cell collections, etc.

Useful protocols for assays involving NK cells can be found in *Natural Killer Cells Protocols* (2000, edited by Campbell KS and Colonna M). Humana Press, pp. 219-238).

The term “tumor associated antigen” (TAA) as used herein means any antigen including but not limited to a protein, glycoprotein, ganglioside, carbohydrate, lipid that is associated with cancer. Such antigen can be expressed on malignant cells or in the tumor microenvironment such as on tumor-associated blood vessels, extracellular matrix, mesenchymal stroma, or immune infiltrates. Expressly included in the term TAA are homologues of a wild-type TAA that differs therefrom as a result of tumor-specific mutations (which can be patient-specific or shared) and that result in altered amino acid sequences, i.e. so-called neoantigens.

A “nucleic acid construct” or “nucleic acid vector” is herein understood to mean a man-made nucleic acid molecule resulting from the use of recombinant DNA technology. The term “nucleic acid construct” therefore does not include naturally occurring nucleic acid molecules although a nucleic acid construct may comprise (parts of) naturally occurring nucleic acid molecules. The terms “expression vector” or expression construct" refer to nucleic acid molecules that are capable of effecting expression of a nucleotide sequence or gene in host cells or host organisms compatible with such expression vectors or constructs. These expression vectors typically include regulatory sequence elements that are operably linked to the nucleotide sequence to be expressed to effect its expression. Such regulatory elements usually at least include suitable transcription regulatory sequences and optionally, 3’ transcription termination signals. Additional elements necessary or helpful in effecting expression may also be present, such as expression enhancer elements. The expression vector will be introduced into a suitable host cell and be able to effect expression of the coding sequence in an in vitro cell culture of the host cell. The expression vector will be suitable for replication in the host cell or organism of the invention whereas an expression construct will usually integrate in the host cell’s genome for it to be maintained. Techniques for the introduction of nucleic acid into cells are well established in the art and any suitable technique may be employed, in accordance with the particular circumstances. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. adenovirus, AAV, lentivirus or vaccinia. For microbial, e.g. bacterial, cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. The introduced nucleic acid may be on an extra-chromosomal vector within the cell or the nucleic acid may be integrated into the genome of the host cell. Integration may be promoted by inclusion of sequences within the nucleic acid or vector which promote recombination with the genome, in accordance with standard techniques. The introduction may be followed by expression of the nucleic acid to produce the encoded fusion protein. In some embodiments, host cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) may be cultured *in vitro* under conditions for expression of the nucleic acid, so that the encoded fusion protein polypeptide is produced, when an inducible promoter is used, expression may require the activation of the inducible promoter.

As used herein, the term “promoter” or “transcription regulatory sequence” refers to a nucleic acid fragment that functions to control the transcription of one or more coding sequences, and is located upstream with respect to the direction of transcription of the transcription initiation site of the coding sequence, and is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and any other DNA sequences, including, but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate the amount of transcription from the promoter. A “constitutive” promoter is a promoter that is active in most tissues under most physiological and developmental conditions. An “inducible” promoter is a promoter that is physiologically or developmentally regulated, e.g. by the application of a chemical inducer.

The term “selectable marker” is a term familiar to one of ordinary skill in the art and is used herein to describe any genetic entity which, when expressed, can be used to select for a cell or cells containing the selectable marker. The term “reporter” may be used interchangeably with marker, although it is mainly used to refer to visible markers, such as green fluorescent protein (GFP). Selectable markers may be dominant or recessive or bidirectional.

As used herein, the term “operably linked” refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For instance, a transcription regulatory sequence is operably linked to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein encoding regions, contiguous and in reading frame.

The terms “protein” or “polypeptide” are used interchangeably and refer to molecules consisting of a chain of amino acids, without reference to a specific mode of action, size, 3-dimensional structure or origin.

The term “signal peptide” (sometimes referred to as signal sequence) is a short peptide (usually 16-30 amino acids long) present at the N-terminus of the majority of newly synthesized proteins that are destined towards the secretory pathway. At the end of the signal peptide there is usually a stretch of amino acids that is recognized and cleaved by signal peptidase either during or after completion of translocation (from the cytosol into the secretory pathway, i.e. ER) to generate a free signal peptide and a mature protein. Signal peptides are extremely heterogeneous, and many prokaryotic and eukaryotic signal peptides are functionally interchangeable even between different species however the efficiency of protein secretion may depend on the signal peptide. Suitable signal peptides are generally known in the art e.g. from Käll et al. (2004 J. Mol. Biol. 338: 1027–1036) and von Heijne (1985, J Mol Biol. 184 (1): 99–105).

The term “gene” means a DNA fragment comprising a region (transcribed region), which is transcribed into an RNA molecule (e.g. an mRNA) in a cell, operably linked to suitable regulatory regions (e.g. a promoter). A gene will usually comprise several operably linked fragments, such as a promoter, a 5’ leader sequence, a coding region and a 3’ non-translated sequence (3’ end) comprising a polyadenylation site. “Expression of a gene” refers to the process wherein a DNA

region which is operably linked to appropriate regulatory regions, particularly a promoter, is transcribed into an RNA, which is biologically active, i.e. which is capable of being translated into a biologically active protein or peptide.

The term “homologous” when used to indicate the relation between a given (recombinant) nucleic acid or polypeptide molecule and a given host organism or host cell, is understood to mean that in nature the nucleic acid or polypeptide molecule is produced by a host cell or organisms of the same species, preferably of the same variety or strain. If homologous to a host cell, a nucleic acid sequence encoding a polypeptide will typically (but not necessarily) be operably linked to another (heterologous) promoter sequence and, if applicable, another (heterologous) secretory signal sequence and/or terminator sequence than in its natural environment. It is understood that the regulatory sequences, signal sequences, terminator sequences, etc. may also be homologous to the host cell. When used to indicate the relatedness of two nucleic acid sequences the term “homologous” means that one single-stranded nucleic acid sequence may hybridize to a complementary single-stranded nucleic acid sequence. The degree of hybridization may depend on a number of factors including the amount of identity between the sequences and the hybridization conditions such as temperature and salt concentration as discussed later.

The term "heterologous" when used with respect to a nucleic acid (DNA or RNA) or protein refers to a nucleic acid or protein that does not occur naturally as part of the organism, cell, genome or DNA or RNA sequence in which it is present, or that is found in a cell or location or locations in the genome or DNA or RNA sequence that differ from that in which it is found in nature. Heterologous nucleic acids or proteins are not endogenous to the cell into which it is introduced but has been obtained from another cell or synthetically or recombinantly produced. Generally, though not necessarily, such nucleic acids encode proteins that are not normally produced by the cell in which the DNA is transcribed or expressed. Similarly exogenous RNA encodes for proteins not normally expressed in the cell in which the exogenous RNA is present. Heterologous nucleic acids and proteins may also be referred to as foreign nucleic acids or proteins. Any nucleic acid or protein that one of skill in the art would recognize as heterologous or foreign to the cell in which it is expressed is herein encompassed by the term heterologous nucleic acid or protein. The term heterologous also applies to non-natural combinations of nucleic acid or amino acid sequences, i.e. combinations where at least two of the combined sequences are foreign with respect to each other.

Detailed description of the invention

We have previously described multispecific antigen binding proteins, which bind to a tumor-associated antigen of interest and/or to an NK cell activating receptor, and which comprise an NK cell-activating cytokine that triggers at least one of the NK cell’s interleukin 21 receptor and 4-1BB, and that are capable of inducing a hyper-functional phenotype in NK cells (see WO2024/056862 and WO2024/056861). We have also previously described multispecific antigen binding proteins, which bind to a tumor-associated antigen of interest and/or to a γδ T cell receptor, and which comprise a γδ T cell-activating agonist (e.g. IL-21), and optionally a γδ T cell co-stimulatory agonist (e.g. 4-1BBL), and that are capable of inducing a hyper-functional phenotype (expansion, activation

and/or innate potency) in γδ T cells (see co-pending applications EP 24208721.1 and EP24208726.0). NK cells and γδ T cells having a hyper-functional phenotype proliferate, are resistant to the tumor microenvironment, have an enhanced capability to mediate lysis of target cells, even in the absence of the original tumor associated antigen targeted, hyper-secrete cytokines (e.g. IFN-γ) when in contact with target cells and have the ability to prolong these capabilities over time. Agents capable of inducing a hyper-functional phenotype in NK cells and/or in γδ T cells are therefore useful in the treatment of cancers and infectious diseases. However, the systemic administration of agents comprising cytokines with pleiotropic effects, such as 4-1BB ligand (4-1BBL), remains challenging because of the risks of toxicity and other undesired side-effects. There remains therefore a need in the art for 4-1BBL treatment modalities that reduce the systemic pleiotropic effect of 4-1BBL, while maintaining their potential when targeted to tumors or sites infected by pathogens. The present invention therefore provides novel muteins of the extracellular domain (ECD) of 4-1BBL having an altered affinity for 4-1BB. For example, 4-1BBL ECD muteins described herein and having a reduced affinity for 4-1BB, and conjugates described herein comprising such 4-1BBL ECD muteins, will have reduced pleiotropic- and undesired side-effects, when present in the bloodstream, while, when present at targeted sites, their avidity will ensure their local efficacy. These novel 4-1BBL ECD muteins can be combined with further cytokines, such as IL-21 muteins having a reduced affinity for IL-21R, in conjugates with antigen-binding proteins. Without being bound by a theory, the 4-1BBL ECD-containing conjugates described herein are designed to utilize the immune potentiating activity of 4-1BBL ECD (which may be prerequisite to address toxicity and off-target immune suppression), to maximize efficacy at targeted loci, and improve the feasibility of dosing in the clinic.

4-1BB ligand and muteins of the extracellular domain of 4-1BB ligand

4-1BB is a member of the tumor necrosis factor receptor family. Its alternative names are tumor necrosis factor receptor superfamily member 9 (TNFRSF9), CD137 and induced by lymphocyte activation (ILA). 4-1BB is encoded by the *TNFRSF9* gene (Entrez Gene ID: 3604). An amino acid sequence for human 4-1BB is described in NCBI accession numbers NP_001552, which is incorporated as SEQ ID NO: 175, wherein the mature 4-1BB corresponds to positions 24 - 255. 4-1BB is known as a co-stimulatory immune checkpoint molecule. 4-1BB is expressed by activated T cells of both the CD4+ and CD8+ lineages, as well as on activated NK cells and on activated γδ T cells. The proliferation and activation of NK cells and γδ T cells at least requires engagement of a costimulatory receptor such as 4-1BB by its ligand 4-1BBL. NK cells and γδ T cells with increased 4-1BB expression are known to be highly active against target cells (e.g. tumor cells) expressing 4-1BB ligand. 4-1BB ligand (4-1BBL), also known as TNFSF9 or CD137L, is a protein that in humans is encoded by the *TNFSF9* gene (Entrez Gene ID: 8744). An amino acid sequence for human 4-1BBL is described in NCBI accession numbers NP_003802, the disclosure of which is incorporated herein by reference. The 4-1BB/4-1BBL complex consists of three monomeric 4-1BBs bound to a trimeric 4-1BBL. Each 4-1BB monomer binds to two 4-1BBLs via cysteine-rich domains (CRDs). The interaction between 4-1BB and the second 4-1BBL is required to stabilize their interactions.

As used herein, an “4-1BB agonist” is an agent that has “agonist” activity at the 4-1BB, which means that the agent that can cause or increase "4-1BB signaling". “4-1BB signaling” refers to an ability of 4-1BB, e.g. when expressed on the surface of T, B and NK cells and triggered by its natural ligand 4-1BBL, to activate or transduce an intracellular signaling pathway. The “natural 4-1BB ligand” is herein understood as the extracellular domain (ECD) of a human wild type 4-1BBL comprising or consisting of an amino acid sequence from position 71 to 254 of the amino acid sequence of human 4-1BBL (i.e. SEQ ID NO: 37). A 4-1BBL extracellular domain (ECD) is herein thus understood as a polypeptide comprising or consisting of an amino acid sequence from positions 71 to 254 of human 4-1BBL, or a fragment thereof having 4-1BB agonist activity.

4-1BB agonist activity, i.e. changes in 4-1BB signaling activity, can be measured, for example, by assays designed to measure changes in the 4-1BB signaling pathways, e.g. by monitoring phosphorylation of signal transduction components, assays to measure the association of certain signal transduction components with other proteins or intracellular structures, or in the biochemical activity of components such as kinases, or indirectly by a downstream effect mediated by 4-1BB (e.g. production of specific cytokines). A suitable cell-based assay for *in vitro* biological activity of a 4-1BB agonist, is e.g. described in Zhang et al. (Clin Cancer Res ,2007;13(9): 2758-2767), using measurement of IL-2 production from splenocytes aseptically removed from BALB/c mice in microtiter plates precoated with an anti-CD3 monoclonal antibody (145-11C clone). Other suitable cell-based assays for *in vitro* biological activity of a 4-1BB agonist, are described in WO2016/075278, Example 6 (see e.g. Example 6.1). The natural 4-1BB ligand, a 4-1BBL ECD trimer as described by Fellermeier et al. (Oncoimmunol. 2016, 5(11): e1238540), e.g. a 4-1BBL ECD trimer comprising the amino acid sequence of SEQ ID NO: 36, or an anti-CD137 agonist antibody (such the antibody 2A, Epstein et al., Tumor necrosis imaging and treatment of solid tumors. In: V. P. Torchilin, editor. Handbook of targeted delivery of imaging agents, Vol. 16. Boca Raton: CRCPress; 1995. p. 259.) can serve as a positive control in an assay for 4-1BB agonist activity and can also be used as a reference for the amount of 4-1BB agonist activity of a given non-natural 4-1BB agonist, such as a multispecific antigen binding protein as described herein comprising a 4-1BB agonist. The data presented herein supports the use of carefully designed 4-1BBL ECD muteins to achieve 4-1BB signalling at the appropriate time and place and to improve pharmacokinetics of therapeutics comprising such 4-1BBL ECD muteins. In addition, the 4-1BBL ECD muteins provided herein are designed to reduce potential unwanted effects in the absence of the target cells, e.g. hepatotoxicity and cytokine release.

In a first aspect, the present disclosure provides muteins of the 4-1BBL ECD comprising at least one substitution, deletion and/or insertion. Amino acid substitutions, deletions and insertions in a 4-1BBL ECD mutein provided herein are indicated relative to the wild-type human 4-1BBL ECD amino acid sequence, which is provided herein as SEQ ID NO: 37. Hence, to allow for allelic variation, a wild-type human 4-1BBL ECD preferably comprises an amino acid sequence having, with increasing preference, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity with SEQ ID NO: 37.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein SEQ ID NO: 49 is

REGPELSPDD PAGLLDLRQG MFAQLVAQNX XLIDGPLSWX SDPXXXGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVXXGEGSGS VSLALHLQPL XSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWXLTX GATVLGLFRV TPEIPAGLPS PRSE (SEQ ID NO: 49),

wherein X represents any amino acid, and wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) by at least one amino acid. The amino acid positions in the amino acid sequence of the 4-1BBL ECD muteins of SEQ ID NO: 49, as referred to herein correspond to the amino acid positions of the full-length 4-1BBL amino acid sequence. Hence, the first amino acid position in SEQ ID NO: 49 is referred to as position 71, from which the subsequent amino acid positions are counted, up to the last amino acid in position 254.

In one embodiment, there is provided a 4-1BBL ECD mutein comprising at least one amino acid substitution, deletion or insertion at a position in SEQ ID NO: 37 selected from the group consisting of the positions: 154, 153, 110, 227, 101, 230, 100, 114, 115, 116, and 171, in decreasing preference. In one embodiment, the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution, deletion or insertion at a position in SEQ ID NO: 37 selected from the group consisting of the positions: 154, 153, 110, 227, 101, , 230, 100, 114, 115, 116, and 171.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein SEQ ID NO: 49 differs from SEQ ID NO: 37 by at least one amino acid at a position designated by X in SEQ ID NO: 49. In one embodiment, the 4-1BBL ECD mutein comprising SEQ ID NO: 49 has, with increasing preference, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.4% sequence identity to SEQ ID NO: 37.

In one embodiment, there is provided a 4-1BBL ECD mutein comprises an amino acid sequence having, with increasing preference, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity with SEQ ID NO: 37, and wherein the amino acid sequence comprises at least one amino acid substitution selected from the group consisting of: A154D; A154E; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; V100T; V100S; V100A; V100G; V100N; V100D; V100E; V100K; V100R; L101E; L101Q; L101D; L101R; L101K; Y110E; Y110N; Y110D; Y110R; Y110K; Y110S; Y110A; Y110G; Y110T; G114K; G114R; G114Q; G114D; G114E; G114N; G114S; G114A; G114G; G114T; L115R; L115K; L115Q; L115N; L115D; L115E; L115S; L115A; L115G; L115T; A116D; A116E; A116R; A116K; A116Q; A116N; A116Y; A116H; V153N; V153R; V153K; V153D; V153E; V153S; V153G; A154R; A154K; A154Q; A154N; A154Y; A154H; G155Q; R171D; R171E; R171Q; R171N; R171S; R171T; R171G; R171A; R171Y; Q227R; Q227K; Q227D; Q227Y; Q227H; Q227G; Q227S; Q227T; Q230S; Q230R; Q230N;

Q230D; Q230E; Q230G; Q230S; Q230T and Q230A. In one embodiment, there is provided a 4-1BBL ECD mutein comprising at least one amino acid substitution selected from the group consisting of: A154D; A154E; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; V100T; V100S; V100A; V100G; V100N; V100D; V100E; V100K; V100R; L101E; L101Q; L101D; L101R; L101K; Y110E; Y110N; Y110D; Y110R; Y110K; Y110S; Y110A; Y110G; Y110T; G114K; G114R; G114Q; G114D; G114E; G114N; G114S; G114A; G114G; G114T; L115R; L115K; L115Q; L115N; L115D; L115E; L115S; L115A; L115G; L115T; A116D; A116E; A116R; A116K; A116Q; A116N; A116Y; A116H; V153N; V153R; V153K; V153D; V153E; V153S; V153G; A154R; A154K; A154Q; A154N; A154Y; A154H; R171D; R171E; R171Q; R171N; R171S; R171T; R171G; R171A; R171Y; Q227R; Q227K; Q227D; Q227Y; Q227H; Q227G; Q227S; Q227T; Q230S; Q230R; Q230N; Q230D; Q230E; Q230G; Q230S; Q230T and Q230A. In one embodiment, the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; V100T; V100S; V100A; V100G; V100N; V100D; V100E; V100K; V100R; L101E; L101Q; L101D; L101R; L101K; Y110E; Y110N; Y110D; Y110R; Y110K; Y110S; Y110A; Y110G; Y110T; G114K; G114R; G114Q; G114D; G114E; G114N; G114S; G114A; G114G; G114T; L115R; L115K; L115Q; L115N; L115D; L115E; L115S; L115A; L115G; L115T; A116D; A116E; A116R; A116K; A116Q; A116N; A116Y; A116H; V153N; V153R; V153K; V153D; V153E; V153S; V153G; A154R; A154K; A154Q; A154N; A154Y; A154H; R171D; R171E; R171Q; R171N; R171S; R171T; R171G; R171A; R171Y; Q227R; Q227K; Q227D; Q227Y; Q227H; Q227G; Q227S; Q227T; Q230S; Q230R; Q230N; Q230D; Q230E; Q230G; Q230S; Q230T; and Q230A.

In one embodiment, there is provided a 4-1BBL ECD mutein comprising at least two, three, four or five amino acid substitutions selected from the group consisting of: A154D; A154E; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; V100T; V100S; V100A; V100G; V100N; V100D; V100E; V100K; V100R; L101E; L101Q; L101D; L101R; L101K; Y110E; Y110N; Y110D; Y110R; Y110K; Y110S; Y110A; Y110G; Y110T; G114K; G114R; G114Q; G114D; G114E; G114N; G114S; G114A; G114G; G114T; L115R; L115K; L115Q; L115N; L115D; L115E; L115S; L115A; L115G; L115T; A116D; A116E; A116R; A116K; A116Q; A116N; A116Y; A116H; V153N; V153R; V153K; V153D; V153E; V153S; V153G; A154R; A154K; A154Q; A154N; A154Y; A154H; R171D; R171E; R171Q; R171N; R171S; R171T; R171G; R171A; R171Y; Q227R; Q227K; Q227D; Q227Y; Q227H; Q227G; Q227S; Q227T; Q230S; Q230R; Q230N; Q230D; Q230E; Q230G; Q230S; Q230T; and Q230A. In one embodiment, the 4-1BBL ECD mutein comprises no other amino acid sequence modification than theat least two, three, four or five amino acid substitutions selected from the group consisting of: A154D; A154E; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; V100T; V100S; V100A; V100G; V100N; V100D; V100E; V100K; V100R; L101E; L101Q; L101D; L101R; L101K; Y110E; Y110N; Y110D; Y110R; Y110K; Y110S; Y110A; Y110G; Y110T; G114K; G114R; G114Q; G114D; G114E; G114N; G114S; G114A; G114G; G114T; L115R; L115K; L115Q; L115N; L115D; L115E; L115S; L115A; L115G; L115T; A116D; A116E; A116R; A116K; A116Q; A116N; A116Y; A116H; V153N; V153R; V153K; V153D; V153E; V153S; V153G; A154R; A154K; A154Q; A154N; A154Y; A154H; R171D; R171E; R171Q; R171N; R171S; R171T; R171G; R171A; R171Y; Q227R; Q227K;

Q227D; Q227Y; Q227H; Q227G; Q227S; Q227T; Q230S; Q230R; Q230N; Q230D; Q230E; Q230G; Q230S; Q230T; and Q230A.

In one embodiment, there is provided a 4-1BBL ECD mutein comprising at least one amino acid substitution at a position in SEQ ID NO: 37 selected from the substitutions listed in Table B.

Table B. Additional substitutions, deletions and insertions for 4-1BBL ECD muteins

Position	Wild type	Preference of additional mutations (with decreasing preference from left to right)
100	V	S, A, G, N, D, E > K, R > all remaining
101	L	E, Q, D > R, K > all remaining
110	Y	E, N, D, R, K > S, A, G, T > all remaining
114	G	R, Q, D, E, N > S, A, G, T > all remaining
115	L	K, Q, N, D, E > S, A, G, T > all remaining
153	V	N, R, K, D, E > S, G > all remaining
171	R	E, Q, N > S, T, G, A > Y > all remaining
227	Q	K, D> Y, H > G, S, T > all remaining
230	Q	R, N, D, E > G, S, T, A > all remaining
116	A	E > R, K, Q, N > Y, H > all remaining
154	A	E > R, K, Q, N > Y, H > all remaining

In one embodiment, there is provided a 4-1BBL ECD mutein comprising at least one amino acid substitution at a position in SEQ ID NO: 37 selected from the group consisting of: A154D, A154E, V153Q, Q227E, L101N, Y110Q, Q230K, and V100Q. In one embodiment, there is provided a 4-1BBL ECD mutein comprising at least one amino acid substitution at a position in SEQ ID NO: 37 selected from the group consisting of: A154D; A154E; V153Q; Q227E; L101N; Y110Q and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising at least one amino acid substitution at a position in SEQ ID NO: 37 selected from the group consisting of: A154D; A154E; V153Q; and Q227E. In one embodiment, there is provided a 4-1BBL ECD mutein comprising at least one amino acid substitution at a position in SEQ ID NO: 37 selected from the group consisting of: A154D and A154E.

In one embodiment, there is provided a 4-1BBL ECD mutein, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at a position corresponding to position 154 of the full-length 4-1BBL amino acid sequence. In one embodiment, the 4-1BBL ECD mutein comprises no other differences from the wild type amino acid sequence than the difference at position 154. In one embodiment, the different amino acid at position 154 is aspartate (D) or glutamate (E), of which aspartate (D) is preferred.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from

the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 154, and wherein preferably the 4-1BBL ECD mutein comprises a A154D or A154E substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a A154D or A154E substitution in combination with at least one, two, three, four or five amino acid substitutions selected from the group consisting of: V100T; V100Q; V100S; V100A; V100G; V100N; V100D; V100E; V100K; V100R; L101N; L101E; L101Q; L101D; L101R; L101K; Y110Q; Y110E; Y110N; Y110D; Y110R; Y110K; Y110S; Y110A; Y110G; Y110T; G114K; G114R; G114Q; G114D; G114E; G114N; G114S; G114A; G114G; G114T; L115R; L115K; L115Q; L115N; L115D; L115E; L115S; L115A; L115G; L115T; A116D; A116E; A116R; A116K; A116Q; A116N; A116Y; A116H; V153Q; V153N; V153R; V153K; V153D; V153E; V153S; V153G; G155Q; R171D; R171E; R171Q; R171N; R171S; R171T; R171G; R171A; R171Y; Q227E; Q227R; Q227K; Q227D; Q227Y; Q227H; Q227G; Q227S; Q227T; Q230S; Q230K; Q230R; Q230N; Q230D; Q230E; Q230G; Q230S; Q230T; and Q230A, of which V153Q; Q227E; L101N; Y110Q; Q230K; and V100Q, are preferred.

In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of: A154D and V153Q; A154D and G155Q; A154D and Q227E; A154D and L101N; A154D and Y110Q; A154D and Q230K; A154D and V100Q; A154D and V100T; A154D and V100S; A154D and V100A; A154D and V100G; A154D and V100N; A154D and V100D; A154D and V100E; A154D and V100K; A154D and V100R; A154D and L101E; A154D and L101Q; A154D and L101D L101R; A154D and L101K; A154D and Y110E; A154D and Y110N; A154D and Y110D; A154D and Y110R; A154D and Y110K; A154D and Y110S; A154D and Y110A; A154D and Y110G; A154D and Y110T; A154D and G114K; A154D and G114R; A154D and G114Q; A154D and G114D; A154D and G114E; A154D and G114N; A154D and G114S; A154D and G114A; A154D and G114G; A154D and G114T; A154D and L115R; A154D and L115K; A154D and L115Q; A154D and L115N; A154D and L115D; A154D and L115E; A154D and L115S; A154D and L115A; A154D and L115G; A154D and L115T; A154D and A116D; A154D and A116E; A154D and A116R; A154D and A116K; A154D and A116Q; A154D and A116N; A154D and A116Y; A154D and A116H; A154D and V153N; A154D and V153R; A154D and V153K; A154D and V153D; A154D and V153E; A154D and V153S; A154D and V153G; A154D and R171D; A154D and R171E; A154D and R171Q; A154D and R171N; A154D and R171S; A154D and R171T; A154D and R171G; A154D and R171A; A154D and R171Y; A154D and Q227R; A154D and Q227K; A154D and Q227D; A154D and Q227Y; A154D and Q227H; A154D and Q227G; A154D and Q227S; A154D and Q227T; A154D and Q230S; A154D and Q230R; A154D and Q230N; A154D and Q230D; A154D and Q230E; A154D and Q230G; A154D and Q230S; A154D and Q230T; A154D and Q230A; A154E and V153Q; A154E and Q227E; A154E and L101N; A154E and Y110Q; A154E and Q230K; A154E and V100Q; A154E and V100T; A154E and V100S; A154E and V100A; A154E and V100G; A154E and V100N; A154E and V100D; A154E and V100E; A154E and V100K; A154E and V100R; A154E and L101E; A154E and L101Q; A154E and L101D L101R; A154E and L101K; A154E and Y110E; A154E and Y110N; A154E and Y110D; A154E and Y110R; A154E and Y110K; A154E and Y110S; A154E and Y110A; A154E and Y110G; A154E and Y110T; A154E and G114K; A154E and G114R; A154E and G114Q; A154E and G114D; A154E and G114E; A154E and

G114N; A154E and G114S; A154E and G114A; A154E and G114G; A154E and G114T; A154E and L115R; A154E and L115K; A154E and L115Q; A154E and L115N; A154E and L115D; A154E and L115E; A154E and L115S; A154E and L115A; A154E and L115G; A154E and L115T; A154E and A116D; A154E and A116E; A154E and A116R; A154E and A116K; A154E and A116Q; A154E and A116N; A154E and A116Y; A154E and A116H; A154E and V153N; A154E and V153R; A154E and V153K; A154E and V153D; A154E and V153E; A154E and V153S; A154E and V153G; A154E and R171D; A154E and R171E; A154E and R171Q; A154E and R171N; A154E and R171S; A154E and R171T; A154E and R171G; A154E and R171A; A154E and R171Y; A154E and Q227R; A154E and Q227K; A154E and Q227D; A154E and Q227Y; A154E and Q227H; A154E and Q227G; A154E and Q227S; A154E and Q227T; A154E and Q230S; A154E and Q230R; A154E and Q230N; A154E and Q230D; A154E and Q230E; A154E and Q230G; A154E and Q230S; A154E and Q230T; and A154E and Q230A.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 100, and wherein preferably the 4-1BBL ECD mutein comprises a V100T or a V100Q substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a V153Q substitution in combination with at least one, two, three, four or five amino acid substitutions selected from the group consisting of: V100T; L101N; Y110Q; G114K; L115R; A116D; R171D; Q227E; Q227R; Q230S; and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of: V153Q and V100T; V153Q and L101N; V153Q and Y110Q; V153Q and G114K; V153Q and L115R; V153Q and A116D; V153QV153Q and R171D; V153Q and Q227E; V153Q and Q227R; V153Q and Q230S; and, V153Q and Q230K.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 100, and wherein preferably the 4-1BBL ECD mutein comprises an L101N substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising an L101N substitution in combination with at least one, two, three, four or five amino acid substitutions selected from the group consisting of: Y110Q; G114K; L115R; A116D; R171D; Q227E; Q227R; Q230S; and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of L101N and Y110Q; L101N and G114K; L101N and L115R; L101N and A116D; L101N and R171D; L101N and Q227E; L101N and Q227R; L101N and Q230S; and, L101N and Q230K.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 100, and wherein preferably the 4-1BBL ECD mutein comprises a Y110Q substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a Y110Q substitution in

combination with at least one, two, three, four or five amino acid substitutions selected from the group consisting of: G114K; L115R; A116D; R171D; Q227E; Q227R; Q230S; and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of Y110Q and G114K; Y110Q and L115R; Y110Q and A116D; Y110Q and R171D; Y110Q and Q227E; Y110Q and Q227R; Y110Q and Q230S; and, Y110Q and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising the combination of substitutions Y110Q, A154D and Q227E.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 100, and wherein preferably the 4-1BBL ECD mutein comprises a G114K substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a G114K substitution in combination with at least one, two, three, four or five amino acid substitutions selected from the group consisting of: L115R; A116D; R171D; Q227E; Q227R; Q230S; and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of G114K and G114K; G114K and L115R; G114K and A116D; G114K and R171D; G114K and Q227E; G114K and Q227R; G114K and Q230S; and, G114K and Q230K.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 100, and wherein preferably the 4-1BBL ECD mutein comprises an L115R substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising an L115R substitution in combination with at least one, two, three, four or five amino acid substitutions selected from the group consisting of: A116D; R171D; Q227E; Q227R; Q230S; and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of L115R and A116D; L115R and R171D; L115R and Q227E; L115R and Q227R; L115R and Q230S; and, L115R and Q230K.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 100, and wherein preferably the 4-1BBL ECD mutein comprises an A116D substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising an A116D substitution in combination with at least one, two, three or four amino acid substitutions selected from the group consisting of: R171D; Q227E; Q227R; Q230S; and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of: A116D and R171D; A116D and Q227E; A116D and Q227R; A116D and Q230S; and, A116D and Q230K.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from

the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 100, and wherein preferably the 4-1BBL ECD mutein comprises an R171D substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising an R171D substitution in combination with at least one or two amino acid substitutions selected from the group consisting of: Q227E; Q227R; Q230S; and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of: R171D and Q227E; R171D and Q227R; R171D and Q230S; and, R171D and Q230K.

In one embodiment, a 4-1BBL ECD mutein provided herein comprises the amino acid sequence of SEQ ID NO: 49, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at position 100, and wherein preferably the 4-1BBL ECD mutein comprises a Q227E or Q227R substitution. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a Q227E substitution in combination with at least one amino acid substitution selected from Q230S and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of: Q227E and Q230S; and, Q227E and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a Q227R substitution in combination with at least one amino acid substitution selected from Q230S and Q230K. In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions selected from the group consisting of: Q227R and Q230S; and, Q227R and Q230K.

In one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions at positions in SEQ ID NO: 37, selected from the group consisting of: Y110Q, V153Q and Q227E; L101N, Y110Q and V153Q; V100Q, Y110Q and V153Q; L101N, V153Q and Q227E; Y110Q, A154D and Q227E; Y110Q and V153Q; V153Q and Q227E; Y110Q and Q227E; and, L101N and Q227E.

Substitutions to aspartate, such A154D, when followed (in an N- to C-terminal direction) by a G-, A- or S-residue, such as G155, can potentially introduce an aspartate isomerization site, which can lead to the instability and/or increased degradation rate of a mutein comprising such isomerization site. In one embodiment therefore, a substitution to aspartate, when followed by a G-, A- or S-residue, the substitution to aspartate is combined with a substitution of the subsequent G-, A- or S-residue to any amino acid residue other than G, A, S or T. Preferably the subsequent G-, A- or S-residue substituted to Q, N, Y, L, V, or F. Hence, in one embodiment, there is provided a 4-1BBL ECD mutein comprising a combination of substitutions at positions in SEQ ID NO: 37, selected from the group consisting of: Y110D and S111X; L115D and A116X; A116D and G117X; V153D and A154X; A154 D and G155X; R171D and S172X; and, Q230D andG231X, wherein X is any to any amino acid residue other than G, A, S or T, whereby preferably X is Q, E, N, D, H, K, R or Y. In a preferred embodiment, any 4-1BBL ECD mutein comprising the A154D substitution is combined with a G155X substitution, wherein X is any to any amino acid residue other than G, A, S or T, whereby preferably X is Q, E, N, D, H, K, R or Y, of which Q is most preferred.

In one embodiment, there is provided a 4-1BBL ECD mutein as structurally defined above, which 4-1BBL ECD mutein binds to its cognate receptor 4-1BB with a reduced affinity, relative to

the affinity of wild-type 4-1BBL ECD for the 4-1BB. In one embodiment, there is provided a 4-1BBL ECD mutein as structurally defined above, which 4-1BBL ECD mutein binds to its cognate receptor human 4-1BB with a reduced affinity, relative to the affinity of wild-type 4-1BBL ECD for the human 4-1BB, e.g. a human 4-1BB having an amino acid sequence comprised in SEQ ID NO: 175.

It is understood herein that a 4-1BBL ECD mutein with reduced affinity for its cognate receptor 4-1BB, as compared to a wild type 4-1BBL ECD mutein, will also have a reduced affinity for 4-1BB when present in a homotrimeric fusion protein comprising three 4-1BBL ECD mutein monomers connected through polypeptide linkers, e.g. $(GGGGS)_4$. Generally, the affinity of a 4-1BBL ECD mutein for its cognate receptor 4-1BB is herein defined as the affinity for 4-1BB as determined when the 4-1BBL ECD mutein is present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, whereby the homotrimer can be present in a conjugate with an antibody (e.g. trastuzumab), which conjugate can further comprise IL-21.

In one embodiment, there is provided a 4-1BBL ECD mutein having a with a reduced affinity for 4-1BB, relative to the affinity of wild-type 4-1BBL ECD, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: V100T; V100Q; L101N; Y110Q; G114K; V153Q; R171D; Q227E; Q227R; Q230S; Q230K; A116D; A154D; and A154E; or wherein the 4-1BBL ECD mutein comprises a combination of substitutions selected from the group consisting of: Y110Q, V153Q and Q227E; L101N, Y110Q and V153Q; V100Q, Y110Q and V153Q; L101N, V153Q and Q227E; Y110Q, A154D and Q227E; Y110Q and V153Q; V153Q and Q227E; A154D and G155Q; Y110Q and Q227E; and, L101N and Q227E.

The 4-1BBL ECD muteins provided herein bind to 4-1BB in a non-covalent and reversible manner. In one embodiment, the binding strength of a 4-1BBL ECD mutein to 4-1BB may be described in terms of its affinity, a measure of the strength of interaction between the binding site of the mutein and 4-1BB. In one embodiment, a 4-1BBL ECD mutein provided herein has a low-affinity for 4-1BB and thus will bind a lesser amount of 4-1BB than a wild type 4-1BBL. In one embodiment, a 4-1BBL ECD mutein provided herein has an equilibrium association constant, K_A, which is, with decreasing preference, at least 10^3 M^{-1},at least 10^4 M^{-1}, at least 10^5 M^{-1}, at least 10^6 M^{-1}, at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, or at least 10^{10} M^{-1}. As understood by the artisan of ordinary skill, K_A can be influenced by factors including pH, temperature and buffer composition.

In one embodiment, the binding strength of a 4-1BBL ECD mutein provided herein to 4-1BB may be described in terms of its affinity, i.e. K_D. K_D is the equilibrium dissociation constant, a ratio of k_{off}/k_{on}, between the 4-1BBL ECD mutein and 4-1BB. K_D and K_A are inversely related. The K_D value relates to the concentration of the mutein (the amount of mutein needed for a particular experiment or application) and so the lower the K_D value (lower concentration needed) the higher the affinity of the mutein. In one embodiment, the binding strength of a 4-1BBL ECD mutein provided herein to 4-1BB may be described in terms of K_D. In one embodiment, the K_D of a 4-1BBL ECD mutein provided herein is about 10^{-3} M, about 10^{-4} M, about 10^{-5} M, about 10^{-6} M, or less. In one embodiment, the K_D of a 4-1BBL ECD mutein provided herein is micromolar, nanomolar, or

picomolar. In one embodiment, the K_D of a 4-1BBL ECD mutein provided herein is within a range of about 10^{-3} to 10^{-4} M, about 10^{-4} to 10^{-5} M, or 10^{-5} to 10^{-6} M, or 10^{-7} to 10^{-8} M, 10^{-8} to 10^{-9} M. In one embodiment, a 4-1BBL ECD mutein provided herein binds to the human 4-1BB with a K_D that is greater than or is about 140 nM. In one embodiment, a 4-1BBL ECD mutein provided herein binds to the human 4-1BB with a K_D of about 100 nM to about 4,000 nM, 200 nM to 4,000 nM, 500 nM to 4,000 nM, 1,000 nM to 4,000 nM, 120 nM to 3,000 nM, 200 nM to 2,000 nM, 500 nM to 2,000 nM, or 1,000 nM to 2,000 nM.

In one embodiment, a 4-1BBL ECD mutein provided herein exhibits a reduction in binding affinity for human 4-1BB. In one embodiment, the 4-1BBL ECD mutein provided herein is a mutein that exhibits at least about a 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, 1000-fold, 2000-fold, 5000-fold or 10.000-fold reduction in binding affinity for 4-1BB, relative to, relative to the affinity of wild-type 4-1BBL ECD for 4-1BB.

In one embodiment, the binding affinity of a 4-1BBL ECD mutein provided herein for 4-1BB is determined by SPR, e.g. as described in the Examples herein. In one embodiment, the binding affinity of a 4-1BBL ECD mutein provided herein for 4-1BB, is thus determined when the 4-1BBL ECD mutein is present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with a monoclonal antibody (e.g. trastuzumab), which conjugate further comprises IL-21.

In one embodiment, a 4-1BBL ECD mutein provided herein exhibits a binding affinity for human 4-1BB expressed as pK_D, that is at least 0.4 lower than the pK_D of a wild-type 4-1BBL ECD for human 4-1BB. pK_D is understood herein to be $-\log_{10}(K_D)$. In one embodiment, the 4-1BBL ECD mutein having at least a 0.4 lower pK_D for human 4-1BB, relative to the affinity of a wild-type 4-1BBL ECD for human 4-1BB, is a 4-1BBL ECD mutein comprising at least one substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; V100T; and A116D; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; V100T; and A116D.

In one embodiment, a 4-1BBL ECD mutein provided herein exhibits a binding affinity for human 4-1BB expressed as pK_D, that is at least 0.5 lower than the pK_D of a wild-type 4-1BBL ECD for human 4-1BB. In one embodiment, the 4-1BBL ECD mutein having at least a 0.5 lower pK_D for human 4-1BB, relative to the pK_D of a wild-type 4-1BBL ECD for human 4-1BB, is a 4-1BBL ECD mutein comprising at least one substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; and V100T; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; Q230K; V100Q; and V100T.

In one embodiment, a 4-1BBL ECD mutein provided herein exhibits a in binding affinity for human 4-1BB expressed as pK_D, that is at least 1.0 lower than the pK_D of a wild-type 4-1BBL ECD

for human 4-1BB. In one embodiment, the 4-1BBL ECD mutein having at least a 1.0 lower pK_D for human 4-1BB, relative to the pK_D of a wild-type 4-1BBL ECD for human 4-1BB, is a 4-1BBL ECD mutein comprising at least one substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; Q230K; and V100Q; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; Q230K; and V100Q.

In one embodiment, a 4-1BBL ECD mutein provided herein exhibits a reduction in activity as measured by a suitable assay for measuring changes in a 4-1BB signaling pathway as described above, relative to the activity of wild-type 4-1BBL ECD under corresponding conditions. In one embodiment, a 4-1BBL ECD mutein provided herein exhibits at least about a 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, 1000-fold, 2000-fold, 5000-fold or 10.000-fold reduction in activity as measured by a 4-1BB signaling assay, relative to the activity of wild-type 4-1BBL ECD under corresponding conditions.

In one embodiment, a 4-1BBL ECD mutein provided herein exhibits at least about a 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, 1,000-fold, 2,000-fold, 5,000-fold, 10,000-fold reduction in activity as measured by a NK cell or γδ T cell proliferation assay, relative to the activity of wild-type 4-1BBL ECD under corresponding conditions. In one embodiment, the NK cell or γδ T cell proliferation assay is a short-term proliferation assay, measuring proliferation over the course of less than one week, e.g. 3, 4, 5 or 6 days, e.g. as described in the Examples herein. In one embodiment, the NK cell or γδ T cell proliferation assay is a long-term proliferation assay, measuring proliferation over the course of more than one week, e.g. at least 10, 12 or 14 days, e.g. as described in the Examples herein.

In one embodiment, a 4-1BBL ECD mutein provided herein, when present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with a monoclonal antibody against a TAA (e.g. trastuzumab), which conjugate further comprises IL-21, exhibits a pEC_{50} for induction of proliferation of NK cells in a 5-day NK cell proliferation assay in the presence of tumor cells expressing the TAA (e.g. SKOV-3 cells), that is not more than 0.25 log less than the pEC_{50} of a corresponding control conjugate comprising wild-type 4-1BBL ECD in the same assay. Hence, in one embodiment, the 4-1BBL ECD mutein comprises at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; Q227R; L101N; Y110Q; and V100Q; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; Q227R; L101N; Y110Q; and V100Q.

In one embodiment, a 4-1BBL ECD mutein provided herein, when present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with trastuzumab, which conjugate further comprises IL-21, exhibits a pEC_{50} for induction of proliferation of NK cells in a 5-

day NK cell proliferation assay in the presence of SKOV3 tumor cells, that is not more than 0.10 log less than the pEC_{50} of a corresponding control conjugate comprising wild-type 4-1BBL ECD in the same assay. Hence, in one embodiment, the 4-1BBL ECD mutein comprises at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; and V100Q; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; and V100Q.

In one embodiment, a 4-1BBL ECD mutein provided herein, when present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with trastuzumab, which conjugate further comprises IL-21, exhibits a pEC_{50} for induction of proliferation of NK cells in a 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, that is not more than 0.05 log less than the pEC_{50} of a corresponding control conjugate comprising wild-type 4-1BBL ECD in the same assay. Hence, in one embodiment, the 4-1BBL ECD mutein comprises at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; and V100Q; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; and V100Q.

In one embodiment, a 4-1BBL ECD mutein provided herein, when present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with trastuzumab, which conjugate further comprises IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is at least 70%, 75%, 80%, 85%, 90% or 95% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and a trimer of wild type 4-1BBL ECD in the same assay. Hence, in one embodiment, the 4-1BBL ECD mutein comprises at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; Q227R; L101N; Y110Q; Q230K; and V100Q; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; V153Q; Q227E; Q227R; L101N; Y110Q; Q230K; and V100Q.

In one embodiment, a 4-1BBL ECD mutein provided herein, when present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with trastuzumab, which conjugate further comprises IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is at least 75% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and a trimer of wild type 4-1BBL ECD in the same assay. Hence, in one embodiment, the 4-1BBL ECD mutein comprises at least one

amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; Q230K; and V100Q; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; Q230K; and V100Q.

In one embodiment, a 4-1BBL ECD mutein provided herein, when present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with trastuzumab, which conjugate further comprises IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is at least 80% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and a trimer of wild type 4-1BBL ECD in the same assay. Hence, in one embodiment, the 4-1BBL ECD mutein comprises at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; and Q230K; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; Q227E; L101N; Y110Q; and Q230K.

In one embodiment, a 4-1BBL ECD mutein provided herein, when present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with trastuzumab, which conjugate further comprises IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is at least 85% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and a trimer of wild type 4-1BBL ECD in the same assay. Hence, in one embodiment, the 4-1BBL ECD mutein comprises at least one amino acid substitution selected from the group consisting of: A154D; A154E; V153Q; and Q227E; or the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one amino acid substitution selected from the group consisting of: A154D; A154E; a combination of A154D and G155Q; V153Q; and Q227E.

In one embodiment, a 4-1BBL ECD mutein provided herein, when present in a homotrimeric fusion protein comprising three identical 4-1BBL ECD mutein monomers connected through $(GGGGS)_4$ polypeptide linkers, which homotrimer is present in a conjugate with trastuzumab, which conjugate further comprises IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is at least 95% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and a trimer of wild type 4-1BBL ECD in the same assay. Hence, in one embodiment, the 4-1BBL ECD mutein comprises at least one amino acid substitution selected from the group consisting of: A154D and A154E; a combination of A154D and G155Q; and the 4-1BBL ECD mutein comprises no other amino acid sequence

modification than the at least one amino acid substitution selected from the group consisting of: A154D; a combination of A154D and G155Q; and A154E.

In one embodiment, there is provided a 4-1BBL ECD mutein as described herein, which, when present in a conjugate with an antigen-binding protein that specifically binds a (target) antigen, as a result of the reduced affinity of the 4-1BBL ECD mutein for 4-1BB, produces reduced (little or no) agonist activity at a 4-1BB expressed at the surface of a cell, in the absence of the antigen or cells carrying the antigen. However, when the conjugate is bound to the antigen or to cells carrying the antigen, the 4-1BBL ECD mutein in the conjugate shows significant agonist activity. This activity is the result of the 4-1BBL ECD mutein being present in high local density on the surface of the target cells, which leads to an enhanced apparent affinity for the 4-1BB on local NK cells (see Figure 1) or γδ T cell, primarily through the mechanism of avidity. As such, a 4-1BBL ECD mutein as described herein in a conjugate with an antigen-binding protein broadens the therapeutic window as compared to a corresponding conjugate comprising a wild type 4-1BBL ECD. The term "therapeutic window" is herein understood as the ratio (or fold-difference) of the EC_{50} values obtained from a functional assay (e.g., a proliferation assay) comparing conditions in which cancer cells are absent to conditions in which they are present. A therapeutic window of 10 (or 1 log) would mean that the EC50 without cancer cells was 10 times higher (a less potent effect) as compared to when the cancer cells were present. Upon systemic administration the conjugate comprising the 4-1BBL ECD mutein will have little or no effect on cells in the periphery, including T-, B- or NK cells, while remaining effective in stimulating these immune cells, in particular NK cells or γδ T cell, at a tumor site or a site of infection or inflammation. This is because the target antigen bound by the antigen-binding protein is present at high local concentrations only in these specific areas, enabling the effect of avidity.

Thus, in one embodiment, there is provided an 4-1BBL ECD mutein as described herein, wherein the 4-1BBL ECD mutein, when present in a conjugate with an antigen binding protein that specifically binds an antigen, wherein the conjugate optionally further comprises IL-21, has an EC_{50} in an NK cell or γδ T cell proliferation assay in the presence of the antigen or cells expressing the antigen that is, with increasing preference, at least a factor 2, 5, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000 or 100,000 lower than the EC_{50} in a corresponding NK cell or γδ T cell proliferation assay in the absence of the antigen or cells expressing the antigen.

In one embodiment, the difference in induction of NK cell or γδ T cell proliferation between the presence and absence of the antigen is determined using a reference multispecific antigen binding protein, such as AVC1 and reference tumor cells such as SK-OV-3 cells expressing HER2. The AVC1 multispecific antigen binding protein consists of a first trastuzumab heavy chain fused to the 4-1BB ligand extracellular domain (SEQ ID NO: 11), a second trastuzumab heavy chain fused to wild type IL-21 (SEQ ID NO: 12) and trastuzumab light chains (SEQ ID NO: 2), wherein the constant regions of the first and second heavy chains are distinguished using knob-in-hole technology (see WO2024/056862). The wild type 4-1BBL ECD amino acid sequence in the first heavy chain amino acid sequence of SEQ ID NO: 11 can be replaced by an amino acid sequence

of an 4-1BBL ECD mutein to be assayed, e.g. for its ability to induce NK cell or γδ T cell proliferation in the presence and absence of tumor cells expressing HER2, such as the SK-OV-3 cells.

In one embodiment, there is provided an 4-1BBL ECD mutein as described herein, wherein the 4-1BBL ECD mutein, when present in a multispecific antigen binding protein consisting of i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 11, wherein the wild type 4-1BBL ECD amino acid sequence is replaced by the amino acid sequence of the 4-1BBL ECD mutein; ii) a second heavy chain comprising the amino acid sequence of SEQ ID NO: 12; and iii) light chains comprising the amino acid sequence of SEQ ID NO: 2, has an EC_{50} in an NK cell or γδ T cell proliferation assay in the presence of SK-OV-3 cells that is, with increasing preference, at least a factor 10, 25, 50, 100, 200, 500, 1,000, 2,000, 5,000, 10,000, 20,000, 50,000 or 100,000 lower than the EC_{50} in a corresponding NK cell or γδ T cell proliferation assay in the absence of the SK-OV-3 cells.

In one embodiment, there is provided an 4-1BBL ECD mutein as described herein, wherein the 4-1BBL ECD mutein, when present in a multispecific antigen binding proteins consisting of i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 11, wherein the wild type 4-1BBL ECD amino acid sequence is replaced by the amino acid sequence of the 4-1BBL ECD mutein; ii) a second heavy chain comprising the amino acid sequence of SEQ ID NO: 12; and iii) light chains comprising the amino acid sequence of SEQ ID NO: 2, produces a fold-difference in EC_{50} in an NK cell or γδ T cell proliferation assay in the presence vs absence of SK-OV-3 cells that is, with increasing preference, at least a factor 2, 5, 10, 20, 50, 100, 200, 500, 1,000, 2,000 or 5,000 higher than the difference in EC_{50} in an NK cell or γδ T cell proliferation assay in the presence vs absence of SK-OV-3 cells as produced by a reference multispecific antigen binding proteins consisting of i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 11; a second heavy chain comprising the amino acid sequence of SEQ ID NO: 12; and iii) light chains comprising the amino acid sequence of SEQ ID NO: 2.

In the above embodiments, the NK cell or γδ T cell proliferation assays are preferably performed using NK cells isolated from healthy donors. In the above embodiments, the EC_{50} values in the NK cell or γδ T cell proliferation assays are preferably determined on the basis of the average values using NK cells or γδ T cells isolated from at least 5 different healthy donors. In the above embodiments, the NK cell or γδ T cell proliferation assays are preferably performed essentially as described in the examples herein.

The reduced affinity of the 4-1BBL ECD muteins described herein when present in a conjugate with an antigen binding protein increases the therapeutic window as compared to a corresponding conjugate comprising a wild type 4-1BBL ECD, while at the same time the ability of a conjugate with an 4-1BBL ECD mutein to induce NK cell or γδ T cell cytotoxicity against cells carrying the antigen that is bound by the antigen binding protein, preferably remains essentially unaffected.

Hence, in one embodiment, there is provided an 4-1BBL ECD mutein as described herein, wherein the 4-1BBL ECD mutein, when present in a multispecific antigen binding protein consisting of i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 11, wherein the wild

type 4-1BBL ECD amino acid sequence is replaced by the amino acid sequence of the 4-1BBL ECD mutein; ii) a second heavy chain comprising the amino acid sequence of SEQ ID NO: 12; and iii) light chains comprising the amino acid sequence of SEQ ID NO: 2, has an EC_{50} in an NK cell or γδ T cell cytotoxicity assay in the presence of SK-OV-3 cells that is, with increasing preference, at least equal to, or at least 2-fold, at least 5-fold or at least 10-fold higher than the EC_{50} of a reference multispecific antigen binding protein consisting of i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 11; ii) a second heavy chain comprising the amino acid sequence of SEQ ID NO: 12; and iii) light chains comprising the amino acid sequence of SEQ ID NO: 2, in the same assay. The NK cell or γδ T cell cytotoxicity assay in the presence of SK-OV-3 cells preferably performed using NK cells or γδ T cells isolated from healthy donors. Preferably, the EC_{50} values in the NK cell or γδ T cell cytotoxicity assays are determined on the basis of the average values using NK cells or γδ T cells isolated from at least 5 different healthy donors. In the above embodiments, the NK cell or γδ T cell cytotoxicity assays are preferably performed essentially as described in the examples herein.

Also the ability of a conjugate between an 4-1BBL ECD mutein as described herein and an antigen binding protein to support long-term expansion of NK cells or γδ T cells in the presence of cells carrying the antigen that is bound by the antigen binding protein, preferably remains essentially unaffected.

Hence, in one embodiment, there is provided an 4-1BBL ECD mutein as described herein, wherein the 4-1BBL ECD mutein, when present in a multispecific antigen binding protein consisting of i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 11, wherein the wild type 4-1BBL ECD amino acid sequence is replaced by the amino acid sequence of the 4-1BBL ECD mutein; ii) a second heavy chain comprising the amino acid sequence of SEQ ID NO: 12; and iii) light chains comprising the amino acid sequence of SEQ ID NO: 2, induces a fold expansion of NK cells in the presence of SK-OV-3 cells in an NK cell or γδ T cell expansion assay, that is, with increasing preference, equal to, or at least 2-fold, at least 5-fold or at least 10-fold higher than the fold expansion induced by a reference multispecific antigen binding protein consisting of i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 11; ii) a second heavy chain comprising the amino acid sequence of SEQ ID NO: 12; and iii) light chains comprising the amino acid sequence of SEQ ID NO: 2, in the same assay. The NK cell or γδ T cell expansion assay in the presence of SK-OV-3 cells preferably performed using NK cells or γδ T cells isolated from healthy donors. Preferably, the fold expansion of NK cells or γδ T cells in the assays is determined on the basis of the average values using NK cells or γδ T cells isolated from at least 5 different healthy donors. In the above embodiments, the NK cell or γδ T cell expansion assays are preferably performed essentially as described in the examples herein.

A further advantage of the 4-1BBL ECD muteins as described herein is that their reduced affinity for 4-1BB the improves pharmaco-kinetics of therapeutics comprising the 4-1BBL ECD muteins. In the body many cells, including T-, B- or NK cells, are present that express 4-1BB molecules at their surfaces. These 4-1BB molecules act as a sink for therapeutics comprising a moiety with affinity for 4-1BB such as a 4-1BBL ECD mutein. Upon binding to a surface expressed

4-1BB, the therapeutic comprising the 4-1BBL-moiety will be internalized and will therefore no longer be available for exerting its therapeutic effect, e.g. in the tumor microenvironment. Hence, the reduced affinity for 4-1BB of the 4-1BBL ECD muteins as described herein reduces or prevents their disappearance in this sink and thereby improves pharmaco-kinetics of therapeutics comprising the 4-1BBL ECD muteins.

In a further aspect, the present disclosure provides a trimeric fusion protein comprising three 4-1BBL ECD monomers, wherein one, two, or three of the monomers are a 4-1BBL ECD mutein as described herein above, fused together in a single polypeptide chain, as e.g. described in Fellermeier et al. (2016, *supra*). In one embodiment, the three 4-1BBL ECD monomers are connected by polypeptide linkers. In one embodiment, the three 4-1BBL ECD monomers are connected by polypeptide linkers selected from the group consisting of $(GGGGS)_1$, $(GGGGS)_2$, $(GGGGS)_3$, $(GGGGS)_4$, $(GGGGS)_5$, GGGSGGG, GGSGGGGSGG and G, of which $(GGGGS)_4$ is preferred. Other suitable flexible polypeptide linker(s) are described herein below. In one embodiment, two or three of the 4-1BBL ECD mutein monomers in the trimeric fusion protein are identical muteins. In one embodiment, two or three monomers of the 4-1BBL ECD mutein in the trimeric fusion protein are different muteins. In the trimeric fusion protein, the 4-1BBL ECD monomer that is not a 4-1BBL ECD mutein as described herein above, can be a wild type 4-1BBL ECD monomer, or a 4-1BBL ECD mutein that is not described herein.

In one embodiment, the present disclosure provides a trimeric fusion protein comprising three monomers of a 4-1BBL ECD mutein as described herein above, fused together in a single polypeptide chain, as e.g. described in Fellermeier et al. (2016, *supra*). In one embodiment, the three 4-1BBL ECD monomers are connected by polypeptide linkers. In one embodiment, the three 4-1BBL ECD monomers are connected by polypeptide linkers selected from the group consisting of $(GGGGS)_1$, $(GGGGS)_2$, $(GGGGS)_3$, $(GGGGS)_4$, $(GGGGS)_5$, GGGSGGG, GGSGGGGSGG and G, of which $(GGGGS)_4$ is preferred. Other suitable flexible polypeptide linker(s) are described herein below. In one embodiment, the three monomers of the 4-1BBL ECD mutein in the trimeric fusion protein are identical muteins. In one embodiment, at least two of the three monomers of the 4-1BBL ECD mutein in the trimeric fusion protein are different muteins.

In one embodiment, a fusion protein comprising three monomers of a 4-1BBL ECD mutein, preferably a 4-1BBL ECD mutein as described herein, fused together in a single polypeptide chain comprise three identical monomers of the 4-1BBL ECD mutein. In another embodiment of the fusion protein at least one of the monomers differs from the other two, or all three monomers differ from each other.

It is to be understood herein that when reference is made to a 4-1BBL ECD mutein as described herein this can also refer to a trimeric fusion protein comprising three monomers of such 4-1BBL ECD muteins.

Conjugates comprising a 4-1BBL ECD mutein

In a second aspect, the present disclosure provides a conjugate comprising one or more of the 4-1BBL ECD muteins as described herein conjugated to a heterologous moiety. It is understood

herein that the term “a conjugate comprising a 4-1BBL ECD mutein as described herein” also includes a conjugate comprising a trimeric fusion protein comprising three 4-1BBL ECD muteins as described herein. As used herein, the term "heterologous moiety" is synonymous with the term "conjugate moiety" and refers to any molecule (chemical or biochemical, naturally-occurring or non-coded) which is different from the 4-1BBL ECD muteins described herein. Exemplary conjugate moieties that can be linked to any of the 4-1BBL ECD muteins described herein include but are not limited to a heterologous peptide or polypeptide (including for example, an immunoglobulin or portion thereof (e.g., variable region, CDR, or Fc region)), a targeting agent, a diagnostic label such as a radioisotope, fluorophore or enzymatic label, a polymer including water soluble polymers, or other therapeutic or diagnostic agents. In some embodiments, a conjugate is provided comprising a 4-1BBL ECD mutein of the present disclosure and an immunoglobulin. The conjugate in some embodiments comprises one or more of the 4-1BBL ECD muteins described herein and one or more of: a peptide or polypeptide (which is distinct from the 4-1BBL ECD muteins described herein), a nucleic acid molecule, an antibody or fragment thereof, a polymer, a quantum dot, a small molecule, a toxin, a diagnostic agent, a carbohydrate, an amino acid.

In one embodiment, there is provided a conjugate wherein the heterologous moiety is attached via non-covalent or covalent bonding to the 4-1BBL ECD mutein as described herein. In exemplary embodiments, the linkage between the 4-1BBL ECD mutein and the heterologous moiety is achieved via covalent chemical bonds, e.g., peptide bonds, disulfide bonds, and the like, or via physical forces, such as electrostatic, hydrogen, ionic, van der Waals, or hydrophobic or hydrophilic interactions. A variety of non-covalent coupling systems may be used, including, e.g., biotin-avidin, ligand/receptor, enzyme/substrate, nucleic acid/nucleic acid binding protein, lipid/lipid binding protein, cellular adhesion molecule partners; or any binding partners or fragments thereof which have affinity for each other.

In one embodiment, there is provided a conjugate wherein the 4-1BBL ECD mutein as described herein is linked to a conjugate moiety via direct covalent linkage by reacting targeted amino acid residues of the 4-1BBL ECD mutein with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of these targeted amino acids. Reactive groups on the 4-1BBL ECD mutein or conjugate moiety include, e.g., an aldehyde, amino, ester, thiol, a-haloacetyl, maleimido or hydrazino group. Derivatizing agents include, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride or other agents known in the art. Alternatively, the conjugate moieties can be linked to the 4-1BBL ECD mutein indirectly through intermediate carriers, such as polysaccharide or polypeptide carriers. Examples of polysaccharide carriers include aminodextran. Examples of suitable polypeptide carriers include polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, e.g., serines, to confer desirable solubility properties on the resultant loaded carrier.

Cysteinyl residues are most commonly reacted with a-haloacetates (and corresponding amines), such as chloroacetic acid, chloroacetamide to give carboxymethyl or carboxyamidomethyl

derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, alpha-bromo- β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7- nitrobenzo-2-oxa-l ,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

Lysinyl and amino-terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4-pentanedione, and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R-N=C=N-R'), where R and R are different alkyl groups, such as l-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or l-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide.

Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), deamidation of asparagine or glutamine, acetylation of the N- terminal amine, and/or amidation or esterification of the C-terminal carboxylic acid group.

Another type of covalent modification involves chemically or enzymatically coupling glycosides to the 4-1BBL ECD mutein. Sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO87/05330 published 11 Sep. 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

In one embodiment, the heterologous moiety is attached to the 4-1BBL ECD mutein as described herein via a linker. In some aspects, the linker comprises a chain of atoms from 1 to about 60, or 1 to 30 atoms or longer, 2 to 5 atoms, 2 to 10 atoms, 5 to 10 atoms, or 10 to 20 atoms long. In some embodiments, the chain atoms are all carbon atoms. In some embodiments, the chain atoms in the backbone of the linker are selected from the group consisting of C, O, N, and S. Chain atoms and linkers may be selected according to their expected solubility (hydrophilicity) so as to provide a more soluble conjugate. In some embodiments, the linker provides a functional group that is subject to cleavage by an enzyme or other catalyst or hydrolytic conditions found in the target tissue or organ or cell. In some embodiments, the length of the linker is long enough to reduce the potential for steric hindrance. If the linker is a covalent bond or a peptidyl bond and the conjugate is a polypeptide, the entire conjugate can be a fusion protein. Such peptidyl linkers may be any length. Exemplary peptidyl linkers are from about 1 to 50 amino acids in length, 5 to 50, 3 to 5, 5 to 10, 5 to 15, or 10 to 30 amino acids in length, and are flexible or rigid. Flexible linkers are usually applied when the joined domains require a certain degree of movement or interaction. They are generally composed of small, non-polar (e.g. Gly) or polar (e.g. Ser or Thr) amino acids. The small size of these amino acids provides flexibility and allows for mobility of the connecting functional domains. The incorporation of Ser or Thr can maintain the stability of the linker in aqueous solutions by forming hydrogen bonds with the water molecules, and therefore reduces the unfavorable interaction between the linker and the protein moieties. Preferred flexible linkers have sequences consisting primarily of stretches of Gly and Ser residues (“GS” linker). An example of preferred (and widely used) flexible linker has the sequence of $(GGGGS)_n$ (SEQ ID NO: 30). By adjusting the copy number “n”, the length of this GS linker can be optimized to achieve appropriate separation of the functional domains, or to maintain necessary inter-domain interactions. The copy number “n” of this GS linker can e.g. be 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Specific examples of GS linkers include $(GGGGS)_4$ (SEQ ID NO: 31), GGGSGGG (SEQ ID NO: 32), GGSGGGGSGG (SEQ ID NO: 33) and G. Besides the GS linkers, many other flexible linkers have been designed for recombinant fusion proteins. These flexible linkers are also rich in small or polar amino acids such as Gly and Ser, but can contain additional amino acids such as Thr and Ala to maintain flexibility, as well as polar amino acids such as Lys and Glu to improve solubility, such as e.g. the flexible linkers KESGSVSSEQLAQFRSLD (SEQ ID NO: 34) and EGKSSGSGSESKST (SEQ ID NO: 35), that have been applied for the construction of a bioactive scFvs.

In one embodiment, the conjugate is a conjugate that has a 4-1BBL ECD mutein-valency that is higher than one that is higher than one, which understood to mean that the conjugate comprises more than one, e.g. two, three, four or five 4-1BBL ECD mutein-moieties. Preferably, more than one 4-1BBL ECD mutein-moieties are identical 4-1BBL ECD mutein-moieties.

In one embodiment, the conjugate is a conjugate that has a valency of trimeric 4-1BBL ECD mutein-fusion proteins that is higher than one that is higher than one, which understood to mean that the conjugate comprises more than one, e.g. two, three, four or five of such trimeric 4-1BBL ECD mutein-fusion proteins. Preferably, more than one trimeric 4-1BBL ECD mutein-fusion proteins are identical trimeric fusion proteins.

Thus, in one embodiment, there is provided a conjugate wherein the heterologous moiety comprises a polypeptide. The polypeptide comprised in the heterologous moiety preferably is a polypeptide distinct from any of the 4-1BBL ECD muteins described herein. In one embodiment, the conjugate is a fusion polypeptide, fusion protein, a chimeric protein or chimeric polypeptide comprising a 4-1BBL ECD mutein or a trimeric 4-1BBL ECD mutein-fusion protein as described herein and an heterologous moiety comprises a polypeptide fused in a single polypeptide chain. Additional descriptions of such conjugates as fusion proteins are provided hereinbelow.

In one embodiment, there is provided a conjugate wherein the heterologous moiety comprises a polypeptide that is an antigen-binding protein or a polypeptide chain of an antigen-binding protein.

In one embodiment, there is provided a conjugate wherein the heterologous moiety comprises an antigen-binding protein or a polypeptide chain of an antigen-binding protein, which antigen-binding protein comprises at least one of: a) at least one of: i) a first antigen-binding region that specifically binds a tumor associated antigen (TAA), that specifically binds an NK cell activating receptor or that specifically binds an epitope of a γδ T cell receptor (TCR), and ii) a second antigen-binding region that specifically binds a TAA, that specifically binds an NK cell activating receptor or that specifically binds an epitope of a γδ TCR; and, b) a third antigen-binding region that has or can have affinity for a surface antigen expressed on natural killer (NK) cells.

An antigen-binding region as used in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, can be derived from any of a variety of immunoglobulin or non-immunoglobulin scaffolds, for example affibodies based on the Z-domain of staphylococcal protein A, engineered Kunitz domains, monobodies or adnectins based on the 10th extracellular domain of human fibronectin III, anticalins derived from lipocalins, DARPins (designed ankyrin repeat domains), Affilins, multimerized LDLR-A module, avimers or cysteine-rich knottin peptides. See, e.g., Gebauer and Skerra (2009) *Current Opinion in Chemical Biology* 13:245-255, the disclosure of which is incorporated herein by reference.

In a preferred embodiment, an antigen-binding region as used in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises or consists of an immunoglobulin variable region. Such immunoglobulin variable regions can comprise or consist of variable domains that are commonly derived from antibodies (immunoglobulin chains), e.g. in the form of associated V_L and V_H domains found on two polypeptide chains, such as present in a Fab. Alternatively, immunoglobulin variable domains can comprise or consist of a single chain antigen-binding domain such as a scFv, a V_H domain, a V_L domain, or an immunoglobulin single variable domain (ISVD) such as a dAb, a V-NAR domain or a V_HH domain. An immunoglobulin variable region to be used in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein can be a human or humanized immunoglobulin variable region or an immunoglobulin single variable domain as herein defined above.

Antigen-binding regions that specifically bind tumor associated antigens

Thus, in one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one of i) a first antigen-binding region that specifically binds a TAA; and, ii) a second antigen-binding region that specifically binds a TAA. In one embodiment, the antigen-binding region that binds a TAA is an antigen-binding region derived from immunoglobulin or non-immunoglobulin scaffolds as defined above. Preferably, the antigen-binding region that specifically binds a TAA comprises or consists of at least one immunoglobulin variable domain. More preferably, the antigen-binding region that specifically binds a TAA comprises or consists of a Fab that specifically binds a TAA or an immunoglobulin single variable domain (ISVD) that specifically binds a TAA. In one embodiment, the antigen-binding region that specifically binds a TAA is an antigen-binding region that binds the TAA with a K_D value of no more than 10^{-4} M, as may be determined as herein described above.

In one embodiment, the antigen-binding region that specifically binds a TAA comprises or consists of a human or humanized immunoglobulin variable region or immunoglobulin single variable region as herein defined above.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises two antigen-binding regions that specifically bind a TAA, i.e. a first and a second antigen-binding region. In an antigen binding protein that comprises two antigen-binding regions that specifically bind a TAA, the two antigen-binding regions can bind one and the same TAA, they can bind at least two different TAAs, or they can bind at least two different epitopes on the same TAA. In one embodiment of an antigen binding protein that comprises two antigen-binding regions that specifically bind a TAA, the two antigen-binding regions are identical. Thus, as regards the two antigen-binding regions that specifically bind a TAA, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein can be a homodimeric or a heterodimeric antigen binding protein.

As used herein, the term tumor-associated antigen (TAA) refers to an antigen that is differentially expressed by cancer/tumor cells as compared to normal, i.e. non-tumoral cells. Alternatively, a TAA can be an antigen that is expressed by non-tumoral cells (e.g. immune cells) having a pro-tumoral effect (e.g. an immunosuppressive effect), and can thereby be exploited in order to target cancer cells. A TAA can thus be any antigen that potentially stimulates apparently tumor-specific immune responses. Some of these antigens are encoded, although not necessarily expressed, or expressed at lower levels or less frequently, by normal cells. These antigens can be characterized as those which are normally silent (i.e., not expressed) in normal cells, those that are expressed only at certain stages of differentiation and those that are temporally expressed such as embryonic and fetal antigens. Other TAAs are encoded by mutant cellular genes, such as oncogenes (e.g., activated ras oncogene), suppressor genes (e.g., mutant p53), fusion proteins resulting from internal deletions or chromosomal translocations, including neo-antigens. Still other TAAs antigens can be encoded by viral genes such as those carried on RNA and DNA tumor viruses. Still other TAAs can be expressed on immune cells capable of contributing to or mediating

a pro-tumoral effect, e.g. cell that contributes to immune evasion, a monocyte or a macrophage, optionally a suppressor T cell, regulatory T cell, or myeloid-derived suppressor cell.

The TAAs are usually normal cell surface antigens which are either overexpressed or expressed at abnormal times or are expressed by a targeted population of cells. Ideally the target TAA is expressed only on proliferative cells (e.g., tumor cells) or pro-tumoral cells (e.g. immune cells having an immunosuppressive effect), however this is rarely observed in practice. As a result, target antigens are in many cases selected on the basis of differential expression between proliferative/disease tissue and healthy tissue.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one antigen-binding region that specifically binds to a TAA selected from the group consisting of: 5T4, ADAM9, ADAM10, ADAM12, ALK, ALPP, ALPP2, ALPPL2, AMHR2, ANGPT2, AXL, Angiopoietin-2, Apelin receptor, B7-H3, B7-H4, B7-H6, B7.1, B7.2, BCMA, BTLA, CA125, CA9, CAIX, CCL2, CCR2, CCR4, CCR5, CCR6, CCR7, CD123, CD133, CD138, CD142, CD147, CD166, CD171, CD19, CD2, CD20, CD205, CD22, CD228, CD24, CD248, CD25, CD27, CD276, CD3, CD30, CD317, CD33, CD37, CD38, CD3E, CD4, CD40, CD44, CD44v6, CD45, CD46, CD47, CD52, CD56, CD70, CD71, CD73, CD74, CD79, CD79B, CD80, CD80/CD86, CDCP1, CDH3, CDH6, CDK4, CEA, CEACAM5, CLDN18, CLEC14A, CLEC4, CSF1R, CSF2, CSPG4, CT-7, CTGF, CTLA4, Cadherin 17, Cadherin 6, CanAg, Claudin 18.2, Claudin 6, Connexin 37, Cripto-1, Crypto, DC3, DKK1, DLK1, DLL3, DLL4, DR5, E-cadherin, E-selectin, EBV-encoded nuclear antigen (EBNA)-I, EDA, EDB, EDNRB, EGF, EGFL7, EGFR, EGFRvIII, ENG, EPCAM, EPHA4, EphA10, EphA2, EphA3, EphB2, EphB4, ExtradomainB (EDB) fibronectin, F3, FAP, FCGR1, FGFR2, FGFR2b, FGFR3, FGFR4, FLT1, FN, FOLH1, FOLR1, FRα, FSHR, FcRL5/FcRH5, Fibronectin extra-domain B, Flt3, Fucosyl, GFRa4, GM3, GPCR5D, GPNMB, GPRC5D, GRP78, GUCY2C, Glycoprotein NMB, Glypican 1, Glypican 2, Glypican 3, GnT-V, HAVCR2, HER-3/ERBB3, HER-4/ERBB4, HER2, HER3, HGF, HLA-G, HSP70, ICAM-1, ICOS, IFNG, IGF-1R, IGF1, IGF1R, IGF2, IL-1 accessory protein, IL-6 receptor, IL-8 receptor, IL13Ra2, IL17A, IL1A, IL1B, IL1RAP, IL2RA, IL3RA, IL6, IL6R, ITGAV, ITGB6, Ig-idiotype, Integrin beta 6, KAAG-1, KDR, KIRD2, KIT, KLK2, KLRC1, Killer Ig-Like Receptor, Killer Ig-Like Receptor 3DL2 (KIR3DL2), L1-CAM, L1CAM, LAG3, LAGE-1, LGR5, LIF, LIV-1, LOXL2, LRRC32, Lewis-Y, MART-1/Melan-A, MET, MIC-A/B, MICB, MIF, MISIIR, MMP2, MS4A1, MSLN, MST1R, MSTN, MUC1, MUC1-C, MUC16, MUM-1, Melanotransferrin, Mesothelin, Mud 6, NAG, NCAM1, NKG2D, NOTCH1, NOTCH2, NOTCH3, NRP1, NT5E, NTRKR1 (EC 2.7.10.1), NaPi2b, Nectin-4, OLR1, OX40, P-cadherin, P1A, PCSK9, PD-L1, PD1, PDGF, PDGF alpha receptor, PDGF beta receptor, PDGFR, PDGFRA, PLAUR, PMEL, PRAME, PSCA, PSMA, PTK7, PTPRC, PVRL4, Plexin-A1, RAGE, ROBO1, ROR1, ROR2, RSPO3, SCP-1, SEZ6, SIRPA, SLAMF7, SLC34A2, SLC3A2, SSTR2, SSX-1, SSX-2 (HOM-MEL-40), SSX-4, SSX-5, STEAP1, STEAP2, Severe, T-cell receptor/CD3-zeta chain, TACSTD2, TGF-alpha, TGFB1, TGFB2, TGFB3, TIGIT, Tissue factor/TF, TLR2, TM4SF1, TMEFF2, TNFRSF10B, TNFRSF17, TNFRSF18, TNFRSF4, TNFRSF8, TNFRSF9, TNFSF11, TNFSF13B, TPBG, TRAILR1, TRAILR2, TROP2, TSHR, TYRP1, VEGF, VEGFA, VEGFR1, VEGFR2, VH1/VL1, VH2/VL2, VH3/VL3, a GAGE-tumor antigen, a GD2

ganglioside, a GM2 ganglioside, a RAET1 protein, a UL16-binding protein (ULBP), a heterodimeric receptor comprised of at least one HER subunit, a human papillomavirus protein, a5β1 integrins, a5β3 integrins, adenomatous polyposis coli protein (APC), adenosine deaminase-binding protein (ADAbp), anti-Müllerian hormone Type II receptor, avB3 integrin, avB6 integrin, avβ6 integrins, bivalent, brain glycogen phosphorylase, c-erbB-2, cMET, colorectal associated antigen (CRC)-C017-1A/GA733, gastrin releasing peptide receptor antigen, gp100, gp75, gpA33, hCG, human papillomavirus protein, integrin receptors, mmp9, muc17, p15, phosphatidylserine, prostate specific antigen (PSA), protein tyrosine kinase 7(PTK7), receptor protein tyrosine kinase 3 (TYRO-3), sVE-cadherin, scatter factor receptor kinase, trivalent, α-catenin, α-fetoprotein, αIIbβ3-integrins, β-catenin, and γ-catenin, although this is not intended to be exhaustive.

In one embodiment therefore, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a combination of complementarity-determining regions (CDRs) CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of: a) the CDR-H1 (SEQ ID NO: 24), CDR-H2 (SEQ ID NO: 25) and CDR-H3 (SEQ ID NO: 26) sequences as comprised in SEQ ID NO: 1, and the CDR-L1 (SEQ ID NO: 27), CDR-L2 (SEQ ID NO: 28) and CDR-L3 (SEQ ID NO: 29) sequences as comprised in SEQ ID NO: 2 (trastuzumab); b) the CDR-H1 (SEQ ID NO: 150), CDR-H2 (SEQ ID NO: 151) and CDR-H3 (SEQ ID NO: 152) sequences as comprised in SEQ ID NO: 59, and the CDR-L1 (SEQ ID NO: 153), CDR-L2 (SEQ ID NO: 154) and CDR-L3 (SEQ ID NO: 155) sequences as comprised in SEQ ID NO: 60 (atezolizumab); c) the CDR-H1 (SEQ ID NO: 156), CDR-H2 (SEQ ID NO: 157) and CDR-H3 (SEQ ID NO: 158) sequences as comprised in SEQ ID NO: 9, and the CDR-L1 (SEQ ID NO: 159), CDR-L2 (SEQ ID NO: 160) and CDR-L3 (SEQ ID NO: 161) sequences as comprised in SEQ ID NO: 10 (avelumab); d) the CDR-H1 (SEQ ID NO: 162), CDR-H2 (SEQ ID NO: 163) and CDR-H3 (SEQ ID NO: 165) sequences as comprised in SEQ ID NO: 61, and the CDR-L1 (SEQ ID NO: 165), CDR-L2 (SEQ ID NO: 166) and CDR-L3 (SEQ ID NO: 167) sequences as comprised in SEQ ID NO: 62 (durvalumab); e) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 3, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 4 (cetuximab); f) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 5, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 6 (rituximab); g) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 7, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 8 (daratumumab); h) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 63, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 64 (cosibelimab); i) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 65, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 66 (margetuximab); j) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 67, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 68 (pertuzumab); k) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 69, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 70 (enoblituzumab); l) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 71, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 72

(necitumumab); m) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 73, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 74 (panitumumab); n) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 75, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 76 (amivantamab EGFR-binding); o) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 77, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 78 (amivantamab cMet-binding); p) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 79, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 80 (zolbetuximab); q) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 81, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 82 (dinutuximab); r) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 83, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 84 (naxitamab); s) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 85, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 86 (enfortumab); t) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 87, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 88 (farletuzumab); u) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 89, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 90 (tisotumab); v) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 91, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 92 (mirvetuximab); w) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 93, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 94 (sacituzumab); x) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 95, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 96 (vobramitamab); y) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 97, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 98 (Onartuzumab); z) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 144, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 145 (sibrotuzumab) aa) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 100, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 101 (olaratumab); ab) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 102, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 103 (rovalpituzumab); ac) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 238, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 239 (adebrelimab); ad) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 240, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 241 (alemtuzumab); ae) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 242, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 243 (belantamab); af) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 244, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 245 (Bevacizumab); ag) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO:

246, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 247 (brentuximab); ah) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 248, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 249 (camrelizumab); ai) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 250, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 251 (cemiplimab); aj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 252, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 253 (dostarlimab); ak) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 254, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 255 (emapalumab); al) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 256, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 257 (enlonstobart); am) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 258, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 259 (gemtuzumab); an) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 260, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 261 (ibritumomab); ao) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 262, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 263 (inotuzumab); ap) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 264, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 265 (ipilimumab); aq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 266, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 267 (isatuximab); ar) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 268, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 269 (loncastuximab); as) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 270, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 271 (mogamulizumab); at) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 272, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 273 (moxetumomab); au) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 274, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 275 (nimotuzumab); av) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 276, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 277 (nivolumab); aw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 278, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 279 (obinutuzumab); ax) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 280, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 281 (ofatumumab); ay) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 282, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 283 (pembrolizumab); az) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 284, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 285 (Penpulimab); ba) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 286, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 287

(polatuzumab); bb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 288, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 289 (prolgolimab); bc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 290, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 291 (pucotenlimab); bd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 292, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 293 (racotumomab) be) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 294, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 295 (ramucirumab); bf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 296, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 297 (relatlimab); bg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 298, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 299 (retifanlimab); bh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 300, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 301 (ripertamab); bi) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 302, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 303 (serplulimab); bj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 304, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 305 (sintilimab); bk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 306, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 307 (socazolimab); bl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 308, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 309 (sugemalimab); bm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 310, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 311 (tafasitamab); bn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 312, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 313 (tagitanlimab); bo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 314, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 315 (tebentafusp); bp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 316, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 317 (Tislelizumab); bq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 318, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 319 (Toripalimab); br) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 320, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 321 (zuberitamab); bs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 322, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 323 (benmelstobart); bt) bu) bv) bw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 324, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 325 (iparomlimab); bx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 326, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 327 (tuvonralimab); by) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 328, and the CDR-L1, CDR-L2

and CDR-L3 sequences as comprised in SEQ ID NO: 329 (anvatabart); bz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 330, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 331 (apamistamab); ca) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 332, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 333 (bemarituzumab); cb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 334, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 335 (cetrelimab); cd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 336, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 337 (cobolimab); ce) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 338, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 339 (datopotamab); cf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 340, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 341 (domvanalimab); cg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 342, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 343 (emactuzumab); ch) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 344, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 345 (favezelimab); ci) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 346, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 347 (felzartamab); cj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 348, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 349 (fianlimab); ck) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 350, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 351 (finotonlimab); cl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 352, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 353 (geptanolimab); cm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 354, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 355 (gotistobart); cn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 356, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 357 (ivuxolimab); co) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 358, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 359 (lemzoparlimab); cp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 360, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 361 (luveltamab); cq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 362, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 363 (magrolimab); cr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 364, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 365 (mecbotamab); cs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 366, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 367 (monalizumab); ct) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 368, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 369 (nofazinlimab); cu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 370, and the CDR-L1, CDR-L2 and CDR-L3

sequences as comprised in SEQ ID NO: 371 (nurulimab); cv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 372, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 373 (ociperlimab); cw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 374, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 375 (oleclumab); cx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 376, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 377 (onfekafusp); cy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 378, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 379 (patritumab); cz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 380, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 381 (pivekimab); da) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 382, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 383 (quavonlimab); db) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 384, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 385 (retlirafusp); dc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 386, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 387 (rosopatamab); dd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 388, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 389 (rulonilimab); de) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 390, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 391 (sabatolimab); df) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 392, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 393 (sasanlimab); dg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 394, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 395 (telisotuzumab); dh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 396, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 397 (tiragolumab); di) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 398, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 399 (tusamitamab); dj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 400, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 401 (vibostolimab); dk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 402, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 403 (vobramitamab); dl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 404, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 405 (zilovertamab); dm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 406, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 407 (suvemcitug); dn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 408, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 409 (becotatug); do) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 410, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 411 (tifcemalimab); dq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 412, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in

SEQ ID NO: 413 (blinatumomab - CD19); dr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 414, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 415 (blinatumomab – CD3); ds) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 416, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 417 (cadonilimab – PD-1); dt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 418, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 419 (cadonilimab – CTLA4); du) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 420, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 421 (disitamab); dv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 422, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 423 (edrecolomab); dw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 424, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 425 (elranatamab – BCMA); dx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 426, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 427 (elranatamab – DC3); dy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 428, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 429 (epcoritamab – CD20); dz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 430, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 431 (epcoritamab – CD3); ea) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 432, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 433 (glofitamab – VH1/VL1); eb); the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 434, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 435 (glofitamab – VH2/VL2); ec) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 436, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 437 (glofitamab – VH3/VL3); ed) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 438, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 439 (mosunetuzumab – CD20); ef) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 440, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 441 (mosunetuzumab – CD3); eg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 442, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 443 (talquetamab - GPCR5D); eh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 444, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 445 (talquetamab – CD3); ei) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 446, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 447 (teclistamab - BCMA); ej) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 448, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 449 (teclistamab – CD3); ek) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 450, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 451 (tositumomab); el) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 452, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 453

(tremelimumab); em) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 454, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 455 (zimberelimab); en) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 456, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 457 (odronextamab – CD20); eo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 458, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 459 (odronextamab – CD3); ep) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 460, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 461 (ivonescimab – PD-1); eq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 462, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 463 (ivonescimab - VEGF); er) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 464, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 465 (anbenitamab – VH1/VL1); es) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 466, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 467 (anbenitamab – VH2/VL2); et) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 468, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 469 (izalontamab – EGFR); eu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 470, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 471 (izalontamab – HER3); ev) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 472, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 473 (linvoseltamab - BCMA); ew) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 474, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 475 (linvoseltamab – CD3); ex) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 476, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 477 (tarlatamab – DLL3); ey) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 478, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 479 (tarlatamab – CD3); ez) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 480, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 481 (zanidatamab – VH1/VL1); fa) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 482, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 483 (zanidatamab – VH2/VL2); fb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 484, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 485 (volrustomig – PD-1); fc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 486, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 487 (volrustomig – CTLA-4); fd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 488, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 489 (zenocutuzumab – HER3); fe) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 490, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 491 (zenocutuzumab – HER2); ff) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 492, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 493

(botensilimab - VH1/VL1); fg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 494, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 495 (botensilimab - VH2/VL2); fh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 496, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 497 (izalontamab - EGFR); fi) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 498, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 499 (izalontamab – HER3); fj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 500, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 501 (rilvegostomig – TIGIT); fk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 502, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 503 (rilvegostomig – PD-1); fl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 701, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 700 (abagovomab); fm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 703, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 702 (abituzumab); fn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 705, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 704 (acasunlimab); fo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 707, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 706 (alnuctamab); fp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 709, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 708 (alomfilimab); fq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 711, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 710 (amatuximab); fr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 713, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 712 (anetumab); fs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 715, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 714 (aplitabart); ft) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 717, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 716 (atigotatug); fu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 719, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 718 (balstilimab); fv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 721, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 720 (bavituximab); fw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 723, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 722 (bavunalimab); fx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 725, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 724 (belrestotug); fy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 727, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 726 (bermekimab); fz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 729, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 728 (bifikafusp); ga) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 731, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised

in SEQ ID NO: 730 (bintrafusp); gb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 733, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 732 (brenetafusp); gc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 735, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 734 (briquilimab); gd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 737, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 736 (brontictuzumab); ge) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 739, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 738 (budigalimab); gf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 741, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 740 (cabiralizumab); gg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 743, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 742 (canakinumab); gh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 745, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 744 (cantuzumab); gi) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 747, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 746 (carlumab); gj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 749, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 748 (carotuximab); gk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 751, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 750 (caxmotabart); gl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 753, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 752 (cergutuzumab); gm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 755, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 754 (cibisatamab); gn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 757, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 756 (cinrebafusp); go) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 759, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 758 (cixutumumab); gp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 761, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 760 (clazakizumab); gq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 763, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 762 (clivatuzumab); gr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 765, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 764 (cofetuzumab); gs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 767, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 766 (coltuximab); gt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 769, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 768 (conatumumab); gu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 771, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 770 (dacetuzumab); gv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO:

773, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 772 (dalutrafusp); gw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 775, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 774 (danburstotug); gx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 777, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 776 (daratumumab); gy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 779, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 778 (demcizumab); gz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 781, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 780 (denintuzumab); ha) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 783, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 782 (denosumab); hb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 785, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 784 (depatuxizumab); hc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 787, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 786 (drozitumab); hd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 789, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 788 (duligotuzumab); he) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 791, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 790 (dusigitumab); hf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 793, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 792 (duvortuxizumab); hg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 795, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 794 (elotuzumab); hh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 797, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 796 (eluvixtamab); hi) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 799, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 798 (enapotamab); hj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 801, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 800 (enoticumab); hk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 803, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 802 (epacmarstobart); hl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 805, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 804 (etentamig); hm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 807, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 806 (falbikitug); hn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 809, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 808 (faricimab); ho) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 811, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 810 (feladilimab); hp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 813, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 812

(ficerafusp); hq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 815, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 814 (ficlatuzumab); hr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 817, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 816 (figitumumab); hs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 819, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 818 (flanvotumab); ht) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 821, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 820 (flotetuzumab); hu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 823, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 822 (forimtamig); hv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 825, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 824 (futuximab); hw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 827, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 826 (ganitumab); hx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 829, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 828 (gevokizumab); hy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 831, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 830 (girentuximab); hz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 833, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 832 (glembatumumab); ia) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 835, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 834 (icrucumab); ib) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 837, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 836 (ifinatamab); ic) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 839, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 838 (iladatuzumab); id) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 841, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 840 (imalumab); ie) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 843, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 842 (imgatuzumab); if) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 845, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 844 (indusatumab); ig) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 847, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 846 (inebilizumab); ih) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 849, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 848 (ispectamab); ii) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 851, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 850 (istiratumab); ij) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 853, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 852 (izeltabart); ik) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 855, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 854 (izuralimab); il) the CDR-

H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 857, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 856 (landogrozumab); im) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 859, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 858 (laprituximab); in) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 861, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 860 (lenzilumab); io) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 863, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 862 (leronlimab); ip) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 865, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 864 (lifastuzumab); iq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 867, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 866 (ligufalimab); ir) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 869, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 868 (lilotomab); is) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 871, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 870 (lintuzumab); it) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 873, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 872 (lirilumab); iu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 875, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 874 (livmoniplimab); iv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 877, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 876 (lorvotuzumab); iw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 879, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 878 (lucatumumab); ix) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 881, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 880 (lumretuzumab); iy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 883, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 882 (matuzumab); iz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 885, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 884 (mipasetamab); ja) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 887, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 886 (modakafusp); jb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 889, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 888 (modotuximab); jc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 891, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 890 (murlentamab); jd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 893, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 892 (nadunolimab); je) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 895, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 894 (naptumomab); jf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 897, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 896

(narlumosbart); jg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 899, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 898 (narnatumab); jh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 901, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 900 (navicixizumab); ji) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 903, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 902 (nesvacumab); jj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 905, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 904 (nisevokitug); jk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 907, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 906 (omburtamab); jl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 909, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 908 (ontuxizumab); jm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 911, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 910 (otlertuzumab); jn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 913, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 912 (pamrevlumab); jo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 915, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 914 (parsatuzumab); jp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 917, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 916 (pavurutamab); jq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 919, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 918 (pemivibart); jr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 921, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 920 (petosemtamab); js) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 923, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 922 (pimivalimab); jt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 925, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 924 (pinatuzumab); ju) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 927, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 926 (plozalizumab); jv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 929, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 928 (pulocimab); jw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 931, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 930 (ragifilimab); jx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 933, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 932 (raludotatug); jy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 935, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 934 (rilotumumab); jz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 937, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 936 (rosmantuzumab); ka) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 939, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO:

938 (runimotamab); kb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 941, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 940 (sabestomig); kc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 943, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 942 (selicrelumab); kd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 945, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 944 (seribantumab); ke) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 947, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 946 (sigvotatug); kf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 949, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 948 (simlukafusp); kg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 951, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 950 (simtuzumab); kh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 953, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 952 (sirexatamab); ki) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 955, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 954 (sofituzumab); kj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 957, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 956 (spartalizumab); kk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 959, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 958 (surzebiclimab); kl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 961, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 960 (tabalumab); km) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 963, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 962 (tafolecimab); kn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 965, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 964 (talacotuzumab); ko) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 967, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 966 (tarextumab); kp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 969, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 968 (tavolimab); kq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 971, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 970 (tebotelimab); kr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 973, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 972 (teprotumumab); ks) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 975, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 974 (tidutamab); kt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 977, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 976 (tigatuzumab); ku) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 979, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 978 (tilvestamab); kv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 981, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 980

(tobemstomig); kw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 983, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 982 (tocilizumab); kx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 985, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 984 (tomaralimab); ky) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 987, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 986 (tovecimig); kz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 989, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 988 (tovetumab); la) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 991, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 990 (tucotuzumab); lb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 993, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 992 (tuparstobart); lc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 995, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 994 (upifitamab); ld) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 997, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 996 (urabrelimab); le) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 999, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 998 (utomilumab); lf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1001, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1000 (vadastuximab); lg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1003, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1002 (vandortuzumab); lh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1005, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1004 (vanucizumab); li) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1007, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1006 (veligrotug); lj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1009, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1008 (verzistobart); lk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1011, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1010 (vesencumab); ll) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1013, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1012 (vofatamab); lm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1015, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1014 (vonlerolizumab); ln) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1017, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1016 (vopikitug); lo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1019, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1018 (vorsetuzumab); lp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1021, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1020 (xaluritamig); lq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1023, and the CDR-L1, CDR-L2 and CDR-L3

sequences as comprised in SEQ ID NO: 1022 (zalutumumab); lr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1025, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1024 (zanolimumab); ls) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1027, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1026 (ivuxolimab-alt); lt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1029, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1028 (inotuzumab-alt); lu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1031, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1030 (moxetumomab-alt); lv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1033, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1032 (luveltamab-alt); lw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1035, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1034 (ibritumomab-alt); lx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1037, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1036 (pivekimab-alt); ly) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1039, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1038 (avelumab-alt); lz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1041, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1040 (sugemalimab-alt); ma) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1043, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1042 (nimotuzumab-alt); mb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1045, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1044 (panitumumab-alt); mc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 540, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 541 (AR46A6); md) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 542, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 543 (KM4097); and me) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 544, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 545 (K5-70).

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one antigen-binding region that specifically binds to TROP2. TROP2 is a transmembrane glycoprotein encoded by the human Tacstd2 gene. The 323 amino acid sequence of human TROP2 described in NCBI accession number NP_002344, the disclosure of which is incorporated herein by reference. The human TROP2 mRNA sequence is described in NCBI accession number NM_002353, the disclosure of which is incorporated herein by reference. TROP2 is an intracellular calcium signal transducer that is differentially expressed in many cancers. TROP2 plays a role in tumor progression by actively interacting with several key molecular signaling pathways traditionally associated with cancer development and progression. Aberrant overexpression of TROP2 has been described in several solid cancers. TROP2 causes cancer cell growth, proliferation, invasion, migration, and survival of cancer cells, which leads to TROP2 being

associated with tumor aggressiveness and poor prognosis. These facts make TROP2 a possible prognostic biomarker to identify high-risk patients, as well as an attractive therapeutic target for (late-stage) diseases.

In one embodiment therefore, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one antigen-binding region that specifically binds TROP2, comprising a combination of complementarity-determining regions (CDRs) CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of: a) a CDR-H1 comprising the sequence of SEQ ID NO: 552, a CDR-H2 comprising the sequence of SEQ ID NO: 553, a CDR-H3 comprising the sequence of SEQ ID NO: 554, a CDR-L1 comprising the sequence of SEQ ID NO: 555, a CDR-L2 comprising the sequence of SEQ ID NO: 556, and a CDR-L3 comprising the sequence of SEQ ID NO: 557 (sacituzumab); b) a CDR-H1 comprising the sequence of SEQ ID NO: 558, a CDR-H2 comprising the sequence of SEQ ID NO: 559, a CDR-H3 comprising the sequence of SEQ ID NO: 560, a CDR-L1 comprising the sequence of SEQ ID NO: 561, a CDR-L2 comprising the sequence of SEQ ID NO: 562, and a CDR-L3 comprising the sequence of SEQ ID NO: 563 (datopotamab); c) a CDR-H1 comprising the sequence of SEQ ID NO: 564, a CDR-H2 comprising the sequence of SEQ ID NO: 565, a CDR-H3 comprising the sequence of SEQ ID NO: 566, a CDR-L1 comprising the sequence of SEQ ID NO: 567, a CDR-L2 comprising the sequence of SEQ ID NO: 568, and a CDR-L3 comprising the sequence of SEQ ID NO: 569 (KM4097); d) a CDR-H1 comprising the sequence of SEQ ID NO: 570, a CDR-H2 comprising the sequence of SEQ ID NO: 571, a CDR-H3 comprising the sequence of SEQ ID NO: 572, a CDR-L1 comprising the sequence of SEQ ID NO: 573, a CDR-L2 comprising the sequence of SEQ ID NO: 574, and a CDR-L3 comprising the sequence of SEQ ID NO: 575 (AR47A6.4.2); and, e) a CDR-H1 comprising the sequence of SEQ ID NO: 576, a CDR-H2 comprising the sequence of SEQ ID NO: 577, a CDR-H3 comprising the sequence of SEQ ID NO: 578, a CDR-L1 comprising the sequence of SEQ ID NO: 579, a CDR-L2 comprising the sequence of SEQ ID NO: 580, and a CDR-L3 comprising the sequence of SEQ ID NO: 581 (K5-70).

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one antigen-binding region that specifically binds to Her2 (Erb2/Neu). The human epidermal growth factor receptor-2 (HER2) receptor (previously called HER2/Neu) is a transmembrane glycoprotein with tyrosine kinase activity is encoded by the *ERBB2* gene and that belongs to the epidermal growth factor receptor family. Amino acid sequences of human HER2 are described in NCBI accession numbers NP_001005862, NP_001276865, NP_001276866, NP_001276867 and NP_004439 NP_002344, the disclosure of which is incorporated herein by reference. Human HER2 mRNA sequences are described in NCBI accession numbers NM_001005862, NM_001289936, NM_001289937, NM_001289938 and NM_004448, the disclosure of which is incorporated herein by reference. Receptors of the epidermal growth factor receptor family are essential in controlling epithelial cell growth and differentiation. Aberrant HER2 protein overexpression has associations with adenocarcinomas, including breast, ovary, endometrium, cervix, as well as lung, esophageal, gastroesophageal junction, gastric, and bladder cancers. HER2 amplification or overexpression occurs in

approximately 20% to 30% of human breast cancers. This protein is strongly associated with increased disease recurrence and is a poor prognostic factor for survival. For this reason, HER2 is an essential target for the therapy of various types of cancer.

In one embodiment therefore, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one antigen-binding region that specifically binds HER2, comprising a combination of complementarity-determining regions (CDRs) CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of: a) a CDR-H1 comprising the sequence of SEQ ID NO: 24, a CDR-H2 comprising the sequence of SEQ ID NO: 25, a CDR-H3 comprising the sequence of SEQ ID NO: 26, a CDR-L1 comprising the sequence of SEQ ID NO: 27, a CDR-L2 comprising the sequence of SEQ ID NO: 28, and a CDR-L3 comprising the sequence of SEQ ID NO: 29 (trastuzumab); b) a CDR-H1 comprising the sequence of SEQ ID NO: 594, a CDR-H2 comprising the sequence of SEQ ID NO: 595, a CDR-H3 comprising the sequence of SEQ ID NO: 596, a CDR-L1 comprising the sequence of SEQ ID NO: 597, a CDR-L2 comprising the sequence of SEQ ID NO: 598, and a CDR-L3 comprising the sequence of SEQ ID NO: 599 (margetuximab); c) a CDR-H1 comprising the sequence of SEQ ID NO: 600, a CDR-H2 comprising the sequence of SEQ ID NO: 601, a CDR-H3 comprising the sequence of SEQ ID NO: 602, a CDR-L1 comprising the sequence of SEQ ID NO: 603, a CDR-L2 comprising the sequence of SEQ ID NO: 604, and a CDR-L3 comprising the sequence of SEQ ID NO: 605 (pertuzumab); d) a CDR-H1 comprising the sequence of SEQ ID NO: 606, a CDR-H2 comprising the sequence of SEQ ID NO: 607, a CDR-H3 comprising the sequence of SEQ ID NO: 608, a CDR-L1 comprising the sequence of SEQ ID NO: 609, a CDR-L2 comprising the sequence of SEQ ID NO: 610, and a CDR-L3 comprising the sequence of SEQ ID NO: 611 (disitamab); e) a CDR-H1 comprising the sequence of SEQ ID NO: 600, a CDR-H2 comprising the sequence of SEQ ID NO: 601, a CDR-H3 comprising the sequence of SEQ ID NO: 602,, a CDR-L1 comprising the sequence of SEQ ID NO: 612, a CDR-L2 comprising the sequence of SEQ ID NO: 613, and a CDR-L3 comprising the sequence of SEQ ID NO: 614 (anbenitamab2); f) a CDR-H1 comprising the sequence of SEQ ID NO: 615, a CDR-H2 comprising the sequence of SEQ ID NO: 616, a CDR-H3 comprising the sequence of SEQ ID NO: 617,, a CDR-L1 comprising the sequence of SEQ ID NO: 618, a CDR-L2 comprising the sequence of SEQ ID NO: 619, and a CDR-L3 comprising the sequence of SEQ ID NO: 620 (zanidatamab2); and, g) a CDR-H1 comprising the sequence of SEQ ID NO: 621, a CDR-H2 comprising the sequence of SEQ ID NO: 622, a CDR-H3 comprising the sequence of SEQ ID NO: 623,, a CDR-L1 comprising the sequence of SEQ ID NO: 624, a CDR-L2 comprising the sequence of SEQ ID NO: 625, and a CDR-L3 comprising the sequence of SEQ ID NO: 626 (zenocutuzumab).

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one antigen-binding region that specifically binds to EGF receptor (EGFR, ERBB1). The human epidermal growth factor receptor-1 (EGFR; ErbB-1; HER1 in humans) is a transmembrane glycoprotein with tyrosine kinase activity is encoded by the ERBB1 gene and that belongs to the epidermal growth factor receptor family. Amino acid sequences of human EGFR are described in NCBI accession numbers NP_001333826, NP_001333827,

NP_001333828, and NP_001333829, the disclosure of which is incorporated herein by reference. Human EGFR mRNA sequences are described in NCBI accession numbers NM_001346897, NM_001346898, NM_001346899, NM_001346900, and NM_001346941, the disclosure of which is incorporated herein by reference. Receptors of the epidermal growth factor receptor family are essential in controlling epithelial cell growth and differentiation. Aberrant EGFR protein overexpression has associations with a wide variety of tumors. Interruption of EGFR signalling, either by blocking EGFR binding sites on the extracellular domain of the receptor or by inhibiting intracellular tyrosine kinase activity, can prevent the growth of EGFR-expressing tumors and improve the patient's condition. For this reason, EGFR is an essential target for the therapy of various types of cancer.

In one embodiment therefore, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one antigen-binding region that specifically binds EGFR, comprising a combination of complementarity-determining regions (CDRs) CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of: a) a CDR-H1 comprising the sequence of SEQ ID NO: 627, a CDR-H2 comprising the sequence of SEQ ID NO: 628, a CDR-H3 comprising the sequence of SEQ ID NO: 629, a CDR-L1 comprising the sequence of SEQ ID NO: 630, a CDR-L2 comprising the sequence of SEQ ID NO: 631, and a CDR-L3 comprising the sequence of SEQ ID NO: 632 (cetuximab); b) a CDR-H1 comprising the sequence of SEQ ID NO: 633, a CDR-H2 comprising the sequence of SEQ ID NO: 634, a CDR-H3 comprising the sequence of SEQ ID NO: 635, a CDR-L1 comprising the sequence of SEQ ID NO: 636, a CDR-L2 comprising the sequence of SEQ ID NO: 637, and a CDR-L3 comprising the sequence of SEQ ID NO: 638 (necitumumab); c) a CDR-H1 comprising the sequence of SEQ ID NO: 639, a CDR-H2 comprising the sequence of SEQ ID NO: 640, a CDR-H3 comprising the sequence of SEQ ID NO: 641, a CDR-L1 comprising the sequence of SEQ ID NO: 642, a CDR-L2 comprising the sequence of SEQ ID NO: 643, and a CDR-L3 comprising the sequence of SEQ ID NO: 644 (panitumumab); d) a CDR-H1 comprising the sequence of SEQ ID NO: 645, a CDR-H2 comprising the sequence of SEQ ID NO: 646, a CDR-H3 comprising the sequence of SEQ ID NO: 647, a CDR-L1 comprising the sequence of SEQ ID NO: 648, a CDR-L2 comprising the sequence of SEQ ID NO: 649, and a CDR-L3 comprising the sequence of SEQ ID NO: 650 (nimotuzumab); e) a CDR-H1 comprising the sequence of SEQ ID NO: 651, a CDR-H2 comprising the sequence of SEQ ID NO: 652, a CDR-H3 comprising the sequence of SEQ ID NO: 653,, a CDR-L1 comprising the sequence of SEQ ID NO: 654, a CDR-L2 comprising the sequence of SEQ ID NO: 655, and a CDR-L3 comprising the sequence of SEQ ID NO: 656 (becotatug); f) a CDR-H1 comprising the sequence of SEQ ID NO: 657, a CDR-H2 comprising the sequence of SEQ ID NO: 658, a CDR-H3 comprising the sequence of SEQ ID NO: 659,, a CDR-L1 comprising the sequence of SEQ ID NO: 660, a CDR-L2 comprising the sequence of SEQ ID NO: 661, and a CDR-L3 comprising the sequence of SEQ ID NO: 662 (amivantamab); and, g) a CDR-H1 comprising the sequence of SEQ ID NO: 663, a CDR-H2 comprising the sequence of SEQ ID NO: 664, a CDR-H3 comprising the sequence of SEQ ID NO: 665,, a CDR-L1 comprising the sequence of SEQ ID NO: 666, a CDR-L2 comprising the sequence of SEQ ID NO: 667, and a CDR-L3 comprising the sequence of SEQ ID NO: 668 (izalontamab).

In one embodiment therefore, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises an immunoglobulin single variable domain (ISVD) comprising a combination of complementarity-determining regions (CDRs) CDR1, CDR2, and CDR3 selected from the group consisting of: a) the CDR1, CDR2, and CDR3 sequences as comprised in SEQ ID NO: 504 (envafolimab); b) the CDR1, CDR2, and CDR3 sequences as comprised in SEQ ID NO: 505 (erfonrilimab – PD-L1); c) the CDR1, CDR2, and CDR3 sequences as comprised in SEQ ID NO: 506 (erfonrilimab – CTLA-4); and, d) the CDR1, CDR2, and CDR3 sequences as comprised in SEQ ID NO: 507 (ozekibart).

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a combination of variable heavy (V_H) and variable light (V_L) domains selected from the group consisting of: a) the V_H sequence as comprised in SEQ ID NO: 39 and the V_L sequence as comprised in SEQ ID NO: 40 (trastuzumab); b) the V_H sequence as comprised in SEQ ID NO: 41 and the V_L sequence as comprised in SEQ ID NO: 42 (cetuximab); c) the V_H sequence as comprised in SEQ ID NO: 43 and the V_L sequence as comprised in SEQ ID NO: 44 (rituximab); d) the V_H sequence as comprised in SEQ ID NO: 45 and the V_L sequence as comprised in SEQ ID NO: 46 (daratumumab); e) the V_H sequence as comprised in SEQ ID NO: 47 and the V_L sequence as comprised in SEQ ID NO: 48 (avelumab); f) the V_H sequence as comprised in SEQ ID NO: 104 and the V_L sequence as comprised in SEQ ID NO: 105 (atezolizumab); g) the V_H sequence as comprised in SEQ ID NO: 106 and the V_L sequence as comprised in SEQ ID NO: 107 (durvalumab); h) the V_H sequence as comprised in SEQ ID NO: 108 and the V_L sequence as comprised in SEQ ID NO: 109 (cosibelimab); i) the V_H sequence as comprised in SEQ ID NO: 110 and the V_L sequence as comprised in SEQ ID NO: 111 (margetuximab); j) the V_H sequence as comprised in SEQ ID NO: 112 and the V_L sequence as comprised in SEQ ID NO: 113 (pertuzumab); k) the V_H sequence as comprised in SEQ ID NO: 114 and the V_L sequence as comprised in SEQ ID NO: 115 (enoblituzumab); l) the V_H sequence as comprised in SEQ ID NO: 116 and the V_L sequence as comprised in SEQ ID NO: 117 (necitumumab); m) the V_H sequence as comprised in SEQ ID NO: 118 and the V_L sequence as comprised in SEQ ID NO: 119 (panitumumab); n) the V_H sequence as comprised in SEQ ID NO: 120 and the V_L sequence as comprised in SEQ ID NO: 121 (amivantamab EGFR-binding); o) the V_H sequence as comprised in SEQ ID NO: 122 and the V_L sequence as comprised in SEQ ID NO: 123 (amivantamab cMet-binding); p) the V_H sequence as comprised in SEQ ID NO: 124 and the V_L sequence as comprised in SEQ ID NO: 125 (zolbetuximab); q) the V_H sequence as comprised in SEQ ID NO: 126 and the V_L sequence as comprised in SEQ ID NO: 127 (dinutuximab); r) the V_H sequence as comprised in SEQ ID NO: 128 and the V_L sequence as comprised in SEQ ID NO: 129 (naxitamab); s) the V_H sequence as comprised in SEQ ID NO: 130 and the V_L sequence as comprised in SEQ ID NO: 131 (enfortumab); t) the V_H sequence as comprised in SEQ ID NO: 132 and the V_L sequence as comprised in SEQ ID NO: 133 (farletuzumab); u) the V_H sequence as comprised in SEQ ID NO: 134 and the V_L sequence as comprised in SEQ ID NO: 135 (tisotumab); v) the V_H sequence as comprised in SEQ ID NO: 136 and the V_L sequence as comprised in SEQ ID NO: 137 (mirvetuximab); w) the V_H sequence as comprised in SEQ ID NO: 138 and the V_L sequence as comprised in SEQ ID NO: 139

(sacituzumab); x) the V_H sequence as comprised in SEQ ID NO: 140 and the V_L sequence as comprised in SEQ ID NO: 141 (vobramitamab); y) the V_H sequence as comprised in SEQ ID NO: 142 and the V_L sequence as comprised in SEQ ID NO: 143 (onartuzumab); z) the V_H sequence as comprised in SEQ ID NO: 144 and the V_L sequence as comprised in SEQ ID NO: 145 (sibrotuzumab); aa) the V_H sequence as comprised in SEQ ID NO: 146 and the V_L sequence as comprised in SEQ ID NO: 147 (olaratumab); ab) the V_H sequence as comprised in SEQ ID NO: 148 and the V_L sequence as comprised in SEQ ID NO: 149 (rovalpituzumab); ac) the V_H sequence as comprised in SEQ ID NO: 238, and the V_L sequence as comprised in SEQ ID NO: 239 (adebrelimab); ad) the V_H sequence as comprised in SEQ ID NO: 240, and the V_L sequence as comprised in SEQ ID NO: 241 (alemtuzumab); ae) the V_H sequence as comprised in SEQ ID NO: 242, and the V_L sequence as comprised in SEQ ID NO: 243 (belantamab); af) the V_H sequence as comprised in SEQ ID NO: 244, and the V_L sequence as comprised in SEQ ID NO: 245 (Bevacizumab); ag) the V_H sequence as comprised in SEQ ID NO: 246, and the V_L sequence as comprised in SEQ ID NO: 247 (brentuximab); ah) the V_H sequence as comprised in SEQ ID NO: 248, and the V_L sequence as comprised in SEQ ID NO: 249 (camrelizumab); ai) the V_H sequence as comprised in SEQ ID NO: 250, and the V_L sequence as comprised in SEQ ID NO: 251 (cemiplimab); aj) the V_H sequence as comprised in SEQ ID NO: 252, and the V_L sequence as comprised in SEQ ID NO: 253 (dostarlimab); ak) the V_H sequence as comprised in SEQ ID NO: 254, and the V_L sequence as comprised in SEQ ID NO: 255 (emapalumab); al) the V_H sequence as comprised in SEQ ID NO: 256, and the V_L sequence as comprised in SEQ ID NO: 257 (enlonstobart); am) the V_H sequence as comprised in SEQ ID NO: 258, and the V_L sequence as comprised in SEQ ID NO: 259 (gemtuzumab); an) the V_H sequence as comprised in SEQ ID NO: 260, and the V_L sequence as comprised in SEQ ID NO: 261 (ibritumomab); ao) the V_H sequence as comprised in SEQ ID NO: 262, and the V_L sequence as comprised in SEQ ID NO: 263 (inotuzumab); ap) the V_H sequence as comprised in SEQ ID NO: 264, and the V_L sequence as comprised in SEQ ID NO: 265 (ipilimumab); aq) the V_H sequence as comprised in SEQ ID NO: 266, and the V_L sequence as comprised in SEQ ID NO: 267 (isatuximab); ar) the V_H sequence as comprised in SEQ ID NO: 268, and the V_L sequence as comprised in SEQ ID NO: 269 (loncastuximab); as) the V_H sequence as comprised in SEQ ID NO: 270, and the V_L sequence as comprised in SEQ ID NO: 271 (mogamulizumab); at) the V_H sequence as comprised in SEQ ID NO: 272, and the V_L sequence as comprised in SEQ ID NO: 273 (moxetumomab); au) the V_H sequence as comprised in SEQ ID NO: 274, and the V_L sequence as comprised in SEQ ID NO: 275 (nimotuzumab); av) the V_H sequence as comprised in SEQ ID NO: 276, and the V_L sequence as comprised in SEQ ID NO: 277 (nivolumab); aw) the V_H sequence as comprised in SEQ ID NO: 278, and the V_L sequence as comprised in SEQ ID NO: 279 (obinutuzumab); ax) the V_H sequence as comprised in SEQ ID NO: 280, and the V_L sequence as comprised in SEQ ID NO: 281 (ofatumumab); ay) the V_H sequence as comprised in SEQ ID NO: 282, and the V_L sequence as comprised in SEQ ID NO: 283 (pembrolizumab); az) the V_H sequence as comprised in SEQ ID NO: 284, and the V_L sequence as comprised in SEQ ID NO: 285 (Penpulimab); ba) the V_H sequence as comprised in SEQ ID NO: 286, and the V_L sequence as comprised in SEQ ID NO: 287

(polatuzumab); bb) the V_H sequence as comprised in SEQ ID NO: 288, and the V_L sequence as comprised in SEQ ID NO: 289 (prolgolimab); bc) the V_H sequence as comprised in SEQ ID NO: 290, and the V_L sequence as comprised in SEQ ID NO: 291 (pucotenlimab); bd) the V_H sequence as comprised in SEQ ID NO: 292, and the V_L sequence as comprised in SEQ ID NO: 293 (racotumomab) be) the V_H sequence as comprised in SEQ ID NO: 294, and the V_L sequence as comprised in SEQ ID NO: 295 (ramucirumab); bf) the V_H sequence as comprised in SEQ ID NO: 296, and the V_L sequence as comprised in SEQ ID NO: 297 (relatlimab); bg) the V_H sequence as comprised in SEQ ID NO: 298, and the V_L sequence as comprised in SEQ ID NO: 299 (retifanlimab); bh) the V_H sequence as comprised in SEQ ID NO: 300, and the V_L sequence as comprised in SEQ ID NO: 301 (ripertamab); bi) the V_H sequence as comprised in SEQ ID NO: 302, and the V_L sequence as comprised in SEQ ID NO: 303 (serplulimab); bj) the V_H sequence as comprised in SEQ ID NO: 304, and the V_L sequence as comprised in SEQ ID NO: 305 (sintilimab); bk) the V_H sequence as comprised in SEQ ID NO: 306, and the V_L sequence as comprised in SEQ ID NO: 307 (socazolimab); bl) the V_H sequence as comprised in SEQ ID NO: 308, and the V_L sequence as comprised in SEQ ID NO: 309 (sugemalimab); bm) the V_H sequence as comprised in SEQ ID NO: 310, and the V_L sequence as comprised in SEQ ID NO: 311 (tafasitamab); bn) the V_H sequence as comprised in SEQ ID NO: 312, and the V_L sequence as comprised in SEQ ID NO: 313 (tagitanlimab); bo) the V_H sequence as comprised in SEQ ID NO: 314, and the V_L sequence as comprised in SEQ ID NO: 315 (tebentafusp); bp) the V_H sequence as comprised in SEQ ID NO: 316, and the V_L sequence as comprised in SEQ ID NO: 317 (Tislelizumab); bq) the V_H sequence as comprised in SEQ ID NO: 318, and the V_L sequence as comprised in SEQ ID NO: 319 (Toripalimab); br) the V_H sequence as comprised in SEQ ID NO: 320, and the V_L sequence as comprised in SEQ ID NO: 321 (zuberitamab); bs) the V_H sequence as comprised in SEQ ID NO: 322, and the V_L sequence as comprised in SEQ ID NO: 323 (benmelstobart); bt) bu) bv) bw) the V_H sequence as comprised in SEQ ID NO: 324, and the V_L sequence as comprised in SEQ ID NO: 325 (iparomlimab); bx) the V_H sequence as comprised in SEQ ID NO: 326, and the V_L sequence as comprised in SEQ ID NO: 327 (tuvonralimab); by) the V_H sequence as comprised in SEQ ID NO: 328, and the V_L sequence as comprised in SEQ ID NO: 329 (anvatabart); bz) the V_H sequence as comprised in SEQ ID NO: 330, and the V_L sequence as comprised in SEQ ID NO: 331 (apamistamab); ca) the V_H sequence as comprised in SEQ ID NO: 332, and the V_L sequence as comprised in SEQ ID NO: 333 (bemarituzumab); cb) the V_H sequence as comprised in SEQ ID NO: 334, and the V_L sequence as comprised in SEQ ID NO: 335 (cetrelimab); cd) the V_H sequence as comprised in SEQ ID NO: 336, and the V_L sequence as comprised in SEQ ID NO: 337 (cobolimab); ce) the V_H sequence as comprised in SEQ ID NO: 338, and the V_L sequence as comprised in SEQ ID NO: 339 (datopotamab); cf) the V_H sequence as comprised in SEQ ID NO: 340, and the V_L sequence as comprised in SEQ ID NO: 341 (domvanalimab); cg) the V_H sequence as comprised in SEQ ID NO: 342, and the V_L sequence as comprised in SEQ ID NO: 343 (emactuzumab); ch) the V_H sequence as comprised in SEQ ID NO: 344, and the V_L sequence as comprised in SEQ ID NO: 345 (favezelimab); ci) the V_H sequence as comprised in SEQ ID NO: 346, and the V_L sequence as comprised in SEQ ID NO: 347 (felzartamab); cj) the V_H sequence as comprised in SEQ ID NO: 348,

and the V_L sequence as comprised in SEQ ID NO: 349 (fianlimab); ck) the V_H sequence as comprised in SEQ ID NO: 350, and the V_L sequence as comprised in SEQ ID NO: 351 (finotonlimab); cl) the V_H sequence as comprised in SEQ ID NO: 352, and the V_L sequence as comprised in SEQ ID NO: 353 (geptanolimab); cm) the V_H sequence as comprised in SEQ ID NO: 354, and the V_L sequence as comprised in SEQ ID NO: 355 (gotistobart); cn) the V_H sequence as comprised in SEQ ID NO: 356, and the V_L sequence as comprised in SEQ ID NO: 357 (ivuxolimab); co) the V_H sequence as comprised in SEQ ID NO: 358, and the V_L sequence as comprised in SEQ ID NO: 359 (lemzoparlimab); cp) the V_H sequence as comprised in SEQ ID NO: 360, and the V_L sequence as comprised in SEQ ID NO: 361 (luveltamab); cq) the V_H sequence as comprised in SEQ ID NO: 362, and the V_L sequence as comprised in SEQ ID NO: 363 (magrolimab); cr) the V_H sequence as comprised in SEQ ID NO: 364, and the V_L sequence as comprised in SEQ ID NO: 365 (mecbotamab); cs) the V_H sequence as comprised in SEQ ID NO: 366, and the V_L sequence as comprised in SEQ ID NO: 367 (monalizumab); ct) the V_H sequence as comprised in SEQ ID NO: 368, and the V_L sequence as comprised in SEQ ID NO: 369 (nofazinlimab); cu) the V_H sequence as comprised in SEQ ID NO: 370, and the V_L sequence as comprised in SEQ ID NO: 371 (nurulimab); cv) the V_H sequence as comprised in SEQ ID NO: 372, and the V_L sequence as comprised in SEQ ID NO: 373 (ociperlimab); cw) the V_H sequence as comprised in SEQ ID NO: 374, and the V_L sequence as comprised in SEQ ID NO: 375 (oleclumab); cx) the V_H sequence as comprised in SEQ ID NO: 376, and the V_L sequence as comprised in SEQ ID NO: 377 (onfekafusp); cy) the V_H sequence as comprised in SEQ ID NO: 378, and the V_L sequence as comprised in SEQ ID NO: 379 (patritumab); cz) the V_H sequence as comprised in SEQ ID NO: 380, and the V_L sequence as comprised in SEQ ID NO: 381 (pivekimab); da) the V_H sequence as comprised in SEQ ID NO: 382, and the V_L sequence as comprised in SEQ ID NO: 383 (quavonlimab); db) the V_H sequence as comprised in SEQ ID NO: 384, and the V_L sequence as comprised in SEQ ID NO: 385 (retlirafusp); dc) the V_H sequence as comprised in SEQ ID NO: 386, and the V_L sequence as comprised in SEQ ID NO: 387 (rosopatamab); dd) the V_H sequence as comprised in SEQ ID NO: 388, and the V_L sequence as comprised in SEQ ID NO: 389 (rulonilimab); de) the V_H sequence as comprised in SEQ ID NO: 390, and the V_L sequence as comprised in SEQ ID NO: 391 (sabatolimab); df) the V_H sequence as comprised in SEQ ID NO: 392, and the V_L sequence as comprised in SEQ ID NO: 393 (sasanlimab); dg) the V_H sequence as comprised in SEQ ID NO: 394, and the V_L sequence as comprised in SEQ ID NO: 395 (telisotuzumab); dh) the V_H sequence as comprised in SEQ ID NO: 396, and the V_L sequence as comprised in SEQ ID NO: 397 (tiragolumab); di) the V_H sequence as comprised in SEQ ID NO: 398, and the V_L sequence as comprised in SEQ ID NO: 399 (tusamitamab); dj) the V_H sequence as comprised in SEQ ID NO: 400, and the V_L sequence as comprised in SEQ ID NO: 401 (vibostolimab); dk) the V_H sequence as comprised in SEQ ID NO: 402, and the V_L sequence as comprised in SEQ ID NO: 403 (vobramitamab); dl) the V_H sequence as comprised in SEQ ID NO: 404, and the V_L sequence as comprised in SEQ ID NO: 405 (zilovertamab); dm) the V_H sequence as comprised in SEQ ID NO: 406, and the V_L sequence as comprised in SEQ ID NO: 407 (suvemcitug); dn) the V_H sequence as comprised in SEQ ID NO: 408, and the V_L sequence as comprised in SEQ ID NO: 409 (becotatug);

do) the V_H sequence as comprised in SEQ ID NO: 410, and the V_L sequence as comprised in SEQ ID NO: 411 (tifcemalimab); dq) the V_H sequence as comprised in SEQ ID NO: 412, and the V_L sequence as comprised in SEQ ID NO: 413 (blinatumomab - CD19); dr) the V_H sequence as comprised in SEQ ID NO: 414, and the V_L sequence as comprised in SEQ ID NO: 415 (blinatumomab – CD3); ds) the V_H sequence as comprised in SEQ ID NO: 416, and the V_L sequence as comprised in SEQ ID NO: 417 (cadonilimab – PD-1); dt) the V_H sequence as comprised in SEQ ID NO: 418, and the V_L sequence as comprised in SEQ ID NO: 419 (cadonilimab – CTLA4); du) the V_H sequence as comprised in SEQ ID NO: 420, and the V_L sequence as comprised in SEQ ID NO: 421 (disitamab); dv) the V_H sequence as comprised in SEQ ID NO: 422, and the V_L sequence as comprised in SEQ ID NO: 423 (edrecolomab); dw) the V_H sequence as comprised in SEQ ID NO: 424, and the V_L sequence as comprised in SEQ ID NO: 425 (elranatamab – BCMA); dx) the V_H sequence as comprised in SEQ ID NO: 426, and the V_L sequence as comprised in SEQ ID NO: 427 (elranatamab – DC3); dy) the V_H sequence as comprised in SEQ ID NO: 428, and the V_L sequence as comprised in SEQ ID NO: 429 (epcoritamab – CD20); dz) the V_H sequence as comprised in SEQ ID NO: 430, and the V_L sequence as comprised in SEQ ID NO: 431 (epcoritamab – CD3); ea) the V_H sequence as comprised in SEQ ID NO: 432, and the V_L sequence as comprised in SEQ ID NO: 433 (glofitamab – VH1/VL1); eb); the V_H sequence as comprised in SEQ ID NO: 434, and the V_L sequence as comprised in SEQ ID NO: 435 (glofitamab – VH2/VL2); ec) the V_H sequence as comprised in SEQ ID NO: 436, and the V_L sequence as comprised in SEQ ID NO: 437 (glofitamab – VH3/VL3); ed) the V_H sequence as comprised in SEQ ID NO: 438, and the V_L sequence as comprised in SEQ ID NO: 439 (mosunetuzumab – CD20); ef) the V_H sequence as comprised in SEQ ID NO: 440, and the V_L sequence as comprised in SEQ ID NO: 441 (mosunetuzumab – CD3); eg) the V_H sequence as comprised in SEQ ID NO: 442, and the V_L sequence as comprised in SEQ ID NO: 443 (talquetamab - GPCR5D); eh) the V_H sequence as comprised in SEQ ID NO: 444, and the V_L sequence as comprised in SEQ ID NO: 445 (talquetamab – CD3); ei) the V_H sequence as comprised in SEQ ID NO: 446, and the V_L sequence as comprised in SEQ ID NO: 447 (teclistamab - BCMA); ej) the V_H sequence as comprised in SEQ ID NO: 448, and the V_L sequence as comprised in SEQ ID NO: 449 (teclistamab – CD3); ek) the V_H sequence as comprised in SEQ ID NO: 450, and the V_L sequence as comprised in SEQ ID NO: 451 (tositumomab); el) the V_H sequence as comprised in SEQ ID NO: 452, and the V_L sequence as comprised in SEQ ID NO: 453 (tremelimumab); em) the V_H sequence as comprised in SEQ ID NO: 454, and the V_L sequence as comprised in SEQ ID NO: 455 (zimberelimab); en) the V_H sequence as comprised in SEQ ID NO: 456, and the V_L sequence as comprised in SEQ ID NO: 457 (odronextamab – CD20); eo) the V_H sequence as comprised in SEQ ID NO: 458, and the V_L sequence as comprised in SEQ ID NO: 459 (odronextamab – CD3); ep) the V_H sequence as comprised in SEQ ID NO: 460, and the V_L sequence as comprised in SEQ ID NO: 461 (ivonescimab – PD-1); eq) the V_H sequence as comprised in SEQ ID NO: 462, and the V_L sequence as comprised in SEQ ID NO: 463 (ivonescimab - VEGF); er) the V_H sequence as comprised in SEQ ID NO: 464, and the V_L sequence as comprised in SEQ ID NO: 465 (anbenitamab – VH1/VL1); es) the V_H sequence as comprised in SEQ ID NO: 466, and the V_L sequence as comprised in SEQ ID NO:

467 (anbenitamab – VH2/VL2); et) the V_H sequence as comprised in SEQ ID NO: 468, and the V_L sequence as comprised in SEQ ID NO: 469 (izalontamab – EGFR); eu) the V_H sequence as comprised in SEQ ID NO: 470, and the V_L sequence as comprised in SEQ ID NO: 471 (izalontamab – HER3); ev) the V_H sequence as comprised in SEQ ID NO: 472, and the V_L sequence as comprised in SEQ ID NO: 473 (linvoseltamab - BCMA); ew) the V_H sequence as comprised in SEQ ID NO: 474, and the V_L sequence as comprised in SEQ ID NO: 475 (linvoseltamab – CD3); ex) the V_H sequence as comprised in SEQ ID NO: 476, and the V_L sequence as comprised in SEQ ID NO: 477 (tarlatamab – DLL3); ey) the V_H sequence as comprised in SEQ ID NO: 478, and the V_L sequence as comprised in SEQ ID NO: 479 (tarlatamab – CD3); ez) the V_H sequence as comprised in SEQ ID NO: 480, and the V_L sequence as comprised in SEQ ID NO: 481 (zanidatamab – VH1/VL1); fa) the V_H sequence as comprised in SEQ ID NO: 482, and the V_L sequence as comprised in SEQ ID NO: 483 (zanidatamab – VH2/VL2); fb) the V_H sequence as comprised in SEQ ID NO: 484, and the V_L sequence as comprised in SEQ ID NO: 485 (volrustomig – PD-1); fc) the V_H sequence as comprised in SEQ ID NO: 486, and the V_L sequence as comprised in SEQ ID NO: 487 (volrustomig – CTLA-4); fd) the V_H sequence as comprised in SEQ ID NO: 488, and the V_L sequence as comprised in SEQ ID NO: 489 (zenocutuzumab – HER3); fe) the V_H sequence as comprised in SEQ ID NO: 490, and the V_L sequence as comprised in SEQ ID NO: 491 (zenocutuzumab – HER2); ff) the V_H sequence as comprised in SEQ ID NO: 492, and the V_L sequence as comprised in SEQ ID NO: 493 (botensilimab - VH1/VL1); fg) the V_H sequence as comprised in SEQ ID NO: 494, and the V_L sequence as comprised in SEQ ID NO: 495 (botensilimab - VH2/VL2); fh) the V_H sequence as comprised in SEQ ID NO: 496, and the V_L sequence as comprised in SEQ ID NO: 497 (izalontamab - EGFR); fi) the V_H sequence as comprised in SEQ ID NO: 498, and the V_L sequence as comprised in SEQ ID NO: 499 (izalontamab – HER3); fj) the V_H sequence as comprised in SEQ ID NO: 500, and the V_L sequence as comprised in SEQ ID NO: 501 (rilvegostomig – TIGIT); fk) the V_H sequence as comprised in SEQ ID NO: 502, and the V_L sequence as comprised in SEQ ID NO: 503 (rilvegostomig – PD-1); fl) the VH sequence as comprised in SEQ ID NO: 701, and the VL sequence as comprised in SEQ ID NO: 700 (abagovomab); fm) the VH sequence as comprised in SEQ ID NO: 703, and the VL sequence as comprised in SEQ ID NO: 702 (abituzumab); fn) the VH sequence as comprised in SEQ ID NO: 705, and the VL sequence as comprised in SEQ ID NO: 704 (acasunlimab); fo) the VH sequence as comprised in SEQ ID NO: 707, and the VL sequence as comprised in SEQ ID NO: 706 (alnuctamab); fp) the VH sequence as comprised in SEQ ID NO: 709, and the VL sequence as comprised in SEQ ID NO: 708 (alomfilimab); fq) the VH sequence as comprised in SEQ ID NO: 711, and the VL sequence as comprised in SEQ ID NO: 710 (amatuximab); fr) the VH sequence as comprised in SEQ ID NO: 713, and the VL sequence as comprised in SEQ ID NO: 712 (anetumab); fs) the VH sequence as comprised in SEQ ID NO: 715, and the VL sequence as comprised in SEQ ID NO: 714 (aplitabart); ft) the VH sequence as comprised in SEQ ID NO: 717, and the VL sequence as comprised in SEQ ID NO: 716 (atigotatug); fu) the VH sequence as comprised in SEQ ID NO: 719, and the VL sequence as comprised in SEQ ID NO: 718 (balstilimab); fv) the VH sequence as comprised in SEQ ID NO: 721, and the VL sequence as comprised in SEQ ID NO: 720

(bavituximab); fw) the VH sequence as comprised in SEQ ID NO: 723, and the VL sequence as comprised in SEQ ID NO: 722 (bavunalimab); fx) the VH sequence as comprised in SEQ ID NO: 725, and the VL sequence as comprised in SEQ ID NO: 724 (belrestotug); fy) the VH sequence as comprised in SEQ ID NO: 727, and the VL sequence as comprised in SEQ ID NO: 726 (bermekimab); fz) the VH sequence as comprised in SEQ ID NO: 729, and the VL sequence as comprised in SEQ ID NO: 728 (bifikafusp); ga) the VH sequence as comprised in SEQ ID NO: 731, and the VL sequence as comprised in SEQ ID NO: 730 (bintrafusp); gb) the VH sequence as comprised in SEQ ID NO: 733, and the VL sequence as comprised in SEQ ID NO: 732 (brenetafusp); gc) the VH sequence as comprised in SEQ ID NO: 735, and the VL sequence as comprised in SEQ ID NO: 734 (briquilimab); gd) the VH sequence as comprised in SEQ ID NO: 737, and the VL sequence as comprised in SEQ ID NO: 736 (brontictuzumab); ge) the VH sequence as comprised in SEQ ID NO: 739, and the VL sequence as comprised in SEQ ID NO: 738 (budigalimab); gf) the VH sequence as comprised in SEQ ID NO: 741, and the VL sequence as comprised in SEQ ID NO: 740 (cabiralizumab); gg) the VH sequence as comprised in SEQ ID NO: 743, and the VL sequence as comprised in SEQ ID NO: 742 (canakinumab); gh) the VH sequence as comprised in SEQ ID NO: 745, and the VL sequence as comprised in SEQ ID NO: 744 (cantuzumab); gi) the VH sequence as comprised in SEQ ID NO: 747, and the VL sequence as comprised in SEQ ID NO: 746 (carlumab); gj) the VH sequence as comprised in SEQ ID NO: 749, and the VL sequence as comprised in SEQ ID NO: 748 (carotuximab); gk) the VH sequence as comprised in SEQ ID NO: 751, and the VL sequence as comprised in SEQ ID NO: 750 (caxmotabart); gl) the VH sequence as comprised in SEQ ID NO: 753, and the VL sequence as comprised in SEQ ID NO: 752 (cergutuzumab); gm) the VH sequence as comprised in SEQ ID NO: 755, and the VL sequence as comprised in SEQ ID NO: 754 (cibisatamab); gn) the VH sequence as comprised in SEQ ID NO: 757, and the VL sequence as comprised in SEQ ID NO: 756 (cinrebafusp); go) the VH sequence as comprised in SEQ ID NO: 759, and the VL sequence as comprised in SEQ ID NO: 758 (cixutumumab); gp) the VH sequence as comprised in SEQ ID NO: 761, and the VL sequence as comprised in SEQ ID NO: 760 (clazakizumab); gq) the VH sequence as comprised in SEQ ID NO: 763, and the VL sequence as comprised in SEQ ID NO: 762 (clivatuzumab); gr) the VH sequence as comprised in SEQ ID NO: 765, and the VL sequence as comprised in SEQ ID NO: 764 (cofetuzumab); gs) the VH sequence as comprised in SEQ ID NO: 767, and the VL sequence as comprised in SEQ ID NO: 766 (coltuximab); gt) the VH sequence as comprised in SEQ ID NO: 769, and the VL sequence as comprised in SEQ ID NO: 768 (conatumumab); gu) the VH sequence as comprised in SEQ ID NO: 771, and the VL sequence as comprised in SEQ ID NO: 770 (dacetuzumab); gv) the VH sequence as comprised in SEQ ID NO: 773, and the VL sequence as comprised in SEQ ID NO: 772 (dalutrafusp); gw) the VH sequence as comprised in SEQ ID NO: 775, and the VL sequence as comprised in SEQ ID NO: 774 (danburstotug); gx) the VH sequence as comprised in SEQ ID NO: 777, and the VL sequence as comprised in SEQ ID NO: 776 (daratumumab); gy) the VH sequence as comprised in SEQ ID NO: 779, and the VL sequence as comprised in SEQ ID NO: 778 (demcizumab); gz) the VH sequence as comprised in SEQ ID NO: 781, and the VL sequence as comprised in SEQ ID NO: 780

(denintuzumab); ha) the VH sequence as comprised in SEQ ID NO: 783, and the VL sequence as comprised in SEQ ID NO: 782 (denosumab); hb) the VH sequence as comprised in SEQ ID NO: 785, and the VL sequence as comprised in SEQ ID NO: 784 (depatuxizumab); hc) the VH sequence as comprised in SEQ ID NO: 787, and the VL sequence as comprised in SEQ ID NO: 786 (drozitumab); hd) the VH sequence as comprised in SEQ ID NO: 789, and the VL sequence as comprised in SEQ ID NO: 788 (duligotuzumab); he) the VH sequence as comprised in SEQ ID NO: 791, and the VL sequence as comprised in SEQ ID NO: 790 (dusigitumab); hf) the VH sequence as comprised in SEQ ID NO: 793, and the VL sequence as comprised in SEQ ID NO: 792 (duvortuxizumab); hg) the VH sequence as comprised in SEQ ID NO: 795, and the VL sequence as comprised in SEQ ID NO: 794 (elotuzumab); hh) the VH sequence as comprised in SEQ ID NO: 797, and the VL sequence as comprised in SEQ ID NO: 796 (eluvixtamab); hi) the VH sequence as comprised in SEQ ID NO: 799, and the VL sequence as comprised in SEQ ID NO: 798 (enapotamab); hj) the VH sequence as comprised in SEQ ID NO: 801, and the VL sequence as comprised in SEQ ID NO: 800 (enoticumab); hk) the VH sequence as comprised in SEQ ID NO: 803, and the VL sequence as comprised in SEQ ID NO: 802 (epacmarstobart); hl) the VH sequence as comprised in SEQ ID NO: 805, and the VL sequence as comprised in SEQ ID NO: 804 (etentamig); hm) the VH sequence as comprised in SEQ ID NO: 807, and the VL sequence as comprised in SEQ ID NO: 806 (falbikitug); hn) the VH sequence as comprised in SEQ ID NO: 809, and the VL sequence as comprised in SEQ ID NO: 808 (faricimab); ho) the VH sequence as comprised in SEQ ID NO: 811, and the VL sequence as comprised in SEQ ID NO: 810 (feladilimab); hp) the VH sequence as comprised in SEQ ID NO: 813, and the VL sequence as comprised in SEQ ID NO: 812 (ficerafusp); hq) the VH sequence as comprised in SEQ ID NO: 815, and the VL sequence as comprised in SEQ ID NO: 814 (ficlatuzumab); hr) the VH sequence as comprised in SEQ ID NO: 817, and the VL sequence as comprised in SEQ ID NO: 816 (figitumumab); hs) the VH sequence as comprised in SEQ ID NO: 819, and the VL sequence as comprised in SEQ ID NO: 818 (flanvotumab); ht) the VH sequence as comprised in SEQ ID NO: 821, and the VL sequence as comprised in SEQ ID NO: 820 (flotetuzumab); hu) the VH sequence as comprised in SEQ ID NO: 823, and the VL sequence as comprised in SEQ ID NO: 822 (forimtamig); hv) the VH sequence as comprised in SEQ ID NO: 825, and the VL sequence as comprised in SEQ ID NO: 824 (futuximab); hw) the VH sequence as comprised in SEQ ID NO: 827, and the VL sequence as comprised in SEQ ID NO: 826 (ganitumab); hx) the VH sequence as comprised in SEQ ID NO: 829, and the VL sequence as comprised in SEQ ID NO: 828 (gevokizumab); hy) the VH sequence as comprised in SEQ ID NO: 831, and the VL sequence as comprised in SEQ ID NO: 830 (girentuximab); hz) the VH sequence as comprised in SEQ ID NO: 833, and the VL sequence as comprised in SEQ ID NO: 832 (glembatumumab); ia) the VH sequence as comprised in SEQ ID NO: 835, and the VL sequence as comprised in SEQ ID NO: 834 (icrucumab); ib) the VH sequence as comprised in SEQ ID NO: 837, and the VL sequence as comprised in SEQ ID NO: 836 (ifinatamab); ic) the VH sequence as comprised in SEQ ID NO: 839, and the VL sequence as comprised in SEQ ID NO: 838 (iladatuzumab); id) the VH sequence as comprised in SEQ ID NO: 841, and the VL sequence as comprised in SEQ ID NO: 840 (imalumab); ie) the VH sequence as

comprised in SEQ ID NO: 843, and the VL sequence as comprised in SEQ ID NO: 842 (imgatuzumab); if) the VH sequence as comprised in SEQ ID NO: 845, and the VL sequence as comprised in SEQ ID NO: 844 (indusatumab); ig) the VH sequence as comprised in SEQ ID NO: 847, and the VL sequence as comprised in SEQ ID NO: 846 (inebilizumab); ih) the VH sequence as comprised in SEQ ID NO: 849, and the VL sequence as comprised in SEQ ID NO: 848 (ispectamab); ii) the VH sequence as comprised in SEQ ID NO: 851, and the VL sequence as comprised in SEQ ID NO: 850 (istiratumab); ij) the VH sequence as comprised in SEQ ID NO: 853, and the VL sequence as comprised in SEQ ID NO: 852 (izeltabart); ik) the VH sequence as comprised in SEQ ID NO: 855, and the VL sequence as comprised in SEQ ID NO: 854 (izuralimab); il) the VH sequence as comprised in SEQ ID NO: 857, and the VL sequence as comprised in SEQ ID NO: 856 (landogrozumab); im) the VH sequence as comprised in SEQ ID NO: 859, and the VL sequence as comprised in SEQ ID NO: 858 (laprituximab); in) the VH sequence as comprised in SEQ ID NO: 861, and the VL sequence as comprised in SEQ ID NO: 860 (lenzilumab); io) the VH sequence as comprised in SEQ ID NO: 863, and the VL sequence as comprised in SEQ ID NO: 862 (leronlimab); ip) the VH sequence as comprised in SEQ ID NO: 865, and the VL sequence as comprised in SEQ ID NO: 864 (lifastuzumab); iq) the VH sequence as comprised in SEQ ID NO: 867, and the VL sequence as comprised in SEQ ID NO: 866 (ligufalimab); ir) the VH sequence as comprised in SEQ ID NO: 869, and the VL sequence as comprised in SEQ ID NO: 868 (lilotomab); is) the VH sequence as comprised in SEQ ID NO: 871, and the VL sequence as comprised in SEQ ID NO: 870 (lintuzumab); it) the VH sequence as comprised in SEQ ID NO: 873, and the VL sequence as comprised in SEQ ID NO: 872 (lirilumab); iu) the VH sequence as comprised in SEQ ID NO: 875, and the VL sequence as comprised in SEQ ID NO: 874 (livmoniplimab); iv) the VH sequence as comprised in SEQ ID NO: 877, and the VL sequence as comprised in SEQ ID NO: 876 (lorvotuzumab); iw) the VH sequence as comprised in SEQ ID NO: 879, and the VL sequence as comprised in SEQ ID NO: 878 (lucatumumab); ix) the VH sequence as comprised in SEQ ID NO: 881, and the VL sequence as comprised in SEQ ID NO: 880 (lumretuzumab); iy) the VH sequence as comprised in SEQ ID NO: 883, and the VL sequence as comprised in SEQ ID NO: 882 (matuzumab); iz) the VH sequence as comprised in SEQ ID NO: 885, and the VL sequence as comprised in SEQ ID NO: 884 (mipasetamab); ja) the VH sequence as comprised in SEQ ID NO: 887, and the VL sequence as comprised in SEQ ID NO: 886 (modakafusp); jb) the VH sequence as comprised in SEQ ID NO: 889, and the VL sequence as comprised in SEQ ID NO: 888 (modotuximab); jc) the VH sequence as comprised in SEQ ID NO: 891, and the VL sequence as comprised in SEQ ID NO: 890 (murlentamab); jd) the VH sequence as comprised in SEQ ID NO: 893, and the VL sequence as comprised in SEQ ID NO: 892 (nadunolimab); je) the VH sequence as comprised in SEQ ID NO: 895, and the VL sequence as comprised in SEQ ID NO: 894 (naptumomab); jf) the VH sequence as comprised in SEQ ID NO: 897, and the VL sequence as comprised in SEQ ID NO: 896 (narlumosbart); jg) the VH sequence as comprised in SEQ ID NO: 899, and the VL sequence as comprised in SEQ ID NO: 898 (narnatumab); jh) the VH sequence as comprised in SEQ ID NO: 901, and the VL sequence as comprised in SEQ ID NO: 900 (navicixizumab); ji) the VH sequence as comprised in SEQ ID NO: 903, and the VL sequence as

comprised in SEQ ID NO: 902 (nesvacumab); jj) the VH sequence as comprised in SEQ ID NO: 905, and the VL sequence as comprised in SEQ ID NO: 904 (nisevokitug); jk) the VH sequence as comprised in SEQ ID NO: 907, and the VL sequence as comprised in SEQ ID NO: 906 (omburtamab); jl) the VH sequence as comprised in SEQ ID NO: 909, and the VL sequence as comprised in SEQ ID NO: 908 (ontuxizumab); jm) the VH sequence as comprised in SEQ ID NO: 911, and the VL sequence as comprised in SEQ ID NO: 910 (otlertuzumab); jn) the VH sequence as comprised in SEQ ID NO: 913, and the VL sequence as comprised in SEQ ID NO: 912 (pamrevlumab); jo) the VH sequence as comprised in SEQ ID NO: 915, and the VL sequence as comprised in SEQ ID NO: 914 (parsatuzumab); jp) the VH sequence as comprised in SEQ ID NO: 917, and the VL sequence as comprised in SEQ ID NO: 916 (pavurutamab); jq) the VH sequence as comprised in SEQ ID NO: 919, and the VL sequence as comprised in SEQ ID NO: 918 (pemivibart); jr) the VH sequence as comprised in SEQ ID NO: 921, and the VL sequence as comprised in SEQ ID NO: 920 (petosemtamab); js) the VH sequence as comprised in SEQ ID NO: 923, and the VL sequence as comprised in SEQ ID NO: 922 (pimivalimab); jt) the VH sequence as comprised in SEQ ID NO: 925, and the VL sequence as comprised in SEQ ID NO: 924 (pinatuzumab); ju) the VH sequence as comprised in SEQ ID NO: 927, and the VL sequence as comprised in SEQ ID NO: 926 (plozalizumab); jv) the VH sequence as comprised in SEQ ID NO: 929, and the VL sequence as comprised in SEQ ID NO: 928 (pulocimab); jw) the VH sequence as comprised in SEQ ID NO: 931, and the VL sequence as comprised in SEQ ID NO: 930 (ragifilimab); jx) the VH sequence as comprised in SEQ ID NO: 933, and the VL sequence as comprised in SEQ ID NO: 932 (raludotatug); jy) the VH sequence as comprised in SEQ ID NO: 935, and the VL sequence as comprised in SEQ ID NO: 934 (rilotumumab); jz) the VH sequence as comprised in SEQ ID NO: 937, and the VL sequence as comprised in SEQ ID NO: 936 (rosmantuzumab); ka) the VH sequence as comprised in SEQ ID NO: 939, and the VL sequence as comprised in SEQ ID NO: 938 (runimotamab); kb) the VH sequence as comprised in SEQ ID NO: 941, and the VL sequence as comprised in SEQ ID NO: 940 (sabestomig); kc) the VH sequence as comprised in SEQ ID NO: 943, and the VL sequence as comprised in SEQ ID NO: 942 (selicrelumab); kd) the VH sequence as comprised in SEQ ID NO: 945, and the VL sequence as comprised in SEQ ID NO: 944 (seribantumab); ke) the VH sequence as comprised in SEQ ID NO: 947, and the VL sequence as comprised in SEQ ID NO: 946 (sigvotatug); kf) the VH sequence as comprised in SEQ ID NO: 949, and the VL sequence as comprised in SEQ ID NO: 948 (simlukafusp); kg) the VH sequence as comprised in SEQ ID NO: 951, and the VL sequence as comprised in SEQ ID NO: 950 (simtuzumab); kh) the VH sequence as comprised in SEQ ID NO: 953, and the VL sequence as comprised in SEQ ID NO: 952 (sirexatamab); ki) the VH sequence as comprised in SEQ ID NO: 955, and the VL sequence as comprised in SEQ ID NO: 954 (sofituzumab); kj) the VH sequence as comprised in SEQ ID NO: 957, and the VL sequence as comprised in SEQ ID NO: 956 (spartalizumab); kk) the VH sequence as comprised in SEQ ID NO: 959, and the VL sequence as comprised in SEQ ID NO: 958 (surzebiclimab); kl) the VH sequence as comprised in SEQ ID NO: 961, and the VL sequence as comprised in SEQ ID NO: 960 (tabalumab); km) the VH sequence as comprised in SEQ ID NO: 963, and the VL sequence as comprised in SEQ ID NO: 962

(tafolecimab); kn) the VH sequence as comprised in SEQ ID NO: 965, and the VL sequence as comprised in SEQ ID NO: 964 (talacotuzumab); ko) the VH sequence as comprised in SEQ ID NO: 967, and the VL sequence as comprised in SEQ ID NO: 966 (tarextumab); kp) the VH sequence as comprised in SEQ ID NO: 969, and the VL sequence as comprised in SEQ ID NO: 968 (tavolimab); kq) the VH sequence as comprised in SEQ ID NO: 971, and the VL sequence as comprised in SEQ ID NO: 970 (tebotelimab); kr) the VH sequence as comprised in SEQ ID NO: 973, and the VL sequence as comprised in SEQ ID NO: 972 (teprotumumab); ks) the VH sequence as comprised in SEQ ID NO: 975, and the VL sequence as comprised in SEQ ID NO: 974 (tidutamab); kt) the VH sequence as comprised in SEQ ID NO: 977, and the VL sequence as comprised in SEQ ID NO: 976 (tigatuzumab); ku) the VH sequence as comprised in SEQ ID NO: 979, and the VL sequence as comprised in SEQ ID NO: 978 (tilvestamab); kv) the VH sequence as comprised in SEQ ID NO: 981, and the VL sequence as comprised in SEQ ID NO: 980 (tobemstomig); kw) the VH sequence as comprised in SEQ ID NO: 983, and the VL sequence as comprised in SEQ ID NO: 982 (tocilizumab); kx) the VH sequence as comprised in SEQ ID NO: 985, and the VL sequence as comprised in SEQ ID NO: 984 (tomaralimab); ky) the VH sequence as comprised in SEQ ID NO: 987, and the VL sequence as comprised in SEQ ID NO: 986 (tovecimig); kz) the VH sequence as comprised in SEQ ID NO: 989, and the VL sequence as comprised in SEQ ID NO: 988 (tovetumab); la) the VH sequence as comprised in SEQ ID NO: 991, and the VL sequence as comprised in SEQ ID NO: 990 (tucotuzumab); lb) the VH sequence as comprised in SEQ ID NO: 993, and the VL sequence as comprised in SEQ ID NO: 992 (tuparstobart); lc) the VH sequence as comprised in SEQ ID NO: 995, and the VL sequence as comprised in SEQ ID NO: 994 (upifitamab); ld) the VH sequence as comprised in SEQ ID NO: 997, and the VL sequence as comprised in SEQ ID NO: 996 (urabrelimab); le) the VH sequence as comprised in SEQ ID NO: 999, and the VL sequence as comprised in SEQ ID NO: 998 (utomilumab); lf) the VH sequence as comprised in SEQ ID NO: 1001, and the VL sequence as comprised in SEQ ID NO: 1000 (vadastuximab); lg) the VH sequence as comprised in SEQ ID NO: 1003, and the VL sequence as comprised in SEQ ID NO: 1002 (vandortuzumab); lh) the VH sequence as comprised in SEQ ID NO: 1005, and the VL sequence as comprised in SEQ ID NO: 1004 (vanucizumab); li) the VH sequence as comprised in SEQ ID NO: 1007, and the VL sequence as comprised in SEQ ID NO: 1006 (veligrotug); lj) the VH sequence as comprised in SEQ ID NO: 1009, and the VL sequence as comprised in SEQ ID NO: 1008 (verzistobart); lk) the VH sequence as comprised in SEQ ID NO: 1011, and the VL sequence as comprised in SEQ ID NO: 1010 (vesencumab); ll) the VH sequence as comprised in SEQ ID NO: 1013, and the VL sequence as comprised in SEQ ID NO: 1012 (vofatamab); lm) the VH sequence as comprised in SEQ ID NO: 1015, and the VL sequence as comprised in SEQ ID NO: 1014 (vonlerolizumab); ln) the VH sequence as comprised in SEQ ID NO: 1017, and the VL sequence as comprised in SEQ ID NO: 1016 (vopikitug); lo) the VH sequence as comprised in SEQ ID NO: 1019, and the VL sequence as comprised in SEQ ID NO: 1018 (vorsetuzumab); lp) the VH sequence as comprised in SEQ ID NO: 1021, and the VL sequence as comprised in SEQ ID NO: 1020 (xaluritamig); lq) the VH sequence as comprised in SEQ ID NO: 1023, and the VL sequence as comprised in SEQ ID NO: 1022 (zalutumumab); lr) the VH sequence as comprised in SEQ ID

NO: 1025, and the VL sequence as comprised in SEQ ID NO: 1024 (zanolimumab); ls) the VH sequence as comprised in SEQ ID NO: 1027, and the VL sequence as comprised in SEQ ID NO: 1026 (ivuxolimab-alt); lt) the VH sequence as comprised in SEQ ID NO: 1029, and the VL sequence as comprised in SEQ ID NO: 1028 (inotuzumab-alt); lu) the VH sequence as comprised in SEQ ID NO: 1031, and the VL sequence as comprised in SEQ ID NO: 1030 (moxetumomab-alt); lv) the VH sequence as comprised in SEQ ID NO: 1033, and the VL sequence as comprised in SEQ ID NO: 1032 (luveltamab-alt); lw) the VH sequence as comprised in SEQ ID NO: 1035, and the VL sequence as comprised in SEQ ID NO: 1034 (ibritumomab-alt); lx) the VH sequence as comprised in SEQ ID NO: 1037, and the VL sequence as comprised in SEQ ID NO: 1036 (pivekimab-alt); ly) the VH sequence as comprised in SEQ ID NO: 1039, and the VL sequence as comprised in SEQ ID NO: 1038 (avelumab-alt); lz) the VH sequence as comprised in SEQ ID NO: 1041, and the VL sequence as comprised in SEQ ID NO: 1040 (sugemalimab-alt); ma) the VH sequence as comprised in SEQ ID NO: 1043, and the VL sequence as comprised in SEQ ID NO: 1042 (nimotuzumab-alt); mb) the VH sequence as comprised in SEQ ID NO: 1045, and the VL sequence as comprised in SEQ ID NO: 1044 (panitumumab-alt); mc) the V_H sequence as comprised in SEQ ID NO: 540, and the V_L sequence as comprised in SEQ ID NO: 541 (AR46A6); md) the V_H sequence as comprised in SEQ ID NO: 542, and the V_L sequence as comprised in SEQ ID NO: 543 (KM4097); and me) the V_H sequence as comprised in SEQ ID NO: 544, and the V_L sequence as comprised in SEQ ID NO: 545 (K5-70).

In one embodiment therefore, an antigen binding protein in a conjugate with an IL-21 mutein as described herein comprises an immunoglobulin single variable domain (ISVD) comprising a VHH domain selected from the group consisting of: a) the VHH sequence as comprised in SEQ ID NO: 504 (envafolimab); b) the VHH sequence as comprised in SEQ ID NO: 505 (erfonrilimab – PD-L1); c) the VHH sequence as comprised in SEQ ID NO: 506 (erfonrilimab – CTLA-4); and, d) the VHH sequence as comprised in SEQ ID NO: 507 (ozekibart).

Antigen-binding regions that specifically bind an NK cell activating receptor

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one or a second antigen-binding region that specifically binds an NK cell activating receptor. In one embodiment, the antigen-binding region that specifically binds an NK cell activating receptor is an agonistic antigen-binding region that activates the NK cell receptor. Preferably, the antigen-binding region comprises at least one immunoglobulin variable region, more preferably, the immunoglobulin variable region comprises or consists of a Fab or an immunoglobulin single variable domain (ISVD). In one embodiment, the antigen-binding region is a human or humanized antigen-binding region.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises two antigen-binding regions that specifically bind an NK cell activating receptor. The two antigen-binding regions can bind the same NK cell activating receptor or they can bind at least two different NK cell activating receptors. In one embodiment, the two antigen binding regions are identical.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein is an antigen binding protein wherein the NK cell activating receptor (that is bound by the antigen-binding region) is selected from the group consisting of: NKp46, NKp30, NKG2D, CD16A, SLAMF7, NKp44, CD94-NKG2C/E, KIR2DS1, KIR2DS3, KIR2DS4, KIR2DS5, KIR2DS2, KIR2DL4, KIR3DS1, CD160, NKp80, DNAM1, 2B4, CRACC, 4-BB, OX40, CRTAM, CD27, PSGL1, CD96, CD100, CEACAM1, CD59, PD-L1, Tim3 and NTB-A, of which NKp46, NKp30, NKG2D, CD16A, and SLAMF7 are preferred.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises the antigen-binding region that specifically binds an NK cell activating receptor that is a natural cytotoxicity receptor (NCR). Natural cytotoxicity receptors are type 1 transmembrane proteins of the immunoglobulin superfamily, which upon stimulation mediate NK killing and release of IFNγ. They bind viral ligands such as hemagglutinins and hemagglutinin neuraminidases, some bacterial ligands and cellular ligands related to tumor growth such as PCNA. Natural Cytotoxicity Receptors include NKp46, NKp44, and NKp30. In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises the antigen-binding region that specifically binds an NK cell activating receptor that is an NCR selected from the group consisting of NKp46, NKp44 and NKp30.

“NKp46” refers to a protein or polypeptide encoded by an *Ncr1* gene or by a cDNA prepared from such a gene. NKp46 has also been designated as NCR1, CD335 (cluster of differentiation, NKP46, NK-p46, and LY94. Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term NKp46 polypeptide (e.g., an NKp46 polypeptide that is at least 90%, at least 95%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 50, or a contiguous sequence of at least 20, at least 30, at least 50, at least 100 or at least 200 amino acid residues thereof). The 304 amino acid residue sequence of human NKp46 (isoform a) is shown in in SEQ ID NO: 50, which corresponds to NCBI accession number NP_004820, the disclosure of which is incorporated herein by reference. The human NKp46 mRNA sequence is described in NCBI accession number NM_004829, the disclosure of which is incorporated herein by reference.

“NKp44” refers to a protein or polypeptide encoded by an *Ncr2* gene or by a cDNA prepared from such a gene. NKp44 has also been designated as NCR2, CD336 (cluster of differentiation 336), NKP44, NK-p44, LY95, and dJ149M18.1. Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term NKp44 polypeptide (e.g., an NKp44 polypeptide that is at least 90%, at least 95%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 51, or a contiguous sequence of at least 20, at least 30, at least 50, at least 100 or at least 200 amino acid residues thereof). The 276 amino acid residue sequence of human NKp46 is shown in SEQ ID NO: 51, which corresponds to NCBI accession number NP_004819, the disclosure of which is incorporated herein by reference. The human NKp46 mRNA sequence is described in NCBI accession number NM_004828, the disclosure of which is incorporated herein by reference.

“NKp30” refers to a protein or polypeptide encoded by an *Ncr3* gene or by a cDNA prepared from such a gene. NKp30 has also been designated as NCR3 and CD337 (cluster of differentiation 337). Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term NKp30

polypeptide (e.g., an NKp30 polypeptide that is at least 90%, at least 95%, at least 98%, at least 99% or 100% identical to SEQ ID NO:52, or a contiguous sequence of at least 20, at least 30, at least 50, at least 100 or at least 200 amino acid residues thereof). The 201 amino acid residue sequence of human NKp30 is shown in below in SEQ ID NO: 52, which corresponds to NCBI accession number NP_667341, the disclosure of which is incorporated herein by reference. The human NKp30 mRNA sequence is described in NCBI accession number NM_147130, the disclosure of which is incorporated herein by reference.

NKG2D is an activating receptor (transmembrane protein) belonging to the NKG2 family of C-type lectin-like receptors. NKG2D is encoded by *KLRK1* gene in humans. NKG2D recognizes induced-self proteins from MIC and RAET1/ULBP families which appear on the surface of stressed, malignant transformed, and infected cells. “NKG2D” refers to a protein or polypeptide encoded by a *KLRK1* gene or by a cDNA prepared from such a gene. NKG2D has also been designated as KLRK1, CD314 (cluster of differentiation 314), D12S2489E, KLR, NKG2-D, natural killer group 2D, killer cell lectin-like receptor K1, killer cell lectin like receptor K1. Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term NKG2D polypeptide (e.g., an NKG2D polypeptide that is at least 90%, at least 95%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 53, or a contiguous sequence of at least 20, at least 30, at least 50, at least 100 or at least 200 amino acid residues thereof). The 216 amino acid residue sequence of human NKG2D is shown in SEQ ID NO: 53, which corresponds to NCBI accession number NP_001186734, the disclosure of which is incorporated herein by reference. The human NKG2D mRNA sequence is described in NCBI accession number NM_007360, the disclosure of which is incorporated herein by reference.

DNAM-1 is a ~65 kDa glycoprotein expressed on the surface of amongst others NK cells. It is a member of the immunoglobulin superfamily containing 2 Ig-like domains of the V-set. DNAM-1 mediates cellular adhesion to other cells bearing its ligands, CD112 and CD155, and cross-linking DNAM-1 with antibodies causes cellular activation. “DNAM-1” refers to a protein or polypeptide encoded by a *KLRK1* gene or by a cDNA prepared from such a gene. DNAM-1 has also been designated as CD226 (cluster of differentiation 226), DNAM-1, DNAM1, PTA1 and TLiSA1. Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term DNAM-1 polypeptide (e.g., an DNAM-1 polypeptide that is at least 90%, at least 95%, at least 98% or 99% identical to SEQ ID NO: 54, or a contiguous sequence of at least 20, at least 30, at least 50, at least 100 or at least 200 amino acid residues thereof). The 336 amino acid residue sequence of human DNAM-1 is shown in SEQ ID NO: 54, which corresponds to NCBI accession number NP_006557, the disclosure of which is incorporated herein by reference. The human DNAM-1 mRNA sequence is described in NCBI accession number NM_006566, the disclosure of which is incorporated herein by reference.

As indicated above, CD16A is an immunoglobulin gamma Fc region receptor (FcγRIIIa) that is expressed on NK cells and through which NK cells recognize IgG that is bound to the surface of a pathogen-infected or TAA-expressing target cell. Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term CD16A polypeptide (e.g., an CD16A polypeptide that is at

least 90%, at least 95%, at least 98%, at least 99% or 100% identical to SEQ ID NO: 55, or a contiguous sequence of at least 20, at least 30, at least 50, at least 100 or at least 200 amino acid residues thereof). The 254 amino acid residue sequence of human CD16A is shown in SEQ ID NO: 55, which corresponds to UniProt accession no. P08637, the disclosure of which is incorporated herein by reference.

"SLAMF7" is a protein that in humans is encoded by the human SLAMF7 gene. Isoform 1 SLAMF7 mediates NK cell activation through a SH2D1A-independent extracellular signal-regulated ERK-mediated pathway. SLAMF7 has also been designated as CD319 (cluster of differentiation 319), 19A, CRACC, and CS1. Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term SLAMF7 polypeptide (e.g., a SLAMF7 polypeptide that is at least 90%, at least 95%, at least 98%, at least or 99% or 100% identical to SEQ ID NO: 56, or a contiguous sequence of at least 20, at least 30, at least 50, at least 100 or at least 200 amino acid residues thereof). The 335 amino acid residue sequence of human SLAMF7 is shown in SEQ ID NO: 56, which corresponds to UniProt accession no. Q9NQ25-1, the disclosure of which is incorporated herein by reference.

In one embodiment of an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, the antigen-binding region that specifically binds an NK cell activating receptor is an agonistic antigen-binding region that activates the NK cell receptor. As used herein, an antigen-binding region that has "agonist" activity at an NK cell activating receptor is an agent that can cause or increase "signaling by the NK cell activating receptor". "Signaling by the NK cell activating receptor" refers to an ability of an NK cell activating receptor to activate or transduce an intracellular signaling pathway. Changes in NK cell activating receptor-signaling activity can be measured, for example, by assays designed to measure changes in NK cell activating receptor-signaling pathways, e.g. by monitoring phosphorylation of signal transduction components, assays to measure the association of certain signal transduction components with other proteins or intracellular structures, or in the biochemical activity of components such as kinases, or assays designed to measure expression of reporter genes under control of NK cell activating receptor-sensitive promoters and enhancers, or indirectly by a downstream effect mediated by the NK cell activating receptor polypeptide (e.g. activation of specific cytolytic machinery in NK cells). Reporter genes can be naturally occurring genes (e.g. monitoring cytokine production) or they can be genes artificially introduced into a cell. Other genes can be placed under the control of such regulatory elements and thus serve to report the level of NK cell activating receptor-signaling activity.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises at least one antigen-binding region that is obtained/obtainable from a monoclonal antibody against NK cell activating receptor as is known in the art. In one embodiment, the at least one antigen-binding region at least comprises the six CDR sequences that are obtained/obtainable from a monoclonal antibody against NK cell activating receptor as is known in the art. In one embodiment, the at least one antigen-binding region at least comprises the variable light (V_L) domain and variable heavy (V_H) domain sequences that are obtained/obtainable from a monoclonal antibody against NK cell activating receptor as is known in the art. Many examples of

monoclonal antibodies against NK cell activating receptor have been described in the art. Anti-NKp46 monoclonal antibodies are described WO 2011/086179, WO 2016/209021 and in Gauthier et al. (2019, Cell 177, 1701–1713) or in WO 2016/207278, such as NKp46-1, -2, -3, -4, -6 or -9. Anti-NKG2D monoclonal antibodies described WO 2009/077483, WO 2018/148447, WO 2019/157366, WO 2018/148445, WO 2018/152518 and WO 2019/195409, which include the heavy and light chain sequences of SEQ ID NO.'s: 16 and 20, respectively. Monoclonal antibodies against NKG2A are e.g. described in WO 2008/009545, WO 2009/092805, WO 2016/032334, WO 2020/094071 and WO 2020/102501. Monoclonal antibodies against NKp30 are e.g. described in WO 2020/172605. Monoclonal antibodies against DNAM-1 are e.g. described in WO 2013/140787. Examples of anti-SLAMF7 monoclonal antibodies include Elotuzumab and others described in US2018208653. Monoclonal antibodies against 4-1BB (CD137) are e.g. described in WO 2005/035584, WO 2006/088464 and US2006188439. Monoclonal antibodies against OX40 are e.g. described in WO 2007/062245, US2010136030, US2019100596, WO 2013/008171 and WO 2013/028231. Monoclonal antibodies against CD96 are e.g. described in WO 2019/091449. Monoclonal antibodies against CD160 are e.g. described in US2012003224 and US2013122006. Monoclonal antibodies against KIR2DS1-5 are e.g. described in WO 2016/031936.

Antigen binding regions binding to γδ T cell receptors

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, can further comprise a third antigen-binding region, which is an antigen-binding region specifically binds an epitope of a γδ T cell receptor (TCR).

The third antigen-binding region as used in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein can be derived from any of a variety of immunoglobulin or non-immunoglobulin scaffolds, as described above for the first and second antigen binding regions. In a preferred embodiment, a third antigen-binding region as used in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises or consists of an immunoglobulin variable region. Such immunoglobulin variable regions can comprise or consist of variable domains derived from antibodies (immunoglobulin chains), e.g. in the form of associated V_L and V_H domains found on two polypeptide chains, such as present in a Fab. Alternatively, immunoglobulin variable domains can comprise or consist of a single chain antigen-binding domain such as a scFv, a V_H domain, a V_L domain, or an immunoglobulin single variable domain (ISVD) such as a dAb, a V-NAR domain or a V_HH domain. An immunoglobulin variable region to be used as third antigen-binding region in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein can be a human or humanized immunoglobulin variable region or an immunoglobulin single variable domain as herein defined above.

In one embodiment, the third antigen-binding region that specifically binds an epitope of a γδ TCR is an antigen-binding region derived from immunoglobulin or non-immunoglobulin scaffolds as defined above. Preferably, the third antigen-binding region comprises or consists of at least one immunoglobulin variable domain. More preferably, the third antigen-binding region comprises or consists of a Fab that specifically binds an epitope of a γδ TCR or an immunoglobulin single variable

domain (ISVD) that specifically binds an epitope of a γδ TCR. In one embodiment, the third antigen-binding region binds the epitope of a γδ TCR with a K_D value of no more than 10^{-3} M or 10^{-4} M, as may be determined as herein described above.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds an epitope of at least one of: a) a variable region (V) of a delta (δ) chain selected from the group consisting of: Vδ1, Vδ2 and Vδ3 chains; b) a V region of a gamma (γ) chain selected from the group consisting of: Vγ2, Vγ3, Vγ4, Vγ5, Vγ8, and Vγ9 chains; c) a constant (C) region of a γ chain; and, d) a C region of a δ chain.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds to a γδ TCR, preferably to any γδ TCR, which antigen-binding region comprises CDR amino acid sequences that are obtained/derived from a monoclonal antibody selected from the group consisting of: clone 5A6.E9 (TCR1061, Thermo Fisher Sci.), clone B1.1 (Thermo Fisher Sci.), clone gamma 3.20 (TCR 1153, Thermo Fisher Sci.), clone IMMU510 (Product No: IM1571U, Beckman Coulter Life Sci.), and clone 11F2 (MUB1809P, Thermo Fisher Sci.). All of these monoclonal antibodies are mouse monoclonal antibodies. Hence, the skilled person will understand that their CDR amino acid sequences preferably are grafted into human framework regions, so as to humanize these monoclonal antibodies for incorporation of into a third antigen-binding region of an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds to a Vδ2 TCR, which antigen-binding region comprises CDR amino acid sequences that are obtained/derived from a monoclonal antibodies selected from the group consisting of: clone 15D (TCR1732, Thermo Fisher Sci.) and clone B6 (MA5-44049, Thermo Fisher Sci.). Both of these monoclonal antibodies are mouse monoclonal antibodies. Hence, the skilled person will understand that their CDR amino acid sequences preferably are grafted into human framework regions, so as to humanize these monoclonal antibodies for incorporation of into a third antigen-binding region of an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds to a Vγ9 TCR, which antigen-binding region comprises CDR amino acid sequences that are obtained/derived from a monoclonal antibodies selected from the group consisting of: clone B3 (MA5-44047, Thermo Fisher Sci.) and clone 7A5 (TCR1720, Thermo Fisher Sci.). Both of these monoclonal antibodies are mouse monoclonal antibodies. Hence, the skilled person will understand that their CDR amino acid sequences preferably are into human framework regions, so as to humanize these monoclonal antibodies for incorporation of into a third antigen-binding region of an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein.

In a preferred embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds an

epitope in the V region of a Vδ1 chain of a γδ TCR. In one embodiment, a third antigen-binding region that specifically binds an epitope in the V region of a Vδ1 chain of a γδ TCR, does not interact with other delta chains such as Vδ2 or Vδ3. In one embodiment, a third antigen-binding region that specifically binds an epitope in the V region of a Vδ1 chain of a γδ TCR, does not interact with gamma chains such as Vγ2, Vγ3, Vγ4, Vγ5, Vγ8, and Vγ9. In one embodiments, a third antigen-binding region that specifically binds an epitope in the V region of a Vδ1 chain of a γδ TCR, also does not bind or interact with other domains found within a γδ TCR, such as TRDJ, TRDC, TRGJ or TRGC.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds to a Vδ1 TCR, which antigen-binding region comprises CDR amino acid sequences that are obtained/derived from a monoclonal antibodies selected from the group consisting of: clone TS8.2 (TCR1730, Thermo Fisher Sci.), clone TS-1 (TCR 1055, Thermo Fisher Sci.), and clone R9.12 (Product No: IM1761, other name: ZAP-70, Beckman Coulter Life Sci.). All three of these monoclonal antibodies are mouse monoclonal antibodies. Hence, the skilled person will understand that their CDR amino acid sequences preferably are grafted into human framework regions, so as to humanize these monoclonal antibodies for incorporation of into a third antigen-binding region of an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds an activating epitope of a γδ T cell. In a preferred embodiment, the third antigen-binding region specifically binds an activating epitope in the V region of a Vδ1 chain of a γδ TCR.

An “activating” epitope can include, for example, stimulating a TCR function, such as cell degranulation, TCR downregulation, cytotoxicity, proliferation, mobilization, increased survival or resistance to exhaustion, intracellular signaling, cytokine or growth factor secretion, phenotypic change, or a change in gene expression. For example, the binding of the activating epitope may stimulate expansion (i.e. proliferation) of the γδ T cell population, preferably the Vδ1+ T cell population. Accordingly, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprising a third antigen-binding region that specifically binds an activating epitope of a γδ T cell, can be used to modulate γδ T cell activation, and, thereby, to modulate the immune response. Therefore, in one embodiment, binding of the activating epitope by the third antigen-binding region downregulates the γδ TCR. In an additional or alternative embodiment, binding of the activating epitope by the third antigen-binding region activates degranulation of the γδ T cell. In a further additional or alternative embodiment, binding of the activating epitope by the third antigen-binding region promotes γδ T cell mediated killing of cells expressing the antigen (e.g. TAA) targeted by the antigen binding protein.

In one embodiment, an activating epitope of TRDV1 is one that, upon being bound by a third antigen-binding region in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, results in down-regulation of the receptor and optionally activates the Vδ1 cell. In some embodiments said down-regulation of the receptor also results in the down-regulation of

associated CD3 molecules. In some embodiments, the activating epitope is one that, upon binding by the third antigen-binding region, upregulates expression of activatory markers on the Vδ1 cell, for example CD107a, CD25, CD69 and/or Ki67. In some embodiments, an activating epitope is one that, upon binding by the third antigen-binding region, upregulates expression of activatory markers on the Vδ1 cell, for example CD107a and CD25, and optionally CD69 and/or Ki67. In some embodiments, upregulation of the one or more activatory markers (such as CD107a) may be upregulation in the presence of cancer cells.

As T-cell receptors are often complexed with other proteins, downregulation of the T-cell receptor via binding of a third antigen-binding region to a Vδ1 domain may cause downregulation of other proteins associated with the T-cell receptor (i.e. the binding of the third antigen-binding region to a Vδ1 domain causes down regulation of the T-cell receptor complex). For example, in some embodiments, an activating epitope of TRDV1 is one that upon binding by a third antigen-binding region, down-regulates the TCR/CD3 receptor complex. In this way, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, may cause indirect downregulation of cell surface proteins that are not bound by the protein, but are complexed to the T-cell receptor. Given T-cells expressing gamma delta 1 chains (i.e. Vδ1 cells) represent only a small number of the total T-cell population, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein can be used to selectively (and indirectly) downregulate proteins in the TCR complex, such as CD3, by only downregulating them in Vδ1 cells.

In one embodiment, a T-cell receptor complex activating epitope is one that upon activation, by being bound by a third antigen-binding region, downregulates the T-cell receptor complex, whilst not downregulating CD3 molecules not associated with said TRDV1 TCR complex.

In one embodiment, a third antigen-binding region preferably binds an epitope that is comprised of at least one extracellular, soluble, hydrophilic or external portion of the Vδ1 chain of a γδ TCR.

In a particular embodiment, a third antigen-binding region binds an epitope that does not comprise an epitope found in a hypervariable region of the Vδ1 chain of the γδ TCR, in particular not in the CDR3 of the Vδ1 chain. In a preferred embodiment, a third antigen-binding region binds an epitope that is located within the non-variable region of the Vδ1 chain of the γδ TCR. It will be appreciated that such binding allows for the unique recognition of the Vδ1 chain without the restriction to the sequences of the TCR which are highly variable (in particular CDR3). Various γδ TCR complexes which recognize antigen may be recognized in this way, solely by presence of the Vδ1 chain. As such, it will be appreciated that any Vδ1 chain-comprising γδ TCR may be recognized using an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, irrespective of the specificity of the γδ TCR. In one embodiment, the third antigen-binding region binds an epitope that comprises one or more amino acid residues within amino acid regions 1-24 and/or 35-90 of SEQ ID NO: 510, e.g. the portions of the Vδ1 chain which are not part of the CDR1 and/or CDR3 sequences. In one embodiment, the third antigen-binding region binds an epitope that does not comprise amino acid residues within amino acid region 91-105 (CDR3) of SEQ ID NO:

510. In some embodiments, the third antigen-binding region binds an the epitope that comprises amino acids in the TRDV-1 CDR2 sequence.

In a similar manner to the well characterized αβ T cells, γδ T cells utilize a distinct set of somatically rearranged variable (V), diversity (D), joining (J), and constant (C) genes, although γδ T cells contain fewer V, D, and J segments than αβ T cells. In one embodiment, the epitope bound by the third antigen-binding region, does not comprise an epitope found in the J region of the Vδ1 chain or in the C-region of the Vδ1 chain. In one embodiment, the epitope bound by the third antigen-binding region binds an epitope found in the N-terminal leader sequence of the Vδ1 chain. The third antigen-binding region may therefore only bind in the V region of the Vδ1 chain. Thus, in one embodiment, the epitope consists of an epitope in the V region of the γδ TCR (e.g. amino acid residues 1-90 of SEQ ID NO: 510).

Reference to the epitope is made in relation to the Vδ1 sequence derived from the sequence described in Luoma et al. (2013) Immunity 39: 1032-1042, and RCSB Protein Data Bank entries: 4MNH and 3OMZ, shown as SEQ ID NO: 510. SEQ ID NO: 510 represents a soluble TCR comprising a V region (also referred to as the variable domain), a D region, a J region and a TCR constant region. The V region comprises amino acid residues 1-90, the D region comprises amino acid residues 91-104, the J region comprises amino acid residues 105-115 and the constant region (derived from T-cell receptor alpha) comprises amino acid residues 116-209. Within the V region, CDR1 is defined as amino acid residues 25-34 of SEQ ID NO: 510, CDR2 is defined as amino acid residues 50-54 of SEQ ID NO: 510, and CDR3 is defined as amino acid residues 93-104 of SEQ ID NO: 510 (Xu et al., PNAS USA 108(6):2414-2419 (2011)).

Therefore, in one embodiment, there is provided an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprising a third antigen-binding region that binds the epitope of a γδ TCR with a binding affinity (K_D) as measured by surface plasmon resonance of less than 1.5×10^{-7} M (i.e.150 nM). In one embodiment, the K_D of the third antigen-binding region for the epitope of a γδ TCR is (with increasing preference) 1.5×10^{-7} M (i.e.150 nM) or less, 1.3×10^{-7} M (i.e.130 nM) or less, 1.0×10^{-7} M (i.e.100 nM) or less, 5.0×10^{-8} M (i.e.50 nM) or less, 2.0×10^{-8} M (i.e. 20 nM) or less, 1.0×10^{-8} M (i.e. 10 nM) or less, 5×10^{-9} M (i.e. 5 nM) or less, 2×10^{-9} M (i.e. 2 nM) or less, or 1×10^{-9} M (i.e. 1 nM) or less.

In one embodiment, there is provided an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprising a third antigen-binding region that binds the epitope of a γδ TCR with a binding affinity (K_D) as measured by surface plasmon resonance of higher than 1.5×10^{-7} M (i.e.150 nM). In one embodiment, the K_D of the third antigen-binding region for the epitope of a γδ TCR is (with increasing preference) 1.5×10^{-7} M (i.e.150 nM) or more, 3×10^{-7} M (i.e. 300 nM) or more, 1.0×10^{-6} M (i.e.1 μM) or more, 2.0×10^{-6} M (i.e. 2 μM) or more, 5.0×10^{-6} M (i.e. 5 μM) or more, 1.0×10^{-5} M (i.e. 10 μM) or more, 2×10^{-5} M (i.e. 20 μM) or more, 5×10^{-5} M (i.e. 50 μM) or more, 1×10^{-4} M (i.e. 100 μM) or more, or 1×10^{-3} M (i.e. 1000 μM) or more.

In one embodiment, the binding affinity of the third antigen-binding region in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, is established by coating the third antigen-binding region or the antigen binding protein comprising the third antigen-

binding region directly or indirectly (e.g. by capture with an antibody) onto the surface of a sensor (e.g. an amine high capacity chip or equivalent), wherein the target bound by the third antigen-binding region (e.g. the Vδ1 chain of a γδ TCR) is flowed over the chip to detect binding. Suitably, a MASS-2 instrument (which may also be referred to as Sierra SPR-32) is used at 25 °C in PBS + 0.02 % Tween 20 running buffer at 30 µl/min.

Unlike anti-Vδ1 antibodies of the prior art which focus on depletion of Vδ1 T-cells or attempt activation of Vδ1 T-cells via the TCR without any appropriate co stimulation or synapse formation, the antigen binding proteins provided herein are useful for the activation of Vδ1 T-cells via the TRDV1-binding third antigen-binding region. Although they may cause downregulation of the TCRs on T-cells to which they bind, they do not cause Vδ1 T-cell depletion, but rather they stimulate the T-cells and hence may be useful in therapeutic settings that would benefit from the activation of this compartment of T-cells. Activation of Vδ1 T-cells is evident through TCR downregulation, CD3 downregulation, changes in activation markers such as CD25 and Ki67 and degranulation marker CD107a. Activation of Vδ1 T-cell in turn triggers release of inflammatory cytokines such as INFɣ and TNFα to promote immune licensing.

In one embodiment, there is provided an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprising a TRDV1-binding third antigen-binding region, characterized in that the antigen binding protein: a. does not exhibit CDC or ADCC; and b. does not deplete Vδ1 T-cells.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein also stimulates Vδ1 T-cell proliferation. T-cell depletion is the process of T cell death, removal or reduction. References to an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein not depleting the Vδ1 T cells refers to a depletion of less than about 30% or less than about 20% (preferably less than about 10%) of the viable Vδ1 T+ cell population when incubated by one or more of the antigen binding proteins as described herein, and as measured by any via suitable means in a controlled study (for example via controlled flow cytometry methodology or via other established controlled assays). ADCC and CDC are mechanisms by which T-cell depletion may occur. Reference to an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein not causing ADCC or CDC refers to a depletion of less than about 30% or less than about 20% (preferably less than about 10%) of the viable Vδ1 T+ cell population via ADCC and/or CDC when incubated by one or more of the antigen binding proteins as described herein, as measured by any via suitable means (for example via controlled flow cytometry methodology or via other established controlled assays).

In one embodiment, there is provided an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, characterized in that it does not induce secretion of IL-17A. IL-17A (Interleukin-17A) is a pro-tumorigenic cytokine which is produced by activated T-cells. IL-17A can enhance tumor growth and dampen the anti- cancer immune response. Reference to an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein not inducing secretion of IL-17A refers to inducing less than about 30%, or less than about 20%, or less than about 10% of the IL-17A secretion induced by equivalent anti-CD3 antigen binding proteins.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds to a Vδ1 TCR, which antigen-binding region comprises a combination of complementarity-determining regions (CDRs) CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of: a) the CDR-H1 sequence of SEQ ID NO: 511, the CDR-H2 sequence of SEQ ID NO: 512, CDR-H3 sequence of SEQ ID NO: 513, the CDR-L1 sequence of SEQ ID NO: 514, the CDR-L2 sequence of SEQ ID NO: 515 (amino acids YDS) and the CDR-L3 sequence of SEQ ID NO: 516; b) the CDR-H1 sequence of SEQ ID NO: 517, the CDR-H2 sequence of SEQ ID NO: 518, CDR-H3 sequence of SEQ ID NO: 519, the CDR-L1 sequence of SEQ ID NO: 520, the CDR-L2 sequence of SEQ ID NO: 521 (amino acids DAS) and the CDR-L3 sequence of SEQ ID NO: 522; c) the CDR-H1 sequence of SEQ ID NO: 523, the CDR-H2 sequence of SEQ ID NO: 524, CDR-H3 sequence of SEQ ID NO: 525, the CDR-L1 sequence of SEQ ID NO: 526, the CDR-L2 sequence of SEQ ID NO: 527 (amino acids VAS) and the CDR-L3 sequence of SEQ ID NO: 528.

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises a third antigen-binding region that specifically binds to a Vδ1 TCR, which antigen-binding region comprises a combination of variable heavy (V_H) and variable light (V_L) domain sequences that have, with increasing preference, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity with the V_H and V_L sequence combinations selected from the group consisting of: a) the V_H sequence as comprised in SEQ ID NO: 529, and the V_L sequence as comprised in SEQ ID NO: 530; b) the V_H sequence as comprised in SEQ ID NO: 531, and the V_L sequence as comprised in SEQ ID NO: 532; c) the V_H sequence as comprised in SEQ ID NO: 533, and the V_L sequence as comprised in SEQ ID NO: 534.

An antigen-binding region with affinity for a surface antigen expressed on NK cells

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, can further comprise a third antigen-binding region, which is an antigen-binding region that has or can have affinity for a surface antigen expressed on NK cells.

In one embodiment of the an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, the third antigen-binding region that has or can have affinity for a surface antigen expressed on NK cells comprises or consists of an immunoglobulin Fc region, or at least a portion thereof that binds the type III Fcγ receptor (FcγRIIIa) as expressed on (human) NK cells, also referred to herein as CD16A. In one embodiment, the immunoglobulin Fc region at least comprises at least one of a CH2 and CH3 domain. In one embodiment, the immunoglobulin Fc region at least comprises at least one of a CH2 and CH3 domain and a hinge region. In one embodiment, the immunoglobulin Fc region comprises or consists of a hinge region and a CH2 and CH3 domain. In one embodiment, the immunoglobulin Fc region is a dimeric Fc region or at least a portion thereof that binds CD16A.

In one embodiment, an Fc region or portion thereof that binds CD16A be a wild-type region or portion thereof.

In one embodiment, an Fc region or portion thereof that binds CD16A can be modified to enhance or reduce its binding affinity to CD16A. Within the Fc region, CD16A binding is mediated by the hinge region and the CH2 domain. For example, within human IgG1, the interaction with CD16 is primarily focused on amino acid residues D265 – E269, N297 – T299, A327 – I332, L 234 – S239, and carbohydrate residue N-acetyl-D-glucosamine in the CH2 domain (see, Sondermann et al., 2000 Nature, 406(6793):267-273). Based on the known domains, mutations can be selected to enhance or reduce the binding affinity to CD16A, such as by using phage-displayed libraries or yeast surface-displayed cDNA libraries or can be designed based on the known three-dimensional structure of the interaction. In one embodiment, the Fc region or portion is IgG2.

Thus, in one embodiment, where the antigen binding protein is intended to have increased affinity for CD16A, an Fc region or portion thereof that binds CD16A, can comprise a modification to increase affinity for CD16A. Thus, an Fc region or portion thereof that binds CD16A, can comprise one or more amino acid modifications (e.g. amino acid substitutions, deletions, insertions) which increase binding to (human) CD16A and optionally another receptor such as FcRn. Typical modifications include modified human IgG1-derived constant regions comprising at least one amino acid modification (e.g. substitution, deletions, insertions), and/or altered types of glycosylation, e.g., hypofucosylation. A modification can, for example, increase binding of an Fc region to FcγRIIIa (CD16A) on NK cells. Examples of modifications are provided in US 10,577,419, the disclosure of which is incorporated herein by reference. Specific mutations (in IgG1 Fc regions) which enhance FcγRIIIa (CD16A) binding, include E333A, S239D/I332E and S239D/A330L/I332E.

In one embodiment, the antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises an Fc region or portion thereof that binds CD16A comprising at least one amino acid modification (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) relative to a wild-type Fc region, such that the molecule has enhanced binding affinity for (human) CD16A relative to a molecule comprising a wild-type Fc region, optionally wherein the variant Fc region comprises a substitution at any one or more of positions 239, 298, 330, 332, 333 and/or 334 (e.g. S239D, S298A, A330L, I332E, E333A and/or K334A substitutions), optionally wherein the variant Fc region comprises a substitution at residues S239 and I332, e.g. a S239D and I332E substitution (Kabat EU numbering).

In one embodiment, the antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises an Fc region or portion thereof that binds CD16A comprising altered glycosylation patterns that increase binding affinity for (human) CD16A. Such carbohydrate modifications can be accomplished by, for example, by expressing a nucleic acid encoding the antigen binding protein in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery are known in the art and can be used as host cells in which to express recombinant antibodies to thereby produce an antibody with altered glycosylation. See, for example. Shields, R.L. et al. (2002) J. *Biol. Chem.* 277:26733-26740; Umana et al. (1999) *Nat. Biotech.* 17:176-1, as well as, European Patent No: EP 1,176,195; WO 06/133148; WO 03/035835; WO 99/54342, each of which is incorporated herein by reference in its entirety. In one embodiment, the antigen binding protein comprises one or more hypofucosylated constant regions. Such an antigen

binding protein can comprise an amino acid alteration or cannot comprise an amino acid alteration and/or may be expressed or synthesized or treated under conditions that result in hypofucosylation. In one embodiment, in a composition comprising the antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, at least 20, at least 30, at least 40, at least 50, at least 60, at least 75, at least 85, at least 90, at least 95% or substantially all of the antigen binding proteins have a constant region comprising a core carbohydrate structure (e.g. complex, hybrid and high mannose structures) which lacks fucose. In one embodiment, there is provided an antigen binding protein which is free of N-linked glycans comprising a core carbohydrate structure having fucose. The core carbohydrate will preferably be a sugar chain at Asn297.

In one embodiment, the antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises an Fc region or portion thereof that binds CD16A that is modified to have increased binding affinity for CD16A, has a binding affinity for human CD16A that is at least 1, 2 or 3 log greater than that of a conventional or wild-type human IgG1 antibody, e.g., as assessed by surface plasmon resonance.

In another embodiment, where an antigen binding protein is intended to have reduced affinity for CD16A, a CH2 and/or CH3 domain, an Fc region or portion thereof that binds CD16A, can comprise a modification to decrease affinity for CD16A. For example, CH2 mutations in a dimeric Fc region protein at reside N297 (Kabat numbering) can eliminate CD16A binding. Other modifications in the Fc region that reduce or eliminate binding to CD16A include the L234A/L235A modification (also known as “LALA”), the L235A/G237A modification (also known as “LAGA” and described in Liu et al., Antibodies. 2020;9(4):64; Szapacs et al., Bioanalysis. 2023;15(16):955–6), and the L234A/L235A/P329G modification (also known as LALAPG) and which more completely abrogates CD16 binding compared to the LALA modification. In embodiments wherein an antigen binding protein in a conjugate with an 4-1BBL ECD mutein as described herein comprises an antigen-binding region that specifically binds an epitope of a γδ TCR, it is preferred that the antigen binding protein comprises an Fc region that is modified to reduce or eliminate binding to CD16A.

Modification of the Fc region that reduce or eliminate its binding to CD16A can be useful to prevent or at least reduce the “sink effect”, wherein at least a fraction of the administered amount of multispecific antigen binding protein is lost by binding to NK cells or monocytes. Modification of the Fc region that reduce or eliminate its binding to CD16A can also be useful in an antigen binding protein to further attenuate apparent affinity to NK cells reducing activity in absence of target cells. Modification of the Fc region that reduce or eliminate its binding to CD16A can also be useful in an antigen binding protein to reduce or avoid NK cell fratricide. The lack of NK cell fratricide can be an advantageous feature for the antigen binding protein described herein. NK cell cross-linking with NK cells or other immune cells is expected to reduce therapeutic efficacy of NK cell-engagement. Most importantly, cross-linking of a NK cell with one or more NK cells or other immune cells through bivalent or multivalent interactions with FcRy or in combination with a second immune cell antigen (e.g. NKp46, NKG2D, NKp30, SLAMF7 or CD38) can cause immune cell activation. This might lead to induction of target cell-driven fratricide or immune cell killing (e.g. NK-NK cell lysis), ultimately resulting in efficient NK cell depletion in vivo, as previously described for a CD16-directed murine

IgG antibody (3G8), the CD38-directed antibody daratumumab and other approaches (Choi et al 2008 Immunology 124 (2) 215-22; DOI: 10.111 l/j.l365-2567.2007.02757.x; Yoshida 2010 Front. Microbiol 1 : 128 DOI: 10.3389/fmicb.2010.00128; Wang et al 2018 Clin Cancer Res, 24(16): 4006-4017; DOI: 10.1158/1078-0432.CCR-17-3117; His et al 2008; Nakamura 2013 PNAS; 110(23) 9421-9426; DOI: 10.1073/pnas.1300140110; Breman et al 2018 Front Immunol, 12(9)2940; DOI: 10.3389/fimmu.2018.02940).

The person of skill in the art will appreciate that other configurations for modification of Fc regions can be implemented. For example, substitutions into human IgG1 or IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 were shown to greatly reduce binding to Fcy receptors and thus ADCC and CDC. Furthermore, Idusogie *et al.* (2000) J. Immunol. 164(8):4178-84 demonstrated that alanine substitution at different positions, including K322, significantly reduced complement activation.

In one embodiment, the antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises an Fc region or portion thereof that binds CD16A that is modified to have reduced binding affinity for CD16A, has a binding affinity for human CD16A that is at least 1, 2 or 3 log less than that of a conventional or wild-type human IgG1 antibody, e.g., as assessed by surface plasmon resonance.

In one embodiment, the antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein comprises an Fc region that has an amino acid sequence having at least 85, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99 or 100% amino acid identity with an Fc region in at least one of SEQ ID NOs: 1, 3, 5, 7, 9, 11 – 19 and 23, and preferably having one or more of the above structural and/or functional features.

In one embodiment, in an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, third antigen-binding region can be an antigen-binding region that specifically binds an NK cell activating receptor, such as described above for the second antigen-binding region. In one embodiment, at least one of the second or third antigen-binding region activates the NK cell activating receptor.

Additional agonists for activation of NK cells, γδ T cells and/or co-stimulation of γδ T cells

In one embodiment, an antigen binding protein in a conjugate with a 4-1BBL ECD mutein as described herein, comprises at least one further agonist, which further agonist can be at least one of: i) a further NK cell-activating cytokine, in addition to the 4-1BBL ECD mutein, ii) a γδ T cell-activating agonist; and iii) a further γδ T cell co-stimulatory agonist, in addition to the 4-1BBL ECD mutein.

In one embodiment of the conjugate, the further agonist is the heterologous moiety. In another embodiment of the conjugate, the further agonist is directly attached to the heterologous moiety, or the further agonist is attached to the heterologous moiety via a linker, preferably a (flexible) peptidyl linker as described above, e.g. $(GGGGS)_n$, wherein the copy number “n” can be 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

In one embodiment of the conjugate, the further NK cell-activating cytokines and/or the γδ T cell-activating agonist can be selected from the group consisting of: an IL-21 receptor agonist, an IL-15 receptor agonist, an IL-2 receptor agonist, a type I interferon (IFN-1) receptor agonist, an IL-12 receptor agonist and an IL-18 receptor agonist, as further detailed below.

In one embodiment of the conjugate, the γδ T cell co-stimulatory agonist, in addition to the 4-1BBL ECD mutein, is selected from the group consisting of: a CD27 agonist and a GITR agonist.

In one embodiment, the conjugate with a 4-1BBL ECD mutein as described herein thus at least comprises an IL-21 receptor (IL-21R) agonist.

Interleukin 21 (IL-21) is a protein that in humans is encoded by the *IL-21* gene (Entrez Gene ID: 59067). IL-21 is a cytokine that has potent regulatory effects on cells of the immune system, including natural killer (NK) cells and γδ T cells, and which induces cell division/proliferation in its target cells. Amino acid sequences for human IL-21 precursor (including its signal sequence) are described in NCBI accession numbers NP_001193935 and NP_068575, the disclosures of which are incorporated herein by reference. IL-21 (mature/processed) comprises amino acids 30 – 153 of NP_001193935 or amino acids 30 – 162 of NP_068575 (i.e. SEQ ID NO: 38). IL-21 exerts its effects on target cells through the IL-21 receptor (IL-21R) is expressed on the surface of T, B and NK cells. IL-21R is similar in structure to the receptors for other type I cytokines like IL-2R or IL-15 and requires dimerization with the common gamma chain (γc) in order to bind IL-21. IL-21R is encoded in humans by the *IL-21R* gene (Entrez Gene ID: 50615). Amino acid sequences for human IL-21R are described in NCBI accession numbers NP_068570, NP_851564 and NP_851565, the disclosures of which are incorporated herein by reference.

As used herein, an “IL-21R agonist” is an agent that has “agonist” activity at the IL-21 receptor, which means that the agent that can cause or increase "IL-21R signaling". “IL-21R signaling” refers to an ability of IL-21R, e.g. when expressed on the surface of T, B and NK cells and triggered by its natural ligand IL-21, to activate or transduce an intracellular signaling pathway. The “natural ligand IL-21” is herein understood as a human wild type IL-21 comprising or consisting of an amino acid sequence as indicated above. When bound to IL-21, the IL-21 receptor acts through the Jak/STAT pathway, utilizing Jak1 and Jak3 and a STAT3 homodimer to activate its target genes. IL-21R agonist activity, i.e. changes in IL-21R signaling activity, can be measured, for example, by assays designed to measure changes in the IL-21R signaling pathways, e.g. by monitoring phosphorylation of signal transduction components, assays to measure the association of certain signal transduction components with other proteins or intracellular structures, or in the biochemical activity of components such as kinases, or assays designed to measure expression of reporter genes under control of IL-21R-sensitive promoters and enhancers, or indirectly by a downstream effect mediated by IL-21R (e.g. activation of specific cytolytic machinery in NK cells or γδ T cell). A suitable cell-based assay for biological activity of an IL-21R agonist, is e.g. described in Maurer et al. (mAbs. 2012; 4(1): 69–83.), wherein a murine pre-B-cell line is transfected with both the human IL-21R and a STAT-responsive luciferase reporter gene. IL-21R agonist activity can be determined using this cell line by measuring the level of STAT3 phosphorylation using anti-pSTAT3 antibody-conjugated beads and/or by detecting luciferase luminescence, upon contacting the cell

line with an IL-21R agonist. The natural ligand IL-21 can serve as a positive control in an assay for IL-21R agonist activity and can also be used as a reference for the amount of IL-21R agonist activity of a given non-natural IL-21R agonist, such as a conjugate as described herein comprising an IL-21R agonist.

In one embodiment, a conjugate as described herein comprises an IL-21R agonist that has reduced IL-21R agonist activity as compared to human wild type IL-21. In one embodiment, the IL-21R agonist has an IL-21R agonist activity that is a factor 2, 5, 10, 20, 50, 100, 200, 500, 1000, 10000, or 100000 less than that of human wild type IL-21.

In one embodiment, a conjugate as described herein comprises an IL-21R agonist that has enhanced IL-21R agonist activity as compared to human wild type IL-21. In one embodiment, the IL-21R agonist has an IL-21R agonist activity that is a factor 2, 5, 10, 20, 50, 100, 200, 500, 1000, 10000, or 100000 higher than that of human wild type IL-21.

In one embodiment, a conjugate as described herein comprises an IL-21R agonist that is an IL-21 polypeptide comprising an amino acid sequence with at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98, at least 99, or 100% sequence identity to SEQ ID NO: 38, and preferably having an IL-21R agonist activity as defined above, and/or preferably having an affinity for the IL-21R as defined below.

In one embodiment, a conjugate as described herein comprises an IL-21R agonist of which the affinity for the IL-21R is reduced or enhanced as compared to human wild type IL-21. The affinity of an IL-21R agonist of the affinity for the IL-21R can be assayed using methods generally known in the art, such as surface plasmon resonance.

In one embodiment, a conjugate as described herein comprises an IL-21R agonist that has reduced affinity for IL-21R as compared to human wild type IL-21. In one embodiment, the affinity of the IL-21R agonist for IL-21R is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 less than that of human wild type IL-21.

In one embodiment, a conjugate as described herein comprises an IL-21R agonist that has enhanced affinity for IL-21R as compared to human wild type IL-21. In one embodiment, the affinity of the IL-21R agonist for IL-21R is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 higher than that of human wild type IL-21.

In one embodiment, a conjugate as described herein comprises an IL-21R agonist that is IL-21 or a fragment thereof that has IL-21R agonist activity. Preferably, the IL-21R agonist is human IL-21 or a fragment thereof that has IL-21R agonist activity. In one embodiment, the IL-21R agonist is an IL-21 mutein with reduced affinity for IL-21R as compared to human wild type IL-21. IL-21 muteins with reduced affinity for IL-21R as compared to human wild type IL-21 are described in one of Shen et al. (Front Immunol. 2020; 11: 832), WO2019/028316, WO2006/111524, WO2008/049920 and the co-pending application by the same applicant with reference no. P62009936EP. Thus, in one embodiment, the IL-21R agonist is an IL-21 mutein with a mutation (i.e. amino acid substitution, deletion or insertion) of one or more amino acids selected from the group consisting of D4, R5, I8, R9, R11, Q12, L13, I14, D15, I16, D18 Q19, L20, K21, Y23, R65,

I66, I67, N68, V69, S70, K72, K73, L74, K75, R76, K77, P78, P79, S80, E100, E109, R110, K112, S113, Q116, K117, I119, H120, and L123 (amino acid positions referring to position in SEQ ID NO: 38 or a corresponding position in an IL-21 allelic variant).

In one embodiment, the IL-21R agonist is an IL-21 mutein comprising at least one amino acid substitution, deletion or insertion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-), L20W, L74D, L20N, I67N, L20S, L13E, I8H, (N63-, E64-, R65- and I66-), L74F, I8V, I8Q, I8F, I8W, I8Y, I8L, D4H, D4R, D4K, D4Q, D4N, R11E, R11Q, R11N, R11Y, Q12K, Q12R, L13S, L13V, L13T, L13G, Q19S, Q19E, Q19K, Q19R, Q19H, Q19G, Q19T, L20D, L20E, L20R, L20K, L20Q, L20H, L20G, K21H, K21N, K21Q, K21E, K21D, I67T, I67D, I67E, I67K, I67R, I67Q, I67S, I67G, L74G, L74E, L74K, L74R, L74N, L74Q, L74S, L74P, K75E, K75Q, K75N, K75S, K75-, K76-, K112H, K112N, K112Q, K112E, K112D, N59-, T60-, G61-, N62-, N63-, E64-, R65-, I66-, (N59-, T60-, G61-, N62-, N63-, E64-, R65- and I66-), (K75- and R76-), (K75-, R76- and R77-), G84 insGGGG, and, K75 insX (wherein X is one, two or three amino acids selected from the group consisting of G, S and D). In one embodiment, the IL-21 mutein comprises no other amino acid sequence modification than the at least one amino acid substitution, deletion or insertion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-), L20W, L74D, L20N, I67N, L20S, L13E, I8H, (N63-, E64-, R65- and I66-), L74F, I8V, I8Q, I8F, I8W, I8Y, I8L, D4H, D4R, D4K, D4Q, D4N, R11E, R11Q, R11N, R11Y, Q12K, Q12R, L13S, L13V, L13T, L13G, Q19S, Q19E, Q19K, Q19R, Q19H, Q19G, Q19T, L20D, L20E, L20R, L20K, L20Q, L20H, L20G, K21H, K21N, K21Q, K21E, K21D, I67T, I67D, I67E, I67K, I67R, I67Q, I67S, I67G, L74G, L74E, L74K, L74R, L74N, L74Q, L74S, L74P, K75E, K75Q, K75N, K75S, K75-, K76-, K112H, K112N, K112Q, K112E, K112D, N59-, T60-, G61-, N62-, N63-, E64-, R65-, I66-, (N59-, T60-, G61-, N62-, N63-, E64-, R65- and I66-), (K75- and R76-), (K75-, R76- and R77-), G84 insGGGG, and, K75 insX (wherein X is one, two or three amino acids selected from the group consisting of G, S and D).

In one embodiment, the IL-21R agonist is an IL-21 mutein with a deletion of at least one, two, three, four, five, six, seven or eight amino acids in the region of N59 to I66, or with a deletion of at least one, two, three, four, five, six, seven or eight amino acids in the region of N82 to R90. In one embodiment, the IL-21R agonist is an IL-21 mutein with the deletions (N59-, T60-, G61-, N62-, N63-, E64-, R65- and I66-), (N63-, E64-, R65- and I66-), (N82-, A83-, G84-, R85-, R86-, Q87- and K88-) and (N82-, A83-, G84-, R85-, R86-, Q87-, K88-, H89- and R90-).

In one embodiment, the IL-21R agonist is an IL-21 mutein with an insertion of at least two, three or four amino acids immediately C-terminal to G84. In one embodiment, the IL-21 mutein comprises an insertion of, with increasing preference, at least one, two, three or four glycine(s) immediately C-terminal to G84.

In one embodiment, the IL-21R agonist is an IL-21 mutein comprising at least one amino acid substitution, deletion or insertion selected from the substitutions, deletions and insertions listed in Table C.

Table C. Additional substitutions, deletions and insertions for IL-21 muteins.

Pos	WT	Preference additional mutations (with decreasing preference from left to right)
4	D	R, K > Q, N > all remaining
8	I	F, W, Y, L > all remaining
11	R	Q, N, Y > all remaining
12	Q	R > all remaining
13	L	S, V, T, G > all remaining
19	Q	E, K, R, H > G, S, T > all remaining
20	L	D, E, R, K > Q, H > G > all remaining
21	K	N, Q, E, D > all remaining
59	N	G, P, S, D > all remaining
60	T	G, P, S, D > all remaining
61	G	P, S, D > all remaining
62	N	G, P, S, D > all remaining
63	N	G, P, S, D > all remaining
64	E	G, P, S > all remaining
65	R	all remaining
66	I	R, K, E, H > Q, N, Y > all remaining
67	I	D, E, K, R > N, Q > S, G > all remaining
74	L	E, K, R > N, Q > S, P > all remaining
75	K	Q, N, S, del > all remaining
84	G	insertion of any combinations of G,S,P,D
112	K	N, Q, E, D > all remaining

In one embodiment, the IL-21 mutein binds to a human IL-21 receptor with a reduced affinity, relative to the affinity of wild-type IL-21 for the human IL-21 receptor, wherein preferably the IL-21 mutein binds with reduced affinity to a human IL-21 receptor having an amino acid sequence of SEQ ID NO: 173 or the IL-21 mutein binds with reduced affinity to an IL-21 receptor gamma chain having an amino acid sequence of SEQ ID NO: 174. Hence, in one embodiment, the IL-21 mutein comprises at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-); L20W; L74D; L20N; I67N; L20S; L13E; I8H; (N63- E64- R65- I66-); and L74F. In one embodiment, the IL-21 mutein comprises no other modification than the at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-), L20W; L74D; L20N; I67N; L20S; L13E; I8H; (N63- E64- R65- I66-); and L74F.

In one embodiment, an IL-21 mutein provided herein exhibits a binding affinity for the human IL-21R, expressed as pK_D, that is at least 0.5 lower than the pK_D of wild-type IL-21 for the IL-21 R.

In one embodiment, the IL-21 mutein having at least a 0.5 lower pK_D for the human IL-21R relative to the pK_D of wild-type IL-21 for the IL-21 R, is an IL-21 mutein comprising at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-); L20W; R5H; I8V; I8Q; Q12K; K73Y; L13E; D4H; I8H; I67N; L74D; L20S; and L20N. In one embodiment, the IL-21 mutein comprises no other amino acid sequence modification than the at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-); L20W; R5H; I8V; I8Q; Q12K; K73Y; L13E; D4H; I8H; I67N; L74D; L20S; and L20N.

In one embodiment, an IL-21 mutein provided herein exhibits a binding affinity for the human IL-21R, expressed in pK_D, that is at least 1.0 lower than the pK_D of wild-type IL-21 for the IL-21 R, In one embodiment, the IL-21 mutein having at least a 1.0 lower pK_D for the human IL-21R relative to the pK_D of wild-type IL-21 for the IL-21 R, is an IL-21 mutein comprising at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-); L20W; R5H; I8Q; Q12K; K73Y; L13E; I67N; L74D; L20S; and L20N. In one embodiment, the IL-21 mutein comprises no other amino acid sequence modification than the at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-); L20W; R5H; I8Q; Q12K; K73Y; L13E; I67N; L74D; L20S; and L20N.

In one embodiment, an IL-21 mutein provided herein exhibits a binding affinity for the human IL-21R, expressed in pK_D, that is at least 1.6 lower than the pK_D of wild-type IL-21 for the IL-21 R, In one embodiment, the IL-21 mutein having at least a 1.6 lower pK_D for the human IL-21R relative to the pK_D of wild-type IL-21 for the IL-21 R, is an IL-21 mutein comprising at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-); L20W; R5H; Q12K; I67N; L74D; L20S; and L20N. In one embodiment, the IL-21 mutein comprises no other amino acid sequence modification than the at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-); L20W; R5H; Q12K; I67N; L74D; L20S; and L20N.

In one embodiment, there is provided an antigen binding protein in a conjugate as described herein comprising a combination of a 4-1BBL ECD mutein as described herein and an IL-21 mutein as described herein, as shown in Table D.

Table D. Combinations of 4-1BBL muteins and IL-21 muteins conjugated to an antigen binding protein.

AVC-ID	4-1BBL ECD mutein	IL-21 mutein
AVC72	V100T	R9E
AVC73	Q227R	R9E
AVC74	Q230S	R9E
AVC75	V100T	R76A
AVC76	Q227R	R76A

AVC77	Q230S	R76A
AVC78	V100T	I8Q
AVC79	Q227R	I8Q
AVC80	Q230S	I8Q
AVC85	Q227E	R9E R76A
AVC86	Q227E	L13E
AVC87	V153Q	L13E I8H K21H D4H
AVC88	V153Q	D4H I8H K21H
AVC89	V153Q	L13E I8H K21H
AVC90	V153Q	L13E D4H I8H
AVC91	V153Q	I8H K21H
AVC92	V153Q	L13E D4H K21H
AVC93	V153Q	D4H I8H
AVC94	V153Q	L13E I8H
AVC95	V153Q	D4H K21H
AVC96	V153Q	L13E K21H
AVC97	V153Q	L13E D4H
AVC98	Y110Q V153Q Q227E	I8H
AVC99	L101N Y110Q V153Q	I8H
AVC100	V100Q Y110Q V153Q	I8H
AVC101	L101N V153Q Q227E	I8H
AVC102	Y110Q A154D Q227E	I8H
AVC103	Y110Q V153Q	I8H
AVC104	V153Q Q227E	I8H
AVC105	Y110Q Q227E	I8H
AVC106	L101N Q227E	I8H
AVC107	V153Q	I8H
AVC176	A154D	L74D
AVC187	A154D	L20N
AVC188	A154D	L20W
AVC189	A154D	L20S

AVC190	A154D	N82- A83- G84- R85- R86- Q87- K88-
AVC285	A154E	N82- A83- G84- R85- R86- Q87- K88-
AVC286	A154D + G155Q	L20W
AVC287	A154E	L20W
AVC288	A154D + G155Q	N82- A83- G84- R85- R86- Q87- K88-

In one embodiment, a conjugate as described herein comprises a combination of a 4-1BBL ECD mutein and an IL-21 mutein, wherein the 4-1BBL ECD mutein is selected from the group consisting of: 4-1BBL mutein A154D; 4-1BBL mutein A154E; 4-1BBL mutein V153Q; 4-1BBL mutein Q227E; 4-1BBL mutein Q227R; 4-1BBL mutein L101N; 4-1BBL mutein Y110Q; 4-1BBL mutein Q230K; and 4-1BBL mutein V100Q; and wherein the IL-21 mutein is selected from the group consisting of: IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); IL-21 mutein L20W; IL-21 mutein L74D; IL-21 mutein L20N; IL-21 mutein I67N; IL-21 mutein L20S; IL-21 mutein L13E; IL-21 mutein I8H; IL-21 mutein (N63- E64- R65- I66-); and IL-21 mutein L74F. The 4-1BBL and IL-21 mutein are hereby understood to at least comprise the indicated substitution or deletion, or to comprise no other amino acid sequence modification than the indicated substitution or deletion. In one embodiment of the conjugate, the 4-1BBL ECD mutein and the IL-21 mutein are each conjugated to an antigen binding protein, whereby preferably, the 4-1BBL ECD mutein is present as a hetero- or homotrimer of 4-1BBL ECDs connected through polypeptide linkers.

In specific embodiments the conjugate comprises a combination of a 4-1BBL ECD mutein and an IL-21 mutein selected from the group consisting of: 4-1BBL mutein A154D and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154D and IL-21 mutein L20W; 4-1BBL mutein A154D and IL-21 mutein L74D; 4-1BBL mutein A154D and IL-21 mutein L20N; 4-1BBL mutein A154D and IL-21 mutein I67N; 4-1BBL mutein A154D and IL-21 mutein L20S; 4-1BBL mutein A154D and IL-21 mutein L13E; 4-1BBL mutein A154D and IL-21 mutein I8H; 4-1BBL mutein A154D and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein A154D and IL-21 mutein L74F; 4-1BBL mutein A154E and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154E and IL-21 mutein L20W; 4-1BBL mutein A154E and IL-21 mutein L74D; 4-1BBL mutein A154E and IL-21 mutein L20N; 4-1BBL mutein A154E and IL-21 mutein I67N; 4-1BBL mutein A154E and IL-21 mutein L20S; 4-1BBL mutein A154E and IL-21 mutein L13E; 4-1BBL mutein A154E and IL-21 mutein I8H; 4-1BBL mutein A154E and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein A154E and IL-21 mutein L74F; 4-1BBL mutein V153Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein V153Q and IL-21 mutein L20W; 4-1BBL mutein V153Q and IL-21 mutein L74D; 4-1BBL mutein V153Q and IL-21 mutein L20N; 4-1BBL mutein V153Q and IL-21 mutein I67N; 4-1BBL mutein V153Q and IL-21 mutein L20S; 4-1BBL mutein V153Q and IL-21 mutein L13E; 4-1BBL mutein V153Q and IL-21 mutein I8H; 4-1BBL mutein V153Q and IL-21

mutein (N63- E64- R65- I66-); 4-1BBL mutein V153Q and IL-21 mutein L74F; 4-1BBL mutein Q227E and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Q227E and IL-21 mutein L20W; 4-1BBL mutein Q227E and IL-21 mutein L74D; 4-1BBL mutein Q227E and IL-21 mutein L20N; 4-1BBL mutein Q227E and IL-21 mutein I67N; 4-1BBL mutein Q227E and IL-21 mutein L20S; 4-1BBL mutein Q227E and IL-21 mutein L13E; 4-1BBL mutein Q227E and IL-21 mutein I8H; 4-1BBL mutein Q227E and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Q227E and IL-21 mutein L74F; 4-1BBL mutein Q227R and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Q227R and IL-21 mutein L20W; 4-1BBL mutein Q227R and IL-21 mutein L74D; 4-1BBL mutein Q227R and IL-21 mutein L20N; 4-1BBL mutein Q227R and IL-21 mutein I67N; 4-1BBL mutein Q227R and IL-21 mutein L20S; 4-1BBL mutein Q227R and IL-21 mutein L13E; 4-1BBL mutein Q227R and IL-21 mutein I8H; 4-1BBL mutein Q227R and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Q227R and IL-21 mutein L74F; 4-1BBL mutein L101N and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein L101N and IL-21 mutein L20W; 4-1BBL mutein L101N and IL-21 mutein L74D; 4-1BBL mutein L101N and IL-21 mutein L20N; 4-1BBL mutein L101N and IL-21 mutein I67N; 4-1BBL mutein L101N and IL-21 mutein L20S; 4-1BBL mutein L101N and IL-21 mutein L13E; 4-1BBL mutein L101N and IL-21 mutein I8H; 4-1BBL mutein L101N and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein L101N and IL-21 mutein L74F; 4-1BBL mutein Y110Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Y110Q and IL-21 mutein L20W; 4-1BBL mutein Y110Q and IL-21 mutein L74D; 4-1BBL mutein Y110Q and IL-21 mutein L20N; 4-1BBL mutein Y110Q and IL-21 mutein I67N; 4-1BBL mutein Y110Q and IL-21 mutein L20S; 4-1BBL mutein Y110Q and IL-21 mutein L13E; 4-1BBL mutein Y110Q and IL-21 mutein I8H; 4-1BBL mutein Y110Q and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Y110Q and IL-21 mutein L74F; 4-1BBL mutein Q230K and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Q230K and IL-21 mutein L20W; 4-1BBL mutein Q230K and IL-21 mutein L74D; 4-1BBL mutein Q230K and IL-21 mutein L20N; 4-1BBL mutein Q230K and IL-21 mutein I67N; 4-1BBL mutein Q230K and IL-21 mutein L20S; 4-1BBL mutein Q230K and IL-21 mutein L13E; 4-1BBL mutein Q230K and IL-21 mutein I8H; 4-1BBL mutein Q230K and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Q230K and IL-21 mutein L74F; 4-1BBL mutein V100Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein V100Q and IL-21 mutein L20W; 4-1BBL mutein V100Q and IL-21 mutein L74D; 4-1BBL mutein V100Q and IL-21 mutein L20N; 4-1BBL mutein V100Q and IL-21 mutein I67N; 4-1BBL mutein V100Q and IL-21 mutein L20S; 4-1BBL mutein V100Q and IL-21 mutein L13E; 4-1BBL mutein V100Q and IL-21 mutein I8H; 4-1BBL mutein V100Q and IL-21 mutein (N63- E64- R65- I66-); and, 4-1BBL mutein V100Q and IL-21 mutein L74F.

In one embodiment, a conjugate comprising a combination of a 4-1BBL ECD mutein and an IL-21 mutein, is a conjugate, which when consisting of trastuzumab to which the IL-21 mutein and the 4-1BBL ECD mutein are conjugated, whereby the 4-1BBL ECD mutein is present as a homotrimer wherein the ECD monomers are connected through $(GGGGS)_4$-linkers, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which

proliferation is at least 60% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and wild type 4-1BBL in the same assay. Hence, in one embodiment, the conjugate comprises a combination of a 4-1BBL ECD mutein and an IL-21 mutein selected from the group consisting of: 4-1BBL mutein A154D and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154D and IL-21 mutein L20W; 4-1BBL mutein A154D and IL-21 mutein L20S; 4-1BBL mutein A154E and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154E and IL-21 mutein L20W; and 4-1BBL mutein A154E and IL-21 mutein L20S.

In one embodiment, a conjugate comprising a combination of a 4-1BBL ECD mutein and an IL-21 mutein, is a conjugate, which when consisting of trastuzumab to which the IL-21 mutein and the 4-1BBL ECD mutein are conjugated, whereby the 4-1BBL ECD mutein is present as a homotrimer wherein the ECD monomers are connected through $(GGGGS)_4$-linkers, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is at least 89% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and wild type 4-1BBL in the same assay. Hence, in one embodiment, the conjugate comprises a combination of a 4-1BBL ECD mutein and an IL-21 mutein selected from the group consisting of: 4-1BBL mutein A154D and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154D and IL-21 mutein L20W; 4-1BBL mutein A154E and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); and 4-1BBL mutein A154E and IL-21 mutein L20W.

In one embodiment, a conjugate comprising a combination of a 4-1BBL ECD mutein and an IL-21 mutein, is a conjugate, which when consisting of trastuzumab to which the IL-21 mutein and the 4-1BBL ECD mutein are conjugated, whereby the 4-1BBL ECD mutein is present as a homotrimer wherein the ECD monomers are connected through $(GGGGS)_4$-linkers, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is at least 105% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and wild type 4-1BBL in the same assay. Hence, in one embodiment, the conjugate comprises a combination of: 4-1BBL mutein A154D and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-).

In one embodiment, a conjugate as described herein comprises an IL-21R agonist that is an antigen-binding region that specifically binds IL-21R and that has IL-21R agonist activity. The antigen-binding region can be an antigen-binding region as described herein above.

In one embodiment, a conjugate as described herein comprises more than one IL-21R agonist as described above. Thus, in one embodiment, the conjugate has an IL-21R agonist-valency that is higher than one. The IL-21R agonist-valency of a conjugate can for example be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more.

In further embodiments, the conjugate as described herein can comprise, in addition to the 4-1BBL ECD mutein, and optionally an IL-21R agonist, a further NK cell-activating cytokine and/or further γδ T cell-activating agonist selected from the group consisting of: an IL-15 receptor agonist, type I interferon (IFN-1) receptor agonist, an IL-2 receptor agonist, an IL-12 receptor agonist and

an IL-18 receptor agonist. In one embodiment, the IL-15 receptor agonist is an IL-15 polypeptide or an agonistic antigen-binding region that specifically binds the IL-15 receptor. In one embodiment, the type I interferon (IFN-1) receptor agonist comprises or consists of an IFN-1 polypeptide or an agonistic antigen-binding region that specifically binds the IFN-α receptor. In one embodiment, the IFN-1 polypeptide is an IFN-1 mutein that is modified to reduce (or enhance) affinity for the IFN-α receptor, relative to a corresponding wild type IFN-1 polypeptide. In one embodiment, the IL-2 receptor agonist is an IL-2 polypeptide or an agonistic antigen-binding region that specifically binds the IL-2 receptor. In one embodiment, the IL-12 receptor agonist is an IL-12 polypeptide or an agonistic antigen-binding region that specifically binds the IL-12 receptor. In one embodiment, the IL-18 receptor agonist is an IL-18 polypeptide or an agonistic antigen-binding region that specifically binds the IL-18 receptor.

In one embodiment, a conjugate as described herein comprises an IL-15 polypeptide comprising an amino acid sequence with at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98, at least 99, or 100% sequence identity to SEQ ID NO: 169 (i.e. the functional domain of IL-15, corresponding to amino acids 49-162 of Uniprot acc. No. P40933). The IL-15 polypeptide preferably has IL-15 receptor agonist activity as can be assayed as described in the art (see e.g. US 20230158164), e.g. by assaying STAT5 phosphorylation in a bio-assay, using reporter cells (e.g. https://nld.promega.com/products/reporter-bioassays/cytokine-and-growth-factor-bioassays/il-15-bioassay/?catNum=JA2011). The IL-15 polypeptide preferably has the ability to specifically bind to an IL-15 receptor, as can be determined using methods generally known in the art, such as surface plasmon resonance.

In one embodiment, the IL-15 polypeptide is an IL-15 mutein that is modified to reduce (or enhance) affinity for IL-15R, relative to a corresponding wild type IL-15 polypeptide. In one embodiment, the IL-15 mutein has reduced affinity for IL-15R, relative to a corresponding wild type IL-15 polypeptide, and wherein the IL-15 mutein has a mutation (i.e. amino acid substitution, deletion or insertion) of one or more amino acids selected from the group consisting of: N1, D8, D30, H32 L45, E46, I50, V49, S51, L42, V63, E64, N65, I68, L69, Q108, M109, N112 (amino acid positions referring to position in SEQ ID NO: 168 or a corresponding position in an IL-15 allelic variant). Preferably, the IL-15 mutein comprises one or more amino acid substitutions selected from the group consisting of: N1G, D8S, D30N, D30T, H32D, H32E, H32N, H32Q, E46G, E46K, V49R, V49D, I50D, V63F, V63A, V63K, V63R, E64Q, E64K, E64R, N65D, N65E, N65K, N65R, I68F, I68H, I68D, I68K, I68R, I68E, I68Q, I68G, L69D, L69E, L69K, L69R, Q108A, Q108D, Q108E, Q108F, Q108H, Q108K, Q108L, Q108M, Q108N, Q108S, Q108T, Q108Y, M109A, M109H, M109R, N112D, N112G, N112P and N112R.

In one embodiment, the IL-15 mutein has reduced affinity for at least one of IL-15Rβ and IL-15Rγ, relative to a corresponding wild type IL-15 polypeptide. IL-15 muteins with an amino acid substitution in D8 or Q108 (e.g. D8S or Q108S) have a reduced affinity for IL-15Rβ and IL-15Rγ, respectively.

In a preferred embodiment, the IL-15 mutein has reduced affinity for IL-15Rβ, relative to a corresponding wild type IL-15 polypeptide. Thus, a preferred IL-15 mutein has an amino acid substitution in at least one of D8 (e.g. D8S), E64, N65, I68, and L69.

In one embodiment, a conjugate as described herein comprises an IL-2 polypeptide comprising an amino acid sequence with at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98, at least 99, or 100% sequence identity to SEQ ID NO: 170 (i.e. the functional domain of IL-2, corresponding to amino acids 21-153 of Uniprot acc. No. P60568). The IL-2 polypeptide preferably has IL-2 receptor agonist activity as can be assayed as described in the art, e.g. by assaying STAT5 phosphorylation in a bio-assay, using reporter cells (e.g. https://nld.promega.com/products/reporter-bioassays/cytokine-and-growth-factor-bioassays/il2-bioassay/?catNum=JA2201)). The IL-2 polypeptide preferably has the ability to specifically bind to an IL-2 receptor, as can be determined using methods generally known in the art, such as surface plasmon resonance. In one embodiment, the IL-2 polypeptide is an IL-2 mutein that is modified to reduce (or enhance) affinity for IL-2R, relative to a corresponding wild type IL-2 polypeptide.

In one embodiment, a conjugate as described herein comprises an IL-12 polypeptide comprising i) an IL-12α polypeptide comprising an amino acid sequence with at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98, at least 99, or 100% sequence identity to SEQ ID NO: 172 (i.e. the functional domain of IL-12 α, corresponding to amino acids 23-219 of Uniprot acc. No. P29459), and, ii) an IL-12β polypeptide comprising an amino acid sequence with at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98, at least 99, or 100% sequence identity to SEQ ID NO: 172 (i.e. the functional domain of IL-12β, corresponding to amino acids 23-328 of Uniprot acc. No. P29460). The IL-12 polypeptide preferably comprises, in an N- to C-terminal direction, an aforementioned IL-12β polypeptide linked to an aforementioned IL-12α polypeptide, whereby the IL-12β polypeptide is linked to the IL-12α polypeptide through a peptidyl linker as herein described, such as $(GGGGS)_n$, whereby n = 3, 4, 5, 6 or 7. The IL-12 polypeptide preferably has IL-12 receptor agonist activity as can be assayed as described in the art, e.g. by assaying STAT4 phosphorylation in a bio-assay, using reporter cells (e.g. https://www.invivogen.com/hek-blue-il12). The IL-12 polypeptide preferably has the ability to specifically bind to an IL-12 receptor, as can be determined using methods generally known in the art, such as surface plasmon resonance. In one embodiment, the IL-12 polypeptide is an IL-12 mutein that is modified to reduce (or enhance) affinity for IL-12R, relative to a corresponding wild type IL-12 polypeptide.

In one embodiment, a conjugate as described herein comprises an IL-18 polypeptide comprising an amino acid sequence with at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98, at least 99, or 100% sequence identity to SEQ ID NO: 173 (i.e. the functional domain of IL-18, corresponding to amino acids 37-193 of Uniprot acc. No. Q14116). The IL-18 polypeptide preferably has IL-18 receptor agonist activity as can be assayed as described in the art, e.g. by assaying NF-

kB activation in a bio-assay, using reporter cells (e.g. https://www.invivogen.com/hek-blue-il18). The IL-18 polypeptide preferably has the ability to specifically bind to an IL-18 receptor, as can be determined using methods generally known in the art, such as surface plasmon resonance. In one embodiment, the IL-18 polypeptide is an IL-18 mutein that is modified to reduce (or enhance) affinity for IL-18R, relative to a corresponding wild type IL-18 polypeptide.

In one embodiment, the conjugate with a 4-1BBL ECD mutein as described herein thus at least comprises a CD27 agonist, e.g. as a further γδ T cell co-stimulatory agonist. CD27 is a member of the tumor necrosis factor receptor family. Its alternative names are tumor necrosis factor receptor superfamily member 7 (TNFRSF7) or S 152. CD274-1BB is encoded by the *TNFRSF7* gene (Entrez Gene ID: 939). An amino acid sequence for human CD27 is described in GenBank Number NP_001233 or Uniprot accession numbers P26842. CD27 known as a co-stimulatory immune checkpoint molecule. CD27 is expressed on both naïve and activated effector T cells as well as NK cells and activated B cells. It is a type I transmembrane protein with cysteine-rich domains. The ligand for CD27 is CD70, also known as TNFSF7 or CD27L.

CD70 is a protein that in humans is encoded by the *TNFSF7* gene (Entrez Gene ID: 970). An amino acid sequence for human CD70 is described in NCBI accession number NP_001243 (SEQ ID NO: 535).

As used herein, a “CD27 agonist” is an agent that has “agonist” activity at the CD27, which means that the agent that can cause or increase "CD27 signaling". “CD27 signaling” refers to an ability of CD27, e.g. when expressed on the surface of T, B and NK cells and triggered by its natural ligand CD70, to activate or transduce an intracellular signaling pathway. The “natural CD27 ligand” is herein understood as the extracellular domain (ECD) of a human wild type CD70 comprising or consisting of an amino acid sequence from position 54 to 193 of the amino acid sequence of human CD70 (i.e. SEQ ID NO: 535). A CD70 extracellular domain (ECD) is herein thus understood as a polypeptide comprising or consisting of an amino acid sequence from positions 54 to 193 of human CD70, which amino acid sequence is depicted in SEQ ID NO: 536, or a fragment thereof having CD27 agonist activity.

The interaction between CD27 and CD70 leads to the recruitment of intracellular adaptor proteins, such as TRAF2 and TRAF5, which then activate signaling pathways, including the NF-κB and JNK pathway. CD27 agonist activity, i.e. changes in CD27 signaling activity, can be measured, for example, by assays designed to measure changes in the CD27 signaling pathways, e.g. by monitoring phosphorylation of signal transduction components, assays to measure the association of certain signal transduction components with other proteins or intracellular structures, or in the biochemical activity of components such as kinases, or indirectly by a downstream effect mediated by CD27 (e.g. production of specific cytokines). A suitable cell-based assay for *in vitro* biological activity of a CD27 agonist, is e.g. described in Wyzgol et al. 2009 (J. Immunol. 183(3):1851-61). Natural CD70 or a CD70 ECD trimer, e.g. a CD70 ECD trimer comprising the amino acid sequence of SEQ ID NO: 537 can serve as a positive control in an assay for CD27 agonist activity and can also be used as a reference for the amount of CD27 agonist activity of a given non-natural CD27

agonist, such as a conjugate with a 4-1BBL ECD mutein as described herein comprising a CD27 agonist.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that has reduced CD27 agonist activity as compared to human wild type CD70. In one embodiment, the CD27 agonist has a CD27 agonist activity that is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 less than that of the ECD of human wild type CD70.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that has enhanced CD27 agonist activity as compared to human wild type CD70. In one embodiment, the CD27 agonist has a CD27 agonist activity that is a factor 2, 5, 10, 20, 50, 100, 200, 500, 1,000 or 10,000 higher than that of the ECD of human wild type CD70.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist comprising at least one CD70 ECD comprising an amino acid sequence with at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98, at least 99, or 100% sequence identity to SEQ ID NO: 536 359, and preferably having an CD27 agonist activity as defined above, and/or preferably having an affinity for CD27 as defined below.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist of which the affinity for the CD27 is reduced or enhanced as compared to the ECD of human wild type CD70. The affinity of a CD27 agonist of the affinity for the CD27 can be assayed using methods generally known in the art, such as surface plasmon resonance.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that has enhanced affinity for CD27 as compared to human wild type CD70 or wild type CD70 ECD. In one embodiment, the affinity of the CD27 agonist for CD27 is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 higher than that of the ECD of human wild type CD70 or wild type CD70 ECD.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that has reduced affinity for CD27 as compared to human wild type CD70 or wild type CD70 ECD. In one embodiment, the affinity of the CD27 agonist for CD27 is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 less than that of the ECD of human wild type CD70 or wild type CD70 ECD.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that is a CD70 ECD mutein as described herein below and that has reduced affinity for CD27 as compared to human wild type CD70.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that comprises or consists of the ECD of CD70 or a fragment thereof that has CD27 agonist activity. In one embodiment, the CD27 agonist in the conjugate is a mutein of the CD70 ECD comprising at least one substitution, deletion and/or insertion. Amino acid substitutions, deletions and insertions in a CD70 ECD mutein provided herein are indicated relative to the wild-type human CD70 ECD amino acid sequence, which is provided herein as SEQ ID NO: 536. Hence, to allow for allelic variation, a wild-type human CD70 ECD preferably comprises an amino acid

sequence having, with increasing preference, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity with SEQ ID NO: 536.

Liu et al. (2021, J. Biol. Chem. 297(4) 101102) disclose amino acid residues of CD70 that reduce its affinity for CD27 as compared to wild type CD70, which residues include Q61, A80, S137, S146 and H148.

Thus, in one embodiment, there is provided a CD70 ECD mutein comprising at least one amino acid substitution, deletion or insertion at a position in SEQ ID NO: 536 selected from the group consisting of: Q61, A80, S137, S146 and H148. In one embodiment, there is provided a CD70 ECD mutein comprising at least one amino acid substitution selected from the group consisting of: Q61A, A80F, A80R, S137A, S137E, S137K, S146A, H148A, H148E and H148D.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that comprises or consists of a fusion protein comprising three CD70 ECD monomers fused together in a single polypeptide chain. It is understood herein that three CD70 ECD monomers fused together in a single polypeptide chain can be three monomers of any of the CD70 ECD muteins described herein. In one embodiment, the three CD70 ECD monomers are connected by polypeptide linkers. In one embodiment, the three CD70 ECD monomers are connected by polypeptide linkers selected from the group consisting of: GGGSGGG, GGSGGGGSGG, $(GGGGS)_n$, $(GGGSS)_n$, $(GGSSS)_n$ and $(GSSSS)_n$, whereby n = 3, 4, 5, 6 or 7, of which 4 is preferred and of which $(GGGGS)_4$ is more preferred. Other suitable flexible polypeptide linker(s) are described herein below. In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that comprises or consists of a fusion protein comprising three CD70 ECD monomers fused together in a single polypeptide chain, e.g. comprising the amino acid sequence of SEQ ID NO: 537.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that comprises three CD70 ECD monomers that are present in more than one polypeptide chain of the conjugate. For example, two CD70 ECD monomers can be fused together in a single polypeptide chain, optionally connected through polypeptide linker as described above, that is part of a first polypeptide chain of the multispecific antigen binding protein and the third CD70 ECD monomer is part of a second polypeptide chain of the conjugate, as e.g. described in WO2016/075278. The first and second polypeptide chains of the antigen binding protein in the conjugate can be the chains comprising the heavy and light chains of an antigen binding protein in the multispecific antigen binding protein, respectively, or *vice versa*, whereby preferably the CD70 ECDs are fused to the N-termini of the variable domains. Alternatively, the first and second polypeptide chains of (an antigen binding protein in) the conjugate can be the two chains comprising the two heavy chains, whereby preferably the CD70 ECDs are fused to the C-termini of the constant domains.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a CD27 agonist that comprises an antigen-binding region that specifically binds CD27 and that has CD27 agonistic activity.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises more than one CD27 agonist as described above. Thus, in one embodiment, the conjugate has a CD27 agonist-valency that is higher than one. The CD27 agonist-valency of a conjugate can for example be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more.

In one embodiment, the conjugate with a 4-1BBL ECD mutein as described herein thus at least comprises a GITR agonist, e.g. as a further γδ T cell co-stimulatory agonist. GITR (glucocorticoid-induced TNFR-related protein) is a member of the tumor necrosis factor receptor family. Its alternative names are tumor necrosis factor receptor superfamily member 18 (TNFRSF18) and CD357. GITR is encoded by the human *TNFRSF18* gene (Entrez Gene ID: 8784). An amino acid sequence for human GITR is described in NCBI accession numbers NP_004186, NP_683699 and NP_683700. GITR is constitutively expressed on $CD25^+CD4^+$ regulatory T cells and its expression is upregulated on all T cell subsets after activation. GITR is also expressed on neutrophils and NK cells. GITR is co-stimulatory surface receptor for T cells and after interaction with GITRL maintain T cell activation, proliferation, cytokine production, and rescue T cells from anti-CD3-induced apoptosis. GITR can be used as Treg marker and its signaling abrogates the suppressive function of regulatory T cells.

GITR ligand (GITRL), also known as TNFSF18, is a protein that in humans is encoded by the *TNFSF18* gene (Entrez Gene ID: 8995). An amino acid sequence for human GITRL is described in NCBI accession number NP_005083 (SEQ ID NO: 538). The human GITR/GITRL complex consists of three monomeric GITRs bound to a trimeric GITRL (Wang et al. 2021, Nature Comm. 12: 1378).

As used herein, an “GITR agonist” is an agent that has “agonist” activity at GITR, which means that the agent that can cause or increase "GITR signaling". “GITR signaling” refers to an ability of GITR, e.g. when expressed on the surface of T, B and NK cells and triggered by its natural ligand GITRL, to activate or transduce an intracellular signaling pathway, which signaling is propagated via recruiting TRAF-family members, specifically TRAF1, TRAF2 and TRAF5, to the GITR-signaling complex. The signaling is then mediated through NF-kB and MAPK pathways. The “natural GITR ligand” is herein understood as the extracellular domain (ECD) of a human wild type GITRL comprising or consisting of an amino acid sequence from position 50 to 177 of the amino acid sequence of human GITRL (i.e. SEQ ID NO: 539). A GITRL extracellular domain (ECD) is herein thus understood as a polypeptide comprising or consisting of an amino acid sequence from positions 50 to 177 of human GITRL which amino acid sequence is depicted in SEQ ID NO: 539, or a fragment thereof having GITR agonist activity.

GITR agonist activity, i.e. changes in GITR signaling activity, can be measured, for example, by assays designed to measure changes in the GITR signaling pathways, e.g. by monitoring phosphorylation of signal transduction components, assays to measure the association of certain signal transduction components with other proteins or intracellular structures, or in the biochemical activity of components such as kinases, or indirectly by a downstream effect mediated by GITR (e.g. production of specific cytokines). A suitable cell-based assay for *in vitro* biological activity of a GITR agonist, is e.g. described in Wang et al. (2021, *supra*), using measurement of IL-2 production

from 3A9 cell lines expressing wild-type hGITRs in microtiter plates precoated with an anti-mCD3 monoclonal antibody (BD Biosciences #553058 or BioLegend #100340). Alternatively, GITR agonist activity can be assayed using NFκB-luciferase reporter cells stably expressing GITR and measuring luminescence, driven by agonism of GITR and subsequent activation of the NFkB pathway, as described in WO2017/025610.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist that has reduced GITR agonist activity as compared to human wild type GITR. In one embodiment, the GITR agonist has a GITR agonist activity that is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 less than that of the ECD of human wild type GITRL.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist that has enhanced GITR agonist activity as compared to human wild type GITRL. In one embodiment, the GITR agonist has a GITR agonist activity that is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 higher than that of the ECD of human wild type GITRL.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist comprising at least one GITRL ECD comprising an amino acid sequence with at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98, at least 99, or 100% sequence identity to SEQ ID NO: 539, and preferably having an GITR agonist activity as defined above, and/or preferably having an affinity for GITR as defined below.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist of which the affinity for the GITR is reduced or enhanced as compared to the ECD of human wild type GITRL. The affinity of a GITR agonist of the affinity for the GITR can be assayed using methods generally known in the art, such as surface plasmon resonance.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist that has reduced affinity for GITR as compared to human wild type GITRL. In one embodiment, the affinity of the GITR agonist for GITR is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 less than that of the ECD of human wild type GITRL.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist that has enhanced affinity for GITR as compared to human wild type GITRL. In one embodiment, the affinity of the GITR agonist for GITR is a factor 2, 5, 10, 20, 50, 100, 200, 500 or 1000 higher than that of the ECD of human wild type GITRL.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist that comprises or consists of the ECD of GITRL or a fragment thereof that has GITR agonist activity. In one embodiment, the GITR agonist in the conjugate is a mutein of the GITRL ECD comprising at least one substitution, deletion and/or insertion. Amino acid substitutions, deletions and insertions in a GITRL ECD mutein provided herein are indicated relative to the wild-type human GITRL ECD amino acid sequence, which is provided herein as SEQ ID NO: 539. Hence, to allow for allelic variation, a wild-type human GITRL ECD preferably comprises an amino acid sequence having, with increasing preference, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity with SEQ ID NO: 539.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist that comprises or consists of a fusion protein comprising three GITRL ECD monomers fused together in a single polypeptide chain. It is understood herein that three GITRL ECD monomers fused together in a single polypeptide chain can be three monomers of any of the GITRL ECD muteins described herein. In one embodiment, the three GITRL ECD monomers are connected by polypeptide linkers. In one embodiment, the three GITRL ECD monomers are connected by polypeptide linkers selected from the group consisting of: GGGSGGG, GGSGGGGSGG, $(GGGGS)_n$, $(GGGSS)_n$, $(GGSSS)_n$ and $(GSSSS)_n$, whereby n = 3, 4, 5, 6 or 7, of which 4 is preferred and of which $(GGGGS)_4$ is more preferred. Other suitable flexible polypeptide linker(s) are described herein below.

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises a GITR agonist that comprises an antigen-binding region that specifically binds GITR and that has GITR agonistic activity. Agonistic antibodies against GITR are e.g. described by Liu et al. (2022, Cell Reports Medicine 3, 100660).

In one embodiment, a conjugate with a 4-1BBL ECD mutein as described herein comprises more than one GITR agonist as described above. Thus, in one embodiment, the conjugate has a GITR agonist-valency that is higher than one. The GITR agonist-valency of a conjugate can for example be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more.

Structure of a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein

In one embodiment, in a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein, the at least one of the first and second antigen-binding regions (that specifically bind a TAA, an NK cell activating receptor, or an epitope of a γδ TCR) is conjugated to the third antigen-binding region that has or can have affinity for a surface antigen expressed on NK cells. Preferably, the at least one of the first and second antigen-binding regions that specifically binds a TAA, an NK cell activating receptor or an epitope of a γδ TCR is conjugated to at least one polypeptide chain of the third antigen-binding region. The conjugation of the two domains/regions is understood to mean that they are covalently linked to each other. The two domains/regions can be chemically cross-linked to each other, using a cross-linker for linking two proteinaceous molecules, as are well-known in the art. There are a number of commercially available crosslinking reagents for preparing protein or peptide bioconjugates. Many of these crosslinkers allow dimeric homo- or heteroconjugation of biological molecules through free amine or sulfhydryl groups in protein side chains. Other crosslinking methods involve coupling through carbohydrate groups with hydrazide moieties. For cross-linking the first and/or second binding regions to the third binding region it is preferred that cross-linkers with heterofunctional specificity are used. In one embodiment, the cross-linker comprises a flexible spacer, to provide flexibility or freedom of motion of the two regions with respect to each other.

In one embodiment, however, the at least one of the first and second antigen-binding regions is conjugated to the third antigen-binding region by being comprised in a single polypeptide chain. As an antigen-binding region can comprise two polypeptide chains, such as a V_H and a V_L chain, in

one embodiment, at least one polypeptide chain in an antigen-binding region that specifically binds a TAA, an NK cell activating receptor or an epitope of a γδ TCR forms a single polypeptide chain with at least one polypeptide chain of the second antigen-binding region. Likewise, as also the third antigen-binding region that has or can have affinity for a surface antigen expressed on NK cells can comprise two polypeptide chains, such as a dimeric Fc region of an antibody, in one embodiment, at least one polypeptide chain of the third antigen-binding region forms a single polypeptide chain with the at least one polypeptide chain in at least one of the first and second antigen-binding region.

Thus, in one embodiment, a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein comprises a single polypeptide chain that comprises in an N- to C-terminal order: i) at least one polypeptide chain in the at least one of the first and second antigen-binding region; ii) optionally a flexible linker; and iii) (at least one polypeptide chain of) the third antigen-binding region. The flexible linker can be an immunoglobulin hinge region or can be linker as described herein below.

In one embodiment, the third antigen-binding region, i.e. the domain that has or can have affinity for a surface antigen expressed on NK cells, is a dimeric immunoglobulin Fc region, wherein each of the two polypeptide chains of the Fc region is linked to a CH1 domain, each of which CH1 domains is linked to an immunoglobulin variable region of the at least one of the first and second antigen-binding region (that specifically binds a TAA, an NK cell activating receptor or an epitope of a γδ TCR). The dimeric immunoglobulin Fc region preferably is a dimer of an Fc region as herein described above. The immunoglobulin variable region can be an scFv, a V_H domain, a V_L domain, or an immunoglobulin single variable domain (ISVD) such as a dAb, a V-NAR domain or a V_HH domain. In one embodiment, the immunoglobulin variable region that is linked to the CH1 domain is a V_H domain that is paired with a V_L domain linked to a Cκ or Cλ domain. In this embodiment, preferably, the V_H and V_L domains together specifically bind the TAA or the NK cell activating receptor.

In one embodiment, the two immunoglobulin variable regions bind the same TAA. In another embodiment, the two immunoglobulin variable regions each bind a different TAA. In one embodiment, the first immunoglobulin variable region binds a TAA and the second immunoglobulin variable region binds an NK cell activating receptor. In one embodiment, the first and the second immunoglobulin variable regions both bind an NK cell activating receptor. The two immunoglobulin variable regions can each bind a different NK cell activating receptor or they can both bind the same NK cell activating receptor. In one embodiment, the first immunoglobulin variable region binds a TAA and the second immunoglobulin variable region binds a γδ TCR. In one embodiment, the first and the second immunoglobulin variable regions both bind a γδ TCR. The two immunoglobulin variable regions can each bind a different a γδ TCR or they can both bind the same a γδ TCR.

With respect to its specificity, a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein can thus be homodimeric, with two identical immunoglobulin variable regions that both bind the same TAA. Alternatively, a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein can thus be heterodimeric with respect to the specificity for TAAs, NK cell activating receptor and/or γδ TCR, wherein each of the two

immunoglobulin variable regions each bind a different TAA, a TAA and an NK cell activating receptor or a TAA and a γδ TCR. In embodiments, wherein the antigen binding protein is bispecific, it is preferred that one of the two immunoglobulin variable regions is an immunoglobulin single variable domain, while the other immunoglobulin variable region is not. Next, the assembly of heterodimeric antibody heavy chains can be accomplished by expressing two different antibody heavy chain sequences in the same cell, which may lead the assembly of homodimers of each antibody heavy chain as well as assembly of heterodimers. Promoting the preferential assembly of heterodimers can be accomplished by incorporating different mutations in the CH3 domain of each antibody heavy chain constant region as shown in US13/494,870, US16/028850, US11/533,709, US12/875,015, US13/289,934, US14/773,418, US12/811,207, US13/866,756, US14/647,480, US 14/830,336 and WO2019/195409. For example, mutations can be made in the CH3 domain based on human IgG1 and incorporating distinct pairs of amino acid substitutions within a first polypeptide and a second polypeptide that allow these two chains to selectively heterodimerize with each other. For example, CH3 domains which comprise amino acid substitutions, wherein the CH3 domain interface of the antibody Fc region is mutated to create altered charge polarity across the Fc dimer interface such that co-expression of electrostatically matched Fc chains supports favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation.

In one embodiment, a “knob-into-holes” approach is used in which the CH3 domain interface of the antibody Fc region is mutated so that the antibodies preferentially form heterodimers (further including the attached light chains). These mutations create altered charge polarity across the Fc dimer interface such that coexpression of electrostatically matched Fc chains support favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation. For example, one heavy chain comprises a T366W substitution and the second heavy chain comprises a T366S, L368A and Y407V substitution, see, e.g. Ridgway et al (1996) *Protein Eng.,* 9, pp. 617-621; Atwell (1997) J. *Mol. Biol.,* 270, pp. 26-35; and W02009/089004, the disclosures of which are incorporated herein by reference. In another approach, one heavy chain comprises a F405L substitution, and the second heavy chain comprises a K409R substitution, see, e.g., Labrijn et al. (2013) *Proc. Natl. Acad. Sci. U.S.A.,* 110, pp. 5145-5150. In another approach, one heavy chain comprises T350V, L351Y, F405A, and Y407V substitutions and the second heavy chain comprises T350V, T366S, K392L, and T394W substitutions, see, e.g. Von Kreudenstein et al., (2013) *mAbs* 5:646-654. In another approach, one heavy chain comprises both K409D and K392D substitutions and the second heavy chain comprises both D399K and E356K substitutions, see, e.g. Gunasekaran et al., (2010) *J. Biol. Chem.* 285:19637-19646. In another approach, one heavy chain comprises D221E, P228E and L368E substitutions and the second heavy chain comprises D221R, P228R, and K409R substitutions, see, e.g. Strop et al., (2012) *J. Mol. Biol.* 420: 204-219. In another approach, one heavy chain comprises S364H and F405A substitutions and the second heavy chain comprises Y349Tand, T394F substitutions, see, e.g. Moore et al., (2011) *mAbs* 3: 546-557. In another approach, one heavy chain comprises a H435R substitution, and the second heavy chain optionally

may or may not comprise a substitution, see, e.g. US Patent no. 8,586,713. When such hetero-multimeric antibodies have Fc regions derived from a human IgG2 or IgG4, the Fc regions of these antibodies can be engineered to contain amino acid modifications that permit CD16 binding or that avoid CD16 binding. In some embodiments, the antibody may comprise mammalian antibody-type N-linked glycosylation at residue N297 (Kabat EU numbering).

In one preferred embodiment, a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein comprises a dimeric immunoglobulin Fc region that is a (homo or hetero) dimer of at least one Fc region as herein described above, wherein each of the two Fc polypeptide chains is operably linked to a Fab that specifically binds a TAA, an NK cell activating receptor or an epitope of a γδ TCR. Apart from the presence of the 4-1BBL ECD mutein (and optional further agonists), the antigen binding protein in a conjugate comprising such a dimeric Fc linked to two Fabs can thus form an immunoglobulin structure, such as a conventional IgG immunoglobulin.

In one embodiment, in a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein, at least one of the 4-1BBL ECD mutein and the further agonists , is conjugated to the at least one of the first and second antigen-binding region that specifically binds a TAA, an NK cell activating receptor or an epitope of a γδ TCR, or to the third antigen-binding region. As indicated above conjugation of two proteinaceous entities is understood to mean that they are covalently linked to each other, which can be done by chemical cross-linking, using cross-linkers for linking two proteinaceous molecules, as are well-known in the art, which cross-linker can comprise a flexible spacer.

In one embodiment, however, the at least one of the 4-1BBL ECD mutein and the further agonist forms a single polypeptide chain with at least one of: i) at least one polypeptide chain in the at least one of the first and a second antigen-binding regions that specifically binds a TAA, NK cell-activating receptor or an epitope of a γδ TCR; and, ii) (at least one polypeptide chain in) the second antigen-binding region that has or can have affinity for a surface antigen expressed on NK cells. In one embodiment, a flexible linker (as described below) is present between the agonist and the region defined in i) or ii).

In one embodiment, the at least one of the 4-1BBL ECD mutein and the further agonist forms a single polypeptide chain with at least one of: i) a light chain in at least one of the two Fabs that specifically bind a TAA, an NK cell activating receptor or an epitope of a γδ TCR; and, ii) at least one of the two Fc chains in the dimeric immunoglobulin Fc region. In one embodiment, a flexible linker (as described below) is present between the at least one of the 4-1BBL ECD mutein and the further agonist and the light chain defined in i) or the Fc chain defined in ii).

In one embodiment, the at least one of the 4-1BBL ECD mutein and the further agonist is fused to at least one of: i) the N-terminus of the light chain in at least one of the two Fabs that specifically bind a TAA, an NK cell activating receptor or an epitope of a γδ TCR, optionally through a flexible linker; ii) the C-terminus of the light chain in at least one of the two Fabs that specifically bind a TAA, an NK cell activating receptor or an epitope of a γδ TCR, optionally through a flexible linker; iii) the N-terminus of the heavy chain in at least one of the two Fabs that specifically bind a

TAA, an NK cell activating receptor or an epitope of a γδ TCR; and, iv) the C-terminus of the heavy chain in at least one of the two Fc chains in the dimeric immunoglobulin Fc region, optionally through a flexible linker, whereby the flexible linker can be as described below.

In one embodiment, wherein the conjugate comprises a (homo- or hetero) dimeric antigen binding protein as described above, the dimer can comprise at least one of the 4-1BBL ECD mutein and the further agonist on only one of the two monomers in the dimer, or the dimer can comprise at least one of the 4-1BBL ECD mutein and the further agonist on each (i.e. both) of the two monomers in the dimer. Thus, in one embodiment, wherein the conjugate comprises an immunoglobulin structure, at least one of the 4-1BBL ECD mutein and the further agonist can be present on at least one or on both sides of the immunoglobulin structure. In embodiments wherein at least one of the 4-1BBL ECD mutein and the further agonist is present on each of the two monomers in the dimer or immunoglobulin structure, the antigen binding protein in the conjugate can for example comprises a 4-1BBL ECD mutein on both monomers, a further agonist (e.g. an IL-21R agonist) on both monomers or, a 4-1BBL ECD mutein on a first monomer and a further agonist (e.g. an IL-21R agonist) on a second monomer. It is understood that when the antigen binding protein in the conjugate comprises heterodimeric heavy chains, the "knob-into-hole" technology as described above can be applied, wherein the CH3 domain of the first chain is modified to have a "protuberance" ("knob") and the second chain is modified to have a corresponding "cavity" ("hole").

Thus, in one embodiment, a conjugate comprising antigen binding protein as described herein is heterodimeric with respect to at least one of: i) the first and second antigen-binding regions; and ii) at least one of the fused 4-1BBL ECD mutein and the fused further agonist, and the dimeric Fc region comprises different first and a second polypeptide chains comprising knob-into-hole modifications promoting association of the first and the second polypeptide chains of the Fc region.

Amino acid sequences of suitable peptidyl linkers for linking the various functional domains and regions in a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein are known in the art as described herein above.

In one embodiment, a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein is a conjugate as exemplified herein, such as e.g. AVC46 – AVC59 (see Table 1.1.10.1) or a derivative thereof wherein the trastuzumab variable heavy (V_H) and variable light (V_L) domains are replaced by variable heavy (V_H) and variable light (V_L) domains from another monoclonal antibody against a TAA or an NK cell activating receptor, for example, from a monoclonal antibody against a TAA known in the art as herein described above (see e.g. Table 1.1.10.2 and 1.1.10.3).

In one embodiment, a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein, and further comprising an IL-21 mutein, is a conjugate as exemplified herein, such as e.g. AVC72 – AVC107, and AVC176, AVC187 - AVC190, AVC323, AVC421, AVC245, AVC267, AVC285 and AVC287 (see Tables 1.1.10.1 and 1.1.10.2) or a derivative thereof wherein the trastuzumab or sacituzumab variable heavy (VH) and variable light (VL) domains are replaced by variable heavy (VH) and variable light (VL) domains from another monoclonal antibody

against a TAA or an NK cell activating receptor , for example, from a monoclonal antibody against a TAA known in the art as herein described above.

In one embodiment, the conjugate comprises a HER2 targeting antigen-binding protein comprising: a) a first heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to any one of SEQ ID NO.'s: 196 – 218, and; b) a second heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to any one of SEQ ID NO.'s: 12, 177 – 195 and 219 - 237; and c) a light chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 2. In a preferred embodiment, the conjugate comprises a HER2 targeting antigen-binding protein comprising: a) a first heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to any one of SEQ ID NO.'s: 209, 675, 202 and 199, and; b) a second heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to any one of SEQ ID NO.'s: 225 or 236; and c) a light chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 2. In a more preferred embodiment, the conjugate comprises a HER2 targeting antigen-binding protein comprising: a) a first heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to any one of SEQ ID NO.'s: 209, 675 and 202; b) a second heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO.'s: 225 or 236; and c) a light chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 2. In a most preferred embodiment, the conjugate comprises a HER2 targeting antigen-binding protein comprising: a) a first heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO.'s: 209 or 675, of which 209 is preferred; b) a second heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO.'s: 225; and c) a light chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 2.

In one embodiment, the conjugate comprises a TROP2 targeting antigen-binding protein comprising: a) a first heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to any one of SEQ ID NO.'s: 585, 591 and 588; b) a second heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to any one of SEQ ID NO.'s: 586, 674, 592 and 589; and c) a light chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to any one of SEQ ID NO.'s: 584, 593 and 590.

In a preferred embodiment, the conjugate comprises: a) a first heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 585; b) a second heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO.'s: 586 or 674; and c) a light chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 584. In a more preferred embodiment, the conjugate comprises: a) a first heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 585; b) a

second heavy chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO.'s: 586; and c) a light chain comprising an amino acid sequence with at least 95, 96, 97, 98, 99 or 100% sequence identity to SEQ ID NO: 584.

Biological activities of the conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein

A conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein can have a one or more biological activities, including e.g. antigen (TAA) binding, binding to an NK cell or a γδ T cell, the ability to direct an NK cell or a γδ T cell to a target cell expressing the TAA, activating an NK cell or a γδ T cell, including inducing hyper-functionality of the NK cell or a γδ T cell, and/or the ability to elicit lysis of target cell by the (activated/hyper-functional) NK cell or a γδ T cell.

In one embodiment, a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein causes an increase in at least one NK cell activity selected from CD107a degranulation, CD69 expression, IFNy production, NK cell proliferation and NK cell cytotoxicity, whereby preferably, the increase is at least a factor 1.0, 1.1, 1.2, 1.5, 2.0, 5.0, 10, 20 50 100, 110, 120, 150, 200, 210, 220, 250 or 300 higher as compared to the increase achieved with the same effector : target cell ratio, with the same NK cells and target cells that are not brought into contact with the conjugate comprising a 4-1BBL ECD mutein or the antigen binding protein.

In one embodiment, a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein cause an increase in at least one NK cell activity selected from CD107a degranulation, CD69 expression, IFNy production, NK cell proliferation and NK cell cytotoxicity, whereby preferably, the increase is at least a factor 1.0, 1.1, 1.2, 1.5, 2.0, 5.0, 10, 20, 50, 100, 110, 120, 150, 200, 210, 220, 250 or 300 higher as compared to the increase achieved with the same effector : target cell ratio, with the same NK cells and target cells that are brought into contact (under otherwise identical conditions) with a reference antigen binding protein.

In one embodiment, the reference antigen binding protein is a conventional human IgG1 monoclonal antibody that binds to the same TAA, preferably, that binds to the same epitope, more preferably that has same TAA-specific antigen-binding region(s) as the conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein. For example, compared to the monoclonal antibody trastuzumab, a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein having a HER2-binding region (preferably having the trastuzumab variable domains), is superior in causing an increase in NK cell activities.

In one embodiment, the reference antigen binding protein is a (multispecific) antigen binding protein comprising at least one antigen-binding region that binds to the same TAA, preferably, that binds to the same epitope, more preferably that has same TAA-specific antigen-binding region(s) as the conjugate comprising a 4-1BBL ECD mutein or the antigen binding protein, and comprising at least one antigen-binding region that specifically binds an NK cell activating receptor such as NKp46, NKp44, NKp30, NKG2D, DNAM1 and CD16A. In one embodiment, the reference antigen binding protein is a (multispecific) antigen binding protein comprising at least one antigen-binding

region that binds to the same TAA, preferably, that binds to the same epitope, more preferably that has same TAA-specific antigen-binding region(s) as the conjugate comprising a 4-1BBL ECD mutein or the antigen binding protein, and comprising at least one NK cell-activating cytokine other than a 4-1BBL ECD mutein. The NK cell-activating cytokine other than a 4-1BBL ECD mutein can be an NK cell activating cytokine is selected from the group consisting of an IL-21R agonist, an IL-15 receptor agonist, a type I interferon (IFN-1) agonist, an IL-2 receptor agonist, an IL-12 receptor agonist and an IL-18 receptor agonist, as described above. For example, an IL21 mutein as described above, or an IL-15 receptor agonist, such as IL15, a human modified IL-15 cross-linker as described in US2018282386 and Vallera et al. (2016, Clin Cancer Res.; 22(14): 3440–3450).

In one embodiment, the reference antigen binding protein is an NK cell engager, such as e.g. described in WO2016/207278, WO 2018/148445, WO2018/152518, WO2019195409 US2018282386, Vallera et al. (2016, *supra*) and Demaria et al. (2021, *supra*). One example of a (multispecific) reference antigen binding protein is for example AVC-006 as described in WO2024/056862, comprising one HER2-binding region and one NKG2D-binding region.

Assays which detect the expression of an NK activation marker or which detects NK cell cytotoxicity, or for detecting NK cell activation and cytotoxicity assays (e.g. short and long term cytotoxicity assays) are described in the Examples herein, as well as for example, in Pessino et al, J. Exp. Med, 1998, 188 (5): 953-960; Sivori et al, Eur J Immunol, 1999. 29:1656-1666; Brando et al, (2005) J. Leukoc. Biol. 78:359-371; El-Sherbiny et al, (2007) Cancer Research 67(18):8444-9; Nolte-'t Hoen et al, (2007) Blood 109:670-673); WO 2016/207278 and WO 2018/148445.

In one embodiment, a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein has the ability to induce hyper-functionality (or a hyper-functional phenotype) in an NK cell or in a population of NK cells. A hyper-functional NK cell phenotype is herein understood as a phenotype that has one or more of the features of the phenotype that is obtained by expanding NK cells obtained from donors *ex vivo* by co-culturing them with irradiated K562 feeder cells modified to express membrane bound IL-21 (mbIL-21) and 4-1BB ligand (FC21 feeder cells) as described by Denman et al. (2012, *supra*). Thus, in one embodiment, *ex vivo* expansion of donor NK cells by co-culturing (e.g. for 7, 14 or 21 days), with a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein, produces a population of NK cells having one or more (or preferably all) of the features selected from the group: a) the fold expansion of the expanded NK cells is at least 0.5, 1.0, 2.0 or 5.0 fold of the fold expansion of expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells; b) the telomere length of the expanded NK cells is increased by at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55% as compared to the telomere length of fresh NK cells, preferably, the percentage telomere length increase of the expanded NK cells as compared to the telomere length of fresh NK cells, is at least 0.5, 1.0, 2.0 or 5.0 fold of the percentage telomere length increase of expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells; c) the expression level of at least one NK cell activating receptor selected from NKG2D, NKp30, NKp44, NKp46 and CD16 on the expanded NK cells is at least 0.5, 1.0, 2.0 or 5.0 fold of the expression level on NK cells obtained upon *ex vivo* expansion in the presence of FC21 feeder cells; d) the

secretion of at least one cytokine of TNF-α, IFN-γ and IL-6 by the expanded NK cells is at least 0.5, 1.0, 2.0 or 5.0 fold of the secretion of the cytokine by NK cells obtained upon *ex vivo* expansion in the presence of FC21 feeder cells; and, e) the cytotoxicity of the expanded NK cells is at least 0.5, 1.0, 2.0 or 5.0 fold of the cytotoxicity of NK cells obtained upon ex vivo expansion in the presence of FC21 feeder cells. In a preferred embodiment, the *ex vivo* expansion of donor NK cells further comprises that the NK cells are co-cultured with tumor cells expressing a TAA specifically bound by the conjugate comprising a 4-1BBL ECD mutein or the antigen binding protein. Protocols for *ex vivo* expansion of donor NK cells and assays for determining fold expansion, telomere length increase, expression level of NK cell activating receptors, cytokine secretion and cytotoxicity (e.g. short term or long term cytotoxicity assays) are described in Denman et al. (2012, *supra*) and in the Examples herein.

In one embodiment, a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein cause an increase in at least one γδ T cell characteristic selected from γδ T cell proliferation, cytokine production, γδ T cell cytotoxicity, γδ T cell differentiation, whereby preferably, the increase is at least a factor 1.0, 2.0, 5.0, 10, 20 50 100, 110, 120, 150, 200, 210, 220, 250 or 300 higher as compared to the increase achieved with the same effector: target cell ratio, with the same γδ T cells and target cells that are not brought into contact with the conjugate or antigen binding protein.

In one embodiment, a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein cause an increase in at least one γδ T cell characteristic selected from γδ T cell proliferation, cytokine production, γδ T cell cytotoxicity, γδ T cell differentiation, whereby preferably, the increase is at least a factor 1.0, 2.0, 5.0, 10, 20 50 100, 110, 120, 150, 200, 210, 220, 250 or 300 higher as compared to as compared to the increase achieved with the same effector : target cell ratio, with the same γδ T cells and target cells that are brought into contact with a reference antigen binding protein, e.g. a conventional human IgG1 monoclonal antibody that has the same TAA-specific antigen-binding regions as the conjugate or antigen binding protein.

The increase in cytokine production preferably is an increase in the production and secretion of inflammatory cytokines such as INFɣ and TNFα, which promote immune licensing. An increase in cytokine production by γδ T cell as induced by a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein can thus be measured by determining the release of at least one of INFɣ and TNFα by methods known per se in the art.

The increase in γδ T cell differentiation can be an increase in γδ T cell cytotoxicity, an increase in the expression of CD56 and/or a change in the ratio of CD27 to CD45RA, all of which can be determined by methods known per se in the art.

In one embodiment, the reference antigen binding protein is a conventional human IgG1 monoclonal antibody that binds to the same TAA, preferably, that binds to the same epitope, more preferably that has same TAA-specific antigen-binding region(s) as the conjugate or antigen binding protein. For example, compared to the monoclonal antibody trastuzumab, a multispecific antigen binding protein having a HER2-binding region (preferably having the trastuzumab variable domains), is superior in causing an increase in γδ T cell activities.

In one embodiment, the reference antigen binding protein is a bispecific antibody binding a γδ TCR and a TAA such as described in WO2022/034562, wherein preferably, the bispecific antibody binds a Vδ1 chain of a γδ TCR, preferably that has same Vδ1 chain-specific antigen-binding region(s) as the conjugate or antigen binding protein described herein, and the same TAA, preferably, that binds to the same epitope, more preferably that has same TAA-specific antigen-binding region(s) as the conjugate or antigen binding protein described herein.

In one embodiment, the reference antigen binding protein is a multispecific antigen binding protein as described herein, which lacks at least one of i) the first and second antigen binding region (binding the target antigen); ii) the third antigen binding region (binding the γδ TCR); iii) the γδ T cell-activating agonist that induces at least one of STAT5 and STAT3 activation; and iv) the co-stimulatory agonist.

In one embodiment, a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein has the ability to induce hyper-functionality or innate potency in a γδ T cell or in a population of γδ T cells, *in vivo* and/or *in vitro*. In one embodiment, γδ T cell innate like potency is determined by measuring the change in the levels of at least one of CD56 and NKp30 present on a Vδ1+ T cell in a model system. These markers are often used as markers of lymphocyte potency (e.g. cytotoxicity against tumor cells or degranulation upon target recognition or T cell receptor engagement with an agonistic agent.) and can be measured following application of a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein described herein to a population of γδ T cells, e.g. by flow cytometry. The change in phenotype of a Vδ1+ T cell or population thereof induced by the conjugate or antigen binding protein as described herein can then be compared to the change in phenotype obtained when a reference antigen binding protein as described above, or an alternative comparator antibody is applied (e.g. OKT-3, TS8.2, etc.) to said equivalent γδ T cells. In one embodiment, the expression level of at least one of CD56 and NKp30 present on Vδ1+ T cells contacted with a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein protein described is increased at least a factor 1.0, 2.0, 5.0, 10, 20 50 100, 110, 120, 150, 200, 210, 220, 250 or 300 higher as compared to the increase achieved with a reference antigen binding protein or comparator antibody as described above, preferably as determined by flow cytometry.

In one embodiment, γδ T cell innate like potency is determined by measuring the killing of TAA negative tumor cells Vδ1+ T cells, after the Vδ1+ T cells have been exposed to a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein as described herein for a minimum of 7 days, preferably 14 days in an artificial culture system and compared to the killing by Vδ1+ T cells that have not been exposed to the conjugate or antigen binding protein, or that have been exposed to a reference antigen binding protein as described above, or an alternative comparator antibody (e.g. OKT-3, TS8.2, etc.). In one embodiment, the cytotoxicity against TAA negative tumor cells of Vδ1+ T cells contacted with a conjugate comprising a 4-1BBL ECD mutein or an antigen binding protein described is increased at least a factor 1.0, 2.0, 5.0, 10, 20 50 100, 110, 120, 150, 200, 210, 220, 250 or 300 higher as compared to the cytotoxicity against TAA negative tumor cells

of Vδ1+ T cells contacted with a reference antigen binding protein or comparator antibody as described above.

Protocols for *ex vivo* proliferation of donor γδ T cells and assays for determining fold expansion, expression level of CD56 and NKp30, cytokine secretion and cytotoxicity (e.g. short term or long term cytotoxicity assays) are described in the Examples herein.

Pharmaceutical compositions

In a further aspect, the invention relates to a pharmaceutical composition comprising a 4-1BBL ECD mutein as described herein or a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein, and a pharmaceutically acceptable carrier (excipient). The pharmaceutically acceptable carrier such as an adjuvant, or vehicle, is for administration of the protein to a subject. Said pharmaceutical composition can be used in the methods of treatment described herein below by administration of an effective amount of the composition to a subject in need thereof. The term "subject", as used herein, refers to all animals classified as mammals and includes, but is not restricted to, primates and humans. The subject is preferably a male or female human of any age or race.

The term "pharmaceutically acceptable carrier", as used herein, is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration (see e.g. “Handbook of Pharmaceutical Excipients”, Rowe et al eds. 7th edition, 2012, www.pharmpress.com). The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) proteins; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter ions such as sodium; metal complexes (e.g. Zn^{2+}-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Supplementary active compounds can also be incorporated into the pharmaceutical composition of the invention. Thus, in a particular embodiment, the pharmaceutical composition of the invention may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a chemotherapeutic agent, a

cytokine, an analgesic agent, a thrombolytic or an immunomodulating agent, e.g. an immunosuppressive agent or an immunostimulating agent. The effective amount of such other active agents depends, among other things, on the amount of the protein of the invention present in the pharmaceutical composition, the type of disease or disorder or treatment, etc.

In one embodiment, the protein of the invention is prepared with carriers that will protect said compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems, e.g. liposomes. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions, including targeted liposomes can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in US 4,522,811 or WO2010/095940.

The administration route of the protein of the invention can be parenteral. The term "parenteral" as used herein includes intravenous, intra-arterial, intralymphatic, intraperitoneal, intramuscular or subcutaneous. The intravenous or intramuscular forms of parenteral administration are preferred. By "systemic administration" is meant intravenous, intraperitoneal and intramuscular administration. The amount of the protein required for therapeutic or prophylactic effect will, of course, vary with the protein chosen, the nature and severity of the condition being treated and the patient. In addition, the protein may suitably be administered by pulse infusion, e.g., with declining doses of the protein. Preferably the dosing is given by injections, most preferably intravenous, intramuscular or subcutaneous injections, depending in part on whether the administration is brief or chronic.

Thus, in a particular embodiment, the pharmaceutical composition of the invention may be in a form suitable for parenteral administration, such as sterile solutions, suspensions or lyophilized products in the appropriate unit dosage form. Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, CremophorEM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyethylene glycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases,

it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol or sodium chloride in the composition.

Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g a protein of the invention) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

In a particular embodiment, said pharmaceutical composition is administered via intravenous (IV), intramuscular (IM) or subcutaneous (SC) route. Adequate excipients can be used, such as bulking agents, buffering agents or surfactants. The mentioned formulations will be prepared using standard methods for preparing parenterally administrable compositions as are well known in the art and described in more detail in various sources, including, for example, “Remington: The Science and Practice of Pharmacy” (Ed. Allen, L. V. 22nd edition, 2012, www.pharmpress.com).

It is especially advantageous to formulate the pharmaceutical compositions, namely parenteral compositions, in dosage unit form for ease administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound (protein of the invention) calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention is dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Generally, for the prevention and/or treatment of the diseases and disorders mentioned herein and depending on the specific disease or condition to be treated and its severity, the potency of the specific protein of the invention to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the protein of the invention will generally be administered in the range of from 0.001 to 1,000 mg/kg body weight/day, preferably about 0.01 to about 100 mg/kg body weight/day, most preferably from about 0.05 to 10 mg/kg body weight/day, such as about 1, 10, 100 or 1000 microgram per kg body weight per day, either continuously (e.g. by infusion), as a single daily dose or as multiple divided doses during the day. The clinician will generally be able to determine a suitable daily dose, depending on the factors mentioned herein. It will also be clear that in specific cases, the clinician may choose to deviate from these amounts, for example on the basis of the factors cited above and his expert judgment. The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Therapeutic use

In another aspect there is provided a 4-1BBL ECD mutein as described herein or a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein for use as a medicament. In one embodiment, the 4-1BBL ECD mutein as described herein or the conjugate as described herein is used as an active ingredient, component or substance in a medicament.

In one aspect, the invention pertains to a use of a 4-1BBL ECD mutein as described herein or a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein for the manufacture of a medicament, e.g. a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate as an active ingredient, for the treatment, prevention or diagnosis of a disease in a subject in need thereof.

In one aspect, the invention pertains to a 4-1BBL ECD mutein as described herein or a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein, or a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate as an active ingredient, for use in the treatment, prevention or diagnosis of a disease in a subject in need thereof.

In one aspect, the invention pertains to a method for the treatment of a disease in a subject in need thereof, wherein the method comprises the step of administering to the subject (an effective amount of) a 4-1BBL ECD mutein as described herein or a conjugate comprising a 4-1BBL ECD mutein and an antigen binding protein as described herein, or a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate as an active ingredient.

The disease to be treated, prevented or diagnosed using the 4-1BBL ECD mutein, the fusion protein or the conjugate can be a cancer, an infectious disease, an inflammatory disease or an autoimmune disease.

In one embodiment, the disease to be treated, prevented or diagnosed using the 4-1BBL ECD mutein, the fusion protein or the conjugate is a cancer, e.g. a cancer as described below. The cancer preferably is a cancer expressing a TAA as described herein above, more preferably the cancer is expressing a TAA that is bound by the antigen binding protein in the conjugate.

In one embodiment, the treatment can comprise the steps of a) identifying a TAA expressed by (tumor) cells in the cancer; b) selection of conjugate comprising an antigen binding protein as described herein that specifically binds the TAA; c) using the conjugate selected in b) in the treatment of the cancer. The cancer can be a cancer as described below.

In one embodiment, the invention pertains to a method for enhancing anti-tumor activity of at least one of an NK cell and a γδ T cell in a subject, the method comprising the step of administering to the subject a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein, or a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate as an active ingredient. In one embodiment, the subject has cancer, e.g. a cancer as described below. Preferably, the cancer comprises tumor cells expressing a TAA, more preferably the tumor cells express a TAA that is bound by the antigen binding protein in the conjugate.

In one embodiment, the invention pertains to a method for expanding and/or inducing hyper-functionality NK cells a in a subject, the method comprising the step of administering to the subject a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein, or a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate as an active ingredient. The fold expansion and the hyper-functionality preferably is as herein described above. In one embodiment, the subject has cancer, preferably a cancer comprising tumor cells expressing the TAA, more preferably the tumor cells express a TAA that is bound by the antigen binding protein in the conjugate.

In one embodiment, there is provided a method for expanding, activating and/or inducing innate potency of γδ T cells in a subject, the method comprising the step of administering to the subject an 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein, or a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate as an active ingredient. The fold expansion, activation and the innate potency of γδ T cells preferably is as herein described above. In one embodiment, the subject has cancer, preferably a cancer comprising tumor cells expressing the TAA.

In one embodiment, there is provided a method for redirecting a γδ T cell to a tumor cell expressing the TAA in a subject, the method comprising the step of administering to the subject an 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein, or a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate as an active ingredient.

Thus, in one embodiment, there is provided a method of modulating an immune response in a subject in need thereof comprising administering a therapeutically effective amount of an 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein, or a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate multispecific antigen binding protein as an active ingredient. In various embodiments, modulating an immune response in a subject comprises binding or targeting γδ T cells, activating γδ T cells, causing or increasing proliferation of γδ T cells, causing or increasing expansion of γδ T cells, causing or increasing γδ T cell degranulation, causing or increasing γδ T cell mediated killing activity, causing or increasing γδ T cell mediated killing activity while sparing healthy cells, causing or increasing γδ T cytotoxicity, causing or increasing γδ T cytotoxicity while sparing healthy cells, causing or increasing γδ T cell mobilization, increasing survival of γδ T cells, or increasing resistance to exhaustion of γδ T cells.

Subjects having cancer, often present with lower numbers of NK cells and/or γδ T cells and/or with exhausted NK cells and/or γδ T cells. The 4-1BBL ECD muteins or the conjugates of the invention can thus be advantageously used to expand the numbers of NK cells and/or γδ T cells and/or to induce hyper-functionality of the NK cells and/or γδ T cells in a subject suffering from cancer. A further advantage of the hyper-functionality of NK cells as induced by the 4-1BBL ECD mutein, the fusion protein or the conjugate of the invention, includes their increased secretion of cytokines such as TNF-α, IFN-γ and IL-6, which help shape adaptive immune response involving DCs and T cells. Indeed, NK cells have been reported to promote the recruitment to the tumor micro environment of a DC subset specializing in the cross-presentation of tumor antigens to $CD8^+$ T

cells, suggesting a crucial role for NK cells in the potentiation of antitumor $CD8^+$ T cell responses (Bottcher et al., Cell, 2018. 172: 1022–1037; and Barry et al., Nat. Med. 2018. 24: 1178–1191). The contribution of NK cells to the orchestration of antitumor T-cell responses has also been confirmed experimentally in mice, demonstrating that, in addition to their direct effector functions, NK cell can promote T-cell responses and long-lasting immune control of tumors (Bonavita et al., Immunity 2020. 53: 1215–1229).

In various embodiment, there is provided the use of a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein for the manufacture of a medicament, for example for the administration to a subject, wherein the subject has cancer, an infectious disease or an inflammatory disease.

In one embodiment, the TAA is a TAA as defined herein above and/or an antigen expressed on the surface of a malignant cell of a type of cancer as described below. A subject to be treated in accordance with the methods described herein can have a cancer selected from the group consisting of: acute lymphoblastic, acute myeloid leukemia, adrenocortical carcinoma, appendix cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma and malignant fibrous histiocytoma, brain stem glioma, brain tumor, brain tumor, brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma, visual pathway and hypothalamic glioma, brain and spinal cord tumors, breast cancer, bronchial tumors, Burkitt lymphoma, carcinoid tumor, gastrointestinal carcinoid tumor, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, central nervous system lymphoma, cerebellar astrocytoma cerebral astrocytoma/malignant glioma, cervical cancer, chordoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, craniopharyngioma, cutaneous T-cell lymphoma, esophageal cancer, Ewing family of tumors, extragonadal germ cell tumor, extrahepatic bile duct cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (gist), germ cell tumor, gestational trophoblastic tumor, glioma, glioma brain stem, glioma cerebral astrocytoma, glioma visual pathway and hypothalamic, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Langerhans cell histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular melanoma, islet cell tumors, kidney (renal cell) cancer, Langerhans cell histiocytosis, laryngeal cancer, acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, lip and oral cavity cancer, liver cancer, non- small cell lung cancer, small cell lung cancer, aids-related lymphoma, Burkitt lymphoma, cutaneous T-cell lymphoma, non-Hodgkin lymphoma, primary central nervous system lymphoma, Waldenstrom macroglobulinemia, malignant fibrous histiocytoma of bone and osteosarcoma, medulloblastoma, melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia

syndrome, multiple myeloma/plasma cell neoplasm, mycosis, fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia, myeloid leukemia, myeloid leukemia acute, multiple myeloma, myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, oral cavity cancer, oropharyngeal cancer, osteosarcoma and malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, pancreatic cancer, papillomatosis, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal parenchymal tumors of intermediate differentiation, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma celt neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, renal pelvis and ureter, respiratory tract carcinoma involving the nut gene on chromosome 15, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma, Ewing family of tumors, Kaposi sarcoma, soft tissue sarcoma, uterine sarcoma, Sezary syndrome, skin cancer (nonmelanoma), skin cancer (melanoma), Merkel cell skin carcinoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, stomach (gastric) cancer, supratentorial primitive neuroectodermal tumors, T-cell lymphoma, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, gestational trophoblastic tumor, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, and Wilms tumor. In various embodiments, the cancer that can be treated by the disclosed methods and compositions is treated while healthy cells are spared.

In various embodiments, the inflammatory diseases that can be treated by the disclosed methods and compositions include, but are not limited to Achalasia, Acute disseminated encephalomyelitis (ADEM), Acute motor axonal neuropathy, Acute respiratory distress syndrome (ARDS), Addison’s disease, Adiposis dolorosa, Adult Still's disease, Adult-onset Still's disease, Agammaglobulinemia, Alopecia Areata, Amyloidosis, Amyotrophic lateral sclerosis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Anti-N- Methyl-D-Aspartate (Anti-NMDA) Receptor Encephalitis, Antiphospholipid syndrome, Antiphospholipid syndrome (APS, APLS), Antisynthetase syndrome, Anti-tubular basement membrane nephritis, Aplastic anemia, Atopic allergy, Atopic dermatitis, Autoimmune angioedema, Autoimmune comorbidities, Autoimmune dysautonomia, Autoimmune encephalomyelitis, Autoimmune enteropathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease (AIED), Autoimmune lymphoproliferative syndrome, Autoimmune myocarditis, Autoimmune neutropenia, Autoimmune oophoritis, Autoimmune orchitis, Autoimmune pancreatitis (AIP), Autoimmune peripheral neuropathy, Autoimmune polyendocrine syndrome (APS) type 1, Autoimmune polyendocrine syndrome (APS) type 2, Autoimmune polyendocrine syndrome (APS) type 3, Autoimmune retinopathy, Autoimmune thrombocytopenic purpura, Autoimmune thyroiditis, Autoimmune urticaria, Autoimmune uveitis, Autoimmune vasculitis, Axonal & neuronal neuropathy (AMAN), Balo concentric sclerosis, Baló disease, Behcet’s disease, Benign mucosal pemphigoid, Bickerstaff's encephalitis, Blau syndrome,

Bullous pemphigoid, Castleman disease (CD), Celiac disease, Chagas disease, Chronic fatigue syndrome, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic obstructive pulmonary disease, Chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss Syndrome (CSS) or Eosinophilic Granulomatosis (EGPA), Cicatricial pemphigoid, Cogan syndrome, Cold agglutinin disease, Complement component 2 deficiency, Complex regional pain syndrome, Congenital heart block, Connective tissue, systemic, and multi-organ, Contact dermatitis, Coxsackie myocarditis, CREST syndrome, Crohn's disease, Cushing's syndrome, Cutaneous leukocytoclastic angiitis, Dego's disease, Dermatitis herpetiformis, Dermatomyositis, Devic'sDisease (neuromyelitis optica), Diabetes mellitus type 1, Digestive system, Discoid lupus, Dressler's syndrome, Drug-induced lupus, Eczema, Endometriosis, Enthesitis-related arthritis, Eosinophilic esophagitis (EoE), Eosinophilic fasciitis, Eosinophilic gastroenteritis, Eosinophilic granulomatosis with polyangiitis (EGPA), Eosinophilic pneumonia, Epidermolysis bullosa acquisita, Erythema nodosum, Erythroblastosis fetalis, Esophageal achalasia, Essential mixed cryoglobulinemia, Evans syndrome, Exocrine, Felty syndrome, Fibrodysplasia ossificans progressiva, Fibromyalgia, Fibrosing alveolitis, Gastritis, Gastrointestinal pemphigoid, Giant cell arteritis (temporal arteritis), Giant cell myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis, Graves' ophthalmopathy, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, Hashimoto's encephalopathy, Hemolytic anemia, Henoch-Schonlein purpura (HSP), Herpes gestationis or pemphigoid gestationis (PG), Hidradenitis Suppurativa (HS) (Acne Inversa), Hypogammaglobulinemia, Idiopathic giant- cell myocarditis, Idiopathic inflammatory demyelinating diseases, Idiopathic pulmonary fibrosis, IgA Nephropathy, IgA vasculitis (IgAV), IgG4-related disease, IgG4-related sclerosing disease, Immune thrombocytopenic purpura (ITP), Inclusion body myositis (IBM), Inflammatory Bowel Disease, Intermediate uveitis, Interstitial cystitis (IC), Interstitial lung disease, IPEX syndrome, Juvenile arthritis, Juvenile diabetes (Type 1 diabetes), Juvenile myositis (JM), Kawasaki disease, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus, Lupus nephritis, Lupus vasculitis, Lyme disease chronic, Majeed syndrome, Meniere's disease, Microscopic polyangiitis (MPA), Mixed connective tissue disease (MCTD), Mooren's ulcer, Morphea, Mucha-Habermann disease, MultifocalMotor Neuropathy (MMN) orMMNCB, Multiple sclerosis, Myasthenia gravis, Myocarditis, Myositis, Narcolepsy, Neonatal Lupus, Nervous system, Neuromyelitis optica, Neuromyotonia, Neutropenia, Ocular cicatricial pemphigoid, Opsoclonus myoclonus syndrome, Optic neuritis, Ord's thyroiditis, Oshtoran syndrome, Palindromic rheumatism (PR), Paraneoplastic cerebellar degeneration (PCD), Paroxysmal nocturnal hemoglobinuria (PNH), Parry–Romberg syndrome, Parsonage–Turner syndrome, ParsPlanitis (peripheral uveitis), Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus (PANDAS), Pelvic Inflammatory Disease (PID), Pemphigus, Pemphigus vulgaris, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia (PA), Pityriasis lichenoides et varioliformis acuta, POEMS syndrome, Polyarteritis nodosa, Polyglandular syndromes type I, II, III, Polymyalgia rheumatica, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Primary biliary cholangitis (PBC), Primary

biliary cirrhosis, Primary immunodeficiency, Primary sclerosing cholangitis, Progesterone dermatitis, Progressive inflammatory neuropathy, Psoriasis, Psoriatic arthritis, Pure red cell aplasia (PRCA), Pyoderma gangrenosum, Rasmussen's encephalitis, Raynaud's phenomenon, Reactive Arthritis, Reflex sympathetic dystrophy, Relapsing polychondritis, Restless legs syndrome (RLS), Retroperitoneal fibrosis, Rheumatic fever, Rheumatoid arthritis, Rheumatoid vasculitis, Sarcoidosis, Schizophrenia, Schmidt syndrome, Schnitzler syndrome, Scleritis, Scleroderma, Serum sickness, Sjögren's Syndrome, Sperm & testicular autoimmunity, Spondyloarthropathy, Stiff person syndrome (SPS), Subacute bacterial endocarditis (SBE), Susac's Syndrome, Sweet's syndrome, Sydenham's chorea, Sympathetic ophthalmia (SO), Systemic lupus erythematosus (SLE), Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombocytopenia, Thrombocytopenic purpura (TTP), Thyroid gland, Tolosa-Hunt syndrome (THS), Transverse myelitis, Type 1 diabetes, Ulcerativecolitis (UC), Undifferentiated connective tissue disease (UCTD), Undifferentiated spondyloarthropathy, Urticarial vasculitis, Uticaria, Uveitis, Vasculitis, Vitiligo, and Vogt-Koyanagi-Harada Disease. In various embodiments, the inflammatory disease that can be treated by the disclosed methods and compositions is treated, while healthy cells are spared.

In various embodiments, the infectious disease that can be treated by the disclosed methods and compositions include, but are not limited to Acinetobacter infection, Actinomycosis, Acute Flaccid Myelitis (AFM), African sleeping sickness (African trypanosomiasis), AIDS (acquired immunodeficiency syndrome), Ameba infection, Amebiasis, Anaplasma phagocytophilum infection, Anaplasmosis, Angiostrongyliasis, Anisakiasis, Anthrax, Arboviral diseases, neuroinvasive and non-neuroinvasive, Arcanobacterium haemolyticum infection, Argentine hemorrhagic fever, Ascariasis, Aspergillosis, Astrovirus infection, Avian Influenza, Babesiosis, Bacillus cereus infection, Bacterial infection, Bacterial meningitis, Bacterial pneumonia, Bacterial vaginosis, Bacteroides infection, Balantidiasis, Bartonellosis, Baylisascaris infection, BK virus infection, Black piedra, Blastocystosis, Bolivian hemorrhagic fever, Botulism, Botulism (foodborne), Botulism (infant), Botulism (other), Botulism (wound), Brazilian hemorrhagic fever, Brucellosis, Bubonic plague, Burkholderia infection, Buruli ulcer, Calicivirus infection (Norovirus and Sapovirus), California serogroup virus diseases, Campylobacter, Campylobacteriosis, Candida auris, clinical, Candidiasis (Moniliasis; Thrush), Capillariasis, Carbapenemase Producing Carbapenem-Resistant Enterobacteriaceae (CP-CRE), Carbapenem-resistant Infection (CRE/CRPA), Carrion's disease, Cat- scratch disease, Cellulitis, Chagas disease (trypanosomiasis), Chancroid, Chickenpox, Chikungunya Virus Infection (Chikungunya), Chlamydia, Chlamydia trachomatis, Chlamydophila pneumoniae infection, Cholera, Chromoblastomycosis, Chytridiomycosis, Ciguatera, Clonorchiasis, Clostridium difficile colitis, Clostridium Difficile Infection, Clostridium perfringens, Coccidioidomycosis fungal infection (Valley fever), Colorado tick fever (CTF), Common cold (Acute viral rhinopharyngitis; Acute coryza), Congenital syphilis, Conjunctivitis, COVID-19 (Coronavirus Disease 2019), CP-CRE, Enterobacter spp., CP-CRE, Escherichia coli (E. coli), CP-CRE, Klebsiella spp., Creutzfeldt-Jacob Disease, transmissible spongiform encephalopathy (CJD), Creutzfeldt–Jakob disease (CJD), Crimean-Congo hemorrhagic fever (CCHF), Crusted Scabies, Cryptococcosis, Cryptosporidiosis (Crypto), Cutaneous larva migrans (CLM), Cyclospora,

Cyclosporiasis, Cysticercosis, Cytomegalovirus infection, Dengue virus infections, Dengue, 1,2,3,4 (Dengue Fever), Dengue-like illness, Desmodesmus infection, Diarrheal Illness, Dientamoebiasis, Diphtheria, Diphyllobothriasis, Dracunculiasis, E. coli, E. coli infection, Shiga toxin-producing (STEC), Eastern equine encephalitis virus disease, Ebola Hemorrhagic Fever (Ebola), Echinococcosis, Ehrlichia chaffeensis infection, Ehrlichia ewingii infection, Ehrlichiosis, Anaplasmosis, Encephalitis, Arboviral or parainfectious, Enterobiasis (Pinworm infection), Enterococcus infection,, Enterovirus Infection, D68 (EV- D68), Enterovirus Infection, Non-Polio (Non-Polio Enterovirus), Epidemic typhus, Epstein–Barr virus infectious mononucleosis (Mono), Erythema infectiosum (Fifth disease), Exanthem subitum (Sixth disease), Fasciolasis, Fasciolopsiasis, Fatal familial insomnia (FFI), Fifth Disease, Filariasis, Flu (Seasonal), Food Poisoning, Food poisoning by Clostridium perfringens, Free-living amebic infection, Fungal infection, Fusobacterium infection, Gas gangrene (Clostridial myonecrosis), Genital Herpes, Genital Warts, Geotrichosis, German Measles, Gerstmann-Sträussler-Scheinker syndrome (GSS), Giardiasis, Glanders, Gnathostomiasis, Gonorrhea, Granuloma inguinale, Granuloma inguinale (Donovanosis), Group A streptococcal infection, Group A Streptococcus, Group B streptococcal infection, Guanarito virus, Haemophilus Influenza disease, Type B (Hib or H-flu), Haemophilus influenzae infection, Hand, foot and mouth disease (HFMD), Hansen's Disease, Hantavirus infection, Hantavirus Pulmonary Syndrome (HPS), Heartland virus disease, Helicobacter pylori infection, Hemolytic Uremic Syndrome (HUS), Hemorrhagic fever with renal syndrome (HFRS), Hendra virus infection, Hepatitis A (Hep A), Hepatitis B (Hep B), Hepatitis C (Hep C), Hepatitis D (Hep D), Hepatitis E (Hep E), Herpes, Herpes B Virus, Herpes simplex, Herpes Zoster, zoster VZV (Shingles), Hib Disease, Histoplasmosis infection (Histoplasmosis), Hookworm infection, HPV (Human Papillomavirus), Human bocavirus infection, Human ewingii ehrlichiosis, Human granulocytic anaplasmosis (HGA), Human Immunodeficiency Virus/AIDS (HIV/AIDS), Human metapneumovirus infection, Human monocytic ehrlichiosis, Human papillomavirus (HPV) infection, Human parainfluenza virus infection, Hymenolepiasis, Impetigo, Influenza (flu), Influenza (Seasonal), Invasive pneumococcal disease, Isosporiasis, Junin virus,, Kawasaki Syndrome, Keratitis, Kingella kingae infection, Kuru, Lassa fever, Lassa virus, Legionellosis (Legionnaires' disease), Leishmaniasis, Leprosy (Hansens Disease), Leptospirosis, Listeriosis (Listeria), Lujo virus, Lyme disease, Lymphatic filariasis (Elephantiasis), Lymphocytic Choriomeningitis (LCMV), Lymphogranuloma venereum infection (LGV), Machupo virus, Malaria, Marburg virus infection, Measles, Melioidosis (Whitmore's disease), Meningitis, Meningitis – Bacterial, Meningitis – Viral, Meningococcal disease, Metagonimiasis, Microsporidiosis, Middle East respiratory syndrome (MERS), Molluscum contagiosum (MC), Monkeypox, Mononucleosis, Mosquito- borne Illness, MRSA, Mumps,, Murine typhus (Endemic typhus), Mycetoma, Mycoplasma genitalium infection, Mycoplasma pneumonia, Myiasis, Neisseria meningitidis, Neonatal conjunctivitis (Ophthalmia neonatorum), Nipah virus infection, Nocardiosis, Norovirus, Onchocerciasis (River blindness), Opisthorchiasis, Orf Virus (Sore Mouth), Paracoccidioidomycosis (South American blastomycosis), Paragonimiasis, Paralytic Shellfish Poisoning (Paralytic Shellfish Poisoning, Ciguatera), Pasteurellosis, PEP, Parasitic infection, Pertussis (whooping cough), Pink Eye, Pneumococcal

Disease, Pneumococcal infection, Pneumocystis pneumonia (PCP), Pneumonia, Pneumonic Plague, Poliomyelitis (Polio), Poliomyelitis, paralytic, Poliovirus infection, Pontiac fever, Powassan virus disease, Prevotella infection, Primary amoebic meningoencephalitis (PAM), Progressive multifocal leukoencephalopathy, Protozoan infection, Psittacosis (Parrot Fever), Pustular Rash diseases (Small pox, monkeypox, cowpox), Rabies, Raccoon Roundworm, Rat Bite Fever, Recreational Water Illnesses, Relapsing fever, Respiratory syncytial virus infection, Reye's Syndrome, Rhinosporidiosis, Rhinovirus infection, Rickettsial infection, Rickettsiosis (Rocky Mountain Spotted Fever), Rift Valley fever (RVF), Ringworm, Rotavirus infection, Rubella, Sabia virus, Salmonella, Salmonella Paratyphi infection, Salmonella Typhi infection, Salmonellosis, SARS (severe acute respiratory syndrome), Scabies, Scarlet fever, Schistosomiasis, Scombroid, Sepsis, Septic Shock, Septicemic Plague, Severe Acute Respiratory Syndrome (SARS), Shiga toxin-producing Escherichia coli, Shigella, Shigellosis, Shingles, Shingles (Herpes zoster), Smallpox, Sore Mouth (Orf Virus), Sporotrichosis, Spotted fever rickettsiosis, St. Louis encephalitis virus disease, Staphyloccal Infection, Staphyloccal Infection (Methicillin-resistant (MRSA)), Staphylococcal food poisoning, Staphylococcal Infection (Vancomycin Intermediate (VISA)), Strep Throat, Streptococcal Disease, Group A (invasive) (Strep A (invasive)), Streptococcal Disease, Group B (Strep-B), Streptococcal toxic shock syndrome, Strongyloidiasis, Subacute sclerosing panencephalitis, Syphilis, Taeniasis, Tetanus Infection, Tickborne Diseases, Tinea barbae, Tinea capitis, Tinea corporis, Tinea cruris, Tinea manum, Tinea nigra, Tinea pedis, Tinea unguium, Tinea versicolor, Toxic shock syndrome, Toxocariasis (ocular larva migrans (OLM)), Toxocariasis (visceral larva migrans (VLM)), Toxoplasmosis, Trachoma, Trichinellosis, Trichomoniasis, Trichonosis Infection (Trichinosis), Trichuriasis (whipworm infection), Tuberculosis (TB), Tularemia (Rabbit fever), Typhoid fever, Typhoid Fever, Group D, Typhus, Typhus fever, Ureaplasma urealyticum infection, Vaginosis, Valley fever, Variant Creutzfeldt–Jakob disease (vCJD, nvCJD), Varicella (Chickenpox), Venezuelan equine encephalitis, Venezuelan hemorrhagic fever, Vibrio cholerae (Cholera), Vibrio parahaemolyticus enteritis, Vibrio vulnificus infection, Vibriosis, Viral infection, Viral hemorrhagic fever, Viral Hemorrhagic Fever (Ebola, Lassa, Marburg), Viral Hemorrhagic Fevers (VHF), Viral pneumonia, West Nile virus disease, Western equine encephalitis virus disease, White piedra (tinea blanca), Whooping Cough, Yellow Fever, Yersenia (Yersinia), Yersinia pseudotuberculosis infection, Yersiniosis, Zeaspora, Zika fever, Zika Virus, Zika virus disease, congenital, Zika virus disease, non-congenital, Zika Virus Infection (Zika), Zika virus infection, congenital, Zika virus infection, non-congenital, and Zygomycosis. In various embodiments, the infectious disease that can be treated by the disclosed methods and compositions is treated, while healthy cells are spared.

In one embodiment, a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein can be used as a monotherapy (i.e. without other therapeutic agents). In another embodiment, a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein can be used in combined treatments.

In one embodiment, a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein is used in combination with another immunotherapy, e.g. a cellular immunotherapy. The 4-

1BBL ECD mutein, the fusion protein or the conjugate can thus be used in combination with the adoptive transfer of immune cells, including the adoptive transfer of T cells, e.g. CAR T cells or γδ T cells, or NK cells. The NK cells or γδ T cells can e.g. be enriched or expanded by methods known in the art or can be *ex vivo* expanded NK cells or γδ T cells as herein described below.

In one embodiment, a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein can be used in combined treatments with one or more other therapeutic agents. The additional therapeutic agent or agents normally utilized for the particular therapeutic purpose for which an antibody against a TAA is being administered. The additional therapeutic agent or agents will normally be administered in amounts and treatment regimens typically used for that agent in a monotherapy for the particular disease or condition being treated. Such therapeutic agents when used in the treatment of cancer, include, but are not limited to anti-cancer agents and chemotherapeutic agents. Exemplary therapeutic agents that may be used as part of a combination therapy in treating cancer, include, for example, radiation, mitomycin, tretinoin, ribomustin, gemcitabine, vincristine, etoposide, cladribine, mitobronitol, methotrexate, doxorubicin, carboquone, pentostatin, nitracrine, zinostatin, cetrorelix, letrozole, raltitrexed, daunorubicin, fadrozole, fotemustine, thymalfasin, sobuzoxane, nedaplatin, cytarabine, bicalutamide, vinorelbine, vesnarinone, aminoglutethimide, amsacrine, proglumide, elliptinium acetate, ketanserin, doxifluridine, etretinate, isotretinoin, streptozocin, nimustine, vindesine flutamide, drogenil, butocin, carmofur, razoxane, sizofilan, carboplatin, mitolactol, tegafur, ifosfamide, prednimustine, picibanil, levamisole, teniposide, improsulfan, enocitabine, lisuride, oxymetholone, tamoxifen, progesterone, mepitiostane, epitiostanol, formestane, interferon-alpha, interferon-2 alpha, interferon-beta, interferon-gamma, colony stimulating factor-1, colony stimulating factor-2, denileukin diftitox, interleukin-2, and luteinizing hormone releasing factor.

An additional class of agents that may be used as part of a combination therapy (including the use of a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein) in treating cancer is immune checkpoint inhibitors. Exemplary immune checkpoint inhibitors include agents that inhibit one or more of (i) cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), (ii) programmed cell death protein 1 (PD1), (iii) PD-L1, (iv) LAG3, (v) B7-H3, (vi) B7-H4, and (vii) TIM3. Yet other agents that may be used as part of a combination therapy in treating cancer are monoclonal antibodies against TAA as described herein above.

In some embodiments the administration of a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein and the other therapeutic agent can elicit an additive or synergistic effect on immunity and/or on therapeutic efficacy.

In one embodiment, a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein is used as at least one of an neoadjuvant therapy and an adjuvant therapy, in addition to a primary therapy comprising e.g. surgery, chemo and/or radiation therapy. As a neoadjuvant therapy, the 4-1BBL ECD mutein, the fusion protein or the conjugate is administered before the primary treatment, e.g. to help reduce the size of a tumor (such that less extensive surgery and/or radiation therapy is required), kill cancer cells that have spread (e.g. micrometastatic disease) and/or reduce the risk of tumor cells spreading post-surgery. As an adjuvant therapy, the 4-1BBL ECD mutein, the

fusion protein or the conjugate is administered after the primary treatment, e.g. to treat minimal residual disease (destroy remaining cancer cells). The 4-1BBL ECD mutein, the fusion protein or the conjugate can also be administered as a maintenance therapy, which is a long-term adjuvant therapy, e.g. administered repeatedly over the course of at least one month or one year. The use of the 4-1BBL ECD mutein or a the conjugate as an neoadjuvant therapy and/or an adjuvant therapy lowers relapse rates. In the neoadjuvant therapy and/or adjuvant therapy, the 4-1BBL ECD mutein, the fusion protein or the conjugate can be used as monotherapy or in in combined treatments as described above.

Ex vivo methods

In a further aspect, the invention relates to methods wherein a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein is used for *ex vivo* (*in vitro*) treatment of an NK cell or a population of NK cells. The method can be a method for at least one of expanding, pre-activating, activating, enhancing cytotoxicity and/or cytokine production, and inducing a hyper-functional phenotype as defined above. The methods at least comprise the step of contacting an NK cell or a population thereof, with a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein or with a composition comprising the 4-1BBL ECD mutein, a fusion protein or the conjugate. In a preferred embodiment, the method comprises the further step of co-culturing the NK cells with tumor cells expressing a TAA specifically bound by the antigen binding protein in the conjugate. Preferably, the NK cells are co-cultured with the tumor cells expressing the TAA specifically bound by the antigen binding protein in the conjugate, in the presence of the conjugate.

An NK cell or a population of NK cells for *ex vivo* treatment can be enriched from peripheral blood mononuclear cells (PBMCs). Methods for enrichment and *ex vivo* treatment of NK cells from PBMCs are e.g. described in Denman et al. (pLoS One. 2012;7(1):e30264) and in US2020/0061115. For example, NK cells enriched from PBMCs can be seeded at 0.1 x 10^6 NK cells/mL in SCGM (CellGenix, Portsmouth, N.H.), supplemented with 10% FBS, 2 mM Glutamax, 100 U/mL IL-2 (Peprotech, Rocky Hill, N.J.) and 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 µg/mL of one or more of the 4-1BBL ECD muteins or a conjugates as described herein. Media with supplements can be refreshed every 2-3 days.

It is understood that the duration of the contact between the NK cells and a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein (i.e. the duration of the pre-activation or activation (i.e., the duration of expanding, pre-activating, activating, enhancing cytotoxicity and/or cytokine production and inducing a hyper-functional phenotype) can be for any length of time necessary to achieve the desired phenotype of the NK cells. For example, the contact can be as little as 1 minute or as much as 7 days (for example, culturing the NK cells in the presence of a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein for 7 days). In one embodiment of the method, the NK cells are contacted with the 4-1BBL ECD mutein, the fusion protein or the conjugate for 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 36, or 48 hours. In one embodiment of the method, the NK cells are contacted with the 4-1BBL ECD mutein, the fusion protein or the conjugate for 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,

20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 days.

In one embodiment, the *ex vivo* treated (expanded) NK cells have one or more features selected from: a) the fold expansion of the expanded NK cells is at least 0.5, 1.0, 2.0 or 5.0 fold of the fold expansion of expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells; b) the telomere length of the expanded NK cells is increased by at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55% as compared to the telomere length of fresh NK cells, preferably, the percentage telomere length increase of the expanded NK cells as compared to the telomere length of fresh NK cells, is at least 0.5, 1.0, 2.0 or 5.0 fold of the percentage telomere length increase of expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells; c) the expression level of at least one NK cell activating receptor selected from NKG2D, NKp30, NKp44, NKp46 and CD16 on the expanded NK cells is at least 0.5, 1.0, 2.0 or 5.0 fold of the expression level on NK cells obtained upon *ex vivo* expansion in the presence of FC21 feeder cells; d) the secretion of at least one cytokine of TNF-α, IFN-γ and IL-6 by the expanded NK cells is at least 0.5, 1.0, 2.0 or 5.0 fold of the secretion of the cytokine by NK cells obtained upon *ex vivo* expansion in the presence of FC21 feeder cells; and, e) the cytotoxicity of the expanded NK cells is at least 0.5, 1.0, 2.0 or 5.0 fold of the cytotoxicity of NK cells obtained upon ex vivo expansion in the presence of FC21 feeder cells.

In yet a further aspect, the invention pertains to a method for the treatment of a disease in a subject in need thereof, wherein the method comprises the step of administering to the subject (an effective amount of) NK cells obtained in the above method for *ex vivo* treatment of an NK cell or a population of NK cells. Once a sufficient number of NK cells with the desired (hyper-functional) phenotype has been expanded by the *ex vivo* treatment, the NK cells can be administered to a subject in need thereof.

In one embodiment, the method for the treatment comprises the administration of the *ex vivo* treated NK cells in combination with a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein, or a pharmaceutical preparation comprising the 4-1BBL ECD mutein, the fusion protein or the conjugate as an active ingredient.

In one embodiment, the method for the treatment comprises the administration of the *ex vivo* treated NK cells in combination with another NK cell engager, such as e.g. described in WO2016/207278, WO 2018/148445, WO2018/152518, WO2019195409 US2018282386, Vallera et al. (2016, *supra*) and Demaria et al. (2021, *supra*), or with a multispecific antigen binding protein as described in the co-pending applications by the same applicant WO2024/056862 and WO2024/056861. One example of another NK cell engager is for example AVC-006 as described in WO2024/056862, comprising one HER2-binding region and one NKG2D-binding region. In a further embodiment, the *ex vivo* treated NK cells can be used in combination with the other engager and with a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein.

The disease to be treated can be a cancer, an infectious disease, an inflammatory disease or an autoimmune disease, as described above. Preferably, the disease to be treated is a cancer,

as described above. The cancer preferably is a cancer expressing a TAA that is specifically bound by the antigen binding protein in the conjugate that is administered in combination with the *ex vivo* treated NK cells. The administration of the *ex vivo* treated NK cells in combination with the conjugate will facilitate targeting of the administered *ex vivo* treated NK cells to tumor cells expressing the TAA that is specifically bound by the antigen binding protein in the conjugate.

In one embodiment, the *ex vivo* treated NK are autologous to the subject. In another embodiment, the *ex vivo* treated NK are allogeneic, e.g. derived from donor PBMCs.

In a further aspect, the invention relates to methods wherein a 4-1BBL mutein, a fusion protein or a conjugate as described herein is used for *ex vivo* (*in vitro*) treatment of a γδ T cell or a population of γδ T cells. The method can be a method for at least one of expanding, pre-activating, activating, enhancing cytotoxicity and/or cytokine production, and inducing a hyper-functional phenotype as defined above. The methods at least comprise the step of contacting a γδ T cell or a population thereof, with a 4-1BBL mutein, a fusion protein or a conjugate as described herein or with a composition comprising the IL-21 mutein, the fusion protein or the conjugate.

A γδ T cell or a population of γδ T cells for *ex vivo* treatment can be enriched from peripheral blood mononuclear cells (PBMCs) from apheresis by depletion of αβ T cells. Methods for enrichment and *ex vivo* treatment of γδ T cells from PBMCs are e.g. described in Almeida et al. (Clin Cancer Res. 2016; 22(23):5795-5804) and Boucher et al. (J Immunother. 2023; 46(1): 5–13). For example, γδ T cells enriched from PBMCs can be seeded at 0.2 x 10^6 cells/mL in RPMI, supplemented with 10% FBS, 2 mM Glutamax, 50 U/mL IL-2 (Peprotech, Rocky Hill, N.J.) and 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 µg/mL of one or more multispecific antigen binding proteins as described herein. Media with supplements can be refreshed every 2-3 days after day 5.

It is understood that the duration of the contact between the γδ T cells and a 4-1BBL mutein, a fusion protein or a conjugate as described herein (i.e. the duration of the pre-activation or activation (i.e., the duration of expanding, pre-activating, activating, enhancing cytotoxicity and/or cytokine production and inducing a hyper-functional phenotype) can be for any length of time necessary to achieve the desired phenotype of the γδ T cells. For example, the contact can be as little as 1 minute or as much as 7 days (for example, culturing the γδ T cells in the presence of a 4-1BBL mutein, a fusion protein or a conjugate as described herein for 7 days). In one embodiment of the method, the γδ T cells are contacted with the IL-21 mutein, the fusion protein or the conjugate for 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 36, or 48 hours. In one embodiment of the method, the γδ T cells are contacted with the IL-21 mutein, the fusion protein or the conjugate for 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 days.

In one embodiment, the *ex vivo* treated (expanded) γδ T cells have one or more features selected from: a) the fold expansion of the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the fold expansion of expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody (e.g. using the protocol of Almeida et al., 2016, *supra*) or by co-culturing with irradiated K562 feeder cells modified

to express a membrane bound version of the γδ T cell-activating agonist and a membrane bound version of the co-stimulatory agonist; b) the telomere length of the expanded γδ T cells is increased by at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55% as compared to the telomere length of fresh γδ T cells, preferably, the percentage telomere length increase of the expanded γδ T cells as compared to the telomere length of fresh γδ T cells, is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the percentage telomere length increase of γδ T cells obtained upon *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells; c) the expression level of at least one γδ T cell activating receptor selected from NKG2D, NKp30, NKp44, NKp46 on the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the expression level on expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells; d) the secretion of at least one cytokine of TNF-α, IFN-γ and IL-6 by the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the secretion of the cytokine by expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells; and, e) the cytotoxicity of the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the cytotoxicity of expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells. It is understood that for appropriate comparison of the fold expansion induced by a 4-1BBL mutein, a fusion protein or a conjugate *vs*. the feeder cells, the membrane bound versions of the γδ T cell-activating agonist and the co-stimulatory agonist expressed on the in with irradiated K562 feeder cells correspond to the γδ T cell-activating agonist and co-stimulatory agonist as comprised in the particular conjugate that is tested. Hence, if a conjugate comprises IL-21 as γδ T cell-activating agonist and a 4-1BBL ECD trimer as co-stimulatory agonist, the feeder cells express membrane bound versions of IL-21 and 4-1BBL.

In yet a further aspect, the invention pertains to a method for the treatment of a disease in a subject in need thereof, wherein the method comprises the step of administering to the subject (an effective amount of) γδ T cells obtained in the above method for *ex vivo* treatment of a γδ T cell or a population of γδ T cells. Once a sufficient number of γδ T cells with the desired (hyper-functional) phenotype has been expanded by the *ex vivo* treatment, the γδ T cells can be administered to a subject in need thereof.

In one embodiment, the method for the treatment comprises the administration of the *ex vivo* treated γδ T cells in combination with a 4-1BBL mutein, a fusion protein or a conjugate as described herein, or a pharmaceutical preparation comprising the 4-1BBL mutein, a fusion protein or the conjugate as an active ingredient.

In one embodiment, the method for the treatment comprises the administration of the *ex vivo* treated γδ T cells in combination with a multispecific antigen binding protein, e.g. a multispecific antigen binding protein as described in the co-pending application by the same applicant with reference no. EP 24208721.1.

The disease to be treated can be a cancer, an infectious disease, an inflammatory disease or an autoimmune disease, as described above. Preferably, the disease to be treated is a cancer, as described above.

In one embodiment, the *ex vivo* treated γδ T cells are autologous to the subject. In another embodiment, the *ex vivo* treated γδ T cells are allogeneic, e.g. derived from donor PBMCs.

Nucleic acids, host cells and methods for producing a 4-1BBL ECD mutein, a fusion protein or a conjugate comprising such mutein

In one aspect, the invention relates to a nucleic acid molecule comprising one or more nucleotide sequences encoding a polypeptide chain of a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein. The nucleotide sequence encoding such a polypeptide chain preferably encodes a signal peptide operably linked to the polypeptide chain. A nucleic acid molecule comprising one or more of the nucleotide sequences encoding a polypeptide chain, further preferably comprises regulatory elements for (or conducive to) the expression of the polypeptide chain in an appropriate host cell, which regulatory elements are operably linked to the nucleotide sequence.

In one aspect, the invention relates to a host cell comprising the nucleic acid molecule comprising one or more nucleotide sequences encoding a polypeptide chain of a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein. In one embodiment, the host cell is an isolated cell or a cultured cell. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram-negative or gram-positive organisms, for example *Escherichia coli* or bacilli. Suitable yeast cells include *Saccharomyces cerevisiae* and *Pichia pastoris*. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-1, COS-7 line of monkey kidney cells (Gluzman et al., 1981, Cell 23:175), L cells, HEK 293 cells (e.g. Expi293, HEK293-F and HEK293-E cells), C127 cells, 3T3 cells, Chinese hamster ovary (CHO) cells (e.g. ExpiCHO cells), HeLa cells, BHK cell lines, e.g. BHK21, BSC-1, Hep G2, 653, SP2/0, and the CVI/EBNA cell line derived from the African green monkey kidney cell line CVI as described by McMahan et al. (1991, EMBO J. 10: 2821). The host cell may be any suitable species or organism capable of producing N-linked glycosylated polypeptides, e.g. a mammalian host cell capable of producing human or rodent IgG type N-linked glycosylation. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985). Host cells comprising the nucleic acid molecule of the invention can be cultured under conditions that promote expression of the polypeptide.

Thus, another aspect the invention relates to a method for producing a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein. The method preferably comprises culturing a host cell as described above such that one or more nucleotide sequences are expressed and the 4-1BBL ECD mutein, the fusion protein or the conjugate is produced. The method preferably comprises the step of cultivating a host cell comprising one or more of the nucleotide sequences

encoding a polypeptide chain of the 4-1BBL ECD mutein, the fusion protein or the conjugate. The host cell is preferably cultured under conditions conducive to expression of the one or more polypeptide chains. The method can further comprise the step of recovering the 4-1BBL ECD mutein, the fusion protein or the conjugate. The 4-1BBL ECD mutein, the fusion protein or the conjugate can be recovered by conventional protein purification procedures, including e.g. protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, size exclusion chromatograpy or affinity chromatography, using e.g. streptavidin/biotin (see e.g. Low et al., 2007, J. Chromatography B, 848:48-63; Shukla et al., 2007, J. Chromatography B, 848:28-39).

In a further aspect, the invention relates to a method for producing a pharmaceutical composition comprising a 4-1BBL ECD mutein, a fusion protein or a conjugate as described herein, the method comprising the steps of a) producing the 4-1BBL ECD mutein, the fusion protein or the conjugate in a method as defined above; and b) formulating the 4-1BBL ECD mutein, the fusion protein or the conjugate with a pharmaceutically acceptable carrier as defined above, to obtain a pharmaceutical composition.

The present invention has been described above with reference to a number of exemplary embodiments as shown in the drawings. Modifications and alternative implementations of some parts or elements are possible, and are included in the scope of protection as defined in the appended claims.

Description of the figures

Figure 1. NK boosters comprising IL-21 or 4-1BBL ECD muteins show impaired capacity to induce proliferation only in the absence of tumor cells. NK booster AVC37 (a conjugate of trastuzumab and the I8H IL-21 mutein and wild type 4-1BBL ECD) and NK booster AVC52 (a conjugate of trastuzumab and wild type IL-21 and the V153Q 4-1BBL ECD mutein) show strongly impaired capacity to induce proliferation of NK cells but only in the absence of SKOV3 tumor cells expressing HER2. In the presence of SKOV3 tumor cells the ability of NK boosters AVC37 and AVC52 is comparable to that of control NK booster AVC1 (a conjugate of trastuzumab and wild type IL-21 and wild type 4-1BBL ECD mutein). NK cells were cultured for 5 days with (dashed lines) or without (solid lines) tumor cells and indicated NK boosters in concentrations as indicated. Data shown is from measurements taken at the end of the co-culture. The darker and lighter grey areas indicate historical averages and the standard deviation obtained with AVC1, with and without tumor cells present, respectively.

Figure 2. Also NK booster AVC54 (a conjugate of trastuzumab and wild type IL-21 and the Q227E 4-1BBL ECD mutein) show strongly impaired capacity to induce proliferation of NK cells but only in the absence of SKOV3 tumor cells expressing HER2. In the presence of SKOV3 tumor cells the ability of NK boosters AVC37 and AVC52 is comparable to that of control NK booster AVC1 (a conjugate of trastuzumab and wild type IL-21 and wild type 4-1BBL ECD mutein). NK cells were

cultured for 5 days with (dashed lines) or without (solid lines) tumor cells and indicated NK boosters in concentrations as indicated. Data shown is from measurements taken at the end of the co-culture.

Figure 3. NK boosters comprising various 4-1BBL ECD as indicated show impaired capacity to induce proliferation as compared to a corresponding control NK booster AVC1 comprising wild type 4-1BBL ECD. NK cells were cultured for 5 days without tumor cells and indicated NK boosters, data shown is from measurements taken at the end of the co-culture. data shown is from measurements taken at the end of the co-culture.

Figure 4. NK cell cytotoxicity against SKOV3 tumor cells, as induced by NK boosters comprising IL-21 or 4-1BBL ECD muteins, is unaffected by IL-21 or 4-1BBL mutations. NK cells were co-cultured for 5 days with tumor cells in the presence of NK booster AVC37 (a conjugate of trastuzumab and the I8H IL-21 mutein and wild type 4-1BBL ECD), NK booster AVC52 (a conjugate of trastuzumab and wild type IL-21 and the V153Q 4-1BBL ECD mutein) or control NK booster AVC1 (a conjugate of trastuzumab and wild type IL-21 and wild type 4-1BBL ECD mutein), respectively, in concentrations as indicated. Data shown is from measurements taken at the end of the co-culture.

Figure 5. The ability of NK boosters comprising IL-21 or 4-1BBL ECD muteins to induce and support long-term NK cell expansion is unaffected. NK cells were expanded for 14 days with SKOV3 tumor cells that were opsonized with indicated NK boosters. Fold expansion of NK cells after 14 days is indicated.

Figure 6. Left hand panels show dose-response curves of NK boosters with 4-1BBL ECD muteins in a 4-1BB reporter cell assay, compared with control booster AVC1 with wild type 4-1BBL. Right-hand panels show the responses fitted at 100 nM booster and EC_{50} values in nM are given on the far-right. A) compares boosters AVC50, AVC51, AVC52, AVC53 and AVC54 to control booster AVC1; B) compares boosters AVC55, AVC56, AVC57, AVC58 and AVC59 to control booster AVC1; and C) compares boosters AVC46, AVC47, AVC48 and AVC49 to control booster AVC1.

Figure 7. pEC_{50} values a 4-1BB reporter cell assay (x-axis) of the NK boosters with 4-1BBL ECD muteins as indicated and of control booster AVC1 with wild type 4-1BBL are plotted against the affinities of the boosters for 4-1BB as determined by surface plasmon resonance (y-axis).

Figure 8. Induction of NK cell expansion by the NK boosters with 4-1BBL ECD muteins as indicated compared to that of control booster AVC1 with wild type 4-1BBL.

Figure 9. Long-term cytotoxicity against SKOV-3 tumor cells by NK cells stimulated with NK booster AVC59 with the 4-1BBL ECD A154D mutein or the wild type control booster AVC1 upon repeated co-culture with SKOV-3 tumor cells. After each 3-day cycle, NK cells were were harvested and used to set up a new cycle of co-culture with fresh target cells at a 1:1 E:T ratio. The boosters AVC1 or

AVC59 or a control protein (a trastuzumab analog) were added at the start of each 3-day cycle to expose NK cells to new booster proteins at the beginning of every cycle. Control wells contained only NK cells and SKOV-3 target cells (NK + SKOV3), or only SKOV-3 target cells (SKOV3 only).

Figure 10. Long-term NK cell cytotoxicity induced by the multispecific antigen binding proteins AVC-001 and AVC-016, against tumor cell lines expressing the respective tumor-associated antigens: (A) AVC-001 (AVC1) with SKOV-3 tumor cells expressing HER2; (B) AVC-016 (AVC16) with BxPC3 tumor cells expressing TROP2; and (C) AVC-020 (AVC20) with A431 tumor cells expressing EGFR. NK cell cytotoxicity induced by the multispecific antigen binding proteins against tumor cells that express the respective antigens (open squares) is followed over time (hours) and compared to the cytotoxicity of NK cells only (solid circles).

Figure 11. Interferon-gamma levels after co-culture with tumor cells that express the respective antigen. Data is shown as fold change over vehicle (NK cells + tumor cells). (A) AVC-001 (AVC1) with SKOV-3 tumor cells expressing HER2; (B) AVC-016 (AVC16) with BxPC3 tumor cells expressing TROP2; and (C) AVC-020 (AVC20) with A431 tumor cells expressing EGFR.

Figure 12. Hyperfunctionality of NK cells, as determined by cytotoxicity against SKOV-3 tumor cells, after 14-days expansion of the NK cells in the presence of control booster AVC1 or booster AVC190 with the IL-21 (N82- A83- G84- R85- R86- Q87- K88-) mutein and the 4-1BBL A154D mutein. Further controls included vehicle (PBS only) or NK cells and SKOV-3 tumor cells without boosters.

Figure 13. Long-term cytotoxicity against (TROP2-expressing) BxPC3 tumor cells by NK cells stimulated with NK boosters AVC16 (based of Fab-fragments from sacituzumab and comprising wild type IL-21 and a trimer of wild type 4-1BBL ECD), AVC267 (based of Fab-fragments from sacituzumab and comprising the IL-21 L20W mutein and a trimer of wild type 4-1BBL ECD) and control protein AVC 137 (a sacituzumab analogue without IL-21 or 4-1BBL cytokines) upon repeated co-culture with BxPC3 tumor cells. After each 3-day cycle, NK cells were were harvested and used to set up a new cycle of co-culture with fresh target cells at a 1:1 E:T ratio. The NK boosters were added at the start of each 3-day cycle to expose NK cells to new booster protein at the beginning of every cycle. Control wells contained only NK cells and BxPC3 target cells (NK + BxPC3), or only BxPC3 target cells (BxPC3 only).

Figure 14. Long-term cytotoxicity against (TROP2-expressing) BxPC3 tumor cells by NK cells stimulated with NK boosters AVC16 (based of Fab-fragments from sacituzumab and comprising wild type IL-21 and a trimer of wild type 4-1BBL ECD), AVC245 (based of Fab-fragments from sacituzumab and comprising the IL-21 DEL7 (N82- A83- G84- R85- R86- Q87- K88-) mutein and a trimer of 4-1BBL ECD A154D mutein) and control protein AVC 137 (a sacituzumab analogue without IL-21 or 4-1BBL cytokines) upon repeated co-culture with BxPC3 tumor cells. After each 4-5 day cycle, NK cells were were harvested and used to set up a new cycle of co-culture with fresh

target cells at a 1:1 E:T ratio. The NK boosters were added at the start of each cycle to expose NK cells to new booster protein at the beginning of every cycle. Control wells contained only NK cells and BxPC3 target cells (NK + BxPC3), NK cells with BxPC3 target cells and PBS (Vehicle), or only BxPC3 target cells (BxPC3 only).

Figure 15. *In vivo* pharmakinetics for NK boosters AVC16 and AVC245 compared to control antibodies AVC137 and trastuzumab. Mice were injected i.v. with 1 mg/kg body weight of the boosters or antibodies. Blood sample for analysis were drawn at indicated times after injection and levels of boosters and control antibodies in mouse serum was determined by ELISA.

Figure 16. Effect of different mutations in IL-21 and/or 41BBL as present in Vδ1 TCR-targeting γδ T-cell boosters on Vδ1 T cell expansion and phenotype. Effects of avidity attenuating mutations in IL-21 and/or 41BBL on Vδ1 T cell expansion (A), PD1 expression on Vδ1 T cells (B) or CD56 expression on Vδ1 T cells (C) after 14 days of expansion. All values have been normalised to AVC160 which uses IL-21 and 41BB-ligand wildtype in its design. A summary of the IL-21 and 41BBL mutations used in these boosters is provide in Table 1.1.10.4.

Figure 17. *In vivo* efficacy of the AVC245 booster in reducing tumor burden in a mouse xenograft model is significantly greater than that of controls of tumor-only, NK cells + tumor or the control antibody AVC137 without the 4-1BBL and IL-21 muteins.

Figure 18. *In vivo* efficacy of the AVC245 booster in inducing expansion of donor NK cells in a mouse xenograft model is significantly greater than that of controls of tumor-only, NK cells + tumor or the control antibody AVC137 without the 4-1BBL and IL-21 muteins.

Examples

Example 1

1.1 Methods and materials

1.1.1 Reagents

NK cell expansion

List of reagents Reagent	Supplier and product code
Buffy Coats	N/A
CD56 lyophilised microbeads	Miltenyi 130-097-042
IL-2	R&D Systems, 202-IL-050
FITC anti-human CD56 (REA196)	Miltenyi, 130-114-549
Hu CD3 BUV395 UCHT1	BD, 563546
PE anti-human CD360 (IL-21R)	Biolegend 359506
APC anti-human CD137 (4-1BB)	Biolegend 309810
Fixable Viability Dye eFluor 780	eBioscience, 65-0865-18
UltraComp beads	Invitrogen, 01-2222-42
LS Columns	Miltenyi, 130-042-401
Pre separation filters, 30 µM	Miltenyi, 130-041-407
Fc block	Miltenyi, 130-059-901
Histopaque	Sigma Aldrich, 10771-500ML
HEPES	Pan Biotech, P05-01100
HBSS	Fisher Sci, 11500476
ACK lysis buffer	Fisher Sci, 11509876

SK-OV-3 cell line medium

Component	Volume (mL)	Cat#
McCoy's 5a Media	418.5	Gibco, 16600082
1% Penicillin Streptomycin	5.0	Hyclone, SV30010
FCS	75.0	Pan BioTech, P30-193602
L-Glutamine	1.5	Gibco, 25030-081

Effector cells (NK) complete media

Component	Volume (mL)	Cat#
RPMI 1640	425.0	Pan BioTech, P04-17500
1% Penicillin Streptomycin	5.0	Hyclone, SV30010
10% AB	50.0	Access Cell Culture, 515-HI
20 mM HEPES	10.0	Pan BioTech, PO5-01100
L- Glutamine	10.0	Gibco, 25030-081

1.1.2 Maintenance of SKOV3 WT cell line SK-OV-3

Split sub-confluent cultures (70-80%)

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2 to 3 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6 to 8 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Sub cultivation Ratio: A sub cultivation ratio of 1:2 to 1:3 is recommended i.e., seeding at 3-6x10,000 cells/cm²

Medium Renewal: Every 2 to 3 days

1.1.3 Media

Growth and Test medium

McCoy's 5a medium modified + 2mM glutamine + 15% FBS + 1% Penicillin Streptomycin

Freezing medium

FCS + 10% DMSO

1.1.4 PBMC sort and NK isolation

Human peripheral blood mononuclear cells (PBMC) were isolated from healthy donors using a density gradient centrifugation method. Briefly, the buffy coat fraction was overlaid onto Histopaque in a falcon tube for density centrifugation. After centrifugation at 500g for 30 min at RT the mononuclear cell fraction was harvest from the interface into a new falcon tube. Cells underwent several washes steps and lysis to remove red blood cell contamination. Subsequently, $CD56^+$ cells were obtained by magnetic cells sorting using the system from Miltenyi Biotec according to the manufacturer's instructions (Miltenyi- 130-097-042). Following separation and an aliquot of sorted NK cells was taken for post-sort purity check.

1.1.5 Irradiation of SK-OV-3

E:T ratio= 1:2 (NK: SK-OV-3)

- $8x10^6$ cells per flask x (X conditions) x (Y Donors)

1. Irradiate extra cells to account for possible cell loss following irradiation. Maintain enough cells to expand for Day 7 restimulation.
2. Resuspend required number of cells @ 5 x 10^6 cells/mL
3. Perform irradiation at 100Gy.
4. Count cells post-irradiation and adjust to 5 x 10^6 cells/mL.

1.1.6 Opsonisation of SK-OV-3 cells

1. Prepare test compounds.
2. Spike in required stock volume to irradiated SK-OV-3 to the required concentration (25 nM) and leave to opsonise for 30 minutes in a flask in 37°C incubator.
3. Wash cells by spinning at 300 x g for 5 mins.
4. Count cells and adjust density to 3.2 x 10^6/mL.

1.1.7 Seeding NK cells for 14-day expansion

1. Adjust NK cells to required density - 1.6 x 10^6/mL in NK media containing IL-2 (50 IU/mL)
2. Seed 2.5 mL of NK cells per flask (4 x 10^6 total cells per flask).
3. Seed 2.5 mL of 3.2 x 10^6/mL compound or vehicle opsonised SK-OV-3 cells according to appropriate flask maps.
4. To all flasks, add 17.5 mL of 2 x IL-2 complete media and 17.5 mL of complete media without IL-2. This is to bring the IL-2 activity to 50 IU/mL.
5. Total final volume per T75 flask will be 40 mL.

1.1.8 Cytotoxicity measurements

NK cells and NucLight-Red (NLR) transduced SKOV3 tumor cells were co-cultured in a 2:1 ratio for 5 days with NK boosters present in different concentrations, using a 7-step 4-fold dilution series starting at 25 nM thus covering a range from 25 nM to 0.01 nM. The number of NLR-positive cells was quantified by an Incucyte automated microscope every 4 hours throughout the duration of culture and compared to the control condition where NK cells were co-cultured with NLR SKOV3 cells in the absence of NK boosters. Cytotoxicity was calculated as follows:

$$\%\ target\ cell\ lysis = 100 - \left(\frac{number\ of\ NLR\ SKOV3\ in\ test\ condition}{number\ of\ NLR\ SKOV3\ in\ control\ condition} \times 100\right)$$

1.1.9 Proliferation measurements

NK cells were labeled with a amine-reactive cell proliferation dye (CPD), for example CellTrace Violet (Thermo Scientific, cat# C34557). Subsequently, labeled NK cells were used either in a co-culture with SKOV3 cells or cultured by themselves with NK boosters present in different concentrations. In the case of a co-culture with SKOV3 cells a 7-step 4-fold dilution series starting at 25 nM thus covering a range from 25 nM to 0.01 nM was used. When only NK cells were cultured

an 8-step 3-fold dilution series which covers a range from 100 nM to 0.01 nM was used instead. After a 5 day (co-)culture, NK cells were analyzed by flow cytometry for cell proliferation dye content, and the percentage of CPD-low cells was used as a read-out for proliferation.

1.1.10 Production of NK boosters

NK cell boosters (also referred to conjugates herein) as listed in Tables 1.1.10.1 – 1.1.10.4, having amino acid sequences as shown in the sequence listing, were prepared, purified and characterized essentially as described in WO2024/056862.

Table 1.1.10.1. Overview of the HER2-targeting NK cell boosters with 4-1BBL and/or IL-21 muteins as indicated and their corresponding amino acid sequences in the sequence listing. AVC1 is the control NK cell booster with wild type 4-1BBL and IL-21.

AVC-ID	HC1 mutation in 4-1BBL numbering	HC1 SEQ ID NO	HC2 mutation in IL-21 numbering	HC2 SEQ ID NO	LC SEQ ID NO
AVC1	-	11	-	12	2
AVC22	-	11	R76K	177	2
AVC23	-	11	R5Q	178	2
AVC24	-	11	R9E	179	2
AVC25	-	11	R76A	180	2
AVC26	-	11	R5H	181	2
AVC27	-	11	I8V	182	2
AVC28	-	11	I8Q	183	2
AVC29	-	11	R11E	184	2
AVC30	-	11	Q12K	185	2
AVC31	-	11	Q19S	186	2
AVC32	-	11	K73Y	187	2
AVC33	-	11	K75E	188	2
AVC34	-	11	L13E	189	2
AVC35	-	11	R76H	190	2
AVC36	-	11	D4H	191	2
AVC37	-	11	I8H	192	2
AVC38	-	11	K21H K112H	193	2
AVC39	-	11	K21H	194	2
AVC40	-	11	K112H	195	2
AVC46	V100T	196	-	12	2
AVC47	V100Q	197	-	12	2
AVC48	L101N	198	-	12	2
AVC49	Y110Q	199	-	12	2
AVC50	G114K	200	-	12	2
AVC51	L115R	201	-	12	2
AVC52	V153Q	202	-	12	2
AVC53	R171D	203	-	12	2
AVC54	Q227E	204	-	12	2
AVC55	Q227R	205	-	12	2
AVC56	Q230S	206	-	12	2

AVC57	Q230K	207	-	12	2
AVC58	A116D	208	-	12	2
AVC59	A154D	209	-	12	2
AVC72	V100T	196	R9E	179	2
AVC73	Q227R	205	R9E	179	2
AVC74	Q230S	206	R9E	179	2
AVC75	V100T	196	R76A	180	2
AVC76	Q227R	205	R76A	180	2
AVC77	Q230S	206	R76A	180	2
AVC78	V100T	196	I8Q	183	2
AVC79	Q227R	205	I8Q	183	2
AVC80	Q230S	206	I8Q	183	2
AVC85	Q227E	204	R9E+R76A		2
AVC86	Q227E	204	L13E	189	2
AVC87	V153Q	202	L13E I8H K21H D4H		2
AVC88	V153Q	202	D4H I8H K21H		2
AVC89	V153Q	202	L13E I8H K21H		2
AVC90	V153Q	202	L13E D4H I8H		2
AVC91	V153Q	202	I8H K21H		2
AVC92	V153Q	202	L13E D4H K21H		2
AVC93	V153Q	202	D4H I8H		2
AVC94	V153Q	202	L13E I8H		2
AVC95	V153Q	202	D4H K21H		2
AVC96	V153Q	202	L13E K21H		2
AVC97	V153Q	202	L13E D4H		2
AVC98	Y110Q V153Q Q227E	210	I8H	192	2
AVC99	L101N Y110Q V153Q	211	I8H	192	2
AVC100	V100Q Y110Q V153Q	212	I8H	192	2
AVC101	L101N V153Q Q227E	213	I8H	192	2
AVC102	Y110Q A154D Q227E	214	I8H	192	2
AVC103	Y110Q V153Q	215	I8H	192	2
AVC104	V153Q Q227E	216	I8H	192	2
AVC105	Y110Q Q227E	217	I8H	192	2
AVC106	L101N Q227E	218	I8H	192	2
AVC107	V153Q	202	I8H	192	2
AVC108	-	11	I14D	219	2
AVC109	-	11	D18A	220	2
AVC110	-	11	E100R	221	2
AVC111	-	11	E109R	222	2
AVC112	-	11	I119D	223	2
AVC113	-	11	E100R E109R	224	2
AVC114	-	11	N82- A83- G84- R85- R86- Q87- K88-	225	2
AVC115	-	11	N82- A83- G84- R85- R86- Q87- K88- H89- R90-	226	2
AVC116	-	11	N63- E64- R65- I66-	227	2
AVC117	-	11	N59- T60- G61- N62- N63- E64- R65- I66-	228	2
AVC118	-	11	I67T	229	2

AVC119	-	11	I67N	230	2
AVC120	-	11	L74G	231	2
AVC121	-	11	L74D	232	2
AVC122	-	11	L74F	233	2
AVC123	-	11	L20S	234	2
AVC124	-	11	L20N	235	2
AVC125	-	11	L20W	236	2
AVC126	-	11	G84 insGGGG	237	2
AVC176	A154D	209	L74D	232	2
AVC187	A154D	209	L20N	235	2
AVC188	A154D	209	L20W	236	2
AVC189	A154D	209	L20S	234	2
AVC190	A154D	209	N82- A83- G84- R85- R86- Q87- K88-	225	2
AVC285	A154E	675	N82- A83- G84- R85- R86- Q87- K88-	225	2
AVC286	A154D G155Q	697	L20W	236	2
AVC287	A154E	675	L20W	236	2
AVC288	A154D G155Q	697	N82- A83- G84- R85- R86- Q87- K88-	225	2

Table 1.1.10.2. Overview of the TROP2-targeting NK cell boosters with 4-1BBL and/or IL-21 muteins and control protein AVC137 (without 4-1BBL and IL-21),, as indicated and their corresponding amino acid sequences in the sequence listing.

AVC-ID	HC1 mutation in 4-1BBL numbering	HC1 SEQ ID NO	HC2 mutation in IL-21 numbering	HC2 SEQ ID NO	LC SEQ ID NO	Based of moAb
AVC16	-		-			Sacituzumab
AVC137	no 4-1BBL	672	no IL-21	673	584	Sacituzumab
AVC221	-	547	-	548	546	AR47A6.4.2
AVC227	-	550	-	551	549	KM4097
AVC245	A154D	585	N82- A83- G84- R85- R86- Q87- K88-	586	587	Sacituzumab
AVC323	A154D	591	N82- A83- G84- R85- R86- Q87- K88-	592	593	KM4097
AVC421	A154D	588	N82- A83- G84- R85- R86- Q87- K88-	589	590	AR47A6.4.2

AVC267	A154D	585	L20W	674	584	Sacituzumab

Table 1.1.10.3. EGFR-targeting NK cell booster with 4-1BBL and/or IL-21 and their corresponding amino acid sequences in the sequence listing.

AVC-ID	HC1 4-1BBL status	HC1 SEQ ID NO	HC2 IL-21 status	HC2 SEQ ID NO	LC SEQ ID NO	Based of moAb
AVC20	wild type	669	-	670	671	cetuximab

Table 1.10.4. Overview of γδ T-cell-boosters comprising anti-Vδ1 TCR V_H and V_L and wild type or mutein 4-BBL and IL-21 as indicated.

AVC ID	**4-1BBL mutation in HC1**	**SEQ ID NO HC1**	**IL-21 mutation in HC2**	**SEQ ID NO HC2**	**SEQ ID NO LC**
AVC160	-	677	-	678	676
AVC276	Y110Q	679	-	680	676
AVC277	A154D	681	-	682	676
AVC278	Q227R	683	-	684	676
AVC279	-	685	L20W	686	676
AVC280	-	687	N82- A83- G84- R85- R86- Q87- K88-	688	676
AVC281	-	689	I67N	690	676
AVC282	-	691	L20S	692	676
AVC283	A154D	693	L20W	694	676
AVC284	Y110Q	695	L20W	696	676

1.1.11 Affinity of NK boosters for 4-1BB or IL-21R

Affinity of the boosters for 4-1BB or IL-21R was analyzed using a Biacore T200 instrument at 25 °C and a flow rate of 50 µl/min. A C1 sensor chip was functionalized for a reversible biotin capture assay in all flow cells (fc1–fc4). The analysis buffer consisted of 10 mM HEPES (pH 7.4), 150 mM NaCl, 0.05% Tween 20, and 3 mM EDTA. Each assay cycle proceeded as follows: the biotin capture reagent was introduced over all surfaces (fc1–fc4), 4-1BB or IL-21R was captured specifically on flow cell 2 (fc2), and the boosters were injected in five serial dilutions (starting at different concentrations) for a 60-second association phase and a 600/1200s dissociation phase (the longer dissociation was applied after the highest concentration). A standard regeneration protocol was then

applied to completely remove the ligand-receptor complex from all surfaces, restoring the chip to baseline before the next cycle.

1.1.12 4-1BB reporter assay

NK boosters were incubated for 6 hours with 4-1BB Effector cells (Promega, cat# JA2351), followed by addition of Bio-Glo reagent and measurement of luciferase activity on a luminometer.

1.1.13 Long-term repeated NK cell cytotoxicity assay

A repeated co-culture system was set up, similar to the set-up used by Thakur et al. (J Cancer Res Clin Oncol. 2020 Aug; 146(8): 2007–2016.) to study repeated cytotoxicity of CAR-Ts with a HER2-EGFR bi-specific binder. Since for these longer term measurements an idea of general cell culture state is desirable, an Incucyte® Live-Cell Analysis System was used. SKOV-3 target cells and NK cells (purified via negative selection using RosetteSep from normal donor buffy coats as above) were used in an E:T ratio of 1:1. The SKOV-3 target cells were lentivirally transduced with Nuclight Red (Sartorius Cat# 4625) to allow read-out of fluorescently labeled target cell counts. Assays were performed in triplicate.

A fixed amount of 10,000 fluorescently labeled target cells in 200 µl medium per well was used to ensure a sufficient amount of nutrients for at least 3 days of culture. Co-culture was performed in the presence of 50 IU/mL IL-2. After 3 days, NK cells were harvested and used to setup a new round of co-culture with fresh target cells in a 1:1 E:T ratio. 25 nM of the NK boosters were added at the start of each 3 day round to expose NK cells to new booster proteins at the beginning of every round. Control wells contained only NK cells and SKOV-3 target cells, or only SKOV-3 target cells. Cells were monitored and fluorescently labeled target cells were counted every 3 hours. The experiment was continued for 6 co-culture rounds, i.e., for 18 days.

1.1.14 Cytotoxicity of NK cells after a 14-day expansion

NK cells (purified via negative selection using RosetteSep from normal donor buffy coats as above) were expanded for 14 days with test articles, followed by a challenge with SK-OV-3 cells. For this challenge, the expanded NK cells were co-cultured for 5 days with Nuclight-red labeled SK-OV-3 tumor cells in a 2:1 ratio, and monitored in an Incucyte throughout this time. Tumor cells were quantified by automated counting and plotted as a percentage of timepoint 0.

1.1.15 *In vivo* pharmakinetics data

Male C57Bl16/J mice (n = 3) were injected i.v. with 1 mg test article per kg body weight. Test articled were NK boosters AVC16 and AVC245 and control antibodies AVC137 and trastuzumab. Blood sample for analysis were drawn 15 minutes, 2 hours and 1, 3 and 7 days after injection. Detection and quantification of booster and control antibodies in the mouse serum was done by sandwich ELISA using TROP2, or in the case of trastuzumab HER2 coated plates. Serum levels were converted from absorbance to ng/mL using the corresponding standard curves of each test article and plotted against elapsed time.

1.1.16 γδ T Cell *ex-vivo* expansion

Protocols used for ex vivo expansion of donor γδ T-cells are adapted from DOT cell expansion protocols as described in Almeida et al. (2016, supra). Briefly, γδ T-cells are enriched from 2 healthy donor derived apheresis through the depletion of αβ T cells. At day 0, αβ T cell depleted cells are seeded at a density of 0.2 x 106 cells/mL in 8mL (24-well GRex) of OpTimizer CTSTM culture medium (Life Technologies) supplemented with OpTimizer CTS T-cell Expansion Supplement, CTS Glutamax (1x) and human heat-inactivated plasma (2.5%) (Octapharm). The media is supplemented with γδ T-cell boosters (see Table 1.1.10.4) at 42ng/mL, 30ng/ml of IL-4, 4.5ng/ml IL-1β and 21ng/mL IFNγ. At day 7, γδ T-cell expansion cultures are fed with multispecific antigen binding proteins at 300ng/mL and 21ng/mL of IL-15. At day 11, γδ T-cell cultures are fed with γδ T-cell boosters at 1µg/mL and 100ng/mL of IL-15. As a control, αβ T cell depleted cells were stimulated using OKT3, as described by Almeida et al. Cultures are harvested, characterized and cryopreserved at day 14.

1.1.17 Phenotypic changes of γδ T cells and differentiation assessment

γδ T-cells were expanded for 14 days as described in method 1. γδ T-cells are collected and analyzed via Flow Cytometry for the following markers: pan γδ TCR, Vδ1 TCR, CD56, DNAM-1 and PD-1.

1.1.18 *In vivo* efficacy data of NK cell booster

Efficacy of AVC245 in *in vivo* induction of expansion of NK cells and reducing tumor burden was assessed in SKOV-3 xenograft mice relative to IgG TROP2-targeting control. Age-matched female NRG mice receive non-lethal low dose total body irradiation at Day -1 with 230 cGy. On Day 0, 1 x 10^6 human donor NK cells in 400 µL per mouse were injected i.p. At Day 7 the mice were engrafted with $5x10^5$ Luciferase-expressing SKOV-3 cells (SKOV-3-Luc) via i.p. injection. For treatments with AVC137 and AVC245, groups of 10 mice were used, and for the tumor-only and NK + tumor controls, groups of 5 mice were used. Beginning on Day 0, AVC137 (5.09 mg/kg), or equimolar AVC245 (8 mg/kg), or vehicle was injected i.p. 3x per week (i.e., Monday, Wednesday, and Friday) for the first week and 2x/week (i.e., Monday and Friday) subsequent weeks. Beginning on Day 0, 5000 IU/mouse of recombinant human IL-2 will be injected i.p. 3x per week (i.e., Monday, Wednesday, and Friday).

At Days 7 and 18, 100 uL of peripheral blood was collected from each mouse and used for staining to determine absolute NK cell numbers per mL of blood via flow cytometry. Blood was collected prior to any treatments scheduled for the day.

Tumor burden was quantified on the first day of each week (Day 7, 14, 21, etc.) via bioluminescent imaging (BLI) using auto exposure. With the exception that the first BLI was conducted on Day -1 in lieu of Day 0 and the final BLI on the day prior to experiment endpoint. BLI was done prior to any injections (i.e., NK cells, AVC, IL-2) that day.

1.2 Results

Figures 1 and 2 show that NK boosters comprising IL-21 and 4-1BBL ECD muteins show impaired capacity to induce proliferation, but only in the absence of tumor cells. Figure 1 shows dose-response curves for NK booster AVC37, NK booster AVC52 and control NK booster AVC1. Figure 2 shows dose-response curves for NK booster AVC54 and control NK booster AVC1. NK boosters AVC37, AVC52 and AVC54 show strongly impaired capacity to induce proliferation of NK cells but only in the absence of SKOV3 tumor cells that express HER2. In the presence of SKOV3 tumor cells the ability of NK boosters AVC37, AVC52 and AVC54 to induce NK cell proliferation is comparable to that of control NK booster AVC1.

Figure 3 shows dose-response curves for various further NK boosters comprising different 4-1BBL ECD muteins as indicated on their ability to induce NK cell proliferation in the absence of tumor cells. As can be seen, the various NK boosters with different 4-1BBL ECD muteins have reduced abilities to different degrees for inducing NK cell proliferation in the absence of tumor cells, as compared to a corresponding control NK booster AVC1 comprising wild type 4-1BBL ECD.

Figure 4 shows that NK cell cytotoxicity against SKOV3 tumor cells, as induced by NK boosters comprising IL-21 and 4-1BBL ECD muteins, is unaffected by the IL-21 or 4-1BBL ECD mutations in AVC37 and AVC54, respectively. The dose-response curves of AVC37 and AVC54 for inducing NK cell cytotoxicity against SKOV3 tumor cells does not significant differ from that of the corresponding control NK booster AVC1 comprising wild types of IL-21 and 4-1BBL ECD.

Figure 5 shows that the ability of NK boosters AVC37 or AVC52, comprising IL-21 or 4-1BBL ECD muteins respectively, to induce and support long-term NK cell expansion is hardly affected.

Table 1.2.1 presents the reduced binding affinities for 4-BB of NK booster comprising 4-1BBL ECD muteins as indicated, as compared to a corresponding control NK booster AVC1, comprising wild type 4-1BBL ECD.

Figure 6A, B and C show that NK boosters with 4-1BBL ECD muteins with reduced affinity for 4-1BB, also have a reduced potency in a 4-1BB reporter cell assay. In fact, Figure 7 shows there is a strong correlation between affinity as measured by SPR and functionality in the 4-1BB reporter assay.

Table 1.2.1. Overview of the reduced binding affinities for 4-1BB of the boosters, and of their ability to induce of proliferation of NK cells in the presence of SKOV3 tumor cells at saturating concentration (25 nM) of boosters comprising trimers of 4-1BBL ECD muteins combined with wt IL-21 as indicated, expressed as percentage of control booster AVC1 (with a trimer of wild type 4-1BBL ECD and wild type IL-21).

AVC ID	4-1BBL mutation	K_D in nM	Log reduction pK_D	%AVC1 of proliferation at 25 nM
AVC1	wild type	39.81	0.00	-
AVC46	V100T	218.78	0.74	-
AVC47	V100Q	776.25	1,29	75%

AVC48	L101N	812.83	1,31	84%
AVC49	Y110Q	1202.26	1,48	81%
AVC50	G114K	57.54	0,16	-
AVC51	L115R	ND		-
AVC52	V153Q	588.84	1,17	92%
AVC53	R171D	61.66	0,19	-
AVC54	Q227E	1819.70	1,66	93%
AVC55	Q227R	>4400	>2.05	71%
AVC56	Q230S	53.70	0,13	-
AVC57	Q230K	1621.81	1,61	80%
AVC58	A116D	107.15	0,43	-
AVC59	A154D	776.25	1,29	97%
AVC285	A154E	830	0.83	-
AVC286	A154D G155Q	2810	5.55	-
AVC287	A154E	732	0.77	-
AVC288	A154D G155Q	3390	5.47	-

Table 1.2.1 presents the reduced binding affinities for 4-1BB of NK boosters comprising trimers of 4-1BBL ECD muteins as indicated, as compared to a corresponding control NK booster AVC1, comprising a trimer of wild type 4-1BBL ECD. Table 1.2.1 further presents the ability of the boosters to induce of proliferation of NK cells in the presence of SKOV3 tumor cells at saturating concentration (25 nM) of the boosters, expressed as percentage of control booster AVC1. As can be seen in Table 1.2.1, the affinity for 4-1BB of many of the NK boosters with 4-1BBL muteins is reduced by at least one order of magnitude compared the AVC1 booster with wild type 4-1BBL. Surprisingly however, in view of the above-mentioned strong correlation between affinity and functionality, despite their reduced affinity for 4-1BB, a subset of these boosters retained much of their ability to induce proliferation of NK cells in the presence of SKOV3 tumor cells to a similar degree as the control booster AVC1 with wild type IL-21, as their ability to induce proliferation is reduced less than 30%, up to even less than 3%, of the control booster.

Most of the NK boosters with 4-1BBL muteins retain their potency to induce NK cell expansion compared to the AVC1 control booster with wild type 4-1BBL (Figure 8). NK cells were seeded in the presence of booster or vehicle (opsonised SK-OV-3 cells) and expansion of NK cells was followed over the course over 14 days as described in Example 1.1.7. As shown in Figure 8, the reduced affinity of the boosters for 4-1BB does not significantly impact expansion compared to AVC1, except for AVC55 which shows lower expansion compared to AVC1 and the other boosters.

Figure 9 shows the results the long-term repeated NK cell cytotoxicity assay as described in Example 1.1.13. NK booster AVC59 comprising the A154D 4-1BBL ECD mutein was compared to control booster AVC1 comprising wild type 4-1BBL ECD. Further controls contained contained vehicle, only NK cells and SKOV-3 target cells, or only SKOV-3 target cells. As can be seen in

Figure 9, the AVC59 booster shows similar efficacy in a long-term cytotoxicity assay as the wild type booster AVC1.

Table 1.2.2. Overview of the reduced binding affinities for IL-21R of selected boosters comprising IL-21 muteins and a trimer of wild type 4-1BBL ECD as compared to control booster AVC1 (with a trimer of wild type 4-1BBL ECD and wild type IL-21).

AVC ID	Mutations	K_D [nM]	pK_D	log reduction pK_D
AVC1	-	0.040	10.39	0.00
AVC34	L13E	0.838	9.08	1.32
AVC36	D4H	0.305	9.52	0.88
AVC37	I8H	0.251	9.60	0.79
AVC114	N82- A83- G84- R85- R86- Q87- K88-	15.85	7.80	2.59
AVC116	N63- E64- R65- I66-	0.1	10.02	0.37
AVC118	I67T	0.04	10.41	-0.02
AVC119	I67N	0.45	9.35	1.05
AVC120	L74G	ND	ND	
AVC121	L74D	>500	<6.3	>4
AVC122	L74F	0.12	9.91	0.48
AVC123	L20S	3.24	8.49	1.91
AVC124	L20N	9.12	8.04	2.35
AVC125	L20W	1.82	8.74	1.65
AVC126	G84GGGGG	0.04	10.36	0.03

In Table 1.2.3, the boosters' ability to induce maximal proliferation of NK cells in the presence of SKOV3 tumor cells is determined at a saturating concentration of 25 nM booster. The boosters' ability to induce maximal NK cell proliferation is determined for boosters comprising IL-21 muteins combined with either wild type 4-1BBL ECD or the 4-1BBL A154D mutein with reduced affinity for its cognate receptor 4-1BB and compared with control booster AVC1. Also presented in Table 1.2.3 for each of the boosters are their pEC_{50} values for the induction of proliferation of NK cells in the presence of SKOV3 tumor cells.

Table 1.2.3. Overview of the ability of induction of proliferation of NK cells in the presence of SKOV3 tumor cells at saturating concentration (25 nM) of boosters comprising IL-21 muteins combined with trimers of wild type 4-1BBL ECD or the 4-1BBL A154D mutein as indicated, expressed as percentage of control booster AVC1 (with a trimer of wild type 4-1BBL ECD and wild type IL-21).

pEC_{50} values of the boosters for the induction of proliferation of NK cells in the presence of SKOV3 tumor cells are also shown.

AVC ID	IL-21	4-1BBL	pEC_{50}	%AVC1 proliferation at 25 nM
AVC1	WT	WT	9.66	100%
AVC114	N82- A83- G84- R85- R86- Q87- K88-	WT	10.42	102%
AVC121	L74D	WT	9.16	95%
AVC123	L20S	WT	10.18	105%
AVC124	L20N	WT	10.25	92%
AVC125	L20W	WT	9.66	95%
AVC187	L20N	A154D	9.20	49%
AVC188	L20W	A154D	9.14	89%
AVC189	L20S	A154D	9.28	61%
AVC190	N82- A83- G84- R85- R86- Q87- K88-	A154D	9.32	108%

As can be seen in Table 1.2.3, the ability to induce proliferation of NK cells in the presence of SKOV3 tumor cells of NK cell boosters comprising a wild type 4-1BBL ECD and a number of selected IL-21 muteins with reduced affinity for IL-21R is hardly affected compared to the corresponding booster with wild type IL-21. The pEC_{50} values are not reduced by no more than log 0.5 compared to the control booster and the maximum induced proliferation at saturating booster concentrations is not reduced by more than 10% compared to the control booster.

However, when the wild type 4-1BBL ECD in these boosters is substituted for the reduced affinity-4-1BBL ECD A154D mutein, the pEC_{50} values are still not reduced by no more than log 0.5 compared to the control booster but the maximum induced proliferation at saturating booster concentration is significantly reduced up to more than 50% compared to control for at least two of the IL-21 muteins. In contrast, the IL-21 muteins L20W retains most of its ability to maximally induce NK cell proliferation in combination with the 4-1BBL mutein, with no more than about 10% reduction in maximally induced proliferation as compared to the corresponding wild type control booster AVC1. And the AVC190 booster, comprising the combination of the 4-1BBL ECD mutein A154D and the IL-21 mutein DEL7 (N82- A83- G84- R85- R86- Q87- K88-) even outperforms the wild type control booster AVC1 in terms of maximum induced proliferation at saturating booster concentration.

Similar results as those obtained above with boosters targeting the HER2 as TAA are also obtained with boosters targeting either TROP2 or EGFR as TAA. The ability to induce NK cell cytotoxicity was compared for boosters AVC1 and AVC16, targeting HER2 and TROP2, respectively. Long-term NK cell cytotoxicity induced by the conjugates AVC1 and AVC16 was determined essentially

as described in Example 1.1.13, except that only a single round co-culture of NK cells and target cells was performed and followed for 96 hours. Briefly, NK cell cytotoxicity was determined against tumor cell lines expressing a TAA specifically bound by the conjugates AVC1 and AVC16 as indicated in Table 1.2.4. A single round of co-culture of NK cells and target cells, in a E:T ratio of 2:1 (20,000 NK cells : 10,000 tumor cells) was followed for 96 hours, in the absence or presence of the multispecific antigen binding proteins at 25 nM. Control wells thus contained only NK cells and the respective target tumor cells. Assays were performed in triplicate. Interferon-gamma levels were determined in the supernatant using the MACSplex cytotoxic IFN-γ kit (cat #130-125-800).

Table 1.2.4 Target tumor cell lines used for determination of NK cell cytotoxicity induced by the multispecific antigen binding proteins AVC1, AVC16 and AVC20.

Multispecific antigen-binding protein	**Target antigen**	**UNIPROT accession**	**Target tumor cell line**
AVC-001	HER2	P04626	SKOV-3
AVC-016	TROP2	P09758	BxPC3
AVC-020	EGFR	P00533	A431

The results for AVC1, AVC16 and AVC20 are shown in Figures 10A, 10B and 10C, respectively. Each of the multispecific antigen binding proteins AVC1, AVC16 and AVC20 significantly induce increased cytotoxicity against tumor cells that express the respective antigens compared to NK cells only. Similarly, Figures 11A, 11B and 11C show that the same set of multispecific antigen binding proteins induce increase interferon-gamma production in response to co-culture with tumor cells that express the respective antigens. Hence, similar stimulatory effects of the multispecific antigen binding proteins, in terms of inducing TAA-targeted NK cell cytotoxicity or induction of interferon-gamma production, are achieved when targeting either HER2, TROP2 or EGFR as tumor-associated antigen.

Figure 12 shows cytotoxicity of NK cells against SKOV-3 tumor cells, after a 14-day expansion of the NK cells in the presence of control booster AVC1 or booster AVC190 with IL-21 and 4-1BBL muteins as indicated above. The experiment was performed as described in Example 1.1.14. Further controls included vehicle (PBS only) or NK cells and SKOV-3 tumor cells without boosters. As can be seen in Figure 12, NK cell hyperfunctionality induced by the AVC190 booster with IL-21 and 4-1BBL muteins is at least as strong, if not slightly stronger, than that induced by the control booster AVC1 with wild type IL-21 and 4-1BBL.

Figure 13 shows a comparison of NK boosters AVC16 (based of Fab-fragments from sacituzumab and comprising wild type IL-21 and a trimer of wild type 4-1BBL ECD), AVC267 (based of Fab-fragments from sacituzumab and comprising the IL-21 L20W mutein and a trimer of the 4-1BBL ECD A154D mutein) and control protein AVC 137 (a sacituzumab analogue without IL-21 or 4-1BBL

cytokines) in the long-term repeated NK cell cytotoxicity assay as described in Example 1.1.13. Further controls only NK cells and BxPC3 target cells (i.e. without Booster) or only BxPC3 target cells. As can be seen in Figure 13, the NK booster AVC267 comprising the IL-21 mutein L20W shows a similar long-term in vitro tumor control as the AVC16 booster with wild type IL-21.

Figure 14 shows a comparison of NK boosters AVC16 (based of Fab-fragments from sacituzumab and comprising wild type IL-21 and a trimer of wild type 4-1BBL ECD), AVC245 (based of Fab-fragments from sacituzumab and comprising the IL-21 DEL7 (N82- A83- G84- R85- R86- Q87- K88-) mutein and a trimer of 4-1BBL ECD A154D mutein) and control protein AVC 137 (a sacituzumab analogue without IL-21 or 4-1BBL cytokines) in the long-term repeated NK cell cytotoxicity assay as described in Example 1.1.13. Further controls only NK cells and BxPC3 target cells (i.e. without Booster) or only BxPC3 target cells. As can be seen in Figure 14, the NK booster AVC245 comprising both IL-21- and 4-1BBL ECD-muteins shows a long-term in vitro tumor control that is even better than the AVC16 booster with wild type IL-21 and 4-1BBL.

Figure 15 shows *in vivo* pharmakinetics for NK boosters AVC16 and AVC245 compared to control antibodies AVC137 and trastuzumab. Mice were injected i.v. with 1 mg/kg body weight of the boosters or antibodies. Blood sample for analysis were drawn 15 minutes, 2 hours and 1, 3 and 7 days after injection and levels of boosters and control antibodies in mouse serum was determined by ELISA. As can be seen in Figure 15, after an initial drop in the first 15 minutes serum levels stabilize for the remainder of the 7-day test period. There is no significant difference between any of the tested boosters and antibodies in this time-frame.

Figure 16 shows the effect of different mutations in IL-21 and/or 41BB-L on Vδ1 expansion and phenotype. γδ T-cell boosters Boosters comprising anti-Vδ1 TCR V_H and V_L and wild type or mutein 4-BBL and IL-21 as indicated in Table 1.1.10.4, were used to induce expansion of of donor γδ T-cells as described in Example 1.1.16. After 14 days expansion of γδ T-cells (Figure 16A), PD1 expression on Vδ1 T cells (Figure 16B) and CD56 expression on Vδ1 T cells (Figure 16C) were determined as described in Example 1.1.17. All values have been normalised to AVC160 which uses IL-21 and 41BB-ligand wildtype in its design. Figure 16A shows that all of the boosters with IL-21 and/or 41BBL muteins are capable of inducing expansion of γδ T-cells, albeit to a somewhat lower rates than the AVC160 booster with wild types IL-21 and 41BBL. However, compared to the AVC160, many of the boosters with muteins show a higher induction of CD56 expression on the expanded γδ T-cells, which is a marker for potency γδ T-cell. Induction of PD1 expression by the mutein boosters is higher compared to than the wild type AVC160 booster.

Figure 17 shows that the *in vivo* efficacy of the AVC245 booster in reducing tumor burden in a mouse xenograft model (see Example 1.1.18) is significantly greater than that of control antibody AVC137 lacking the 4-1BBL and IL-21 muteins. Table 1.2.5 confirms the statistical significance of

the AVC245's efficacy in reducing tumor burden over the control antibody AVC137, as well as the tumor-only and NK cells + tumor controls.

Table 1.2.5. 2-way ANOVA + Tukey's multiple comparisons test.

	Tumor only	NK	NK + AVC245
NK	ns		
NK + AVC245	**** (<0.0001)	**** (<0.0001)	
NK + AVC137	*** (0.0006)	**** (<0.0001)	** (0.0083)

Figure 18 shows that the *in vivo* efficacy of the AVC245 booster in inducing expansion of donor NK cells in a mouse xenograft model (see Example 1.1.18) is significantly greater than that of control antibody AVC137 lacking the 4-1BBL and IL-21 muteins. Hence, Figures 17 and 18 convincingly demonstrate the therapeutic efficacy of conjugates comprising an antigen-binding protein against a TAA and a combination of 4-1BBL and IL-21 muteins as described herein, in treating a tumor expressing the TAA.

Numbered embodiments

1. A mutein of a 4-1BB ligand (4-1BBL) extracellular domain (ECD) comprising the amino acid sequence:
   REGPELSPDD PAGLLDLRQG MFAQLVAQNX XLIDGPLSWX SDPXXXGVSL TGGLSYKEDT
   KELVVAKAGV YYVFFQLELR RVXXGEGSGS VSLALHLQPL XSAAGAAALA LTVDLPPASS
   EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWXLTX GATVLGLFRV TPEIPAGLPS
   PRSE (SEQ ID NO: 49), representing amino acids 71 – 254 of the full-length 4-1BBL, wherein X represents any amino acid, and wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD of SEQ ID NO: 37 by at least one amino acid.
2. A 4-1BBL ECD mutein according to embodiment 1, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, A154E, V153Q, Q227E, L101N, Y110Q, Q230K, V100Q, V100T, V100S, V100A, V100G, V100N, V100D, V100E, V100K, V100R, L101E, L101Q, L101D L101R, L101K, Y110E, Y110N, Y110D, Y110R, Y110K, Y110S, Y110A, Y110G, Y110T, G114K, G114R, G114Q, G114D, G114E, G114N, G114S, G114A, G114G, G114T, L115R, L115K, L115Q, L115N, L115D, L115E, L115S, L115A, L115G, L115T, A116D, A116E, A116R, A116K, A116Q, A116N, A116Y, A116H, V153N, V153R, V153K, V153D, V153E, V153S, V153G, A154R, A154K, A154Q, A154N, A154Y, A154H, G155Q; R171D, R171E, R171Q, R171N, R171S, R171T, R171G, R171A, R171Y, Q227R, Q227K, Q227D, Q227Y, Q227H, Q227G, Q227S, Q227T, Q230S, Q230R, Q230N, Q230D, Q230E, Q230G, Q230S, Q230T and Q230A or wherein the

4-1BBL ECD mutein comprises no other amino acid sequence modification than the aforementioned amino acid substitutions.

3. A 4-1BBL ECD mutein according to embodiment 1 or 2, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, A154E, a combination of A154D and G155Q, V153Q, Q227E, Q227R, L101N, Y110Q, Q230K, and V100Q.

4. A 4-1BBL ECD mutein according to any one of embodiments 1 – 3, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, A154E, a combination of A154D and G155Q, V153Q, Q227E.

5. A 4-1BBL ECD mutein according to any one of embodiments 1 – 4, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, a combination of A154D and G155Q, and A154E.

6. A 4-1BBL ECD mutein according to embodiment 1, wherein the 4-1BBL ECD mutein amino acid sequence differs from the amino acid sequence of the wild type human 4-1BBL ECD (SEQ ID NO: 37) at least at a position corresponding to position 154 of the full-length 4-1BBL amino acid sequence, and wherein preferably, the 4-1BBL ECD mutein comprises no other differences from the wild type amino acid sequence than the difference at position 154.

7. A 4-1BBL ECD mutein according to embodiment 6, wherein the amino acid at position 154 is aspartate (D) or glutamate (E), of which aspartate (D) is preferred.

8. A 4-1BBL ECD mutein according to any one of the preceding embodiments, wherein the 4-1BBL ECD mutein binds to a human 4-1BB with a reduced affinity, wherein preferably, the 4-1BBL ECD mutein binds with a reduced affinity to a human 4-1BB having an amino acid sequence comprised in SEQ ID NO: 175, relative to the affinity of a wild-type 4-1BBL ECD for the human 4-1BB.

9. A 4-1BBL ECD mutein according to embodiment 8, wherein the 4-1BBL ECD mutein exhibits at least a 2-fold 5-fold, 10-fold, 20-fold 45-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold reduction in binding affinity for human 4-1BB, relative to the affinity of a wild-type 4-1BBL ECD for human 4-1BB.

10. A 4-1BBL ECD mutein according to embodiment 8 or 9, wherein the 4-1BBL ECD mutein exhibits no more than a 1000-fold, 500-fold, 200-fold, 100-fold, or 50-fold reduction in binding affinity for human 4-1BB, relative to the affinity of a wild-type 4-1BBL ECD for human 4-1BB.

11. A 4-1BBL ECD mutein according to any one of the preceding embodiments, wherein the 4-1BBL ECD mutein exhibits at least 2-fold reduction in activity as measured by an assay for measuring changes in a 4-1BB signaling pathway, relative to the activity of wild-type 4-1BBL ECD under corresponding conditions.

12. A 4-1BBL ECD mutein according to any one of the preceding embodiments, wherein the 4-1BBL ECD mutein exhibits a binding affinity for human 4-1BB, expressed in pK_D, that is at least 0.4, 0.5, or 1.0 lower than the pK_D of a wild-type 4-1BBL ECD for human 4-1BB.

13. A 4-1BBL ECD mutein according to embodiment 12, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, A154E, a combination of A154D and G155Q, V153Q, Q227E, L101N, Y110Q, Q230K, and V100Q.
14. A 4-1BBL ECD mutein according to embodiment 12 or 13, wherein the 4-1BBL ECD mutein, when present in a conjugate with trastuzumab as part of a homotrimer of the 4-1BBL ECD mutein, which conjugate further comprises a human IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is not less than 80% of the proliferation induced by a corresponding control conjugate comprising a trimer of wild type 4-1BBL ECD in the same assay.
15. A 4-1BBL ECD mutein according to embodiment 14, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, A154E, a combination of A154D and G155Q, V153Q, Q227E, L101N, Y110Q, and Q230K.
16. A 4-1BBL ECD mutein according to any one of embodiments 12 – 15, wherein the 4-1BBL ECD mutein, when present in a conjugate with trastuzumab as part of a homotrimer of the 4-1BBL ECD mutein, which conjugate further comprises a human IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is not less than 90% of the proliferation induced by a corresponding control conjugate comprising a trimer of wild type 4-1BBL ECD in the same assay.
17. A 4-1BBL ECD mutein according to embodiment 16, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, A154E, a combination of A154D and G155Q, V153Q, and Q227E.
18. A 4-1BBL ECD mutein according to any one of embodiments 12 – 17, wherein the 4-1BBL ECD mutein, when present in a conjugate with trastuzumab as part of a homortrimer of the 4-1BBL ECD mutein, which conjugate further comprises a human IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is not less than 94% of the proliferation induced by a corresponding control conjugate comprising a trimer of wild type 4-1BBL ECD in the same assay.
19. A 4-1BBL ECD mutein according to embodiment 18, wherein the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, a combination of A154D and G155Q, and A154E.
20. A fusion protein comprising three 4-1BBL ECD monomers, wherein at least one or two of the monomers is a 4-1BBL ECD mutein according to any one of the previous embodiments, fused together in a single polypeptide chain, and wherein, optionally, the three 4-1BBL ECD monomers are connected by polypeptide linkers.
21. A fusion protein according to embodiment 20, wherein each of the three monomers is a 4-1BBL ECD mutein according to any one of the previous embodiments.
22. A fusion protein according to embodiment 21, wherein the three monomers are identical.

23. A conjugate comprising a 4-1BBL ECD mutein according to any one of the preceding embodiments and a heterologous moiety.

24. A conjugate according to embodiment 23, wherein the 4-1BBL ECD mutein is directly attached to the heterologous moiety or wherein the 4-1BBL ECD mutein is attached to the heterologous moiety via a linker.

25. A conjugate according to embodiment 24, wherein the linker comprises a peptide, preferably a flexible linker peptide.

26. A conjugate according to embodiment 23 or 24, wherein the conjugate has a 4-1BBL ECD mutein-valency that is higher than one and/or wherein the conjugate comprises a fusion protein according to any one of embodiments 20 - 22.

27. A conjugate according to any one of embodiments 23 – 26, wherein the heterologous moiety comprises a polypeptide.

28. A conjugate according to embodiment 26, wherein the polypeptide is an antigen-binding protein or a polypeptide chain of an antigen-binding protein.

29. A conjugate according to embodiment 28, wherein the antigen-binding protein comprises at least one of:

a) at least one of:

i) a first antigen-binding region that specifically binds a tumor associated antigen (TAA), that specifically binds an NK cell activating receptor or that specifically binds an epitope of a γδ T cell receptor (TCR), and

ii) a second antigen-binding region that specifically binds a TAA, that specifically binds an NK cell activating receptor or that specifically binds an epitope of a γδ TCR; and,

b) a third antigen-binding region that has affinity for a surface antigen expressed on natural killer (NK) cells.

30. A conjugate according to embodiment 29, wherein the first and second antigen-binding region comprise at least one immunoglobulin-derived antigen-binding region.

31. A conjugate according to embodiment 30, wherein the immunoglobulin-derived antigen-binding region comprises or consists of a Fab or an immunoglobulin single variable domain (ISVD).

32. A conjugate according to any one of embodiments 28 – 30, wherein the first and second antigen-binding regions are human or humanized antigen-binding regions.

33. A conjugate according to any one of embodiments 28 – 32, wherein the first and second antigen-binding regions bind the same TAA, at least two different TAAs, or at least two different epitopes of the same TAA.

34. A conjugate according to embodiment 33, wherein the first and second antigen-binding regions are identical.

35. A conjugate according to any one of embodiments 28 – 34, wherein the TAA is selected from the group consisting of: 5T4, ADAM9, ADAM10, ADAM12, ALK, ALPP, ALPP2, ALPPL2, AMHR2, ANGPT2, AXL, Angiopoietin-2, Apelin receptor, B7-H3, B7-H4, B7-H6, B7.1, B7.2, BCMA, BTLA, CA125, CA9, CAIX, CCL2, CCR2, CCR4, CCR5, CCR6, CCR7, CD123,

CD133, CD138, CD142, CD147, CD166, CD171, CD19, CD2, CD20, CD205, CD22, CD228, CD24, CD248, CD25, CD27, CD276, CD3, CD30, CD317, CD33, CD37, CD38, CD3E, CD4, CD40, CD44, CD44v6, CD45, CD46, CD47, CD52, CD56, CD70, CD71, CD73, CD74, CD79, CD79B, CD80, CD80/CD86, CDCP1, CDH3, CDH6, CDK4, CEA, CEACAM5, CLDN18, CLEC14A, CLEC4, CSF1R, CSF2, CSPG4, CT-7, CTGF, CTLA4, Cadherin 17, Cadherin 6, CanAg, Claudin 18.2, Claudin 6, Connexin 37, Cripto-1, Crypto, DC3, DKK1, DLK1, DLL3, DLL4, DR5, E-cadherin, E-selectin, EBV-encoded nuclear antigen (EBNA)-I, EDA, EDB, EDNRB, EGF, EGFL7, EGFR, EGFRvIII, ENG, EPCAM, EPHA4, EphA10, EphA2, EphA3, EphB2, EphB4, ExtradomainB (EDB) fibronectin, F3, FAP, FCGR1, FGFR2, FGFR2b, FGFR3, FGFR4, FLT1, FN, FOLH1, FOLR1, FRα, FSHR, FcRL5/FcRH5, Fibronectin extra-domain B, Flt3, Fucosyl, GFRa4, GM3, GPCR5D, GPNMB, GPRC5D, GRP78, GUCY2C, Glycoprotein NMB, Glypican 1, Glypican 2, Glypican 3, GnT-V, HAVCR2, HER-3/ERBB3, HER-4/ERBB4, HER2, HER3, HGF, HLA-G, HSP70, ICAM-1, ICOS, IFNG, IGF-1R, IGF1, IGF1R, IGF2, IL-1 accessory protein, IL-6 receptor, IL-8 receptor, IL13Ra2, IL17A, IL1A, IL1B, IL1RAP, IL2RA, IL3RA, IL6, IL6R, ITGAV, ITGB6, Ig-idiotype, Integrin beta 6, KAAG-1, KDR, KIRD2, KIT, KLK2, KLRC1, Killer Ig-Like Receptor, Killer Ig-Like Receptor 3DL2 (KIR3DL2), L1-CAM, L1CAM, LAG3, LAGE-1, LGR5, LIF, LIV-1, LOXL2, LRRC32, Lewis-Y, MART-1/Melan-A, MET, MIC-A/B, MICB, MIF, MISIIR, MMP2, MS4A1, MSLN, MST1R, MSTN, MUC1, MUC1-C, MUC16, MUM-1, Melanotransferrin, Mesothelin, Mud 6, NAG, NCAM1, NKG2D, NOTCH1, NOTCH2, NOTCH3, NRP1, NT5E, NTRKR1 (EC 2.7.10.1), NaPi2b, Nectin-4, OLR1, OX40, P-cadherin, P1A, PCSK9, PD-L1, PD1, PDGF, PDGF alpha receptor, PDGF beta receptor, PDGFR, PDGFRA, PLAUR, PMEL, PRAME, PSCA, PSMA, PTK7, PTPRC, PVRL4, Plexin-A1, RAGE, ROBO1, ROR1, ROR2, RSPO3, SCP-1, SEZ6, SIRPA, SLAMF7, SLC34A2, SLC3A2, SSTR2, SSX-1, SSX-2 (HOM-MEL-40), SSX-4, SSX-5, STEAP1, STEAP2, Severe, T-cell receptor/CD3-zeta chain, TACSTD2, TGF-alpha, TGFB1, TGFB2, TGFB3, TIGIT, Tissue factor/TF, TLR2, TM4SF1, TMEFF2, TNFRSF10B, TNFRSF17, TNFRSF18, TNFRSF4, TNFRSF8, TNFRSF9, TNFSF11, TNFSF13B, TPBG, TRAILR1, TRAILR2, TROP2, TSHR, TYRP1, VEGF, VEGFA, VEGFR1, VEGFR2, VH1/VL1, VH2/VL2, VH3/VL3, a GAGE-tumor antigen, a GD2 ganglioside, a GM2 ganglioside, a RAET1 protein, a UL16-binding protein (ULBP), a heterodimeric receptor comprised of at least one HER subunit, a human papillomavirus protein, a5β1 integrins, a5β3 integrins, adenomatous polyposis coli protein (APC), adenosine deaminase-binding protein (ADAbp), anti-Müllerian hormone Type II receptor, avB3 integrin, avB6 integrin, avβ6 integrins, bivalent, brain glycogen phosphorylase, c-erbB-2, cMET, colorectal associated antigen (CRC)-C017-1A/GA733, gastrin releasing peptide receptor antigen, gp100, gp75, gpA33, hCG, human papillomavirus protein, integrin receptors, mmp9, muc17, p15, phosphatidylserine, prostate specific antigen (PSA), protein tyrosine kinase 7(PTK7), receptor protein tyrosine kinase 3 (TYRO-3), sVE-cadherin, scatter factor receptor kinase, trivalent, α-catenin, α-fetoprotein, αIIbβ3-integrins, β-catenin, and γ-catenin.

36. A conjugate according to embodiment 35, wherein at least one of the first and second antigen-binding regions comprises a combination of complementarity-determining regions (CDRs) CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of: a) the CDR-H1 (SEQ ID NO: 24), CDR-H2 (SEQ ID NO: 25) and CDR-H3 (SEQ ID NO: 26) sequences as comprised in SEQ ID NO: 1, and the CDR-L1 (SEQ ID NO: 27), CDR-L2 (SEQ ID NO: 28) and CDR-L3 (SEQ ID NO: 29) sequences as comprised in SEQ ID NO: 2 (trastuzumab); b) the CDR-H1 (SEQ ID NO: 150), CDR-H2 (SEQ ID NO: 151) and CDR-H3 (SEQ ID NO: 152) sequences as comprised in SEQ ID NO: 59, and the CDR-L1 (SEQ ID NO: 153), CDR-L2 (SEQ ID NO: 154) and CDR-L3 (SEQ ID NO: 155) sequences as comprised in SEQ ID NO: 60 (atezolizumab); c) the CDR-H1 (SEQ ID NO: 156), CDR-H2 (SEQ ID NO: 157) and CDR-H3 (SEQ ID NO: 158) sequences as comprised in SEQ ID NO: 9, and the CDR-L1 (SEQ ID NO: 159), CDR-L2 (SEQ ID NO: 160) and CDR-L3 (SEQ ID NO: 161) sequences as comprised in SEQ ID NO: 10 (avelumab); d) the CDR-H1 (SEQ ID NO: 162), CDR-H2 (SEQ ID NO: 163) and CDR-H3 (SEQ ID NO: 165) sequences as comprised in SEQ ID NO: 61, and the CDR-L1 (SEQ ID NO: 165), CDR-L2 (SEQ ID NO: 166) and CDR-L3 (SEQ ID NO: 167) sequences as comprised in SEQ ID NO: 62 (durvalumab); e) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 3, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 4 (cetuximab); f) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 5, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 6 (rituximab); g) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 7, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 8 (daratumumab); h) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 63, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 64 (cosibelimab); i) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 65, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 66 (margetuximab); j) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 67, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 68 (pertuzumab); k) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 69, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 70 (enoblituzumab); l) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 71, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 72 (necitumumab); m) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 73, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 74 (panitumumab); n) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 75, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 76 (amivantamab EGFR-binding); o) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 77, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 78 (amivantamab cMet-binding); p) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 79, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 80

(zolbetuximab); q) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 81, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 82 (dinutuximab); r) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 83, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 84 (naxitamab); s) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 85, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 86 (enfortumab); t) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 87, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 88 (farletuzumab); u) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 89, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 90 (tisotumab); v) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 91, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 92 (mirvetuximab); w) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 93, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 94 (sacituzumab); x) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 95, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 96 (vobramitamab); y) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 97, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 98 (Onartuzumab); z) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 144, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 145 (sibrotuzumab) aa) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 100, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 101 (olaratumab); ab) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 102, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 103 (rovalpituzumab); ac) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 238, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 239 (adebrelimab); ad) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 240, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 241 (alemtuzumab); ae) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 242, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 243 (belantamab); af) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 244, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 245 (Bevacizumab); ag) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 246, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 247 (brentuximab); ah) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 248, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 249 (camrelizumab); ai) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 250, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 251 (cemiplimab); aj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 252, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ

ID NO: 253 (dostarlimab); ak) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 254, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 255 (emapalumab); al) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 256, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 257 (enlonstobart); am) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 258, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 259 (gemtuzumab); an) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 260, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 261 (ibritumomab); ao) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 262, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 263 (inotuzumab); ap) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 264, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 265 (ipilimumab); aq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 266, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 267 (isatuximab); ar) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 268, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 269 (loncastuximab); as) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 270, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 271 (mogamulizumab); at) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 272, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 273 (moxetumomab); au) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 274, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 275 (nimotuzumab); av) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 276, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 277 (nivolumab); aw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 278, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 279 (obinutuzumab); ax) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 280, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 281 (ofatumumab); ay) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 282, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 283 (pembrolizumab); az) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 284, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 285 (Penpulimab); ba) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 286, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 287 (polatuzumab); bb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 288, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 289 (prolgolimab); bc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 290, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 291 (pucotenlimab); bd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in

SEQ ID NO: 292, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 293 (racotumomab) be) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 294, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 295 (ramucirumab); bf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 296, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 297 (relatlimab); bg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 298, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 299 (retifanlimab); bh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 300, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 301 (ripertamab); bi) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 302, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 303 (serplulimab); bj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 304, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 305 (sintilimab); bk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 306, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 307 (socazolimab); bl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 308, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 309 (sugemalimab); bm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 310, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 311 (tafasitamab); bn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 312, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 313 (tagitanlimab); bo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 314, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 315 (tebentafusp); bp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 316, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 317 (Tislelizumab); bq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 318, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 319 (Toripalimab); br) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 320, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 321 (zuberitamab); bs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 322, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 323 (benmelstobart); bt) bu) bv) bw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 324, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 325 (iparomlimab); bx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 326, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 327 (tuvonralimab); by) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 328, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 329 (anvatabart); bz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 330, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO:

331 (apamistamab); ca) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 332, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 333 (bemarituzumab); cb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 334, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 335 (cetrelimab); cd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 336, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 337 (cobolimab); ce) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 338, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 339 (datopotamab); cf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 340, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 341 (domvanalimab); cg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 342, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 343 (emactuzumab); ch) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 344, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 345 (favezelimab); ci) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 346, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 347 (felzartamab); cj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 348, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 349 (fianlimab); ck) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 350, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 351 (finotonlimab); cl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 352, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 353 (geptanolimab); cm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 354, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 355 (gotistobart); cn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 356, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 357 (ivuxolimab); co) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 358, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 359 (lemzoparlimab); cp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 360, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 361 (luveltamab); cq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 362, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 363 (magrolimab); cr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 364, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 365 (mecbotamab); cs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 366, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 367 (monalizumab); ct) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 368, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 369 (nofazinlimab); cu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ

ID NO: 370, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 371 (nurulimab); cv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 372, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 373 (ociperlimab); cw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 374, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 375 (oleclumab); cx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 376, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 377 (onfekafusp); cy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 378, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 379 (patritumab); cz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 380, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 381 (pivekimab); da) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 382, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 383 (quavonlimab); db) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 384, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 385 (retlirafusp); dc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 386, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 387 (rosopatamab); dd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 388, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 389 (rulonilimab); de) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 390, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 391 (sabatolimab); df) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 392, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 393 (sasanlimab); dg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 394, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 395 (telisotuzumab); dh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 396, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 397 (tiragolumab); di) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 398, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 399 (tusamitamab); dj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 400, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 401 (vibostolimab); dk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 402, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 403 (vobramitamab); dl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 404, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 405 (zilovertamab); dm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 406, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 407 (suvemcitug); dn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 408, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 409 (becotatug); do) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in

SEQ ID NO: 410, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 411 (tifcemalimab); dq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 412, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 413 (blinatumomab - CD19); dr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 414, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 415 (blinatumomab – CD3); ds) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 416, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 417 (cadonilimab – PD-1); dt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 418, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 419 (cadonilimab – CTLA4); du) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 420, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 421 (disitamab); dv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 422, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 423 (edrecolomab); dw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 424, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 425 (elranatamab – BCMA); dx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 426, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 427 (elranatamab – DC3); dy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 428, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 429 (epcoritamab – CD20); dz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 430, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 431 (epcoritamab – CD3); ea) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 432, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 433 (glofitamab – VH1/VL1); eb); the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 434, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 435 (glofitamab – VH2/VL2); ec) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 436, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 437 (glofitamab – VH3/VL3); ed) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 438, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 439 (mosunetuzumab – CD20); ef) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 440, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 441 (mosunetuzumab – CD3); eg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 442, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 443 (talquetamab - GPCR5D); eh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 444, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 445 (talquetamab – CD3); ei) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 446, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 447 (teclistamab - BCMA); ej) the

CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 448, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 449 (teclistamab – CD3); ek) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 450, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 451 (tositumomab); el) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 452, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 453 (tremelimumab); em) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 454, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 455 (zimberelimab); en) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 456, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 457 (odronextamab – CD20); eo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 458, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 459 (odronextamab – CD3); ep) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 460, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 461 (ivonescimab – PD-1); eq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 462, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 463 (ivonescimab - VEGF); er) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 464, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 465 (anbenitamab – VH1/VL1); es) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 466, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 467 (anbenitamab – VH2/VL2); et) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 468, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 469 (izalontamab – EGFR); eu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 470, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 471 (izalontamab – HER3); ev) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 472, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 473 (linvoseltamab - BCMA); ew) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 474, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 475 (linvoseltamab – CD3); ex) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 476, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 477 (tarlatamab – DLL3); ey) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 478, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 479 (tarlatamab – CD3); ez) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 480, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 481 (zanidatamab – VH1/VL1); fa) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 482, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 483 (zanidatamab – VH2/VL2); fb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 484, and the CDR-L1, CDR-L2 and CDR-L3 sequences as

comprised in SEQ ID NO: 485 (volrustomig – PD-1); fc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 486, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 487 (volrustomig – CTLA-4); fd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 488, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 489 (zenocutuzumab – HER3); fe) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 490, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 491 (zenocutuzumab – HER2); ff) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 492, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 493 (botensilimab - VH1/VL1); fg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 494, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 495 (botensilimab - VH2/VL2); fh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 496, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 497 (izalontamab - EGFR); fi) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 498, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 499 (izalontamab – HER3); fj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 500, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 501 (rilvegostomig – TIGIT); fk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 502, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 503 (rilvegostomig – PD-1); fl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 701, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 700 (abagovomab); fm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 703, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 702 (abituzumab); fn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 705, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 704 (acasunlimab); fo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 707, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 706 (alnuctamab); fp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 709, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 708 (alomfilimab); fq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 711, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 710 (amatuximab); fr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 713, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 712 (anetumab); fs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 715, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 714 (aplitabart); ft) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 717, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 716 (atigotatug); fu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 719, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO:

718 (balstilimab); fv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 721, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 720 (bavituximab); fw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 723, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 722 (bavunalimab); fx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 725, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 724 (belrestotug); fy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 727, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 726 (bermekimab); fz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 729, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 728 (bifikafusp); ga) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 731, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 730 (bintrafusp); gb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 733, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 732 (brenetafusp); gc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 735, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 734 (briquilimab); gd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 737, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 736 (brontictuzumab); ge) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 739, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 738 (budigalimab); gf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 741, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 740 (cabiralizumab); gg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 743, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 742 (canakinumab); gh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 745, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 744 (cantuzumab); gi) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 747, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 746 (carlumab); gj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 749, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 748 (carotuximab); gk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 751, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 750 (caxmotabart); gl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 753, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 752 (cergutuzumab); gm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 755, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 754 (cibisatamab); gn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 757, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 756 (cinrebafusp); go) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 759, and the CDR-

L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 758 (cixutumumab); gp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 761, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 760 (clazakizumab); gq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 763, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 762 (clivatuzumab); gr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 765, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 764 (cofetuzumab); gs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 767, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 766 (coltuximab); gt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 769, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 768 (conatumumab); gu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 771, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 770 (dacetuzumab); gv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 773, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 772 (dalutrafusp); gw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 775, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 774 (danburstotug); gx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 777, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 776 (daratumumab); gy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 779, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 778 (demcizumab); gz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 781, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 780 (denintuzumab); ha) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 783, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 782 (denosumab); hb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 785, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 784 (depatuxizumab); hc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 787, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 786 (drozitumab); hd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 789, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 788 (duligotuzumab); he) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 791, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 790 (dusigitumab); hf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 793, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 792 (duvortuxizumab); hg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 795, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 794 (elotuzumab); hh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 797, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO:

796 (eluvixtamab); hi) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 799, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 798 (enapotamab); hj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 801, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 800 (enoticumab); hk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 803, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 802 (epacmarstobart); hl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 805, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 804 (etentamig); hm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 807, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 806 (falbikitug); hn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 809, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 808 (faricimab); ho) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 811, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 810 (feladilimab); hp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 813, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 812 (ficerafusp); hq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 815, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 814 (ficlatuzumab); hr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 817, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 816 (figitumumab); hs) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 819, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 818 (flanvotumab); ht) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 821, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 820 (flotetuzumab); hu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 823, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 822 (forimtamig); hv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 825, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 824 (futuximab); hw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 827, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 826 (ganitumab); hx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 829, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 828 (gevokizumab); hy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 831, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 830 (girentuximab); hz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 833, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 832 (glembatumumab); ia) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 835, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 834 (icrucumab); ib) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 837, and the CDR-L1, CDR-L2 and CDR-L3

sequences as comprised in SEQ ID NO: 836 (ifinatamab); ic) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 839, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 838 (iladatuzumab); id) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 841, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 840 (imalumab); ie) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 843, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 842 (imgatuzumab); if) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 845, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 844 (indusatumab); ig) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 847, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 846 (inebilizumab); ih) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 849, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 848 (ispectamab); ii) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 851, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 850 (istiratumab); ij) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 853, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 852 (izeltabart); ik) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 855, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 854 (izuralimab); il) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 857, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 856 (landogrozumab); im) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 859, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 858 (lapriximab); in) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 861, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 860 (lenzilumab); io) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 863, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 862 (leronlimab); ip) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 865, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 864 (lifastuzumab); iq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 867, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 866 (ligufalimab); ir) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 869, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 868 (lilotomab); is) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 871, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 870 (lintuzumab); it) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 873, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 872 (lirilumab); iu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 875, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 874 (livmoniplimab); iv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 877, and the

CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 876 (lorvotuzumab); iw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 879, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 878 (lucatumumab); ix) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 881, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 880 (lumretuzumab); iy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 883, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 882 (matuzumab); iz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 885, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 884 (mipasetamab); ja) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 887, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 886 (modakafusp); jb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 889, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 888 (modotuximab); jc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 891, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 890 (murlentamab); jd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 893, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 892 (nadunolimab); je) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 895, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 894 (naptumomab); jf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 897, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 896 (narlumosbart); jg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 899, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 898 (narnatumab); jh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 901, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 900 (navicixizumab); ji) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 903, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 902 (nesvacumab); jj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 905, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 904 (nisevokitug); jk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 907, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 906 (omburtamab); jl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 909, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 908 (ontuxizumab); jm) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 911, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 910 (otlertuzumab); jn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 913, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 912 (pamrevlumab); jo) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 915, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 914 (parsatuzumab); jp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised

in SEQ ID NO: 917, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 916 (pavurutamab); jq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 919, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 918 (pemivibart); jr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 921, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 920 (petosemtamab); js) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 923, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 922 (pimivalimab); jt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 925, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 924 (pinatuzumab); ju) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 927, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 926 (plozalizumab); jv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 929, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 928 (pulocimab); jw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 931, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 930 (ragifilimab); jx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 933, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 932 (raludotatug); jy) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 935, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 934 (rilotumumab); jz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 937, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 936 (rosmantuzumab); ka) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 939, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 938 (runimotamab); kb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 941, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 940 (sabestomig); kc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 943, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 942 (selicrelumab); kd) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 945, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 944 (seribantumab); ke) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 947, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 946 (sigvotatug); kf) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 949, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 948 (simlukafusp); kg) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 951, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 950 (simtuzumab); kh) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 953, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 952 (sirexatamab); ki) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 955, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ

ID NO: 954 (sofituzumab); kj) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 957, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 956 (spartalizumab); kk) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 959, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 958 (surzebiclimab); kl) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 961, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 960 (tabalumab); km) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 963, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 962 (tafolecimab); kn) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 965, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 964 (talacotuzumab); ko) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 967, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 966 (tarextumab); kp) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 969, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 968 (tavolimab); kq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 971, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 970 (tebotelimab); kr) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 973, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 972 (teprotumumab); ks) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 975, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 974 (tidutamab); kt) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 977, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 976 (tigatuzumab); ku) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 979, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 978 (tilvestamab); kv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 981, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 980 (tobemstomig); kw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 983, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 982 (tocilizumab); kx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 985, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 984 (tomaralimab); ky) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 987, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 986 (tovecimig); kz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 989, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 988 (tovetumab); la) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 991, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 990 (tucotuzumab); lb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 993, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 992 (tuparstobart); lc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ

ID NO: 995, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 994 (upifitamab); Id) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 997, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 996 (urabrelimab); Ie) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 999, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 998 (utomilumab); If) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1001, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1000 (vadastuximab); Ig) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1003, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1002 (vandortuzumab); Ih) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1005, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1004 (vanucizumab); Ii) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1007, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1006 (veligrotug); Ij) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1009, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1008 (verzistobart); Ik) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1011, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1010 (vesencumab); Il) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1013, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1012 (vofatamab); Im) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1015, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1014 (vonlerolizumab); In) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1017, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1016 (vopikitug); Io) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1019, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1018 (vorsetuzumab); Ip) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1021, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1020 (xaluritamig); Iq) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1023, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1022 (zalutumumab); Ir) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1025, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1024 (zanolimumab); Is) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1027, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1026 (ivuxolimab-alt); It) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1029, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1028 (inotuzumab-alt); Iu) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1031, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1030 (moxetumomab-alt); Iv) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1033, and the CDR-L1, CDR-L2 and CDR-L3

sequences as comprised in SEQ ID NO: 1032 (luveltamab-alt); lw) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1035, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1034 (ibritumomab-alt); lx) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1037, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1036 (pivekimab-alt); ly) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1039, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1038 (avelumab-alt); lz) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1041, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1040 (sugemalimab-alt); ma) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1043, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1042 (nimotuzumab-alt); mb) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 1045, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 1044 (panitumumab-alt); mc) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 540, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 541 (AR46A6); md) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 542, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 543 (KM4097); and me) the CDR-H1, CDR-H2 and CDR-H3 sequences as comprised in SEQ ID NO: 544, and the CDR-L1, CDR-L2 and CDR-L3 sequences as comprised in SEQ ID NO: 545 (K5-70).

37. A conjugate according to embodiment 26, wherein at least one of the first and second antigen-binding region comprises a combination of variable light (V_L) and variable heavy (V_H) domains selected from the group consisting of: a) the V_H sequence as comprised in SEQ ID NO: 39 and the V_L sequence as comprised in SEQ ID NO: 40 (trastuzumab); b) the V_H sequence as comprised in SEQ ID NO: 41 and the V_L sequence as comprised in SEQ ID NO: 42 (cetuximab); c) the V_H sequence as comprised in SEQ ID NO: 43 and the V_L sequence as comprised in SEQ ID NO: 44 (rituximab); d) the V_H sequence as comprised in SEQ ID NO: 45 and the V_L sequence as comprised in SEQ ID NO: 46 (daratumumab); e) the V_H sequence as comprised in SEQ ID NO: 47 and the V_L sequence as comprised in SEQ ID NO: 48 (avelumab); f) the V_H sequence as comprised in SEQ ID NO: 104 and the V_L sequence as comprised in SEQ ID NO: 105 (atezolizumab); g) the V_H sequence as comprised in SEQ ID NO: 106 and the V_L sequence as comprised in SEQ ID NO: 107 (durvalumab); h) the V_H sequence as comprised in SEQ ID NO: 108 and the V_L sequence as comprised in SEQ ID NO: 109 (cosibelimab); i) the V_H sequence as comprised in SEQ ID NO: 110 and the V_L sequence as comprised in SEQ ID NO: 111 (margetuximab); j) the V_H sequence as comprised in SEQ ID NO: 112 and the V_L sequence as comprised in SEQ ID NO: 113 (pertuzumab); k) the V_H sequence as comprised in SEQ ID NO: 114 and the V_L sequence as comprised in SEQ ID NO: 115 (enoblituzumab); l) the V_H sequence as comprised in SEQ ID NO: 116 and the V_L sequence as comprised in SEQ ID NO: 117 (necitumumab); m) the V_H sequence as comprised in SEQ ID NO: 118 and the V_L sequence as comprised in SEQ ID

NO: 119 (panitumumab); n) the V_H sequence as comprised in SEQ ID NO: 120 and the V_L sequence as comprised in SEQ ID NO: 121 (amivantamab EGFR-binding); o) the V_H sequence as comprised in SEQ ID NO: 122 and the V_L sequence as comprised in SEQ ID NO: 123 (amivantamab cMet-binding); p) the V_H sequence as comprised in SEQ ID NO: 124 and the V_L sequence as comprised in SEQ ID NO: 125 (zolbetuximab); q) the V_H sequence as comprised in SEQ ID NO: 126 and the V_L sequence as comprised in SEQ ID NO: 127 (dinutuximab); r) the V_H sequence as comprised in SEQ ID NO: 128 and the V_L sequence as comprised in SEQ ID NO: 129 (naxitamab); s) the V_H sequence as comprised in SEQ ID NO: 130 and the V_L sequence as comprised in SEQ ID NO: 131 (enfortumab); t) the V_H sequence as comprised in SEQ ID NO: 132 and the V_L sequence as comprised in SEQ ID NO: 133 (farletuzumab); u) the V_H sequence as comprised in SEQ ID NO: 134 and the V_L sequence as comprised in SEQ ID NO: 135 (tisotumab); v) the V_H sequence as comprised in SEQ ID NO: 136 and the V_L sequence as comprised in SEQ ID NO: 137 (mirvetuximab); w) the V_H sequence as comprised in SEQ ID NO: 138 and the V_L sequence as comprised in SEQ ID NO: 139 (sacituzumab); x) the V_H sequence as comprised in SEQ ID NO: 140 and the V_L sequence as comprised in SEQ ID NO: 141 (vobramitamab); y) the V_H sequence as comprised in SEQ ID NO: 142 and the V_L sequence as comprised in SEQ ID NO: 143 (onartuzumab); z) the V_H sequence as comprised in SEQ ID NO: 144 and the V_L sequence as comprised in SEQ ID NO: 145 (sibrotuzumab); aa) the V_H sequence as comprised in SEQ ID NO: 146 and the V_L sequence as comprised in SEQ ID NO: 147 (olaratumab); ab) the V_H sequence as comprised in SEQ ID NO: 148 and the V_L sequence as comprised in SEQ ID NO: 149 (rovalpituzumab); ac) the V_H sequence as comprised in SEQ ID NO: 238, and the V_L sequence as comprised in SEQ ID NO: 239 (adebrelimab); ad) the V_H sequence as comprised in SEQ ID NO: 240, and the V_L sequence as comprised in SEQ ID NO: 241 (alemtuzumab); ae) the V_H sequence as comprised in SEQ ID NO: 242, and the V_L sequence as comprised in SEQ ID NO: 243 (belantamab); af) the V_H sequence as comprised in SEQ ID NO: 244, and the V_L sequence as comprised in SEQ ID NO: 245 (Bevacizumab); ag) the V_H sequence as comprised in SEQ ID NO: 246, and the V_L sequence as comprised in SEQ ID NO: 247 (brentuximab); ah) the V_H sequence as comprised in SEQ ID NO: 248, and the V_L sequence as comprised in SEQ ID NO: 249 (camrelizumab); ai) the V_H sequence as comprised in SEQ ID NO: 250, and the V_L sequence as comprised in SEQ ID NO: 251 (cemiplimab); aj) the V_H sequence as comprised in SEQ ID NO: 252, and the V_L sequence as comprised in SEQ ID NO: 253 (dostarlimab); ak) the V_H sequence as comprised in SEQ ID NO: 254, and the V_L sequence as comprised in SEQ ID NO: 255 (emapalumab); al) the V_H sequence as comprised in SEQ ID NO: 256, and the V_L sequence as comprised in SEQ ID NO: 257 (enlonstobart); am) the V_H sequence as comprised in SEQ ID NO: 258, and the V_L sequence as comprised in SEQ ID NO: 259 (gemtuzumab); an) the V_H sequence as comprised in SEQ ID NO: 260, and the V_L sequence as comprised in SEQ ID NO: 261 (ibritumomab); ao) the V_H sequence as comprised in SEQ ID NO: 262, and the V_L sequence as comprised in SEQ ID NO: 263 (inotuzumab); ap) the V_H sequence as comprised in SEQ

ID NO: 264, and the V_L sequence as comprised in SEQ ID NO: 265 (ipilimumab); aq) the V_H sequence as comprised in SEQ ID NO: 266, and the V_L sequence as comprised in SEQ ID NO: 267 (isatuximab); ar) the V_H sequence as comprised in SEQ ID NO: 268, and the V_L sequence as comprised in SEQ ID NO: 269 (loncastuximab); as) the V_H sequence as comprised in SEQ ID NO: 270, and the V_L sequence as comprised in SEQ ID NO: 271 (mogamulizumab); at) the V_H sequence as comprised in SEQ ID NO: 272, and the V_L sequence as comprised in SEQ ID NO: 273 (moxetumomab); au) the V_H sequence as comprised in SEQ ID NO: 274, and the V_L sequence as comprised in SEQ ID NO: 275 (nimotuzumab); av) the V_H sequence as comprised in SEQ ID NO: 276, and the V_L sequence as comprised in SEQ ID NO: 277 (nivolumab); aw) the V_H sequence as comprised in SEQ ID NO: 278, and the V_L sequence as comprised in SEQ ID NO: 279 (obinutuzumab); ax) the V_H sequence as comprised in SEQ ID NO: 280, and the V_L sequence as comprised in SEQ ID NO: 281 (ofatumumab); ay) the V_H sequence as comprised in SEQ ID NO: 282, and the V_L sequence as comprised in SEQ ID NO: 283 (pembrolizumab); az) the V_H sequence as comprised in SEQ ID NO: 284, and the V_L sequence as comprised in SEQ ID NO: 285 (Penpulimab); ba) the V_H sequence as comprised in SEQ ID NO: 286, and the V_L sequence as comprised in SEQ ID NO: 287 (polatuzumab); bb) the V_H sequence as comprised in SEQ ID NO: 288, and the V_L sequence as comprised in SEQ ID NO: 289 (prolgolimab); bc) the V_H sequence as comprised in SEQ ID NO: 290, and the V_L sequence as comprised in SEQ ID NO: 291 (pucotenlimab); bd) the V_H sequence as comprised in SEQ ID NO: 292, and the V_L sequence as comprised in SEQ ID NO: 293 (racotumomab) be) the V_H sequence as comprised in SEQ ID NO: 294, and the V_L sequence as comprised in SEQ ID NO: 295 (ramucirumab); bf) the V_H sequence as comprised in SEQ ID NO: 296, and the V_L sequence as comprised in SEQ ID NO: 297 (relatlimab); bg) the V_H sequence as comprised in SEQ ID NO: 298, and the V_L sequence as comprised in SEQ ID NO: 299 (retifanlimab); bh) the V_H sequence as comprised in SEQ ID NO: 300, and the V_L sequence as comprised in SEQ ID NO: 301 (ripertamab); bi) the V_H sequence as comprised in SEQ ID NO: 302, and the V_L sequence as comprised in SEQ ID NO: 303 (serplulimab); bj) the V_H sequence as comprised in SEQ ID NO: 304, and the V_L sequence as comprised in SEQ ID NO: 305 (sintilimab); bk) the V_H sequence as comprised in SEQ ID NO: 306, and the V_L sequence as comprised in SEQ ID NO: 307 (socazolimab); bl) the V_H sequence as comprised in SEQ ID NO: 308, and the V_L sequence as comprised in SEQ ID NO: 309 (sugemalimab); bm) the V_H sequence as comprised in SEQ ID NO: 310, and the V_L sequence as comprised in SEQ ID NO: 311 (tafasitamab); bn) the V_H sequence as comprised in SEQ ID NO: 312, and the V_L sequence as comprised in SEQ ID NO: 313 (tagitanlimab); bo) the V_H sequence as comprised in SEQ ID NO: 314, and the V_L sequence as comprised in SEQ ID NO: 315 (tebentafusp); bp) the V_H sequence as comprised in SEQ ID NO: 316, and the V_L sequence as comprised in SEQ ID NO: 317 (Tislelizumab); bq) the V_H sequence as comprised in SEQ ID NO: 318, and the V_L sequence as comprised in SEQ ID NO: 319 (Toripalimab); br) the V_H sequence as comprised in SEQ ID NO: 320, and the V_L sequence as comprised in SEQ ID NO: 321 (zuberitamab);

bs) the V_H sequence as comprised in SEQ ID NO: 322, and the V_L sequence as comprised in SEQ ID NO: 323 (benmelstobart); bt) bu) bv) bw) the V_H sequence as comprised in SEQ ID NO: 324, and the V_L sequence as comprised in SEQ ID NO: 325 (iparomlimab); bx) the V_H sequence as comprised in SEQ ID NO: 326, and the V_L sequence as comprised in SEQ ID NO: 327 (tuvonralimab); by) the V_H sequence as comprised in SEQ ID NO: 328, and the V_L sequence as comprised in SEQ ID NO: 329 (anvatabart); bz) the V_H sequence as comprised in SEQ ID NO: 330, and the V_L sequence as comprised in SEQ ID NO: 331 (apamistamab); ca) the V_H sequence as comprised in SEQ ID NO: 332, and the V_L sequence as comprised in SEQ ID NO: 333 (bemarituzumab); cb) the V_H sequence as comprised in SEQ ID NO: 334, and the V_L sequence as comprised in SEQ ID NO: 335 (cetrelimab); cd) the V_H sequence as comprised in SEQ ID NO: 336, and the V_L sequence as comprised in SEQ ID NO: 337 (cobolimab); ce) the V_H sequence as comprised in SEQ ID NO: 338, and the V_L sequence as comprised in SEQ ID NO: 339 (datopotamab); cf) the V_H sequence as comprised in SEQ ID NO: 340, and the V_L sequence as comprised in SEQ ID NO: 341 (domvanalimab); cg) the V_H sequence as comprised in SEQ ID NO: 342, and the V_L sequence as comprised in SEQ ID NO: 343 (emactuzumab); ch) the V_H sequence as comprised in SEQ ID NO: 344, and the V_L sequence as comprised in SEQ ID NO: 345 (favezelimab); ci) the V_H sequence as comprised in SEQ ID NO: 346, and the V_L sequence as comprised in SEQ ID NO: 347 (felzartamab); cj) the V_H sequence as comprised in SEQ ID NO: 348, and the V_L sequence as comprised in SEQ ID NO: 349 (fianlimab); ck) the V_H sequence as comprised in SEQ ID NO: 350, and the V_L sequence as comprised in SEQ ID NO: 351 (finotonlimab); cl) the V_H sequence as comprised in SEQ ID NO: 352, and the V_L sequence as comprised in SEQ ID NO: 353 (geptanolimab); cm) the V_H sequence as comprised in SEQ ID NO: 354, and the V_L sequence as comprised in SEQ ID NO: 355 (gotistobart); cn) the V_H sequence as comprised in SEQ ID NO: 356, and the V_L sequence as comprised in SEQ ID NO: 357 (ivuxolimab); co) the V_H sequence as comprised in SEQ ID NO: 358, and the V_L sequence as comprised in SEQ ID NO: 359 (lemzoparlimab); cp) the V_H sequence as comprised in SEQ ID NO: 360, and the V_L sequence as comprised in SEQ ID NO: 361 (luveltamab); cq) the V_H sequence as comprised in SEQ ID NO: 362, and the V_L sequence as comprised in SEQ ID NO: 363 (magrolimab); cr) the V_H sequence as comprised in SEQ ID NO: 364, and the V_L sequence as comprised in SEQ ID NO: 365 (mecbotamab); cs) the V_H sequence as comprised in SEQ ID NO: 366, and the V_L sequence as comprised in SEQ ID NO: 367 (monalizumab); ct) the V_H sequence as comprised in SEQ ID NO: 368, and the V_L sequence as comprised in SEQ ID NO: 369 (nofazinlimab); cu) the V_H sequence as comprised in SEQ ID NO: 370, and the V_L sequence as comprised in SEQ ID NO: 371 (nurulimab); cv) the V_H sequence as comprised in SEQ ID NO: 372, and the V_L sequence as comprised in SEQ ID NO: 373 (ociperlimab); cw) the V_H sequence as comprised in SEQ ID NO: 374, and the V_L sequence as comprised in SEQ ID NO: 375 (oleclumab); cx) the V_H sequence as comprised in SEQ ID NO: 376, and the V_L sequence as comprised in SEQ ID NO: 377 (onfekafusp); cy) the V_H sequence as comprised in SEQ ID NO: 378, and the V_L sequence as comprised in

SEQ ID NO: 379 (patritumab); cz) the V_H sequence as comprised in SEQ ID NO: 380, and the V_L sequence as comprised in SEQ ID NO: 381 (pivekimab); da) the V_H sequence as comprised in SEQ ID NO: 382, and the V_L sequence as comprised in SEQ ID NO: 383 (quavonlimab); db) the V_H sequence as comprised in SEQ ID NO: 384, and the V_L sequence as comprised in SEQ ID NO: 385 (retlirafusp); dc) the V_H sequence as comprised in SEQ ID NO: 386, and the V_L sequence as comprised in SEQ ID NO: 387 (rosopatamab); dd) the V_H sequence as comprised in SEQ ID NO: 388, and the V_L sequence as comprised in SEQ ID NO: 389 (rulonilimab); de) the V_H sequence as comprised in SEQ ID NO: 390, and the V_L sequence as comprised in SEQ ID NO: 391 (sabatolimab); df) the V_H sequence as comprised in SEQ ID NO: 392, and the V_L sequence as comprised in SEQ ID NO: 393 (sasanlimab); dg) the V_H sequence as comprised in SEQ ID NO: 394, and the V_L sequence as comprised in SEQ ID NO: 395 (telisotuzumab); dh) the V_H sequence as comprised in SEQ ID NO: 396, and the V_L sequence as comprised in SEQ ID NO: 397 (tiragolumab); di) the V_H sequence as comprised in SEQ ID NO: 398, and the V_L sequence as comprised in SEQ ID NO: 399 (tusamitamab); dj) the V_H sequence as comprised in SEQ ID NO: 400, and the V_L sequence as comprised in SEQ ID NO: 401 (vibostolimab); dk) the V_H sequence as comprised in SEQ ID NO: 402, and the V_L sequence as comprised in SEQ ID NO: 403 (vobramitamab); dl) the V_H sequence as comprised in SEQ ID NO: 404, and the V_L sequence as comprised in SEQ ID NO: 405 (zilovertamab); dm) the V_H sequence as comprised in SEQ ID NO: 406, and the V_L sequence as comprised in SEQ ID NO: 407 (suvemcitug); dn) the V_H sequence as comprised in SEQ ID NO: 408, and the V_L sequence as comprised in SEQ ID NO: 409 (becotatug); do) the V_H sequence as comprised in SEQ ID NO: 410, and the V_L sequence as comprised in SEQ ID NO: 411 (tifcemalimab); dq) the V_H sequence as comprised in SEQ ID NO: 412, and the V_L sequence as comprised in SEQ ID NO: 413 (blinatumomab - CD19); dr) the V_H sequence as comprised in SEQ ID NO: 414, and the V_L sequence as comprised in SEQ ID NO: 415 (blinatumomab – CD3); ds) the V_H sequence as comprised in SEQ ID NO: 416, and the V_L sequence as comprised in SEQ ID NO: 417 (cadonilimab – PD-1); dt) the V_H sequence as comprised in SEQ ID NO: 418, and the V_L sequence as comprised in SEQ ID NO: 419 (cadonilimab – CTLA4); du) the V_H sequence as comprised in SEQ ID NO: 420, and the V_L sequence as comprised in SEQ ID NO: 421 (disitamab); dv) the V_H sequence as comprised in SEQ ID NO: 422, and the V_L sequence as comprised in SEQ ID NO: 423 (edrecolomab); dw) the V_H sequence as comprised in SEQ ID NO: 424, and the V_L sequence as comprised in SEQ ID NO: 425 (elranatamab – BCMA); dx) the V_H sequence as comprised in SEQ ID NO: 426, and the V_L sequence as comprised in SEQ ID NO: 427 (elranatamab – DC3); dy) the V_H sequence as comprised in SEQ ID NO: 428, and the V_L sequence as comprised in SEQ ID NO: 429 (epcoritamab – CD20); dz) the V_H sequence as comprised in SEQ ID NO: 430, and the V_L sequence as comprised in SEQ ID NO: 431 (epcoritamab – CD3); ea) the V_H sequence as comprised in SEQ ID NO: 432, and the V_L sequence as comprised in SEQ ID NO: 433 (glofitamab – VH1/VL1); eb); the V_H sequence as comprised in SEQ ID NO: 434, and the V_L sequence as comprised in SEQ ID NO: 435 (glofitamab –

VH2/VL2); ec) the V_H sequence as comprised in SEQ ID NO: 436, and the V_L sequence as comprised in SEQ ID NO: 437 (glofitamab – VH3/VL3); ed) the V_H sequence as comprised in SEQ ID NO: 438, and the V_L sequence as comprised in SEQ ID NO: 439 (mosunetuzumab – CD20); ef) the V_H sequence as comprised in SEQ ID NO: 440, and the V_L sequence as comprised in SEQ ID NO: 441 (mosunetuzumab – CD3); eg) the V_H sequence as comprised in SEQ ID NO: 442, and the V_L sequence as comprised in SEQ ID NO: 443 (talquetamab - GPCR5D); eh) the V_H sequence as comprised in SEQ ID NO: 444, and the V_L sequence as comprised in SEQ ID NO: 445 (talquetamab – CD3); ei) the V_H sequence as comprised in SEQ ID NO: 446, and the V_L sequence as comprised in SEQ ID NO: 447 (teclistamab - BCMA); ej) the V_H sequence as comprised in SEQ ID NO: 448, and the V_L sequence as comprised in SEQ ID NO: 449 (teclistamab – CD3); ek) the V_H sequence as comprised in SEQ ID NO: 450, and the V_L sequence as comprised in SEQ ID NO: 451 (tositumomab); el) the V_H sequence as comprised in SEQ ID NO: 452, and the V_L sequence as comprised in SEQ ID NO: 453 (tremelimumab); em) the V_H sequence as comprised in SEQ ID NO: 454, and the V_L sequence as comprised in SEQ ID NO: 455 (zimberelimab); en) the V_H sequence as comprised in SEQ ID NO: 456, and the V_L sequence as comprised in SEQ ID NO: 457 (odronextamab – CD20); eo) the V_H sequence as comprised in SEQ ID NO: 458, and the V_L sequence as comprised in SEQ ID NO: 459 (odronextamab – CD3); ep) the V_H sequence as comprised in SEQ ID NO: 460, and the V_L sequence as comprised in SEQ ID NO: 461 (ivonescimab – PD-1); eq) the V_H sequence as comprised in SEQ ID NO: 462, and the V_L sequence as comprised in SEQ ID NO: 463 (ivonescimab - VEGF); er) the V_H sequence as comprised in SEQ ID NO: 464, and the V_L sequence as comprised in SEQ ID NO: 465 (anbenitamab – VH1/VL1); es) the V_H sequence as comprised in SEQ ID NO: 466, and the V_L sequence as comprised in SEQ ID NO: 467 (anbenitamab – VH2/VL2); et) the V_H sequence as comprised in SEQ ID NO: 468, and the V_L sequence as comprised in SEQ ID NO: 469 (izalontamab – EGFR); eu) the V_H sequence as comprised in SEQ ID NO: 470, and the V_L sequence as comprised in SEQ ID NO: 471 (izalontamab – HER3); ev) the V_H sequence as comprised in SEQ ID NO: 472, and the V_L sequence as comprised in SEQ ID NO: 473 (linvoseltamab - BCMA); ew) the V_H sequence as comprised in SEQ ID NO: 474, and the V_L sequence as comprised in SEQ ID NO: 475 (linvoseltamab – CD3); ex) the V_H sequence as comprised in SEQ ID NO: 476, and the V_L sequence as comprised in SEQ ID NO: 477 (tarlatamab – DLL3); ey) the V_H sequence as comprised in SEQ ID NO: 478, and the V_L sequence as comprised in SEQ ID NO: 479 (tarlatamab – CD3); ez) the V_H sequence as comprised in SEQ ID NO: 480, and the V_L sequence as comprised in SEQ ID NO: 481 (zanidatamab – VH1/VL1); fa) the V_H sequence as comprised in SEQ ID NO: 482, and the V_L sequence as comprised in SEQ ID NO: 483 (zanidatamab – VH2/VL2); fb) the V_H sequence as comprised in SEQ ID NO: 484, and the V_L sequence as comprised in SEQ ID NO: 485 (volrustomig – PD-1); fc) the V_H sequence as comprised in SEQ ID NO: 486, and the V_L sequence as comprised in SEQ ID NO: 487 (volrustomig – CTLA-4); fd) the V_H sequence as comprised in SEQ ID NO: 488, and the V_L sequence as comprised in SEQ ID

NO: 489 (zenocutuzumab – HER3); fe) the V_H sequence as comprised in SEQ ID NO: 490, and the V_L sequence as comprised in SEQ ID NO: 491 (zenocutuzumab – HER2); ff) the V_H sequence as comprised in SEQ ID NO: 492, and the V_L sequence as comprised in SEQ ID NO: 493 (botensilimab - VH1/VL1); fg) the V_H sequence as comprised in SEQ ID NO: 494, and the V_L sequence as comprised in SEQ ID NO: 495 (botensilimab - VH2/VL2); fh) the V_H sequence as comprised in SEQ ID NO: 496, and the V_L sequence as comprised in SEQ ID NO: 497 (izalontamab - EGFR); fi) the V_H sequence as comprised in SEQ ID NO: 498, and the V_L sequence as comprised in SEQ ID NO: 499 (izalontamab – HER3); fj) the V_H sequence as comprised in SEQ ID NO: 500, and the V_L sequence as comprised in SEQ ID NO: 501 (rilvegostomig – TIGIT); fk) the V_H sequence as comprised in SEQ ID NO: 502, and the V_L sequence as comprised in SEQ ID NO: 503 (rilvegostomig – PD-1); fl) the VH sequence as comprised in SEQ ID NO: 701, and the VL sequence as comprised in SEQ ID NO: 700 (abagovomab); fm) the VH sequence as comprised in SEQ ID NO: 703, and the VL sequence as comprised in SEQ ID NO: 702 (abituzumab); fn) the VH sequence as comprised in SEQ ID NO: 705, and the VL sequence as comprised in SEQ ID NO: 704 (acasunlimab); fo) the VH sequence as comprised in SEQ ID NO: 707, and the VL sequence as comprised in SEQ ID NO: 706 (alnuctamab); fp) the VH sequence as comprised in SEQ ID NO: 709, and the VL sequence as comprised in SEQ ID NO: 708 (alomfilimab); fq) the VH sequence as comprised in SEQ ID NO: 711, and the VL sequence as comprised in SEQ ID NO: 710 (amatuximab); fr) the VH sequence as comprised in SEQ ID NO: 713, and the VL sequence as comprised in SEQ ID NO: 712 (anetumab); fs) the VH sequence as comprised in SEQ ID NO: 715, and the VL sequence as comprised in SEQ ID NO: 714 (aplitabart); ft) the VH sequence as comprised in SEQ ID NO: 717, and the VL sequence as comprised in SEQ ID NO: 716 (atigotatug); fu) the VH sequence as comprised in SEQ ID NO: 719, and the VL sequence as comprised in SEQ ID NO: 718 (balstilimab); fv) the VH sequence as comprised in SEQ ID NO: 721, and the VL sequence as comprised in SEQ ID NO: 720 (bavituximab); fw) the VH sequence as comprised in SEQ ID NO: 723, and the VL sequence as comprised in SEQ ID NO: 722 (bavunalimab); fx) the VH sequence as comprised in SEQ ID NO: 725, and the VL sequence as comprised in SEQ ID NO: 724 (belrestotug); fy) the VH sequence as comprised in SEQ ID NO: 727, and the VL sequence as comprised in SEQ ID NO: 726 (bermekimab); fz) the VH sequence as comprised in SEQ ID NO: 729, and the VL sequence as comprised in SEQ ID NO: 728 (bifikafusp); ga) the VH sequence as comprised in SEQ ID NO: 731, and the VL sequence as comprised in SEQ ID NO: 730 (bintrafusp); gb) the VH sequence as comprised in SEQ ID NO: 733, and the VL sequence as comprised in SEQ ID NO: 732 (brenetafusp); gc) the VH sequence as comprised in SEQ ID NO: 735, and the VL sequence as comprised in SEQ ID NO: 734 (briquilimab); gd) the VH sequence as comprised in SEQ ID NO: 737, and the VL sequence as comprised in SEQ ID NO: 736 (brontictuzumab); ge) the VH sequence as comprised in SEQ ID NO: 739, and the VL sequence as comprised in SEQ ID NO: 738 (budigalimab); gf) the VH sequence as comprised in SEQ ID NO: 741, and the VL sequence as comprised in SEQ ID NO: 740 (cabiralizumab); gg) the VH sequence

as comprised in SEQ ID NO: 743, and the VL sequence as comprised in SEQ ID NO: 742 (canakinumab); gh) the VH sequence as comprised in SEQ ID NO: 745, and the VL sequence as comprised in SEQ ID NO: 744 (cantuzumab); gi) the VH sequence as comprised in SEQ ID NO: 747, and the VL sequence as comprised in SEQ ID NO: 746 (carlumab); gj) the VH sequence as comprised in SEQ ID NO: 749, and the VL sequence as comprised in SEQ ID NO: 748 (carotuximab); gk) the VH sequence as comprised in SEQ ID NO: 751, and the VL sequence as comprised in SEQ ID NO: 750 (caxmotabart); gl) the VH sequence as comprised in SEQ ID NO: 753, and the VL sequence as comprised in SEQ ID NO: 752 (cergutuzumab); gm) the VH sequence as comprised in SEQ ID NO: 755, and the VL sequence as comprised in SEQ ID NO: 754 (cibisatamab); gn) the VH sequence as comprised in SEQ ID NO: 757, and the VL sequence as comprised in SEQ ID NO: 756 (cinrebafusp); go) the VH sequence as comprised in SEQ ID NO: 759, and the VL sequence as comprised in SEQ ID NO: 758 (cixutumumab); gp) the VH sequence as comprised in SEQ ID NO: 761, and the VL sequence as comprised in SEQ ID NO: 760 (clazakizumab); gq) the VH sequence as comprised in SEQ ID NO: 763, and the VL sequence as comprised in SEQ ID NO: 762 (clivatuzumab); gr) the VH sequence as comprised in SEQ ID NO: 765, and the VL sequence as comprised in SEQ ID NO: 764 (cofetuzumab); gs) the VH sequence as comprised in SEQ ID NO: 767, and the VL sequence as comprised in SEQ ID NO: 766 (coltuximab); gt) the VH sequence as comprised in SEQ ID NO: 769, and the VL sequence as comprised in SEQ ID NO: 768 (conatumumab); gu) the VH sequence as comprised in SEQ ID NO: 771, and the VL sequence as comprised in SEQ ID NO: 770 (dacetuzumab); gv) the VH sequence as comprised in SEQ ID NO: 773, and the VL sequence as comprised in SEQ ID NO: 772 (dalutrafusp); gw) the VH sequence as comprised in SEQ ID NO: 775, and the VL sequence as comprised in SEQ ID NO: 774 (danburstotug); gx) the VH sequence as comprised in SEQ ID NO: 777, and the VL sequence as comprised in SEQ ID NO: 776 (daratumumab); gy) the VH sequence as comprised in SEQ ID NO: 779, and the VL sequence as comprised in SEQ ID NO: 778 (demcizumab); gz) the VH sequence as comprised in SEQ ID NO: 781, and the VL sequence as comprised in SEQ ID NO: 780 (denintuzumab); ha) the VH sequence as comprised in SEQ ID NO: 783, and the VL sequence as comprised in SEQ ID NO: 782 (denosumab); hb) the VH sequence as comprised in SEQ ID NO: 785, and the VL sequence as comprised in SEQ ID NO: 784 (depatuxizumab); hc) the VH sequence as comprised in SEQ ID NO: 787, and the VL sequence as comprised in SEQ ID NO: 786 (drozitumab); hd) the VH sequence as comprised in SEQ ID NO: 789, and the VL sequence as comprised in SEQ ID NO: 788 (duligotuzumab); he) the VH sequence as comprised in SEQ ID NO: 791, and the VL sequence as comprised in SEQ ID NO: 790 (dusigitumab); hf) the VH sequence as comprised in SEQ ID NO: 793, and the VL sequence as comprised in SEQ ID NO: 792 (duvortuxizumab); hg) the VH sequence as comprised in SEQ ID NO: 795, and the VL sequence as comprised in SEQ ID NO: 794 (elotuzumab); hh) the VH sequence as comprised in SEQ ID NO: 797, and the VL sequence as comprised in SEQ ID NO: 796 (eluvixtamab); hi) the VH sequence as comprised

in SEQ ID NO: 799, and the VL sequence as comprised in SEQ ID NO: 798 (enapotamab); hj) the VH sequence as comprised in SEQ ID NO: 801, and the VL sequence as comprised in SEQ ID NO: 800 (enoticumab); hk) the VH sequence as comprised in SEQ ID NO: 803, and the VL sequence as comprised in SEQ ID NO: 802 (epacmarstobart); hl) the VH sequence as comprised in SEQ ID NO: 805, and the VL sequence as comprised in SEQ ID NO: 804 (etentamig); hm) the VH sequence as comprised in SEQ ID NO: 807, and the VL sequence as comprised in SEQ ID NO: 806 (falbikitug); hn) the VH sequence as comprised in SEQ ID NO: 809, and the VL sequence as comprised in SEQ ID NO: 808 (faricimab); ho) the VH sequence as comprised in SEQ ID NO: 811, and the VL sequence as comprised in SEQ ID NO: 810 (feladilimab); hp) the VH sequence as comprised in SEQ ID NO: 813, and the VL sequence as comprised in SEQ ID NO: 812 (ficerafusp); hq) the VH sequence as comprised in SEQ ID NO: 815, and the VL sequence as comprised in SEQ ID NO: 814 (ficlatuzumab); hr) the VH sequence as comprised in SEQ ID NO: 817, and the VL sequence as comprised in SEQ ID NO: 816 (figitumumab); hs) the VH sequence as comprised in SEQ ID NO: 819, and the VL sequence as comprised in SEQ ID NO: 818 (flanvotumab); ht) the VH sequence as comprised in SEQ ID NO: 821, and the VL sequence as comprised in SEQ ID NO: 820 (flotetuzumab); hu) the VH sequence as comprised in SEQ ID NO: 823, and the VL sequence as comprised in SEQ ID NO: 822 (forimtamig); hv) the VH sequence as comprised in SEQ ID NO: 825, and the VL sequence as comprised in SEQ ID NO: 824 (futuximab); hw) the VH sequence as comprised in SEQ ID NO: 827, and the VL sequence as comprised in SEQ ID NO: 826 (ganitumab); hx) the VH sequence as comprised in SEQ ID NO: 829, and the VL sequence as comprised in SEQ ID NO: 828 (gevokizumab); hy) the VH sequence as comprised in SEQ ID NO: 831, and the VL sequence as comprised in SEQ ID NO: 830 (girentuximab); hz) the VH sequence as comprised in SEQ ID NO: 833, and the VL sequence as comprised in SEQ ID NO: 832 (glembatumumab); ia) the VH sequence as comprised in SEQ ID NO: 835, and the VL sequence as comprised in SEQ ID NO: 834 (icrucumab); ib) the VH sequence as comprised in SEQ ID NO: 837, and the VL sequence as comprised in SEQ ID NO: 836 (ifinatamab); ic) the VH sequence as comprised in SEQ ID NO: 839, and the VL sequence as comprised in SEQ ID NO: 838 (iladatuzumab); id) the VH sequence as comprised in SEQ ID NO: 841, and the VL sequence as comprised in SEQ ID NO: 840 (imalumab); ie) the VH sequence as comprised in SEQ ID NO: 843, and the VL sequence as comprised in SEQ ID NO: 842 (imgatuzumab); if) the VH sequence as comprised in SEQ ID NO: 845, and the VL sequence as comprised in SEQ ID NO: 844 (indusatumab); ig) the VH sequence as comprised in SEQ ID NO: 847, and the VL sequence as comprised in SEQ ID NO: 846 (inebilizumab); ih) the VH sequence as comprised in SEQ ID NO: 849, and the VL sequence as comprised in SEQ ID NO: 848 (ispectamab); ii) the VH sequence as comprised in SEQ ID NO: 851, and the VL sequence as comprised in SEQ ID NO: 850 (istiratumab); ij) the VH sequence as comprised in SEQ ID NO: 853, and the VL sequence as comprised in SEQ ID NO: 852 (izeltabart); ik) the VH sequence as comprised in SEQ ID NO: 855, and the VL sequence as comprised in SEQ ID NO: 854 (izuralimab); il)

the VH sequence as comprised in SEQ ID NO: 857, and the VL sequence as comprised in SEQ ID NO: 856 (landogrozumab); im) the VH sequence as comprised in SEQ ID NO: 859, and the VL sequence as comprised in SEQ ID NO: 858 (laprituximab); in) the VH sequence as comprised in SEQ ID NO: 861, and the VL sequence as comprised in SEQ ID NO: 860 (lenzilumab); io) the VH sequence as comprised in SEQ ID NO: 863, and the VL sequence as comprised in SEQ ID NO: 862 (leronlimab); ip) the VH sequence as comprised in SEQ ID NO: 865, and the VL sequence as comprised in SEQ ID NO: 864 (lifastuzumab); iq) the VH sequence as comprised in SEQ ID NO: 867, and the VL sequence as comprised in SEQ ID NO: 866 (ligufalimab); ir) the VH sequence as comprised in SEQ ID NO: 869, and the VL sequence as comprised in SEQ ID NO: 868 (lilotomab); is) the VH sequence as comprised in SEQ ID NO: 871, and the VL sequence as comprised in SEQ ID NO: 870 (lintuzumab); it) the VH sequence as comprised in SEQ ID NO: 873, and the VL sequence as comprised in SEQ ID NO: 872 (lirilumab); iu) the VH sequence as comprised in SEQ ID NO: 875, and the VL sequence as comprised in SEQ ID NO: 874 (livmoniplimab); iv) the VH sequence as comprised in SEQ ID NO: 877, and the VL sequence as comprised in SEQ ID NO: 876 (lorvotuzumab); iw) the VH sequence as comprised in SEQ ID NO: 879, and the VL sequence as comprised in SEQ ID NO: 878 (lucatumumab); ix) the VH sequence as comprised in SEQ ID NO: 881, and the VL sequence as comprised in SEQ ID NO: 880 (lumretuzumab); iy) the VH sequence as comprised in SEQ ID NO: 883, and the VL sequence as comprised in SEQ ID NO: 882 (matuzumab); iz) the VH sequence as comprised in SEQ ID NO: 885, and the VL sequence as comprised in SEQ ID NO: 884 (mipasetamab); ja) the VH sequence as comprised in SEQ ID NO: 887, and the VL sequence as comprised in SEQ ID NO: 886 (modakafusp); jb) the VH sequence as comprised in SEQ ID NO: 889, and the VL sequence as comprised in SEQ ID NO: 888 (modotuximab); jc) the VH sequence as comprised in SEQ ID NO: 891, and the VL sequence as comprised in SEQ ID NO: 890 (murlentamab); jd) the VH sequence as comprised in SEQ ID NO: 893, and the VL sequence as comprised in SEQ ID NO: 892 (nadunolimab); je) the VH sequence as comprised in SEQ ID NO: 895, and the VL sequence as comprised in SEQ ID NO: 894 (naptumomab); jf) the VH sequence as comprised in SEQ ID NO: 897, and the VL sequence as comprised in SEQ ID NO: 896 (narlumosbart); jg) the VH sequence as comprised in SEQ ID NO: 899, and the VL sequence as comprised in SEQ ID NO: 898 (narnatumab); jh) the VH sequence as comprised in SEQ ID NO: 901, and the VL sequence as comprised in SEQ ID NO: 900 (navicixizumab); ji) the VH sequence as comprised in SEQ ID NO: 903, and the VL sequence as comprised in SEQ ID NO: 902 (nesvacumab); jj) the VH sequence as comprised in SEQ ID NO: 905, and the VL sequence as comprised in SEQ ID NO: 904 (nisevokitug); jk) the VH sequence as comprised in SEQ ID NO: 907, and the VL sequence as comprised in SEQ ID NO: 906 (omburtamab); jl) the VH sequence as comprised in SEQ ID NO: 909, and the VL sequence as comprised in SEQ ID NO: 908 (ontuxizumab); jm) the VH sequence as comprised in SEQ ID NO: 911, and the VL sequence as comprised in SEQ ID NO: 910 (otlertuzumab); jn) the VH sequence as comprised in SEQ ID NO: 913, and the VL sequence as comprised in SEQ

ID NO: 912 (pamrevlumab); jo) the VH sequence as comprised in SEQ ID NO: 915, and the VL sequence as comprised in SEQ ID NO: 914 (parsatuzumab); jp) the VH sequence as comprised in SEQ ID NO: 917, and the VL sequence as comprised in SEQ ID NO: 916 (pavurutamab); jq) the VH sequence as comprised in SEQ ID NO: 919, and the VL sequence as comprised in SEQ ID NO: 918 (pemivibart); jr) the VH sequence as comprised in SEQ ID NO: 921, and the VL sequence as comprised in SEQ ID NO: 920 (petosemtamab); js) the VH sequence as comprised in SEQ ID NO: 923, and the VL sequence as comprised in SEQ ID NO: 922 (pimivalimab); jt) the VH sequence as comprised in SEQ ID NO: 925, and the VL sequence as comprised in SEQ ID NO: 924 (pinatuzumab); ju) the VH sequence as comprised in SEQ ID NO: 927, and the VL sequence as comprised in SEQ ID NO: 926 (plozalizumab); jv) the VH sequence as comprised in SEQ ID NO: 929, and the VL sequence as comprised in SEQ ID NO: 928 (pulocimab); jw) the VH sequence as comprised in SEQ ID NO: 931, and the VL sequence as comprised in SEQ ID NO: 930 (ragifilimab); jx) the VH sequence as comprised in SEQ ID NO: 933, and the VL sequence as comprised in SEQ ID NO: 932 (raludotatug); jy) the VH sequence as comprised in SEQ ID NO: 935, and the VL sequence as comprised in SEQ ID NO: 934 (rilotumumab); jz) the VH sequence as comprised in SEQ ID NO: 937, and the VL sequence as comprised in SEQ ID NO: 936 (rosmantuzumab); ka) the VH sequence as comprised in SEQ ID NO: 939, and the VL sequence as comprised in SEQ ID NO: 938 (runimotamab); kb) the VH sequence as comprised in SEQ ID NO: 941, and the VL sequence as comprised in SEQ ID NO: 940 (sabestomig); kc) the VH sequence as comprised in SEQ ID NO: 943, and the VL sequence as comprised in SEQ ID NO: 942 (selicrelumab); kd) the VH sequence as comprised in SEQ ID NO: 945, and the VL sequence as comprised in SEQ ID NO: 944 (seribantumab); ke) the VH sequence as comprised in SEQ ID NO: 947, and the VL sequence as comprised in SEQ ID NO: 946 (sigvotatug); kf) the VH sequence as comprised in SEQ ID NO: 949, and the VL sequence as comprised in SEQ ID NO: 948 (simlukafusp); kg) the VH sequence as comprised in SEQ ID NO: 951, and the VL sequence as comprised in SEQ ID NO: 950 (simtuzumab); kh) the VH sequence as comprised in SEQ ID NO: 953, and the VL sequence as comprised in SEQ ID NO: 952 (sirexatamab); ki) the VH sequence as comprised in SEQ ID NO: 955, and the VL sequence as comprised in SEQ ID NO: 954 (sofituzumab); kj) the VH sequence as comprised in SEQ ID NO: 957, and the VL sequence as comprised in SEQ ID NO: 956 (spartalizumab); kk) the VH sequence as comprised in SEQ ID NO: 959, and the VL sequence as comprised in SEQ ID NO: 958 (surzebiclimab); kl) the VH sequence as comprised in SEQ ID NO: 961, and the VL sequence as comprised in SEQ ID NO: 960 (tabalumab); km) the VH sequence as comprised in SEQ ID NO: 963, and the VL sequence as comprised in SEQ ID NO: 962 (tafolecimab); kn) the VH sequence as comprised in SEQ ID NO: 965, and the VL sequence as comprised in SEQ ID NO: 964 (talacotuzumab); ko) the VH sequence as comprised in SEQ ID NO: 967, and the VL sequence as comprised in SEQ ID NO: 966 (tarextumab); kp) the VH sequence as comprised in SEQ ID NO: 969, and the VL sequence as comprised in SEQ ID NO: 968 (tavolimab); kq) the VH sequence as

comprised in SEQ ID NO: 971, and the VL sequence as comprised in SEQ ID NO: 970 (tebotelimab); kr) the VH sequence as comprised in SEQ ID NO: 973, and the VL sequence as comprised in SEQ ID NO: 972 (teprotumumab); ks) the VH sequence as comprised in SEQ ID NO: 975, and the VL sequence as comprised in SEQ ID NO: 974 (tidutamab); kt) the VH sequence as comprised in SEQ ID NO: 977, and the VL sequence as comprised in SEQ ID NO: 976 (tigatuzumab); ku) the VH sequence as comprised in SEQ ID NO: 979, and the VL sequence as comprised in SEQ ID NO: 978 (tilvestamab); kv) the VH sequence as comprised in SEQ ID NO: 981, and the VL sequence as comprised in SEQ ID NO: 980 (tobemstomig); kw) the VH sequence as comprised in SEQ ID NO: 983, and the VL sequence as comprised in SEQ ID NO: 982 (tocilizumab); kx) the VH sequence as comprised in SEQ ID NO: 985, and the VL sequence as comprised in SEQ ID NO: 984 (tomaralimab); ky) the VH sequence as comprised in SEQ ID NO: 987, and the VL sequence as comprised in SEQ ID NO: 986 (tovecimig); kz) the VH sequence as comprised in SEQ ID NO: 989, and the VL sequence as comprised in SEQ ID NO: 988 (tovetumab); la) the VH sequence as comprised in SEQ ID NO: 991, and the VL sequence as comprised in SEQ ID NO: 990 (tucotuzumab); lb) the VH sequence as comprised in SEQ ID NO: 993, and the VL sequence as comprised in SEQ ID NO: 992 (tuparstobart); lc) the VH sequence as comprised in SEQ ID NO: 995, and the VL sequence as comprised in SEQ ID NO: 994 (upifitamab); ld) the VH sequence as comprised in SEQ ID NO: 997, and the VL sequence as comprised in SEQ ID NO: 996 (urabrelimab); le) the VH sequence as comprised in SEQ ID NO: 999, and the VL sequence as comprised in SEQ ID NO: 998 (utomilumab); lf) the VH sequence as comprised in SEQ ID NO: 1001, and the VL sequence as comprised in SEQ ID NO: 1000 (vadastuximab); lg) the VH sequence as comprised in SEQ ID NO: 1003, and the VL sequence as comprised in SEQ ID NO: 1002 (vandortuzumab); lh) the VH sequence as comprised in SEQ ID NO: 1005, and the VL sequence as comprised in SEQ ID NO: 1004 (vanucizumab); li) the VH sequence as comprised in SEQ ID NO: 1007, and the VL sequence as comprised in SEQ ID NO: 1006 (veligrotug); lj) the VH sequence as comprised in SEQ ID NO: 1009, and the VL sequence as comprised in SEQ ID NO: 1008 (verzistobart); lk) the VH sequence as comprised in SEQ ID NO: 1011, and the VL sequence as comprised in SEQ ID NO: 1010 (vesencumab); ll) the VH sequence as comprised in SEQ ID NO: 1013, and the VL sequence as comprised in SEQ ID NO: 1012 (vofatamab); lm) the VH sequence as comprised in SEQ ID NO: 1015, and the VL sequence as comprised in SEQ ID NO: 1014 (vonlerolizumab); ln) the VH sequence as comprised in SEQ ID NO: 1017, and the VL sequence as comprised in SEQ ID NO: 1016 (vopikitug); lo) the VH sequence as comprised in SEQ ID NO: 1019, and the VL sequence as comprised in SEQ ID NO: 1018 (vorsetuzumab); lp) the VH sequence as comprised in SEQ ID NO: 1021, and the VL sequence as comprised in SEQ ID NO: 1020 (xaluritamig); lq) the VH sequence as comprised in SEQ ID NO: 1023, and the VL sequence as comprised in SEQ ID NO: 1022 (zalutumumab); lr) the VH sequence as comprised in SEQ ID NO: 1025, and the VL sequence as comprised in SEQ ID NO: 1024 (zanolimumab); ls) the VH sequence as comprised in SEQ ID NO: 1027, and the VL sequence as comprised in SEQ ID NO: 1026

(ivuxolimab-alt); lt) the VH sequence as comprised in SEQ ID NO: 1029, and the VL sequence as comprised in SEQ ID NO: 1028 (inotuzumab-alt); lu) the VH sequence as comprised in SEQ ID NO: 1031, and the VL sequence as comprised in SEQ ID NO: 1030 (moxetumomab-alt); lv) the VH sequence as comprised in SEQ ID NO: 1033, and the VL sequence as comprised in SEQ ID NO: 1032 (luveltamab-alt); lw) the VH sequence as comprised in SEQ ID NO: 1035, and the VL sequence as comprised in SEQ ID NO: 1034 (ibritumomab-alt); lx) the VH sequence as comprised in SEQ ID NO: 1037, and the VL sequence as comprised in SEQ ID NO: 1036 (pivekimab-alt); ly) the VH sequence as comprised in SEQ ID NO: 1039, and the VL sequence as comprised in SEQ ID NO: 1038 (avelumab-alt); lz) the VH sequence as comprised in SEQ ID NO: 1041, and the VL sequence as comprised in SEQ ID NO: 1040 (sugemalimab-alt); ma) the VH sequence as comprised in SEQ ID NO: 1043, and the VL sequence as comprised in SEQ ID NO: 1042 (nimotuzumab-alt); mb) the VH sequence as comprised in SEQ ID NO: 1045, and the VL sequence as comprised in SEQ ID NO: 1044 (panitumumab-alt); mc) the V_H sequence as comprised in SEQ ID NO: 540, and the V_L sequence as comprised in SEQ ID NO: 541 (AR46A6); md) the V_H sequence as comprised in SEQ ID NO: 542, and the V_L sequence as comprised in SEQ ID NO: 543 (KM4097); and me) the V_H sequence as comprised in SEQ ID NO: 544, and the V_L sequence as comprised in SEQ ID NO: 545 (K5-70).

38. A conjugate according to any one of embodiments 28 – 37, wherein at least one of the first and second antigen-binding region specifically binds an NK cell activating receptor, and wherein preferably at least one of the first and second antigen-binding region is an agonistic antigen-binding region that activates the NK cell receptor.

39. A conjugate according to any one of embodiments 28 – 38, wherein the antigen binding protein comprises two antigen-binding regions that specifically bind an NK cell activating receptor.

40. A conjugate according to embodiment 39, wherein the two antigen-binding regions bind the same NK cell activating receptor or at least two different NK cell activating receptors.

41. A conjugate according to embodiment 40, wherein the two antigen binding regions are identical.

42. A conjugate according to any one of embodiments 28 – 41, wherein the NK cell activating receptor is selected from the group consisting of NKp46, NKp30, NKG2D, CD16A, SLAMF7, NKp44, CD94-NKG2C/E, KIR2DS1, KIR2DS3, KIR2DS4, KIR2DS5, KIR2DS2, KIR2DL4, KIR3DS1, CD160, NKp80, DNAM1, 2B4, CD59, PD-L1, Tim3, CRACC, 4-BB, OX40, CRTAM, CD27, PSGL1, CD96, CD100, CEACAM1, and NTB-A.

43. A conjugate according to any one of embodiments 28 – 37, wherein at least one of the first and second antigen-binding region specifically binds specifically binds an epitope of a γδ TCR.

44. A conjugate according to embodiment 43, at least one first antigen-binding region specifically binds a TAA and at least one second antigen-binding region specifically binds an epitope of a γδ TCR.

45. A conjugate according to any one of embodiments 28 – 44, wherein the antigen-binding region that specifically binds an epitope of a γδ TCR, binds an epitope of at least one of:

a) a variable region (V) of a delta (δ) chain selected from the group consisting of: Vδ1, Vδ2 and Vδ3 chains;

b) a V region of a gamma (γ) chain selected from the group consisting of: Vγ2, Vγ3, Vγ4, Vγ5, Vγ8, and Vγ9 chains;

c) a constant (C) region of a γ chain; and,

d) a C region of a δ chain.

46. A conjugate according to any one of embodiments 28 – 45, wherein the antigen-binding region that specifically binds an epitope of a γδ TCR, binds an epitope in the V region of a Vδ1 chain of a γδ TCR.

47. A conjugate according to any one of embodiments 28 – 46, wherein the antigen-binding region that specifically binds an epitope of a γδ TCR, binds an activating epitope of a γδ T cell.

48. A conjugate according to embodiment 47, wherein binding of the antigen-binding region to the activating epitope: (i) downregulates the γδ TCR; (ii) activates degranulation of the γδ T cell; (iii) induces proliferation in a γδ T cell; and/or (iv) promotes γδ T cell mediated killing.

49. A conjugate according to embodiment 47 or 48, wherein the antigen-binding region specifically binds an epitope that upregulates expression of CD107a, CD25, CD56, CD69 and/or Ki67.

50. A conjugate according to any one of embodiments 28 – 49, wherein the antigen-binding region that specifically binds an epitope of a γδ TCR, binds the epitope of a γδ TCR with a binding affinity (K_D) as measured by surface plasmon resonance of less than 1.5×10^{-7} M, preferably less than 1.0×10^{-7} M or less, such as less than 5.0×10^{-8} M, in particular less than 1.0×10^{-8} M.

51. A conjugate according to any one of embodiments 28 – 50, wherein the antigen-binding region that specifically binds an epitope of a γδ TCR, binds the epitope of a γδ TCR with a K_D of more than 1.5×10^{-7} M, preferably more than 1.0×10^{-6} M, such as more than 5.0×10^{-6} M, in particular more than 1.0×10^{-5} M.

52. A conjugate according to any one of embodiments 46 – 51, wherein the antigen-binding region that specifically binds an epitope of a γδ TCR the third antigen-binding region comprises a combination of complementarity-determining regions (CDRs) CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of:

a) the CDR-H1 sequence of SEQ ID NO: 511, the CDR-H2 sequence of SEQ ID NO: 512, CDR-H3 sequence of SEQ ID NO: 513, the CDR-L1 sequence of SEQ ID NO: 514, the CDR-L2 sequence of SEQ ID NO: 515 and the CDR-L3 sequence of SEQ ID NO: 516;

b) the CDR-H1 sequence of SEQ ID NO: 517, the CDR-H2 sequence of SEQ ID NO: 518, CDR-H3 sequence of SEQ ID NO: 519, the CDR-L1 sequence of SEQ ID NO: 520, the CDR-L2 sequence of SEQ ID NO: 521 and the CDR-L3 sequence of SEQ ID NO: 522;

c) the CDR-H1 sequence of SEQ ID NO: 523, the CDR-H2 sequence of SEQ ID NO: 524, CDR-H3 sequence of SEQ ID NO: 525, the CDR-L1 sequence of SEQ ID NO: 526, the CDR-L2 sequence of SEQ ID NO: 527 and the CDR-L3 sequence of SEQ ID NO: 528,

or wherein the third antigen-binding region comprises a combination of variable light (V_L) and variable heavy (V_H) domains selected from the group consisting of:

d) the V_H sequence as comprised in SEQ ID NO: 529, and the V_L sequence as comprised in SEQ ID NO: 530;

e) the V_H sequence as comprised in SEQ ID NO: 531, and the V_L sequence as comprised in SEQ ID NO: 532;

f) the V_H sequence as comprised in SEQ ID NO: 533, and the V_L sequence as comprised in SEQ ID NO: 534.

53. A conjugate according to any one of embodiments 28 – 52, wherein the third antigen-binding region comprises or consists of i) an immunoglobulin Fc region or ii) an antigen-binding region that specifically binds a surface antigen expressed on NK cells.

54. A conjugate according to embodiment 53, wherein the surface antigen expressed on NK cells is an NK cell activating receptor.

55. A conjugate according to embodiment 54, wherein the Fc region is a dimeric Fc region.

56. A conjugate according to any one of embodiments 53 – 55, wherein the Fc region binds to CD16A.

57. A conjugate according to embodiment 56, wherein the Fc region is modified to reduce or enhance affinity for CD16A, relative to a corresponding wild-type Fc region.

58. A conjugate according to embodiment 57, wherein the Fc region is modified to reduce or enhance NK cell activation through CD16A binding, relative to a corresponding wild-type Fc region.

59. A conjugate according to any one of embodiments 52 – 58, wherein the third antigen-binding region activates the NK cell activating receptor.

60. A conjugate according to any one of embodiments 43 – 52, wherein the third antigen-binding region comprises or consists of an immunoglobulin Fc region, preferably is dimeric Fc region, that is modified to reduce or abolish affinity for CD16A, relative to a corresponding wild-type Fc region.

61. A conjugate according to any one of embodiments 13 – 60, comprising, in addition to the 4-1BBL ECD mutein, at least one further NK cell activating cytokine.

62. A conjugate according to embodiment 61, wherein the further NK cell activating cytokine is the heterologous moiety.

63. A conjugate according to embodiment 61, wherein the further NK cell activating cytokine is directly attached to the heterologous moiety or wherein the further NK cell activating cytokine is attached to the heterologous moiety via a linker.

64. A conjugate according to any one of embodiments 61 – 63, wherein the further agonist is at least one of:

i) a further NK cell activating cytokine selected from the group consisting of: an IL-21 receptor agonist, an IL-15 receptor agonist, a type I interferon (IFN-1) receptor agonist, an IL-2 receptor agonist, an IL-12 receptor agonist and an IL-18 receptor agonist;

ii) a γδ T cell-activating agonist selected from the group consisting of: an IL-21 receptor agonist, an IL-15 receptor agonist, a type I interferon (IFN-1) receptor agonist, an IL-2 receptor agonist, an IL-12 receptor agonist and an IL-18 receptor agonist; and,

iii) a further γδ T cell co-stimulatory agonist selected from the group consisting of: a CD27 agonist and a GITR agonist.

65. A conjugate according to embodiment 64, wherein:

i) the IL-21R agonist comprises or consist of an IL-21 polypeptide or an agonistic antigen-binding region that specifically binds IL-21R; or,

ii) the IL-15R agonist comprises or consist of an IL-15 polypeptide or an agonistic antigen-binding region that specifically binds IL-15R;

iii) the type I interferon (IFN-1) receptor agonist comprises or consists of an IFN-1 polypeptide or an agonistic antigen-binding region that specifically binds the IFN-α receptor;

iv) the IL-2R agonist comprises or consists of an IL-2 polypeptide or an agonistic antigen-binding region that specifically binds IL-2R;

v) the IL-12R agonist comprises or consists of an IL-12 polypeptide or an agonistic antigen-binding region that specifically binds IL-12R;

vi) the IL-18R agonist comprises or consists of an IL-18 polypeptide or an agonistic antigen-binding region that specifically binds IL-18R;

vii) the CD27 agonist comprises or consists of at least one CD70 extracellular domain (ECD) or agonistic antigen-binding region that specifically binds CD27; and,

viii) the GITR agonist comprises or consists of at least one GIRTL extracellular domain (ECD) or at least one agonistic antigen-binding region that specifically binds GITR.

66. A conjugate according to embodiment 65, wherein:

i) the IL-21 polypeptide comprises an amino acid sequence with at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 38, and preferably has at least one of IL-21R agonist activity and affinity for the IL-21R; or,

ii) the IL-15 polypeptide comprises an amino acid sequence with at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 169, and preferably has at least one of IL-15R agonist activity and affinity for the IL-15R;

iiii) the IL-2 polypeptide comprises an amino acid sequence with at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 351, and preferably has at least one of IL-2R agonist activity and affinity for the IL-2R;

iv) the IL-12 polypeptide comprises an IL-12α polypeptide comprising an amino acid sequence with at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 352, and an IL-12β polypeptide comprising an amino acid sequence with at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100%

sequence identity to SEQ ID NO: 353, wherein preferably, in an N- to C-terminal direction, the IL-12β polypeptide is linked, optionally through a peptidyl linker, to the IL-12α polypeptide, and wherein the IL-12 polypeptide preferably has at least one of IL-12R agonist activity and affinity for the IL-12R;

v) the IL-18 polypeptide comprises an amino acid sequence with at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 354, and preferably has at least one of IL-18R agonist activity and affinity for the IL-18R;

vi) the CD70 ECD comprises an amino acid sequence with at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 536, and preferably has at least one of CD27 agonist activity and affinity for CD27; and,

vii) the GITRL ECD comprises an amino acid sequence with at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 539, and preferably has at least one of GITR agonist activity and affinity for GITR.

67. A conjugate according to embodiment 65 or 66, wherein:

i) the IL-21 polypeptide is an IL-21 mutein that is modified to enhance or preferably reduce or affinity for IL-21R, relative to a corresponding wild type IL-21 polypeptide; or,

ii) the IL-15 polypeptide is an IL-15 mutein that is modified to enhance or preferably reduce affinity for IL-15R, relative to a corresponding wild type IL-15 polypeptide;

iii) the IFN-1 polypeptide is an IFN-1 mutein that is modified to reduce (or enhance) affinity for the IFN-α receptor, relative to a corresponding wild type IFN-1 polypeptide;

iv) the IL-2 polypeptide is an IL-2 mutein that is modified to reduce (or enhance) affinity for IL-2R, relative to a corresponding wild type IL-2 polypeptide;

v) the IL-12 polypeptide is an IL-12 mutein that is modified to reduce (or enhance) affinity for IL-12R, relative to a corresponding wild type IL-12 polypeptide;

vi) the IL-18 polypeptide is an IL-18 mutein that is modified to reduce (or enhance) affinity for IL-18R, relative to a corresponding wild type IL-18 polypeptide;

vii) the CD70 ECD is a mutein that is modified to reduce or enhance affinity for CD27, improve stability or improve expression, relative to a corresponding wild type CD70 ECD; and,

viii) the GITRL ECD is a mutein that is modified to reduce or enhance affinity for GITR, improve stability or improve expression, relative to a corresponding wild type GITRL ECD.

68. A conjugate according to embodiment 67, wherein:

i) the IL-21 mutein comprises at least one amino acid substitution, deletion or insertion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-), L20W, L74D, L20N, I67N, L20S, L13E, I8H, (N63-, E64-, R65- and I66-), L74F, I8V, I8Q, I8F, I8W, I8Y, I8L, D4H, D4R, D4K, D4Q, D4N, R11E, R11Q, R11N, R11Y, Q12K, Q12R, L13S, L13V, L13T, L13G, Q19S, Q19E, Q19K, Q19R, Q19H, Q19G, Q19T, L20D, L20E, L20R, L20K, L20Q, L20H, L20G, K21H, K21N, K21Q, K21E, K21D, I67T, I67D, I67E, I67K, I67R, I67Q, I67S, I67G, L74G, L74E, L74K, L74R, L74N, L74Q, L74S, L74P, K75E, K75Q, K75N, K75S, K75-, K76-, K112H, K112N, K112Q, K112E, K112D, N59-, T60-,

G61-, N62-, N63-, E64-, R65-, I66-, (N59-, T60-, G61-, N62-, N63-, E64-, R65- and I66-), (K75- and R76-), (K75-, R76- and R77-), G84 insGGGG, and, K75 insX (wherein X is one, two or three amino acids selected from the group consisting of G, S and D), or wherein the IL-21 mutein comprises no other amino acid sequence modification than the aforementioned amino acid substitutions, deletions or insertions;

ii) the IL-15 mutein comprises a mutation in at least one amino acid position selected from the group consisting of: D8, E64, N65, I68, and L69; and,

iii) the CD70 ECD mutein comprises at least one amino acid substitution selected from the group consisting of: Q61A, A80F, A80R, S137A, S137E, S137K, S146A, H148A, H148E and H148D.

69. A conjugate according to embodiment 68, wherein the IL-21 mutein comprises at least one amino acid substitution or deletion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-); L20W; L74D; L20N; I67N; L20S; L13E; I8H; (N63- E64- R65- I66-); and L74F, or wherein the IL-21 mutein comprises no other amino acid sequence modification than the aforementioned amino acid substitutions or deletions.

70. A conjugate according to embodiment 69, wherein the IL-21 mutein comprises at least one amino acid substitution selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-), L20W; L74D; L20N; I67N; L20S; and L13E, or wherein the IL-21 mutein comprises no other amino acid sequence modification than the aforementioned amino acid substitutions.

71. A conjugate according to embodiment 70, wherein the IL-21 mutein comprises at least one amino acid substitution selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-), L20W; L74D; L20N; and I67N, or wherein the IL-21 mutein comprises no other amino acid sequence modification than the aforementioned amino acid substitutions.

72. A conjugate according to embodiment 71, wherein the IL-21 mutein, when present in a conjugate with trastuzumab, which conjugate further comprises a trimer of an 4-1BBL ECD mutein comprising a substitution A154D, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of SKOV3 tumor cells, which proliferation is not less than 89% of the proliferation induced by a corresponding control conjugate comprising wild-type IL-21 and a trimer of wild type 4-1BBL ECD in the same assay.

73. A conjugate according to embodiment 72, wherein the IL-21 mutein comprises a (N82- A83- G84- R85- R86- Q87- K88-) deletion, or an L20W amino acid substitution, or wherein the IL-21 mutein comprises no other modification than the (N82- A83- G84- R85- R86- Q87- K88-) deletion, or the L20W substitution.

74. A conjugate according to embodiment 73, wherein the IL-21 mutein comprises a (N82- A83- G84- R85- R86- Q87- K88-) deletion or wherein the IL-21 mutein comprises no other modification than the (N82- A83- G84- R85- R86- Q87- K88-) deletion.

75. A conjugate according to any one of embodiments 65 – 74, wherein the conjugate comprises a combination of a 4-1BBL ECD mutein and an IL-21 mutein, wherein the 4-1BBL ECD

mutein is selected from the group consisting of: 4-1BBL mutein A154D; 4-1BBL mutein A154E; 4-1BBL mutein V153Q; 4-1BBL mutein Q227E; 4-1BBL mutein Q227R; 4-1BBL mutein L101N; 4-1BBL mutein Y110Q; 4-1BBL mutein Q230K; and 4-1BBL mutein V100Q; and wherein the IL-21 mutein is selected from the group consisting of: IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); IL-21 mutein L20W; IL-21 mutein L74D; IL-21 mutein L20N; IL-21 mutein I67N; IL-21 mutein L20S; IL-21 mutein L13E; IL-21 mutein I8H; IL-21 mutein (N63- E64- R65- I66-); and IL-21 mutein L74F.

76. A conjugate according to embodiment 75, wherein the 4-1BBL ECD mutein and the IL-21 mutein are each conjugated to an antigen binding protein, whereby preferably, the 4-1BBL ECD mutein is present as a hetero- or homotrimer of 4-1BBL ECDs connected through polypeptide linkers.

77. A conjugate according to embodiment 75 or 76, wherein the conjugate comprises a combination of a 4-1BBL ECD mutein and an IL-21 mutein selected from the group consisting of: 4-1BBL mutein A154D and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154D and IL-21 mutein L20W; 4-1BBL mutein A154D and IL-21 mutein L74D; 4-1BBL mutein A154D and IL-21 mutein L20N; 4-1BBL mutein A154D and IL-21 mutein I67N; 4-1BBL mutein A154D and IL-21 mutein L20S; 4-1BBL mutein A154D and IL-21 mutein L13E; 4-1BBL mutein A154D and IL-21 mutein I8H; 4-1BBL mutein A154D and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein A154D and IL-21 mutein L74F; 4-1BBL mutein A154E and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154E and IL-21 mutein L20W; 4-1BBL mutein A154E and IL-21 mutein L74D; 4-1BBL mutein A154E and IL-21 mutein L20N; 4-1BBL mutein A154E and IL-21 mutein I67N; 4-1BBL mutein A154E and IL-21 mutein L20S; 4-1BBL mutein A154E and IL-21 mutein L13E; 4-1BBL mutein A154E and IL-21 mutein I8H; 4-1BBL mutein A154E and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein A154E and IL-21 mutein L74F; 4-1BBL mutein V153Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein V153Q and IL-21 mutein L20W; 4-1BBL mutein V153Q and IL-21 mutein L74D; 4-1BBL mutein V153Q and IL-21 mutein L20N; 4-1BBL mutein V153Q and IL-21 mutein I67N; 4-1BBL mutein V153Q and IL-21 mutein L20S; 4-1BBL mutein V153Q and IL-21 mutein L13E; 4-1BBL mutein V153Q and IL-21 mutein I8H; 4-1BBL mutein V153Q and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein V153Q and IL-21 mutein L74F; 4-1BBL mutein Q227E and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Q227E and IL-21 mutein L20W; 4-1BBL mutein Q227E and IL-21 mutein L74D; 4-1BBL mutein Q227E and IL-21 mutein L20N; 4-1BBL mutein Q227E and IL-21 mutein I67N; 4-1BBL mutein Q227E and IL-21 mutein L20S; 4-1BBL mutein Q227E and IL-21 mutein L13E; 4-1BBL mutein Q227E and IL-21 mutein I8H; 4-1BBL mutein Q227E and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Q227E and IL-21 mutein L74F; 4-1BBL mutein Q227R and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Q227R and IL-21 mutein L20W; 4-1BBL mutein Q227R and IL-21 mutein L74D; 4-1BBL mutein Q227R and IL-21 mutein L20N; 4-1BBL mutein Q227R and IL-21 mutein I67N; 4-1BBL mutein Q227R and IL-21 mutein L20S; 4-1BBL mutein Q227R and IL-21 mutein

L13E; 4-1BBL mutein Q227R and IL-21 mutein I8H; 4-1BBL mutein Q227R and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Q227R and IL-21 mutein L74F; 4-1BBL mutein L101N and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein L101N and IL-21 mutein L20W; 4-1BBL mutein L101N and IL-21 mutein L74D; 4-1BBL mutein L101N and IL-21 mutein L20N; 4-1BBL mutein L101N and IL-21 mutein I67N; 4-1BBL mutein L101N and IL-21 mutein L20S; 4-1BBL mutein L101N and IL-21 mutein L13E; 4-1BBL mutein L101N and IL-21 mutein I8H; 4-1BBL mutein L101N and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein L101N and IL-21 mutein L74F; 4-1BBL mutein Y110Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Y110Q and IL-21 mutein L20W; 4-1BBL mutein Y110Q and IL-21 mutein L74D; 4-1BBL mutein Y110Q and IL-21 mutein L20N; 4-1BBL mutein Y110Q and IL-21 mutein I67N; 4-1BBL mutein Y110Q and IL-21 mutein L20S; 4-1BBL mutein Y110Q and IL-21 mutein L13E; 4-1BBL mutein Y110Q and IL-21 mutein I8H; 4-1BBL mutein Y110Q and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Y110Q and IL-21 mutein L74F; 4-1BBL mutein Q230K and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Q230K and IL-21 mutein L20W; 4-1BBL mutein Q230K and IL-21 mutein L74D; 4-1BBL mutein Q230K and IL-21 mutein L20N; 4-1BBL mutein Q230K and IL-21 mutein I67N; 4-1BBL mutein Q230K and IL-21 mutein L20S; 4-1BBL mutein Q230K and IL-21 mutein L13E; 4-1BBL mutein Q230K and IL-21 mutein I8H; 4-1BBL mutein Q230K and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Q230K and IL-21 mutein L74F; 4-1BBL mutein V100Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein V100Q and IL-21 mutein L20W; 4-1BBL mutein V100Q and IL-21 mutein L74D; 4-1BBL mutein V100Q and IL-21 mutein L20N; 4-1BBL mutein V100Q and IL-21 mutein I67N; 4-1BBL mutein V100Q and IL-21 mutein L20S; 4-1BBL mutein V100Q and IL-21 mutein L13E; 4-1BBL mutein V100Q and IL-21 mutein I8H; 4-1BBL mutein V100Q and IL-21 mutein (N63- E64- R65- I66-); and, 4-1BBL mutein V100Q and IL-21 mutein L74F.

78. A conjugate according to any one of embodiments 64 – 68, wherein:

i) the CD27 agonist comprises or consists of a fusion protein comprising three CD70 ECD monomers fused together in a single polypeptide chain, and wherein, optionally, the three CD70 ECD monomers are connected by polypeptide linkers; and,

ii) the GITR agonist comprises or consists of a fusion protein comprising three GITRL ECD monomers fused together in a single polypeptide chain, and wherein, optionally, the three GITRL ECD monomers are connected by polypeptide linkers.

79. A conjugate to any one of embodiments 64 – 78, wherein the conjugate has:

i) an IL-21R agonist-valency that is higher than one;

ii) an IL-15 receptor agonist-valency that is higher than one;

iii) a type I interferon (IFN-1) receptor agonist-valency that is higher than one;

iv) an IL-2 receptor agonist-valency that is higher than one;

v) an IL-12 receptor agonist-valency that is higher than one;

vi) an IL-18 receptor agonist-valency that is higher than one;

vii) a CD27 agonist-valency that is higher than one; and,

viii) a GITR agonist-valency that is higher than one.

80. A conjugate according to any one of the embodiments 19 – 60, wherein at least one of the first and second antigen-binding regions is conjugated to the third antigen-binding region.

81. A conjugate according to embodiment 80, wherein at least one polypeptide chain in the at least one of the first and second antigen-binding regions forms a single polypeptide chain with at least one polypeptide chain of the third antigen-binding region.

82. A conjugate according to embodiment 81, wherein the single polypeptide chain comprises in an N- to C-terminal order: i) at least one polypeptide chain in the at least one of the first and second antigen-binding region; ii) optionally a flexible linker; and iii) the third antigen-binding region.

83. A conjugate according to embodiment 81 or 82, wherein the third antigen-binding region is a dimeric Fc region, wherein each of the two polypeptide chains of the dimeric Fc region is linked to a CH1 domain, each of which CH1 domains is linked to an immunoglobulin-derived antigen-binding region that specifically binds a TAA, an NK cell activating receptor or an epitope of a γδ TCR.

84. A conjugate according to embodiment 83, wherein the two immunoglobulin-derived antigen-binding regions bind the same TAA, or wherein the two immunoglobulin-derived antigen-binding region each bind a different TAA.

85. A conjugate according to embodiment 83 or 84, wherein the protein comprises a dimeric Fc region, wherein each of the two Fc polypeptide chains is operably linked to a Fab that specifically binds a TAA, an NK cell activating receptor or an epitope of a γδ TCR.

86. A conjugate according to any one of embodiments 80 – 85, wherein at least one of the 4-1BBL ECD mutein and the further agonist, is conjugated to the at least one antigen-binding region that specifically binds a TAA, an NK cell activating receptor or an epitope of a γδ TCR, or to the third antigen-binding region.

87. A conjugate according to embodiment 86, wherein at least one of the 4-1BBL ECD mutein and the further agonist forms a single polypeptide chain with at least one of:

i) at least one polypeptide chain in at least one of the first and second antigen-binding regions; and,

ii) at least one polypeptide chain in the third antigen-binding region;

wherein optionally, a flexible linker is present between the at least one of the 4-1BBL ECD mutein and the further agonist and the at least one polypeptide chain in the region defined in i) or ii).

88. A conjugate according to any one of embodiments 85 – 87, wherein at least one of the 4-1BBL ECD mutein and the further agonist forms a single polypeptide chain with at least one of:

i) a light chain in at least one of the two Fabs that specifically bind a TAA, an NK cell activating receptor or an epitope of a γδ TCR; and,

ii) at least one of the two Fc chains in the dimeric Fc region;

wherein optionally, a flexible linker is present between the at least one of the 4-1BBL ECD mutein and the further agonist and the light chain defined in i) or the Fc chain defined in ii).

89. A conjugate according to embodiment 88, wherein at least one of the 4-1BBL ECD mutein and the further agonist is fused to at least one of:

i) the N-terminus of the light chain in at least one of the two Fab's that specifically bind a TAA, an NK cell activating receptor or an epitope of a γδ TCR, optionally through a flexible linker;

ii) the C-terminus of the light chain in at least one of the two Fab's that specifically bind a TAA, an NK cell activating receptor or an epitope of a γδ TCR, optionally through a flexible linker;

iii) the N-terminus of the heavy chain in at least one of the two Fab's that specifically bind a TAA, an NK cell activating receptor or an epitope of a γδ TCR; and,

iv) the C-terminus of the heavy chain in at least one of the two Fc chains in the dimeric immunoglobulin Fc domain, optionally through a flexible linker.

90. A conjugate according to embodiment 88 or 89, wherein at least one of the 4-1BBL ECD mutein and the further agonist is present on at least one or on both sides of the immunoglobulin structure.

91. A conjugate according to any one of embodiments 83 – 90, wherein the conjugate is heterodimeric with respect to at least one of: i) the first and second antigen-binding regions; and ii) at least one of the fused 4-1BBL ECD mutein and the fused further agonist, and wherein the dimeric Fc region comprises different first and a second polypeptide chains comprising knob-into-hole modifications promoting association of the first and the second polypeptide chains of the Fc region.

92. A conjugate according to any one of embodiments 13 – 91, wherein the conjugate has at least one biological activity selected from:

a) the conjugate causes an increase in at least one NK cell activity selected from CD107a degranulation, CD69 expression, IFNy production, NK cell proliferation and NK cell cytotoxicity, whereby preferably, the increase is at least a factor 1.01 higher as compared to the increase achieved with the same effector : target cell ratio, with the same NK cells and target cells that are not brought into contact with the conjugate;

b) the conjugate causes an increase in at least one NK cell activity selected from CD107a degranulation, CD69 expression, IFNy production, NK cell proliferation and NK cell cytotoxicity, whereby preferably, the increase is at least a factor 1.01 higher as compared to the increase achieved with the same effector : target cell ratio, with the same NK cells and target cells that are brought into contact with a conventional human IgG1 monoclonal antibody that has the same TAA-specific antigen-binding regions as the conjugate;

c) the conjugate causes an increase in at least one γδ T cell activity selected from γδ T cell proliferation, cytokine production, γδ T cell cytotoxicity, γδ T cell differentiation, whereby preferably, the increase is at least a factor 1.01 higher as compared to the

increase achieved with the same effector : target cell ratio, with the same γδ T cells and target cells that are not brought into contact with the conjugate; and,

b) the conjugate causes an increase in at least one γδ T cell activity selected from γδ T cell proliferation, cytokine production, γδ T cell cytotoxicity, γδ T cell differentiation, whereby preferably, the increase is at least a factor 1.01 higher as compared to the increase achieved with the same effector : target cell ratio, with the same γδ T cells and target cells that are brought into contact with a conventional human IgG1 monoclonal antibody that has the same TAA-specific antigen-binding regions as the conjugate binding protein.

93. A conjugate according to any one of embodiments 13 – 92, wherein *ex vivo* expansion of donor NK cells by co-culturing with the conjugate, produces a population of expanded NK cells having one or more features selected from:

a) the fold expansion of the expanded NK cells is at least 0.5, 1.0, 2.0, or 5.0 fold of the fold expansion of expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated K562 feeder cells modified to express membrane bound IL-21 (mbIL-21) and 4-1BB ligand (FC21 feeder cells);

b) the telomere length of the expanded NK cells is increased by at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55% as compared to the telomere length of fresh NK cells, preferably, the percentage telomere length increase of the expanded NK cells as compared to the telomere length of fresh NK cells, is at least 0.5, 1.0, 2.0, or 5.0 fold of the percentage telomere length increase of NK cells obtained upon *ex vivo* expansion in the presence of FC21 feeder cells;

c) the expression level of at least one NK cell activating receptor selected from NKG2D, NKp30, NKp44, NKp46 and CD16 on the expanded NK cells is at least 0.5, 1.0, 2.0, or 5.0 fold of the expression level on expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells;

d) the secretion of at least one cytokine of TNF-α, IFN-γ and IL-6 by the expanded NK cells is at least 0.5, 1.0, 2.0, or 5.0 fold of the secretion of the cytokine by expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells; and,

e) the cytotoxicity of the expanded NK cells is at least 0.5, 1.0, 2.0, or 5.0 fold of the cytotoxicity of expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells,

wherein, preferably the NK cells are co-cultured with tumor cells expressing a TAA specifically bound by the conjugate,or wherein *ex vivo* expansion of donor γδ T cells by co-culturing with the conjugate, produces a population of expanded NK cells having one or more features selected from:

a) the fold expansion of the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the fold expansion of expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with irradiated K562 feeder cells modified to express a membrane

bound version of the IL-21 mutein and a membrane bound version of the co-stimulatory agonist;

b) the telomere length of the expanded γδ T cells is increased by at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55% as compared to the telomere length of fresh γδ T cells, preferably, the percentage telomere length increase of the expanded NK cells as compared to the telomere length of fresh γδ T cells, is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the percentage telomere length increase of γδ T cells obtained upon *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells;

c) the expression level of at least one γδ T cell activating receptor selected from NKG2D, NKp30, NKp44, NKp46 and CD16 on the expanded NK cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the expression level on expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells;

d) the secretion of at least one cytokine of TNF-α, IFN-γ and IL-6 by the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the secretion of the cytokine by expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells; and,

e) the cytotoxicity of the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the cytotoxicity of expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells;

wherein, preferably the γδ T cells are co-cultured with tumor cells expressing a TAA specifically bound by the conjugate.

94. A pharmaceutical composition comprising an 4-1BBL ECD mutein according to any one of embodiments 1 – 19, a fusion protein according to any one of embodiments 20 – 22, or a conjugate according to any one of embodiments 23 – 93, and a pharmaceutically acceptable carrier.

95. An *ex vivo* method for expansion of NK cells, the method comprising the step of contacting an NK cell with a conjugate according to any one of embodiments 23 – 93, or with a composition according to embodiment 94, and wherein preferably, the expanded NK cells have one or more features selected from:

a) the fold expansion of the expanded NK cells is at least 0.5, 1.0, 2.0, or 5.0 fold of the fold expansion of expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated K562 feeder cells modified to express membrane bound IL-21 (mbIL-21) and 4-1BB ligand (FC21 feeder cells);

b) the telomere length of the expanded NK cells is increased by at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55% as compared to the telomere length of fresh NK cells, preferably, the percentage telomere length increase of the expanded NK cells as compared to the

telomere length of fresh NK cells, is at least 0.5, 1.0, 2.0, or 5.0 fold of the percentage telomere length increase of NK cells obtained upon *ex vivo* expansion in the presence of FC21 feeder cells;

c) the expression level of at least one NK cell activating receptor selected from NKG2D, NKp30, NKp44, NKp46 and CD16 on the expanded NK cells is at least 0.5, 1.0, 2.0, or 5.0 fold of the expression level on expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells;

d) the secretion of at least one cytokine of TNF-α, IFN-γ and IL-6 by the expanded NK cells is at least 0.5, 1.0, 2.0, or 5.0 fold of the secretion of the cytokine by expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells; and,

e) the cytotoxicity of the expanded NK cells is at least 0.5, 1.0, 2.0, or 5.0 fold of the cytotoxicity of expanded NK cells obtained by *ex vivo* expansion by co-culturing with irradiated FC21 feeder cells,

wherein, preferably the method comprising the further step of co-culturing the NK cells with tumor cells expressing a TAA specifically bound by the conjugate.

96. An *ex vivo* method for expansion of γδ T cells, the method comprising the step of contacting a γδ T cell with conjugate according to any one of embodiments 23 – 93, or with a composition according to embodiment 94, and wherein preferably, the expanded γδ T cells have one or more features selected from:

a) the fold expansion of the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the fold expansion of expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with irradiated K562 feeder cells modified to express a membrane bound version of the IL-21 mutein and a membrane bound version of the co-stimulatory agonist;

b) the telomere length of the expanded γδ T cells is increased by at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or 55% as compared to the telomere length of fresh γδ T cells, preferably, the percentage telomere length increase of the expanded NK cells as compared to the telomere length of fresh γδ T cells, is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the percentage telomere length increase of γδ T cells obtained upon *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells;

c) the expression level of at least one γδ T cell activating receptor selected from NKG2D, NKp30, NKp44, NKp46 and CD16 on the expanded NK cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the expression level on expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells;

d) the secretion of at least one cytokine of TNF-α, IFN-γ and IL-6 by the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the secretion of the cytokine by expanded γδ T cells obtained by *ex vivo* expansion by αβ

T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells; and,

e) the cytotoxicity of the expanded γδ T cells is at least 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 fold of the cytotoxicity of expanded γδ T cells obtained by *ex vivo* expansion by αβ T cell depletion and stimulation with OKT3 antibody or by co-culturing with the modified irradiated K562 feeder cells.

97. An 4-1BBL ECD mutein according any one of embodiments 1 – 19, a fusion protein according to any one of embodiments 20 – 22, or a conjugate according to any one of embodiments 23 – 93, a composition according to embodiment 94, an *ex vivo* expanded NK cell obtained in a method according to embodiment 95, optionally in combination with the 4-1BBL ECD mutein, the fusion protein or the conjugate, or an *ex vivo* expanded γδ T cell obtained in a method according to embodiment 96, optionally in combination with the 4-1BBL ECD mutein, the fusion protein or the conjugate, for use as a medicament.

98. An 4-1BBL ECD mutein according any one of embodiments 1 – 19, a fusion protein according to any one of embodiments 20 – 22, or a conjugate according to any one of embodiments 23 – 93, a composition according to embodiment 94, an *ex vivo* expanded NK cell obtained in a method according to embodiment 95, optionally in combination with the 4-1BBL ECD mutein, the fusion protein or the conjugate, or an *ex vivo* expanded γδ T cell obtained in a method according to embodiment 96, optionally in combination with the 4-1BBL ECD mutein, the fusion protein or the conjugate, for use in the treatment of a cancer, an infectious disease or an inflammatory disease, wherein preferably the cancer is a cancer comprising tumor cells expressing the TAA.

99. An 4-1BBL ECD mutein according any one of embodiments 1 – 19, a fusion protein according to any one of embodiments 20 – 22, or a conjugate according to any one of embodiments 23 – 93, a composition according to embodiment 94, for use in the treatment of a cancer, preferably a cancer comprising tumor cells expressing the TAA, wherein the 4-1BBL ECD mutein, the conjugate or the composition is used in combination with an adoptive transfer of immune cells, wherein preferably the immune cells are selected from T cells and NK cells, wherein the T cells are preferably γδ T cells.

100. An 4-1BBL ECD mutein according any one of embodiments 1 – 19, a fusion protein according to any one of embodiments 20 – 22, or a conjugate according to any one of embodiments 23 – 93 or a composition according to embodiment 94, for a use according to embodiment 98 or 99, wherein at least one of:

a) the 4-1BBL ECD mutein, the conjugate or the composition is administered as a neoadjuvant therapy before a primary therapy comprising at least one of surgery and radiation therapy of the cancer; and,

b) the 4-1BBL ECD mutein, the conjugate or the composition is administered as an adjuvant therapy after a primary therapy comprising at least one of surgery and radiation therapy of the cancer.

101. A method for enhancing anti-tumor activity of at least one of an NK cell and a γδ T cell in a subject, the method comprising the step of administering to the subject an 4-1BBL ECD mutein according any one of embodiments 1 – 19, a fusion protein according to any one of embodiments 20 – 22, or a conjugate according to any one of embodiments 23 – 93, a composition according to embodiment 94, or an ex vivo expanded NK cell or γδ T cell obtained in a method according to embodiment 95 or 96, optionally, in combination with the 4-1BBL ECD mutein, the fusion protein or the conjugate, or a combination of the 4-1BBL ECD mutein, the fusion protein or the conjugate and an immune cell selected from T cells and NK cells, wherein the T cells are preferably γδ T cells.

102. The method of embodiment 101, wherein the subject has cancer, preferably a cancer comprising tumor cells expressing the TAA.

103. The method of embodiment 102, wherein at least one of:

a) the 4-1BBL ECD mutein, the conjugate or the composition is administered as a neoadjuvant therapy before a primary therapy comprising at least one of surgery and radiation therapy of the cancer; and,

b) the 4-1BBL ECD mutein, the conjugate or the composition is administered as an adjuvant therapy after a primary therapy comprising at least one of surgery and radiation therapy of the cancer.

104. A nucleic acid molecule comprising one or more nucleotide sequences encoding an 4-1BBL ECD mutein according any one of embodiments 1 – 19, a fusion protein according to any one of embodiments 20 – 22, or a polypeptide chain of a conjugate according to any one of embodiments 23 – 93.

105. A nucleic acid molecule according to embodiment 104, wherein the one or more nucleotide sequences are operably linked to regulatory sequences for expression of the one or more polypeptide chains in a host cell.

106. A host cell comprising a nucleic acid molecule according to embodiment 104 or 105.

107. A method for producing an 4-1BBL ECD mutein according any one of embodiments 1 – 19, a fusion protein according to any one of embodiments 20 – 22, or a conjugate according to any one of embodiments 23 – 93, the method comprising culturing a host cell according to embodiment 106 such that the one or more nucleotide sequences are expressed, and the 4-1BBL ECD mutein, the fusion protein or the conjugate is produced.

108. A method according to embodiment 107, further comprising the steps of: recovery of the 4-1BBL ECD mutein, the fusion protein or the conjugate, and, optionally, formulation of the 4-1BBL ECD mutein, the fusion protein or the conjugate with a pharmaceutically acceptable carrier.

Claims

1. A mutein of a 4-1BB ligand (4-1BBL) extracellular domain (ECD), wherein the 4-1BBL ECD mutein exhibits a binding affinity for human 4-1BB, expressed in pK_D, that is at least 1.0 lower than the pK_D of a wild-type 4-1BBL ECD for human 4-1BB, wherein the 4-1BBL ECD mutein, when present as part of a homotrimer of the 4-1BBL ECD mutein in a conjugate with an antibody that specifically binds a tumor-associated antigen (TAA), which conjugate further comprises a human IL-21, induces a maximal proliferation of NK cells at a saturating concentration of 25 nM of the conjugate in a normalized 5-day NK cell proliferation assay in the presence of tumor cells expressing the TAA, which proliferation is not less than 75% of the proliferation induced by a corresponding control conjugate comprising a trimer of wild type 4-1BBL ECD in the same assay, wherein the amino acid sequence of the 4-1BBL ECD mutein differs from a wild type human 4-1BBL ECD amino acid sequence of SEQ ID NO: 37 in that the 4-1BBL ECD mutein comprises at least one substitution selected from the group consisting of: A154D, A154E, a combination of A154D and G155Q, V153Q, Q227E, L101N, Y110Q, Q230K, and V100Q.

2. A 4-1BBL ECD mutein according to claim 1, wherein the 4-1BBL ECD mutein comprises no other amino acid sequence modification than the at least one substitution selected from the group consisting of: A154D, A154E, a combination of A154D and G155Q, V153Q, Q227E, L101N, Y110Q, Q230K, and V100Q.

3. A fusion protein comprising three 4-1BBL ECD monomers, wherein one, two or three of the monomers are a 4-1BBL ECD mutein according to claim 1 or 2, wherein the three 4-1BBL ECD monomers are fused together in a single polypeptide chain, and wherein, optionally, the three 4-1BBL ECD monomers are connected by polypeptide linkers.

4. A conjugate comprising: i) a 4-1BBL ECD mutein according to claim 1 or 2, or a fusion protein according to claim 3; and ii) a heterologous moiety, wherein preferably, the heterologous moiety comprises a polypeptide, and wherein more preferably, the polypeptide is an antigen-binding protein or a polypeptide chain of an antigen-binding protein.

5. A conjugate according to claim 4, wherein the antigen-binding protein comprises at least one of:

   a) at least one of:

      i) a first antigen-binding region that specifically binds a TAA, that specifically binds an NK cell activating receptor or that specifically binds an epitope of a γδ T cell receptor (TCR), and

      ii) a second antigen-binding region that specifically binds a TAA, that specifically binds an NK cell activating receptor or that specifically binds an epitope of a γδ TCR; and,

b) a dimeric Fc region that binds to CD16A, or wherein the dimeric Fc region is modified to reduce affinity for CD16A, relative to a corresponding wild-type Fc region.

6. A conjugate according to claim 5, wherein the TAA is selected from the group consisting of: 5T4, ADAM9, ADAM10, ADAM12, ALK, ALPP, ALPP2, ALPPL2, AMHR2, ANGPT2, AXL, Angiopoietin-2, Apelin receptor, B7-H3, B7-H4, B7-H6, B7.1, B7.2, BCMA, BTLA, CA125, CA9, CAIX, CCL2, CCR2, CCR4, CCR5, CCR6, CCR7, CD123, CD133, CD138, CD142, CD147, CD166, CD171, CD19, CD2, CD20, CD205, CD22, CD228, CD24, CD248, CD25, CD27, CD276, CD3, CD30, CD317, CD33, CD37, CD38, CD3E, CD4, CD40, CD44, CD44v6, CD45, CD46, CD47, CD52, CD56, CD70, CD71, CD73, CD74, CD79, CD79B, CD80, CD80/CD86, CDCP1, CDH3, CDH6, CDK4, CEA, CEACAM5, CLDN18, CLEC14A, CLEC4, CSF1R, CSF2, CSPG4, CT-7, CTGF, CTLA4, Cadherin 17, Cadherin 6, CanAg, Claudin 18.2, Claudin 6, Connexin 37, Cripto-1, Crypto, DC3, DKK1, DLK1, DLL3, DLL4, DR5, E-cadherin, E-selectin, EBV-encoded nuclear antigen (EBNA)-I, EDA, EDB, EDNRB, EGF, EGFL7, EGFR, EGFRvIII, ENG, EPCAM, EPHA4, EphA10, EphA2, EphA3, EphB2, EphB4, ExtradomainB (EDB) fibronectin, F3, FAP, FCGR1, FGFR2, FGFR2b, FGFR3, FGFR4, FLT1, FN, FOLH1, FOLR1, FRα, FSHR, FcRL5/FcRH5, Fibronectin extra-domain B, Flt3, Fucosyl, GFRa4, GM3, GPCR5D, GPNMB, GPRC5D, GRP78, GUCY2C, Glycoprotein NMB, Glypican 1, Glypican 2, Glypican 3, GnT-V, HAVCR2, HER-3/ERBB3, HER-4/ERBB4, HER2, HER3, HGF, HLA-G, HSP70, ICAM-1, ICOS, IFNG, IGF-1R, IGF1, IGF1R, IGF2, IL-1 accessory protein, IL-6 receptor, IL-8 receptor, IL13Ra2, IL17A, IL1A, IL1B, IL1RAP, IL2RA, IL3RA, IL6, IL6R, ITGAV, ITGB6, Ig-idiotype, Integrin beta 6, KAAG-1, KDR, KIRD2, KIT, KLK2, KLRC1, Killer Ig-Like Receptor, Killer Ig-Like Receptor 3DL2 (KIR3DL2), L1-CAM, L1CAM, LAG3, LAGE-1, LGR5, LIF, LIV-1, LOXL2, LRRC32, Lewis-Y, MART-1/Melan-A, MET, MIC-A/B, MICB, MIF, MISIIR, MMP2, MS4A1, MSLN, MST1R, MSTN, MUC1, MUC1-C, MUC16, MUM-1, Melanotransferrin, Mesothelin, Mud 6, NAG, NCAM1, NKG2D, NOTCH1, NOTCH2, NOTCH3, NRP1, NT5E, NTRKR1 (EC 2.7.10.1), NaPi2b, Nectin-4, OLR1, OX40, P-cadherin, P1A, PCSK9, PD-L1, PD1, PDGF, PDGF alpha receptor, PDGF beta receptor, PDGFR, PDGFRA, PLAUR, PMEL, PRAME, PSCA, PSMA, PTK7, PTPRC, PVRL4, Plexin-A1, RAGE, ROBO1, ROR1, ROR2, RSPO3, SCP-1, SEZ6, SIRPA, SLAMF7, SLC34A2, SLC3A2, SSTR2, SSX-1, SSX-2 (HOM-MEL-40), SSX-4, SSX-5, STEAP1, STEAP2, Severe, T-cell receptor/CD3-zeta chain, TACSTD2, TGF-alpha, TGFB1, TGFB2, TGFB3, TIGIT, Tissue factor/TF, TLR2, TM4SF1, TMEFF2, TNFRSF10B, TNFRSF17, TNFRSF18, TNFRSF4, TNFRSF8, TNFRSF9, TNFSF11, TNFSF13B, TPBG, TRAILR1, TRAILR2, TROP2, TSHR, TYRP1, VEGF, VEGFA, VEGFR1, VEGFR2, VH1/VL1, VH2/VL2, VH3/VL3, a GAGE-tumor antigen, a GD2 ganglioside, a GM2 ganglioside, a RAET1 protein, a UL16-binding protein (ULBP), a heterodimeric receptor comprised of at least one HER subunit, a human papillomavirus protein, a5β1 integrins, a5β3 integrins, adenomatous polyposis coli protein (APC), adenosine deaminase-binding protein (ADAbp), anti-Müllerian hormone Type II receptor, avB3 integrin, avB6 integrin, avβ6 integrins, bivalent, brain

glycogen phosphorylase, c-erbB-2, cMET, colorectal associated antigen (CRC)-C017-1A/GA733, gastrin releasing peptide receptor antigen, gp100, gp75, gpA33, hCG, human papillomavirus protein, integrin receptors, mmp9, muc17, p15, phosphatidylserine, prostate specific antigen (PSA), protein tyrosine kinase 7(PTK7), receptor protein tyrosine kinase 3 (TYRO-3), sVE-cadherin, scatter factor receptor kinase, trivalent, α-catenin, α-fetoprotein, αIIbβ3-integrins, β-catenin, and γ-catenin.

7. A conjugate according to claim 5 or 6, wherein the dimeric Fc region is modified to reduce affinity for CD16A, relative to a corresponding wild-type Fc region and wherein the antigen-binding region that specifically binds an epitope of a γδ TCR, binds an epitope in the V region of a Vδ1 chain, a Vδ2 chain or a Vγ9 chain of a γδ TCR.

8. A conjugate according to any one of claims 4 – 8, comprising, in addition to the 4-1BBL ECD mutein or the fusion protein, at least one further agonist that is at least one of:

i) a further NK cell activating cytokine selected from the group consisting of: an IL-21 receptor agonist, an IL-15 receptor agonist, a type I interferon (IFN-1) receptor agonist, an IL-2 receptor agonist, an IL-12 receptor agonist and an IL-18 receptor agonist;

ii) a γδ T cell-activating agonist selected from the group consisting of: an IL-21 receptor agonist, an IL-15 receptor agonist, a type I interferon (IFN-1) receptor agonist, an IL-2 receptor agonist, an IL-12 receptor agonist and an IL-18 receptor agonist; and,

iii) a further γδ T cell co-stimulatory agonist selected from the group consisting of: a CD27 agonist and a GITR agonist,

wherein preferably:

i) the IL-21R agonist comprises or consist of an IL-21 polypeptide or an agonistic antigen-binding region that specifically binds IL-21R; or,

ii) the IL-15R agonist comprises or consist of an IL-15 polypeptide or an agonistic antigen-binding region that specifically binds IL-15R;

iii) the type I interferon (IFN-1) receptor agonist comprises or consists of an IFN-1 polypeptide or an agonistic antigen-binding region that specifically binds the IFN-α receptor;

iv) the IL-2R agonist comprises or consists of an IL-2 polypeptide or an agonistic antigen-binding region that specifically binds IL-2R;

v) the IL-12R agonist comprises or consists of an IL-12 polypeptide or an agonistic antigen-binding region that specifically binds IL-12R;

vi) the IL-18R agonist comprises or consists of an IL-18 polypeptide or an agonistic antigen-binding region that specifically binds IL-18R;

vii) the CD27 agonist comprises or consists of at least one CD70 extracellular domain (ECD) or agonistic antigen-binding region that specifically binds CD27; and,

viii) the GITR agonist comprises or consists of at least one GIRTL extracellular domain (ECD) or at least one agonistic antigen-binding region that specifically binds GITR.

9. A conjugate according to claim 8, wherein the IL-21 polypeptide is an IL-21 mutein comprises at least one amino acid substitution, deletion or insertion selected from the group consisting of: (N82- A83- G84- R85- R86- Q87- K88-), L20W, L74D, L20N, I67N, L20S, L13E, I8H, (N63-, E64-, R65- and I66-), L74F, I8V, I8Q, I8F, I8W, I8Y, I8L, D4H, D4R, D4K, D4Q, D4N, R11E, R11Q, R11N, R11Y, Q12K, Q12R, L13S, L13V, L13T, L13G, Q19S, Q19E, Q19K, Q19R, Q19H, Q19G, Q19T, L20D, L20E, L20R, L20K, L20Q, L20H, L20G, K21H, K21N, K21Q, K21E, K21D, I67T, I67D, I67E, I67K, I67R, I67Q, I67S, I67G, L74G, L74E, L74K, L74R, L74N, L74Q, L74S, L74P, K75E, K75Q, K75N, K75S, K75-, K76-, K112H, K112N, K112Q, K112E, K112D, N59-, T60-, G61-, N62-, N63-, E64-, R65-, I66-, (N59-, T60-, G61-, N62-, N63-, E64-, R65- and I66-), (K75- and R76-), (K75-, R76- and R77-), G84 insGGGG, and, K75 insX (wherein X is one, two or three amino acids selected from the group consisting of G, S and D).

10. A conjugate according to claim 9, wherein the conjugate comprises a combination of a 4-1BBL ECD mutein and an IL-21 mutein, wherein the 4-1BBL ECD mutein is selected from the group consisting of: 4-1BBL mutein A154D; 4-1BBL mutein A154E; 4-1BBL mutein V153Q; 4-1BBL mutein Q227E; 4-1BBL mutein L101N; 4-1BBL mutein Y110Q; 4-1BBL mutein Q230K; and 4-1BBL mutein V100Q; and wherein the IL-21 mutein is selected from the group consisting of: IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); IL-21 mutein L20W; IL-21 mutein L74D; IL-21 mutein L20N; IL-21 mutein I67N; IL-21 mutein L20S; IL-21 mutein L13E; IL-21 mutein I8H; IL-21 mutein (N63- E64- R65- I66-); and IL-21 mutein L74F, and wherein preferably, the 4-1BBL ECD mutein and the IL-21 mutein are each conjugated to an antigen binding protein, whereby more preferably, the 4-1BBL ECD mutein is present as a hetero- or homotrimer of 4-1BBL ECDs connected through polypeptide linkers.

11. A conjugate according to claim 10, wherein the conjugate comprises a combination of a 4-1BBL ECD mutein and an IL-21 mutein selected from the group consisting of: 4-1BBL mutein A154D and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154D and IL-21 mutein L20W; 4-1BBL mutein A154D and IL-21 mutein L74D; 4-1BBL mutein A154D and IL-21 mutein L20N; 4-1BBL mutein A154D and IL-21 mutein I67N; 4-1BBL mutein A154D and IL-21 mutein L20S; 4-1BBL mutein A154D and IL-21 mutein L13E; 4-1BBL mutein A154D and IL-21 mutein I8H; 4-1BBL mutein A154D and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein A154D and IL-21 mutein L74F; 4-1BBL mutein A154E and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein A154E and IL-21 mutein L20W; 4-1BBL mutein A154E and IL-21 mutein L74D; 4-1BBL mutein A154E and IL-21 mutein L20N; 4-1BBL mutein A154E and IL-21 mutein I67N; 4-1BBL mutein A154E and IL-21 mutein L20S; 4-1BBL mutein A154E and IL-21 mutein L13E; 4-1BBL mutein A154E and IL-21 mutein I8H; 4-1BBL mutein A154E and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein A154E and IL-21 mutein L74F; 4-1BBL mutein A154D + G155Q and IL-21 mutein (N82- A83- G84- R85-

R86- Q87- K88-); 4-1BBL mutein A154D + G155Q and IL-21 mutein L20W; 4-1BBL mutein A154D + G155Q and IL-21 mutein L74D; 4-1BBL mutein A154D + G155Q and IL-21 mutein L20N; 4-1BBL mutein A154D + G155Q and IL-21 mutein I67N; 4-1BBL mutein A154D + G155Q and IL-21 mutein L20S; 4-1BBL mutein A154D + G155Q and IL-21 mutein L13E; 4-1BBL mutein A154D + G155Q and IL-21 mutein I8H; 4-1BBL mutein A154D + G155Q and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein A154D + G155Q and IL-21 mutein L74F; 4-1BBL mutein V153Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein V153Q and IL-21 mutein L20W; 4-1BBL mutein V153Q and IL-21 mutein L74D; 4-1BBL mutein V153Q and IL-21 mutein L20N; 4-1BBL mutein V153Q and IL-21 mutein I67N; 4-1BBL mutein V153Q and IL-21 mutein L20S; 4-1BBL mutein V153Q and IL-21 mutein L13E; 4-1BBL mutein V153Q and IL-21 mutein I8H; 4-1BBL mutein V153Q and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein V153Q and IL-21 mutein L74F; 4-1BBL mutein Q227E and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Q227E and IL-21 mutein L20W; 4-1BBL mutein Q227E and IL-21 mutein L74D; 4-1BBL mutein Q227E and IL-21 mutein L20N; 4-1BBL mutein Q227E and IL-21 mutein I67N; 4-1BBL mutein Q227E and IL-21 mutein L20S; 4-1BBL mutein Q227E and IL-21 mutein L13E; 4-1BBL mutein Q227E and IL-21 mutein I8H; 4-1BBL mutein Q227E and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Q227E and IL-21 mutein L74F; 4-1BBL mutein L101N and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein L101N and IL-21 mutein L20W; 4-1BBL mutein L101N and IL-21 mutein L74D; 4-1BBL mutein L101N and IL-21 mutein L20N; 4-1BBL mutein L101N and IL-21 mutein I67N; 4-1BBL mutein L101N and IL-21 mutein L20S; 4-1BBL mutein L101N and IL-21 mutein L13E; 4-1BBL mutein L101N and IL-21 mutein I8H; 4-1BBL mutein L101N and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein L101N and IL-21 mutein L74F; 4-1BBL mutein Y110Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Y110Q and IL-21 mutein L20W; 4-1BBL mutein Y110Q and IL-21 mutein L74D; 4-1BBL mutein Y110Q and IL-21 mutein L20N; 4-1BBL mutein Y110Q and IL-21 mutein I67N; 4-1BBL mutein Y110Q and IL-21 mutein L20S; 4-1BBL mutein Y110Q and IL-21 mutein L13E; 4-1BBL mutein Y110Q and IL-21 mutein I8H; 4-1BBL mutein Y110Q and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Y110Q and IL-21 mutein L74F; 4-1BBL mutein Q230K and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein Q230K and IL-21 mutein L20W; 4-1BBL mutein Q230K and IL-21 mutein L74D; 4-1BBL mutein Q230K and IL-21 mutein L20N; 4-1BBL mutein Q230K and IL-21 mutein I67N; 4-1BBL mutein Q230K and IL-21 mutein L20S; 4-1BBL mutein Q230K and IL-21 mutein L13E; 4-1BBL mutein Q230K and IL-21 mutein I8H; 4-1BBL mutein Q230K and IL-21 mutein (N63- E64- R65- I66-); 4-1BBL mutein Q230K and IL-21 mutein L74F; 4-1BBL mutein V100Q and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); 4-1BBL mutein V100Q and IL-21 mutein L20W; 4-1BBL mutein V100Q and IL-21 mutein L74D; 4-1BBL mutein V100Q and IL-21 mutein L20N; 4-1BBL mutein V100Q and IL-21 mutein I67N; 4-1BBL mutein V100Q and IL-21 mutein L20S; 4-1BBL mutein V100Q and IL-21 mutein L13E; 4-1BBL mutein V100Q and IL-21 mutein I8H; 4-1BBL mutein V100Q and IL-21 mutein (N63- E64- R65- I66-); and,

4-1BBL mutein V100Q and IL-21 mutein L74F; of which the combinations: 4-1BBL mutein A154D and IL-21 mutein (N82- A83- G84- R85- R86- Q87- K88-); and, 4-1BBL mutein A154D and IL-21 mutein L20W, are preferred.

12. A conjugate according to any one of claims 4 – 11, wherein the first antigen-binding region that specifically binds a TAA and the second antigen-binding region that specifically binds a TAA form an immunoglobulin structure with the dimeric Fc region, and wherein at least one of: i) the 4-1BBL ECD mutein or the fusion protein; and ii) the further agonist, is present on at least one or on both sides of the immunoglobulin structure.

13. A pharmaceutical composition comprising a 4-1BBL ECD mutein according to claim 1 or 2, a fusion protein according to claim 3, or a conjugate according to any one of claims 4 – 12, and a pharmaceutically acceptable carrier.

14. A 4-1BBL ECD mutein according to claim 1 or 2, a fusion protein according to claim 3, a conjugate according to any one of claims 4 – 12, or a composition according to claim 13, for use in the treatment of a cancer, preferably a cancer comprising tumor cells expressing the TAA, wherein optionally, the 4-1BBL ECD mutein, the conjugate or the composition is used in combination with an adoptive transfer of immune cells, wherein preferably the immune cells are selected from T cells and NK cells.

15. A 4-1BBL ECD mutein according to claim 1 or 2, a fusion protein according to claim 3, a conjugate according to any one of claims 4 – 12 or a composition according to claim 13, for a use according to claim 14, wherein at least one of:

a) the 4-1BBL ECD mutein, the conjugate or the composition is administered as a neoadjuvant therapy before a primary therapy comprising at least one of surgery and radiation therapy of the cancer; and,

b) the 4-1BBL ECD mutein, the conjugate or the composition is administered as an adjuvant therapy after a primary therapy comprising at least one of surgery and radiation therapy of the cancer.