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(54) Titre : ANTICORPS BISPECIFIQUES ANTI-V β 17/ANTI-CD123

(54) Title: ANTI-V β 17/ANTI-CD123 BISPECIFIC ANTIBODIES

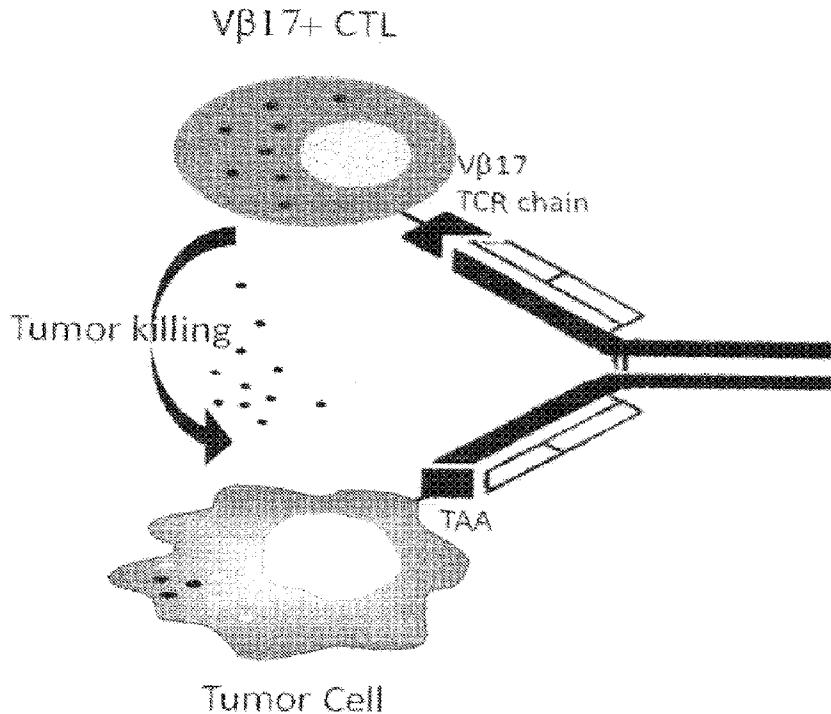


FIG. 1

(57) Abrégé/Abstract:

The present disclosure relates to V β 17 /CD123 bispecific antibodies. The antibodies are characterized by their CDRs sequences. Toxicity experiments are performed on Kasumi-3 cells (used to perform acute myeloblastic leukaemia pathologies)

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(54) Title: ANTI-V(BETA17)/ANTI-CD123 BISPECIFIC ANTIBODIES

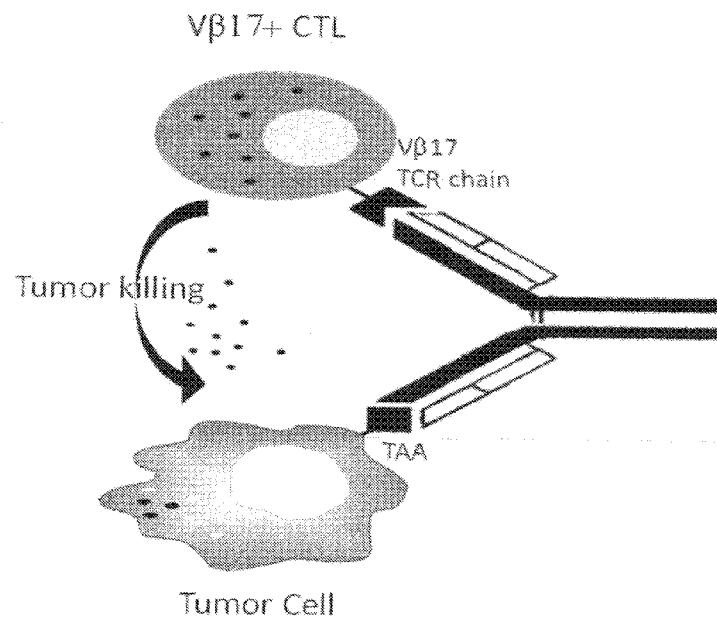


FIG. 1

(57) Abstract: The present disclosure relates to V β 17 /CD123 bispecific antibodies. The antibodies are characterized by their CDRs sequences. Toxicity experiments are performed on Kasumi-3 cells (used to perform acute myeloblastic leukaemia pathologies)

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ANTI-V β 17/ANTI-CD123 BISPECIFIC ANTIBODIES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/816,464, filed on March 11, 2019, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to monoclonal anti-V β 17/anti-CD123 bispecific antibodies, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors, and compositions comprising the antibodies. Methods of making the antibodies, and methods of using the antibodies to kill cancer cells, are also provided.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name “PRD4012WOPCT Sequence Listing” and a creation date of March 11, 2020 and having a size of 85 kb. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] Cytotoxic T cells (e.g., CD8+ T cells) can be utilized to directly kill cancer cells. Finding a way to direct cytotoxic T cells to a cancer cell could lead to the killing of such cells and an inhibition of cancer cell propagation. It has been demonstrated that cytotoxic T cells can be activated against cancer cells expressing cancer-associated antigens, by bringing said cytotoxic T cells into close proximity to the cancer cells for an extended period of time using a bispecific antibody that binds both the cytotoxic T cell and the cancer cell. A variety of potential complications to this approach of killing cancer cells exist, such as selecting T cell and cancer cell antigens that mediate T cell activation, selecting parental antibodies that will have adequate affinity to mediate binding in the context of a bispecific antibody, and choosing a cancer cell antigen that will activate T cells to act specifically against cancer cells, rather than elicit nonspecific T cell activation. These complications are only compounded in the context of attempting to activate T cells to destroy cancer cells in an animal subject.

BRIEF SUMMARY OF THE INVENTION

[0005] Provided herein are bispecific antibodies capable of binding V β 17, an antigen associated with T cells, and CD123, an antigen associated with cancer cells. Cytotoxic T cells express T cell receptors that consist of α - and β -chains, such as V β 17. It is hypothesized that a bispecific antibody binding to V β 17 and a cancer-associated antigen, such as CD123, may direct a cytotoxic T cell to an antigen-expressing cancer cell. Utilizing a bispecific antibody of this sort to recruit, or redirect, the cytotoxic T cell to an antigen-expressing cancer cell and could allow the T cell to kill the cancer cell.

[0006] In one general aspect, the present disclosure relates to isolated bispecific antibodies or antigen-binding fragments thereof that bind to V β 17 and CD123.

[0007] Provided herein are isolated V β 17 bispecific antibodies or antigen-binding fragments thereof. The isolated V β 17 bispecific antibody or antigen-binding fragment thereof comprises:

- a. a first heavy chain (HC1);
- b. a second heavy chain (HC2);
- c. a first light chain (LC1); and
- d. a second light chain (LC2),

wherein HC1 is associated with LC1 and HC2 is associated with LC2, and wherein HC1 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and LC1 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively, to form a binding site for a first antigen, and wherein HC2 and LC2 form a binding site for a second antigen. In certain embodiments, the binding site for the first antigen binds to a V β 17 on the surface of a CD8 $+$ or CD4 $+$ T cell. In certain embodiments, the binding site for the second antigen binds to a tumor antigen present on the surface of a cancer cell.

[0008] In certain embodiments, the binding of the bispecific antibody to V β 17 present on the surface of the CD8 $+$ or CD4 $+$ T cell and the binding of the tumor antigen present on the surface of the cancer cells results in the killing of the cancer cell.

[0009] In certain embodiments, HC2 and LC2 bind to CD123.

[0010] In certain embodiments, the bispecific antibody or antigen-binding fragment thereof is an IgG isotype, such as IgG4.

[0011] In certain embodiments, the bispecific antibody or antigen-binding fragment thereof induces CD8+ or CD4+ T-cell dependent cytotoxicity of a cancer cell *in vitro* with an EC₅₀ of less than about 0.2 pM.

[0012] Also provided are isolated anti-V β 17/anti-CD123 bispecific antibodies or antigen-binding fragments thereof. The anti-V β 17/anti-CD123 bispecific antibodies or antigen-binding fragments thereof comprise:

- a. a first heavy chain (HC1);
- b. a second heavy chain (HC2)
- c. a first light chain (LC1); and
- d. a second light chain (LC2),

wherein HC1 is associated with LC1 and HC2 is associated with LC2, and wherein HC1 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and LC1 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively, to form a binding site for a first antigen that specifically binds V β 17, and wherein HC2 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, respectively, and LC2 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39, respectively, to form a binding site for a second antigen that specifically binds CD123. In certain embodiments, the HC1 comprises the amino acid sequence of SEQ ID NO:13 and LC1 comprises the amino acid sequence of SEQ ID NO:14, and the HC2 comprises the amino acid sequence of SEQ ID NO:15 and LC2 comprises the amino acid sequence of SEQ ID NO:16. In certain embodiments, the V β 17 is on the surface of a CD8+ or CD4+ T cell. In certain embodiments, the CD123 is on the surface of a cancer cell. In certain embodiments, the bispecific antibody or antigen-binding fragment thereof induces CD8+ or CD4+ T-cell dependent cytotoxicity of a cancer cell *in vitro* with an EC₅₀ of less than about 0.2 pM.

[0013] In certain embodiments, the anti-V β 17/anti-CD123 bispecific antibodies or antigen-binding fragments thereof are chimeric, partially humanized, or fully humanized.

[0014] Also provided are isolated humanized V β 17 monoclonal antibodies or antigen-binding fragments thereof. The isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof can comprise an amino acid sequence with at least 95% identity to the amino

acid sequence of SEQ ID NO:28. In certain embodiments, the isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof comprises an amino acid sequence of SEQ ID NO:28.

[0015] Also provided are isolated nucleic acids encoding the monoclonal antibodies or antigen-binding fragments thereof and the bispecific antibodies or antigen-binding fragments thereof disclosed herein.

[0016] Also provided are vectors comprising the isolated nucleic acids encoding the monoclonal antibodies or antigen-binding fragments thereof and the bispecific antibodies or antigen-binding fragments thereof disclosed herein.

[0017] Also provided are host cells comprising the vectors comprising the isolated nucleic acids disclosed herein.

[0018] Also provided are methods of directing a V β 17-expressing CD8+ or CD4+ T cell to a cancer cell. The methods comprise contacting a V β 17-expressing CD8+ or CD4+ T cell with a anti-V β 17/anti-CD123 bispecific antibodies or antigen-binding fragments thereof disclosed herein. Contacting the V β 17-expressing CD8+ or CD4+ T cell with the anti-V β 17/anti-CD123 bispecific antibodies or antigen-binding fragments thereof can direct the V β 17-expressing CD8+ or CD4+ T cell to a cancer cell.

[0019] Also provided are methods for inhibiting growth or proliferation of cancer cells. The methods comprise contacting the cancer cells with the bispecific antibodies disclosed herein. Contacting the cancer cells with the described antibodies can, for example, inhibit the growth or proliferation of the cancer cells, or promote T cell mediated killing of the cancer cells.

[0020] Also provided are methods of producing the bispecific antibodies or antigen-binding fragments thereof disclosed herein. The methods comprise culturing a cell comprising a nucleic acid encoding one heavy and light chain pair of the bispecific antibody under conditions to produce the heavy and light chains or an antigen-binding fragment thereof, and recovering the heavy and light chains of the bispecific antibody or an antigen-binding fragment thereof from the cell or culture. Following collection of heavy and light chains for both arms of the bispecific antibody, the heavy and light chain pairs are mixed in conditions suitable to allow for self-assembly, after which the self-assembled bispecific antibodies are collected.

[0021] Also provided are methods of producing compositions comprising the bispecific antibodies or antigen-binding fragments disclosed herein, such as buffered compositions or purified compositions and the like. For example, the methods may comprise combining the bispecific antibody or antigen-binding fragment thereof with a buffer acceptable that is acceptable for storage and use of the bispecific antibody.

[0022] Also provided are kits comprising bispecific antibodies or antigen-binding fragments thereof disclosed herein and packaging for the same.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The foregoing summary, as well as the following detailed description of preferred embodiments of the present application, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the application is not limited to the precise embodiments shown in the drawings.

[0024] **FIG. 1** shows a schematic demonstrating the binding of an anti-V β 17/anti-tumor antigen bispecific antibody to recruit T-cells to a cancer cell and to induce cancer cell death.

[0025] **FIGS. 2A-2B** shows that V β 17+ CD8+ T cells exist in healthy subjects and upon culture with M1 peptide these cells can be expanded *in vitro*. **FIG. 2A** shows FACS histograms of gated peripheral blood mononuclear cells (PBMCs) for CD8+ T cells expressing V β 17 (V β 17+) on the cells surface from healthy subjects. **FIG. 2B** shows HLA sub-type of various donors and presence of percent V β 17+ CD8+ T cells identified as day 0, and after *in vitro* expansion with M1 peptide for 14 days (Day 14).

[0026] **FIGS. 3** shows V β 17+ CD8+ T cells have hallmarks of killer cytotoxic cells. Bar graph indicates expression of CD107a, CD69, Granzyme B (Gzb) and Interferon- γ (IFN γ) on gated PBMCs for CD8+ T cells expressing V β 17 (V β 17+) on the cell surface at day 0 (no M1) and at day 14 after stimulation with M1 peptide (+M1).

[0027] **FIG. 4** shows binding of VB11 [anti-V β 17/anti-CD123] bispecific as well as VB13 [V β 17 null control bispecific] antibodies to CD8+ T cells. Data presented from CD8+ T cells isolated from PBMCs from 3 different donors (D203517, HPU09381 and HPU08694). The table below each graph presents EC₅₀ values for binding in nM.

[0028] **FIG. 5** shows binding of V β 17 and CD123 bispecific (VB11) as well as V β 17 null control bispecific (VB13) antibodies to AML cancer cell line. Data presented shows binding of bispecific antibodies to Kasumi3 AML cell line. The table below the graph presents EC₅₀ values for binding in nM.

[0029] **FIG. 6** shows redirection of V β 17+ T cells by bispecific antibodies that induce efficient killing of AML cancer cells. Data in the left graph shows killing of Kasumi3 cancer cells at an effector to target (E:T) ratio 0.5:1 and dose titration of bispecific antibodies. Data in the middle graph shows killing of Kasumi3 cancer cells at an E:T ratio 1:1 and dose titration of bispecific antibodies. Data in the right graph shows killing of Kasumi3 cancer cells at an E:T ratio 5:1 and

dose titration of bispecific antibodies. The table below the graphs shows EC₅₀ values calculated from the above graphs given in pM.

[0030] **FIGS. 7A-7B** show specific binding of an anti-V β 17/anti-CD123 bispecific antibody (VB11) and a V β 17 null bispecific antibody (VB13) to CD8+ T cells isolated from PBMCs. **FIG. 7A** shows FACS histograms of gated PBMCs for CD8+ T cells expressing V β 17 (V β 17+) on the cell surface from healthy subjects (left graph, V β 17 non-depleted) and from PBMCs that were depleted of V β 17+ T cells using negative selection (right graph, V β 17 depleted). **FIG 7B** shows specific binding of an anti-V β 17/anti-CD123 bispecific antibody (VB11) and a V β 17 null bispecific antibody (VB13) to CD8+ T cells from **FIG 7A**. A dose response of bispecific antibodies is shown in the figure. The table below the graph shows EC₅₀ values for binding calculated from the above graph given in nM.

[0031] **FIG. 8** shows specific recruitment of V β 17 T cells by a V β 17-bispecific antibody for killing of Kasumi3 cancer cells. Left figure shows killing of Kasumi3 AML cell line when effectors cells were isolated from PBMCs containing CD8+ T cells expressing V β 17 (V β 17+) on the cell surface (untouched CD8 T cells). Insert shows presence of 10.1% V β 17+ CD8 T cells in the effector cell population. Right figure shows killing of Kasumi3 AML cell line when effector CD8+ T cells were isolated from PBMCs, but V β 17+ T cells were depleted by negative selection. Insert shows presence of a minor population (0.086%) V β 17+ CD8+ T cells in the effector cell population.

[0032] **FIGS. 9A-9B** show that there is no pan activation of T cells when using V β 17 bispecific antibodies. **FIG. 9A** shows FACS plots of V β 17+ and V β 17- gated CD8+ T cells. When T cells were activated with V β 17 bispecific antibody there was high level of upregulation of CD69 (62.5%) on V β 17+ as compared to V β 17- CD8+ T cells (1.80%). **FIG. 9B** shows a bar graph for upregulation of CD69 on V β 17+ and V β 17- gated CD8+ T cells when activated using V β 17 bispecific antibody.

[0033] **FIG. 10** shows that V β 17+ T cells from HLA A2 negative donor are also effector killer cells and no pre-stimulation of V β 17+ cell required. Efficient cytotoxicity mediated by V β 17 bispecific antibody of Kasumi3 cancer cells is shown from PBMCs containing V β 17+ T cells from HLA A2 negative donor (HPU 09381).

DETAILED DESCRIPTION OF THE INVENTION

[0034] Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention.

Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

[0035] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification.

[0036] Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term “about.” Thus, a numerical value typically includes $\pm 10\%$ of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0037] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

[0038] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

[0039] As used herein, the terms “comprises,” “comprising,” “includes,” “including,” “has,” “having,” “contains” or “containing,” or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers and are intended to be non-exclusive or open-ended. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but can include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, “or” refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0040] As used herein, the conjunctive term “and/or” between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by “and/or,” a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without

the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or” as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or.”

[0041] It should also be understood that the terms “about,” “approximately,” “generally,” “substantially,” and like terms, used herein when referring to a dimension or characteristic of a component of the preferred invention, indicate that the described dimension/characteristic is not a strict boundary or parameter and does not exclude minor variations therefrom that are functionally the same or similar, as would be understood by one having ordinary skill in the art. At a minimum, such references that include a numerical parameter would include variations that, using mathematical and industrial principles accepted in the art (e.g., rounding, measurement or other systematic errors, manufacturing tolerances, etc.), would not vary the least significant digit.

[0042] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences (e.g., anti-V β 17/anti-CD123 bispecific antibodies and polynucleotides that encode them, V β 17 polypeptides and V β 17 polynucleotides that encode them, CD123 polypeptides and CD123 polynucleotides that encode them), refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

[0043] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0044] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally, Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

[0045] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and Altschul *et al.* (1997) *Nucleic Acids Res.* 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased.

[0046] Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N= -4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

[0047] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0048] A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a

polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

[0049] Antibodies

[0050] Described herein are isolated anti-V β 17 bispecific antibodies or antigen-binding fragments thereof, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors, and compositions comprising the antibodies. The invention additionally relates to isolated anti-V β 17/anti-CD123 bispecific antibodies or antigen-binding fragments thereof, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors, and compositions comprising the bispecific antibodies. Methods of making the antibodies, and methods of using the antibodies to treat diseases, including cancer, are also provided. The antibodies disclosed herein possess one or more desirable functional properties, including but not limited to high-affinity binding to V β 17 and/or CD123, high specificity to V β 17 and/or CD123, and the ability to treat or prevent cancer when administered alone or in combination with other anti-cancer therapies.

[0051] As used herein, the term “antibody” is used in a broad sense and includes immunoglobulin or antibody molecules including human, humanized, composite and chimeric antibodies and antibody fragments that are monoclonal or polyclonal. In general, antibodies are proteins or peptide chains that exhibit binding specificity to a specific antigen. Antibody structures are well known. Immunoglobulins can be assigned to five major classes (i.e., IgA, IgD, IgE, IgG and IgM), depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Antibody light chains of vertebrate species can be assigned to one of two clearly distinct types, namely kappa and lambda, based on the amino acid sequences of their constant domains. Accordingly, the antibodies of the invention can contain a kappa or lambda light chain constant domain. According to particular embodiments, the antibodies disclosed herein include heavy and/or light chain constant regions from mouse or human antibodies. In addition to the heavy and light constant domains, antibodies contain an antigen-binding region that is made up of a light chain variable region and a heavy chain variable region, each of which contains three domains (i.e., complementarity determining regions 1-3; CDR1, CDR2, and CDR3). The light chain variable region domains are alternatively referred to as LCDR1, LCDR2, and LCDR3, and the heavy chain variable region domains are alternatively referred to as HCDR1, HCDR2, and HCDR3.

[0052] As used herein, the term an “isolated antibody” refers to an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to V β 17 is substantially free of antibodies that do not bind to V β 17; an isolated antibody that specifically binds to CD123 is substantially free of antibodies that do not bind to CD123). In addition, an isolated antibody is substantially free of other cellular material and/or chemicals.

[0053] As used herein, the term “monoclonal antibody” refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that can be present in minor amounts. The monoclonal antibodies disclosed herein can be made by the hybridoma method, phage display technology, single lymphocyte gene cloning technology, or by recombinant DNA methods. For example, the monoclonal antibodies can be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, such as a transgenic mouse or rat, having a genome comprising a human heavy chain transgene and a light chain transgene.

[0054] As used herein, the term “antigen-binding fragment” refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), a single domain antibody (sdab) an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment binds. According to particular embodiments, the antigen-binding fragment comprises a light chain variable region, a light chain constant region, and an Fd segment of the heavy chain. According to other particular embodiments, the antigen-binding fragment comprises Fab and F(ab').

[0055] As used herein, the term “humanized antibody” refers to a non-human antibody that is modified to increase the sequence homology to that of a human antibody, such that the antigen-binding properties of the antibody are retained, but its antigenicity in the human body is reduced.

[0056] As used herein, the term “multispecific antibody” refers to an antibody that comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, the first and second epitopes do not overlap or do not substantially

overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a multispecific antibody comprises a third, fourth, or fifth immunoglobulin variable domain. In an embodiment, a multispecific antibody is a bispecific antibody molecule, a trispecific antibody molecule, or a tetraspecific antibody molecule.

[0057] As used herein, the term “bispecific antibody” refers to a multispecific antibody that binds no more than two epitopes or two antigens. A bispecific antibody is characterized by a first immunoglobulin heavy and light chain pair which has binding specificity for a first epitope (*e.g.*, an epitope on a V β 17 antigen) and a second immunoglobulin heavy and light chain pair that has binding specificity for a second epitope (*e.g.*, an epitope on a CD123 antigen). In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a bispecific antibody comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a scFv, or fragment thereof, having binding specificity for a first epitope, and a scFv, or fragment thereof, having binding specificity for a second epitope. In an embodiment, the first epitope is located on V β 17 and the second epitope is located on CD123. In an embodiment, the first epitope is located on V β 17 and the second epitope is located on PD-1, PD-L1, CTLA-4, EGFR, HER-2, CD19, CD20, CD3 and/or other tumor associated immune suppressors or surface antigens.

[0058] The term "half antibody" as used herein refers to one immunoglobulin heavy chain associated with one immunoglobulin light chain. An exemplary half-antibody is depicted in SEQ ID NO: 28. One skilled in the art will readily appreciate that a half-antibody can encompass a fragment thereof and can also have an antigen binding domain consisting of a single variable domain, *e.g.*, originating from a camelidae.

[0059] As used herein, the term “V β 17” refers to a T cell receptor, which is expressed in response to an immune response on a cytotoxic T cell. V β 17-expressing CD8+ T cells are commonly produced in response to influenza A virus exposure in a subject. V β 17-expressing CD8+ T cells provide great recall in response to influenza exposure in the subject. The term “V β 17” includes any V β 17 variant, isoform, and species homolog, which is naturally expressed by cells (including T cells) or can be expressed on cells transfected with genes or cDNA

encoding the polypeptide. Unless noted, preferably the V β 17 is a human V β 17. A human V β 17 amino acid sequence is provided by GenBank Accession Number AAB49730.1.

[0060] The term “CD123” refers to a molecule that is found on cells which helps transmit the signal of interleukin-3, a soluble cytokine that is important in the immune system. CD123 can also be referred to as the “interleukin-3 receptor.” The receptor belongs to the type I cytokine receptor family and is a heterodimer with a unique alpha chain paired with the common beta subunit (beta c or CD131). The CD123 receptor can be found on pluripotent progenitor cells and can induce tyrosine phosphorylation within the cell and promote proliferation and differentiation within hematopoietic cell lines. CD123 can also be expressed in acute myeloid leukemia (AML) subtypes. The term “CD123” includes any CD123 variant, isoform, and species homolog, which is naturally expressed by cells (including T cells) or can be expressed on cells transfected with genes or cDNA encoding those polypeptides, unless noted, preferably the “CD123” is a human CD123. A human CD123 amino acid sequence is provided by GenBank Accession Number AY789109.1.

[0061] As used herein, an antibody that “specifically binds to V β 17” refers to an antibody that binds to a V β 17, preferably a human V β 17, with a KD of 1×10^{-7} M or less, preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term “KD” refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods in the art in view of the present disclosure. For example, the KD of an antibody can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as an Octet RED96 system.

[0062] As used herein, an antibody that “specifically binds to CD123” refers to an antibody that binds to a CD123, preferably a human CD123, with a KD of 1×10^{-7} M or less, preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term “KD” refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods in the art in view of the present disclosure. For example, the KD of an antibody can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as an Octet RED96 system.

[0063] The smaller the value of the KD of an antibody, the higher affinity that the antibody binds to a target antigen.

[0064] According to a particular aspect, the invention relates to an isolated V β 17 bispecific antibody or antigen-binding fragment thereof comprising (a) a first heavy chain (HC1); (b) a second heavy chain (HC2); (c) a first light chain (LC1); and (d) a second light chain (LC2). The HC1 can be associated with the LC1 and the HC2 can be associated with LC2. The HC1 can comprise a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and LC1 can comprise a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.

[0065] The HC1 and LC1 form a binding site for a first antigen, and the HC2 and LC2 form a binding site for a second antigen. By way of an example, the binding site for the first antigen can bind to a V β 17 on a CD8+ or CD4+ T cell, and the binding site for the second antigen can, for example, bind a tumor antigen present on the surface of a cancer cell. The binding of the V β 17 bispecific antibody to V β 17 present on the surface of the CD8+ or CD4+ T cell, and the binding of the tumor antigen present on the surface of the cancer cells can, for example, result in the killing of the cancer cell.

[0066] Also provided herein are anti-V β 17/anti-CD123 bispecific antibodies or antigen-binding fragments thereof comprising an anti-V β 17 antibody or an antigen-binding fragment thereof and an anti-CD123 antibody or antigen-binding fragment thereof. In certain embodiments the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof comprises (a) a first heavy chain (HC1); (b) a second heavy chain (HC2); (c) a first light chain (LC1); and a second light chain (LC2). The HC1 is associated with the LC1 and the HC2 is associated with the LC2. In certain embodiments, the HC1 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and LC1 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively. In certain embodiments, the HC2 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, respectively, and LC2 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39, respectively.

[0067] In certain embodiments, the HC1 can, for example, comprise an amino acid sequence of SEQ ID NO:13 and the LC1 can, for example, comprise an amino acid sequence of SEQ ID

NO:14 to form a binding site for a first antigen that specifically binds V β 17. The HC2 can, for example, comprise an amino acid sequence of SEQ ID NO:15 and the LC2 can, for example, comprise an amino acid sequence of SEQ ID NO:16 to form a binding site for a second antigen that specifically binds CD123.

[0068] In certain embodiments, the V β 17 is on the surface of a CD8 $^{+}$ or CD4 $^{+}$ T cell. In certain embodiments, the CD123 is on the surface of a cancer cell (e.g., a leukemia cell).

[0069] In some embodiments, the bispecific antibodies disclosed herein can take the form of a diabody, a cross-body, or a bispecific antibody obtained via a controlled Fab arm exchange as described herein.

[0070] In some embodiments, the bispecific antibodies include IgG-like molecules with complementary CH3 domains that promote heterodimerization; recombinant IgG-like dual targeting molecules, wherein the two sides of the molecule each contain the Fab fragment or part of the Fab fragment of at least two different antibodies; IgG fusion molecules, wherein full length IgG antibodies are fused to an extra Fab fragment or parts of Fab fragment; Fc fusion molecules, wherein single chain Fv molecules or stabilized diabodies are fused to heavy-chain constant-domains, Fc-regions or parts thereof; Fab fusion molecules, wherein different Fab-fragments are fused together; ScFv- and diabody-based and heavy chain antibodies (e.g., domain antibodies, nanobodies) wherein different single chain Fv molecules or different diabodies or different heavy-chain antibodies (e.g. domain antibodies, nanobodies) are fused to each other or to another protein or carrier molecule.

[0071] In some embodiments, IgG-like molecules with complementary CH3 domains molecules include the Triomab/Quadroma (Trion Pharma/Fresenius Biotech), the Knobs-into-Holes (Genentech), CrossMAbs (Roche) and the electrostatically-matched (Amgen), the LUZ-Y (Genentech), the Strand Exchange Engineered Domain body (SEEDbody) (EMD Serono), the Biclonic (Merus) and the DuoBody (Genmab A/S).

[0072] In some embodiments, recombinant IgG-like dual targeting molecules include Dual Targeting (DT)-Ig (GSK/Domainis), Two-in-one Antibody (Genentech), Cross-linked Mabs (Karmanos Cancer Center), mAb2 (F-Star) and CovX-body (CovX/Pfizer).

[0073] In some embodiments, IgG fusion molecules include Dual Variable Domain (DVD)-Ig (Abbott), IgG-like Bispecific (InnClone/Eli Lilly), Ts2Ab (MedImmune/AZ) and BsAb (ZymoGenetics), HERCULES (Biogen Idec) and TvAb (Roche).

[0074] In some embodiments, Fc fusion molecules can include ScFv/Fc Fusions (Academic Institution), SCORPION (Emergent BioSolutions/Trubion, ZymoGenetics/BMS), Dual Affinity Retargeting Technology (Fc-DART) (MacroGenics) and Dual(ScFv)₂-Fab (National Research Center for Antibody Medicine--China).

[0075] In some embodiments, Fab fusion bispecific antibodies include F(ab)₂ (Medarex/AMGEN), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock (DNL) (ImmunoMedics), Bivalent Bispecific (Biotecnol) and Fab-Fv (UCB-Celltech). ScFv-, diabody-based, and domain antibodies, include but are not limited to, Bispecific T Cell Engager (BiTE) (Micromet), Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology (DART) (MacroGenics), Single-chain Diabody (Academic), TCR-like Antibodies (AIT, ReceptorLogics), Human Serum Albumin ScFv Fusion (Merrimack) and COMBODY (Epigen Biotech), dual targeting nanobodies (Ablynx), dual targeting heavy chain only domain antibodies.

[0076] Full length bispecific antibodies disclosed herein can be generated for example using Fab arm exchange (or half molecule exchange) between two mono specific bivalent antibodies by introducing substitutions at the heavy chain CH3 interface in each half molecule to favor heterodimer formation of two antibody half molecules having distinct specificity either *in vitro* in cell-free environment or using co-expression. The Fab arm exchange reaction is the result of a disulfide-bond isomerization reaction and dissociation-association of CH3 domains. The heavy-chain disulfide bonds in the hinge regions of the parent mono specific antibodies are reduced. The resulting free cysteines of one of the parent monospecific antibodies form an inter heavy-chain disulfide bond with cysteine residues of a second parent mono specific antibody molecule and simultaneously CH3 domains of the parent antibodies release and reform by dissociation-association. The CH3 domains of the Fab arms can be engineered to favor heterodimerization over homodimerization. The resulting product is a bispecific antibody having two Fab arms or half molecules, each binding a distinct epitope, i.e. an epitope on V β 17 and an epitope on a tumor antigen.

[0077] “Homodimerization” as used herein refers to an interaction of two heavy chains having identical CH3 amino acid sequences. “Homodimer” as used herein refers to an antibody having two heavy chains with identical CH3 amino acid sequences.

[0078] “Heterodimerization” as used herein refers to an interaction of two heavy chains having non-identical CH3 amino acid sequences. “Heterodimer” as used herein refers to an antibody having two heavy chains with non-identical CH3 amino acid sequences.

[0079] The “knob-in-hole” strategy (see, e.g., PCT Inti. Publ. No. WO 2006/028936) can be used to generate full length bispecific antibodies. Briefly, selected amino acids forming the interface of the CH3 domains in human IgG can be mutated at positions affecting CH3 domain interactions to promote heterodimer formation. An amino acid with a small side chain (hole) is introduced into a heavy chain of an antibody specifically binding a first antigen and an amino acid with a large side chain (knob) is introduced into a heavy chain of an antibody specifically

binding a second antigen. After co-expression of the two antibodies, a heterodimer is formed as a result of the preferential interaction of the heavy chain with a “hole” with the heavy chain with a “knob”. Exemplary CH3 substitution pairs forming a knob and a hole are (expressed as modified position in the first CH3 domain of the first heavy chain/modified position in the second CH3 domain of the second heavy chain): T366Y/F405A, T366W/ F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S_L368A_Y407V.

[0080] Other strategies such as promoting heavy chain heterodimerization using electrostatic interactions by substituting positively charged residues at one CH3 surface and negatively charged residues at a second CH3 surface can be used, as described in US Pat. Publ. No. US2010/0015133; US Pat. Publ. No. US2009/0182127; US Pat. Publ. No. US2010/028637; or US Pat. Publ. No. US2011/0123532. In other strategies, heterodimerization can be promoted by the following substitutions (expressed as modified position in the first CH3 domain of the first heavy chain/modified position in the second CH3 domain of the second heavy chain):

L351Y_F405AY407V/T394W, T366I_K392M_T394W/F405A_Y407V,
 T366L_K392M_T394W/F405A_Y407V, L351Y_Y407A/T366A_K409F,
 L351Y_Y407A/T366V K409F Y407A/T366A_K409F, or T350V_L351Y_F405A
 Y407V/T350V_T366L_K392L_T394W as described in U.S. Pat. Publ. No. US2012/0149876 or
 U.S. Pat. Publ. No. US2013/0195849.

[0081] In addition to methods described above, bispecific antibodies disclosed herein can be generated *in vitro* in a cell-free environment by introducing asymmetrical mutations in the CH3 regions of two mono specific homodimeric antibodies and forming the bispecific heterodimeric antibody from two parent monospecific homodimeric antibodies in reducing conditions to allow disulfide bond isomerization according to methods described in International Pat. Publ. No. WO2011/131746. In the methods, the first monospecific bivalent antibody (e.g., anti-CD33 antibody) and the second monospecific bivalent antibody (e.g., anti-CD3 antibody) are engineered to have certain substitutions at the CH3 domain that promotes heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions can optionally be restored to non-reducing conditions. Exemplary reducing agents that may be used are 2-mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris (2-carboxyethyl) phosphine (TCEP), L-cysteine and beta-mercaptoethanol, preferably a reducing agent selected from the group consisting of: 2-mercaptoethylamine, dithiothreitol and tris (2-carboxyethyl) phosphine. For example, incubation for at least 90 min at a temperature of at least 20° C in the presence of at

least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

[0082] In certain embodiments, the anti-V β 17 antibody or antigen-binding fragment thereof comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequence of:

- a. SEQ ID NOs:1, 2, 3, 4, 5, and 6, respectively;

and the anti-CD123 antibody or antigen-binding fragment thereof comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequence of:

- 1. SEQ ID NOs:34, 35, 36, 37, 38, and 39, respectively.

[0083] According to another particular aspect, the invention relates to an isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof that induces antibody-dependent cell-mediated cytotoxicity (ADCC). The bispecific antibody or antigen-binding fragment thereof can, for example, induce ADCC *in vitro*. The bispecific antibody or antigen-binding fragment thereof can induce ADCC with an EC₅₀ of less than about 1 pM. In certain embodiments, the EC₅₀ is less than about 1 pM, less than about 0.9 pM, less than about 0.8 pM, less than about 0.7 pM, less than about 0.6 pM, less than about 0.5 pM, less than about 0.4 pM, less than about 0.300 pM, less than about 0.2 pM, less than about 0.19 pM, less than about 0.18 pM, less than about 0.17 pM, less than about 0.16 pM, less than about 0.15 pM, less than about 0.14 pM, less than about 0.13 pM, less than about 0.12 pM, less than about 0.11 pM, less than about 0.1 pM, less than about 0.09 pM, less than about 0.08 pM, less than about 0.07 pM, less than about 0.06 pM, less than about 0.05 pM, less than about 0.04 pM, less than about 0.03 pM, less than about 0.02 pM, or less than about 0.01 pM. In certain embodiments, the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof comprises an IgG1, IgG2, IgG3, or IgG4 backbone. In one such embodiment, the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof has an antibody backbone of the IgG4 isotype.

[0084] In some embodiments described herein, immune effector properties of the anti-V β 17/anti-CD123 bispecific antibodies can be enhanced or silenced through Fc modifications by techniques known to those skilled in the art. For example, Fc effector functions such as Clq binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. may be provided and/or controlled by modifying residues in the Fc responsible for these activities.

[0085] “Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a cell-mediated reaction in which non-specific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell.

[0086] The ability of antibodies to induce ADCC can be enhanced by engineering their oligosaccharide component. Human IgG1 or IgG3 are N-glycosylated at Asn297 with the majority of the glycans in the well-known biantennary G0, G0F, G1, G1F, G2 or G2F forms. Antibodies produced by non-engineered CHO cells typically have a glycan fucose content of about at least 85%. The removal of the core fucose from the biantennary complex-type oligosaccharides attached to the Fc regions enhances the ADCC of antibodies via improved Fc γ RIIIa binding without altering antigen-binding or CDC activity. Such Abs can be achieved using different methods reported to lead to the successful expression of relatively high defucosylated antibodies bearing the biantennary complex-type of Fc oligosaccharides such as control of culture osmolality (Konno et al., Cytotechnology 64:249-65, 2012), application of a variant CHO line Lec13 as the host cell line (Shields et al., J Biol Chem 277:26733-26740, 2002), application of a variant CHO line EB66 as the host cell line (Olivier et al., MAbs; 2(4), 2010; Epub ahead of print; PMID:20562582), application of a rat hybridoma cell line YB2/0 as the host cell line (Shinkawa et al., J Biol Chem 278:3466-3473, 2003), introduction of small interfering RNA specifically against the α -1,6-fucosyltransferase (FUT8) gene (Mori et al., Biotechnol Bioeng 88:901-908, 2004), or coexpression of β -1,4-N-acetylglucosaminyltransferase III and golgi α -mannosidase II or a potent alpha-mannosidase I inhibitor, kifunensine (Ferrara et al., J Biol Chem 281:5032-5036, 2006, Ferrara et al., Biotechnol Bioeng 93:851-861, 2006; Xhou et al., Biotechnol Bioeng 99:652-65, 2008).

[0087] In some embodiments described herein, ADCC elicited by the anti-V β 17/anti-CD123 bispecific antibodies can also be enhanced by certain substitutions in the antibody Fc. Exemplary substitutions include, for example, substitutions at amino acid positions 256, 290, 298, 312, 356, 330, 333, 334, 360, 378 or 430 (residue numbering according to the EU index) as described in U.S. Pat. No. 6,737,056.

[0088] According to another particular aspect, the invention relates to an isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof capable of inducing T-cell dependent cytotoxicity in V β 17-expressing cells and/or CD123-expressing cells. The bispecific antibody or antigen-binding fragment thereof can, for example, induce T-cell dependent cytotoxicity in V β 17-expressing cells and/or CD123-expressing cells *in vitro* with an EC₅₀ value of less than about 2nM. In certain embodiments, the EC₅₀ is less than about 2.0nM,

less than about 1.9nM, less than about 1.8nM, less than about 1.7nM, less than about 1.6nM, less than about 1.5nM, less than about 1.4nM, less than about 1.3nM, less than about 1.2nM, less than about 1.1nM, less than about 1.0nM, less than about 0.9nM, less than about 0.8nM, less than about 0.7nM, less than about 0.6nM, less than about 0.5nM, less than about 0.4nM, less than about 0.3nM, less than about 0.2nM, and less than about 0.1nM.

[0089] According to another particular aspect, the invention relates to an isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof, wherein the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof is chimeric.

[0090] According to another particular aspect, the invention relates to an isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof, wherein the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof is human or humanized.

[0091] In another general aspect, the invention relates to an isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof. The isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof comprises an amino acid sequence with at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO:28. In certain embodiments, the humanized V β 17 monoclonal antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NO:28.

[0092] In another general aspect, the invention relates to isolated nucleic acids encoding the monoclonal antibodies or antigen-binding fragments thereof disclosed herein. In another general aspect, the invention relates to isolated nucleic acids encoding the bispecific antibodies or antigen-binding fragments thereof disclosed herein. It will be appreciated by those skilled in the art that the coding sequence of a protein can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that nucleic acid sequences encoding monoclonal antibodies and/or bispecific antibodies disclosed herein can be altered without changing the amino acid sequences of the proteins.

[0093] In another general aspect, the invention relates to vectors comprising the isolated nucleic acids disclosed herein. Any vector known to those skilled in the art in view of the present disclosure can be used, such as a plasmid, a cosmid, a phage vector or a viral vector. In some embodiments, the vector is a recombinant expression vector such as a plasmid. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter, ribosome binding element, terminator, enhancer, selection marker, and

origin of replication. The promoter can be a constitutive, inducible or repressible promoter. A number of expression vectors capable of delivering nucleic acids to a cell are known in the art and can be used herein for production of an antibody or antigen-binding fragment thereof in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments disclosed herein. Such techniques are well known to those skilled in the art in view of the present disclosure.

[0094] In another general aspect, the invention relates to host cells comprising the isolated nucleic acids encoding the monoclonal antibodies and/or bispecific antibodies or antigen-binding fragments thereof disclosed herein. Any host cell known to those skilled in the art in view of the present disclosure can be used for recombinant expression of antibodies or antigen-binding fragments thereof disclosed herein. In some embodiments, the host cells are *E. coli* TG1 or BL21 cells (for expression of, e.g., an scFv or Fab antibody), CHO-DG44 or CHO-K1 cells or HEK293 cells (for expression of, e.g., a full-length IgG antibody). According to particular embodiments, the recombinant expression vector is transformed into host cells by conventional methods such as chemical transfection, heat shock, or electroporation, where it is stably integrated into the host cell genome such that the recombinant nucleic acid is effectively expressed.

[0095] In another general aspect, the invention relates to a method of producing a bispecific antibody or antigen-binding fragment thereof disclosed herein. The methods comprise culturing a cell comprising a nucleic acid encoding the bispecific antibody or antigen-binding fragment thereof under conditions to produce a bispecific antibody or antigen-binding fragment thereof disclosed herein, and recovering the antibody or antigen-binding fragment thereof from the cell or cell culture (e.g., from the supernatant). Expressed antibodies or antigen-binding fragments thereof can be harvested from the cells and purified according to conventional techniques known in the art and as described herein.

Methods of use

[0096] In another general aspect, the invention relates to a method of targeting CD123 on the surface of a cancer cell, the method comprising exposing the cancer cell to an anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof.

[0097] The functional activity of bispecific antibodies and antigen-binding fragments thereof that bind V β 17 and/or CD123 can be characterized by methods known in the art and as described herein. Methods for characterizing antibodies and antigen-binding fragments thereof that bind V β 17 and/or CD123 include, but are not limited to, affinity and specificity assays including Biacore, ELISA, and OctetRed analysis; binding assays to detect the binding of antibodies to CD123 on cancer cells by FACS; binding assays to detect the binding of antibodies to V β 17 on

CD8+ or CD4+ T cells. According to particular embodiments, the methods for characterizing antibodies and antigen-binding fragments thereof that bind V β 17 and/or CD123 include those described below.

[0098] In another general aspect, the invention relates to a method of directing V β 17-expressing CD8+ or CD4+ T cells to a cancer cell. The methods comprise contacting the V β 17-expressing CD8+ or CD4+ T cell with a anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof, wherein the antibody or antibody fragment directs the V β 17-expressing CD8+ or CD4+ T cell to a cancer cell having CD123 on its surface.

[0099] In another general aspect, the invention relates to a method for inhibiting growth or proliferation of cancer cells. The methods comprise contacting the V β 17-expressing CD8+ T cells with a anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof, wherein contacting the cancer cells with the antibody or antibody fragment inhibits the growth or proliferation of the cancer cells.

[00100] According to embodiments of the invention, the described anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof can be provided in a buffered composition for storage or use. Suitable buffers for the storage of the described anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof would serve to maintain the stability of the antibody or antibody fragment by minimizing deterioration while stored, not promoting aggregation of the antibody or antibody fragment, or minimizing adhesion to the storage vessel.

EMBODIMENTS

[00101] This invention provides the following non-limiting embodiments.

[00102] Embodiment 1 is an isolated V β 17 bispecific antibody or antigen-binding fragment thereof, the isolated V β 17 bispecific antibody or antigen-binding fragment thereof comprising:

- a. a first heavy chain (HC1);
- b. a second heavy chain (HC2);
- c. a first light chain (LC1); and
- d. a second light chain (LC2),

wherein HC1 is associated with LC1 and HC2 is associated with LC2, and wherein HC1 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and LC1 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and

SEQ ID NO:6, respectively, to form a binding site for a first antigen, and wherein HC2 and LC2 form a binding site for a second antigen.

[00103] Embodiment 2 is the V β 17 bispecific antibody or antigen-binding fragment thereof of embodiment 1, wherein the binding site for the first antigen binds to V β 17 on a CD8+ or CD4+ T cell.

[00104] Embodiment 3 is the V β 17 bispecific antibody or antigen-binding fragment thereof of embodiment 1 or 2, wherein the binding site for the second antigen binds to a tumor antigen present on the surface of a cancer cell.

[00105] Embodiment 4 is the V β 17 bispecific antibody or antigen-binding fragment of any one of embodiments 1 to 3, wherein HC1 and LC1 are humanized.

[00106] Embodiment 5 is the V β 17 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 4, wherein HC2 and LC2 bind to CD123.

[00107] Embodiment 6 is the V β 17 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 5, wherein the bispecific antibody or antigen-binding fragment thereof is a IgG isotype.

[00108] Embodiment 7 is the V β 17 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 6, wherein the bispecific antibody or antigen-binding fragment thereof is a IgG4 isotype.

[00109] Embodiment 8 is the V β 17 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 7, wherein the bispecific antibody or antigen-binding fragment thereof induces CD8+ or CD4+ T-cell dependent cytotoxicity of a cancer cell *in vitro* with an EC₅₀ of less than about 0.2 pM.

[00110] Embodiment 9 is an isolated nucleic acid encoding HC1 and LC1 of the V β 17 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 8.

[00111] Embodiment 10 is an isolated nucleic acid encoding HC2 and LC2 of the V β 17 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 8.

[00112] Embodiment 11 is a vector comprising the isolated nucleic acid of embodiment 9 or embodiment 10.

[00113] Embodiment 12 is a host cell comprising the vector of embodiment 11.

[00114] Embodiment 13 is a buffered composition comprising the isolated V β 17 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 8 and a buffered solution.

[00115] Embodiment 14 is an isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof comprising:

- a. a first heavy chain (HC1);
- b. a second heavy chain (HC2)
- c. a first light chain (LC1); and
- d. a second light chain (LC2),

wherein HC1 is associated with LC1 and HC2 is associated with LC2, and wherein HC1 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and LC1 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively, to form a binding site for a first antigen that specifically binds V β 17, and wherein HC2 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, respectively, and LC2 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39, respectively, to form a binding site for a second antigen that specifically binds CD123.

[00116] Embodiment 15 is the isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment of embodiment 14, wherein HC1 comprises the amino acid sequence of SEQ ID NO:13 and LC1 comprises the amino acid sequence of SEQ ID NO:14, and wherein HC2 comprises the amino acid sequence of SEQ ID NO:15 and LC2 comprises the amino acid sequence of SEQ ID NO:16.

[00117] Embodiment 16 is the isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of embodiment 14 or embodiment 15, wherein the V β 17 is on the surface of a CD8+ or CD4+ T cell.

[00118] Embodiment 17 is the isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 14 to 16, wherein the CD123 is on the surface of a cancer cell.

[00119] Embodiment 18 is the isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 14 to 17, wherein bispecific antibody or antigen-binding fragment thereof induces CD8+ or CD4+ T-cell dependent cytotoxicity of a cancer cell in vitro with an EC₅₀ of less than about 0.2 pM.

[00120] Embodiment 19 is an isolated nucleic acid encoding the HC1 and LC1 of the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 14 to 18.

[00121] Embodiment 20 is an isolated nucleic acid encoding the HC2 and LC2 of the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 14 to 18.

[00122] Embodiment 21 is a vector comprising the isolated nucleic acid of embodiment 19 or embodiment 20.

[00123] Embodiment 22 is a host cell comprising the vector of embodiment 21.

[00124] Embodiment 23 is a buffered composition comprising the isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 14 to 18 and a buffered solution.

[00125] Embodiment 24 is a method of directing a V β 17-expressing CD8 $+$ or CD4 $+$ T cell to a cancer cell, the method comprising contacting a V β 17-expressing CD8 $+$ or CD4 $+$ T cell with the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 8 or 14 to 18, wherein contacting the V β 17-expressing CD8 $+$ or CD4 $+$ T cell with the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof directs the V β 17-expressing CD8 $+$ or CD4 $+$ T cell to a cancer cell having CD123 on its surface.

[00126] Embodiment 24(a) is the method of embodiment 24, wherein the V β 17-expressing CD8 $+$ or CD4 $+$ T cell is contacted with an anti-V β 17/anti-CD123 bispecific antibody of any one of embodiments 1 to 8 or 14 to 18.

[00127] Embodiment 24(b) is the method of embodiment 24, wherein the V β 17-expressing CD8 $+$ or CD4 $+$ T cell is contacted with an anti-V β 17/anti-CD123 bispecific antibody fragment of any one of embodiments 1 to 8 or 14 to 18.

[00128]

[00129] Embodiment 25 is a method for inhibiting growth or proliferation of cancer cells expressing CD123 on its surface, the method comprising contacting the cancer cells with the anti-V β 17/anti-CD123 bispecific antibody or fragment thereof with any one of embodiments 1 to 8 or 14 to 18, wherein contacting the cancer cells with said antibody or antibody fragment inhibits the growth or proliferation of the cancer cells.

[00130] Embodiment 25(a) is the method of embodiments 25, wherein the CD123-expressing cancer cell is in the presence of a V β 17-expressing CD8 $+$ T cell while in contact with an anti-V β 17/anti-CD123 bispecific antibody or fragment thereof.

[00131] Embodiment 25(b) is the method of embodiment 25 or 25(a), wherein the CD123-expressing cancer cell is contacted with an anti-V β 17/anti-CD123 bispecific antibody of any one of embodiments 1 to 8 or 14 to 18.

[00132] Embodiment 25(c) is the method of embodiment 25 or 25(a), wherein the CD123-expressing cancer cell is contacted with an anti-V β 17/anti-CD123 bispecific antibody fragment of any one of embodiments 1 to 8 or 14 to 18.

[00133] Embodiment 26 is a kit comprising a V β 17 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1 to 8 and packaging for the same.

[00134] Embodiment 27 is a kit comprising an anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 14 to 18 and packaging for the same.

[00135] Embodiment 28 is a method of producing a V β 17 bispecific antibody or antigen-binding fragment thereof, comprising culturing the host cell of embodiment 12 under conditions to produce the V β 17 bispecific antibody or antigen-binding fragment thereof, and recovering the V β 17 bispecific antibody or antigen-binding fragment thereof from the cell or culture.

[00136] Embodiment 29 is a method of producing an anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 14 to 18, comprising culturing the host cell of embodiment 22 under conditions to produce the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof, and recovering the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof from the cell or culture.

[00137] Embodiment 30 is an isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof, the V β 17 monoclonal antibody or antigen-binding fragment thereof comprising an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO:28.

[00138] Embodiment 31 is isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof of embodiment 30, wherein the V β 17 monoclonal antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NO:28.

[00139] Embodiment 32 is an isolated nucleic acid encoding the humanized V β 17 monoclonal antibody or antigen-binding fragment thereof of embodiment 30 or embodiment 31.

[00140] Embodiment 33 is a vector comprising the isolated nucleic acid of embodiment 32.

[00141] Embodiment 34 is a host cell comprising the vector of embodiment 33.

[00142] Embodiment 35 is a buffered composition comprising the isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof of embodiment 30 or embodiment 31.

EXAMPLES

[00143] The following examples are based on the premise that influenza virus derived peptide M1 is capable of expanding a select set of T cells. These cells express TCR- haplotype-V β 17

and majority of these cells exhibit efficient cytotoxicity of tumor target cells. This ability is then harnessed using bispecific antibodies constructed such that one arm binds to the V β 17 structure and the other arm binds to an antigen expressed by the cancer cells. Thus, the bispecific antibody bridges the effector and target cells together- resulting in cancer cell killing. This mechanism of action is described in the schematic outlined in FIG. 1.

[00144] The subsequent examples can be divided into the following categories: (1) Generation of bispecific antibodies capable of binding to the V β 17 arm of T-cell receptors (TCR) on CTL (Examples 1 and 2); and (2) Evidence for bispecific antibody-enabled target cell killing by CTL expanded *in vitro* (Example 3).

[00145] Example 1: Human Framework Adaptation of anti-V β 17 mAb E17.5F

[00146] The mouse IgG1 anti-human T cell receptor V β 17 clone E17.5F was obtained from Beckman Coulter, Inc. (Brea, CA). Sample preparation and LC/MSMS analysis were performed at Protea Bioscience Inc. (Morgantown, WV). The sample was reduced and alkylated, divided into seven aliquots, and proteolytically digested with Trypsin/LysC, Chymotrypsin, LysC, Pepsin, and AspN, Elastase, and Proteinase K enzymes. Resulting peptides were desalted using a ZipTip C18 Pipette Tips and separated on-line using reverse phase chromatography. Mass spectrometry was performed on Thermo Q-Exactive spectrometer using HCD fragmentation. MS data sets were analyzed using PEAKS software by matching *de novo* sequence tags to an IMGT-based antibody sequences database. Gaps in the sequence were assigned using Contig sequence assembly of *de novo* identified peptides. All CDRs and hyper-mutations were confirmed by inspecting the MS/MS spectra

[00147] The sequences obtained are shown in Tables 1 and 2.

[00148] Table 1: CDR Sequences of TCR V β 17 clone E17.5F.

Antibody	HCDR1	SEQ ID NO:	HCDR2	SEQ ID NO:	HCDR3	SEQ ID NO:
E17.5F	GYSITSGYFWN	1	YISYDGGSNN	2	PSPGTGYAVDY	3
Antibody	LCDR1	SEQ ID NO:	LCDR2	SEQ ID NO:	LCDR3	SEQ ID NO:
E17.5F	RSSQSLVHSNGNTYLN	4	KVSNRFS	5	SQSTHVPFT	6

[00149] Table 2: Heavy chain and light chain sequences of TCR V β 17 clone E17.5F.

mAb ID	Heavy Chain Amino Acid Sequence	SEQ ID NO:
B17B01	NVQLQESGPGLVKPSQLSLTCVAGYSITSGYFWNWIRQFPGNKLEWMGYISYDGNNYNPSLKNRISITRDTSKNQFFLKNLSVTTEDTATYYCASPSPGTGYAVDYWGQGTSVTVSSAKTTPPSVYPLAPGSSAQTNMSVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLLSSVTVPSSWPSQTVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPPKPDVLITLTPKTCVVVDISKDDPEVQFSWFVDDVEVHTAQTKPREEQINSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTIKYGRPKAPQVYTIPPPKEQMAKDKVSLTCMITNFFPEDITVEWQWNGQPAENYKNTQPIMTDGSYFVYSKLNQKSNWEAGNTFTCSVLEG	7

	LHNHHTEKSLSHSPGK	
	Light Chain Amino Acid Sequence	SEQ ID NO:
B17B01	NVVMTQTPLSLPVSLGDQASISCRSSQLVHSNGNTYLHWYLQKPGQSPKFLIY KVSNRFSGPDRFSGGGSGTEFTLKISRVEAEDLGVYFCSQSTHVPFTFGSGTK LEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVWKWIDGSERQN GVLNSWTDQDSKDSTYSMSSTLTKEYERHNSYTCEATHKTSTPIVKSFN RNEC	8

[00150] Changes were made in the sequences for the preparation of bispecific antibodies (Table 3). The changes include the following: (1) a framework mutation Asn1 of the heavy chain was not conserved, so the sequence has been modified to have the DVQLW sequence; (2) another mutation identified in the Fc, K337Y, was deemed uncharacteristic, and, thus, a construct without this mutation was synthesized; and (3) a potential secondary glycosylation site on the heavy chain was observed, and, thus, two versions of this mAb with and without the N-linked site (N82a, based on Chothia numbering) were synthesized.

[00151] Table 3: Heavy and Light Chain sequences for V β 17 clone E17.5F antibody variants

mAb ID	Heavy Chain Amino Acid Sequence	SEQ ID NO:
B17B1	NVQLQESGPLVKPSQLSLTCVAGYSITSGYFWNWRQFPGNKLEWMGYIS YDGSNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCASPSPGTGYAV DYWGQGTSVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVVDHKPSNTK VDKRVESKGPPCCPCPAPEAAGGPSVFLFPPPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYV р DGVEV р HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKV р NKGLPSSIEKTISKAKGQPREPQV р LPPSQEEMTKNQV р SLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPV р LSDGSFLLYSKLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLSLGK	9
B17B2	DVQLKESGPLVKPSQLSVTCSVTGYSITSGYYWNWYRQFPGNKLEWMGYI SYDGSNYNPSLKNRISITRDTSKNQILLKLTYVTTEDTATYYCASPSPGTGYA VDYWGQGTLTVTSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVVDHKPSNTK VDKRVESKGPPCCPCPAPEAAGGPSVFLFPPPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYV р DGVEV р HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKV р NKGLPSSIEKTISKAKGQPREPQV р LPPSQEEMTKNQV р SLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPV р LSDGSFLLYSKLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLSLGK	11
	Light Chain Amino Acid Sequence	SEQ ID NO:
B17B1	NVVMTQTPLSLPVSLGDQASISCRSSQLVHSNGNTYLHWYLQKPGQSPKFLIY KVSNRFSGPDRFSGGGSGTEFTLKISRVEAEDLGVYFCSQSTHVPFTFGSGTK LEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVWKWIDGSERQN GVLNSWTDQDSKDSTYSMSSTLTKEYERHNSYTCEATHKTSTPIVKSFN RNEC	10
B17B2	DIVMTQSPDSLAVSLGERATINCRSSQLVHSNGNTYLHWYQQKPGQPPKLLI YKVSNRFSGPDRFSGSGSGTDFLTISLQAEDVAVYYCSQSTHVPFTFGQGT KVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVWKWIDGSERQ NGVLNSWTDQDSKDSTYSMSSTLTKEYERHNSYTCEATHKTSTPIVKSFN NRNEC	12

[00152] The two antibodies (B17B1 and B17B2) were expressed in HEK293Expi cells. The supernatants were tested for V β 17 binding (B17B1 and B17B2) and only B17B1 demonstrated binding. Thus, B17B1 was expressed having an IgG4 constant region with Fc substitutions.

[00153] The anti-human TCR V β 17 mouse mAb B17B1 was humanized using the Human Framework Adaptation (HFA) method (Fransson J, et al. *J. Mol. Biol.* 2010; 398:214-231). To find the best combination of humanized heavy and light chains, several human V-region sequences were selected for testing (Table 4). Selection of human germlines was based solely on the overall sequence similarity to the mouse antibody in the framework (FR) region. Neither the CDR sequences, nor their length or canonical structures, were considered in this selection.

[00154] The CDR definition used in HFA is described in (Fransson J, et al. *J. Mol. Biol.* 2010; 398:214-231) and corresponds to the Martin's definition (Abhinandan KR and Martin AC. *Mol. Immunol.* 2008; 45:3832-3839). The CDRs (Table 1) were defined as described below (using the Chothia numbering scheme [Chothia C, and Lesk A. *J. Mol. Biol.* 1987; 196:901-917]):

HCDR1 (SEQ ID NO: 1)	26-35
HCDR2 (SEQ ID NO: 2)	50-58
HCDR3 (SEQ ID NO: 3)	95-102
LCDR1 (SEQ ID NO: 4)	24-34
LCDR2 (SEQ ID NO: 5)	50-56
LCDR3 (SEQ ID NO: 6)	89-97

[00155] The selected human germlines are provided in Table 4 (in the IMGT notation).

[00156] Table 4: VH and VL variants

Ab VH	Sequence	SEQ ID NO:
B17H1	<u>NVQLQESGPGLVKPSQLSLTCSVAGYSITSGYFWNWIRQFPGNKLEWMG</u> <u>YISYDGSNYYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCASPSPG</u> <u>TGYAVDYWGQGTSVTVSS</u>	25
B17H3	<u>EVQLLESGGGLVQPGGSLRLSCAASGYSITSGYFWNWVRQAPGKGLEWVS</u> <u>YISYDGSNYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPSP</u> <u>GTGYAVDYWGQGTLTVSS</u>	19
B17H4	<u>EVQLLESGGGLVQPGGSLRLSCAASGYSITSGYFWNWVRQAPGKGLEWVS</u> <u>YISYDGSNYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCASPSPG</u> <u>TGYAVDYWGQGTLTVSS</u>	20
B17H5	<u>QVQLQESGPGLVKPSETSLTCTVSGYSITSGYFWNWIRQPPGKGLEWIGYIS</u> <u>YDGSNYYNPSLKSRTISRDTSKNQFSKLSSVTAADTAVYYCASPSPGTGY</u> <u>AVDYWGQGTLTVSS</u>	21
Ab VL	Sequence	SEQ ID NO:
B17L1	<u>NVVMTQTPLSLPVSLGDQASISCRSSQLVHSNGNTYLHWYLQKPGQSPKFLIY</u> <u>KVSNRFGVPDRFSGGGSGTEFTLKRISRVEADLGVYFCSQSTHVPFTFGSGTKL</u> EIK	26
B17L3	<u>DIQMTQSPSSLASAVGDRVTITCRSSQLVHSNGNTYLHWYQQKPGKAPKLLIY</u> <u>KVSNRFGVPSRFSGSGTDFLTISSLQPEDFATYYCSQSTHVPFTFGQGTL</u> EIK	22

B17L4	DIQMTQSPSSLSAVGDRVTITCRSSQLVHSNGNTYLHWYQQKPGKAPKFLIY <u>KVSNRFSGVPSRFSGSGTDFTLTISLQPEDFATYYCSQSTHVPFTFGQGTLK</u> EIK	23
B17L5	DVVMTQSPLSLPVTLGQPASISCRSSQLVHSNGNTYLHWFQQRPGQSPRFLIY <u>KVSNRFSGVPDRFSGSGTDFTLKISRVEAEDVGVYYCSQSTHVPFTFGQGTLK</u> EIK	24

CDRs1-3 are underlined

[00157] “Back mutations” in several variants were introduced at FR positions that are known to be important for VL/VH pairing and CDR conformation. The selected human germlines are provided in Table 5 (in the IMGT notation), with the back mutations noted.

[00158] Table 5: The selected J-regions

J-region	Sequence	SEQ ID NO:
IGHJ1*01 HC	WGQGTLVTVSS	42
IGKJ2*01 LC	FGQGTLKLEIK	43

[00159] Amino acid sequences of all nine pairwise combinations of three heavy chains and three light chains were back-translated to DNA, and cDNA was prepared using gene synthesis techniques (U.S. Pat. No. 6,670,127; U.S. Pat. No. 6,521,427). Heavy chain (HC) variable regions were subcloned onto human IgG4 constant region using an in-house expression vector with the CMV promoter using standard molecular biology techniques. Light chain (LC) variable regions were subcloned onto a human Lambda (λ) constant regions using an in-house expression vector with the CMV promoter using standard molecular biology techniques. Resulting plasmids were transfected into HEK EXPI cells (LifeTechnologies; Carlsbad, CA) and mAbs were expressed. Purification was by standard methods using a Protein A column (hiTrap MAbSelect SuRe column). After elution, the pools were dialyzed into D-PBS, pH 7.2.

[00160] Table 6: Heavy and Light chains of nine humanized V β 17 antibodies

mAb	Hc	SEQ ID NO:	Lc	SEQ ID NO:	Concentration (μ g/mL)
B17B14	B17H3	19	B17L3	22	686.3
B17B15	B17H3	19	B17L4	23	13.8
B17B16	B17H3	19	B17L5	24	14.6
B17B17	B17H4	20	B17L3	22	335.1
B17B18	B17H4	20	B17L4	23	45.2
B17B19	B17H4	20	B17L5	24	27.5
B17B20	B17H5	21	B17L3	22	602.1
B17B21	B17H5	21	B17L4	23	570.9
B17B22	B17H5	21	B17L5	24	320.5

[00161] The humanized antibodies were screened for binding to a TCRV β 17 (SEQID NO:27)/Va10.2-Fc (SEQ ID NO:44) fusion protein by ELISA. Biotinylated TCRV β 17/Va10.2-

Fc fusion protein was added to a streptavidin-coated ELISA plate. Unbound protein was washed away and mAb was added at a range of concentrations (0.01-10 µg/mL). Plates were washed and anti-kappa:HRP detection antibody was added. Plates were washed, chemiluminescent detection reagent was added, and the plates were read on a Perkin Elmer EnVision plate reader for luminescence. B17B20 and B17B21 showed positive binding to the TCR-V β 17 protein. B17B22 showed weak binding to this protein. These antibodies were then purified as described above for further studies. B17B21 demonstrated the best binding to recombinant TCR-V β 17 protein and to M1-stimulated T-cells and was thus chosen as the molecule for further functional studies, specifically T-cell re-directed cancer cell killing as a bispecific antibody.

[00162] Thus, the variable region sequence of B17B21 (anti-V β 17) and I3RB217 (anti-CD123 antibody) was used to generate a bispecific antibody to be tested for T-cell re-directed killing of acute myeloid leukemia (AML) cells.

[00163] Example 2. Preparation of anti-V β 17/anti-CD123 bispecific antibodies

[00164] VB11 (anti-V β 17/anti-CD123) and VB13 (V β 17 x Null) bispecific antibodies were produced as full-length antibodies in the knob-into-hole format as human IgG4, as previously described (Atwell et al. J. Mol. Biol. 270: 26-35, 1997). Nucleic acid sequences encoding variable regions were subcloned into a custom mammalian expression vectors containing constant region of IgG4 expression cassettes using standard PCR restriction enzyme based cloning techniques. The bispecific antibodies were expressed by transient transfection in Chinese hamster ovary cell line. The antibodies were initially purified by Mab Select SuRe Protein A column (GE healthcare, Piscataway, New Jersey) (Brown, Bottomley et al. 1998). The column was equilibrated with Phosphate Buffer Saline (PBS), pH 7.2 and loaded with fermentation supernatant at a flow rate of 2 mL/min. After loading, the column was washed with PBS (4 CV) followed by elution in 30 mM sodium acetate, pH 3.5. Fractions containing protein peaks as monitored by Absorbance at 280 nm in Akta Explorer (GE healthcare) were pooled together and were neutralized to pH 5.0 by adding 1% of 3M sodium acetate, pH 9.0. As a polishing step, the antibodies were purified on a preparative size exclusion chromatography (SEC) using a Superdex 200 column (GE healthcare). The integrity of the sample was assessed by endotoxin measurement and SDS polyacrylamide gel electrophoresis under reducing and non-reducing conditions. The final protein concentrations were 0.48 mg/ml for anti-V β 17/anti-CD123 and 0.24 mg/mL for V β 17 x Null. The final EU levels of anti-V β 17/anti-CD123 and V β 17 x Null based on these protein concentrations were 2.053 EU/mg and 4.219 EU/mg, respectively.

[00165] Table 7: Sequences of half antibodies expressed in CHO cells

mAb ID	'Knob' arm and 'hole' arm amino acid sequence	SEQ ID NO:

B17B21 (V β 17 half Ab)	MAVVWTLLFLMAAAQSIQADIQMTQSPSSLASAVGDRVTITCRSSQLVHS NGNTYLHWYQQKPGKAPKFLIYKVSNRFGVPNSRFGSGSGTDFTLTISLQ PEDFATYYCSQSTHVPFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTEGK SSGSGSESKESTGGSQVQLQESGPGLVKPSETSLTCTVSGYSITSGYFWNWIR QPPGKGLEWIGYISYDGNSNNYNPSLKSRTISRTSKNQFLKLSSVTAADT AVYYCASPSPGTGYAVDYWGQGTLVTVSSASTKGPSVFLAPCSRSTSESTA ALGCLVKDYFPEPVTVWSNSGALTSGVHTFPALQSSGLYSLSSVTVPS LGTKTYTCNVDHKPSNTKVDKRVESKGPPCPAPEAAGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPV YTLPPSQEEMTKNQVSLSCAVKGFPYPSDIAVEWESNGQPENNYKTPPVLD DGSFFLVSRLTVDKSRWQEGNVFSCVMHEALHNRTQKSLSLGK	28
I3RB217 (CD123 half Ab)	MAVVWTLLFLMAAAQSIQAEIVLTQSPGTLSLPGERATLSCRASQSVSSY LAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQDYGFPWTGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTSKADYEK HKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTEGKSSGGS ESKSTGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWISWVRQMPGK GLEWMIIDPSDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYY CARGDGSTDLDYWQGQGTLVTVSSASTKGPSVFLAPCSRSTSESTAALGCLV KDYFPEPVTVWSNSGALTSGVHTFPALQSSGLYSLSSVTVPSQLGKTY TCNVDHKPSNTKVDKRVESKGPPCPAPEAAGGPSVFLFPPKPDTLMI SRTPEVTCVVVDVQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPVYTLPPS EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCVMHEALHNHTQKSLSLGK	30
B23B49 (Null half Ab)	MAVVWTLLFLMAAAQSIQAEIVLTQSPGTLSLPGERATLSCRASQSVSSY LAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQDYGFPWTGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTSKADYEK HKVYACEVTHQGLSSPVTKSFNRGECGGSEGKSSGSGSESKSTEGKSSGGS ESKSTGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWISWVRQMPGK GLEWMIIDPSDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYY CARGDGSTDLDYWQGQGTLVTVSSASTKGPSVFLAPCSRSTSESTAALGCLV KDYFPEPVTVWSNSGALTSGVHTFPALQSSGLYSLSSVTVPSQLGKTY TCNVDHKPSNTKVDKRVESKGPPCPAPEAAGGPSVFLFPPKPDTLMI SRTPEVTCVVVDVQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPVYTLPPS EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCVMHEALHNHTQKSLSLGK	
Half Antibody DNA sequence		
B17B21 (V β 17 half Ab)	ATGGCCTGGGTGGACCTGCTGTTCTGATGGCCGCCGCCAGAGCAT CCAGGGCGACATCCAGATGACCCAGAGCCCAAGCAGCTGAGCGCCAGC GTGGGCGACCGCGTGACCATCACCTGCCGAGCAGCCAGAGCTGGTGC ACAGCAACGGAACACACTACCTGCACTGGTACCAGCAGAACGCCAGG GGCCCCAAAGTCTCTGATCTACAAGGTGAGCAACCGCTCAGCGCGTG CCAAGCCGCTTCAGCGGAGCGGAGCGGACCCGACTTCACCTGACCA TCAGCAGCTGAGCCAGAGGACTTCGCCACCTACTACTGAGCCAGAG CACCCACGTGCCATTACCTTCCGGCCAGGGCACCAAGCTGGAGATCAAG CGCACCGTGGCCGCCAACCGTGTTCATCTTCCCACCAAGCGACGAGC AGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCTGTAACAACCTCTA CCCACCGCAGGCCAACGGTGCAGTGGAGGTGACAACGCCCTGAGCAG CGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGC CTACAGCCTGAGCAGCACCTGACCTGAGCAAGGCCAGCTGAGCAGCC CACAAGGTGTACGCCCTGCGAGGTGACCCACCAGGGCTGAGCAGCC TGACCAAGAGCTCAACCGCGCGAGTGCAGCGGCCAGCGAGGGCAAG GCAGCGGCAGCGAGAGCAAGAGCACCGAGCGAGGGCAAGAGCAGC GCAGCGGCAGCGAGAGCAAGAGCACCGAGCGAGGGCAAGAGCAGC AGGAGAGCGGCCAGGCCTGGTGAAGCCAAGCGAGACCTGAGCCTGA CCTGCACCGTGAGCGGCTACAGCATCACAGCGGCTACTTCTGGA ACTG	29

GATCCGCCAGCCACCAGGCAAGGGCCTGGAGTGGATCGGCTACATCAGC TACGACGGCAGCAACAACATACAACCCAAGCCTGAAGAGCCCGTGTACCA TCAGCCCGACACCAGCAAGAACCAAGCAGTTCAGCTGAAGCTGAGCAGCGT GACCGCCGCCGACACCGCCGTACTACTGCGCCAGCCCAGCCAGGC ACCGGCTACGCCGTGGACTACTGGGCCAGGGCACCCCTGGTACCGTGA GCAGCGCCAGCACAAGGGCCAAGCGTGTCCCAGTGGCTGGTACCGTGA CCGCAGCACCAGCAGGAGAGCACCGCCCTGGTGCCTGGTGAAGGAC TACTTCCCAGAGCCAGTGAACGTGAGCTGGAACAGCGGGCCCTGACCA GCGGCGTGCACACCTTCCCAGCCGTGCTGCAGAGCAGCGGCCTGTACAG CCTGAGCAGCGTGGTACCGTGCAGCAGCAGCCTGGCACCAAGACC TACACCTGCAACGTGGACCACAAGCCAAGCAACACCAAGGTGGACAAG CGCGTGGAGAGCAAGTACGGCCCACCATGCCACCATGCCAGCCCCAG AGGCCGCCGGCCCAAGCGTGTCTGGTACCGAAGGCCAAGGAAAGGAA CACCTGATGATCAGCCGACCCAGAGGTGACCTGCCTGGTGGAC GTGAGCCAGGAGGACCCAGAGGTGACCTGAACTGGTACGTGGACGGCG TGGAGGTGCACAACGCCAAGACCAAGGCCACGGCAGGAGCAGTTCAACA GCACCTACCGCGTGGTGAGCGTGTGACCGTGTGACCCAGGACTGGCT GAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGGCCTGCCAAG CAGCATCGAGAAAGACCATCAGCAAGGCCAAGGGCCAGGCCACGCCAG ACAGGTGTACACCTGCCACCAAGGCCAGGAGGAGATGACCAAGAACCA GGTAGCCTGAGCTGCGCCGTGAAGGGCTTCTACCCAAAGCGACATCGCC GTGGAGTGGGAGAGCAACGGCCAGCCAGAGAACAAACTACAAGACCACC CCACCACTGCTGGACAGCGACGGCAGCTTCTGGTGAAGCCGCCCTGA CCGTGGACAAGAGCCGCTGGCAGGAGGGCAACGTGTTCACTGAGCTGAGCGT GATGCAAGAGGCCCTGCACAACCGCTTCACCCAGAAGAGCCTGAGCCTG AGCCTGGCAAGATGGCCTGGGTGAGCGCTGCTGTTCTGATGGCCG CCGCCCAGAGCATCCAGGGCGACATCCAGATGACCCAGAGGCCAAGCAG CCTGAGCGCCAGCGTGGCGACCGCGTGAACATCACCTGCCAGCAGC CAGAGCCTGGTGACAGCAACGGCAACACCTACCTGCACTGGTACCA AGAACGCCAGGCAAGGCCAAAGTCTGATCTACAAGGTGAGCAACCG CTTCAGCGGCGTCCAAGGCCCTGAGCGGCAAGGGCAGCGGAGCGACCCGAC TTCACCCCTGACCATCAGCACCTGCAAGCCAGGAGACTTCGCCACCTACTA CTGCAGCCAGAGCACCCACGTGCCATTCACCTCGGCCAGGGCACCAAG CTGGAGATCAAGCGCACCGTGGCCCAAGCGTGTTCATCTTCCACC AAGCGACGAGCAGCTGAAGAGCGGCCAGGTGCACTGGAAGGTGGACAACG CCCTGCAGAGCGGCAACAGCCAGGGAGAGCGTGAACCGAGGACAGCA AGGACAGCACCTACAGCCTGAGCAGCACCCCTGACCGTGAAGCAAGGCC CTACGAGAAGCACAGGTGTACGCCCTGCAAGGTGACCCACCAAGGGCCTG AGCAGGCCAGTGAACAGAGCTCAACCGCGGAGTGCAGTGGAGTGGACAACG GAGGGCAAGAGCAGCGGAGCGGAGCGAGAGCAAGAGCAGGCCAGGGC AAGAGCAGCGGAGCGGAGCGAGAGCAAGAGCAGGCCAGGGCGGAGCCAG GTGCAGCTGCAGGAGAGCGGCCAGGCCAGGTGGTAAGCCAAGCGAGACC CTGAGCCTGACCTGCAACCGTGAAGCGGCTACAGCATCACAGCGGCTACT TCTGGAACTGGATCCGCCAGCCACCGAGCAAGGGCCTGGAGTGGATCGG CTACATCAGCTACGACGGCAGCAACAACCTACAACCCAAGCCTGAAGAGC CGCGTGAACCATCAGCCGACACCAGCAAGAACCAAGCTGCTGAAGC TGAGCAGCGTACCGCCGCCAGGCCAGGTGACTACTGCGCCAGCCC AAGCCCAGGCACCGCTACGCCGTGGACTACTGGGCCAGGGCACCC GTGACCGTGAGCAGCGCCAGCACCAAGGGCCAAGCGTGTGGAAACAGCGG GCCCTGACCAGCGGCGTGCACACCTTCCAGCCGTGCTGCAGAGCAGCG GCCTGTACAGCCTGAGCAGCGTGGTGAACCGTGCAGCAGCGCTGG CACCAAGACCTACACCTGCAACGTGGACCAAGCCAAGCAACACCAAG GTGGACAAGCGCGTGGAGAGCAAGTACGGCCCACCATGCCACCATGCC CAGCCCCAGAGGCCGCCGGCGGCCAAGCGTGTCTGGTACCGTGA GCCAAAGGACACCCCTGATGATCAGCCGACCCAGAGGTGACCTGCGT GTGGTGGACGTGAGCCAGGAGGCCAGAGGTGCAAGTCAACTGGTACG TGGACGGCGTGGAGGTGACAACGCCAAGACCAAGGCCACCGCAGGGAGC AGTTCAACAGCACCTACCGCGTGGTGAACGTGCTGACCGTGTGACCA GGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTGAGCAACAAGGG	
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	CCTGCCAAGCAGCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCC ACCGAGCCACAGGTGTACACCCTGCCACCAAGCCAGGAGGAGATGACC AAGAACCGAGGTGAGCCTGAGCTGCCCGTGAAGGGCTTCTACCCAAGCG ACATGCCGTGGAGTGGAGAGCAACGCCAGCCAGAGAACAACTACA AGACCACCCACACTGCTGGACAGCGACGCCAGCTTCTCTGGTGA CCGCCTGACCGTGGACAAGAGGCCCTGGCAGGAGGGCAACGTGTTCAGC TGCAGCGTGTGACGAGGCCCTGCACAACCGCTTCACCCAGAAGAGCC TGAGCCTGAGCCTGGCAAGTGATAG	
I3RB217 (CD123 half Ab)	ATGGCCTGGGTGGACCTGCTGTTCTGATGGCCGCCAGAGCAT CCAGGCCGAGATCGTGTGACCCAGAGCCCAGGCACCCCTGAGCCTGAGC CCAGGCAGCGCCACCCCTGAGCTGCCGCCAGCCAGAGCGTGTGAGCA GCAGCTACCTGGCCTGGTACCAAGCAGAAGCCAGGCCAGGCCCCACGCCT GCTGATCTACGGGCCAGCAGCCGCCACCCGATCCAGACCCGCTTC AGCGCAGCGGCAGCGGACCCGACTCACCCTGACCATCAGCCGCCCTGG AGCCAGAGGACTTCGCCGTGTACTACTGCCAGCAGGACTACGGCTTCCC ATGGACCTTCGCCAGGGACCAAGGTGGAGATCAAGCGCACCGTGGCC GCCCAAGCGTGTTCATCTCCCACCAAGCAGCAGCAGCTGAAGAGCG GCACCGCCAGCGTGGTGCCTGCTGAACAACCTCTACCCACGCGAGGC CAAGGTGCAGTGBAAGGTGGACAACGCCCTGCCAGAGCGGCAACAGCCA GGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTGAG CAGCACCCCTGACCCCTGAGCAAGGCCAGTACCGAGAAGCACAAAGGTGTAC GCCTGCGAGGTGACCCACCCAGGGCTGAGCAGCCCAGTGACCAAGAGCT TCAACCGCGCGAGTGCggcgccagcggagggcaagagcagcggcagcggcagcag caccggggcaagagcagcggcagcggcagcggagggcaagagcaccggcggcagcGGAGGTGCAGCT GGTCAGAGCGCGCCAGGTGAAGAAGCCAGGCAGAGAGCCTGAAGAT CAGCTGAAGGGCAGCGGCTACAGCTTACCCAGCTACTGGATCAGCTGG GTGCGCCAGATGCCAGGCAGGGCTGGAGTGGATGGCATCATCGACC CAAGCGACAGCGACACCCGCTACAGCCCAAGCTTCCAGGGCCAGGTGAC CATCAGCGCCGACAAGAGCATCAGCACCGCCTACCTGCAGTGGAGCAGC CTGAAGGCCAGCGACACCCGCTACAGCTTACCTGCGCCCGCGACGGCA GCACCGACCTGGACTACTGGGCCAGGGCACCCCTGGTACCGTGAGCAG CGCCAGCACCAAGGCCAACCGTGTCCCACGGCCATGCAGCCGC AGCACCAAGCGAGAGCACCGCCGCCCTGGCTGCTGGTGAAGGACTACT TCCCAGAGCCAGTGACCGTGAGCTGGAACAGCGGCCCTGACCGAGCG CGTGCACACCTTCCCAGCGTGTGAGCAGAGCAGCGGCCCTGACCGCTG AGCAGCGTGTGACCGTGCAAGCAGCAGCGCTGGCACCAAGACCTACA CCTGCAACAGTGGACCAAGCCAAGCAACACCAAGGTGGACAAGCGCG TGGAGAGCAAGTACGCCACCATGCCACCATGCCACGCCAGGGCAGAGGC CGCCGGCGGCCAACCGTGTCTGGTACCGTGTGACCGTGCTGACCGACTGGCTGAAC CTGATGATCAGCGCACCCAGAGGTGACCTGCGTGGTGGACGTGA GCCAGGAGGACCCAGAGGTGCACTTCAACTGGTACGTGGACGGCGTGG GGTGCACAACGCCAAGACCAAGCCACGCGAGGAGCAGTTCAACACGAC CTACCGCGTGTGAGCGTGTGACCGTGCTGACCGACTGGCTGAAC GGCAAGGAGTACAAGTCAAGGTGAGCAACAAGGCCCTGCCAGCAGC ATCGAGAAGACCATCAGCAAGGCCAACGGCCAGCCACGCCAGCAG GTGTACACCTGCCACCAAGCCAGGAGGAGATGACCAAGAACCAAGGTG AGCCTGTGTGCTGGTGAAGGGCTTCTACCCAGCGACATGCCGTGG AGTGGAGAGCAACGCCAGCCAGAGAACAACTACAAGACCACCCAC CACTGCTGGACAGCGACGCCAGCTTCTCTGTACAGCCCTGACCGT GGACAAGAGGCCCTGGCAGGAGGGCAACGTGTTCACTGCGACCGTGATG CACGAGGCCCTGCACAACCAACTACACCCAGAAGAGCCTGAGCCTGAGCC TGGGCAAG	31
B23B49 (Null half Ab)	ATGGCCTGGGTGGACCTGCTGTTCTGATGGCCGCCAGAGCAT CCAGGCCGACATCGTGTGACCCAGAGCCCAGACAGCTGCCGTGAGC CTGGCGAGCGCCACCATCAACTGCCGCCAGCCAGAGCGTGGACT ACAACGGCATCAGCTACATGCACTGGTACCGAGCAGAACGCCAGGCC ACCAAAGCTGCTGATCTACGCCGCCAGCAACCCAGAGAGCGCGTGGCCA GACCGCTTCAGCGGCAGCGGCAGCGGCCACCGACTTACCCCTGACCATCA GCAGCCTGCGAGGCCAGGACGTGGCGTGTACTACTGCCAGCAGATCAT CGAGGACCCATGGACCTTCGCCAGGGCACCAAGGTGGAGATCAAGCGC ACCGTGGCCGCCAACCGTGTTCATCTCCCACCAAGCGACGAGCAGC TGAAGAGCGGCACCGCCAGCGTGGTGCCTGCTGAACAACCTTACCC	

	ACCGGAGGCCAAGTGCAGTGGAAAGGTGACAACGCCCTGCAGAGCGG CAACAGCCAGGAGAGCGTGAACGAGCAGCAAGGACAGCACCTA CAGCCTGAGCAGCACCTGACCCCTGAGCAAGGCCACTACGAGAAC AAGGTGTACGCCTGCGAGGTGACCCACCAGGGCCTGAGCAGCCCAGTGA CCAAGAGCTTCAACCGCGCGAGTCGGCGAGCGAGGGCAAGAGCA CGGGCAGCGAGAGCAAGAGCACCAGGAGGGCAAGAGCAGCGGCA CGGGCAGCGAGAGCAAGAGCACCAGGAGGGCAAGAGCAGCGGCA AGAGCGGCCAACCCCTGGTGAAGCCAACCCAGACCCCTGACCTGAC CACCTCAGCGCTTCAGCCTGAGCACCAAGCGGATGGCGTGAGCTGG ATCCGCCAGCCACCAGGCAAGGCCCTGGAGTGGCTGGCCCACATCTACT GGGACGACGACAAGCGCTACAACCCAAGCCTGAAGAGGCCCTGAC CACCAAGGACACCAGCAAGAACCAAGGTGGTGTGACCATGACCAACATG GACCCAGTGGACACGCCACCTACTACTGCGCCCGCCTGTACGGCTTCAC CTACGGCTTCGCTTAAGGGCCAGGGCACCCCTGGTACCGTGAGCAGCAGC GCCAGCACCAAGGGCCCAAGCGTGTCCCCTGGCCCATGCGAC GCACCAAGCGAGAGCACCGCCGCCCTGGGCTGCCTGGTGAAGGACTACT CCCAGAGCCAGTGAACCGTGAGCTGGAACAGCGGCCCTGACCGCGC GTGCACACCTTCCCAGCCGTGCTGCAGAGCAGCGGCCCTGTACAGCCTGA GCAGCGTGGTACCGTGCCAAGCAGCAGCCTGGGACCAAGACCTACAC CTGCAACGTGGACCACAAGCCAAGCAACACCAAGGTGGACAAGCGCGT GGAGAGCAAGTACGGCCCACCATGCCCACCATGCCCAGCCCCAGAGGCC GCCGGCGGCCAAGCGTGTCCCCTGTTCCCACCAAGCCAAGGCCAC TGATGATCAGCCGCACCCAGAGGTGACCTCAACTGGTACGTGGAC CCAGGAGGACCCAGAGGTGACGTTCAACTGGTACGTGGAC GTGCACAACGCCAAGACCAAGCCACCGCAGGAGCAGTTCAACAGCAC ACCGCGTGGTACCGTGCTGACCGTGCTGCACCAAGGACTGGCTAAC CAAGGAGTACAAGTCAAGGTGAGCAACAAGGCCCTGCCAAGCAGC CGAGAAAGACCATCAGCAAGGCCAAGGGCCAGGCCACCGCAGGCCAC GTACACCCCTGCCACCAAGCCAGGAGGAGATGACCAAGAAC CAGGTGAG CCTGTGGTGCCTGGTGAAGGGCTTCTACCCAAGCGACATGCCGTGGAG TGGGAGAGCAACGCCAGGCCAGAGAACAACTACAAGACCAC GTGCTGGACAGCGACGGCAGCTCTCCTGTACAGCCGCTGACCGTGG ACAAGAGCCGCTGGCAGGAGGGCAACGTGTTCAGCTGCAGCGTATGCA CGAGGCCCTGCACAACCACCAAGAGGCCCTGACGCCCTGAGCCTGG GGCAAG	
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[00166] Table 8: Heavy and Light Chain Sequences for V β 17 bispecific antibodies

Bispecific Antibody		Amino Acid Sequence
Anti-V β 17/ anti-CD123	Heavy chain 1 B17B21 (SEQ ID NO: 13)	QVQLQESGPGLVKPSETLSLTCTVSGYSITSGYFWNWRQPP GKGLEWIGIYISYDGSNNYNPSLKSRTISRTDSKNQFLKLS SVTAADTAVYYCASPSPGTGYAVDYWGQCTLTVSSASTK GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVWSNSGAL TSGVHTFPALVQSSGLYSLSSVVTPVSSSLGTKTYTCNVDHK PSNTKVDKRVESKYGPPCPCPAPEAAGGPSVFLFPPPKD LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN AKTK PREEQFNSTYR VVSVLT VLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQP REPQVYTL PPSQEEMTKNQV SLSCAVKGF YPSDIAVE WESNGQ PENNYK TTPPV LSDGS FFL VSRL TVD KSRW QEGNV FSC SVM HEAL HN RFT QK SL SL LGK
	Light Chain 1 B17B21 (SEQ ID NO: 14)	DIQMTQSPSSLSASVGDRVTITCRSSQLVHSNGNTY LHWY QQKPGKAPKFLIYKVS NRFSGVPSRFSGSG GTDF TL TISS LQ PEDF ATYYCSQ STH VP FTFG QGT KLE IKRT VA APS V F IF PP SD EQL KSGT AS VV C L NN F P REAK V Q W K V D N A L Q S G N Q S E V T E Q D S K D S T Y S L S S T L T S K A D Y E K H K V Y A C E V T H Q G L S S P V T K F S N R G E C
	Heavy chain 2 I3RB217 (SEQ ID NO: 15)	EVQLVQSGA EV KK PG GE SL K I S C K G S G Y S F T S Y W I S W V R Q M P G K G L E W M G I D P S D T R Y S P S F Q G Q V T I S A D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R G D G S T D L D Y W G Q G T L V T V S S A S T K G P S V F P L A P C S R S T S E S T A A L G C L V K D Y F P E P V T V W S N G A L T

		SGVHTFPALQSSGLYSLSSVVTVPSSSLGKTYTCNVVDHKP SNTKVDKRVESKYGPPCPPCPAPEAAGGPSVLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHTQKSLSLSLGK
	Light Chain 2 I3RB217 (SEQ ID NO: 16)	EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQDYGFPWTFGQGKTVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
V β 17 x Null	Heavy chain 1 B17B21 (SEQ ID NO: 13)	QVQLQESGPGLVKPSETLSLTCTVSGYSITSGYFWNWRQPP GKGLEWIGIYISYDGNNYNPSLKSRTVISRTDSKNQFLKLS SVTAADTAVYYCASPSPGTGYAVDYWGQGTLTVSSASTK GPSVPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPALQSSGLYSLSSVVTVPSSSLGKTYTCNVVDHK PSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLCAVGF YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTV KSRWQEGNVFSCSVMHEALHNRTQKSLSLSLGK
	Light Chain 1 B17B21 (SEQ ID NO: 14)	DIQMTQSPSSLSASVGDRVTITCRSSSQLVHSNGNTYLHWY QQKPGKAPKFLIYKVSNRSGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCSQSTHVPFTFGQGKTLEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
	Heavy chain 2 Null (SEQ ID NO: 17)	QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPP GKALEWLAHIYWDDDKRYNPSLKSRLTITKDTDSKNQVVL MTNMDPVDTATYYCARLYGFTYGFAYWGQGTLTVSSAS TKGPSVPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG ALTSVGVHTFPALQSSGLYSLSSVVTVPSSSLGKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN TKPREEQFNSTYRVSVLTVLHQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWCLV GFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTV DKSRWQEGNVFSCSVMHEALHNHTQKSLSLSLGK
	Light Chain 2 Null (SEQ ID NO: 18)	DIVMTQSPDSLAVSLGERATINCRAQSVDYNGISYMHWYQ QKPGQPPKLLIYAASNPESGVPDRFSGSGSGTDFTLTISSLQA EDVAVYYCQQIIEDPWTFGQGKTVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC

[00167] Example 3. Evaluation of binding and cytotoxic properties of anti-V β 17/ anti-CD123 bispecific antibody using Kasumi-3 cells and human CD8 $^{+}$ T cells

[00168] Stimulation and expansion of V β 17 $^{+}$ CD8 $^{+}$ T cells from total PBMCs

[00169] To expand V β 17 $^{+}$ CD8 $^{+}$ T cells, whole PBMCs from HLA-A2 donor (HPU-08694) were stimulated with 1 μ g/mL FLU MP 58 peptide (in DMSO). Frequency of V β 17 $^{+}$ cells among total CD8 $^{+}$ T cells was determined on day 8 and 14 of the culture period. To enumerate the frequency of V β 17 $^{+}$ cells among total CD8 $^{+}$ T cells, total live PBMCs were initially gated,

doublets were excluded, total CD8⁺ T cells were gated and then V β 17⁺ cells were gated (FIG. 3A). Compared to the frequency of V β 17⁺ cells among total CD8⁺ T cells on day 0, a substantial expansion of these cells were observed at day 8 of the culture period (FIG. 3B). A larger fraction of CD8⁺ T cells on day 8 were V β 17⁺ cells (FIG. 3B) in this donor.

[00170] *anti-V β 17/anti-CD123 bispecific antibody binding assay*

[00171] On Kasumi-3 cells

[00172] To understand the binding kinetics of the anti-V β 17/anti-CD123 bispecific antibody, Kasumi-3 cells were incubated with the anti-V β 17/anti-CD123 bispecific antibody and V β 17XNULL arm control at various concentrations (concentration range from 5 μ g/mL to 0 μ g/mL). Cell bound bispecific antibody was detected with mouse anti-human IgG4 Fc-PE secondary antibody. Table 9 shows the frequency of Kasumi-3 cells positive for PE (secondary antibody) when treated with different concentration of bispecific antibodies. The EC₅₀ for anti-V β 17/anti-CD123 and NULLXCD123 was determined as 6 and 42.7 nM respectively (Table 9).

[00173] Table 9: Binding affinity of Kasumi-3 cells for bispecific antibodies.

Conc.(μ g/mL)	anti-V β 17/ anti-CD123	V β 17XNULL	NULLXCD123
5	87.37	-0.02	47.47
1.667	74.37	0.09	16.37
0.556	24.07	0.15	1.2
0.185	1.65	0.35	0.31
0.062	0.32	0.12	0.04
0.021	0.16	0.14	0.05
0.007	0.23	0.19	-0.06
0.002	0.04	-0.09	-0.04
0.001	0.09	0	-0.18
EC ₅₀ (μ g/mL)	0.9	ND	6.4
EC ₅₀ (nM)	6	ND	42.7

[00174] Bispecific antibody binding affinities to Kasumi-3 cells were determined by flow cytometry. Half maximal effective concentration (EC₅₀) values were calculated as the bispecific concentration that generates 50% of maximal Binding (PE positive cells). ND: Not determined.

[00175] On enriched CD8⁺ T cells

[00176] Enriched FLU MP 58 peptide stimulated CD8⁺ T (from day 14 culture) cells were incubated with various concentrations of anti-V β 17/anti-CD123 bispecific and V β 17XNULL arm control antibodies. Mouse anti-human IgG4 Fc-PE secondary antibody was used to detect the bispecific antibody. Table 10 shows the frequency of CD8⁺ T cells positive for PE (secondary antibody) when treated with different concentration of bispecific antibodies. The

EC₅₀ for anti-Vβ17/anti-CD123, Vβ17XNULL, was determined as 9.0 nm, 18.7 nm respectively (Table 10).

[00177] Table 10: Binding activity of CD8⁺ T cell for bispecific antibodies.

Conc.(µg/mL)	anti-Vβ17/ anti-CD123	Vβ17XNULL	NULLXCD123
20	74.7	76.2	0.1
10	72.4	75.4	0.4
5	70.8	64.6	0.3
2.5	64.8	42.5	0.5
1.25	38.0	32.4	0.4
0.625	41.4	21.7	-0.1
0.3125	26.5	11.8	0.8
0.15625	19.9	3.4	0.6
0.078125	10.8	1.8	0.7
EC ₅₀ (µg/mL)	1.35	2.80	ND
EC ₅₀ (nM)	9	18.7	ND

[00178] Bispecific antibody binding affinities to CD8⁺ T cell were determined by flow cytometry. Half maximal effective concentration (EC₅₀) values were calculated as the antibody concentration that generates 50% of the maximal binding (PE positive cells). ND: Not Determined

[00179] *Bispecific mediated cytotoxicity assay*

[00180] In order to analyze the potency of the anti-Vβ17/anti-CD123 bispecific antibody mediated cytotoxicity, CFSE labelled target (Kasumi-3) cells were co-cultured with stimulated CD8⁺ T cells (effectors) from day 14 of culture at an effector to target (ET) ratio 0.5:1, 1:1, 5:1 for 14 and 24 hours with various concentrations of anti-Vβ17/anti-CD123 bispecific and Vβ17XNULL arm control antibody. CD123 expression on target Kasumi-3 cells were checked by using a commercially available anti-CD123 antibody. Target cells (Kasumi-3) were labelled with CFSE to identify them as CFSE⁺ during flow cytometry analysis. Post co-culture period, 7-AAD was added to analyze the percentage of 7-AAD⁺ CFSE⁺ cells as a measure of cytotoxicity. Basal cytotoxicity observed in the absence of bispecific antibody was subtracted to obtain specific cytotoxicity in response to bispecific antibody. The assay was performed once with a single donor (HPU-08694). The EC₅₀ for the anti-Vβ17/anti-CD123 bispecific antibody at 0.5:1, 1:1 and 5:1 ET ratios for 14-hour time point were 3.7, 0.1 and 0.133 pM respectively (Table 11).

[00181] Table 11: Summary of EC₅₀ values for various bispecific antibodies upon co-culturing FLU MP 58 peptide stimulated CD8⁺ T cell with Kasumi-3 cells at ET ratios 0.5:1, 1:1 and 5:1 for 14 hours.

Bispecific Ab	EC ₅₀ (ng/mL)		
	E:T Ratio	E:T Ratio	E:T Ratio

	(0.5:1)	(1:1)	(5:1)
NULLXCD123	UD	UD	UD
V β 17XNULL	UD	UD	UD
Anti-V β 17/anti-CD123	0.55	0.015	0.02

Bispecific Ab	EC ₅₀ (pM)		
	E:T Ratio (0.5:1)	E:T Ratio (1:1)	E:T Ratio (5:1)
NULLXCD123	UD	UD	UD
V β 17XNULL	UD	UD	UD
anti-V β 17/anti-CD123	3.7	0.1	0.133

UD: Undetectable, as the activity was too low for proper curve fitting.

[00182] The EC₅₀ for the anti-V β 17/anti-CD123 bispecific at 0.5:1, 1:1 and 5:1 ET ratio for 24-hour time point were 0.4, 0.2 and 1.0 pM respectively (Table 12).

[00183] Table 12: Summary of EC₅₀ values for various bispecific antibodies upon co-culturing FLU MP 58 peptide stimulated CD8 $^{+}$ T cells with Kasumi-3 cells at ET ratios 0.5:1, 1:1 and 5:1 for 24 hours.

Bispecific Ab	EC ₅₀ (ng/mL)		
	E:T Ratio (0.5:1)	E:T Ratio (1:1)	E:T Ratio (5:1)
NULLXCD123	UD	UD	UD
V β 17XNULL	UD	UD	UD
anti-V β 17/anti-CD123	0.06	0.03	0.15

Bispecific Ab	EC ₅₀ (pM)		
	E:T Ratio (0.5:1)	E:T Ratio (1:1)	E:T Ratio (5:1)
NULLXCD123	UD	UD	UD
V β 17XNULL	UD	UD	UD
anti-V β 17/anti-CD123	0.4	0.2	1.0

UD: Undetectable

[00184] Similarly, anti-V β 17/anti-CD123 bispecific mediated unstimulated CD8 $^{+}$ T cell cytotoxicity was tested at ET ratio 0.5:1, 1:1, 5:1 for 14 (Table 13) and 24 (Table 14) hours. At 5 ng/ml anti-V β 17/anti-CD123 bispecific concentration and 14-hour time point, unstimulated CD8 $^{+}$ T cells at 0.5:1 and 1:1 ET ratio showed 2.8% and 9.8% target cell cytotoxicity respectively (Table 13), compared to 77% and 73% cytotoxicity by stimulated CD8 $^{+}$ T cells. At 5:1 ET ratio, unstimulated CD8 $^{+}$ T cells exhibited 31.65% target cytotoxicity, compared to 70.9% by stimulated CD8 $^{+}$ T cells. Similar results were obtained from 24-hour time point (Table 12, 15, 16, and 17). At highest concentration (5 ng/ml) of anti-V β 17/anti-CD123 bispecific

tested, unstimulated CD8⁺ T cells exhibited higher cytotoxicity towards target cells at a higher ET ratio.

[00185] Table 13: Cytotoxicity assay with unstimulated CD8⁺ T cells at various ET ratios for 14 hours. Frequency of CFSE and 7-AAD positive cells when treated with different concentrations of bispecific antibodies.

Bispecific Ab	Conc (ng/mL)	E:T Ratio (0.5:1)	E:T Ratio (1:1)	E:T Ratio (5:1)
NULLXCD123	5	1.3	-0.6	-0.45
	0.005	1.3	-0.5	0.45
Vβ17XNULL	5	-0.6	-0.8	3.25
	0.005	-0.3	1	-1.35
anti-Vβ17/anti-CD123	5	2.8	9.8	31.65
	0.005	0.1	3.2	12.25

[00186] Table 14: Cytotoxicity assay with unstimulated CD8⁺ T cells at various ET ratios for 24 hours. Frequency of CFSE and 7-AAD positive cells when treated with different concentrations of bispecific antibodies.

Unstimulated CD8+ T cells				
	Conc (ng/mL)	E:T Ratio (0.5:1)	E:T Ratio (1:1)	E:T Ratio (5:1)
NULLXCD123	5	-1.3	-0.55	-4.85
	0.005	-2	-0.85	-2.95
Vβ17XNULL	5	-0.8	-1.35	10.85
	0.005	-1	-1.05	-1.95
anti-Vβ17/anti-CD123	5	4.8	11.55	30.65
	0.005	1.5	1.75	10.95

[00187] Table 15: Cytotoxicity assay at 0.5:1 ET ratio (stimulated CD8⁺ T cell: Kasumi-3 cells) upon incubation for 14 hrs. Frequency of CFSE and 7-AAD positive cells when treated with different concentrations of bispecific antibodies at 0.5:1 ET ratio for 14 hrs.

Conc.(ng/mL)	NULLXCD123	Vβ17XNULL	anti-Vβ17/anti-CD123
50	3.47	2.57	77.07
5	2.67	2.17	77.47
0.5	-0.73	1.87	46.77
0.05	0.77	1.67	3.77
0.005	-0.03	1.47	1.97
0.0005	0.67	1.07	2.17
0.00005	1.67	0.17	0.17
0.000005	-0.43	3.27	0.87
EC ₅₀ (ng/mL)	ND	ND	0.55
EC ₅₀ (pM)	ND	ND	3.7

[00188] Half maximal effective concentration (EC₅₀) values were calculated as the antibody concentration that generates 50% of maximal cytotoxicity (CFSE⁺ 7AAD⁺) cells. ND: Not Determined.

[00189] Table 16: Cytotoxicity assay at 1:1 ET ratio (stimulated CD8⁺ T cell: Kasumi-3 cells) upon incubation for 14 hrs. Frequency of CFSE and 7-AAD positive cells when treated with different concentrations of bispecific antibodies at 11 ET ratio for 14 hrs.

Conc.(ng/mL)	NULLXCD123	Vβ17XNULL	anti-Vβ17/ anti-CD123
50	0.6	-0.5	76.0
5	0.1	0.9	73.0
0.5	1.0	0.9	77.9
0.05	0.4	1.8	59.4
0.005	1.8	0.9	36.8
0.0005	0.9	1.1	18.0
0.00005	0.7	0.9	6.1
0.000005	1.6	0.8	1.9
EC ₅₀ (ng/mL)	ND	ND	0.015
EC ₅₀ (pM)	ND	ND	0.1

[00190] Half maximal effective concentration (EC₅₀) values were calculated as the antibody concentration that generates 50% of maximal cytotoxicity (CFSE⁺ 7AAD⁺) cells. ND: Not Determined

[00191] Table 17: Cytotoxicity assay at 5:1 E:T ratio (stimulated CD8⁺ T cell: Kasumi-3 cells) upon incubation for 14 hrs. Frequency of CFSE and 7-AAD positive cells when treated with different concentrations of bispecific antibodies at 5:1 ET ratio for 14 hrs.

Conc.(ng/mL)	NULLXCD123	Vβ17XNULL	anti-Vβ17/ anti-CD123
50	2.3	0.0	70.2
5	3.1	2.7	70.9
0.5	1.8	4.4	74.5
0.05	3.0	1.5	73.2
0.005	2.1	1.6	2.5
0.0005	2.9	3.5	1.4
0.00005	3.2	5.5	2.5
0.000005	4.1	4.4	4.8
EC ₅₀ (ng/mL)	UD	UD	0.02
EC ₅₀ (pM)	UD	UD	0.13

[00192] Half maximal effective concentration (EC₅₀) values were calculated as the antibody concentration that generates 50% of maximal cytotoxicity (CFSE⁺ 7AAD⁺) cells. UD: Undetectable.

[00193] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.

SEQ ID NO:	Type	Species	Description	Sequence
1	PRT	mouse	B17B01-HCDR1	GY SITSGYFWN
2	PRT	mouse	B12B01-HCDR2	YISYDG SNN
3	PRT	mouse	B12B01-HCDR2	PSPGTGYAVDY
4	PRT	mouse	B17B01-LCDR1	RSSQSLVHSNGNTY LH
5	PRT	mouse	B12B01-LCDR2	KVSNRFS
6	PRT	mouse	B12B01-LCDR2	SQSTHVPFT
7	PRT	mouse	B17B01-HC	NVQLQESGPLVKPSQLSLTCVAGYSITSGYF WNWIRQFPGNKLEWMGYISYDGNNYNPSLKN RISITRDTSKNQFFLKLNSVTTEDTATYYCASPSP GTGYAVDYWGQGTSVTVSSAKTTPPSVYPLAP GSAAQTNMSMVTLGCLVKGYFPEPVTVWNSGS LSSGVHTFPAVLQSDLYT LSSS VTPSSTWPSQT VTCNVAHPASSTKVDKKIVPRDCGCKPCICTV EVSSVIFPPKPKDVL TITLTPK VTCVVVDISKDD PEVQFSWFVDDVEVHTAQTKPREEQINSTFRSV SELPIMHQDWLNGKEFKCRVNSAAFPAPIEK KTYGRP KAPQVYTIPPKEQMAKDKVSLTCMIT NFPEDITVEWQWNGQPAENYKNTQPIMDTDG SYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHN HHTEKSLSHSPGK
8	PRT	mouse	B17B01-LC	NVVMQTPLSLPVSLGDQASISCRSSQLVHSN GNTYLHWYLQKPGQSPKFLIYKVSNRFSGV PDR FSGGGSGTEFTL KISRVEAEDLGVYFCSQSTHVP FTFGSGTKLEIKRADAAPTVSIFPPSSEQLTSGGA SVVCFLNNFYPKDINVKWKIDGSERQNGVLNS WTDQDSK D STYSMSSTLT KDEYERHNSYTC EATHKTSTSPIVKSFNRNEC
9	PRT	mouse	B17B1-HC	NVQLQESGPLVKPSQLSLTCVAGYSITSGYF WNWIRQFPGNKLEWMGYISYDGNNYNPSLKN RISITRDTSKNQFFLKLNSVTTEDTATYYCASPSP GTGYAVDYWGQGTSVTVSSASTKGPSVPLAP CSRSTSESTAALGCLVKDYFPEPVTVWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKT YTCNVDHKPSNTKVDKRVESKYGPPCP PCAP AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS LTCLVKGFYPSDIAVEWESNGQ PENNYK TTPPV LSDGSFL YSKLTVDKSRWQEGNVFSCSVMH EALHNHYTQKSLSLSLGK
10	PRT	mouse	B17B1-LC	NVVMQTPLSLPVSLGDQASISCRSSQLVHSN GNTYLHWYLQKPGQSPKFLIYKVSNRFSGV PDR FSGGGSGTEFTL KISRVEAEDLGVYFCSQSTHVP FTFGSGTKLEIKRADAAPTVSIFPPSSEQLTSGGA SVVCFLNNFYPKDINVKWKIDGSERQNGVLNS WTDQDSK D STYSMSSTLT KDEYERHNSYTC EATHKTSTSPIVKSFNRNEC
11	PRT	mouse	B17B2-HC	DVQLKESGPLVKPSQLSLTCVGTGYSITSGYY WNWYRQFPGNKLEWMGYISYDGNNYNPSLKN NRISITRDTSKNQILLKLYVTTEDTATYYCTRP SPGTGYAVDYWGQGTL VTVSSASTKGPSVPL

				APCSRSTSESTAALGCLVKDYFPEPVTVWSNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT KTYTCNDHKPSNTKVDKRVESKYGPPCPCPAPEAAGGPSVFLFPPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNAKTPREEQFN STYRVSVLTVLHQDWLNGKEYKCKVSNKGLP SIEKTISKAKGQPREPVYTLPPSQEEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTPP VLDSDGSFLYSKLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGK
12	PRT	mouse	B17B2-LC	DIVMTQSPDSLAVSLGERATINCSSQLVHSNG NTYLHWYQQKPGQPKLIIYKVSNRFSGVPDFR SGSGSGTDFTLTISLQAEVAVYYCSQSTHVPFT TGFQGTKVEIKRADAAPTVSIFPPSSEQLTSGGA SVVCFLNNFYPKDINVWKIDGSRQNGVLNS WTDQDSKDSTYSMSSTLTLDKDEYERHNSYTC EATHKTSTSPIVKSFNRNEC
13	PRT	artificial	B17B21-HC	QVQLQESGPGLVKPSETSLTCTVSGYSITSGYF WNWIRQPPGKGLEWIGIISYDGNNYNPSLCSR VTISRDTSKNQFSKLSSVTAADTAVYYCASPSP GTGYAVDYWGQGTLTVVSSASTKGPSVFPLAP CSRSTSESTAALGCLVKDYFPEPVTVWSNGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKT YTCNDHKPSNTKVDKRVESKYGPPCPCPAPE AAGGPSVFLFPPPKPKDTLMISRTPEVTCVVVD SQEDPEVQFNWYVDGVEVHNAKTPREEQFNS TYRVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPVYTLPPSQEEMTKNQVS LSCAVKGFYPSDIAVEWESNGQPENNYKTPP LSDGSFFLVSRLTVDKSRWQEGNVFSCSVMH EALHNRFTQKSLSLSLGK
14	PRT	artificial	B17B21-LC	DIQMTQSPSSLSASVGDRVTITCRSSQLVHSNG NTYLHWYQQKPGKAPKFLIIYKVSNRFSGVPSRF SGSGSGTDFTLTISLQPEDFATYYCSQSTHVPFT FGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEHKVVACEV THQGLSSPVTKSFNRGEC
15	PRT	human	I3RB217-HC	EVQLVQSGAEVKPGESLKISCKGSGYSFTSYW ISWVRQMPGKGLEWMIGIIDPSDSDTRYSPSFQG QVTISADKSISTAYLQWSSLKASDTAMYCAR DGSTDLDYWGQGTLTVVSSASTKGPSVFPLAPC SRSTSESTAALGCLVKDYFPEPVTVWSNGALT SGVHTFPAVLQSSGLYSLSSVTVPSSSLGKT YTCNDHKPSNTKVDKRVESKYGPPCPCPAPEA AAGGPSVFLFPPPKPKDTLMISRTPEVTCVVVD QEDPEVQFNWYVDGVEVHNAKTPREEQFN STYRVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPVYTLPPSQEEMTKNQVS WCLVKGFYPSDIAVEWESNGQPENNYKTPP LSDGSFFLVSRLTVDKSRWQEGNVFSCSVMH EALHNRFTQKSLSLSLGK
16	PRT	human	I3RB217-LC	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQQDYGFPTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEHKVVACEV THQGLSSPVTKSFNRGEC
17	PRT	human	Null-HC	QITLKESGPTLVKPTQTLTLCFTSGFSLSTSGM GVSWIRQPPGKALEWLAHIYWDDDKNYPSLK SRLTITKDTSKNQVVLMTNMDPVDTATYYCA

				RLYGFTYGFAYWGQGTLTVSSASTKGPSVFPL APCSRSTSESTAALGCLVKDYFPEPVTVWSNG ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT KTYTCNVDHKPSNTKVDKRVESKYGPPCPCPA PEAAGGSPSVFLFPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNAKTPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP SSIEKTISKAKGQPREQVYTLPPSQEEMTKNQV SLWCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSSFLYSRLTVDKSRWQEGNVFSCSV HEALHNHYTQKSLSLSGK
18	PRT	human	Null-LC	DIVMTQSPDSLAVSLGERATINCRASQSVDYN G I SYMHWYQQKPGQPPKLLIY A A S N P E S G V P D R F S G S G T D F T L T I S S L Q A E D V A V Y Y C Q Q I I E D P W T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V E N G Q P E N N Y K T T P P V L D S D G S F F L Y S R L T V D K S R W Q E G N V F S C S V M H N Y T Q K S L S L G K
19	PRT	artificial	B17H3	EVQLLESGGGLVQPGGSLRLSCAASGY S I T S G Y F W N W V R Q A P G K G L E W V S Y I S Y D G S N N Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A K P S P G T G Y A V D Y W G Q G T L V T V S S
20	PRT	artificial	B17H4	EVQLLESGGGLVQPGGSLRLSCAASGY S I T S G Y F W N W V R Q A P G K G L E W V S Y I S Y D G S N N Y A D S V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A S P S P G T G Y A V D Y W G Q G T L V T V S S
21	PRT	artificial	B17H5	QVQLQESGPGLVKPSETLSLTCTVSGYSITSGY F W N W I R Q P P G K G L E W I G Y I S Y D G S N N Y N P S L K S R V T I S R D T S K N Q F S L K L S S V T A A D T A V Y Y C A S P S P G T G Y A V D Y W G Q G T L V T V S S
22	PRT	artificial	B17L3	DIQMTQSPSSLSASVGDRVTITCRSSQLVHSNG N T Y L H W Y Q Q K P G K A P K L I Y K V S N R F S G V P S R F S G S G T D F T L T I S S L Q P E D F A T Y Y C S Q S T H V P F G Q G T K L E I K
23	PRT	artificial	B17L4	DIQMTQSPSSLSASVGDRVTITCRSSQLVHSNG N T Y L H W Y Q Q K P G K A P K F L I Y K V S N R F S G V P S R F S G S G T D F T L K I S R V E A E D V G V Y Y C S Q S T H V P F G Q G T K L E I K
24	PRT	artificial	B17L5	DVVMQTPLSLPVSLGQPA S I C R S S Q S L V H S N G N T Y L H W F Q Q R P G Q S P R F L I Y K V S N R F S G V P D R F S G G S G T E F T L K I S R V E A E D L G V Y F C S Q S T H V P F T F G S G T K L E I K
25	PRT	artificial	B17H1	NVQLQESGPGLVKPSQLSLTC S V A G Y S I T S G Y F W N W I R Q F P G N K L E W M G Y I S Y D G S N N Y N P S L K N R I S T R D T S K N Q F F L K L N S V T E D T A T Y Y C A S P S P G T G Y A V D Y W G Q G T S V T V S S
26	PRT	artificial	B17L1	NVVMQTPLSLPVSLGQAS I C R S S Q S L V H S N G N T Y L H W Y L Q K P G Q S P K F L I Y K V S N R F S G V P D R F S G G S G T E F T L K I S R V E A E D L G V Y F C S Q S T H V P F T F G S G T K L E I K
27	PRT	human	TCR-V β 17	MAWWVTLFLMAAAQSIQAVDGGITQSPKYL F R K E G Q N V T L S C E Q N L N H D A M Y W Y R Q D P G Q G L R L I Y Y S Q I V N D F Q K G D I A E G Y S V S R E K K E S F P L T V T E D L K N V F P P E V A V F E P S E A E I S H T Q K A T L V C L A T G F Y P D H V E L S W W V N G K E V H S G V S T D P Q P L K E Q P A L N D S R Y S L S S R L R V S A T F W Q N P R N H F R C Q V Q F Y G L S E N D E W T Q D R A P V T Q I V S A E A W G R A

				DepkscdkthtoppcpapeLLggpsvlfppkpkdtlmisrtpcv vvDvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltv hqdwlngkeykckvsnkalpapiektskakgqprepqvylp emtknqvsILclvkgfypsdiavewesngqpenyLtWppvldsd gsfflyskltvdksrwqggnvfcscvmhealhnhytqkslspsg
28	PRT	artificial	B17B21 half antibody	MAWWVWTLLFLMAAAQSIQADIQMTQSPSSLSA SVGDRVTITCRSSQSLVHSNGNTYLHWYQQKP GKAPKFLIYKVSNRFSGVPSRFSGSGSGTDFLT SSLQPEDFATYYCSQSTHVPFTFGQGTKLEIKRT VAAPSVFIFPPSDEQLKSGTASVVCLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTL SKADYEKHKVYACEVTHQGLSSPVTKS FNRGECGGSEGKSSGSGSESKSTEGKSSGSGSES KSTGGSQVQLQESGPGLVKPSETLSLTCTVSGY SITSGYFWNWIRQPPGKGLEWIGYISYDGSNYY NPSLKS RVTISRDTSKNQFSLKLSSVTAADTAVY YCASPSPGTGYAVDYWGQGTLVTVSSASTKGP SVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPALQSSGLYSLSSVTVPS SSLGTTKTYTCNVVDHKPSNTKVDKRVESKYGPPC PPCPAPEAAGGPSVFLFPPPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVSVLTVLHQDWLNGKEYKCKV SNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLSCAVKGFYPSDIAVEWESNGQOPEN NYKTPPPVLDSDGSFFLVSRLTVDKSRWQEGNV FSCSVMHEALHNFTQKSLSLSLGK
29	DNA	artificial	B17B21 half antibody	ATGGCCCTGGGTGTGGACCCCTGCTGTTCCCTGAT GGCCGCCGCCAGAGCATCCAGGCCGACATC CAGATGACCCAGAGCCAAGCAGCCTGAGCG CCAGCGTGGCGACCCGCGTACCATCACCTG CCGCAGCACGCCAGAGCCTGGTCACAGCAAC GGCACACACCTACCTGACTGGTACCAAGCAGA AGCCAGGCAAGGCCCAAAGTTCCTGATCTA CAAGGTGAGCAACCGCTTCAGCGCGTGCAC AGCCGCTTCAGCGGAGCGGCAGCGCACCG ACTTCACCCCTGACCATCAGCAGCCTGCAGCCA GAGGA CTTCGCCACCTACTACTGCAGCCAGA GCACCCACGTGCCATTCA CCTCGGCCAGGGC ACCAAGCTGGAGATCAAGCGCACCGTGGCCG CCCCAAGCGTGTTCATCTTCCCACCAAGCGAC GAGCAGCTGAAGAGCGGCACCGCCAGCGTGG TGTGCCTGCTGAACA ACTTCA CCCACGCGAG GCCAAGGTGCAGTGGAAAGGTGGACAACGCC TGCAGAGCGGCAACAGCCAGGAGAGCGTGAC CGAGCAGGACAGCAAGGACAGCACCTACAGC CTGAGCAGCACCCCTGACCCCTGAGCAAGGCC ACTACGAGAAGCACAAGGTGTACGCCCTGCGA GGTGACCCACCAAGGGCTGAGCAGCCCAGTG ACCAAGAGCTTCAACCGCGGCAGTGCAGCG GCAGCGAGGGCAAGAGCAGCGCAGCGCA GCGAGAGCAAGAGCACCAGGGCAAGAGCA GCGGCAGCGGAGCGAGAGCAAGAGCACCG GCGGCAGCCAGGTGCAGCTGAGCGAGACCG CCCAGGCCTGGTGAAGCCAAGCGAGACCC AGCCTGACCTGCACCGTGAGCGGCTACAGCA TCACCA GCGGCTACTTCTGGAACTGGATCCGC CAGCCACCAAGGCAAGGGCTGGAGTGATCG GCTACATCAGCTACGACGGCAGCAACA CAACCCAAGCCTGAAGAGCGCGTGACCATC AGCCCGACACCAGCAAGAACCAAGTTCA TGAAGCTGAGCAGCGTGACCGCCGCCACAC

				CGCCGTGTACTACTGCCAGCCCCAAGCCCCA GGCACCGGCTACGCCGTGGACTACTGGGGCC AGGGCACCCCTGGTACCGTGAGCAGCGCCAG CACCAAGGGCCAAGCGTGTCCACTGGCC CCATGCAGCCGAGCACCAAGCGAGAGCACCG CCGCCCTGGGCTGCCTGGTGAAGGACTACTTC CCAGAGCCAGTGACCGTGAGCTGGAACAGCG GCCGCCCTGACCAGCGCGTGACACCTTCCA GCCGTGCTGCAGAGCAGCGGCCGTACAGCC TGAGCAGCGTGGTGAACCGTGCCAAGCAGCAG CCTGGGACCCAAGACCTACACCTGCAACGTG GACCACAAGCCAAGCAACACCAAGGTGGACA AGCGCGTGGAGAGCAAGTACGCCACCATG CCCACCATGCCAGCCCCAGAGGCCGCC GCCCCAAGCGTGTCCCTGTTCCAAGGCC AAAGGACACCCCTGATGATCAGCCGACCCCA GAGGTGACCTGCGTGGTGGACGTGAGCC AGGAGGACCCAGAGGTGCACTTCAACTGGTA CGTGGACGGCGTGGAGGTGACAACGCCAAG ACCAAGCCACGCGAGGAGCAGTTAACAGCA CCTACCGCGTGGTGAGCGTGCTGACCGTGCTG CACCAGGACTGGCTGAACGCCAAGGAGTACA ATGCAAGGTGAGCAACAAGGCCCTGCAAG CAGCATCGAGAAAGACCATCAGCAAGGCCAAG GCCAGCCACGCGAGGCCACAGGTGTACACCC TGCCACCAAGCCAGGAGGAGATGACCAAGAA CCAGGTGACGCTGAGCTGCGCCGTGAAGGGC TTCTACCCAAAGCAGCATGCCGTGGAGTGGG AGAGCAACGGCCAGCCAGAGAACAACTACAA GACCACCCCACCAAGTGCTGGACAGCGACGGC AGCTTCTTCTGGTGAGCCGCTGACCGTGGA CAAGAGCCGCTGGCAGGAGGGCAACGTGTT AGCTGCAGCGTGATGCACGAGGCCCTGCACA ACCGCTTCACCCAGAACAGCCTGAGCCTGAG CCTGGCAAGATGGCTGGGTGTGGACCCCTG CTGTTCTGATGGCCGCCAGAGCATCCA GGCCGACATCCAGATGACCCAGAGGCCAAGC AGCCTGAGCGCCAGCGTGGCGACCGCGTGA CCATCACCTGCCAGCAGCCAGAGCCTG GCACAGCAACGGCAACACCTACCTGACTGG TACAGCAGAACAGCCAGGCAAGGCCCAAAGT TCCTGATCTACAAGGTGAGCAACCGCTTCAGC GGCGTGCCAAGCCGCTTCAGCGCAGCGCA GCGGCACCGACTTCACCCCTGACCATCAGCAG CCTGCAGCCAGAGGACTTCGCCACCTACTACT GCAGCCAGAGCACCCACGTGCCATTACCTTC GCCAGGGACCAAGCTGGAGATCAAGCGCA CCGTGGCCGCCCAAGCGTGTTCATCTTCCA CCAAGCGACGAGCAGCTGAAGAGCGGCACCG CCAGCGTGGTGTGCCTGCTGAACAACCTTAC CCACCGAGGCCAACGGTGCAGTGGAGGTGG ACAACGCCCTGCAGAGCGGAACAGCCAGGA GAGCGTGACCGAGCAGGACAGCAAGGACAGC ACCTACAGCCTGAGCAGCACCCCTGACCCCTGA GCAAGGCCACTACGAGAACAGCACAGGTGTA CGCCTGCGAGGTGACCCACCAGGGCCTGAGC AGCCCAAGTACCAAGAGCTTCAACCGCGCG AGTGCGGCCGCAGCGAGGGCAAGAGCAGCG GCAGCGGCAGCGAGAGCAAGAGCACCGAGG GCAAGAGCAGCGGCAGCGGCAGCGAGAGCA AGAGCACCGGCGGCAGCCAGGTGCAAGCTGCA GGAGAGCGGCCAGGCCCTGGTGAAGCCAAGC
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				GAGACCCCTGAGCCTGACCTGCACCGTGAGCG GCTACAGCATCACCAAGCGGCTACTTCTGGAAC TGGATCCGCCAGCCACCAGGCAAGGGCTGG AGTGGATCGGCTACATCAGCTACGACGGCAG CAACAACCTACAACCCAAAGCCTGAAGAGCCGC GTGACCACATAGCCGCCACCCAGCAAGAAC AGTTCAACCTGAAGCTGAGCAGCGTACCGC CGCCGACACCGCCGTACTACTGCGCCAGCC CAAGCCCAGGCACCGGCTACGCCGTGGACTA CTGGGGCCAGGGCACCCCTGGTACCGTGAGC AGCGCCAGCACCAAGGGCCAAGCGTGTCC CACTGGCCCCATGCAGCCGCAGCACCAAGCGA GAGCACCGGCCGCCCTGGCTGCCTGGTGAAG GAECTACTTCCCAGAGCCAGTGACCGTGAGCT GGAACAGCGGCCCTGACCAGCGCGTGCA CACCTTCCCAGCCGTGCTGCAGAGCAGCGC CTGTACAGCCTGAGCAGCGTGGTACCGTG CAAGCAGCAGCCTGGCACCAAGACCTACAC CTGCAACGTGGACCACAAGCCAAGCAACACC AAGGTGGACAAGCGCGTGGAGAGAGCAAGTACG GCCACCAGCCCACCATGCCAGCCCCAGA GGCCGCCGGCGGCCAACCGTGTCCCTGTTCC CACCAAAGCAAAGGACACCCGTATGATCAG CCGCACCCCCAGAGGTGACCTGCGTGGTGGT GACGTGAGCCAGGAGGACCCAGAGGTGCA TCAACTGGTACGTGGACGGCGTGGAGGTGCA CAACGCCAAGACCAAGGCCACCGGAGGAGCAG TTCAACAGCACCTACCGCGTGGTACCGTG GACCGTGTGCTGCACCAGGACTGGCTGAACGGC AAGGAGTACAAGTGCAAGGTGAGCAACAAGG GCCTGCCAACAGCATCGAGAACGACCATCAG CAAGGCCAACGGCCAGCCACCGGAGCCACAG GTGTACACCCCTGCCACCAAGCCAGGAGGAGA TGACCAAGAACCAAGGTCAGCTGAGCTGCGC CGTGAAGGCTTCTACCCAAGCGACATGCC GTGGAGTGGAGAGCAACGGCCAGCCAGAGA ACAACATAAACAGCACCCCCACCAAGTGTGGA CAGCGACGGCAGCTTCTCTGGTACGCCAGG TGACCGTGGACAAGAGCCGCTGGCAGGAGGG CAACGTGTTCACTGAGCGTGTGACCGAG GCCCTGCACAACCGCTTCACCCAGAACAGGCC TGAGCCTGAGCCTGGCAAGTGATAG
30	PRT	artificial	I3RB217 half antibody	MAVVWTLLFLMAAAQSIQAEIVLTQSPGTLSSL PGERATLSCRASQSVSSSYLAWYQQKPGQAPRL LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQDYGFPWTFQGQTKVEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDKSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRG ECGGSEGKSSSGSGSESKSTEGKSSSGSGSESKSTG GSEVQLVQSGAEVKKPGESLKISCKGSGYSFTS YWISWVRQMPGKGLEWMGIIDPSDSDTRYSPSF QQQVTISADKSISTAYLQWSSLKASDTAMYCA RGDGSTDLDYWQGQTLVTVSSASTKGPSVFPL APCSRSTSESTAALGCLVKDYFPEPVTVWSNSG ALTSGVHTFPAVLQSSGLYSLSSVTVPSLGLT KTYTCNVVDHKPSNTKVDKRVESKYGPPCPCPA PEAAGGPSVFLFPPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV SLWCLVKGFYPSDIAVEWESNGQPENNYKTPP

				VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGK
31	DNA	artificial	I3RB217 half antibody	ATGGCCTGGGTGTGGACCCCTGCTGTTCCCTGAT GGCCGCCGCCAGAGCATCCAGGCCAGATC GTGCTGACCCAGAGGCCAGGCACCCTGAGCC TGAGCCCAGGCAGCGCGCCACCCTGAGCTG CCGCGCCAGCCAGAGCGTGAGCAGCAGCTAC CTGGCCTGGTACCAAGCAGAAGCCAGGCCAGG CCCCACGCCTGCTGATCTACGGCGCCAGCAGC CGCGCCACCGGCATCCCAGACCCTCAGCG GCAGCGGCAGCGGCACCCTACCCCTGAC CATCAGCCGCCTGGAGGCCAGAGGACTTCGCC GTGTACTACTGCCAGCAGGACTACGGCTTCCC ATGGACCTTCGGCCAGGGCACCAAGGTGGAG ATCAAGCGCACCGTGGCCGCCAAGCGTGT TCATCTCCACCAAGCAGCAGCAGCTGAA GAGCGGCACCGCCAGCGTGGTGTGCCTGCTG AACAACTTCTACCCACCGCAGGCCAAGGTGC AGTGGAAAGGTGGACAACGCCCTGCAGACGG CAACAGCCAGGAGAGCGTGACCGAGCAGGAC AGCAAGGACAGCACCTACAGCCTGAGCAGCA CCCTGACCCCTGAGCAAGGCCGACTACGAGAA GCACAAGGTGTACGCCCTGCGAGGTGACCCAC CAGGGCCTGAGCAGCCAGTGACCAAGAGCT TCAACCGCGCGAGTGCGggggcagcgaggcaagac agccgcagccgcagcagcagcagcaccggcggcagcGAGGTGC AGCTGGTGCAGAGCGCGCCAGGTGAAGAA GCCAGGGAGAGCCTGAAGATCAGCTGCAAG GGCAGCGGCTACAGCTTACCCAGCTACTGGA TCAGCTGGGTGCCTGAGATGCCAGGCAAGGG CCTGGAGTGGATGGGCATCATCGACCCAAAG GACAGCGACACCCGCTACAGCCCAAGCTTCC AGGGCCAGGTGACCATCAGCGCCGACAAGAG CATCAGCACCGCCTACCTGCAGTGGAGCAGC CTGAAGGCCAGCGACACCGCATGTACTACT GCGCCCGCCGCGACGGCAGCAGCAGCTGG CTACTGGGCCAGGGCACCTGGTGACCGTG AGCAGCGCCAGCACCAAGGGCCAAGCGTGT TCCCACGGCCCCATGCAGCCGAGCACCAG CGAGAGCACCGCCGCCCTGGCTGCCTGGT AAGGACTACTCCCAGAGCCAGTGACCGTGA GCTGGAACAGCGCGCCCTGACCGAGCGCG GCACACCTTCCCAGCCGTGCTGCAGAGCAGC GGCCTGTACAGCCTGAGCAGCGTGGTACCG TGCCAAGCAGCAGCCTGGCACCAAGACCTA CACCTGCAACGTGGACCACAAGCCAAGCAAC ACCAAGGTGGACAAGCGCTGGAGAGCAAGT ACGGCCCACCATGCCACCATGCCAGCCCC AGAGGCCGCCGGCGGGCCAAGCGTGTCTG TTCCCAACCAAGCCAAGGACACCCCTGATGA TCAGCCGCACCCAGAGGTGACCTGCGTGGT GGTGGACGTGAGCCAGGAGGACCCAGAGGTG CAGTTCAACTGGTACGTGGACGGCGTGGAGG TGACAACGCCAAGACCAAGCCACCGCAGGA GCAGTTCAACAGCACCTACCGCGTGGTGAGC GTGCTGACCGTGTGCTGCACCAAGGACTGGCTGA ACGGCAAGGAGTACAAGTGAAGGTGAGCAA CAAGGGCCTGCCAACAGCAGCATCGAGAAC ATCAGCAAGGCCAAGGGCCAGCCACGCCAG CACAGGTGTACACCCCTGCCACCAAGGCCAGGA GGAGATGACCAAGAACCAAGGTGAGCCTGTGG

				TGCCCTGGTGAAGGGCTTCTACCCAAGCGACAT CGCCGTGGAGTGGGAGAGCAACGGCCAGCCA GAGAACAACTACAAGACCACCCACCAGTGC TGACAGCGACGGCAGCTTCTCTGTACAGC CGCCTGACCGTGGACAAGAGCCGCTGGCAGG AGGGCAACGTGTTAGCTGCACCGTGTAGCA CGAGGCCCTGCACAACCAACTACACCCAGAAG AGCCTGAGCCTGAGCCTGGGCAAG
32	PRT	artificial	B23B49 half antibody	MAVVWTLFLMAAQSIQAEIVLTQSPGTLSSL PGERATLSCRASQSVSSSYLAWYQQKPGQAPRL LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQDYGFPWTFGQGTTKVEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRG ECGGSEKGSSSGSGSESKSTEGKSSSGSGSESKSTG GSEVQLVQSGAEVKKPGESLKISCKGSGYSFTS YWISWVRQMPGKGLEWMGIIDPSDSDTRYSPSF QQVTISADKSISTAYLQWSSLKASDTAMYCA RGDGSTDLDYWQGTLTVTSSASTKGPSVFPL APCSRTSESTAALGCLVKDYFPEPVTVWSNG ALTSGVHTFPAAVLQSSGLYSLSSVTVPSSSLGT KTYTCNVDHKPSNTKVDKRVESKYGPPCPCPA PEAAGGPSVFLFPPPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNATKPREEQFN STYRVSVSLTVLHQDWLNGKEYKCKVSNKGLP SSIEKTISKAKGQPREPVYTLPPSQEEMTKNQV SLWCLVKGFYPSDIAVEWESNGQPENNYKTPP VLSDGSFFLYSRLTVDKSRWQEGNVFSCVM HEALHNHYTQKSLSLSGK
33	DNA	artificial	B23B49 half antibody	ATGGCCTGGGTGTGGACCCCTGCTGTTCCCTGAT GCCGCCGCCAGAGCATCCAGGCCGACATC GTGATGACCCAGAGGCCAGACAGCCTGGCC TGAGCCTGGCGAGCGCGCCACCATCAACTG CCGCGCCAGCCAGAGCGTGGACTACAACGGC ATCAGCTACATGCACTGGTACCCAGCAGAAC CAGGCCAGCCACCAAAGCTGCTGATCTACGC CGCCAGCAACCCAGAGAGCGGGTGCCAGAC CGCTTCAGCGGCAGCGGCAGCGGCACCGACT TCACCCCTGACCATCAGCAGCCTGCAGGCCGA GGACGTGGCCGTGTAAGCTGCTGAGATCAGAC ATCGAGGACCCATGGACCTTCGGCCAGGGCA CCAAGGTGGAGATCAAGCGCACCGTGGCCGC CCAAGCGTGTTCATCTTCCCACCAAGCGACG AGCAGCTGAAGAGCGGCACCGCCAGCGTGGT GTGCCTGCTGAACAACCTCTACCCACGCGAGG CCAAGGTGCAGTGGAAAGGTGGACAACGCCCT GCAGAGCGGCAACAGCCAGGAGAGCGTGACC GAGCAGGACAGCAAGGACAGCACCTACAGCC TGAGCAGCACCTGACCCCTGAGCAAGGCCGA CTACGAGAACACAAGGTGTACGCCCTGCGAG GTGACCCACCAAGGGCTGAGCAGCCAGTGA CCAAGAGCTCAACCGCGCGAGTGCAGGGCG CAGCGAGGGCAAGACGAGCGGGCAGCGGCAG CGAGAGCAAGAGCACCGAGGGCAAGAGCAG CGGCAGCGGCAGCGAGAGCAAGAGCACCGGC GGCAGCCAGATCACCTGAAGGGAGAGCGGCC CAACCCCTGGTGAAGCCAACCCAGACCTGAC CCTGACCTGCACCTTCAGCGGCTTCAGCCTGA GCACCAGCGGCATGGCGTGAGCTGGATCCG CCAGCCACCAAGGCAAGGCCCTGGAGTGGCTG GCCCACATCTACTGGGACGACGACAAGCGCT

				ACAAACCCAAGCCTGAAGAGCCGCCTGACCAT CACCAAGGACACCAGCAAGAACCCAGGTGGTG CTGACCATGACCAACATGGACCCAGTGGACA CCGCCACCTACTACTGCGCCGCCTGTACGGC TTCACCTACGGCTTCCCTACTGGGCCAGGG CACCCCTGGTACCGTGAGCAGGCCAGCACC AAGGGGCCAAGCGTGTCCCACAGGCCCAT GCAGCCGCAGCACCGAGAGCACGCCGC CCTGGGCTGCCCTGGTGAAGGACTACTTCCAG AGCCAGTACCGTGAGCTGAAACAGCGGCC CCTGACCAGCGCGTGACACCTTCCAGCCG TGCTGCAGAGCAGCGCCCTGTACAGCCTGAG CAGCGTGGTACCGTGCCAAGCAGCAGCCTG GGCACCAAGACCTACACCTGCAACGTGGACC ACAAGCCAAGCAACACCAAGGTGGACAAGCG CGTGGAGAGCAAGTACGGCCACCATGCCA CCATGCCAGCCCCAGAGGCCGCCGCC CAAGCGTGTCCCTGTTCCCACCAAAGCCAAAG GACACCTGATGATCAGCCGCACCCAGAGG TGACCTGCGTGGTGGACGTGAGCCAGGA GGACCCAGAGGTGCAGTTCAACTGGTACGTG GACGGCGTGGAGGTGACAACGCCAAGACCA AGCCACGCCAGGAGCAGTCAACAGCACCTA CCCGCTGGTGGACGTGCTGACCGTGTGCACC AGGACTGGCTGAACGGCAAGGAGTACAAGTG CAAGGTGAGCAACAAGGGCTGCCAAGCAGC ATCGAGAAGACCATCAGCAAGGCCAAGGGCC AGCCACGCCAGGCCACAGGTGTACACCCTGCC ACCAAGCCAGGAGGAGATGACCAAGAACAG GTGAGCCTGTGGTGCCTGGTGAAGGGCTCTA CCCAAGCGACATGCCGTGGAGTGGAGAGC AACGGCCAGGCCAGAGAACAACTACAAGACCA CCCCACCAGTGCTGGACAGCGACGGCAGCTT CTTCCTGTACAGCCGCCTGACCGTGGACAAGA GCCGCTGGCAGGAGGGCAACGTGTTAGCTG CAGCGTGATGACGAGGCCCTGCACAACCAC TACACCCAGAAGAGCCTGAGCCTGAGCCTGG GCAAG
34	PRT	Artificial	HCDR1	SYWIS
35	PRT	Artificial	HCDR2	IIDPSDSDTRYSPSFQG
36	PRT	Artificial	HCDR3	GDGSTLDY
37	PRT	Artificial	LCDR1	RASQSVSSSYL
38	PRT	Artificial	LCDR2	GASSRAT
39	PRT	Artificial	LCDR3	QQDYGFPWT
40	PRT	Artificial	HC	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYW ISWVRQMPGKLEWMGIIDPSDSDTRYSPSFQG QVTISADKSISTAYLQWSSLKASDTAMYCARG DGSTDLDYWQGQTLTVVSS
41	PRT	Artificial	LC	EIVLTQSPGTLSSLPGERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQQDYGFPWTFGQ GTKVEIK
42	PRT	Artificial	IGHJ1*01 HC	WGQGTLTVVSS
43	PRT	Artificial	IGKJ2*01 LC	FGQGTKLEIK
44	PRT	Artificial	Va10.2_Fc	MAWWWTLLFLMAAAQSIQALLEQSPQFLSIQE GENLTVYCNSSSVFSSLQWYRQEPGEGPVLLVT VVTGGEVKKLKRLTFQFGDARKDSSLHITAAQP GDTGLYLCAGAGSQGNLIFGKGTKLSVKPNIQN PDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQS KDSDVYITDKTVLDMRSMDFKNSAVAWSNKS

				DFACANAFNNSIIPEDTFFPSepkscdkthcpcpapeL LggpsvflfppkpkdtlmisrtpevtcvvvDvshedpevkfnwyvdg vevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalp apiektiskakgqprepqvyVYppsreemtknqvsItclvkgfypsdi avewesngqpenmykttppvldsdgsfAIVskltvdksrwqqgnvfs csvmhealhnhytqkslslspg
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CLAIMS

1. An isolated V β 17 bispecific antibody or antigen-binding fragment thereof, the isolated V β 17 bispecific antibody or antigen-binding fragment thereof comprising:

- a. a first heavy chain (HC1);
- b. a second heavy chain (HC2);
- c. a first light chain (LC1); and
- d. a second light chain (LC2),

wherein HC1 is associated with LC1 and HC2 is associated with LC2, and wherein HC1 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and LC1 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively, to form a binding site for a first antigen, and wherein HC2 and LC2 form a binding site for the second antigen.

2. The V β 17 bispecific antibody or antigen-binding fragment thereof of claim 1, wherein the binding site for the first antigen binds to V β 17 on a CD8+ or CD4+ T cell.

3. The V β 17 bispecific antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the binding site for the second antigen binds to a tumor antigen present on the surface of a cancer cell.

4. The V β 17 bispecific antibody or antigen-binding fragment of any one of claims 1 to 3, wherein HC1 and LC1 are humanized.

5. The V β 17 bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 4, wherein HC2 and LC2 bind to CD123.

6. The V β 17 bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 5, wherein the bispecific antibody or antigen-binding fragment thereof is a IgG isotype.

7. The V β 17 bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 6, wherein the bispecific antibody or antigen-binding fragment thereof is a IgG4 isotype.

8. The V β 17 bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 7, wherein the bispecific antibody or antigen-binding fragment thereof induces CD8+ or CD4+ T-cell dependent cytotoxicity of a cancer cell *in vitro* with an EC₅₀ of less than about 0.2 pM.

9. An isolated nucleic acid encoding the HC1 and the LC1 of the V β 17 bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 8.

10. An isolated nucleic acid encoding the HC2 and the LC2 of the V β 17 bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 8.
11. A vector comprising the isolated nucleic acid of claim 9 or claim 10.
12. A host cell comprising the vector of claim 11.
13. An isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof comprising:
 - a. a first heavy chain (HC1);
 - b. a second heavy chain (HC2)
 - c. a first light chain (LC1); and
 - d. a second light chain (LC2),

wherein HC1 is associated with LC1 and HC2 is associated with LC2, and wherein HC1 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and LC1 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively, to form a binding site for a first antigen that specifically binds V β 17, and wherein HC2 comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, respectively, and LC2 comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39, respectively, to form a binding site for a second antigen that specifically binds CD123.

14. The isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of claim 13, wherein HC1 comprises the amino acid sequence of SEQ ID NO:13 and LC1 comprises the amino acid sequence of SEQ ID NO:14, and wherein HC2 comprises the amino acid sequence of SEQ ID NO:15 and LC2 comprises the amino acid sequence of SEQ ID NO:16.
15. The isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of claim 13 or 14, wherein the V β 17 is on the surface of a CD8+ or CD4+ T cell.
16. The isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of claims 13 to 15, wherein the CD123 is on the surface of a cancer cell or a CD34+ stem cell.
17. The isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of claims 13 to 16, wherein bispecific antibody or antigen-binding fragment

thereof induces CD8+ or CD4+ T-cell dependent cytotoxicity of a cancer cell *in vitro* with an EC₅₀ of less than about 0.2 pM.

18. An isolated nucleic acid encoding the HC1 and LC1 of the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of claims 13 to 17.
19. An isolated nucleic acid encoding the HC2 and LC2 of the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of claims 13 to 18.
20. A vector comprising the isolated nucleic acid of claim 18 or 19.
21. A host cell comprising the vector of claim 20.
22. A buffered composition comprising the isolated anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of claims 13 to 17.
23. A method of directing a V β 17-expressing CD8+ or CD4+ T cell to a cancer cell, the method comprising contacting a V β 17-expressing CD8+ or CD4+ T cell with the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of claim 13, wherein contacting the V β 17-expressing CD8+ or CD4+ T cell with the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof directs the V β 17-expressing CD8+ or CD4+ T cell to a cancer cell.
24. A method for inhibiting growth or proliferation of cancer cells, the method comprising contacting the cancer cells with the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of claim 13, wherein contacting the cancer cells with the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof inhibits the growth or proliferation of the cancer cells.
25. The method of claim 23 or 24, wherein the cancer cell is a CD123-expressing cancer cell.
26. A kit comprising a V β 17 bispecific antibody or antigen-binding fragment thereof of any one of claims 1 to 8 and packaging for the same.
27. A kit comprising an anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof of any one of claims 13 to 17 and packaging for the same.
28. A method of producing a V β 17 bispecific antibody or antigen-binding fragment thereof comprising culturing the host cell of claim 12 to produce the V β 17 bispecific antibody or antigen-binding fragment thereof, and recovering the V β 17 bispecific antibody or antigen-binding fragment thereof from the cell or culture.
29. A method of producing an anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof comprising culturing the host cell of claim 21 to produce the anti-V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof, and recovering the anti-

V β 17/anti-CD123 bispecific antibody or antigen-binding fragment thereof from the cell or culture.

30. An isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof, the humanized V β 17 monoclonal antibody or antigen-binding fragment thereof comprising an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO:28.
31. The isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof of claim 30, wherein the humanized V β 17 monoclonal antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NO:28.
32. An isolated nucleic acid encoding the isolated humanized V β 17 monoclonal antibody or antigen-binding fragment thereof of claim 30 or 31.
33. A vector comprising the isolated nucleic acid of claim 32.
34. A host cell comprising the vector of claim 33.

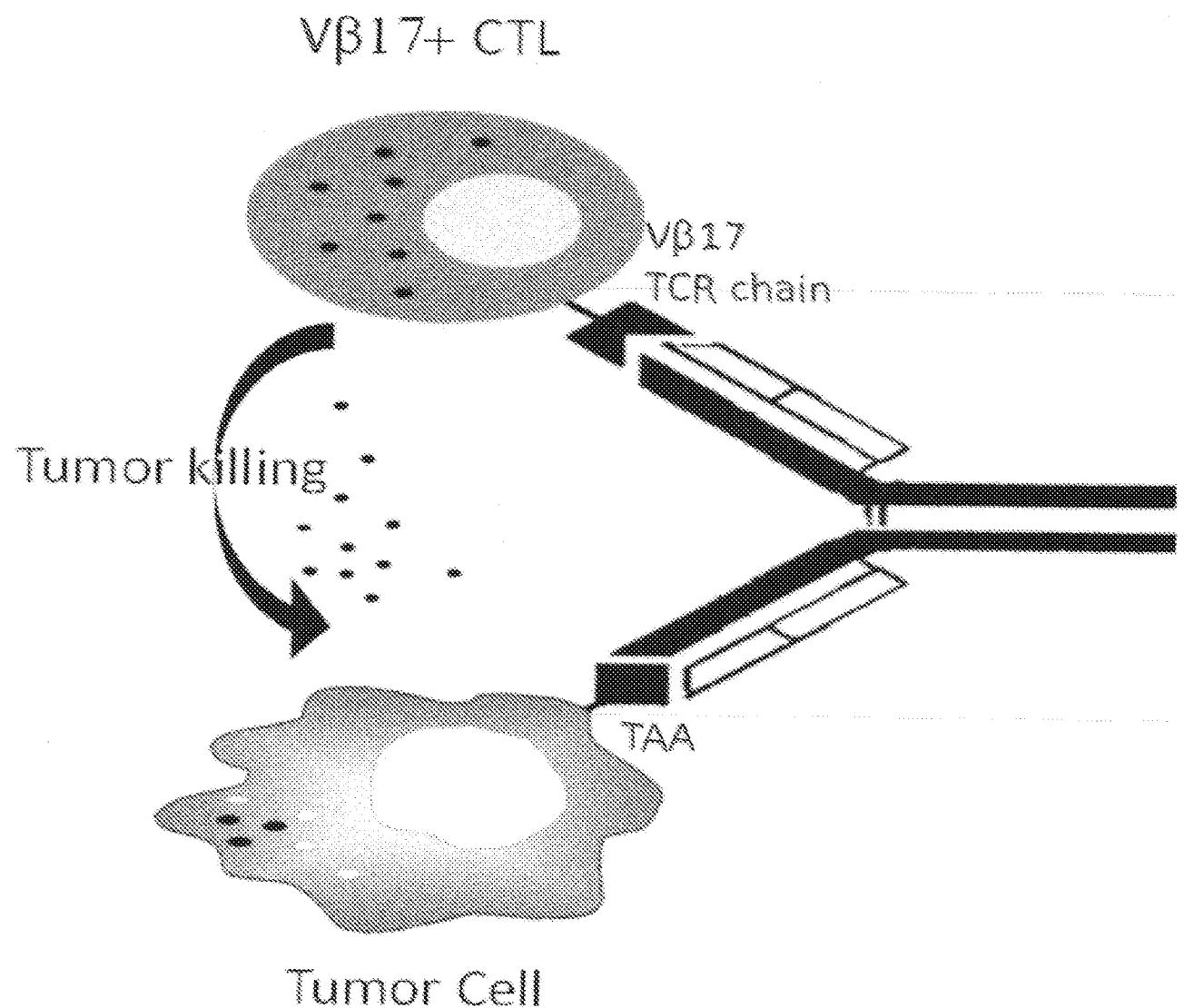


FIG. 1

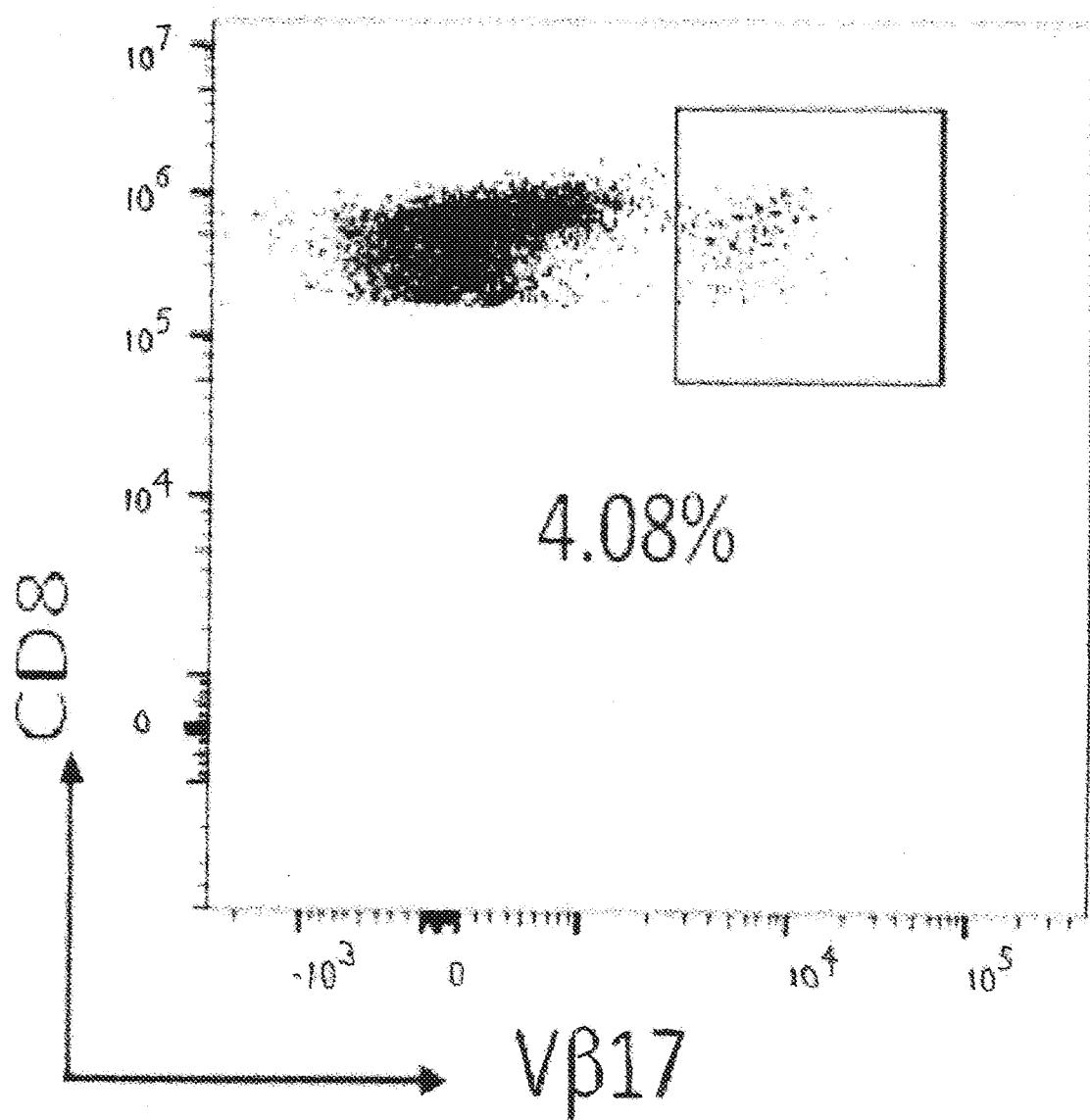


FIG. 2A

Donor	HLA status	% of V β 17 $^{+}$ in CD8 T cells (Day 0)	% of V β 17 $^{+}$ in CD8 T cells (Day 14)
HPU-03033	A2	5.45	14.4
HPU-07540	A2	3.70	34.4
HPU-08694	A2	3.88	75
17043595	A*0201	2	15
14035473	A*0201	2.8	40.7
15036948	A*0201	3.64	43.0

FIG. 2B

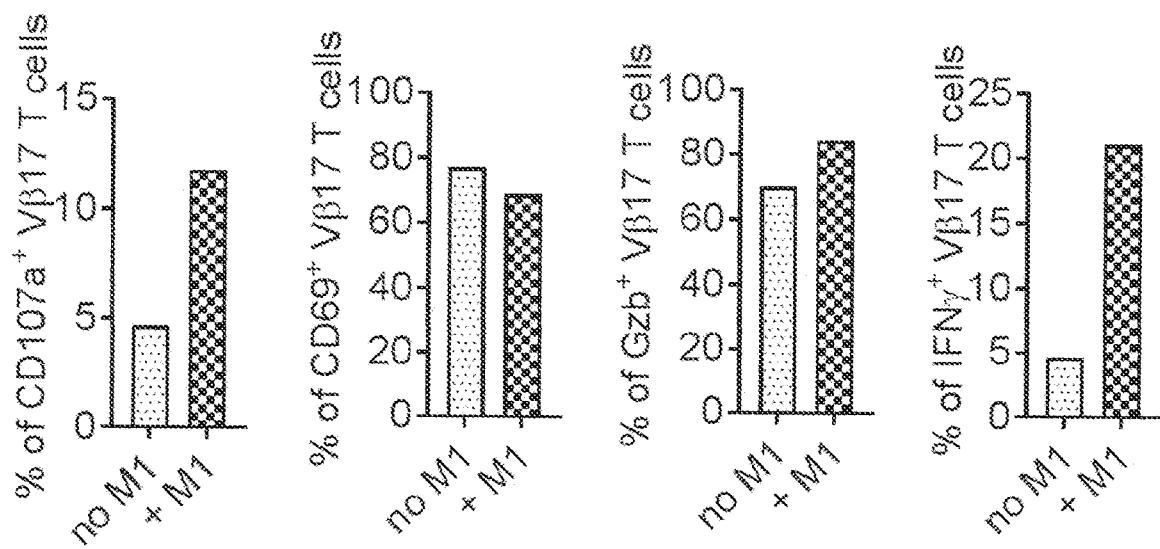


FIG. 3

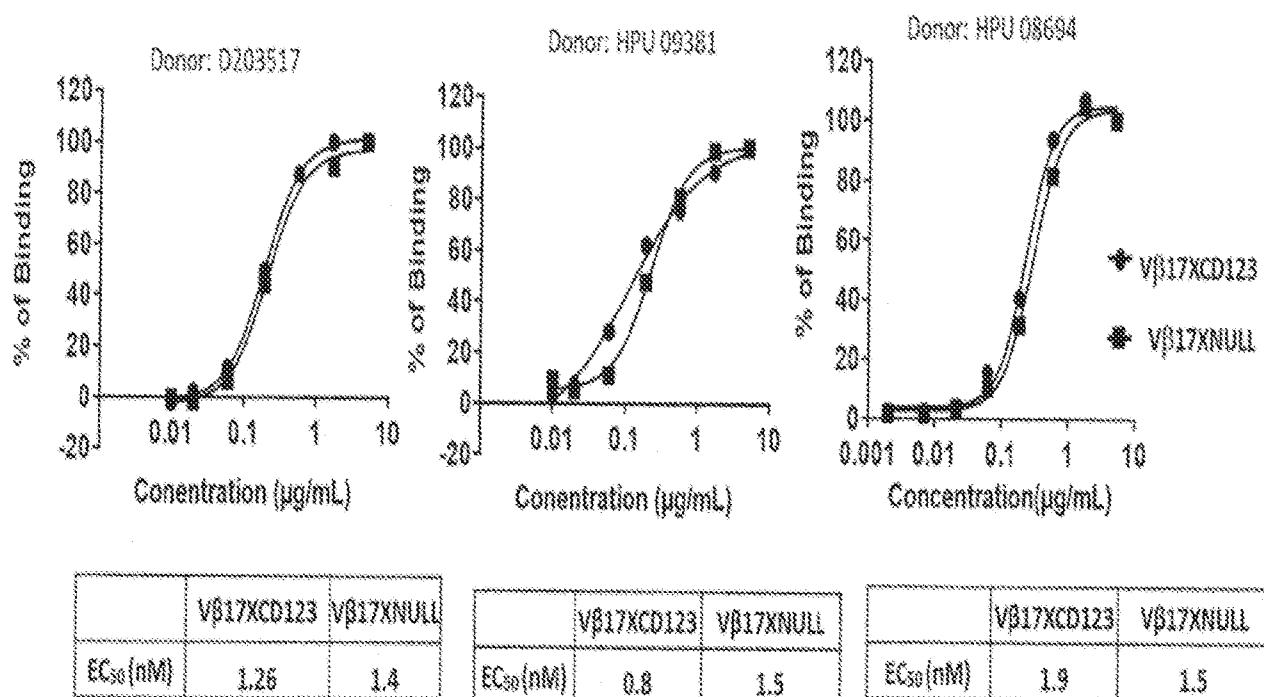
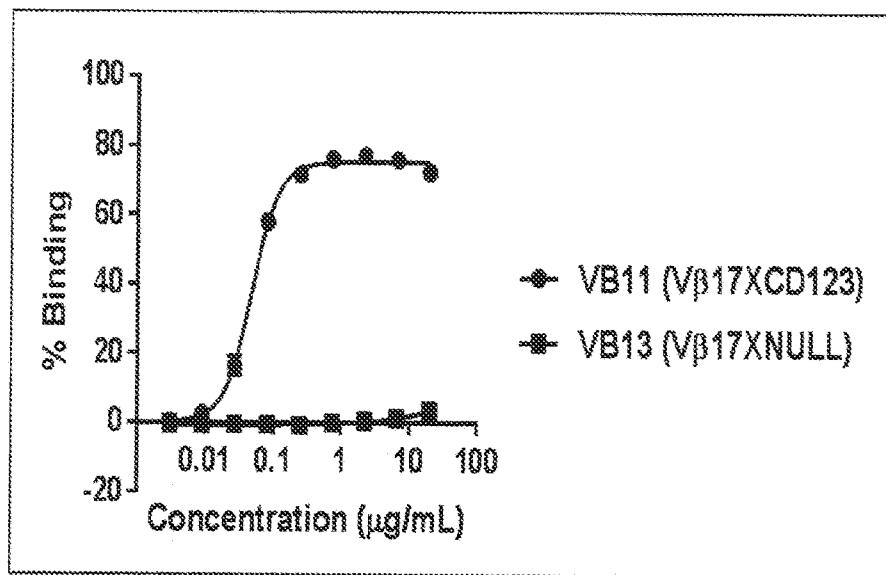


FIG. 4



	V β 17XCD123	V β 17XNULL
EC ₅₀ (nM)	0.33	ND

FIG. 5

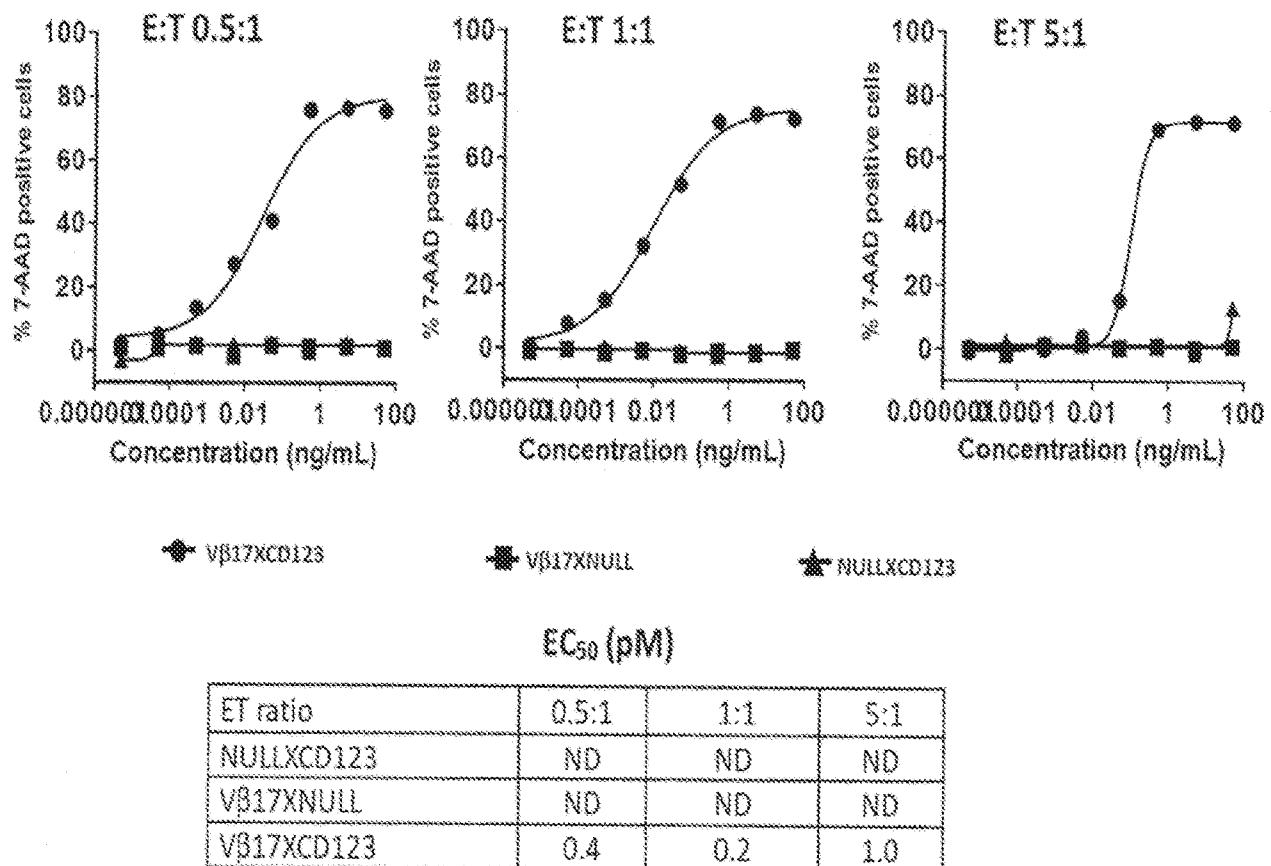


FIG. 6

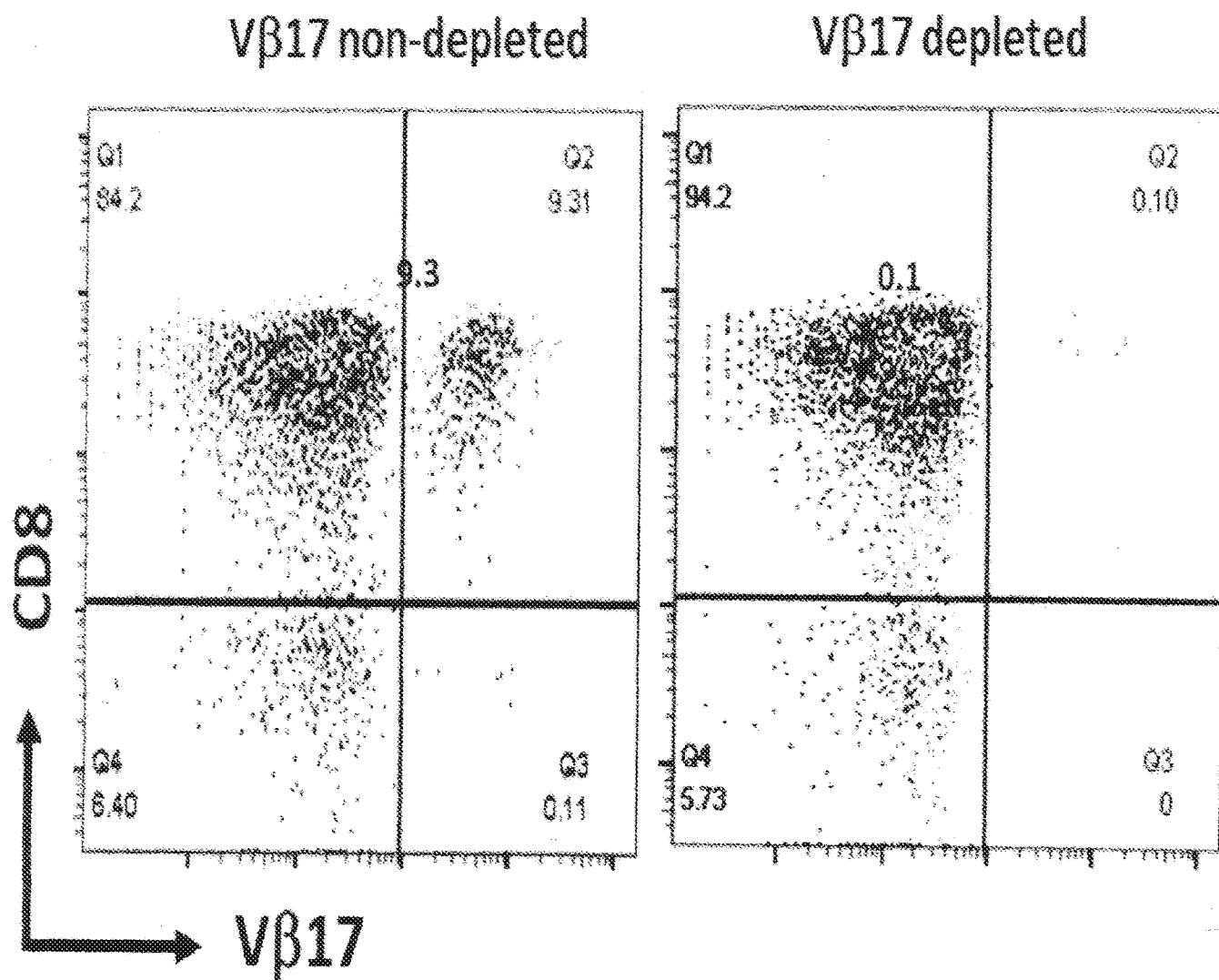


FIG. 7A

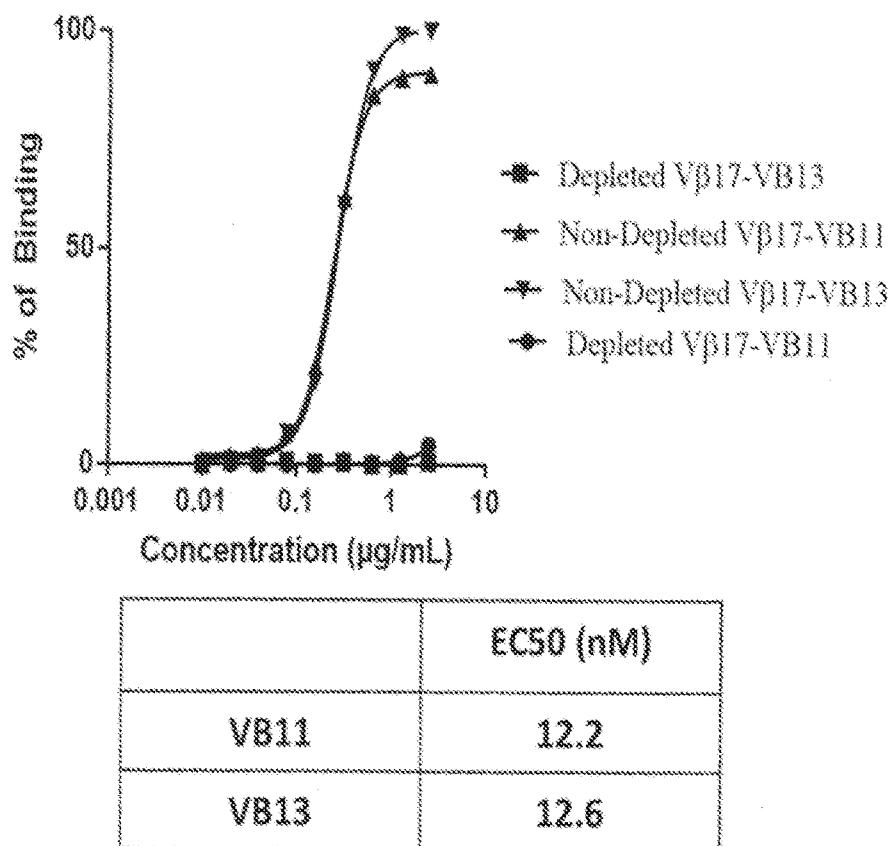


FIG. 7B

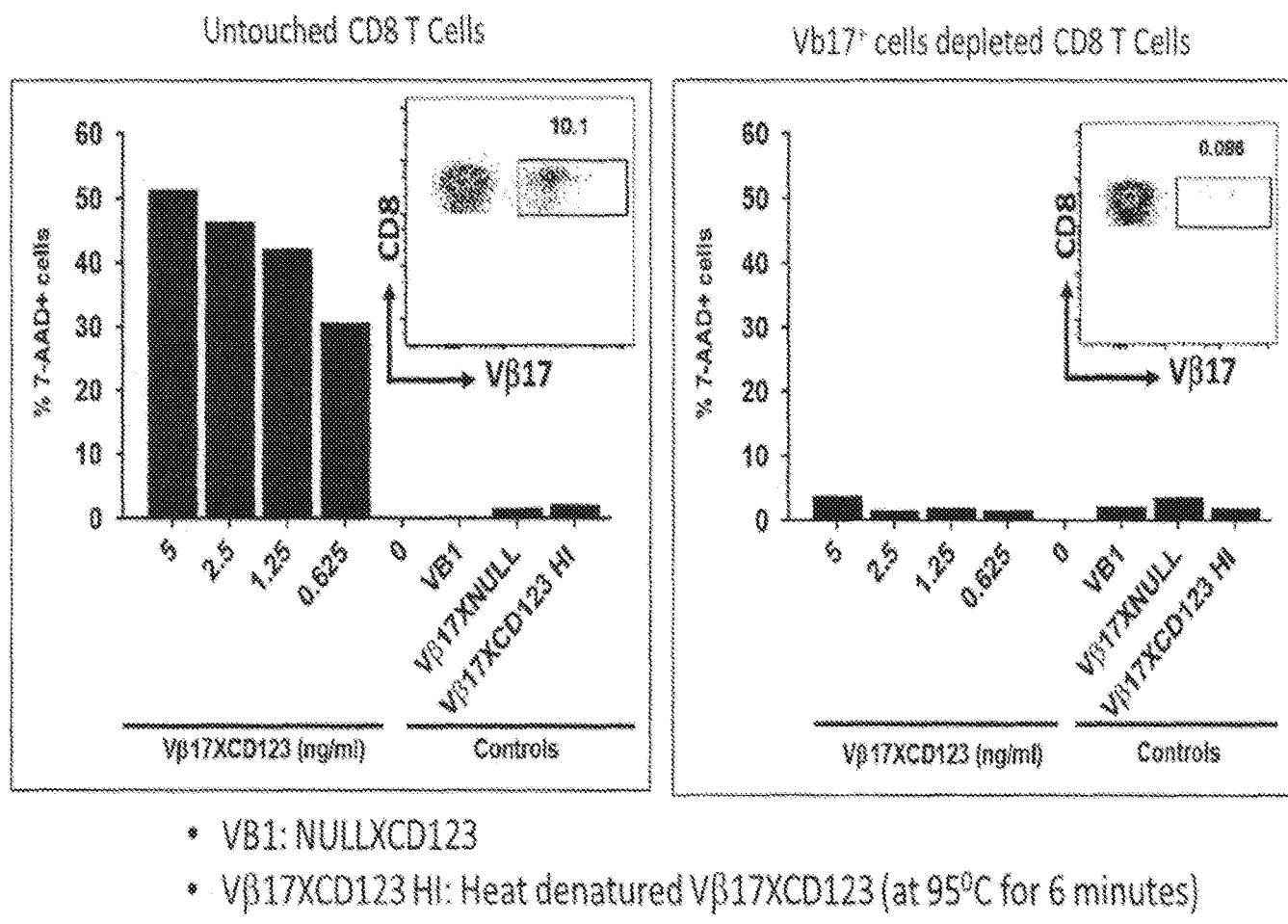


FIG. 8

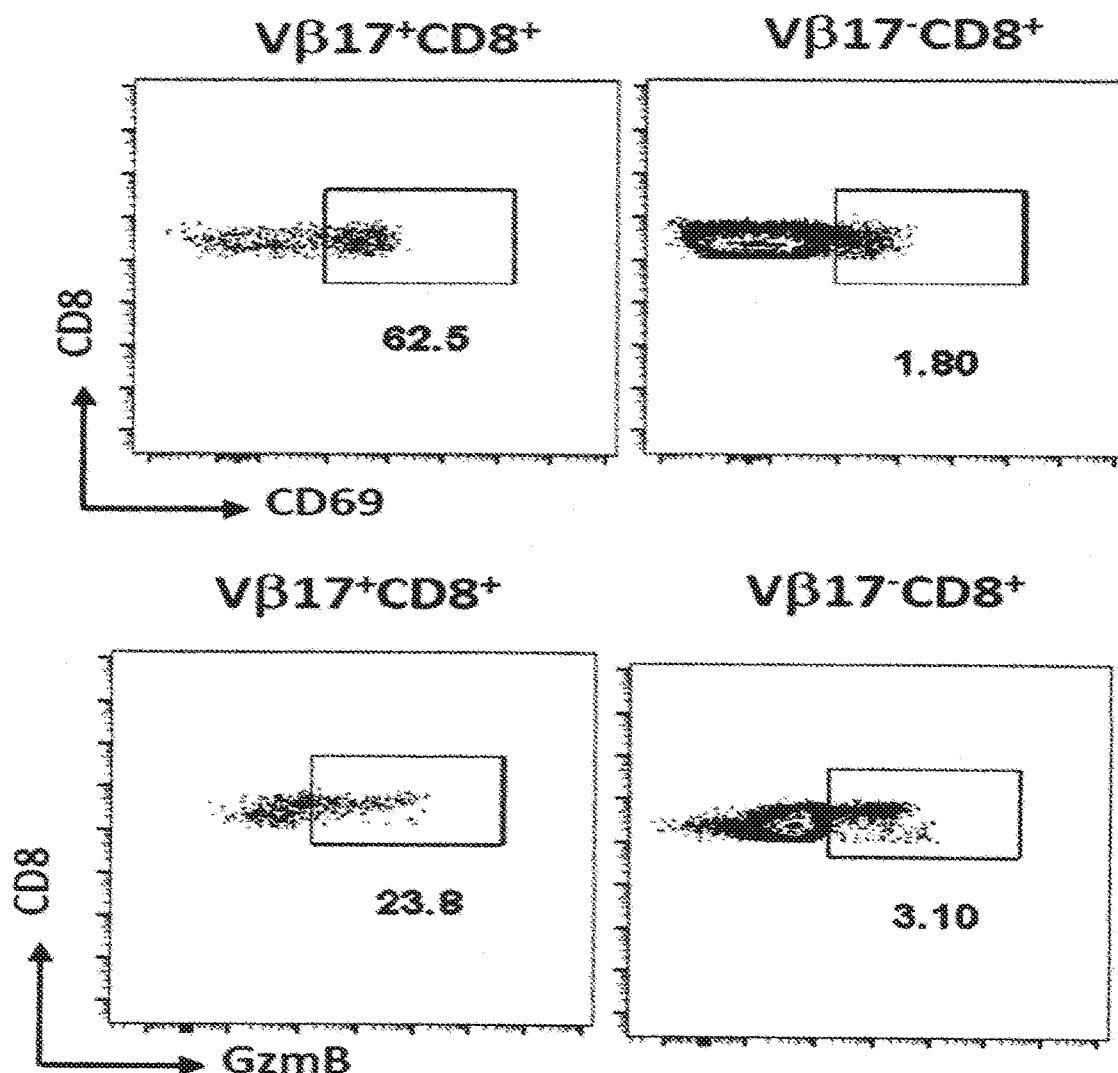


FIG. 9A

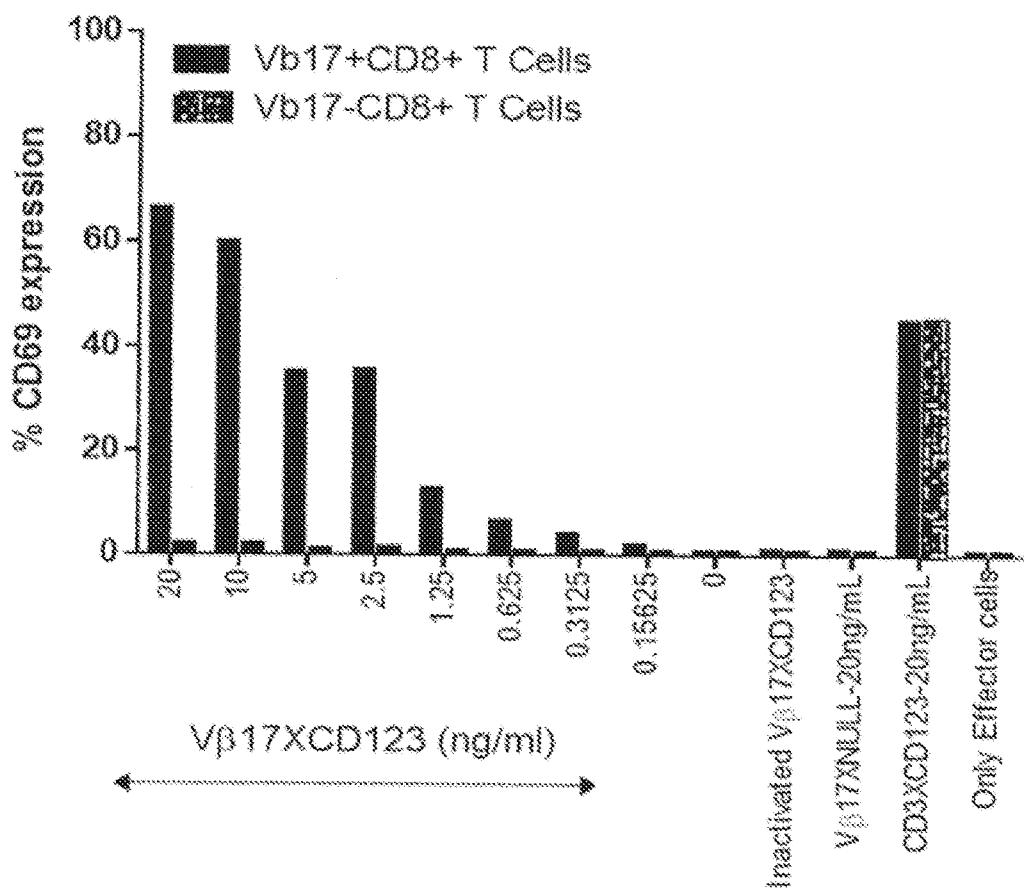


FIG. 9B

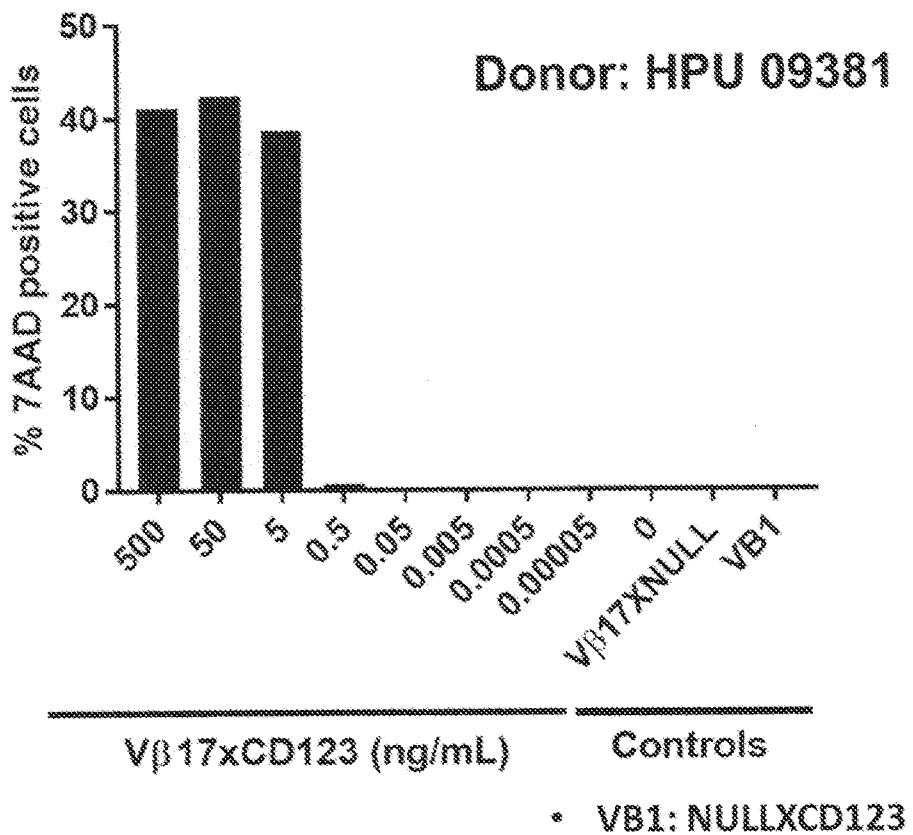


FIG. 10

$\text{V}\beta 17+$ CTL

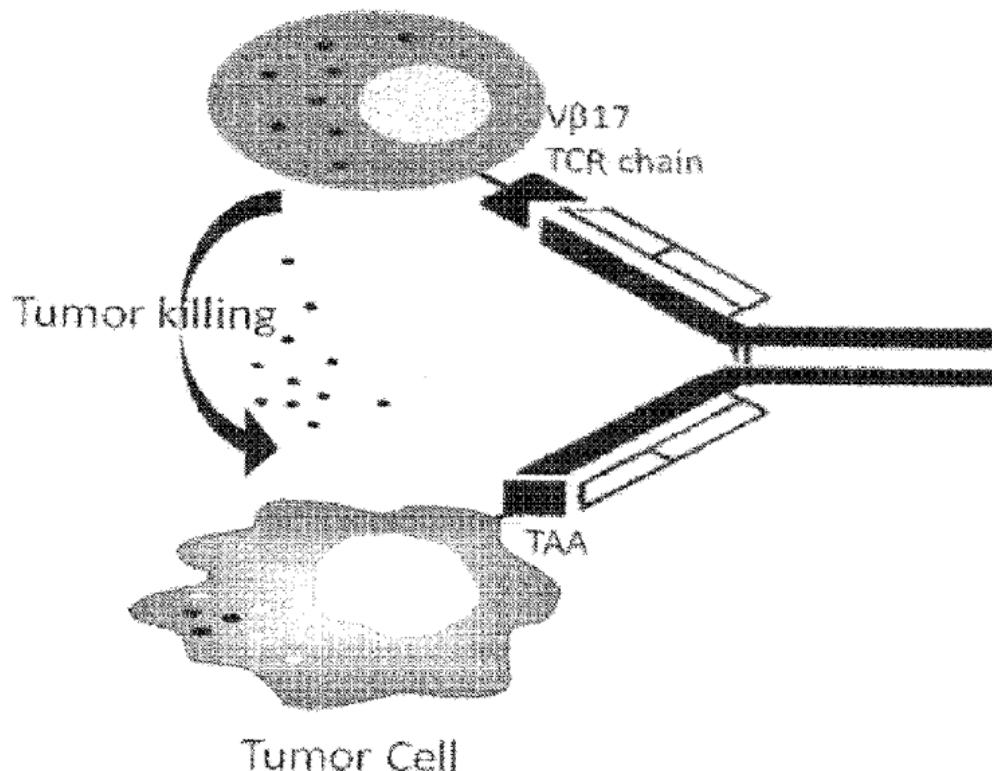


FIG. 1