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(54) Title: NOVEL HUMAN ANTIBODIES BINDING TO HUMAN CD3 EPSILON

(57) Abstract: The present disclosure provides novel fully human antibodies and antibody fragments specific for human CD3 epsilon.

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NOVEL HUMAN ANTIBODIES BINDING TO HUMAN CD3 EPSILON

FIELD OF THE INVENTION

The present disclosure relates to a fully human antibody as well as improved variants thereof which bind to human CD3 epsilon. The present disclosure also provides multispecific antibody formats comprising such human CD3 specific antibodies and antibody fragments. The disclosure also relates to nucleic acids, vectors and host cells capable of expressing such antibodies, pharmaceutical compositions comprising said antibodies and uses of said antibodies and pharmaceutical compositions for the treatment of specific diseases.

BACKGROUND

CD3 is a homodimeric or heterodimeric antigen expressed on T cells in association with the T cell receptor complex (TCR) and is required for T cell activation. CD3 is formed by the dimeric association of two of four different chains: epsilon, zeta, delta and gamma. The CD3 dimeric arrangements include gamma/epsilon, delta/epsilon and zeta/zeta.

Antibodies binding to CD3 have been shown to cluster CD3 on T cells, thereby causing T cell activation in a manner similar to the engagement of the T-Cell receptor (TCR) by peptide-loaded MHC molecules. CD3 specific antibodies have been proposed for therapies involving the activation of T cells. Accordingly, bi-, or multispecific antibody formats that co-engage CD3 and one or more cancer associated antigens (CAA) have been developed to redirect T-cells to attack and lyse cancer cells. While the CD3 targeting approach has shown considerable promise, the therapeutic use of such multispecific antibody formats described in the art share some common downsides. First, the absence of specific tumor antigens. Indeed, cancer associated antigens which are usually targeted are often shared by tumors and non-malignant cells. Second, a major problem of multispecific antibody formats relying on the use of CD3 specific antibody fragments (e.g. single-chain variable fragment (scFv)) is the inherent ability of these fragments to activate T cells, irrespective of binding to the target antigen (e.g. the cancer associated antigen). Indeed, many side effects observed in context of CD3 based antibody therapy appear to be associated with errant T cell function (such as the associated production of cytokines which may lead to a toxic cytokine release syndrome).

A novel approach for a multispecific antibody format which overcomes the aforementioned shortcomings for antibody based therapeutics that co-engage CD3 and cancer associated antigens is described in WO2013/104804 [JULIUS-MAXIMILIANS-UNIVERSITÄT WÜRZBURG] and refers to "DUAL ANTIGEN-INDUCED BIPARTITE FUNCTIONAL COMPLEMENTATION". This approach or multispecific antibody format is referred herein as "CyCAT®" (Cytotoxic cell activation at tumour).

As part of the aforementioned approach, two polypeptides are designed, each composed of an targeting moiety (e.g. a single-chain variable fragment (scFv) or an antibody Fab fragment) fused to either the variable light chain domain (VL) or the complementary variable heavy chain (VH) domain of a T cell-activating anti-CD3 antibody. Such dissected or splitted Fv domain is referred herein as "Split Effector Domain" whereas the re-assembled Fv domain reflects the "Effector Domain".

The unpaired VH or VL domain (e.g. the split CD3 VH domain and the split CD3 VL domain) is not able to bind to CD3 alone. However, once the aforementioned two polypeptides bind to their target antigen on the surface of a cell via their targeting moiety, the complementary VL and VH domain of the CD3 specific antibody come in close proximity and interact with each other to reconstitute the original CD3 antibody (e.g. the CD3 specific antibody Fv domain). The thus on-target cell formed trispecific heterodimeric molecule engages and stimulates T cells for cancer cell destruction such as a conventional trivalent bi-or trispecific antibody formats.

One of the critical aspects in the aforementioned approach relies in the identification of a suitable CD3 specific antibody. Such antibody does not only has to address the requirements for a conventional bispecific antibody format in terms of species cross-reactivity, affinity, potency, safety (e.g. therapeutic window) and immunogenicity but also the specific requirements of the CyCAT format.

As described above, in the CyCAT approach the employed CD3 specific Fv domain must be splittable or dissectible. This feature implies several specific requirements for the CD3 antibody.

First, the VH and VL domain of the splitted Fv domain must be producible in the CyCAT format (e.g. as unpaired CyCAT polypeptides) with acceptable purity, acceptable yield as well as acceptable monomer content. This is in particular challenging as unpaired VH or VL domains tend to form strong aggregates after production. This aggregation tendency is i.a. dependent on the particular antibody sequence and appears to be more critical for polypeptides carrying the unpaired VH domain. Indeed, the inventors of the presently claimed new CD3 specific antibodies discovered that even only slight variations in the CDR sequences of antibody variants derived from one parental antibody reveal strong differences in producibility in the CyCAT format.

Secondly, the CD3 specific antibody (e.g. the Fv domain) must retain its functional activity in terms of activating T cells and/or redirecting T cell killing to cancer cells once the unpaired CD3 specific VH and VL domain present in the set of CyCAT polypeptides are paired.

Thirdly, the unpaired CD3 specific VH or VL domain or the respective unpaired CyCAT polypeptides comprising the same should not bind to CD3 alone.

Fourthly, the unpaired CD3 specific VH or VL domain or the respective unpaired CyCAT polypeptides comprising the same should not activate T-cells and/or mediate redirected T-cell killing alone.

And finally, the unpaired CD3 specific VH or VL domain or the respective unpaired CyCAT polypeptides comprising the same should not pair before the two polypeptides bind to their respective cancer associated antigens on a target cell. Such unwanted pre-pairing (or hetero-association) may occur in patient serum after separate administration of the two polypeptides (e.g. as part of two pharmaceutical compositions) or when both polypeptides are part of one pharmaceutical composition. Unwanted pre-pairing is thought to be i.a. concentration dependent as well as dependent of the affinity of the splitted VH and VL domains to each other.

A plethora of antibodies binding to CD3 are described in the art. However, the majority of these antibodies with intended therapeutic use are derived (e.g. humanized) from a small set of rodent anti-CD3 antibodies discovered in the early 1980s and 1990s. Of these, SP34 (Yoshino N. et al., Exp. Anim 49:97-110, 2000; Conrad ML. et al., Cytometry 71A:925-33, 2007) is probably the most used antibody for humanization approaches because it is one of the few CD3 specific antibodies with cross-reactivity to cynomolgus monkey CD3 (cynomolgus CD3 or cyno CD3) and as such can be used both for preclinical evaluation of safety, activity and/or pharmacokinetic profile in primates and - in the identical form - as drugs in humans. SP34 recognizes an N-terminal 1 – 27 amino acid residue polypeptide fragment of the extracellular domain of human CD3 epsilon. However, the inventors of the present disclosure have found that at least three prior-art antibodies of which two are derived from SP34 were not suited to be used in the discussed CyCAT format based on their unfavorable production properties and/or functional activity.

Interestingly, de novo in vitro generation of fully human antibodies binding to CD3 recognizing the N-terminal extracellular region of human CD3 epsilon and thus being cross-reactive to cynomolgus CD3 appears challenging and has been at least to the knowledge of the present inventors not reported so far.

Accordingly, the present disclosure provides novel antibodies and antibody fragments specific for CD3 epsilon which are superior to the CD3 specific antibodies reported in the art.

In particular, the antibodies or antibody fragments specific for CD3 epsilon according to the present disclosure are fully human antibodies which also cross-react with cynomolgus CD3 with favorable affinity and combine desirable producibility, functionality and safety properties never observed before.

These features makes the antibodies and antibody fragments of the present disclosure highly desirable for therapeutic use in multispecific antibody formants, in particular in the CyCAT format as described herein.

SUMMARY OF THE DISCLOSURE

Isolated human antibodies or antibody fragments as well as antibody variable domains specific for human CD3 epsilon and their respective CDRs according to the present disclosure as well as CyCAT polypeptides comprising the same are summarized in Tables 2 – 10 and Tables 17 - 18 of the present disclosure.

Preferred improved variable heavy chains (VH) and their HCDRs 1 – 3 according to the present disclosure are summarized in Table 4. Preferred improved variable light chains (VL) and their LCDRSs 1 – 3 according to the present disclosure are summarized in Table 5.

Preferred isolated human antibodies or antibody fragments specific for human CD3 epsilon according to the present disclosure are summarized in Table 10. Preferred combinations of improved variable heavy chains (VH) and improved variable light chains (VL) specific for human CD3 epsilon are summarized in Table 10. Most preferred isolated human antibodies or antibody fragments specific for human CD3 epsilon according to the present disclosure are summarized in Table 17. Most preferred combinations of improved variable heavy chains (VH) and improved variable light chains (VL) specific for human CD3 epsilon are summarized in Table 17.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising 6 CDRs disclosed in Table 2 and Table 3 of the present disclosure. In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising the 6 CDRs of an antibody disclosed in Table 2 or Table 3 of the present disclosure.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising the 3 HCDRs of an variable heavy chain (VH) disclosed in Table 4 and the 3 LCDRs of an antibody light chain (VL) disclosed in Table 5 of the present disclosure.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising the HCDRs of any one of the antibodies or variable domains disclosed in Table 4 and the LCDRs of any one of the antibodies or variable domains disclosed in Table 5 of the present disclosure.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising a combination of a variable heavy chain (VH) and a variable light chain (VL) as disclosed in Table 2, or Table 3 and Tables 8 – 10 of the present disclosure.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising a variable heavy chain (VH) and a variable light chain (VL) of any one of the antibodies disclosed in Table 2, 3 and Tables 8 – 10 of the present disclosure. In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising a variable heavy chain (VH) disclosed in Table 4 and a variable light chain (VL) disclosed in Table 5 of the present disclosure. In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising a combination of a variable heavy chain (VH) disclosed in Table 4 and a variable light chain (VL) disclosed in Table 5 of the present disclosure. In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon disclosed in Table 10 of the present disclosure. In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon disclosed in Table 17 of the present disclosure.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon comprising a combination of a variable heavy chain (VH) disclosed and a variable light chain (VL) disclosed in Table 10 of the present disclosure.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of GFTFX₁SX₂X₃MX₄, wherein X₁ is S, K or R; X₂ is Y or H; X₃ is W or Y; and X₄ is S or T (SEQ ID NO: 90);
- b) an HCDR2 region comprising the amino acid sequence of NIX₁X₂X₃X₄X₅X₆X₇YYX₈X₉SVKG, wherein X₁ is K or D; X₂ is Q or Y; X₃ is D, Q or E; X₄ is S or G; X₅ is S, Q or T; X₆ is E, H or R; X₇ is K, A or T; X₈ is V or A; and X₉ is D or E (SEQ ID NO: 91);
- c) an HCDR3 region comprising the amino acid sequence of GYSAEFAHRSGLDV (SEQ ID NO: 39);
- d) a LCDR1 region comprising the amino acid sequence of SGSSSNIGX₁X₂YVY, wherein X₁ is S, I, A, K or Q; and X₂ is N or T (SEQ ID NO: 92);

- e) a LCDR2 region comprising the amino acid sequence of RNX_1X_2RPS , wherein X_1 is N, K, S, H, T or Y; and X_2 is Q, I or K (SEQ ID NO: 93); and
- f) a LCDR3 region comprising the amino acid sequence of $AX_1WDX_2X_3X_4X_5GAV$, wherein X_1 is A or G; X_2 is H or R; X_3 is H or R; X_4 is R, S or L; and X_5 is S or H (SEQ ID NO: 94).

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of GFTFSSYWMS (SEQ ID NO: 40), GFTFKSYMS (SEQ ID NO: 41) or GFTFRSHYMT (SEQ ID NO: 42);
- b) an HCDR2 region comprising the amino acid sequence of NIKQDGSEKYYVDSVKG (SEQ ID NO: 43), NIDYQSQHAYYAESVKG (SEQ ID NO: 44) or NIDYEGTRTYAESVKG (SEQ ID NO: 45);
- c) an HCDR3 region comprising the amino acid sequence of GYSAEFAHRSGLDV (SEQ ID NO: 39);
- d) a LCDR1 region comprising the amino acid sequence of SGSSSNIGSNYVY (SEQ ID NO: 46), SGSSSNIGINYVY (SEQ ID NO: 47), SGSSSNIGANYVY (SEQ ID NO: 48), SGSSSNIGQTYVY (SEQ ID NO: 49), or SGSSSNIGKNYVY (SEQ ID NO: 50);
- e) a LCDR2 region comprising the amino acid sequence of RNNQRPS (SEQ ID NO: 51), RNNIRPS (SEQ ID NO: 52), RNNKRPS (SEQ ID NO: 53), RNKKRPS (SEQ ID NO: 54), RNKQRPS (SEQ ID NO: 55), RNSQRPS (SEQ ID NO: 56), RNHIRPS (SEQ ID NO: 57), RNTQRPS (SEQ ID NO: 58), or RNYQRPS (SEQ ID NO: 59); and
- f) a LCDR3 region comprising the amino acid sequence of AAWDHHRSGAV (SEQ ID NO: 60), AAWDRHSHGAV (SEQ ID NO: 61) or AGWDRRLHGAV (SEQ ID NO: 62).

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25,

SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable heavy chain (VH) and a variable light chain (VH) selected from the group consisting of:

- a) the VH comprising the amino acid sequence of SEQ ID NO: 13 and the VL comprising the amino acid sequence of SEQ ID NO: 16,
- b) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 16,
- c) the VH comprising the amino acid sequence of SEQ ID NO: 13 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- d) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- e) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- f) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 19,
- g) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 20,
- h) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 21,

- i) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 22,
- j) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 23,
- k) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 24,
- l) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 25,
- m) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 26,
- n) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 27,
- o) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 28,
- p) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 29,
- q) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 30,
- r) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 31,
- s) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 32,
- t) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 33,
- u) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 34,
- v) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 35,
- w) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 36,
- x) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 37,
- y) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 38,
- z) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 17,

- aa) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- bb) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 19,
- cc) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 20,
- dd) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 21,
- ee) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 22,
- ff) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 23,
- gg) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 24,
- hh) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 25,
- ii) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 26,
- jj) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 27,
- kk) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 28,
- ll) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 29,
- mm) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 30,
- nn) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 31,
- oo) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 32,
- pp) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 33,
- qq) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 34,
- rr) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 35,

- ss) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 36,
- tt) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 37, and
- uu) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 38.

In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure cross-reactively binds to cynomolgus CD3 epsilon. In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is cross-reactive to cynomolgus CD3 epsilon.

In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is a recombinant antibody or antibody fragment. In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is a monoclonal antibody or antibody fragment.

In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is an antibody fragment selected from the group consisting of Fab, Fv, and scFv. In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is a Fab fragment. In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is a single chain Fv (scFv) fragment. In an embodiment, the isolated antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is a Fv domain. In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is an IgG. In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is a full-length IgG. In an embodiment, said isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is a full-length IgG of an isotype selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.

In an embodiment, said isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is an IgG comprising an Fc region that has reduced effector function relative to that of a wild type Fc-receptor. "**Effector function**" as used in this context refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-

mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

In an embodiment, said isolated human antibody or antibody fragment specific for human CD3 epsilon, comprises a Fc region, wherein in at least 5 amino acids in the positions corresponding to positions L234, L235, D237, N330, P331 in a human IgG1 heavy chain, are mutated to A, E, A, S, and S, respectively. In an embodiment, said isolated human antibody or antibody fragment specific for human CD3 epsilon, comprises a Fc region, wherein in at least 5 amino acids in the positions corresponding to positions L234, L235, G237, N330, P331 in a human IgG1 heavy chain, are mutated to A, E, A, S, and S, respectively.

In an embodiment, the present disclosure provides a multispecific antibody comprising the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure. In an embodiment of the present disclosure, said multispecific antibody also specifically binds to a cancer associated antigen.

In an embodiment, the present disclosure provides a multispecific antibody comprising the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure and a second antibody or antibody fragment which binds to a different antigen than the antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure. In an embodiment, said second antibody fragment binds to a cell surface target antigen. In an embodiment, said cell surface target antigen is a tumor antigen. In an embodiment, said second antibody fragment binds to a cancer associated antigen on a cancer cell. In an embodiment, the present disclosure provides a multispecific antibody comprising a first antibody fragment of the human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure and a second antibody fragment which binds to a different antigen than said first antibody fragment. In an embodiment, said multispecific antibody comprises a third antibody fragment which binds to a different antigen than said first and second antibody fragment. In an embodiment, said multispecific antibody comprises a third antibody fragment which binds to the same antigen than said second antibody fragment. In an embodiment, said second and third antibody fragment binds to a cell surface target antigen. In an embodiment, said cell surface target antigen is a tumor antigen. In an embodiment, said second and third antibody fragment binds to a cancer associated antigen on a cancer cell.

In an embodiment, the present disclosure provides a nucleic acid composition comprising a nucleic acid sequence or a plurality of nucleic acid sequences encoding the human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure or the multispecific antibody according to the present disclosure. In an embodiment, the present disclosure provides a vector composition comprising a vector or a plurality of vectors

comprising the nucleic acid sequence or plurality of nucleic acid sequences according to the present disclosure. In an embodiment, the present disclosure provides a host cell comprising the vector composition according to the present disclosure. In an embodiment, said host cell is mammalian cell. In an embodiment, said host cell is prokaryotic cell. In another embodiment, the disclosure provides a method of producing an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure or the multispecific antibody according to the present disclosure for use as a medicament. In further embodiments, the present disclosure provides an isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure for use in the treatment of a subject in need thereof.

In an embodiment, the present disclosure also provides methods for treating patients suffering from a disease, such as an proliferative disease, by administering to said patient an effective amount of an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure. In an embodiment, the present disclosure provides a pharmaceutical composition comprising the human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure or the multispecific antibody according to the present disclosure and a pharmaceutically acceptable carrier or excipient.

In an embodiment, the present disclosure provides a method for re-directing cytotoxic activity of a T cell to a cancer cell comprising contacting said cancer cell in the presence of a T cell with the multispecific antibody according to the present disclosure.

There is utility in the claimed antibodies or antibody fragments. Furthermore, there is utility in the claimed method to generate such antibodies or antibody fragments. Utilization of the claimed antibodies or antibody fragments is to target T cells expressing CD3, and for stimulating T cell activation, e.g. under circumstances where T cell mediated killing is beneficial or desirable. In particular the claimed antibodies or antibody fragments are for therapeutic use, such as the treatment of cancer.

FIGURE LEGENDS

Figure 1: Cell binding of mammalian produced CD3 specific MAB-1 IgG. FIGURE 1A shows binding as a function of IgG concentration as determined on human derived PBMCs expressing human CD3 by flow cytometry. As negative control, an IgG against hen egg lysozyme was included. FIGURE 1B depicts the same as FIGURE 1A with the difference that binding on cynomolgus monkey derived PBMCs expressing cynomolgus CD3 is shown.

Figure 2: Schematic principle of the CyCAT format: A set of polypeptides is designed, each composed of an targeting moiety (e.g. antibody Fab fragment) fused to either the variable light chain domain (VL) or the complementary variable heavy chain (VH) domain of a T cell activating anti-CD3 antibody. The unpaired VH or VL domain are not able to bind to CD3. Once the aforementioned two polypeptides bind to their antigen on the surface of a cell via their targeting moiety, the complementary VL and VH domains come in close proximity and interact with each other to reconstitute the original CD3 antibody fragment (e.g. the CD3 specific Fv domain). The thus on-target cell formed trispecific heterodimeric molecule engages and stimulates T cells for tumor cell destruction such as a conventional trivalent bi- or trispecific antibody format.

DETAILED DESCRIPTION OF THE DISCLOSURE

Definitions

The term "**CD3**" refers to an antigen which is expressed on T cells as part of the multi-molecular T cell receptor (TCR) and which consists of a homodimer or heterodimer formed from the association of two of four receptor chains: CD3-epsilon, CD3-delta, CD3-zeta, and CD3-gamma.

Human CD3 epsilon including signal sequence (underlined) has the amino acid sequence of UniProt P07766:

MQSGTHWRVLGLCLLSVGWVGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSE
 ILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYL
 YLRARVCENCMEMDVMSVATIVIVDICITGLLLLVYYWSKNRKAKAKPVTRGAGAG
 GRQRGQNKERPPPVPNPDYEPYIRKGQRDLYSGLNQRRRI (SEQ ID NO: 1)

Cynomolgus CD3 epsilon including signal sequence (underlined) has the amino acid sequence of UniProt Q95LI5:

MQSGTRWRVLGLCLLSIGWVGQDGNEEMGSITQTPYQVSISGTTVILTCQHLGSE
 AQWQHNGKNKEDSGDRLFLPEFSEMEQSGYYVCYPRGSNPEDASHHLYLKARVC
 ENCMEMDVMAVATIVIVDICITLGLLLLLVYYWSKNRKAKAKPVTRGAGAGGRQRGQN
 KERPPPVPNPDYEPYIRKGQQDLYSGLNQRRRI (SEQ ID NO: 2)

The terms "**antigen**" or "target antigen" as used herein refers to any molecule of interest that can be bound by one of the binding sites present in an antigen binding molecule according to

the present disclosure. Typically, an antigen is a peptide, a protein or any other proteinaceous molecule. Alternatively, an antigen may be any other organic or inorganic molecule, such as carbohydrate, fatty acid, lipid, dye or fluorophore.

The term "**antigen binding molecule**" as used herein, refers in its broadest sense to a proteinaceous molecule that specifically binds to at least one antigen. An antigen binding molecule may be composed of one or more polypeptides. Examples of antigen binding molecules are immunoglobulins and derivatives and/or fragments thereof. Antigen binding molecules according to the present disclosure may be based on a regular immunoglobulin (e.g. IgG), in particular of half IgG molecules. The antigen binding molecule as disclosed herein are composed of at least a targeting moiety (such as an antibody Fab fragment) and an additional VH or VL domain of an antibody Fv domain wherein neither the VH or VL domain is able to bind to its antigen alone. Accordingly, an antigen binding molecule according to the present disclosure incorporates a half Fv domain ($\frac{1}{2}$ Fv domain) or a half binding site and a full binding site and thus can be also denoted as a $1 + \frac{1}{2}$ or $1 \frac{1}{2}$ antigen binding molecule.

The term "**antibody**" as used herein refers to a protein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds which interacts with an antigen. Each heavy chain is comprised of a heavy chain variable region or domain (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region or domain (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FR's arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The term "antibody" includes for example, monoclonal antibodies, human antibodies, humanized antibodies, camelised antibodies and chimeric antibodies. The antibodies can be of any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass. Both the light and heavy chains are divided into regions of structural and functional homology.

The term "**antibody fragment**", as used herein, refers to one or more portions of an antibody that retain the ability to specifically interact with (e.g., by binding, steric hindrance, stabilizing spatial distribution) an antigen. Examples of **antibody fragments** include, but are not limited

to, a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment (or Fv domain) consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment (or Fv domain), VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al., (1988) Science 242:423-426; and Huston et al., (1988) Proc. Natl. Acad. Sci. 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antibody fragment". These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Antibody fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, (2005) Nature Biotechnology 23:1126-1136). Antibody fragments can be grafted into scaffolds based on polypeptides such as Fibronectin type III (Fn3) (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies). Antibody fragments can be incorporated into single chain molecules comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen-binding sites (Zapata et al., (1995) Protein Eng. 8:1057-1062; and U.S. Pat. No. 5,641,870).

A "**human antibody**" or "**human antibody fragment**", as used herein, includes antibodies and antibody fragments having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such sequences. Human origin includes, e.g., human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik et al., (2000) J Mol Biol 296:57-86). Thereby said human antibody can be obtained from technology platforms which comprise antibodies derived from human germline genes either generated by PCR-amplification of VHA/L repertoire isolated from B-cells or are generated synthetically. Technology platforms include library based approaches comprising human immunoglobulin genes displayed on phage, ribosome or yeast. Respective display technologies are standard in the scientific community. Furthermore immunization of a transgenic mouse carrying human immunoglobulin repertoire is another approach to generate human antibodies against an antigen of interest. Antibodies or fragments thereof selected from an antibody library based on the MorphoSys HuCAL®

concept (Knappik et al., (2000) J Mol Biol 296:57-86) as well as the from the MorphoSys Ylanthia® library (Tiller et al. mAbs 5:3, 1–26; May/June (2013) and U.S. Patent No. 8,728,981) are considered as fully human.

A "**humanized antibody**" or "**humanized antibody fragment**" is defined herein as an antibody or antibody fragment which has constant antibody regions derived from sequences of human origin and the variable antibody regions or parts thereof or only the CDRs are derived from another species. For example a humanized antibody can be CDR-grafted, wherein the CDRs of the variable domain are from a non-human origin, while one or more frameworks of the variable domain are of human origin and the constant domain (if any) is of human origin.

The structures and locations of immunoglobulin variable domains, e.g., CDRs, may be defined using well known numbering schemes, e.g., the Kabat numbering scheme, the Chothia numbering scheme, or a combination of Kabat and Chothia (see, e.g., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services (1991), eds. Kabat et al.; Lazikani et al., (1997) J. Mol. Bio. 273:927-948); Kabat et al., (1991) Sequences of Proteins of Immunological Interest, 5th edit., NIH Publication no. 91-3242 U.S. Department of Health and Human Services; Chothia et al., (1987) J. Mol. Biol. 196:901-917; Chothia et al., (1989) Nature 342:877-883; and Al-Lazikani et al., (1997) J. Mol. Biol. 273:927-948.

The term "**isolated**" refers to a compound, which can be e.g. an antibody or antibody fragment, that is substantially free of other antibodies or antibody fragments having different antigenic specificities. Thus, in some aspects, antibodies provided are isolated antibodies which have been separated from antibodies with a different specificity. An isolated antibody may be a monoclonal antibody. An isolated antibody may be a recombinant monoclonal antibody. An isolated antibody that specifically binds to an epitope, isoform or variant of a target may, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., species homologs).

The term "**recombinant antibody**", as used herein, includes all antibodies that are prepared, expressed, created or segregated by means not existing in nature. For example antibodies isolated from a host cell transformed to express the antibody, antibodies selected and isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences or antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom. Preferably, such recombinant antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human

Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. A recombinant antibody may be a monoclonal antibody.

As used herein, the term "**monoclonal antibody**" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies as disclosed herein may be made by the hybridoma method as described in Kohler et al.; Nature, 256:495 (1975) or may be isolated from phage libraries using the techniques as described herein, for example. Other methods for the preparation of clonal cell lines and of monoclonal antibodies expressed thereby are well known in the art (see, for example, Chapter 11 in: Short Protocols in Molecular Biology, (2002) 5th Ed., Ausubel et al., eds., John Wiley and Sons, New York). Other exemplary methods of producing other monoclonal antibodies are provided in the Examples herein.

As used herein, an antibody "**binds specifically to**", "**specifically binds to**", is "**specific to/for**" or "**specifically recognizes**" an antigen if such antibody is able to discriminate between such antigen and one or more reference antigen(s), since binding specificity is not an absolute, but a relative property. For example, a standard ELISA assay can be carried out. The scoring may be carried out by standard color development (e.g. secondary antibody with horseradish peroxidase and tetramethyl benzidine with hydrogen peroxide). The reaction in certain wells is scored by the optical density, for example, at 450 nm. Typical background (=negative reaction) may be 0.1 OD; typical positive reaction may be 1 OD. This means the difference positive/negative can be more than 10-fold. Typically, determination of binding specificity is performed by using not a single reference antigen, but a set of about three to five unrelated antigens, such as milk powder, BSA, transferrin or the like.

As used herein, "**an antibody that binds to CD3**" or an "**anti-CD3 antibody**" or "**antibody specific for CD3**" includes antibodies and antibody fragments that specifically recognize one or more CD3 receptor chain (or subunits) (e.g., epsilon, delta, gamma or zeta), as well as antibodies and antibody fragments that specifically recognize a dimeric complex of two CD3 receptor chains (e.g., gamma/epsilon, delta/epsilon, and zeta/zeta CD3 dimers). The antibodies and antibody fragments of the present disclosure may bind soluble CD3 and/or cell surface expressed CD3. Soluble CD3 includes natural CD3 proteins as well as recombinant CD3 protein variants such as, e.g., monomeric and dimeric CD3 constructs, that lack a transmembrane domain or are otherwise unassociated with a cell membrane. Use of the term "**antibody that binds to CD3**" or an "**anti-CD3 antibody**" or a "**CD3 specific antibody**" herein is intended to include both, monospecific antibodies as well as bispecific or multispecific antibodies.

"Cell surface-expressed CD3" includes CD3 proteins contained within the context of a functional T cell receptor in the membrane of a cell. The expression **"cell surface-expressed CD3"** also includes CD3 protein expressed as part of a homodimer or heterodimer on the surface of a cell (e.g., gamma/epsilon, delta/epsilon, and zeta/zeta CD3 receptor chain dimers). The expression, **"cell surface-expressed CD3"** also includes a CD3 receptor chain (e.g., CD3-epsilon, CD3-delta or CD3-gamma or CD3-zeta) that is expressed by itself, without other CD3 chain types, on the surface of a cell. A **"cell surface-expressed CD3"** can comprise or consist of a CD3 protein expressed on the surface of a cell which normally expresses CD3 protein. Alternatively, **"cell surface-expressed CD3"** can comprise or consist of CD3 protein expressed on the surface of a cell that normally does not express human CD3 on its surface but has been artificially engineered to express CD3 on its surface.

The term **"cross-reactively binds"** or the term **"cross-reactive"** are used herein interchangeably and refers to an antibody which has the ability to specifically bind to more than one antigen. For example, in the present disclosure the antibody cross-reactively binds to cynomolgus CD3 or cynomolgus CD3 epsilon.

As used herein, the term **"affinity"** refers to the strength of interaction between the polypeptide and its target at a single site. Within each site, the binding region of the polypeptide interacts through weak non-covalent forces with its target at numerous sites; the more interactions, the stronger the affinity. The term **"K_D"**, as used herein, refers to the dissociation constant, which is obtained from the ratio of K_d to K_a (i.e. K_d/K_a) and is expressed as a molar concentration (M). K_D values for antigen binding moieties like e.g. monoclonal antibodies can be determined using methods well established in the art. Methods for determining the K_D of an antigen binding moiety like e.g. a monoclonal antibody are SET (soluble equilibrium titration) or surface plasmon resonance using a biosensor system such as a Biacore® system. In the present disclosure an antibody specific to the human CD3 epsilon polypeptide typically has a dissociation rate constant (K_D) (K_{off}/K_{on}) of less than 5x10⁻²M, less than 10⁻²M, less than 5x10⁻³M, less than 10⁻³M, less than 5x10⁻⁴M, less than 10⁻⁴M, less than 5x10⁻⁵M, less than 10⁻⁵M, less than 5x10⁻⁶M, less than 10⁻⁶M, less than 5x10⁻⁷M, less than 10⁻⁷M, less than 5x10⁻⁸M, less than 10⁻⁸M, less than 5x10⁻⁹M, less than 10⁻⁹M, less than 5x10⁻¹⁰M, less than 10⁻¹⁰M, less than 5x10⁻¹¹M, less than 10⁻¹¹M, less than 5x10⁻¹²M, less than 10⁻¹²M, less than 5x10⁻¹³M, less than 10⁻¹³M, less than 5x10⁻¹⁴M, less than 10⁻¹⁴M, less than 5x10⁻¹⁵M, or less than 10⁻¹⁵M or lower.

Compositions of the present disclosure may be used for therapeutic or prophylactic applications. The present disclosure, therefore, includes a **pharmaceutical composition** containing an antibody (or functional antibody fragment) as disclosed herein and a pharmaceutically acceptable carrier or excipient therefor. In a related aspect, the present disclosure provides a method for treating cancer. Such method contains the steps of

administering to a subject in need thereof an effective amount of the pharmaceutical composition that contains an antibody (or functional antibody fragment) as described herein.

The present disclosure provides therapeutic methods comprising the administration of a therapeutically effective amount of a human antibody or antibody fragment specific for CD3 as disclosed herein to a subject in need of such treatment. A "**therapeutically effective amount**" or "**effective amount**", as used herein, refers to the amount of an CD3 specific antibody necessary to elicit the desired biological response. In accordance with the disclosure, the therapeutic effective amount is the amount of a CD3 specific antibody or antibody fragment necessary to treat and/or prevent a disease.

The terms "**cell proliferative disease**" and "proliferative disease" refer to diseases that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disease is cancer. In one embodiment, the cell proliferative disease is a tumor.

The terms "**cancer**" and "**cancerous**" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth.

As used herein, "**delaying progression**" of a disease or disease means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease or disease (e.g., a cell proliferative disease, e.g., cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

The term "**IC₅₀**", as used herein, refers to the concentration of an inhibitor (e.g. an antibody or antibody fragment) that inhibits a response in an assay half way between the maximal response and the baseline. It represents the antibody concentration that reduces a given response by 50%.

The terms "**inhibition**" or "**inhibit**" or "**reduction**" or "**reduce**" or "**neutralization**" or "**neutralize**" refer to a decrease or cessation of any phenotypic characteristic (such as binding, a biological activity or function) or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. The "inhibition", "reduction" or "neutralization" needs not to be complete as long as it is detectable using an appropriate assay. In some embodiments, by "reduce" or "inhibit" is meant the ability to cause a decrease of 20% or greater. In another embodiment, by "reduce" or "inhibit" is meant the ability to cause a decrease of 50% or greater. In yet another embodiment, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater.

"Administered" or **"administration"** includes but is not limited to delivery of a drug by an injectable form, such as, for example, an intravenous, intramuscular, intradermal or subcutaneous route or mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestible solution, capsule or tablet. Preferably, the administration is by an injectable form.

As used herein, **"treatment"**, **"treat"** or **"treating"** and the like refers to clinical intervention in an attempt to alter the natural course of a disease in the subject being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies or antibody fragments according to the preset disclosure are used to delay development of a disease or to slow the progression of a disease.

The term **"multispecific"** means that an antibody is able to specifically bind to two or more different antigens. Typically, a multispecific antigen-binding molecule comprises of two or more antigen-binding sites, each of which is specific for a different antigen or epitope.

The term **"bispecific"** means that an antibody is able to specifically bind to two different antigens. Typically, a bispecific antigen-binding molecule comprises two antigen-binding sites, each of which is specific for a different antigen or epitope.

As used herein, the terms **"first"** and **"second"** with respect to a Fab and/or Fv region or the like are used for distinguishing when there is more than one of each type of component. Use of these terms is not intended to confer a specific order or orientation unless explicitly so stated.

As used herein, **"amino acid residues"** or **"amino acid"** will be indicated either by their full name or according to the standard three-letter or one-letter amino acid code. "Natural occurring amino acids" means the following amino acids:

Table 1: Natural occurring amino acids

Amino acid	Three letter code	One letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C

Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

The term "**polypeptide**" as used herein refer to a polymer of amino acid residues and does not refer to a specific length of a product. The term applies to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. Unless otherwise indicated, a particular amino acid sequence of a polypeptide also implicitly encompasses conservatively modified variants thereof (e.g. by replacing an amino acid residue with another amino acid residue having similar structural and/or chemical properties). A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including chemical synthesis. A polypeptide may include one or more disulfide bonds or be chemically modified.

The term "**domain**", as used herein, refers to a linear molecular chain of amino acids that includes the amino acid sequence of an entire polypeptide or a portion of a polypeptide

A "**functional domain**", as used herein in the context of a CyCAT molecule according to the present disclosure, is a domain that is capable of fulfilling a certain function, such as specific binding to a certain binding partner or antigen, specific activation of a certain receptor, mediation of toxic effects, or fluorescence upon excitation with light of an appropriate wavelength.

The term "**a fragment of a domain**" or "**a fragment of a functional domain**", as used herein in the context of a CyCAT molecule according to the present disclosure, refers to a linear molecular chain of amino acids that corresponds to a part of a domain, but not the entire domain or functional domain.

The term "**moiety**", as used herein in the context of a CyCAT molecule according to the present disclosure, refers to a linear molecular chain of amino acids that includes the amino acid sequence of an entire polypeptide or a portion of a polypeptide. A "**targeting moiety**" is used to refer to a moiety that specifically binds to an antigen. Non-limiting examples of targeting moieties are antibodies, antibody fragments, a natural ligand of an antigen, a mutein of such ligand or a mimetic of such ligand,

"**Not associated**" in the context of a CyCAT molecule according to present disclosure means not functionally associated with respect of the function of a functional domain, i.e. not allowing a first fragment of said functional domain and a second fragment of said functional domain to form said functional domain; or with respect of the function of the Fv domain, i.e. not allowing the VH and VL to form a functional Fv domain.

As used herein, "**non-covalent association**" refers to molecular interactions that do not involve an interatomic bond. Noncovalent interactions involve, for example, ionic bonds, hydrogen bonds, hydrophobic interactions, and van der Waals forces.

As used herein, "**covalent bond**" refers to an interatomic bond characterized by sharing of electrons.

Embodiments

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of GFTFX₁SX₂X₃MX₄, wherein X₁ is S, K or R; X₂ is Y or H; X₃ is W or Y; and X₄ is S or T (SEQ ID NO: 90);
- b) an HCDR2 region comprising the amino acid sequence of NIX₁X₂X₃X₄X₅X₆X₇YYX₈X₉SVKG, wherein X₁ is K or D; X₂ is Q or Y; X₃ is D, Q or E; X₄ is S or G; X₅ is S, Q or T; X₆ is E, H or R; X₇ is K, A or T; X₈ is V or A; and X₉ is D or E (SEQ ID NO: 91);
- c) an HCDR3 region comprising the amino acid sequence of GYSAEFAHRSGLDV (SEQ ID NO: 39);

- d) a LCDR1 region comprising the amino acid sequence of SGSSSNIGX₁X₂YVY, wherein X₁ is S, I, A, K or Q; and X₂ is N or T (SEQ ID NO: 92);
- e) a LCDR2 region comprising the amino acid sequence of RNX₁X₂RPS, wherein X₁ is N, K, S, H, T or Y; and X₂ is Q, I or K (SEQ ID NO: 93); and
- f) a LCDR3 region comprising the amino acid sequence of AX₁WDX₂X₃X₄X₅GAV, wherein X₁ is A or G; X₂ is H or R; X₃ is H or R; X₄ is R, S or L; and X₅ is S or H (SEQ ID NO: 94).

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of GFTFSSYWMS (SEQ ID NO: 40), GFTFKSYMS (SEQ ID NO: 41) or GFTFRSHYMT (SEQ ID NO: 42);
- b) an HCDR2 region comprising the amino acid sequence of NIKQDGSEKYYVDSVKG (SEQ ID NO: 43), NIDYQSQHAYYAESVKG (SEQ ID NO: 44) or NIDYEGTRTYAESVKG (SEQ ID NO: 45);
- c) an HCDR3 region comprising the amino acid sequence of GYSAEFAHRSGLDV (SEQ ID NO: 39);
- d) a LCDR1 region comprising the amino acid sequence of SGSSSNIGSNYVY (SEQ ID NO: 46), SGSSSNIGINYVY (SEQ ID NO: 47) SGSSSNIGANYVY (SEQ ID NO: 48), SGSSSNIGQTYVY (SEQ ID NO: 49), or SGSSSNIGKNYVY (SEQ ID NO: 50);
- e) a LCDR2 region comprising the amino acid sequence of RNNQRPS (SEQ ID NO: 51), RNNIRPS (SEQ ID NO: 52), RNNKRPS (SEQ ID NO: 53), RNKKRPS (SEQ ID NO: 54), RNKQRPS (SEQ ID NO: 55), RNSQRPS (SEQ ID NO: 56), RNHIRPS (SEQ ID NO: 57), RNTQRPS (SEQ ID NO: 58), or RNYQRPS (SEQ ID NO: 59); and
- f) a LCDR3 region comprising the amino acid sequence of AAWDHHRSGAV (SEQ ID NO: 60), AAWDRHSHGAV (SEQ ID NO: 61) or AGWDRRLHGAV (SEQ ID NO: 62).

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 40;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 43;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;

- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 46;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 60.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 46;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 60.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 40;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 43;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 46;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 46;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 52; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 53; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 54; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;

- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 55; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 49;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 56; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 57; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and

- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 55; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 58; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 50;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 59; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 46;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 49;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 58; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;

- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 53; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 57; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 55; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 58; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 49;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 58; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 41;

- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 44;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 52; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 46;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 52; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;

- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 53; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 54; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 55; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 49;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 56; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 57; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;

- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 55; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 58; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 50;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 59; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 46;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and

- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 49;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 58; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 61.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 53; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 57; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 51; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 48;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 55; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;

- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 58; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 49;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 58; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:

- a) an HCDR1 region comprising the amino acid sequence of SEQ ID NO: 42;
- b) an HCDR2 region comprising the amino acid sequence of SEQ ID NO: 45;
- c) an HCDR3 region comprising the amino acid sequence of SEQ ID NO: 39;
- d) a LCDR1 region comprising the amino acid sequence of SEQ ID NO: 47;
- e) a LCDR2 region comprising the amino acid sequence of SEQ ID NO: 52; and
- f) a LCDR3 region comprising the amino acid sequence of SEQ ID NO: 62.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 15.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment

comprises a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 14.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 15.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO:

29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 29.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable heavy chain (VH) and a variable light chain (VL) selected from the group consisting of:

- a) the VH comprising the amino acid sequence of SEQ ID NO: 13 and the VL comprising the amino acid sequence of SEQ ID NO: 16,
- b) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 16,
- c) the VH comprising the amino acid sequence of SEQ ID NO: 13 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- d) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- e) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- f) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 19,
- g) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 20,
- h) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 21,
- i) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 22,
- j) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 23,

- k) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 24,
- l) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 25,
- m) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 26,
- n) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 27,
- o) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 28,
- p) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 29,
- q) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 30,
- r) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 31,
- s) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 32,
- t) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 33,
- u) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 34,
- v) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 35,
- w) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 36,
- x) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 37,
- y) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 38,
- z) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- aa) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- bb) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 19,

- cc) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 20,
- dd) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 21,
- ee) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 22,
- ff) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 23,
- gg) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 24,
- hh) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 25,
- ii) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 26,
- jj) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 27,
- kk) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 28,
- ll) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 29,
- mm) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 30,
- nn) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 31,
- oo) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 32,
- pp) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 33,
- qq) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 34,
- rr) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 35,
- ss) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 36,
- tt) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 37, and

uu) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 38.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises a variable heavy chain (VH) and a variable light chain (VL) selected from the group consisting of:

- a) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- b) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- c) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- d) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 19,
- e) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 23,
- f) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 24,
- g) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 25,
- h) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 26,
- i) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 27,
- j) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 29,
- k) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- l) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 27, and
- m) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 29.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment

comprises a variable heavy chain (VH) and a variable light chain (VL) selected from the group consisting of:

- a) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- b) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- c) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- d) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 24,
- e) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 25, abd
- f) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 29.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 13 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 16.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 16.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 13 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 17.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 17.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 18.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 19.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 20.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 21.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 22.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 23.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 24.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 25.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 26.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment

comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 27.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 28.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 29.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 30.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 31.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 32.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 33.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 34.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 35.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 36.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 37.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 14 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 38.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 17.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 18.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 19.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 20.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 21.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment

comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 22.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 23.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 24.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 25.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 26.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 27.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 28.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 29.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 30.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 31.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 32.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 33.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 34.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 35.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 36.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 37.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises the variable heavy chain (VH) comprising an amino acid sequence of SEQ ID NO: 15 and the variable light chain (VL) comprising the amino acid sequence of SEQ ID NO: 38.

Specificity

In an embodiment, the present disclosure provides an isolated human antibody or and antibody fragment specific for CD3 according to the present disclosure. In an embodiment, the said isolated human antibody or and antibody fragment specific for CD3 is specific for human CD3. In an embodiment, the said isolated human antibody or and antibody fragment specific for CD3 is specific for cynomolgus CD3. In an embodiment, the said isolated human antibody or antibody fragment specific for CD3 is specific for human and cynomolgus CD3.

In an embodiment, the isolated human antibody or and antibody fragment specific for CD3 according to the present disclosure is specific for human CD3 epsilon. In an embodiment, the isolated human antibody or and antibody fragment according to the present disclosure specific for CD3 according to the present disclosure is specific for cynomolgus CD3 epsilon. In an embodiment, the isolated human antibody or and antibody fragment specific for CD3 according to the present disclosure is specific for human and cynomolgus CD3 epsilon. In an embodiment, the isolated human antibody or and antibody fragment according to the present disclosure is specific for human and cynomolgus CD3 epsilon.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3, wherein said antibody or antibody fragment cross-reactively binds to cynomolgus CD3. In an embodiment, the present disclosure provides an isolated human antibody or and antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment cross-reactively binds to cynomolgus CD3 epsilon.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for CD3, wherein said antibody or antibody fragment specifically binds to human CD3 epsilon. In an embodiment, said isolated human antibody or antibody fragment specific for CD3, specifically binds to human and cynomolgus CD3 epsilon.

In an embodiment, the present disclosure provides an isolated antibody or antibody fragment specific for human CD3 epsilon, wherein said human CD3 epsilon comprises the amino acid sequence SEQ ID NO: 1.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for cynomolgus CD3 epsilon, whereon said cynomolgus CD3 epsilon comprises the amino acid sequence SEQ ID NO: 2.

In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a human CD3 epsilon polypeptide comprising the amino acid sequence of SEQ ID NO: 1. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure

specifically binds to a human CD3 epsilon polypeptide comprising the amino acid sequence of SEQ ID NO: 3. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a human CD3 epsilon polypeptide comprising the amino acid sequence of SEQ ID NO: 5. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a human CD3 epsilon polypeptide comprising the amino acid sequence of SEQ ID NO: 7 or SEQ ID NO: 8. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a human CD3 epsilon polypeptide comprising any of the amino acid sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, and 8.

In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to the extracellular region human CD3 epsilon. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to the extracellular region human and cynomolgus CD3 epsilon.

In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a cynomolgus CD3 epsilon polypeptide comprising the amino acid sequence of SEQ ID NO: 2. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a cynomolgus CD3 epsilon polypeptide comprising the amino acid sequence of SEQ ID NO: 4. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a cynomolgus CD3 epsilon polypeptide comprising the amino acid sequence of SEQ ID NO: 6. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a cynomolgus CD3 epsilon polypeptide comprising the amino acid sequence of SEQ ID NO: 104. In an embodiment, the isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure specifically binds to a cynomolgus CD3 epsilon polypeptide comprising any of the amino acid sequence selected from the group consisting of SEQ ID NO: 2, 4, 6, and 104.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for a polypeptide encoded by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 104 or SEQ ID NO: 8.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for a polypeptide encoded by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3,

SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 104 and SEQ ID NO: 8.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment according to the present disclosure which specifically binds to a polypeptide encoded by SEQ ID NO: 1, 3, 5, 7, or 8 and to a polypeptide encoded by SEQ ID NO: 2, 4, 6, or 104.

In an embodiment, the isolated human antibody or antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is cross-reactive to cynomolgus CD3 epsilon. In an embodiment, the isolated human antibody or antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure cross-reactively binds to cynomolgus CD3 epsilon.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said antibody or antibody fragment has a monovalent affinity for a human CD3 epsilon peptide comprising SEQ ID NO: 7 or SEQ ID NO: 8 with a K_D of 10 nM or less, such as 8 nM or less, 7 nM or less, 6 nM or less, 5 nM or less, 4 nM or less, 3 nM or less, 2 nM or less, 1 nM or less, 0.1 nM or less, 0.2 nM, or 0.1 nM less.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said antibody or antibody fragment has a monovalent affinity for a human CD3 epsilon peptide comprising SEQ ID NO: 7 or SEQ ID NO: 8 with a K_D between 0.1 nM and 10 nM.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said antibody or antibody fragment has a monovalent affinity for a cynomolgus CD3 epsilon peptide comprising SEQ ID NO: 104 with a K_D of 10 nM or less, such as 8 nM or less, 7 nM or less, 6 nM or less, 5 nM or less, 4 nM or less, 3 nM or less, 2 nM or less, 1 nM or less, 0.1 nM or less, 0.2 nM, or 0.1 nM less.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said antibody or antibody fragment has a monovalent affinity for a cynomolgus CD3 epsilon peptide comprising SEQ ID NO: 104 with a K_D between 0.1 nM and 10 nM.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said antibody or antibody fragment has a monovalent affinity for a human CD3 epsilon peptide

comprising SEQ ID NO: 7 or SEQ ID NO: 8 and for a cynomolgus CD3 epsilon peptide comprising SEQ ID NO: 104 with a K_D between 0.1 nM and 10 nM.

In an embodiment, the present disclosure pertains to an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said antibody or antibody fragment has a monovalent affinity for a human CD3 epsilon peptide comprising SEQ ID NO: 8 with a K_D between 0.1 nM and 5 nM and for a cynomolgus CD3 epsilon peptide comprising SEQ ID NO: 104 with a K_D between 0.1 nM and 1 nM.

In certain embodiments, said monovalent affinity is determined in an antibody Fv or Fab format. In an embodiment, said monovalent affinity is determined as described herein in Example 10 or Example 13.

In alternative embodiments, the present disclosure pertains to an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said antibody or antibody fragment specifically binds to human and/or cynomolgus derived PBMCs with an EC_{50} concentration between 1 nM and 10 nM as determined in a FACS assay as described herein in Example 14. In an embodiment, said EC_{50} concentration is determined in the Fab format. In an embodiment, said antibody fragment is a Fab.

In an embodiment, said isolated human antibody or antibody fragment specific for human CD3 epsilon comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

Method of Treatment

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure for use as a medicament.

In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure for use in

stimulating T cell activation. In an embodiment, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure for use in enhancing immune function in a subject having a cell proliferative disease. In an embodiment, the present disclosure provides a method of treating or delaying the progression of a cell proliferative disease in a subject in need thereof, the method comprising administering to said subject an effective amount of an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure. In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure can be for use in treating or delaying progression of a cell proliferative disease in a subject in need thereof. In an embodiment, said cell proliferative disease is a cancer.

In an embodiment, the cancer is selected from the group consisting of esophageal cancer, stomach cancer, small intestine cancer, large intestine cancer, colorectal cancer, breast cancer, non-small cell lung cancer, non-Hodgkin's lymphoma (NHL), B cell lymphoma, B cell leukemia, multiple myeloma, renal cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, germinal-center B-cell-like (GCB) DLBCL, activated B-cell-like (ABC) DLBCL, follicular lymphoma (FL), mantle cell lymphoma (MCL), acute myeloid leukemia (AML), chronic lymphoid leukemia (CLL), marginal zone lymphoma (MZL), small lymphocytic leukemia (SLL), lymphoplasmacytic lymphoma (LL), Waldenstrom macroglobulinemia (WM), central nervous system lymphoma (CNSL), Burkitt's lymphoma (BL), B-cell prolymphocytic leukemia, Splenic marginal zone lymphoma, Hairy cell leukemia, Splenic lymphoma/leukemia, unclassifiable, Splenic diffuse red pulp small B-cell lymphoma, Hairy cell leukemia variant, Waldenstrom macroglobulinemia, Plasma cell myeloma, Solitary plasmacytoma of bone, Extravascular plasmacytoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), Nodal marginal zone lymphoma, Pediatric nodal marginal zone lymphoma, Pediatric follicular lymphoma, Primary cutaneous follicle centre lymphoma, T-cell/histiocyte rich large B-cell lymphoma, Primary DLBCL of the CNS, Primary cutaneous DLBCL, leg type, EBV-positive DLBCL of the elderly, DLBCL associated with chronic inflammation, Lymphomatoid granulomatosis, Primary mediastinal (thymic) large B-cell lymphoma, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, Primary effusion lymphoma: B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, and B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma.

In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure can be for use as a medicament. In an embodiment, the present disclosure refers to the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure for use in medicine. In an embodiment, the present disclosure refers to the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure for use in the preparation of a medicament. In an embodiment, the present disclosure provides the use of the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure for the manufacture of a medicament.

In an embodiment, the present disclosure provides the use of the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure for the manufacture of a medicament for treating or delaying progression of a cell proliferative disease. In an embodiment, the present disclosure provides the use of the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure for the manufacture of a medicament for enhancing immune function in a subject having a cell proliferative disease.

In an embodiment, the present disclosure provides a method of treating a subject in need thereof with the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure.

In embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure or a pharmaceutical composition comprising said antibody or antibody fragment is administered subcutaneously, intravenously, intramuscularly, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In an embodiment, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure or pharmaceutical composition according to the present disclosure is administered intravenously.

In an embodiment, said isolated human antibody or antibody fragment specific for human CD3 epsilon comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO:

29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

Safety

In certain embodiments, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure that does not activate T cells. In certain embodiments, the present disclosure provides an isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure that does not induce upregulation of CD69 expression on CD4 positive and/or CD8 positive T cells.

In an embodiment, said isolated human antibody or antibody fragment specific for human CD3 epsilon comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

Composition

In an embodiment, the present disclosure provides a pharmaceutical composition comprising the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure and a pharmaceutically acceptable carrier. Such carriers are well known in the art, and the skilled artisan will find a formulation and a route of administration best suited to treat a subject with the antibodies or antibody fragments according to the present disclosure.

In an embodiment, the present disclosure refers to the use of said pharmaceutical compositions comprising the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure in the preparation of a medicament for the treatment of a disease. In an embodiment the present disclosure refers to the use of said pharmaceutical composition for the treatment of a disease.

In an embodiment, the present disclosure provides a method of treating a cell proliferative disease in a subject, said method comprising administering a pharmaceutical composition

comprising a therapeutically effective amount of the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure.

In an embodiment, the present disclosure provides a pharmaceutical composition comprising a combination of the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure and a second therapeutic agent. In an embodiment, said second therapeutic agent is any agent that is advantageously combined with said human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure.

The present disclosure provides therapeutic methods for stimulating T cell activation using the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein the therapeutic methods comprise administering a therapeutically effective amount of an pharmaceutical composition comprising said isolated human antibody or antibody fragment specific for CD3 epsilon according to the present disclosure to a subject in need thereof.

The present disclosure also provides therapeutic methods for redirecting T cell killing to a cancerous cell using the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising said antibody or antibody fragment according to the present disclosure to a subject in need thereof.

In an embodiment, said isolated human antibody or antibody fragment specific for human CD3 epsilon comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

Multispecific antibodies

The human CD3 epsilon specific human antibody or antibody fragment according to the present disclosure is preferentially to be used in multispecific antibody formats to target T cells

expressing CD3 and to stimulate T cell activation, e.g., under circumstances where T cell mediated killing of specific cell types such as tumor cells is beneficial or desirable.

The isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or antibody fragment can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bispecific or multispecific antibody with an e.g. second or third binding specificity. Bispecific or multispecific antibodies, capable of binding to two or more antigens are of great interest for therapeutic applications, as they allow for the simultaneous binding and inactivation of two or more target antigens, and as such represent an alternative approach to conventional combination therapies.

Accordingly, in certain embodiments, the present disclosure provides an isolated human antibody or antibody fragments specific for human CD3 epsilon, wherein said antibody or antibody fragment is a monospecific, bispecific or multispecific antibody or antibody fragment.

A multispecific antibody may contain antibody fragments being specific for different epitopes on the same target antigen or may contain antibody fragments specific for more than one target antigen. In the context of bispecific or multispecific antibodies according to the present disclosure, the cell surface target antigen can be a cancer-associated antigen (CAA). Non-limiting examples of cancer-associated antigen include, e.g., an antigen that is expressed on the surface of a tumor or cancerous cell.

Exemplary multispecific antibody formats that can be used in the context of the present disclosure include, without limitation, e.g., scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (e.g., common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab₂ bispecific formats (see, e.g., Klein et al. 2012, mAbs 4:6, 1-11). Preferably, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is used in a format as described in WO 2020/115115. Most preferably, the isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure is used in the CyCAT format as described herein or in WO 2013/104804.

In an embodiment, the present disclosure provides a bispecific or multispecific antibody comprising a first antibody fragment specific for human CD3 epsilon and a second antibody fragment which binds a different antigen than said first antibody fragment, wherein said first antibody fragment specific for CD3 comprises any one of the antibodies or antibody fragments

specific for human CD3 epsilon according to the present disclosure. In an embodiment, said first antibody fragment specific for CD3 comprises any one of the antibodies or antibody fragments specific for human CD3 epsilon listed in Table 10.

In an embodiment, the present disclosure provides a multispecific antibody comprising a first antibody fragment specific for human CD3 epsilon and a second antibody fragment which binds a different antigen than said first antibody fragment, wherein said first antibody fragment specific for human CD3 epsilon comprises any one of the human antibodies or antibody fragments specific for human CD3 epsilon according to the present disclosure. In an embodiment, said first antibody fragment specific for CD3 comprises any one of the antibodies or antibody fragments specific for human CD3 epsilon listed in Table 10.

In an embodiment, said second antibody fragment specifically binds to a cell surface antigen. In an embodiment, said first antibody fragment binds to CD3 present on an immune effector cell. In an embodiment, the cell surface target antigen is a cancer-associated antigen. In an embodiment, said immune effector cell is a T cell. In an embodiment, said T cell is a cytotoxic T lymphocyte.

In an embodiment, the present disclosure provides a multispecific antibody comprising the human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein the multispecific antibody mediates redirected T cell killing of target antigen expressing cells. In certain embodiments, said target cell killing can be determined by the method as described herein in Example 11. In an embodiment, the multispecific antibody according to the present disclosure specifically binds a) CD3 expressed on a T cell and b) a second antigen present on a target cell other than the T-cell. In certain embodiments, said multispecific antibody activates the T cell following binding to (a) and (b). In some embodiments, the activated T cell is capable of exerting a cytotoxic effect and/or an apoptotic effect on a target cell.

In an embodiment, the present disclosure provides a multispecific antibody as disclosed herein, comprising the human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said multispecific antibody does not induce upregulation of CD69 expression on CD4 positive and CD8 positive T cells in the absence of a cell surface antigen expressing cell. In an embodiment, the present disclosure provides a multispecific antibody comprising the human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure, wherein said multispecific antibody induces human T cell proliferation in the presence of a cell surface target antigen expressing cell. In an embodiment, the present disclosure provides a multispecific antibody as disclosed herein comprising the human antibody or antibody fragment specific for human CD3 epsilon

according to the present disclosure, wherein said multispecific antibody does not activate T cells in the absence of cell surface antigen expressing cells.

In an embodiment, said human antibody or antibody fragment specific for human CD3 epsilon comprises

- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

CyCAT®

The isolated human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure may be used in the CyCAT approach as described herein or as described in WO 2013/104804 (which is incorporated herein in its entirety).

As parts of the aforementioned approach, two polypeptides are designed, each composed of an antibody fragment (e.g. a single-chain variable fragment (scFv) or an antibody Fab fragment) fused to either the variable light chain domain (VL) or the complementary variable heavy chain (VH) domain of a T cell activating anti-CD3 antibody. The unpaired VH or VL domain alone are not able to bind to CD3 and thus are not functional. However, once the two CyCAT polypeptides bind to their respective antigen on the surface of a cell, the complementary VL and VH domains come in close proximity and interact with each other to reconstitute the original CD3 antibody fragment (e.g. Fv domain) and become functional. The thus on-target cell formed trispecific heterodimer engages and stimulates T-cells for tumor cell destruction such as a conventional multispecific antibodies.

Accordingly, the present disclosure provides the CyCAT format comprising the human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure.

Thus, in an embodiment, the present disclosure provides a set of polypeptides comprising:

- a) a first polypeptide comprising
 - (i) a first targeting moiety, wherein said first targeting moiety specifically binds to a first antigen, and

(ii) a first fragment of a functional domain,

wherein neither said first fragment of the functional domain by itself nor said first polypeptide by itself is functional with respect to the function of said functional domain,

and

b) a second polypeptide comprising

(i) a second targeting moiety,

wherein said second targeting moiety specifically binds to a second antigen, and

(ii) a second fragment of said functional domain,

wherein neither said second fragment of the functional domain by itself nor said second polypeptide by itself is functional with respect to the function of said functional domain,

wherein said first polypeptide and said second polypeptide are not associated with each in the absence of a cell that has both, said first and second antigen at its cell surface,

wherein, upon dimerization of said first fragment of said first polypeptide with said second fragment of said second polypeptide, the resulting dimer is functional with respect to the function of said functional domain,

and wherein said first fragment of said functional domain comprises the VL of the human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure and said second fragment of said functional domain comprises the VH of the same human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure;

or wherein said first fragment of said functional domain comprises the VH of the human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure and said second fragment of said functional domain comprises the VL of the same human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure,

and wherein said functional domain is or comprises a Fv domain.

In an embodiment, said functional domain is a Fv domain.

In an embodiment, the cell carrying both, the first and second antigen at its cell surface induces dimerization of the first fragment of said functional domain of said first polypeptide with the second fragment of said functional domain of said second polypeptide, whereas a cell which

does not carry both, the first and second antigen at its cell surface does not induce dimerization of the first fragment of said functional domain of said first polypeptide with the second fragment of said functional domain of said second polypeptide.

In an alternative embodiment, the present disclosure provides a set of polypeptides comprising:

a) a first polypeptide comprising

- (i) a first targeting moiety, wherein said first targeting moiety specifically binds to a first antigen, and
- (ii) a VH or VL of a Fv domain,

wherein neither said VH or VL of said Fv domain by itself nor said first polypeptide by itself is functional with respect to the function of said Fv domain,

and

b) a second polypeptide comprising

- (i) a second targeting moiety, wherein said second targeting moiety specifically binds to a second antigen, and
- (ii) the complementary VH or VL of said Fv domain,

wherein neither said complementary VH or VL of said Fv domain by itself nor said second polypeptide by itself is functional with respect to the function of said Fv domain,

wherein said first polypeptide and said second polypeptide are not associated with each in the absence of a cell that has said first antigen and said second antigen at its cell surface,

wherein, upon dimerization of said VH of said first polypeptide with said VL of said second polypeptide, the resulting dimer is functional with respect to the function of said Fv domain, or

wherein, upon dimerization of said VL of said first polypeptide with said VH of said second polypeptide, the resulting dimer is functional with respect to the function of said Fv domain,

and wherein said VL comprises a VL of an human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure and said VH domain comprises the VH domain of the same human antibody or antibody fragment specific for human CD3 epsilon according to the present disclosure.

In an embodiment, the cell carrying both, the first and second antigen at its cell surface induces dimerization of the VH of said first polypeptide with the VL of said second polypeptide, whereas a cell which does not carry both, the first and second antigen at its cell surface does not induce dimerization of the VH of said first polypeptide with the VL of said second polypeptide or

wherein, the cell carrying both, the first and second antigen at its cell surface induces dimerization of the VL of said first polypeptide with the VH of said second polypeptide, whereas a cell which does not carry both, the first and second antigen at its cell surface does not induce dimerization of the VL of said first polypeptide with the VH of said second polypeptide.

In an embodiment, said Fv comprises an VH and VL selected from the group consisting of:

- a) the VH comprising an amino acid sequence of SEQ ID NO: 13 and the VL comprising an amino acid sequence of SEQ ID NO: 16,
- b) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 16,
- c) the VH comprising an amino acid sequence of SEQ ID NO: 13 and the VL comprising an amino acid sequence of SEQ ID NO: 17,
- d) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 17,
- e) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 18,
- f) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 19,
- g) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 20,
- h) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 21,
- i) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 22,
- j) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 23,
- k) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 24,
- l) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 25,
- m) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 26,

- n) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 27,
- o) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 28,
- p) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 29,
- q) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 30,
- r) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 31,
- s) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 32,
- t) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 33,
- u) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 34,
- v) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 35,
- w) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 36,
- x) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 37,
- y) the VH comprising an amino acid sequence of SEQ ID NO: 14 and the VL comprising an amino acid sequence of SEQ ID NO: 38,
- z) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 17,
- aa) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 18,
- bb) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 19,
- cc) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 20,
- dd) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 21,
- ee) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 22,

- ff) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 23,
- gg) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 24,
- hh) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 25,
- ii) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 26,
- jj) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 27,
- kk) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 28,
- ll) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 29,
- mm) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 30,
- nn) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 31,
- oo) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 32,
- pp) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 33,
- qq) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 34,
- rr) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 35,
- ss) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 36,
- tt) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 37, and
- uu) the VH comprising an amino acid sequence of SEQ ID NO: 15 and the VL comprising an amino acid sequence of SEQ ID NO: 38.

In an embodiment, said VH is selected from the group consisting of SEQ ID NO 13, SEQ ID NO 14 and SEQ ID NO 15. In an embodiment, said VL is selected from the group consisting of SEQ ID NO 16 to SEQ ID NO: SEQ ID NO: 38. In an embodiment, said VH is selected from the group consisting of SEQ ID NO 13, SEQ ID NO 14 and SEQ ID NO 15 and said VL is selected from the group consisting of SEQ ID NO 16 to SEQ ID NO: 38.

In an embodiment, said VH is selected from the group consisting of SEQ ID NO: 14 or SEQ ID NO: 15 and said VL is selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 29.

In an embodiment, said first antigen and/or said second antigen is an antigen expressed on the surface of cells of a tumor or on the surface of progenitor/precursor cells of a tumor. In an embodiment, said first antigen and/or said second antigen is a cancer associated antigen expressed on the surface of cells of a tumor or on the surface of progenitor/precursor cells of a tumor.

In an embodiment, said first antigen and said second antigen is the same antigen. In an embodiment, said first antigen and said antigen are different antigen. In an embodiment, the combination of the first antigen and the second antigen is only found on cancerous cells, and not on cells that are not cancerous. In an embodiment, the combination of the first antigen and the second antigen is specific for cancerous cells of a certain type of cancer.

In an embodiment, said first targeting moiety and/or said second targeting moiety comprises a scFv, a Fab, a F(ab')₂ fragment of an antibody or a VHH antibody.

In an embodiment, the set of polypeptides according to the present disclosure are for use in the treatment of a patient who is suffering from a cancer or for use in diagnosis in a patient who is suffering from a cancer.

In an embodiment, the present disclosure provides a pharmaceutical composition comprising the set of polypeptides according to the present disclosure, said pharmaceutical composition further comprises a pharmaceutically acceptable carrier. In an embodiment, the present disclosure provides a two pharmaceutical composition each of them comprising one of the two polypeptides of the set of polypeptides according to the present disclosure, said pharmaceutical compositions further comprises a pharmaceutically acceptable carrier.

In an embodiment, the present disclosure a kit comprising the set of polypeptides according to the present disclosure. The set of polypeptides according to the present disclosure may be used for the prevention and treatment of diseases, which are mediated by biological pathways in which a target antigen of interest is involved.

In an embodiment, the present disclosure provides a method for inducing lysis of a cancer cell, comprising contacting said cancer target cell expressing said first and second antigen in the presence of a cytotoxic T cell with set of polypeptides according to the present disclosure.

In an embodiment, the present disclosure provides a method for inhibition of proliferation of a cancer cell comprising contacting said cancer cell expressing said first and second antigen in

the presence of a cytotoxic T cell with an with set of polypeptides according to the present disclosure.

In an embodiment, the present disclosure provides a method for killing a cancer cell comprising contacting said cancer cell expressing said first and second antigen, in the presence of a cytotoxic T cell with a set of polypeptides according to the present disclosure.

In an embodiment, the present disclosure provides a method for inducing a cellular response in a cytotoxic T cell, comprising contacting said cytotoxic T cell in the presence of a cancer cell expressing said first and second antigen, with a set of polypeptides according to the present disclosure. In an embodiment, said cellular response is selected from the group consisting of: proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of activation markers.

In an embodiment, the present disclosure provides a method for inducing T cell proliferation in the presence of a cancer cell, comprising contacting said cancer cell expressing said first and second antigen, in the presence of a T-cell with set of polypeptides according to the present disclosure. In an embodiment, the present disclosure provides a method for stimulating a primary T cell response in the presence of cancer cells, comprising contacting said cancer cells expressing said first and second antigen, in the presence of said T cell with set of polypeptides according to the present disclosure. In an embodiment, the present disclosure provides a method for re-directing cytotoxic activity of a T cell to a cancer cell, comprising contacting said cancer cell expressing said first and second antigen, in the presence of said T cell with a set of polypeptides according to the present disclosure

In an embodiment, the present disclosure provides the use of a set of polypeptides according to the present disclosure for the treatment of cancer, comprising cancer cells expressing said first and second cancer associated antigen, in a subject, comprising:

- (a) selecting a subject who is afflicted with a cancer,
- (b) collecting one or more biological samples from the subject,
- (c) identifying the said first and second cancer associated antigen expressing cancer cells in the one or more samples; and
- (d) administering to the subject an effective amount of a set of polypeptides according to the present disclosure.

Further provided herein are combinations or sets of a first antigen binding molecule and a second antigen binding molecule, wherein each of the two antigen binding molecules is composed of a targeting moiety with specificity for a target antigen, fused to either an unpaired

VL or VH domain of an antibody Fv domain specific for human CD3 epsilon and wherein the two antigen binding molecules are not associated by a covalent bond.

In an embodiment, the present disclosure pertains to an antigen binding molecule consisting from its N-terminus to its C-terminus of

- a) a targeting moiety specific for a first antigen,
- b) a peptide linker, and
- c) either the VH domain of an antibody Fv domain specific for human CD3 epsilon selected from the group consisting of SEQ ID NO 13, SEQ ID NO 14 and SEQ ID NO 15 or the VL domain of the antibody Fv domain specific for human CD3 epsilon selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38,

wherein the targeting moiety is fused to the N-terminus of either the VH or the VL domain of the antibody Fv domain specific for human CD3 epsilon via the peptide linker.

In an embodiment, the targeting moiety is fused via the peptide linker to the N-terminus of the VH domain of the antibody Fv domain specific for human CD3 epsilon. In an embodiment, the targeting moiety is fused via the peptide linker to the N-terminus of the VL domain of the antibody Fv domain specific for human CD3 epsilon.

In an embodiment, the targeting moiety is an antibody or antibody fragment. In an embodiment, the targeting moiety is selected from the group consisting of a Fab, scFab, Fab', scFv, dsFv, and VHH. In an embodiment, the targeting moiety is a Fab.

In an embodiment of the present disclosure, the antigen binding molecule according to the present disclosure consists of two polypeptides, wherein

- a) the first polypeptide consists of the light chain of a Fab, and
- b) the second polypeptide consists from its N-terminus to its C-terminus
 - I. the heavy chain of a Fab
 - II. the peptide linker and

- III. either the VH domain of the antibody Fv domain specific for human CD3 epsilon selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO 15 or the VL domain of the antibody Fv domain specific for human CD3 epsilon selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.

In an embodiment, the present disclosure pertains to a set of antigen binding molecules consisting of

- a) a first antigen binding molecule consisting from its N-terminus to its C-terminus of
- i. a first targeting moiety specific for a first antigen,
 - ii. a first peptide linker and
 - iii. either the VH domain of an antibody Fv domain specific for human CD3 epsilon selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO 15 or the VL domain of the antibody Fv domain specific for human CD3 epsilon, selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38,

wherein the first targeting moiety is fused to the N-terminus of either the VH or VL domain of the antibody Fv domain specific for human CD3 epsilon of the first antigen binding molecule via the first peptide linker, and

- b) a second antigen binding molecule consisting from its N-terminus to its C-terminus of
- I. a second targeting moiety specific for a second antigen,
 - II. a second peptide linker, and

- III. either the VL domain of the antibody Fv domain specific for human CD3 epsilon selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38. if the first antigen binding molecule comprises the VH domain of an antibody Fv domain specific for human CD3 epsilon or the VH domain of the antibody Fv domain specific for human CD3 epsilon selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO 15, if the first antigen binding molecule comprises the VL domain of the antibody Fv domain specific for human CD3 epsilon,

wherein the second targeting moiety is fused to the N-terminus of either the VL or VH domain of the antibody Fv domain specific for human CD3 epsilon of the second antigen binding molecule via the second peptide linker.

In an embodiment, the first targeting moiety is fused via the first peptide linker to the N-terminus of the VH domain of the antibody Fv domain specific for human CD3 epsilon of the first antigen binding molecule. In an embodiment, the first targeting moiety is fused via the first peptide linker to the N-terminus of the VL domain of the antibody Fv domain specific for human CD3 epsilon of the first antigen binding molecule.

In an embodiment, the second targeting moiety is fused via the second peptide linker to the N-terminus of the VH domain of the antibody Fv domain specific for human CD3 epsilon of the second antigen binding molecule. In an embodiment, the second targeting moiety is fused via the second peptide linker to the N-terminus of the VL domain of the antibody Fv domain specific for human CD3 epsilon of the second antigen binding molecule.

In an embodiment, the targeting moiety is an antibody or antibody fragment. In an embodiment, the targeting moiety is selected from the group consisting of Fab, scFab, Fab', scFv, dsFv, and VHH. In an embodiment, the targeting moiety is a Fab.

In an embodiment, the peptide linker used in an antigen binding molecules according to the present disclosure has a length of 5 to 20 amino acids residues. In an embodiment, the peptide linker used in antigen binding molecules according to the present disclosure has a length of 5 amino acids residues. In an embodiment, the peptide linker comprises the amino acid sequence of: GQPSG (SEQ ID NO: 105). In an embodiment, the peptide linker consists of the amino acid sequence of: GQPSG (SEQ ID NO: 105).

In an embodiment of the present disclosure, in the set of antigen binding molecules according to the present disclosure, the first antigen binding molecule and the second antigen binding molecule are not linked by a covalent bond. In an embodiment, the first antigen binding molecule and the second antigen binding molecule according to the present disclosure are capable of forming a heteromeric molecule. In an embodiment, the amount of the heteromeric molecule formed under conditions in which cells expressing the first antigen and the second antigen are present is higher than that under conditions in which the cells are not present or in which cells expressing either the first or the second antigen are present.

In an embodiment of the present disclosure, the VH and VL domain of the antibody Fv domain specific for human CD3 epsilon are capable of non-covalently associating thereby forming the antibody Fv domain specific for human CD3 epsilon. In an embodiment, the non-covalent association of said VH and VL domain specific human CD3 epsilon results in the formation of the antibody Fv domain specific for human CD3 epsilon. In an embodiment, the non-covalent association of the VH and VL domain specific human CD3 epsilon dimerizes the first and second antigen binding molecule. In an embodiment, said non-covalently association dimerizes the first and second antigen binding molecule resulting in the formation of a trispecific heteromeric antibody. In an embodiment, said formation of a trispecific heteromeric antibody occurs on-cell. In an embodiment, said formation of a trispecific heteromeric antibody occurs in vivo. In an embodiment, said formation of a trispecific heteromeric antibody molecule occurs in vitro. In an embodiment of the present disclosure, the either VH or VL domain of the antibody Fv domain specific for human CD3 epsilon of the first antigen binding molecule and the complementary VH or VL domain specific for human CD3 epsilon of the second antigen binding molecule are capable of non-covalently associating thereby forming an antibody Fv domain specific for human CD3 epsilon. In an embodiment of the present disclosure, the non-covalent association of the VH and VL domain specific human CD3 epsilon preferentially occurs when cells expressing the first antigen and the second antigen are present. In an embodiment, said non-covalent association of the VH and VL domain specific human CD3 epsilon preferentially occurs when cells expressing the first antigen and the second antigen are present and when both antigen binding molecules are bound to their antigen on the cell.

In an embodiment, said on-cell formed trispecific heterodimeric antibody has monovalent binding for the first antigen, monovalent binding to the second antigen and monovalent binding to human CD3 epsilon. In an embodiment of the present disclosure, the on-cell formed trispecific heterodimeric antibody is capable of engaging and stimulating cytotoxic T-cells for destruction of the target cell.

In an embodiment of the present disclosure, the first antigen and the second antigen are present on the same cell. In an embodiment, said cell is a tumor cell. In an embodiment, the first antigen and the second antigen are tumor associated antigens.

A set of antigen binding molecules according to the present disclosure may be used for the prevention and treatment of diseases, which are mediated by biological pathways in which target antigens of interest are involved. This may be preferably achieved by recruiting cytotoxic immune cells, such as T-cells, to cells expressing the target antigens, preferably the CAAs. In an embodiment, the present disclosure provides a method for inducing lysis of a target cell, such as a tumor cell, comprising contacting said cell in the presence of a cytotoxic T-cell with a set of antigen binding molecule according to the present disclosure.

In an embodiment, the present disclosure provides a method for inhibition of signaling in a target cell, such as a tumor cell, comprising contacting said cell in the presence of a cytotoxic T-cell with a set of antigen binding molecule according to the present disclosure. In an embodiment, the present disclosure provides a method for inhibition of proliferation of a target cell, such as a tumor cell, comprising contacting said cell in the presence of a cytotoxic T-cell with a set of antigen binding molecules according to the present disclosure. In an embodiment, the present disclosure provides a method for inducing a cellular response in cytotoxic T-cells, comprising contacting said cytotoxic T-cell in the presence of a target cell, such as a tumor cell, with a set of antigen binding molecules according to the present disclosure. In an embodiment, said cellular response is selected from the group consisting of: proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of activation markers.

In an embodiment, the present disclosure provides a method for inducing human T-cell proliferation in the presence of a target cell, such as a tumor cell, comprising contacting said cell in the presence of a T-cell with a set of antigen binding molecules according to the present disclosure. In an embodiment, the present disclosure provides a method for stimulating a primary T-cell response in the presence of a target cell, such as a tumor cell, comprising contacting said cell in the presence of said T-cell with a set of antigen binding molecules according to the present disclosure. In an embodiment, the present disclosure provides a method for re-directing cytotoxic activity of a T-cell to a target cell, such as a tumor cell, comprising contacting said cancer cells in the presence of said T-cell with a set of antigen binding molecule according to the present disclosure.

In an embodiment, the present disclosure provides the use of a set of antigen binding molecules according to the present disclosure for the treatment of cancer that is positive for at least two cancer associated antigen (CAA) in a subject, comprising:

- (a) selecting a subject who is afflicted with a cancer,
- (b) collecting one or more biological samples from the subject,
- (c) identifying the at least two tumor associated antigens expressing cancer cells in the one or more samples, and
- (d) administering to the subject an effective amount of a set of antigen binding molecule according to the present disclosure.

In an embodiment, said cancer cell expresses a first and second CAA. In a preferred embodiment, said first and second CAA are different. In an embodiment, in the set of antigen binding molecules according to the present disclosure, the first antigen binding molecule binds to a first CAA and the second antigen binding molecule binds to a second CAA.

Sequences

Table 2: Antibody variable domains and CDR sequences of the parental CD3 specific antibody MAB-1 (CDRs are provided in combined Kabat + Chothia annotation)

Antibody	VH/VL/ CDR	SEQ ID NO:	[aa]
MAB-1 (parental)	VH	13	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKG LEWVANIKQDGSEKYYVDSVKGRFTISRDNKNSLYLQMNSLRAED TAVYYCARGYSAEFAHRSGLDVWGQGTLVTVSS
	VL	16	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYYVYQQLPGTAPK LLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAW DHHRSGAVFGGGTKLTVLGQ
	HCDR1	40	GFTFSSYWMS
	HCDR2	43	NIKQDGSEKYYVDSVKG
	HCDR3	39	GYSAEFAHRSGLDV
	LCDR1	46	SGSSSNIGSNYYVY
	LCDR2	51	RNNQRPS
	LCDR3	60	AAWDHHRSGAV

Table 3: Affinity matured variants with improved variable domains of MAB-1 (CDRs are provided in combined Kabat + Chothia annotation)

Antibody	VH/VL/ CDR	SEQ ID NO:	[aa]
MAB-1_25	VH	14	EVQLVESGGGLVQPGGSLRLSCAASGFTFKSYMSWVRQAPG KGLEWVANIDYQSQHAYYAESVKGRFTISRDNKNSLYLQMNSL RAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLVTVSS
	VL	16	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYYVYQQLPGTA PKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYY CAAWDHHRSGAVFGGGTKLTVLGQ
	HCDR1	41	GFTFKSYMS
	HCDR2	44	NIDYQSQHAYYAESVKG

Antibody	VH/VL/ CDR	SEQ ID NO:	[aa]
MAB-1_2	HCDR3	39	GYSAEFAHRSGLDV
	LCDR1	46	SGSSSNIGSNYYVY
	LCDR2	51	RNNQRPS
	LCDR3	60	AAWDHHRSGAV
	VH	13	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPG KGLEWVANIKQDGSEKYYYVDSVKGRFTISRDNKNSLYLQMN RAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLTVSS
	VL	17	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYYVYQQLPGTA PKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEADYY CAAWDRHSHGAVFGGGTKLTVLGQ
	HCDR1	40	GFTFSSYWMS
	HCDR2	43	NIKQDGSEKYYYVDSVKG
	HCDR3	39	GYSAEFAHRSGLDV
	LCDR1	46	SGSSSNIGSNYYVY
	LCDR2	51	RNNQRPS
	LCDR3	61	AAWDRHSHGAV

Table 4: Improved VH domains and CDR sequences of MAB-1 (CDRs are provided in combined Kabat + Chothia annotation)

Antibody	VH/ CDR	SEQ ID NO:	[aa]
MAB-1_1_VH (parental)	VH	13	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAP GKLEWVANIKQDGSEKYYYVDSVKGRFTISRDNKNSLYLQM NSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLTVSS
	HCDR1	40	GFTFSSYWMS
	HCDR2	43	NIKQDGSEKYYYVDSVKG
	HCDR3	39	GYSAEFAHRSGLDV
MAB-1_25_VH	VH	14	EVQLVESGGGLVQPGGSLRLSCAASGFTFKSYMSWVRQAP GKLEWVANIDYQSQAAYAESVKGRFTISRDNKNSLYLQM NSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLTVSS
	HCDR1	41	GFTFKSYMS
	HCDR2	44	NIDYQSQAAYAESVKG
	HCDR3	39	GYSAEFAHRSGLDV
MAB-1_24_VH	VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQAP GKLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLYLQMN SLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLTVSS
	HCDR1	42	GFTFRSHYMT
	HCDR2	45	NIDYEGTRTYAESVKG
	HCDR3	39	GYSAEFAHRSGLDV

Table 5: Improved VL domains and CDR sequences of MAB-1 (CDRs are provided in combined Kabat + Chothia annotation)

Antibody	VL/ CDR	SEQ ID NO:	[aa]
MAB-1_1_VL parental	VL	16	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYVYWYQQLPGT APKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDHHRSGAVFGGGTKLTVLGQ
	LCDR1	46	SGSSSNIGSNYVY
	LCDR2	51	RNNQRPS
	LCDR3	60	AAWDHHRSGAV
MAB-1_2_VL	VL	17	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYVYWYQQLPGT APKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	46	SGSSSNIGSNYVY
	LCDR2	51	RNNQRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_3_VL	VL	18	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYWYQQLPGT APKLLIYRNNIRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	52	RNNIRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_4_VL	VL	19	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYWYQQLPGT APKLLIYRNNKRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	53	RNNKRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_5_VL	VL	20	QSVLTQPPSASGTPGQRTISCSGSSSNIGANYVYWYQQLPGT APKLLIYRNKKRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	48	SGSSSNIGANYVY
	LCDR2	54	RNKKRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_6_VL	VL	21	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYWYQQLPGT APKLLIYRNKQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	55	RNKQRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_7_VL	VL	22	QSVLTQPPSASGTPGQRTISCSGSSSNIGQTYVYWYQQLPGT APKLLIYRNSQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	49	SGSSSNIGQTYVY
	LCDR2	56	RNSQRPS
	LCDR3	61	AAWDRHSHGAV

Antibody	VL/ CDR	SEQ ID NO:	[aa]
MAB-1_8_VL	VL	23	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLPGT APKLLIYRNHIRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	57	RNHIRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_9_VL	VL	24	QSVLTQPPSASGTPGQRTISCSGSSSNIGANYVYQQLPGT APKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	48	SGSSSNIGANYVY
	LCDR2	51	RNNQRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_10_VL	VL	25	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLPGT APKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	51	RNNQRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_11_VL	VL	26	QSVLTQPPSASGTPGQRTISCSGSSSNIGANYVYQQLPGT APKLLIYRNKQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	48	SGSSSNIGANYVY
	LCDR2	55	RNKQRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_12_VL	VL	27	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLPGT APKLLIYRNTQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	58	RNTQRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_13_VL	VL	28	QSVLTQPPSASGTPGQRTISCSGSSSNIGKNYVYQQLPGT APKLLIYRNYQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ
	LCDR1	50	SGSSSNIGKNYVY
	LCDR2	59	RNYQRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_14_VL	VL	29	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYVYQQLPGT APKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ
	LCDR1	46	SGSSSNIGSNYVY
	LCDR2	51	RNNQRPS
	LCDR3	62	AGWDRRLHGAV
MAB-1_15_VL	VL	30	QSVLTQPPSASGTPGQRTISCSGSSSNIGQTYVYQQLPGT APKLLIYRNTQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDRHSHGAVFGGGTKLTVLGQ

Antibody	VL/ CDR	SEQ ID NO:	[aa]
	LCDR1	49	SGSSSNIGQTYVY
	LCDR2	58	RNTQRPS
	LCDR3	61	AAWDRHSHGAV
MAB-1_16_VL	VL	31	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLPGT APKLLIYRNNKRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	53	RNNKRPS
	LCDR3	62	AGWDRRLHGAV
MAB-1_17_VL	VL	32	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLPGT APKLLIYRNHIRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	57	RNHIRPS
	LCDR3	62	AGWDRRLHGAV
MAB-1_18_VL	VL	33	QSVLTQPPSASGTPGQRTISCSGSSSNIGANYVYQQLPGT APKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ
	LCDR1	48	SGSSSNIGANYVY
	LCDR2	51	RNNQRPS
	LCDR3	62	AGWDRRLHGAV
MAB-1_19_VL	VL	34	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLPGT APKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	51	RNNQRPS
	LCDR3	62	AGWDRRLHGAV
MAB-1_20_VL	VL	35	QSVLTQPPSASGTPGQRTISCSGSSSNIGANYVYQQLPGT APKLLIYRNKQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ
	LCDR1	48	SGSSSNIGANYVY
	LCDR2	55	RNKQRPS
	LCDR3	62	AGWDRRLHGAV
MAB-1_21_VL	VL	36	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLPGT APKLLIYRNTQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	58	RNTQRPS
	LCDR3	62	AGWDRRLHGAV
MAB-1_22_VL	VL	37	QSVLTQPPSASGTPGQRTISCSGSSSNIGQTYVYQQLPGT APKLLIYRNTQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ

Antibody	VL/ CDR	SEQ ID NO:	[aa]
	LCDR1	49	SGSSSNIGQTYVY
	LCDR2	58	RNTQRPS
	LCDR3	62	AGWDRRLHGAV
MAB-1_23_VL	VL	38	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYVYQQLPGT APKLLIYRNNIRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAGWDRRLHGAVFGGGTKLTVLGQ
	LCDR1	47	SGSSSNIGINYVY
	LCDR2	52	RNNIRPS
	LCDR3	62	AGWDRRLHGAV

Table 6: Sequences of CyCAT polypeptides carrying the improved VL domains of MAB-1 (according Table 5)

CyCAT polypeptide [FabTrastuzumab-HC]- anti-CD3-VL]	SEQ ID NO:	[aa]
CyCAT_MAB-1_1_VL	63	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTISCSGS SSNIGSNYVYVYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDHHRSGAVFGGGTKLTV LGQ
CyCAT_MAB-1_2_VL	64	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTISCSGS SSNIGSNYVYVYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_3_VL	65	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTISCSGS SSNIGINYVYVYQQLPGTAPKLLIYRNNIRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_4_VL	66	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTISCSGS SSNIGINYVYVYQQLPGTAPKLLIYRNNKRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_5_VL	67	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS

CyCAT polypeptide [FabTrastuzumab-HC]- anti-CD3-VL]	SEQ ID NO:	[aa]
		LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGANYVYVYQQLPGTAPKLLIYRNKKRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_6_VL	68	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYVYQQLPGTAPKLLIYRNKQRPSPGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_7_VL	69	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGQTYVYVYQQLPGTAPKLLIYRNSQRPSPGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_8_VL	70	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYVYQQLPGTAPKLLIYRNHIRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_9_VL	71	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGANYVYVYQQLPGTAPKLLIYRNNQRPSPGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_10_VL	72	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYVYQQLPGTAPKLLIYRNNQRPSPGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_11_VL	73	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS

CyCAT polypeptide [FabTrastuzumab-HC]- anti-CD3-VL]	SEQ ID NO:	[aa]
		SSNIGANYVYWYQQLPGTAPKLLIYRNKQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_12_VL	74	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYWYQQLPGTAPKLLIYRNTQRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_13_VL	75	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGKNYVYWYQQLPGTAPKLLIYRNYQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_14_VL	76	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGSNYVYWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_15_VL	77	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGQTYVYWYQQLPGTAPKLLIYRNTQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_16_VL	78	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYWYQQLPGTAPKLLIYRNNKRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_17_VL	79	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYWYQQLPGTAPKLLIYRNHIRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTVL GQ

CyCAT polypeptide [FabTrastuzumab-HC]- anti-CD3-VL]	SEQ ID NO:	[aa]
CyCAT_MAB-1_18_VL	80	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGANYVYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_19_VL	81	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_20_VL	82	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGANYVYQQLPGTAPKLLIYRNKQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_21_VL	83	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYQQLPGTAPKLLIYRNTQRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTVL GQ
CyCAT_MAB-1_22_VL	84	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGQTYVYQQLPGTAPKLLIYRNTQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTV LGQ
CyCAT_MAB-1_23_VL	85	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCGQPSGQSVLTQPPSASGTPGQRTVISCSSGS SSNIGINYVYQQLPGTAPKLLIYRNNIRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAGWDRRLHGAVFGGGTKLTVL GQ
Fab-Light Chain (VL- CL) of Trastuzumab	86	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWFQQKPGK APKLLIYSASFLYSGVPSRFSGSRSGTDFLTISSLQPEDFATY YCQQHYTTPPTFGGQTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD

CyCAT polypeptide [FabTrastuzumab-HC]- anti-CD3-VL]	SEQ ID NO:	[aa]
		STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C

Table 7: Sequences of CyCAT polypeptides carrying improved VH domains of MAB-1 (according Table 4)

CyCAT polypeptide [Fab-Trastuzumab- HC]-anti-CD3-VL]	SEQ ID NO:	[aa]
CyCAT_MAB-1_1_VH	87	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQ APGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNT AYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PVPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCGQPSGEVQL VESGGGLVQPGGSLRLSCAASGFTFSSYWMWVRQAP GKGLEWVANIKQDGSEKYYVDSVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
CyCAT_MAB-1_24_VH	88	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQ APGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNT AYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PVPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCGQPSGEVQL VESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQAPG KGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLYLQ MNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
CyCAT_MAB-1_25_VH	89	QVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQ APGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNT AYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PVPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCGQPSGEVQL VESGGGLVQPGGSLRLSCAASGFTFKSYMSWVRQAPG KGLEWVANIDYQSQAAYAESVKGRFTISRDNKNSLYL QMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
Fab-Light Chain (VL- CL) of Trastuzumab	86	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWFQQK PGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFLT LTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC

Table 8: Combinations of improved CD3 specific VH and VL domains incorporated into CyCAT polypeptides (Fab light chain of Trastuzumab is not shown for CyCAT polypeptides)

CyCAT	Paired CyCAT polypeptides [Fab_Trastuzumab_HC]- Linker-[CD3-VL] x [Fab_Trastuzumab_HC]- Linker-[CD3-VH]	SEQ ID NO:	New anti-CD3 antibody		
			CD3 VL	SEQ ID NO:	anti-CD3
			CD3 VH		
CYCAT-7	CyCAT_MAB-1_2_VL	64	MAB-1_2_VL	17	MAB-7
	CyCAT_MAB-1_25_VH	89	MAB-1_25_VH	14	
CYCAT-8	CyCAT_MAB-1_2_VL	64	MAB-1_2_VL	17	MAB-8
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-9	CyCAT_MAB-1_3_VL	65	MAB-1_3_VL	18	MAB-9
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-10	CyCAT_MAB-1_4_VL	66	MAB-1_4_VL	19	MAB-10
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-11	CyCAT_MAB-1_8_VL	70	MAB-1_8_VL	23	MAB-11
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-12	CyCAT_MAB-1_9_VL	71	MAB-1_9_VL	24	MAB-12
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-13	CyCAT_MAB-1_10_VL	72	MAB-1_10_VL	25	MAB-13
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-14	CyCAT_MAB-1_11_VL	73	MAB-1_11_VL	26	MAB-14
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-15	CyCAT_MAB-1_12_VL	74	MAB-1_12_VL	27	MAB-15
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-16	CyCAT_MAB-1_14_VL	76	MAB-1_14_VL	29	MAB-16
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-17	CyCAT_MAB-1_3_VL	65	MAB-1_3_VL	18	MAB-17
	CyCAT_MAB-1_25_VH	89	MAB-1_25_VH	14	
CYCAT-18	CyCAT_MAB-1_12_VL	74	MAB-1_12_VL	27	MAB-18
	CyCAT_MAB-1_25_VH	89	MAB-1_25_VH	14	
CYCAT-19	CyCAT_MAB-1_14_VL	76	MAB-1_14_VL	29	MAB-19
	CyCAT_MAB-1_25_VH	89	MAB-1_25_VH	14	

Table 9: Additional combinations of further improved CD3 specific VH and VL domains domains incorporated into CyCAT polypeptides

CyCAT	Paired CyCAT polypeptides [Fab_Trastuzumab_HC]- Linker-[CD3-VL] x [Fab_Trastuzumab_HC]- Linker-[CD3-VH]	SEQ ID NO:	New anti-CD3 antibody		
			CD3_VL	SEQ ID NO:	anti-CD3
			CD3_VH		
CYCAT-20	CyCAT_MAB-1_16_VL	78	MAB-1_16_VL	31	MAB-20
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-21	CyCAT_MAB-1_17_VL	79	MAB-1_17_VL	32	MAB-21
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	

CyCAT	Paired CyCAT polypeptides [Fab_Trastuzumab_HC]- Linker-[CD3-VL] x [Fab_Trastuzumab_HC]- Linker-[CD3-VH]	SEQ ID NO:	New anti-CD3 antibody		
			CD3_VL	SEQ ID NO:	anti-CD3
			CD3_VH		
CYCAT-22	CyCAT_MAB-1_18_VL	80	MAB-1_18_VL	33	MAB-22
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-23	CyCAT_MAB-1_19_VL	81	MAB-1_19_VL	34	MAB-23
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-24	CyCAT_MAB-1_20_VL	82	MAB-1_20_VL	35	MAB-24
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-25	CyCAT_MAB-1_21_VL	83	MAB-1_21_VL	36	MAB-25
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-26	CyCAT_MAB-1_22_VL	84	MAB-1_22_VL	37	MAB-26
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	
CYCAT-27	CyCAT_MAB-1_23_VL	85	MAB-1_23_VL	38	MAB-27
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15	

Table 10: New improved human CD3 specific human antibodies according to the present invention

Anti-CD3 Antibody	VH / VL domains	SEQ ID NO:	[aa]
MAB-7	MAB-1_2_VL	17	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYYVYQQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB1_25_VH	14	EVQLVESGGGLVQPGGSLRLSCAASGFTFKSYMSWVRQAPGKGLEWVANIDYQSQHAYYAESVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLVTVSS
MAB-8	MAB-1_2_VL	17	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYYVYQQQLPGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQAPGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLVTVSS
MAB-9	MAB-1_3_VL	18	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYYVYQQQLPGTAPKLLIYRNNIRPSGVPDRFSGSKSGTSASLAISGLRSEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQAPGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLVTVSS
MAB-10	MAB-1_4_VL	19	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYYVYQQQLPGTAPKLLIYRNNKRPSGVPDRFSGSKSGTSASLAISGLRSEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQAPGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLVTVSS

Anti-CD3 Antibody	VH / VL domains	SEQ ID NO:	[aa]
MAB-11	MAB-1_8_VL	23	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLP GTAPKLLIYRNHIRPSGVPDRFSGSKSGTSASLAISGLRSED EADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNANKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLV TVSS
MAB-12	MAB-1_9_VL	24	QSVLTQPPSASGTPGQRTISCSGSSSNIGANYVYQQLP PGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRS EDEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNANKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLV TVSS
MAB-13	MAB-1_10_VL	25	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLP GTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSE DEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNANKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLV TVSS
MAB-14	MAB-1_11_VL	26	QSVLTQPPSASGTPGQRTISCSGSSSNIGANYVYQQLP PGTAPKLLIYRNKQRPSGVPDRFSGSKSGTSASLAISGLRS EDEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNANKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLV TVSS
MAB-15	MAB-1_12_VL	27	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLP GTAPKLLIYRNTQRPSGVPDRFSGSKSGTSASLAISGLRSE DEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNANKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLV TVSS
MAB-16	MAB-1_14_VL	29	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNYVYQQLP PGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRS EDEADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNANKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLV TVSS
MAB-17	MAB-1_3_VL	18	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLP GTAPKLLIYRNIRPSGVPDRFSGSKSGTSASLAISGLRSED EADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_25_VH	14	EVQLVESGGGLVQPGGSLRLSCAASGFTFKSYMSWVRQ APGKGLEWVANIDYQSQHAYYAESVKGRFTISRDNANKNSL YLQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLV TVSS
MAB-18	MAB-1_12_VL	27	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYQQLP GTAPKLLIYRNTQRPSGVPDRFSGSKSGTSASLAISGLRSE DEADYYCAAWDRHSHGAVFGGGTKLTVLGQ
	MAB-1_25_VH	14	EVQLVESGGGLVQPGGSLRLSCAASGFTFKSYMSWVRQ APGKGLEWVANIDYQSQHAYYAESVKGRFTISRDNANKNSL YLQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGTLLV TVSS

Anti-CD3 Antibody	VH / VL domains	SEQ ID NO:	[aa]
MAB-19	MAB-1_14_VL	29	QSVLTQPPSASGTPGQRTVISCSSSSNIGSNYVYWYQQL PGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRS EDEADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_25_VH	14	EVQLVESGGGLVQPGGSLRLSCAASGFTFKSYMSWVRQ APGKGLEWVANIDYQSQHAYYAESVKGRFTISRDNKNSL YLMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
MAB-20	MAB-1_16_VL	31	QSVLTQPPSASGTPGQRTVISCSSSSNIGINYVYWYQQLP GTAPKLLIYRNNKRPSGVPDRFSGSKSGTSASLAISGLRSE DEADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
MAB-21	MAB-1_17_VL	32	QSVLTQPPSASGTPGQRTVISCSSSSNIGINYVYWYQQLP GTAPKLLIYRNHIRPSGVPDRFSGSKSGTSASLAISGLRSE EADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
MAB-22	MAB-1_18_VL	33	QSVLTQPPSASGTPGQRTVISCSSSSNIGANYVYWYQQL PGTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRS EDEADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
MAB-23	MAB-1_19_VL	34	QSVLTQPPSASGTPGQRTVISCSSSSNIGINYVYWYQQLP GTAPKLLIYRNNQRPSGVPDRFSGSKSGTSASLAISGLRSE DEADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
MAB-24	MAB-1_20_VL	35	QSVLTQPPSASGTPGQRTVISCSSSSNIGANYVYWYQQL PGTAPKLLIYRNKQRPSGVPDRFSGSKSGTSASLAISGLRS EDEADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
MAB-25	MAB-1_21_VL	36	QSVLTQPPSASGTPGQRTVISCSSSSNIGINYVYWYQQLP GTAPKLLIYRNTQRPSGVPDRFSGSKSGTSASLAISGLRSE DEADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS
MAB-26	MAB-1_22_VL	37	QSVLTQPPSASGTPGQRTVISCSSSSNIGQTYVYWYQQL PGTAPKLLIYRNTQRPSGVPDRFSGSKSGTSASLAISGLRS EDEADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LTVSS

Anti-CD3 Antibody	VH / VL domains	SEQ ID NO:	[aa]
MAB-27	MAB-1_23_VL	38	QSVLTQPPSASGTPGQRTISCSGSSSNIGINYVYWYQQLP GTAPKLLIYRNNIRPSGVPDRFSGSKSGTSASLAISGLRSED EADYYCAGWDRRLHGAVFGGGTKLTVLGQ
	MAB-1_24_VH	15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHYMTWVRQ APGKGLEWVANIDYEGTRTYAESVKGRFTISRDNKNSLY LQMNSLRAEDTAVYYCARGYSAEFAHRSGLDVWGQGT LVTVSS

Working Examples

Example 1: Antigen generation and quality control

Amino acid sequences of human CD3 epsilon and cynomolgus monkey CD3 epsilon were retrieved from publicly available sources (e.g. Uniprot), verified and produced in-house or by external service providers.

Antigens

As antigens for the initial pannings and screenings and later binding studies, linear peptides covering the N-terminal extracellular region of human and cynomolgus CD3 epsilon as well as fusion proteins of human CD3 epsilon and human CD3 delta were used. Peptides were either chemically synthesized or recombinantly produced. Chemically synthesized peptides were synthesized with a biotin tag, RP-HPLC purified and delivered as lyophilized material. The lyophilized peptides were stored at -80°C.

Recombinant antigens

Cell lines

The HEK293-6E cell line was developed by the National Research Council of Canada (NRC). HEK293-6E cells were maintained in Freestyle F17 medium (Thermo Scientific) in a humidified CO₂-incubator at 37 °C and 6 % CO₂. HKB11 (Parental clone: US patent 6,136,599. J. Biomed. Sci. 2002; 9:631-638) is a human hybrid cell line resulting from a fusion of HEK293 human embryonic kidney and 2B8 Burkitt lymphoma cells. HKB11 #52 cells were maintained in MAC1.0 medium containing 1 % FCS in a humidified CO₂ incubator at 37 °C and 6 % CO₂.

Recombinant antigen production

HKB11#52 or HEK293-6E cells were transiently transfected one day post seeding with a commercially available transfection reagent according to the manufacturer's instructions. The cells were cultured for 3 days and the conditioned cell culture supernatant was harvested by centrifugation followed by sterile filtration (0.22 µm filter). Stably transfected HKB11#52 pools

were generated by transfection of cells followed by selection with 800 µg/mL G418 (Thermo Scientific). Expression of antigens from stable pools was done for 4 days post seeding.

CD3 FLAG-chLys-Avi-tagged antigens

DNA encoding the N-terminal extracellular region (ECD) of human or cynomolgus monkey CD3 epsilon or the fusion proteins of CD3 epsilon ECD with CD3 delta ECD including the natural leader sequence was cloned in frame with a C-terminal FLAG-chLys-Avi-tag into a pMAX expression vector, which is a modified expression vector based on pcDNA3.1 (Thermo Fisher). The antigens were purified by antibody affinity chromatography using self-made MOR003207 columns. After binding and washing the proteins were eluted with 0.1 M Glycine, pH 3.0. All affinity chromatography steps were performed using ÄKTA Express (GE Healthcare) chromatography systems. The samples were subsequently neutralized (with 3 M Tris pH 8), buffer-exchanged to PBS using PD10 columns (GE Healthcare) and sterile filtered (0.2 µm pore size).

CD3 Fc-tagged antigens

DNA encoding the N-terminal sequence of human or cynomolgus monkey CD3 epsilon including the natural leader sequence was cloned in frame with a C-terminal human IgG Fc-tag into a pMAX expression vector, which is a modified expression vector based on pcDNA3.1 (Thermo Fisher). The antigens were purified by Protein A chromatography using HiTrap MabSelect SuRe columns (GE Healthcare). After binding and washing the proteins were eluted with 100 mM Glycine pH 3.0. All affinity chromatography steps were performed using ÄKTA Express (GE Healthcare) chromatography systems. The samples were subsequently neutralized (with 3 M Tris pH 8), buffer-exchanged to PBS using PD10 columns (GE Healthcare) and sterile filtered (0.2 µm pore size). Biotinylation of CD3 antigens was performed by biotinylation using the BirA Kit (Avidity) followed by a preparative SEC using a Superdex 200 column (GE Healthcare). Protein concentrations were determined by UV-spectrophotometry. The quality of the samples was analyzed by denaturing, reducing or non-reducing SDS-PAGE, Streptavidin-Shift Assay, HP-SEC and DLS.

Table 11: Sequences of produced or synthesized CD3 antigens (without signal sequences).

CD3 antigens	SEQ ID NO:	[aa]
Mature human CD3e (22-41)-bio (synthetic peptide)	3	Pyr-DGNEEMGGITQTPYKVSIS-Biotin
Mature cynomolgus CD3e(22-41)-bio (synthetic peptide)	4	Pyr-DGNEEMGSITQTPYQVSIS-Biotin
Mature human CD3e(1-49) ₂ Fc2(K105-K330)	5	QDGNEEMGGITQTPYKVSISGTTVILTCDIKHTCPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV

CD3 antigens	SEQ ID NO:	[aa]
		LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
Mature cyno CD3e(1-49)_Fc2(K105-K330)	6	QDGNEEMGSITQTPYQVSISGTTVILTCDIKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
Mature human CD3e(1-118)_FLAG-chLys_Avi	7	QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVDIDYKDDDDKIEGRMDKVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCACKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCRLVNSRGLNDIFEAQKIEWHE
Mature human CD3e(1-118)_FLAG-chLys_Avi-bio	8	QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVDIDYKDDDDKIEGRMDKVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCACKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCRLVNSRGLNDIFEAQKIEWHE-BIOTIN
Mature human CD3e(1-118)-GS-PAS-CD3d(22-92)_FLAG-chLys_Avi-bio	9	QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVGGGGSGGGGSGGGGASPAAFKIPIEELEDVRFVNCNTSITWVEGTGVTLLSDITRLDLGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMDIDYKDDDDKIEGRMDKVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCACKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCRLVNSRGLNDIFEAQKIEWHE-BIOTIN
Mature cyno CD3e(1-109)-GS-PAS-CD3d(22-92)_FLAG_chLys_Avi	10	QDGNEEMGSITQTPYQVSISGTTVILTCSQHLGSEAQWQHNGKNKEDSGDRLFLPEFSEMEQSGYYVCYPRGSNPEDASHHLYLKARVIDYKGGGGSGGGGSGGGGASPAAFKIPVEELEDVRFVNCNTSVTWVEGTGVTLLTNTRLDLGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMDIDYKDDDDKIEGRMDKVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCACKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCRLVNSRGLNDIFEAQKIEWHE
Mature cyno CD3e(1-109)-GS-PAS-CD3d(22-92)_FLAG_chLys_Avi-bio	11	QDGNEEMGSITQTPYQVSISGTTVILTCSQHLGSEAQWQHNGKNKEDSGDRLFLPEFSEMEQSGYYVCYPRGSNPEDASHHLYLKARVIDYKGGGGSGGGGSGGGGASPAAFKIPVEELEDVRFVNCNTSVTWVEGTGVTLLTNTRLDLGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMDIDYKDDDDKIEGRMDKVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCACKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCRLVNSRGLNDIFEAQKIEWHE-BIOTIN

CD3 antigens	SEQ ID NO:	[aa]
Mature cyno CD3e(1-108)_FLAG-chLys_Avi	104	QDGNEEMGSITQTPYQVSISGTTVILTCSQHLGSEAQW QHNGKNKEDSGDRLFLPEFSEMEQSGYYVCYPRGSN PEDASHHLYLKARVVIDYKDDDDKIEGRMDKVFGRCCL AAAMKRHGLDNRYRGYSLGNWVCAAKFESNFNTQATN RNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCS ALLSSDITASVNC AKKIVSDGNGMNAWVAWRNRCKGT DVQAWIRGCRLVNSRGLNDIFEAQKIEWHE

Example 2: Generation of fully human antibodies binding to human and cynomolgus CD3 epsilon from the MorphoSys Ylanthia® library

For antibody generation, the MorphoSys Ylanthia® library was used to select Fab fragments against human and cynomolgus CD3. The MorphoSys Ylanthia® library (Tiller et al. mAbs 5:3, 1–26; May/June (2013) and U.S. Patent No. 8,728,981) is a commercially available phagemid library and employs the CysDisplay® technology for displaying the Fab on the phage surface (Lohning et al., WO2001/05950).

To identify human/cynomolgus CD3 cross-reactive antibodies various panning strategies were performed. Thereby different synthetic and recombinant human and cynomolgus CD3 epsilon antigens representing the N-terminal extracellular region of CD3 epsilon were used. This region aligns to the binding region recognized by the human / cynomolgus CD3 epsilon cross-reactive prior-art antibody SP34. To identify CD3 specific antibodies, solution and semi-solution based panning strategies were performed using human and cynomolgus CD3 antigen material as indicated above to select species cross-reactive antibodies. Each conducted panning comprised of at least 3 individual rounds of selection.

The identification of human / cynomolgus CD3 cross-reactive antibodies turned out to be very challenging, with the parental antibody MAB-1 being the only clone revealing specific binding to recombinant human and cynomolgus CD3 epsilon as well as cell binding to human and cynomolgus PBMCs (see Figure 2). Surprisingly, MAB-1 was found suited to be used in the multispecific CyCAT format described herein.

MAB-1 was subjected to HCDR1+2 affinity maturation of the variable heavy chain (VH) and LCDR3 affinity maturation of the variable light chain (VL) using soluble CD3 epsilon antigens as described above and/or Jurkat cells with endogenous expression of human CD3 to further increase affinity and specificity for human and cynomolgus CD3 and to improve functional activity.

Accordingly, several hundred clones were screened for affinity. Functionality was rigorously tested in *in vitro* assays comprising e.g.

- Binding to human T-cells and Jurkat cells with endogenous expression of CD3 and J.RT-T3.5 cells without CD3 expression in a HER2 x CD3 bispecific antibody construct.
- Functional NFAT Reporter Gene Assay in a HER2 x CD3 bispecific antibody constructs
- Functional cytotoxicity assay as HER2 x CD3 bispecific constructs

Out of these studies, 7 most promising affinity matured variants of MAB-1 were further characterized for their suitability in the CyCAT format.

Example 3: Identification of improved fully human CD3 specific antibodies suited for the CyCAT format

In order to determine the suitability of the newly identified affinity matured CD3 specific human antibodies to be used in the CyCAT format, VH and VL domains of parental MAB-1, or of its affinity matured variants, as well as certain CD3-specific reference prior art antibodies were cloned into the set of polypeptides of the CyCAT format.

As exemplified herein, each polypeptide in the set of two polypeptides of the CyCAT format is composed of an antibody Fab fragment with specificity for the cancer target HER2 and either the VL or the VH domain of an anti-CD3 antibody of interest. The fusion between the Fab portion and the VL or VH domain is achieved by short peptide linkers between the C-terminus of the Fab heavy chain and the N-terminus of the CD3 specific VH or VL domain, respectively.

For HER2 binding, nucleotide sequences encoding the VH and VL domains from "Trastuzumab" (HERCEPTIN®) as described by Baselga et al. 1998, Cancer Res 58(13): 2825-2831) were used. Trastuzumab and its method of preparation are described in US 5,821,337.

A summary of the protein sequences of the produced CyCAT polypeptides carrying the improved CD3 specific VH or VL domains made in accordance with the examples described herein are set forth in Tables: 6 – 7.

Gene Synthesis

All nucleic acid sequences or desired gene segments either were generated by PCR using appropriate templates or were gene synthesized as linear DNA fragments with appropriate flanking regions (e.g. suitable restriction enzyme recognition sites, linker sequences) in-house or by an external provider. The nucleic acid sequences or gene segments flanked by singular restriction endonuclease cleavage sites were cloned into respective mammalian expression vectors using standard molecular biology methods. When intended for use in mammalian expression vectors, all constructs were designed with a 5'-end DNA sequence coding for a

leader peptide which targets proteins for secretion in eukaryotic cells. The DNA sequence of the subcloned gene fragments was confirmed by DNA by double strand sequencing

Example 4: Recombinant production of CyCAT polypeptides

For expression of CyCAT polypeptides, exponentially growing eukaryotic HEK293-6E cells were transfected with a mammalian expression vector encoding all components of a CyCAT polypeptide, resulting in a 1:1 heterodimer of the polypeptide comprising the Fab heavy chain of Trastuzumab fused to the VH or VL domain of the CD3 antibody of interest and the Fab light chain of Trastuzumab, respectively.

Cell culture supernatants were harvested on day 6 post transfection and subjected to anti-CH1 affinity chromatography (Capture Select IgG-CH1 or CH1-XL | ThermoFisher Scientific). Buffer exchange was performed to 1x Dulbecco's PBS (pH 7.2 | Invitrogen) and samples were sterile filtered (0.2 µm pore size). Protein concentrations were determined by UV-spectrophotometry and purities of the constructs were analyzed under denaturing, reducing and non-reducing conditions using CE-SDS (LabChip GX Touch | Perkin Elmer | USA). UHP-SEC was performed to analyze individual unpaired CyCAT polypeptide preparations in native state.

Results:

VH and VL domains of 3 CD3 specific prior art antibodies (Ref-MAB-1, Ref-MAB-2, Ref-MAB-3) were produced as CyCAT polypeptides. Ref-MAB-2, Ref-MAB-3 are prior-art antibodies based on SP34 and are cross-reactive to cynomolgus CD3. Ref-MAB-2 was first described in WO2008/119567, Ref-MAB-2 was first described in WO2019/034580. Ref-MAB-1 binds a distinct epitope in CD3 and is not cross-reactive to cynomolgus CD3.

In addition, the VH and VL domain of the newly identified parental fully human antibody MAB-1 as well as 7 affinity matured variable domains of MAB-1 were produced as CyCAT polypeptides.

Results of the quality control of the mammalian produced CyCAT polypeptides are summarized in Table 12. This data reveal a strong CD3 specific antibody variable domain dependent producibility of the CyCAT polypeptides. In general, polypeptides carrying an unpaired CD3 specific VH domain appeared prone for aggregation when compared to polypeptides carrying an (unpaired) CD3 specific VL domain. .

Overall, CyCAT polypeptides carrying the CD3 specific VH domains of the prior art reference antibodies (Ref-MAB-1, Ref-MAB-2, Ref-MAB-3) revealed poor producibility in terms of

monomer content and purity. Surprisingly, both variable domains of the newly identified parental CD3 specific human antibody MAB-1 could be produced as unpaired CyCAT polypeptides with acceptable yield (data not shown), monomer content and purity (see Table 12). However, two produced polypeptides each carrying an affinity matured VH domain of MAB-1 (VH of MAB-2 and VH of MAB-3) failed in production, but 5 polypeptides carrying other affinity matured VH domains of MAB-1 (VH of MAB-4, MAB-5, MAB-6, and MAB-1_25) or the affinity matured VL domain of MAB-1 (VL of MAB-1_2) could be produced in an acceptable manner.

These results indicate the difficulty in finding suitable pairs of CD3 specific variable domains which can be actually produced in the CyCAT format.

Table 12: Summary quality control after production: Monomer content and impurities of produced CyCAT polypeptides

Antibody	Source	CyCAT polypeptides [Fab_Trastuzumab- CD3-VL] or [Fab_Trastuzumab- CD3-VH]	Un- paired CD3 VH or VL	SEC Monomer Content [%]	SEC High molecular weight impurities [%]	SEC Low molecular weight impurities [%]
Ref-MAB-1	Prior art	CyCAT_Ref-MAB-1_VL	VL	89,3	10,6	0,2
		CyCAT_Ref-MAB-1_VH	VH	78,9	10,4	10,7
Ref-MAB-2	Prior art	CyCAT_Ref-MAB-2_VL	VL	87,9	12,1	0,0
		CyCAT_Ref-MAB-2_VH	VH	81,9	11,2	7,0
Ref-MAB-3	Prior art	CyCAT_Ref-MAB-3_VL	VL	92,4	4,7	3,1
		CyCAT_Ref-MAB-3_VH	VH	88,0	11,4	0,6
MAB-1	parental	CyCAT_MAB-1_VL	VL	95,1	4,4	0,4
		CyCAT_MAB-1_VH	VH	82,9	16,4	0,7
MAB-2	LCDR3 maturation	CyCAT_MAB-2_VL	VL	81,6	10,7	10,7
	HCDR1/2 maturation	CyCAT_MAB-2_VH	VH	<i>nep</i>	<i>nep</i>	<i>nep</i>
MAB-3*	HCDR1/2 maturation	CyCAT_MAB-3_VH	VH	<i>nep</i>	<i>nep</i>	<i>nep</i>
MAB-4*	HCDR1/2 maturation	CyCAT_MAB-4_VH	VH	85,9	9,1	5,1
MAB-5*	HCDR1/2 maturation	CyCAT_MAB-5_VH	VH	80,7	10,3	9,1
MAB-1_25*	HCDR1/2 maturation	CyCAT_MAB-1_25_VH	VH	78,6	19,1	2,3
MAB-6*	HCDR1/2 maturation	CyCAT_MAB-6_VH	VH	78,1	15,2	6,8
MAB-1_2**	LCDR3 maturation	CyCAT_MAB-1_2_VL	VL	96,3	3,3	0,4

* VH affinity matured descendants share the same VL of parental antibody MAB-1

** VL affinity matured descendant shares the same VH of parental antibody MAB-1

nep = no evaluation possible.

Produced CyCAT polypeptides carrying optimized CD3 specific variable domains derived from MAB-1 were then further characterized for affinity and functional activity in cytotoxicity assays as described herein in Example 10 and Example 11 by combining a CyCAT polypeptide carrying the VH of an CD3 specific antibody with a CyCAT polypeptide carrying the VL of an CD3 specific antibody.

Out of these studies, a new improved human CD3 specific antibody named MAB-7 was identified comprising the affinity matured VH domain of MAB-1_25 (MAB-1_25_VH (SEQ ID NO: 14)) and the affinity matured VL domain of MAB-1_2 (MAB-1_2_VL (SEQ ID NO: 17)).

An overview of the functional characteristics for the MAB-7 antibody (CyCAT-7) is shown in Table 15 (first row) and reveal that MAB-7 binds to human CD3 epsilon with an K_D of 5 nM and mediates efficient T-cell killing of HER-2 expressing SW-480 colon cancer cells when tested in the bispecific CyCAT format with IC50 values in the single digit nM range.

Example 5: Identification of further improved human antibodies specific for CD3.

To identify further improved human CD3 specific variable domains of MAB-1 and MAB-7 in terms of affinity, potency and producibility in the CyCAT format, various strategies were pursued:

1. Additional LCDR1+2 affinity maturation of the variable light chain of MAB-7 (MAB-1_2_VL) using human and cynomolgus CD3 antigens, as well as Jurkat cells expressing native CD3.
2. Identification of new LCDR3 variants of parental antibody MAB-1 based on Next Generation Sequencing (NGS) via analysis of complete LCDR3 affinity maturation outputs from the first affinity maturation campaign described in Example 2.
3. Identification of new HCDR1+2 variants of parental MAB-1 based on NGS analysis of complete affinity maturation outputs from the first affinity maturation campaign described in Example 2.

Out of these studies, 81 most promising clones in terms of their e.g. affinity were produced as unpaired CyCAT polypeptides as described in Example 4.

Example 6: Production of unpaired CyCAT polypeptides carrying improved CD3 specific variable domains of Example 5.

Out of the 81 clones of Example 5, variable domains of 57 affinity matured antibodies could be successfully produced as unpaired CyCAT polypeptides at least as good as the unpaired CyCAT polypeptides carrying either the VH domain of MAB-7 (MAB-1_25_VH; (SEQ ID NO: 14) or the VL domain of MAB-7 (MAB-1_2_VL; (SEQ ID NO: 17)). This included 6 of only 8 newly identified affinity matured VH domains.

Results:

Overall, production data revealed that a larger number of acidic amino acids in the CDRs of the CD3 specific variable domains were associated with better producibility of the CyCAT polypeptides. A summary of the quality control of produced CyCAT polypeptides carrying 13 improved CD3 specific VL domains (MAB-1_3_VL to MAB-1_15_VL) and 1 improved VH domain (MAB-1_24_VH) of parental MAB-1 is shown in Table 13.

These polypeptides could be produced in high yield (data not shown) and revealed excellent monomer content and purity.

In particular, the CyCAT polypeptides carrying the affinity matured VH domain of MAB-1_24 (MAB-1_24_VH; SEQ ID NO: 15) could be produced with a high monomer content of ~90% which is significantly increased when compared to the formerly determined monomer content of its parental antibody MAB-1, which was only in the range of 80% (see Table 12).

MAB-1_24_VH was independently identified by a novel rational design approach and was based on the sequences of 100 unique affinity screening hits after H-CDR1+2 maturation. Amino acid frequency for each position of the affinity matured HCDR1 + 2 sequences was analyzed via Next Generation Sequencing. For each position the amino acid residue which showed best enrichment compared to the maturation module design was selected. If two or more amino acids were similarly enriched, acidic amino acids were preferred.

Table 13: Summary quality control of the produced CyCAT polypeptides carrying further improved CD3 specific variable domains. Monomer content and impurities of produced CyCAT polypeptides are shown.

Improved Anti-CD3 Variable Domain	SEQ ID NO: CD3 VH/VL	CyCAT polypeptide	SEQ ID NO: CyCAT polypept.	SEC Monomer Content [%]	SEC High molecular weight impurities [%]	SEC Low molecular weight impurities [%]
MAB-1_3_VL	18	CyCAT_MAB-1_3_VL	65	94,1	5,4	0,6
MAB-1_4_VL	19	CyCAT_MAB-1_4_VL	66	96,0	3,7	0,3

MAB-1_5_VL	20	CyCAT_MAB-1_5_VL	67	90,0	9,7	0,3
MAB-1_6_VL	21	CyCAT_MAB-1_6_VL	68	90,9	8,4	0,7
MAB-1_7_VL	22	CyCAT_MAB-1_7_VL	69	97,1	2,5	0,4
MAB-1_8_VL	23	CyCAT_MAB-1_8_VL	70	95,3	4,2	0,5
MAB-1_9_VL	24	CyCAT_MAB-1_9_VL	71	97,2	2,8	0,0
MAB-1_10_VL	25	CyCAT_MAB-1_10_VL	72	96,9	3,0	0,1
MAB-1_11_VL	26	CyCAT_MAB-1_11_VL	73	95,3	4,0	0,8
MAB-1_12_VL	27	CyCAT_MAB-1_12_VL	74	94,4	5,6	0,0
MAB-1_13_VL	28	CyCAT_MAB-1_13_VL	75	92,2	7,3	0,5
MAB-1_14_VL	29	CyCAT_MAB-1_14_VL	76	94,1	5,9	0,0
MAB-1_15_VL	30	CyCAT_MAB-1_15_VL	77	96,1	3,1	0,8
MAB-1_24_VH	15	CyCAT_MAB-1_24_VH	88	90,2	9,5	0,3

Example 7: Characterization of paired CyCAT polypeptides carrying improved CD3 specific variable domains of Examples 5 and 6.

52 successfully produced CyCAT polypeptides of Example 6 were subjected to affinity determination as described in Example 10 and functional testing as described in Example 11 by combining them with CyCAT polypeptides carrying either the VH of MAB-7 (MAB-1_25_VH (SEQ ID NO: 14)) or the VL of MAB-7 (MAB-1_2_VL (SEQ ID NO: 17)).

These studies resulted in the identification of 13 improved and most preferred CD3 specific VL domains and 1 CD3 specific VH domain derived from of MAB-1 or MAB-7 of Example 6 (as summarized in Table 13).

An overview of the functional characteristics of the preferred combinations of the CD3 specific VL and VH domains are summarized in Table 15 and reveal that the newly identified CD3 specific antibodies according to the present disclosure bind to human CD3 epsilon with an K_D of 5 nM or lower and mediate efficient T cell killing of SW-480 colon cancer cells in the CyCAT format with IC_{50} values mostly in the subnanomolar range.

Out of these VH/VL combinations, MAB-7, MAB-8, MAB-9, MAB-12, MAB-13 and MAB-16 were selected as the CD3 specific lead antibodies for use in T-cell engaging multispecific antibody formats.

Example 8: Production of unpaired CyCAT polypeptides carrying further improved CD3 specific variable domains of MAB-1 or MAB-7.

As a final step of optimization, 8 cross-clones were generated by combining the LCDR1 + 2 sequences of the best LCDR1 + 2 affinity matured variants of MAB-7 (see Table 13) with the LCDR3 sequence of MAB-1_14_VL (SEQ ID NO: 29). MAB-1_14_VL was identified as described in Example 5 (option 2 of the numbered list) as a consensus sequence based on the enrichment of particular amino acid residues at certain positions in the LCDR3 when compared to the design of the affinity maturation module used. CyCAT polypeptides were then produced as described in Example 4.

Results

A summary of the quality control of the produced CyCAT polypeptides carrying the further improved CD3 specific VL domains of the 8 cross-clones is shown in Table 14. These produced polypeptides revealed an overall less favorable monomer content and a higher degree of high molecular weight impurities after production when compared the polypeptides carrying the improved CD3 specific variable domains of Table 15. Therefore, the polypeptides were subjected to a preparatory size exclusion chromatography (Prep-SEC). Prep-SEC preparations resulted in a very high monomer content for the CyCAT polypeptides as shown in Table 14.

Table 14: Summary quality control of produced CyCAT polypeptides carrying further improved cross-cloned CD3 specific variable light chain domains before and after preparatory size exclusion chromatography. Monomer content and impurities of produced CyCAT polypeptides are shown.

Further improved Anti-CD3 Variable Domain (X-clones)	SEQ ID NO:	CyCAT polypeptide	SEQ ID NO:	SEC Monomer Content [%]*	SEC High molecular weight impurities [%]	SEC Low molecular weight impurities [%]	Polished SEC Monomer Content [%]*
MAB-1_16_VL	31	CyCAT_MAB-1_16_VL	78	89,8	10,2	0,0	97,0
MAB-1_17_VL	32	CyCAT_MAB-1_17_VL	79	nep*	nep*	nep*	99,0
MAB-1_18_VL	33	CyCAT_MAB-1_18_VL	80	83,7	14,8	1,5	99,7
MAB-1_19_VL	34	CyCAT_MAB-1_19_VL	81	92,4	7,6	0,0	99,0
MAB-1_20_VL	35	CyCAT_MAB-1_20_VL	82	nep*	nep*	nep*	99,2
MAB-1_21_VL	36	CyCAT_MAB-1_21_VL	83	84,9	13,7	1,4	99,4
MAB-1_22_VL	37	CyCAT_MAB-1_22_VL	84	81,3	17,1	1,5	92,9
MAB-1_23_VL	38	CyCAT_MAB-1_23_VL	85	90,1	9,9	0,0	99,2

nep* = no evaluation possible

Example 9: Characterization of paired CyCAT polypeptides carrying improved cross-cloned CD3 specific variable light chain domains of Examples 8.

The produced CyCAT polypeptides of Example 8 were subjected to affinity determination as described in Example 10 and functional testing as described in Example 11 by combining them with the CyCAT polypeptides carrying the VH domain of MAB-7 (MAB-1_25_VH (SEQ ID NO: 14)) or the newly identified optimized VH domain of MAB-1_24 (MAB-1_24_VH (SEQ ID NO: 15)).

An overview of the functional characteristics of the further improved CD3 specific VL and VH domains are summarized in Table 16. This data reveal that the paired CyCAT polypeptides comprising the further improved CD3 specific VL domains bind to human CD3 epsilon with an K_D of below 1 nM. However, no further improved T-cell mediated killing of SW-480 colon cancer cells in the CyCAT format could be observed (data not shown).

Example 10: Affinity determination for paired CD3 VH/VL domains in the CyCAT format on human CD3 epsilon

Produced unpaired CyCAT polypeptides carrying either a VH or VL domain of an CD3 specific antibody according to the present disclosure were combined to allow for the formation of paired and functional CD3 specific Fv domains. Functional complementation of the CD3-specific VH and VL domains occurs once each of the two CyCAT polypeptides is bound to its target antigen via the Fab portion and the two polypeptides come in close proximity.

Method

K_D determination for paired VH and VL domains was performed on an Octet HTX (FortéBIO, Sartorius AG) instrument at 27 °C.

Streptavidin-coated biosensors (FortéBIO, Sartorius AG) were loaded with purified and biotinylated human HER2_his_Avi_bio (ACROBiosystems, HE2-H82E2) in immobilization buffer (DPBS, 0.05% (v/v) Tween 20) resulting in a high density human HER2 sensor surface. Afterwards, CyCAT polypeptides were diluted in Octet buffer (DPBS, 0.05% (v/v) Tween-20, 0.1% (w/v) BSA) and sequentially injected until a loading threshold value of approx. 0.5 nm was reached for CyCAT_VH_CD3 and CyCAT_VL_CD3, respectively. Kinetic measurements were done using 3 different human CD3 epsilon (CD3e (1-118)_FLAG_chLys_AVI-bio (MorphoSys AG) (SEQ ID NO: 8) concentrations (4-fold serial dilution, 120 to 7.5 nM in Octet buffer) with 300 s association time and 720 s dissociation time. After each dissociation step the sensors were regenerated to remove bound CyCAT molecules (2x30 s Gly/HCl, pH 1.5).

All data were referenced with Streptavidin-coated biosensors loaded with human HER2_his_Avi_bio incubated in Octet buffer instead of analyte containing buffer. The sensorgrams were fitted using Octet Data Analysis Software 10.0 (FortéBio, Sartorius AG) to determine k_{on} and k_{off} rate constants (using a 1:1 binding model), which were used to calculate K_D .

Results

A summary of the determined affinities on human CD3 epsilon for preferred VH and VL pairs is provided in Table 15 and Table 16 and reveal monovalent affinities for the newly identified CD3 specific antibodies according to the present disclosure on human CD3 epsilon in the single digit nanomolar to triple digit picomolar range.

As expected, testing of unpaired not combined CyCAT polypeptides of CyCAT_MAB-1_24_VH, CyCAT_MAB-1_3_VL, CyCAT_MAB-1_14_VL and CyCAT_MAB-1_16_VL to CyCAT_MAB-1_23_VL up to a concentration of 500 nM revealed no binding to CD3 epsilon due to lack of formation of a functional CD3 specific Fv binding fragment.

Example 11: T cell mediated killing of cancer cells induced by paired CyCAT polypeptides

The produced unpaired CyCAT polypeptides were combined to allow for the formation of a CyCAT molecule comprising a newly formed CD3 specific Fv binding domain. Functional complementation of the CD3 specific VH and VL domains occurs once each of the two CyCAT polypeptides is bound via its Fab portion to its target antigen on the cancer cell and the two polypeptides are in close proximity. Redirected killing of cancer cells via immune cells is mediated by binding of the newly formed CD3 specific antibody Fv domain to T cells.

Methods

Isolation of human T-cells

Human whole blood from healthy donors was collected e.g. in Li-Heparin containing S-Monovette containers (Sarstedt). 20 mL blood were transferred to 50 mL conical tubes, mixed with 1 mL of RosetteSep Human CD8+ Enrichment Cocktail (Stemcell Technologies, #15063) and incubated for 20 min at room temperature. Blood containing RosetteSep human CD8+ enrichment cocktail was diluted with an equal volume of PBS containing 2% fetal bovine serum (Sigma, #F7524) and 2 mM EDTA. Diluted blood was transferred to SepMate-50 tubes (Stemcell Technologies, #85450) containing 15 mL of Lymphoprep density gradient medium (Stemcell Technologies, #07811) and centrifuged for 20 min at 1200 x g at room temperature.

Supernatant was transferred into a 50 mL conical tube, diluted to 45 mL with PBS containing 2% fetal bovine serum and 2 mM EDTA and centrifuged for 5 min at 800 x g. Supernatant was discarded, cell pellet resuspended in 1 mL PBS containing 2 % fetal bovine serum. Cell suspensions were pooled and transferred to a 50 mL tube and diluted to 30 mL PBS containing 2 % fetal bovine serum. Cells were pelleted by centrifugation for 5 min at 800 x g. The cell pellet was resuspended in 2 mL of 1x Pharm Lyse Red Blood Cell lysing buffer (BD, #555899) and incubated at 4°C for 10 min. PBS containing 2 % fetal bovine serum was added to a final volume of 15 mL. Cells were pelleted for 10 min at 120 x g and the supernatant decanted. The cells were washed twice with PBS containing 2 % fetal bovine serum and counted (CASY TT device, Beckmann Coulter).

Assay Method

7,5000 HER-2 expressing SW-480 colon cancer cells (ATCC® CCL-228™) were suspended in culture medium supplemented with 10% FCS, seeded in black 96 well assay plates (Corning) and incubated over night at 37°C and 5% CO₂ and humidity.

CellToxGreen dye (Promega, #G8731), serially diluted CyCAT polypeptides (final concentration: 0.00001 – 100 nM) and purified human T cells (E:T ratio 1:10) or human PBMCs (E:T ratio of 1:30), all diluted in assay medium comprising RPMI 1640 w/o Phenol red (Gibco, #32404-014), GlutaMAX (Gibco 35050-038) and 10% fetal bovine serum, were added to the cells and incubated for 48 or 72 hrs at 37°C and 5% CO₂ and humidity.

Cytotoxic activity was assessed by measuring incorporated CellToxGreen fluorescence at 485 nm excitation and 535 nm emission using a Tecan Infinite F500 device.

Results

The results of the experiments for selected CyCAT polypeptides carrying the newly identified variable domains of the CD3 specific antibodies according of the present disclosure are summarized in Table 15.

Co-cultivation of T-cells with a combination of CyCAT polypeptides with specificity for HER-2 and CD3 induced killing of HER-2 positive SW-480 target cells in a dose dependent manner. As expected, a negative control combination of CyCAT polypeptides induced no killing activity (data not shown). Co-cultivation of T-cells with unpaired and not combined CyCAT polypeptide with specificity for HER-2 and CD3 did not induced killing of HER-2 positive SW480 target cells. In the presence of HER-2 negative cells, none of the tested HER-2 specific combinations of CyCAT polypeptides induced cytotoxic activity (data not shown).

These results clearly demonstrate that the CD3 specific VH and VL domains comprised in each of the unpaired CyCAT polypeptides are complemented to a functional CD3 specific antibody

Fv domain once both CyCAT polypeptides are bound via their Fab portion to the target antigen on the cancer cell and the two polypeptides get in close proximity. Accordingly, the newly identified CD3 specific human antibodies according to the present disclosure can be used in various approaches to efficiently re-direct T-cells to kill tumor expressing cells.

Summary affinities and functional activity of the new CD3 specific antibodies according to the present disclosure.

Table 15: Summary affinities of CD3 specific antibodies (according Examples 5 to 7) and re-directed T-cell killing of cancer cells by corresponding CyCAT molecules with specificity for HER2 and CD3.

CyCAT	Paired CyCAT polypeptides	SEQ ID NO:	New anti-CD3 antibody			Affinity CD3	T cell Killing 48h	T cell Killing 72h
			CD3_VL	SEQ ID NO:	Anti-CD3 Anti-body	K _D [nM] hCD3e (1-118)	IC ₅₀ [nM] SW-480	IC ₅₀ [nM] SW-480
			CD3_VH					
CYCAT-7	CyCAT_MAB-1_2_VL	64	MAB-1_2_VL	17	MAB-7	5	2,72 5,70 N/A 17,46	0,54 0,43 N/A 9,03
	CyCAT_MAB-1_25_VH	89	MAB-1_25_VH	14				
CYCAT-8	CyCAT_MAB-1_2_VL	64	MAB-1_2_VL	17	MAB-8	1,4	0,80 0,93	0,35 0,61
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				
CYCAT-9	CyCAT_MAB-1_3_VL	65	MAB-1_3_VL	18	MAB-9	0,2 0,7	0,54 1,70	0,32 1,07
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				
CYCAT-10	CyCAT_MAB-1_4_VL	66	MAB-1_4_VL	19	MAB-10	1,2	0,36 3,19	0,28 1,37
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				
CYCAT-11	CyCAT_MAB-1_8_VL	70	MAB-1_8_VL	23	MAB-11	1,1	1,09 1,59	0,67 0,85
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				
CYCAT-12	CyCAT_MAB-1_9_VL	71	MAB-1_9_VL	24	MAB-12	0,8	0,30 1,40	0,23 0,98
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				
CYCAT-13	CyCAT_MAB-1_10_VL	72	MAB-1_10_VL	25	MAB-13	0,9	0,46 1,36	0,28 0,84
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				
CYCAT-14	CyCAT_MAB-1_11_VL	73	MAB-1_11_VL	26	MAB-14	1,2	1,43 2,95	0,57 1,28
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				
CYCAT-15	CyCAT_MAB-1_12_VL	74	MAB-1_12_VL	27	MAB-15	0,3	0,58 1,29	0,42 0,91
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				

CyCAT	Paired CyCAT polypeptides	SEQ ID NO:	New anti-CD3 antibody			Affinity CD3	T cell Killing 48h	T cell Killing 72h
			CD3_VL	SEQ ID NO:	Anti-CD3 Anti-body	K _D [nM] hCD3e (1-118)	IC ₅₀ [nM] SW-480	IC ₅₀ [nM] SW-480
			CD3_VH					
CYCAT-16	CyCAT_MAB-1_14_VL	76	MAB-1_14_VL	29	MAB-16	0,9 0,9	0,33 0,35 0,56	0,22 0,23 0,33
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15				
CYCAT-17	CyCAT_MAB-1_3_VL	65	MAB-1_3_VL	18	MAB-17	1,1	1,91 20,53	0,59 2,40
	CyCAT_MAB-1_25_VH	89	MAB-1_25_VH	14				
CYCAT-18	CyCAT_MAB-1_12_VL	74	MAB-1_12_VL	27	MAB-18	1,6	1,56	0,45
	CyCAT_MAB-1_25_VH	89	MAB-1_25_VH	14				
CYCAT-19	CyCAT_MAB-1_14_VL	76	MAB-1_14_VL	29	MAB-19	2,6	15,36	0,37
	CyCAT_MAB-1_25_VH	89	MAB-1_25_VH	14				

Table 16: Summary affinities of CD3 specific antibodies with cross-cloned variable light chain domains according Example 8.

CyCAT	Paired CyCAT polypeptides [Fab_Trastuzumab]- Linker-[CD3-VL] x [Fab_Trastuzumab]- Linker-[CD3-VH]	SEQ ID NO:	New anti-CD3 antibody			Affinity CD3
			CD3_VL	SEQ ID NO:	CD3 anti- body	K _D [nM] hCD3e (1-118)
			CD3_VH			
CYCAT-20	CyCAT_MAB-1_16_VL	78	MAB-1_16_VL	31	MAB-20	0,5
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15		
CYCAT-21	CyCAT_MAB-1_17_VL	79	MAB-1_17_VL	32	MAB-21	0,4
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15		
CYCAT-22	CyCAT_MAB-1_18_VL	80	MAB-1_18_VL	33	MAB-22	0,6
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15		
CYCAT-23	CyCAT_MAB-1_19_VL	81	MAB-1_19_VL	34	MAB-23	0,4
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15		
CYCAT-24	CyCAT_MAB-1_20_VL	82	MAB-1_20_VL	35	MAB-24	0,6
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15		
CYCAT-25	CyCAT_MAB-1_21_VL	83	MAB-1_21_VL	36	MAB-25	0,3
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15		
CYCAT-26	CyCAT_MAB-1_22_VL	84	MAB-1_22_VL	37	MAB-26	0,1*
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15		
CYCAT-27	CyCAT_MAB-1_23_VL	85	MAB-1_23_VL	38	MAB-27	0,5
	CyCAT_MAB-1_24_VH	88	MAB-1_24_VH	15		

*KD restriction; dissociation reached assay limit

Example 12: Reconversion and production of newly identified and optimized CD3 specific VH/VL pairs into conventional Fab and IgG antibody backbones.

In order to determine the suitability of the newly identified and optimized CD3 specific VH / VL pairs to be used in conventional antibody formats, most preferred VH / VL pairs (see Table 17) were cloned into the mammalian IgG1f-AEASS (effector silent) or FabCys expression vectors for IgG or Fab production, respectively.

All nucleic acid sequences or desired gene segments were generated by PCR using appropriate templates or were gene synthesized as linear DNA fragments with appropriate flanking regions for Gibson Assembly, (e.g. suitable restriction enzyme recognition sites and 21bp overlap sequence) in-house or by an external provider. The nucleic acid sequences were cloned into respective Ylanthia® FabCys mammalian expression vectors.

For IgG expression, antibody variable domain encoding FabCys vectors were enzymatically digested and the resulting inserts were ligated with the Ylanthia® mammalian expression cassette and further sub-cloned into the respective mammalian human silent IgG vectors.

Table 17: Selected optimized CD3 specific human antibodies re-converted from the CyCAT into the Fab-Cys and the human IgG1-AEASS format .

Anti-CD3 Antibody	VH / VL domains	SEQ ID NO:
MAB-7	MAB-1_2_VL	17
	MAB1_25_VH	14
MAB-8	MAB-1_2_VL	17
	MAB-1_24_VH	15
MAB-9	MAB-1_3_VL	18
	MAB-1_24_VH	15
MAB-12	MAB-1_9_VL	24
	MAB-1_24_VH	15
MAB-13	MAB-1_10_VL	25
	MAB-1_24_VH	15
MAB-16	MAB-1_14_VL	29
	MAB-1_24_VH	15

Table 18: Human FabCys and human IgG1f-AEASS amino acid sequences of produced CD3 specific antibodies according to the present disclosure.

Antibody#		SEQ ID NO:	[aa]
MAB-7	h_IgG1-AEASS Heavy chain	95	EVQLVESGGGLVQPGGSLRLSCAASGFTFKSYY MSWVRQAPGKGLEWVANIDYQSQHAYYAESVK GRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDWVGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCDKHT CPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEV TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	h_IgG1f Light Chain (VL+CL)	96	QSVLTQPPSASGTPGQRTVISCSSSSNIGSNYV YWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
	FabCys Heavy chain	97	EVQLVESGGGLVQPGGSLRLSCAASGFTFKSYY MSWVRQAPGKGLEWVANIDYQSQHAYYAESVK GRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDWVGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC
	FabCys Light chain (VL+CL)	96	QSVLTQPPSASGTPGQRTVISCSSSSNIGSNYV YWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
MAB-8	h_IgG1-AEASS Heavy chain	98	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDWVGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCDKHT CPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEV TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	h_IgG1f Light Chain (VL+CL)	96	QSVLTQPPSASGTPGQRTVISCSSSSNIGSNYV YWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP

Antibody#		SEQ ID NO:	[aa]
			SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
	FabCys Heavy chain	99	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDVWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC
	FabCys Light chain (VL+CL)	96	QSVLTQPPSASGTPGQRTVISCSSSSNIGSNYV YWYQQLPGTAPKLLIYRNNQRPSPGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
MAB-9	h_IgG1-AEASS Heavy chain	98	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDVWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEV TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYCKKVS NKAIPSSIEKTIKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSGDSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	h_IgG1f Light Chain (VL+CL)	100	QSVLTQPPSASGTPGQRTVISCSSSSNIGINYV YWYQQLPGTAPKLLIYRNNIRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDRHSHGAVF GGGKTLTVLGQPKAAPSVTLFPPSSEELQANKAT LVCLISDFYPGAVTVAWKADSSPVKAGVETTPS KQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHE GSTVEKTVAPTECS
	FabCys Heavy chain	99	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDVWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC
	FabCys Light chain (VL+CL)	100	QSVLTQPPSASGTPGQRTVISCSSSSNIGINYV YWYQQLPGTAPKLLIYRNNIRPSGVPDRFSGSKS GTSASLAISGLRSEDEADYYCAAWDRHSHGAVF GGGKTLTVLGQPKAAPSVTLFPPSSEELQANKAT LVCLISDFYPGAVTVAWKADSSPVKAGVETTPS KQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHE GSTVEKTVAPTECS
MAB-12	h_IgG1-AEASS Heavy chain	98	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDVWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSS

Antibody#		SEQ ID NO:	[aa]
			LGTQTYICNVNHHKPSNTKVDKRVEPKSCDKTHT CPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEV TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	h_IgG1f Light Chain (VL+CL)	101	QSVLTQPPSASGTPGQRTVISCSSSSNIGANYV YWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
	FabCys Heavy chain	99	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDWWGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHHKPSNTKVDKRVEPKSC
	FabCys Light chain (VL+CL)	101	QSVLTQPPSASGTPGQRTVISCSSSSNIGANYV YWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
MAB-13	h_IgG1-AEASS Heavy chain	98	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDWWGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHHKPSNTKVDKRVEPKSCDKTHT CPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEV TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	h_IgG1f Light Chain (VL+CL)	102	QSVLTQPPSASGTPGQRTVISCSSSSNIGINYV YWYQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
	FabCys Heavy chain	99	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDWWGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHHKPSNTKVDKRVEPKSC

Antibody#		SEQ ID NO:	[aa]
	FabCys Light chain (VL+CL)	102	QSVLTQPPSASGTPGQRTISCSSSSNIGINYV YWYQQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAAWDRHSHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
MAB-16	h_IgG1-AEASS Heavy chain	98	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDVWGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCDKHT CPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEV TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYCKKVS NKALPSSIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	h_IgG1f Light Chain (VL+CL)	103	QSVLTQPPSASGTPGQRTISCSSSSNIGSNYV YWYQQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAGWDRRLHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS
	FabCys Heavy chain	99	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSHY MTWVRQAPGKGLEWVANIDYEGTRTYAESVK GRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR GYSAEFAHRSGLDVWGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC
	FabCys Light chain (VL+CL)	103	QSVLTQPPSASGTPGQRTISCSSSSNIGSNYV YWYQQQLPGTAPKLLIYRNNQRPSGVPDRFSGSK SGTSASLAISGLRSEDEADYYCAGWDRRLHGAV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAGVETTP SKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS

Exploratory Scale Production of IgG

Eukaryotic HEK293 cells were transfected with mammalian expression vector DNA encoding both heavy and light chains of IgG. Cell culture supernatants were harvested on day 3 post transfection and subjected to standard Protein A affinity chromatography (MabSelect SURE | GE Healthcare). If not stated otherwise, buffer exchange was performed to 1x Dulbecco's PBS (pH 7.2 | Invitrogen) and samples were sterile filtered (0.2 µm pore size).

Protein concentrations were determined by UV-spectrophotometry and purity of IgG was analyzed under denaturing, reducing and non-reducing conditions using CE-SDS (LabChip GXII | Perkin Elmer | USA). HP-SEC was performed to analyze IgG preparations in native state.

Exploratory Scale Production of Tagless FabCys

Eukaryotic HEK293 cells were transfected with mammalian expression vector DNA encoding both heavy and light chains of disulfide-bridged FabCys. Cell culture supernatants were harvested on day 3 post transfection and subjected to CH1 affinity chromatography (Capture Select CH1-XL | Thermo Scientific). If not stated otherwise, buffer exchange was performed to 1x Dulbecco's PBS (pH 7.2 | Invitrogen) and samples were sterile filtered (0.2 µm pore size).

Protein concentrations were determined by UV-spectrophotometry and purity of FabCys was analyzed under denaturing, reducing and non-reducing conditions using CE-SDS (LabChip GXII | Perkin Elmer | USA). HP-SEC was performed to analyze FabCys preparations in native state.

Production Results:

A summary of the quality control of the mammalian produced FabCys and human IgG1s are summarized in Tables 19 - 21.

This data reveal that the antibodies could be produced in acceptable yields, monomer content and purity. For 3 IgGs, the monomer content was determined in the range of 85% to 90%. All produced IgGs were subjected to preparatory size exclusion chromatography (Prep-SEC). Prep-SEC preparations resulted in monomer content of greater 93% for all produced IgGs as shown in Table 21.

Table 19: Production results of selected CD3 specific antibodies of the present disclosure produced in the monovalent human FabCys format.

Antibody	CE-SDS		UHP-SEC
	Purity red. [%]	Purity non-red. [%]	Monomer [%]
MAB-7_h_FabCys	>95	>80	99,3
MAB-8_h_FabCys	>95	>80	97,7
MAB-9_h_FabCys	>95	>80	97,8
MAB-12_h_FabCys	>95	>80	98,2
MAB-13_h_FabCys	>95	>80	97,1
MAB-16_h_FabCys	>95	>80	98,3

Table 20: Production results of selected CD3 specific antibodies of the present disclosure produced in the silent bivalent human IgG1_AEASS format before preparatory size exclusion chromatography.

Antibody	Volumetric Yield [mg/L]	CE-SDS		UHP-SEC
		Purity red. [%]	Purity non-red. [%]	Monomer [%]
MAB-7_h_IgG1f_AEASS	83,8	>95	>80	96,5
MAB-8_h_IgG1f_AEASS	57,4	>95	>80	93,5
MAB-9_h_IgG1f_AEASS	23,9	>95	>80	84,5
MAB-12_h_IgG1f_AEASS	46,0	>95	>80	89,9
MAB-13_h_IgG1f_AEASS	32,0	>95	>80	86,5
MAB-16_h_IgG1f_AEASS	50,0	>95	>80	90,4

Table 21: Production results of selected CD3 specific antibodies of the present disclosure produced in the silent human IgG1-AEASS format after preparatory size-exclusion chromatography

Antibody	CE-SDS		UHP-SEC
	Purity red. [%]	Purity non-red. [%]	Monomer [%]
MAB-7_h_IgG1f_AEASS	>95	>80	99,5
MAB-8_h_IgG1f_AEASS	>95	>80	98,2
MAB-9_h_IgG1f_AEASS	>95	>80	93,3
MAB-12_h_IgG1f_AEASS	>95	>80	95,3
MAB-13_h_IgG1f_AEASS	>95	>80	94,3
MAB-16_h_IgG1f_AEASS	>95	>80	95,2

Example 13: K_D determination on human and cynomolgus CD3 epsilon by Biolayer Interferometry (BLI)

For K_D determinations, monomer fractions of antibody protein (Fab-Cys) were used (at least 90% monomer content, as analyzed by analytical SEC). Affinity determination by determining kinetic rate constants was performed on an Octet HTX (FortéBIO, Sartorius AG) instrument as described below.

The different Fab-Cys samples diluted in assay buffer (D-PBS, 0.05 % (v/v) PS 20, 0.1 % (w/v) BSA) were captured onto Fab-specific BLI sensors with a loading level of approx. 0.5 nm. For analysis, human CD3 epsilon antigen hCD3e(1-118)_F-chLys_avi (SEQ ID NO: 7) and cynomolgus monkey CD3 epsilon antigen cyCD3e(1-109)_F-chLys_avi (SEQ ID NO: 104) were diluted with assay buffer to concentrations ranging from 1.56 nM to 100 nM. A blank sample with assay buffer was included for referencing, i.e. correcting for dissociation of captured antibody. The association phase was recorded for 300 s, followed by a dissociation phase of 900 s. After each cycle, the biosensors were regenerated two times with 10 mM Glycine HCl pH 1.7 to remove bound ligand/antibody complex, while maintaining the integrity of the capture surface. Between regeneration steps, biosensors were washed with assay buffer for 20 s.

The sensorgrams were fitted using Octet Data Analysis Software 10.0 (FortéBio, Sartorius AG) to determine k_{on} and k_{off} rate constants (using a 1:1 binding model), which were used to calculate K_D .

The results of the K_D determination are summarized in Table 22. The newly identified CD3 specific antibodies exhibited K_D values in a comparable range for the binding to recombinant human and cynomolgus monkey CD3 epsilon antigens.

Table 22: K_D values of mammalian produced FabCys determined on human and cynomolgus CD3 epsilon antigens.

Fab	hCD3e(1-118)_F-chLys_avi			cyCD3e(1-109)_F-chLys_avi		
	k_{on} [1/Ms]	k_{off} [1/s]	K_D [nM]	k_{on} [1/Ms]	k_{off} [1/s]	K_D [nM]
MAB-7	4.99E+05	6.27E-04	1.3	3.70E+05	1.58E-04	0.4
MAB-8	8.02E+05	2.58E-04	0.3	4.64E+05	5.07E-05*	0.1
MAB-9	7.24E+05	1.22E-04	0.2	4.46E+05	5.00E-05*	0.1
MAB-12	6.50E+05	1.88E-04	0.3	4.45E+05	5.00E-05*	0.1
MAB-13	7.05E+05	1.34E-04	0.2	4.13E+05	5.00E-05*	0.1
MAB-16	8.52E+05	2.72E-04	0.3	4.78E+05	7.92E-05	0.2

* Dissociation approaching assay limit of the capture setup. Rate constants below 5E-05 1/s were set to this value (i.e. for these samples, the dissociation rate is maximally 5E-05 1/s). Therefore, K_D values were formatted with grey italic and should be considered as less precise, but may still serve as good estimation.

Example 14: Cell binding of CD3 specific antibodies to human and cynomolgus derived PBMCs***Isolation of human and cynomolgus PBMCs***

Human whole blood from healthy donors (in house) and whole blood from cynomolgus monkey (retrieved from LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany) was collected in Li-Heparin containing S-Monovette containers (Sarstedt). Blood was transferred to 50ml conical tubes and mixed with an equal volume of PBS containing 2% fetal bovine serum (Sigma, #F7524) and 2 mM EDTA. Diluted blood was transferred to SepMate-50 tubes (StemCell Technologies, #86450) containing 15 ml Biocoll solution (Biochrom, #L6115) and centrifuged for 10 min at 1200 xg. Supernatant was transferred into a 50 ml conical tube, diluted to 45 ml with PBS and centrifuged for 8 min at 300 xg. Supernatant was discarded, cell pellet resuspended in 1 ml PBS and cells counted using a Neubauer chamber.

Cell binding Assays

CD3 specific FabCys molecules according the present disclosure and an in-house prepared Fab-Cys of the prior art anti-CD3 antibody UCHT-1 (known to be not cross-reactive to cynomolgus monkey CD3) were tested by FACS for their ability to bind to human and cynomolgus derived PBMCs.

200,000 purified human or cynomolgus PBMCs (pre-blocked with in house produced recombinant human Fc2-H and goat anti-human IgG (Jackson Immuno Research, # 109-005-097) or recombinant cynomolgus monkey IgG1 (R&D Systems, # 9315-HG) and mouse anti-monkey IgG (Southern Biotech, SB108a), respectively) were mixed with antibodies serially diluted (final concentration: 0.012 nM – 200 nM) in D-PBS (Gibco) containing 3% fetal bovine serum (Sigma, #F7524) and incubated for 1 h on ice. Bound antibodies were detected using goat anti human IgG-AF647 (Jackson Immuno Research, # 109-606-097).

Antibody staining was measured using a multiformat Flow Cytometer (NovoCyte, Agilent Technologies) and analyzed using NovoExpress software (Version 1.5.0). Lymphocytes were identified by morphological gating of forward and side scatters. EC₅₀ values were calculated using 4-parameter non-linear regression analysis in Prism software (GraphPad Software Inc., version 8.4.3).

The results of the experiments are shown in Table 23 and reveal that the newly identified CD3 specific antibodies exhibited comparable binding to human and cynomolgus PBMCs. As expected, control Fab of UCHT-1 showed binding to human but no binding to cynomolgus monkey PBMCs at a concentration of 200 nM.

Table 23: Cell binding of CD3 specific FabCys antibodies to human and cynomolgus monkey PBMCs expressing CD3. Shown are EC₅₀ values. Control FabCys UCHT-1 was tested at a single concentration of 200 nM.

	EC ₅₀ [nM]	
	human PBMCs	cyno PBMCs
MAB-7_h_FabCys	6,1	3,7
MAB-8_h_FabCys	5,5	3,2
MAB-9_h_FabCys	7,7	4,7
MAB-12_h_FabCys	5,7	4,1
MAB-13_h_FabCys	8,3	5,2
MAB-16_h_FabCys	3,3	2,0
UCHT-1_FabCys	binding	no binding

We claim

1. An isolated human antibody or antibody fragment specific for human CD3 epsilon, wherein said antibody or antibody fragment comprises:
 - a) an HCDR1 region comprising the amino acid sequence of GFTFX₁SX₂X₃MX₄, wherein X₁ is S, K or R; X₂ is Y or H; X₃ is W or Y; and X₄ is S or T (SEQ ID NO: 90);
 - b) an HCDR2 region comprising the amino acid sequence of NIX₁X₂X₃X₄X₅X₆X₇YYX₈X₉SVKG, wherein X₁ is K or D; X₂ is Q or Y; X₃ is D, Q or E; X₄ is S or G; X₅ is S, Q or T; X₆ is E, H or R; X₇ is K, A or T; X₈ is V or A; and X₉ is D or E (SEQ ID NO: 91);
 - c) an HCDR3 region comprising the amino acid sequence of GYSAEFAHRSGLDV (SEQ ID NO: 39);
 - d) a LCDR1 region comprising the amino acid sequence of SGSSSNIGX₁X₂YVY, wherein X₁ is S, I, A, K or Q; and X₂ is N or T (SEQ ID NO: 92);
 - e) a LCDR2 region comprising the amino acid sequence of RNX₁X₂RPS, wherein X₁ is N, K, S, H, T or Y; and X₂ is Q, I or K (SEQ ID NO: 93); and
 - f) a LCDR3 region comprising the amino acid sequence of AX₁WDX₂X₃X₄X₅GAV, wherein X₁ is A or G; X₂ is H or R; X₃ is H or R; X₄ is R, S or L; and X₅ is S or H (SEQ ID NO: 94).

2. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to claim 1, wherein said antibody or antibody fragment comprises:
 - a) an HCDR1 region comprising the amino acid sequence of GFTFSSYWMS (SEQ ID NO: 40), GFTFKSYYS (SEQ ID NO: 41) or GFTFRSHYMT (SEQ ID NO: 42);
 - b) an HCDR2 region comprising the amino acid sequence of NIKQDGSEKYYVDSVKG (SEQ ID NO: 43), NIDYQSQHAYYAESVKG (SEQ ID NO: 44) or NIDYEGTRTYAESVKG (SEQ ID NO: 45);
 - c) an HCDR3 region comprising the amino acid sequence of GYSAEFAHRSGLDV (SEQ ID NO: 39);
 - d) a LCDR1 region comprising the amino acid sequence of SGSSSNIGSNYVY (SEQ ID NO: 46), SGSSSNIGINYVY (SEQ ID NO: 47), SGSSSNIGANYVY (SEQ ID NO: 48), SGSSSNIGQTYVY (SEQ ID NO: 49), or SGSSSNIGKNYVY (SEQ ID NO: 50);
 - e) a LCDR2 region comprising the amino acid sequence of RNNQRPS (SEQ ID NO: 51), RNNIRPS (SEQ ID NO: 52), RNNKRPS (SEQ ID NO: 53), RNKKRPS (SEQ ID NO: 54), RNKQRPS (SEQ ID NO: 55), RNSQRPS (SEQ ID NO: 56), RNHIRPS (SEQ ID NO: 57), RNTQRPS (SEQ ID NO: 58), or RNYQRPS (SEQ ID NO: 59); and

- f) a LCDR3 region comprising the amino acid sequence of AAWDHHRSGAV (SEQ ID NO: 60), AAWDRHSHGAV (SEQ ID NO: 61) or AGWDRRLHGAV (SEQ ID NO: 62).
3. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to claim 1 or claim 2, wherein said antibody or antibody fragment comprises a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15.
4. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to any one of the preceding claims, wherein said antibody or antibody fragment comprises a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.
5. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to any one of the preceding claims, wherein said antibody or antibody fragment comprises
- a) a variable heavy chain (VH) comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15; and
- b) a variable light chain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38.
6. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to any one of the preceding claims, wherein the variable heavy chain and variable light chain are selected from the group consisting of:
- a) the VH comprising the amino acid sequence of SEQ ID NO: 13 and the VL comprising the amino acid sequence of SEQ ID NO: 16,
- b) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 16,

- c) the VH comprising the amino acid sequence of SEQ ID NO: 13 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- d) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- e) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- f) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 19,
- g) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 20,
- h) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 21,
- i) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 22,
- j) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 23,
- k) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 24,
- l) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 25,
- m) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 26,
- n) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 27,
- o) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 28,
- p) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 29,
- q) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 30,
- r) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 31,
- s) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 32,
- t) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 33,

- u) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 34,
- v) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 35,
- w) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 36,
- x) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 37,
- y) the VH comprising the amino acid sequence of SEQ ID NO: 14 and the VL comprising the amino acid sequence of SEQ ID NO: 38,
- z) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 17,
- aa) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 18,
- bb) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 19,
- cc) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 20,
- dd) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 21,
- ee) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 22,
- ff) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 23,
- gg) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 24,
- hh) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 25,
- ii) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 26,
- jj) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 27,
- kk) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 28,
- ll) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 29,

- mm) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 30,
 - nn) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 31,
 - oo) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 32,
 - pp) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 33,
 - qq) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 34,
 - rr) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 35,
 - ss) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 36,
 - tt) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 37, and
 - uu) the VH comprising the amino acid sequence of SEQ ID NO: 15 and the VL comprising the amino acid sequence of SEQ ID NO: 38.
7. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to any one of the preceding claims, wherein said antibody or antibody fragment cross-reactively binds to cynomolgus CD3 epsilon.
 8. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to any one of the preceding claims, wherein said antibody or antibody fragment is a recombinant antibody or antibody fragment.
 9. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to any one of the preceding claims, wherein said antibody or antibody fragment is a monoclonal antibody or antibody fragment.
 10. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to any one of the preceding claims, wherein the antibody fragment is selected from the group consisting of a Fab, Fv, and scFv.
 11. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to claims 1 – 9, wherein the antibody is an IgG.

12. A multispecific antibody comprising the isolated human antibody or antibody fragment specific for human CD3 epsilon according to any one of the preceding claims.
13. The multispecific antibody according to claim 12, wherein the multispecific antibody also specifically binds to a cancer associated antigen.
14. A pharmaceutical composition comprising the isolated human antibody or antibody fragment specific for human CD3 epsilon according to claims 1 – 11 or the multispecific antibody according to claims 12 – 13 and a pharmaceutically acceptable carrier or excipient.
15. The isolated human antibody or antibody fragment specific for human CD3 epsilon according to claims 1 – 11, the multispecific antibody according to claims 12 - 13 or the pharmaceutical composition according claim 13 for use as a medicament.

FIGURE 1

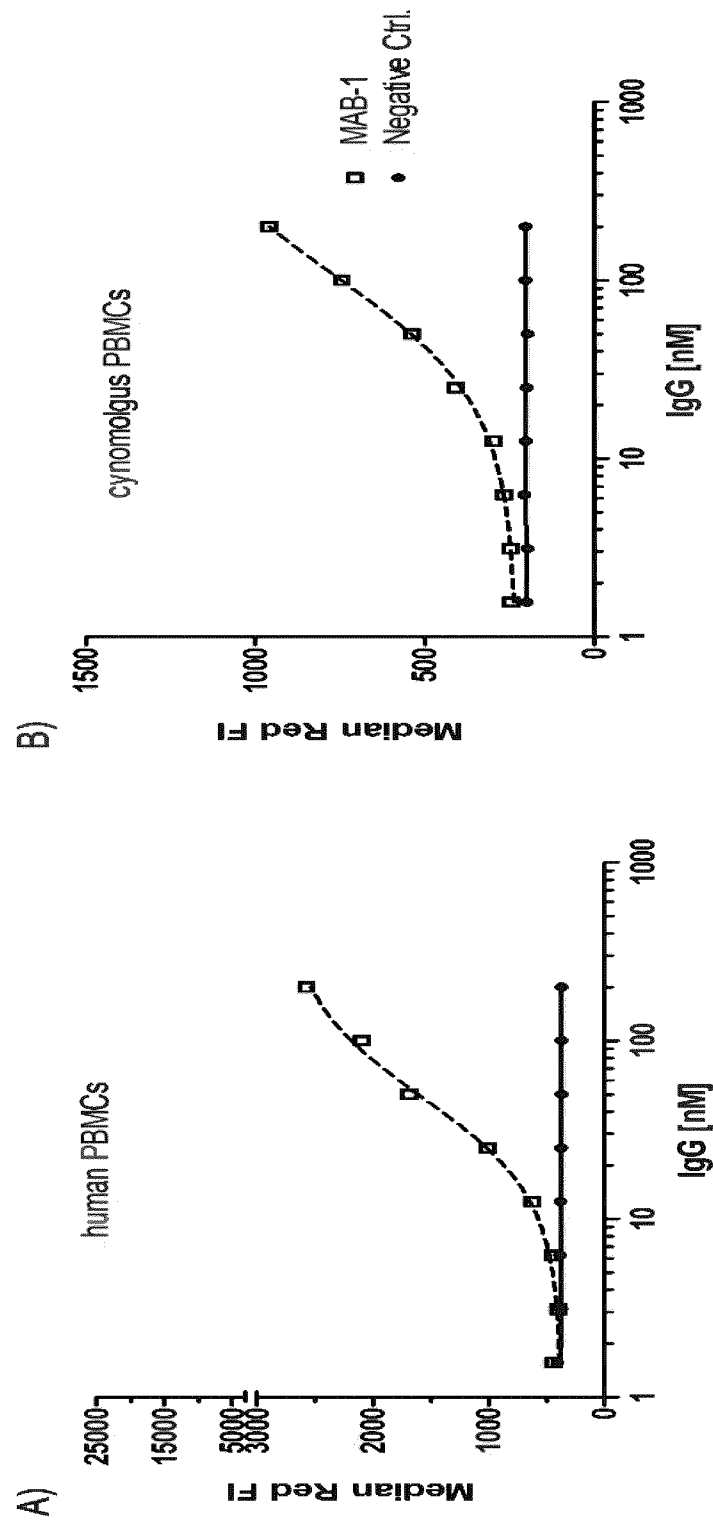
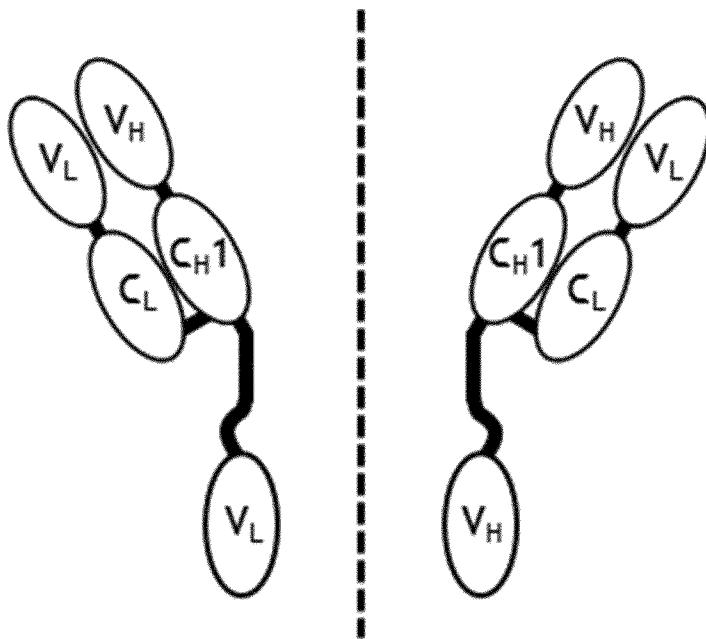


Figure 2



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/076052

A. CLASSIFICATION OF SUBJECT MATTER INV. C07K16/28 C07K16/32 C07K16/46 A61P35/00 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C07K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, EMBASE, BIOSIS, Sequence Search		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/181098 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 3 December 2015 (2015-12-03) page 25 - page 27; claim 14 -----	1-15
X	WO 2019/034580 A1 (MORPHOSYS AG [DE]) 21 February 2019 (2019-02-21) page 14; examples 1, 7; table 10 page 53 -----	1-15
X	WO 2008/119567 A2 (MICROMET AG [DE]; EBERT EVELYN [DE] ET AL.) 9 October 2008 (2008-10-09) claims 1-3, 11, 12 ----- -/--	1-15
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 6 February 2022		Date of mailing of the international search report 16/02/2022
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Saame, Tina

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2021/076052

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/104804 A2 (UNIV WUERZBURG J MAXIMILIANS [DE]) 18 July 2013 (2013-07-18) cited in the application figure 2 -----	1-15
A	SHIRA WARSZAWSKI ET AL: "Optimizing antibody affinity and stability by the automated design of the variable light-heavy chain interfaces", PLOS COMPUTATIONAL BIOLOGY, vol. 15, no. 8, 23 August 2019 (2019-08-23), page e1007207, XP055680871, DOI: 10.1371/journal.pcbi.1007207 the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2021/076052

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2021/076052

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 3907241 A1	10-11-2021
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-15 (partially)

Independent claim 1 relates to human antibodies or antibody fragments specific for human CD3 epsilon wherein the antibodies are furthermore defined by the sequences of HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 regions, allowing for a significant amount of variability within each of HCDR1, HCDR2, LCDR1, LCDR2 and LCDR3.

Similarly, dependent claim 2 allows for a choice of different possibilities for each of HCDR1, HCDR2, LCDR1, LCDR2 and LCDR3.

Claim 1 (and to a certain extent claim 2 as well) thus relates to a large number of possible products. Support and disclosure within the meaning of Articles 5 and 6 PCT are to be found, however, for only a very small proportion of these products, namely antibodies comprising the combinations of six CDRs of exemplified antibodies as depicted in tables 2-5 of the application.

The examiner is unable to search the plethora of combinations claimed without undue burden, furthermore it is apparent that such a search would not be useful, since only an antibody defined by its exact 6 CDR sequences has any defined technical properties.

In the interest of clarity and for the purposes of a partial search, the applicant was requested in an invitation to provide informal clarification dated January 05th, 2022, to identify the subject-matter to be searched in more detail, e.g. with reference to antibodies defined by a complete set of exact CDR sequences. Applicant was informed that their comments would be considered when selecting the subject-matter of the search.

Applicant was also informed that in the event of failure to reply to the request for clarification, the search would be established for subject-matter relating to the first antibody detailed in the description, i.e. a human antibody or antibody fragment thereof specific for human CD3 characterized by the six CDR regions of exemplified antibody MAB-1 as depicted in table 2.

In reply to the invitation to provide informal clarification dated January 14th, 2022, applicant had identified the subject-matter to be searched as six exemplified antibodies, namely MAB-7, MAB8, MAB9, MAB-12, MAB-13 and MAB-16, each defined by a complete set of 6 CDR sequences and corresponding VH/VL sequences. Of note, applicant failed to specify whether the subject-matter to be searched consisted of antibodies comprising the combined 6 CDRs of the exemplified antibodies or of antibodies comprising the combined VH/VL regions thereof. However, this search authority was unable to identify a basis in the application as originally filed for antibodies merely comprising the 6 CDRs of antibodies MAB-7, MAB8, MAB9, MAB-12, MAB-13 and MAB-16. Table 17 which had been referred to by applicant in the letter of January 14th, 2022, relates to antibodies comprising the complete combined VH/VL sequences of MAB-7, MAB8, MAB9, MAB-12, MAB-13 and MAB-16.

The following opinion is thus limited to the claims insofar as they relate to antibodies having

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the VH/VL combinations of exemplified antibodies MAB-7, MAB8, MAB9, MAB-12, MAB-13 and MAB-16.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) PCT declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2021/076052

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2021/076052

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: **1-15 (partially)**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.