Humanized or chimeric CD3 antibodies

Field of invention

The present invention relates to a humanized or chimeric antibody binding to human CD3, compositions comprising said humanized or chimeric antibody, and use of said humanized or chimeric antibodies in treatment of a disease.

Background

The Cluster of Differentiation 3 (CD3) has been known for many years and therefore has been subject of interest in many aspects. Specifically antibodies raised against CD3 or the T cell Receptor Complex, which CD3 is part of, are known. An *in vitro* characterization of recombinant chimeric CD3 isotype variants as well as a number of humanized OKT3 effector function variant antibodies has been described [1].

CD3 antibodies, e.g. muromonab-CD3, have been widely used in the treatment of acute allograft rejection. In addition, treatment with the anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improved C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes in absence of continued immunosuppressive medications [2].

A promising approach to improve targeted antibody therapy is by delivering cytotoxic cells specifically to the antigen-expressing cancer cells. This concept of using T cells for efficient killing of tumor cells has been described [3]. However, initial clinical studies were rather disappointing mainly due to low efficacy, severe adverse effects (cytokine storm) and immunogenicity of the bispecific antibodies [4]. Advances in the design and application of bispecific antibodies have partially overcome the initial barrier of cytokine storm and improved clinical effectiveness without dose-limiting toxicities [5].

For example, certain bispecific antibodies targeting with one arm the antigen on the tumor cell and with the other arm for instance CD3 on T cells, and containing an active Fc fragment providing Fc receptor binding have been shown to induce tumor cell killing. Upon binding, a complex of T cells, tumor cells and effector cells that bind the antibody Fc region is potentially formed, leading to killing of the tumor cells [4]. Catumaxomab consists of a mouseIgG2a/ratIgG2b heavy chain heterodimer and has been found successful for the treatment of cancer-associated ascites after intraperitoneal application [6]. However, the mouse/rat hybrid is immunogenic [7] and cannot be applied for long-term treatment in humans. Frequent treatment-related adverse events attributed to catumaxomab included cytokine-release-related symptoms (i.e. pyrexia, nausea,

vomiting, chills, tachycardia and hypotension) [8]-[9], which relate the potent polyclonal T cell activation by catumaxomab due to its active Fc fragment. Another antibody is ertumaxomab (HER2xCD3), which induces cytotoxicity in cell lines with HER2 expression. Ertumaxomab has been in Phase II clinical development for metastatic breast cancer [10]-[11].

Efficacy of CD3 bispecific antibodies and other CD3 bispecific antibody-based formats is dependent on several properties of bispecific antibodies, such as the affinity of the CD3 arm and/or the affinity to the target of the second arm and the target copy number on target cells. Some CD3 bispecific antibodies show high efficacy when the CD3 affinity is low (EpCamxCD3 - Bortoletto 2002 PMID 12385030, MT103/Blinatumomab vs TandAb - Molhoj 2007 PMID 17083975), while other CD3 bispecifics demonstrate high efficacy using a high CD3 affinity (Reusch 2015, Mabs, PMID 25875246). In some cases high CD3 affinity is required, for example when arming ex vivo expanded activated T cells from patients with a bispecific antibody comprised of an anti-CD3 targeting arm and a second arm directed at a selected tumor-associated antigen. In the latter case, CD3 affinity should be high to retain the interaction with the expanded T cell when the product is infused back into the patient to mediate cytolysis of tumor cells (Reusch 2006 Clin Cancer Res PMID 16397041). However, high affinity antibodies to CD3, in contrast to low affinity ligands, are much less effective in TCR triggering at low copy number, since they display a stoichiometry of ~1:1 and a linear dose–response curve, indicative of a single cycle rather than a serial triggering mode of T cell response (Viola 1996 Science, PMID 8658175). In other words, low affinity of CD3 arm can allow T cells to flexibly move from one target and/or target cell to the other (Hoffman 2005, PMID: 15688411).

Low CD3 affinity can potentially prevent the biased localization of the bispecific antibody to T cells (due to first encounter in circulation) and thus improve biodistribution and minimize interference with normal T cell immune responses. Depending on the target of the second arm and target copy number, the indication and/or administration route, desirable CD3 affinity can be customized to enhance a product's maximum efficacy. A panel of CD3 variants covering a range of CD3 affinity can be essential to suit these specific tailor-made needs per antibody product.

CD3 antibodies cross-reactive to cynomolgus and/or rhesus monkey CD3 have been described [12]-[13], however, further improvements for such cross-reactive antibodies are needed.

Summary of invention

The the object of the present invention is to provide humanized or chimeric CD3 antibodies with an optimized affinity to CD3. Thus it is an object of the present invention to provide humanized or chimeric CD3 antibodies which are optimized compared to a reference antibody such as an antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8. Hence, such antibodies may have a reduced or increased affinity to CD3 compared to a reference antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8. It is a further object of the present invention to provide antibodies with a lower binding affinity to CD3 than the antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8. The inventors found that antibodies with a reduced binding affinity to the CD3 peptide as set forth in SEQ ID NO: 402 compared to a reference antibody having the VH region sequence set forth in SEQ ID NO: 4 manitain the same or similar cytotoxic activit in vitro and in vivo compared to the reference antibody. Another object of the present invention is to provide CD3 antibodies with reduced binding affinity to CD3 compared to a reference antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8, but retaining the same cytolytic activity as the reference antibody. It is yet another object of the present invention to provide antibodies with a higher binding affinity to CD3 than the antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8.

The present invention provides in one aspect a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region, wherein said VH region comprises a mutation in one of the three CDR sequences of a reference antibody having the VH CDR sequences set forth in CDR1 SEQ ID NO: 1, CDR2 SEQ ID NO: 2 and CDR3 SEQ ID NO: 3, which mutation is in one of the positions selected from the group consisting of: T31M, T31P, N57, H101, G105, S110 and Y114, wherein the positions are numbered according to the reference sequence of the SEQ ID NO: 4. The amino acids in SEQ ID NO: 4 are numbered accoding to a direct numerical numbering scheme from the first amino acid to number 125 in the direction from N-terminus to the C-terminus. The numerical numbering of positions corresponding to SEQ ID NO:4 is illustrated in figure 2. Further, the VH CDR regions have been annotated according to the IMGT definitions.

In one embodiment of the invention, the antibody has a reduced or increased binding affinity to human CD3 compared to the reference antibody having the VH CDR sequences set forth in CDR1 SEQ ID NO: 1, CDR2 SEQ ID NO: 2 and CDR3 SEQ ID NO: 3.

In some embodiements of the invention, an antibody with reduced bining affinity to a human CD3 molecule, such as a CD3 peptide e.g. SEQ ID NO:402, compared to a reference antibody may manintain the same cytolytic activity against a target cell as the reference antibody.

In one embodiment of the invention the antibody comprises a mutation in the position corresponding to N57 of SEQ ID NO: 4. In one embodiment the mutation is N57E.

In one embodiment of the invention the antibody comprises a mutation in the position corresponding to H101G of SEQ ID NO: 4. In one embodiment the mutation is H101G or H101N.

In one embodiment of the invention the antibody comprises a mutation in the position corresponding to G105 of SEQ ID NO: 4. In one embodiment the mutation is G105P.

In one embodiment of the invention the antibody comprises a mutation in the position corresponding to Y114 of SEQ ID NO: 4. In one embodiment the mutation is Y114M, Y114R or Y114V.

In one embodiment, the present invention provides a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising heavy chain variable (VH) region, wherein said VH region comprises theCDR1, CDR2, and CDR3 regions having the CDR sequences selected from one of the groups consisting of;

a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 12, 2, 3;
b) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 14, 2, 3;
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 16, 2, 3;
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 18, 2, 3;
e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 20, 2, 3;
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 22, 2, 3;
g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 24, 2, 3;
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 26, 2, 3;
i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 28, 2, 3;
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 30, 2, 3;
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 32, 2, 3;
l) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 34, 2, 3;
m) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 36, 2, 3;

n) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 38, 2, 3;
o) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 40, 2, 3;
p) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 42, 2, 3;
q) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 44, 2, 3;
r) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 46, 2, 3;
s) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 48, 2, 3;
t) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 50, 2, 3;
u) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 52, 2, 3;
v) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3;
w) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 56, 2, 3;
x) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3;
y) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 60, 2, 3;
z) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 62, 2, 3;
aa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 64, 2, 3;
bb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 66, 2, 3;
cc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 68, 2, 3;
dd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 70, 2, 3;
ee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 72, 2, 3;
ff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 74, 2, 3;
gg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 76, 2, 3;
hh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 78, 2, 3;
ii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 80, 2, 3;
jj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 82, 2, 3;
kk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 84, 2, 3;
ll) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 86, 2, 3;
mm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 88, 2, 3;
nn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 90, 2, 3;
oo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 92, 2, 3;
pp) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 94, 2, 3;
qq) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 96, 2, 3;
rr) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 98, 2, 3;
ss) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 100, 3;
tt) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 102, 3;
uu) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 104, 3;
vv) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3;

ww) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 108, 3;

xx) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 110, 3;

yy) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 112, 3;

zz) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 114, 3;

aaa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 116, 3;

bbb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 118, 3;

ccc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 120, 3;

ddd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 122, 3;

eee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 124, 3;

fff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 126, 3;

ggg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 128, 3;

hhh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 130, 3;

iii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 132, 3;

jjj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 134, 3;

kkk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 136, 3;

lll) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 138, 3;

mmm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 140, 3;

nnn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 142, 3;

ooo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 144, 3;

ppp) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 146, 3;

qqq) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 148, 3;

rrr) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 150, 3;

sss) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 152, 3;

ttt) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 154, 3;

uuu) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 156, 3;

vvv) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 158, 3;

www) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 160, 3;

xxx) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 162, 3;

yyy) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 164, 3;

zzz) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 166, 3;

aaaa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 168, 3;

bbbb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 170;

cccc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 172;

dddd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 174;

eeee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176;

ffff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 178;

gggg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 180;

hhhh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 182;

iiii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184;

jjjj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 186;

kkkk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 188;

llll) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 190;

mmmm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 192;

nnnn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 194;

oooo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 196;

pppp) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 198;

qqqq) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 200;

rrrr) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 202;

ssss) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 204;

tttt) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 206;

uuuu) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 208;

vvvv) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 210;

wwww) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 212;

xxxx) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 214;

yyyy) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 216;

zzzz) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 218;

aaaaa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220;

bbbbb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 222;

ccccc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 224;

ddddd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 226;

eeeee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 228;

fffff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 230;

ggggg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 232;

hhhhh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 234;

iiiii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236;

jjjjj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 238;

kkkkk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 240;

lllll) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 242;

mmmmm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244;

nnnnn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 246;

ooooo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 248;

ppppp) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 250;

qqqqq) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 252;

rrrrr) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 254;

sssss) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 256;

ttttt) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 258;

uuuuu) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 260;

vvvvv) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 262;

wwwww) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 264;

xxxxx) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 266;

yyyyy) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 268;

zzzzz) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 270;

aaaaaa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 272;

bbbbbb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 274;

cccccc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 276;

dddddd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 278;

eeeeee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 280;

ffffff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 282;

gggggg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284;

hhhhhh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 286;

iiiiii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 288;

jjjjjj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 290;

kkkkkk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292;

llllll) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 294;

mmmmmm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 296;

nnnnnn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 and

oooooo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 300.

That is, the inventors of the present invention in a first aspect of the invention found that humanized or chimeric antibodies of said sequences had an optimized binding affinity to a CD3 peptide SEQ ID NO: 402 compared to a reference antibody such as an antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8. The reference antibody specified by SEQ ID NO:4 and the VL sequence SEQ ID NO:8 has a binding affinity to the CD3 peptide of SEQ ID NO:402 of $1.5x10^{-8}$ M as illustrated by example 7. In some embodiments of the present invention the antibodies have a lower binding affinity to the CD3 peptide of SEQ ID NO:402 than $1.5x10^{-8}$ M such as a binding affinity from $1.6\ x10^{-8}$ M to $9.9x10^{-8}$ M or such as a binding affinity from $1.0x10^{-7}$ to $9.9x10^{-7}$ M when determined by Bio-Layer Interferometry as described in Table 6 in example 7. In some embodiments of the present invention the antibodies have a higher binding affinity to CD3 peptide of SEQ ID NO: 402 than $1.5x10^{-8}$ M, such as from $1.4\ x10^{-8}$ to $1.0\ x10^{-8}$ M, such as $9.9x10^{-9}$ to $1\ x10^{-9}$ M or such as $9.9x10^{-9}$ to $1\ x10^{-9}$ M . The binding affinity corresponds to the K_D value.

In one aspect of the present invention, the present invention relates to a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 regions having the CDR sequences selected from one of the groups consisting of;

a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M];
b) CDR1, CDR2 and CDR3 sequence set forth in SEQ ID NO: 58, 2, 3 [T31P];
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];

i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V] and
l) CDR1, CDR2 and CDR3 sequences having at least 90% or at least 95% amino acid sequence identity, in total across the three CDR sequences, to any one of the three CDR sequences as set forth in a) to k), provided that the CDR1, CDR2 and CDR3 sequences do not have the sequences as set forth in SEQ ID NO: 1, 2, 3.

In another aspect, the present invention relates to a humanized or chimeric antibody, wherein said binding region comprises a variable light chain (VL) region, wherein said VL region comprises CDR1, CDR2, and CDR3 regions having the CDR as set forth in SEQ ID NO: 6, GTN, 7.

In a further aspect, the present invention relates to a method of reducing the binding affinity of an antibody binding to human CD3 compared to a reference antibody comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 3, which method comprises introducing a mutation in one of the three CDR sequences of the said reference antibody selected from a mutation in one of the positions selected from the group of T31M, T31P, N57, H101, S110 and Y114, wherein the positions are numbered according to the reference sequence of the SEQ ID NO: 4.

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR1 region sequence corresponding to T31M or T31P. In another embodiment of the present invention the method comprises introducing a mutation in the VH region CDR2 region corresponding to N57E. In a further embodiment of the present invention the method comprises introducing a mutation in the VH region CDR3 region selected from H101G, H101N, G105P, S110A, S110G, Y114M, Y114R or Y114V.

In one embodiement CD3 is human CD3 epsilon..

In another aspect, the present invention relates to a bispecific antibody comprising a first binding region of an antibody according to the invention, and a second binding region which binds a different target than said first antigen binding region.

In another aspect, the present invention relates to a nucleic acid construct encoding one or more amino acid sequences according to the invention.

In another aspect, the present invention relates to an expression vector comprising (i) a nucleic acid sequence encoding a heavy chain sequence of a humanized or chimeric antibody according to the invention, (ii) a nucleic acid sequence encoding a light chain sequence of a humanized or chimeric antibody according to the invention, or (iii) both (i) and (ii).

In another aspect, the present invention relates to a host cell comprising an expression vector according to the invention.

In another aspect, the present invention relates to a composition comprising the antibody or bispecific antibody according to the invention.

In another aspect, the present invention relates to a pharmaceutical composition comprising the antibody or bispecific antibody according to the invention and a pharmaceutical acceptable carrier.

In another aspect, the present invention relates to the antibody or bispecific antibody, the composition, or the pharmaceutical composition according to the invention for use as a medicament.

In another aspect, the present invention relates to the antibody or bispecific antibody, the composition, or the pharmaceutical composition according to the invention for use in the treatment of a disease.

In another aspect, the present invention relates to a method of treatment of a disease comprising administering the antibody or bispecific antibody, the composition, or the pharmaceutical composition according to the invention, to a subject in need thereof.

In another aspect, the present invention relates to a metod of administering the antibody or bispecific antibody, wherein the antibody or bispecific antibody is administered by subcutaneuous or local administration.

In one aspect, the present invention relates to a method of diagnosing a disease characterized by involvement or accumulation of CD3-expressing cells, comprising administering the humanized or chimeric antibody, the composition or the pharmaceutical composition according to the invention to a subject, optionally wherein said humanized or chimeric antibody is labeled with a detectable agent.

In another aspect, the present invention relates to a method for producing an antibody or a bispecific antibody according to the invention, comprising the steps of a) culturing a host cell according to the invention, and b) purifying the antibody from the culture media.

In another aspect, the present invention relates to a diagnostic composition comprising an antibody or bispecific antibody according to any one of the embodiments as disclosed herein.
In one embodiment, the diagnostic composition is a companion diagnostic which is used to screen and select those patients who will benefit from treatment with the bispecific antibody.

In another aspect, the present invention relates to a method for detecting the presence of CD3 antigen, or a cell expressing CD3, in a sample comprising the steps of a) contacting the sample with an antibody or bispecific antibody according to the invention, under conditions that allow for formation of a complex between the antibody or bispecific antibody and CD3, and b) analyzing whether a complex has been formed.

In another aspect, the present invention relates to a kit for detecting the presence of CD3 antigen, or a cell expressing CD3, in a sample comprising i) an antibody or bispecific antibody according to the invention, and ii) instructions for use of the kit.

In another aspect, the present invention relates to an anti-idiotypic antibody or a pair of anti-idiotypic antibodies which bind to an antibody according to the invention.

Brief description of figures

Figure 1: Heat plot with binding ratios of mutant versus wt UniTE-huCD3-H1L1-T41K molecules. Ratios above 1 indicate stronger binding than wt, while ratios below 1 indicate weaker binding than wt. Binding was determined on Freestyle 293-F cells transfected with CD3/TCR-LC13.

Figure 2: Alignment of selected CD3 affinity variants in the generated library with mutations in the VH. CDRs are underlined in the humanized wild type sequence (SEQ ID NO:4) HuCD3-H1. Highlighted amino acids are the substitutions.

Figure 3: T cell binding curves of selected VH affinity variants of humanized CD3 (UniTE-huCD3-H1L1-T41K) antibodies as determined by flow cytometry Affinity variants depicted cover a broad range of T cell binding capacity between the wild type response and undetectable response.

Figure 4: T cell binding curves of selected VH affinity variants of humanized CD3 (UniTE-huCD3-H1L1-T41K) antibodies as determined by flow cytometry showing a very low, undetectable T cell binding.

Figure 5: T cell binding curves of selected VH affinity variants of humanized CD3 (BisG1-huCD3-H1L1-X-FEAL/1014-Herceptin-FEAR) antibodies as determined by flow cytometry. Affinity variants depicted cover a broad range of T cell binding capacity between the wild type response and undetectable response .

Figure 6: Cytotoxicity of CD3 affinity variants on solid tumor cell lines measured by alamar blue assay. (A) NCI-N87 cells, Effector cells (T cells): Tumor cell (NCI-N87 cell) ratio = 3:1 , 48 hours of incubation, n=2 donors (B) SKOV3 cells, T cells: SKOV3 cell ratio = 4:1 , 48 hours of incubation, n=2 donors (C) MDA-MB-231 cells, T cells : MDA-MB-231 cell ratio = 8:1 , 48 hours of incubation, n=2 donors. The tested affinity variants depicted cover a broad range of cytotoxicity between wild type response and no observed cytotoxicity for all tested tumor cell lines.

Figure 7: Cytotoxicity of CD3 affinity variants on a hematological (Daudi) cell line measured by chromium release assay. T cell: Daudi celll ratio = 10:1, 24 hours of incubation, 1 donor. The tested affinity variants depicted cover a broad range of cytotoxicity between wild type response and no observed cytotoxicity for the tested tumor cell line.

Figure 8: Cytotoxicity of CD3xHER2 bispecific antibodies in a NCI-N87, human PBMC co-engraftment model in NOD-SCID mice. HLA-A-matched human unstimulated PBMCs, as a source of human T cells, were co-inoculated with NCI-N87 tumor cells in NOD-SCID mice at two different dose levels of CD3 affinity antibodies (0.5 and 0.05 mg/kg). Humanized WT CD3 (huCD3) and 4 different CD3 affinity variants (N57E, H101K, S110A, Y114M) were tested. (A) Average tumor volume followed over time after treatment with 0.05 mg/kg of antibody (n=4 per group). (B) Average tumor volume followed over time after treatment with 0.5 mg/kg of antibody (n=4 per group). (C) Average tumor volume at day 44 after treatment with 0.05 mg/kg of antibody at day 0 (n=4 per group). (D) Average tumor volume at day 44 after treatment with 0.5 mg/kg of antibody at day 0 (n=4 per group). Statistics has been performed on data for C and D.

Detailed description

In one aspect, the present invention relates to a humanized or chimeric antibody binding to human CD3 with an optimized affinity to CD3. Thus it is an object of the present invention to provide humanized or chimeric CD3 antibodies which are optimized compared to a reference antibody such as the antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8. It is a further object of the invention to provide antibodies with optimized in vivo efficacy compared to a reference antibody such as the antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8. It is a further object of the present invention to provide antibodies with a lower binding affinity to CD3 than the antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8. It is yet another object of the present invention to provide antibodies with a higher binding affinity to CD3 than the antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8.

In one aspect the invention relaes to a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region, wherein said VH region comprises a mutation in one of the three CDR sequences of a reference antibody having the CDR sequences set forth in CDR1 SEQ ID NO: 1, CDR2 SEQ ID NO: 2 and CDR3 SEQ ID NO: 3, which mutation is in one of the positions selected from the group consisting of: T31M, T31P, N57, H101, G105, S110 and Y114, wherein the positions are numbered according to the reference sequence of the SEQ ID NO: 4. The amino acids in SEQ ID NO: 4 are numbered accoding to a direct numerical numbering scheme from the first amino acid to number 125 in the direction from N-terminus to the C-terminus. The numerical numbering of positions corresponding to SEQ ID NO: 4 is illustrated in figure 2. Further, The CDR regions have been annotated according to the IMGT defnitions.

In one embodiment of the invention, the antibody has a reduced or increased binding affinity to human CD3 compared to the reference antibody having the VH CDR sequences set forth in CDR1 SEQ ID NO: 1, CDR2 SEQ ID NO: 2 and CDR3 SEQ ID NO: 3.

In some embodiemtns of the invention, an antibody with reduced bining affinity to a CD3 molecule, such a CD3 peptide e.g. SEQ ID NO:402, compared to a reference antibody may manintain the same cytolytic activity against a target cell as the reference antibody.

In one embodiment of the invention the antibody comprises a T31M or T31P mutation. Position T31 is in accordance to SEQ ID NO:4.

In one embodiment of the invention the antibody comprises a mutation in the position N57. Position N57 is in accordance to SEQ ID NO:4. In one embodiment the mutation is N57E.

In one embodiment of the invention the antibody comprises a mutation in the position H101. Position H101 is in accordance to SEQ ID NO: 4. In one embodiment the mutation is H101G or H101N.

In one embodiment of the invention the antibody comprises a mutation in the position G105 . Position G105 is in accordance to SEQ ID NO: 4. In one embodiment the mutation is G105P.

In one embodiment of the invention the antibody comprises a mutation in the position Y114. Position Y114 is in accordance to of SEQ ID NO: 4. In one embodiment the mutation is Y114M, Y114R or Y114V.

The reference antibody specified by SEQ ID NO:4 and the VL sequence SEQ ID NO:8 has a binding affinity to the CD3 peptide of SEQ ID NO:402 corresponding to a K_D value of $1.5x10^{-8}$ M as illustrated by example 7.

In some embodiments of the present invention the antibodies have a lower binding affinity to the CD3 peptide of SEQ ID NO:402 than $1.5x10^{-8}$ M, such as a binding affinity from 1.6 $x10^{-8}$ M to $9.9x10^{-8}$ M or such as a binding affinity from $1.0x10^{-7}$ to $9.9x10^{-7}$ M when determined by Bio-Layer Interferometry as described in Example 7. In some embodiments of the present invention the antibodies have a higher binding affinity to the CD3 peptide of SEQ ID NO:402 than $1.5x10^{-8}$ M such as from $1.4x10^{-8}$ to $1.0x10^{-8}$ M, such as, such as such as $9.9x10^{-9}$ to 1.0 $x10^{-9}$ M.

In one embodiment, the present invention relates to a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 having the CDR sequences selected from one of the groups consisting of;

a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 12, 2, 3;
b) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 14, 2, 3;
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 16, 2, 3;
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 18, 2, 3;

e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 20, 2, 3;
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 22, 2, 3;
g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 24, 2, 3;
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 26, 2, 3;
i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 28, 2, 3;
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 30, 2, 3;
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 32, 2, 3;
l) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 34, 2, 3;
m) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 36, 2, 3;
n) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 38, 2, 3;
o) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 40, 2, 3;
p) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 42, 2, 3;
q) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 44, 2, 3;
r) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 46, 2, 3;
s) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 48, 2, 3;
t) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 50, 2, 3;
u) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 52, 2, 3;
v) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3;
w) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 56, 2, 3;
x) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3;
y) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 60, 2, 3;
z) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 62, 2, 3;
aa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 64, 2, 3;
bb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 66, 2, 3;
cc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 68, 2, 3;
dd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 70, 2, 3;
ee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 72, 2, 3;
ff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 74, 2, 3;
gg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 76, 2, 3;
hh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 78, 2, 3;
ii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 80, 2, 3;
jj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 82, 2, 3;
kk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 84, 2, 3;
ll) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 86, 2, 3;
mm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 88, 2, 3;
nn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 90, 2, 3;

oo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 92, 2, 3;
pp) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 94, 2, 3;
qq) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 96, 2, 3;
rr) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 98, 2, 3;
ss) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 100, 3;
tt) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 102, 3;
uu) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 104, 3;
vv) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3;
ww) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 108, 3;
xx) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 110, 3;
yy) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 112, 3;
zz) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 114, 3;
aaa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 116, 3;
bbb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 118, 3;
ccc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 120, 3;
ddd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 122, 3;
eee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 124, 3;
fff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 126, 3;
ggg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 128, 3;
hhh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 130, 3;
iii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 132, 3;
jjj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 134, 3;
kkk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 136, 3;
lll) CDR sequences set forth in SEQ ID NO: 1, 138, 3;
mmm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 140, 3;
nnn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 142, 3;

ooo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 144, 3;

ppp) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 146, 3;

qqq) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 148, 3;

rrr) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 150, 3;

sss) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 152, 3;

ttt) CDR sequences set forth in SEQ ID NO: 1, 154, 3;

uuu) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 156, 3;

vvv) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 158, 3;

www) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 160, 3;

xxx) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 162, 3;

yyy) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 164, 3;

zzz) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 166, 3;

aaaa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 168, 3;

bbbb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 170;

cccc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 172;

dddd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 174;

eeee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176;

ffff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 178;

gggg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 180;

hhhh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 182;

iiii) CDR sequences set forth in SEQ ID NO: 1, 2, 184;

jjjj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 186;

kkkk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 188;

llll) CDR sequences set forth in SEQ ID NO: 1, 2, 190;

mmmm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 192;

nnnn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 194;

oooo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 196;

pppp) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 198;

qqqq) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 200;

rrrr) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 202;

ssss) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 204;

tttt) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 206;

uuuu) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 208;

vvvv) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 210;

wwww) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 212;

xxxx) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 214;

yyyy) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 216;

zzzz) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 218;

aaaaa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220;

bbbbb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 222;

ccccc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 224;

ddddd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 226;

eeeee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 228;

fffff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 230;

ggggg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 232;

hhhhh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 234;

iiiii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236;

jjjjj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 238;

kkkkk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 240;

lllll) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 242;

mmmmm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244;

nnnnn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 246;

ooooo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 248;

ppppp) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 250;

qqqqq) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 252;

rrrrr) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 254;

sssss) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 256;

ttttt) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 258;

uuuuu) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 260;

vvvvv) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 262;

wwwww) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 264;

xxxxx) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 266;

yyyyy) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 268;

zzzzz) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 270;

aaaaaa) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 272;

bbbbbb) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 274;

cccccc) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 276;

dddddd) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 278;

eeeeee) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 280;

ffffff) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 282;

gggggg) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284;

hhhhhh) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 286;

iiiiii) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 288;

jjjjjj) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 290;

kkkkkk) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292;

llllll) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 294;

mmmmmm) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 296;

nnnnnn) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 and

oooooo) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 300.

In one embodiment, the present invention relates to a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 regions having the CDR sequences selected from one of the groups consisting of;

a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
b) CDR1, CDR2 and CDR3 sequence set forth in SEQ ID NO: 58, 2, 3 [T31P];
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V] and
l) CDR1, CDR2 and CDR3 sequences having at least 90% or at least 95% amino acid sequence identity, in total across the three CDR sequences, to

any one of the three CDR sequences as set forth in a) to k), provided that the CDR1, CDR2 and CDR3 sequences do not have the sequences as set forth in SEQ ID NO: 1, 2, 3.

In one embodiment, the present invention relates to a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 regions having the CDR sequences selected from one of the groups consisting of;

a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
b) CDR1, CDR2 and CDR3 sequence set forth in SEQ ID NO: 58, 2, 3 [T31P];
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V]; and
    a. and
l) CDR1, CDR2 and CDR3 sequences as specified in a) to k) having at most 5 further mutations or substitutions, at most 4 further mutations or substitutions, at most 3 further mutations or substitutions, at most 2 further mutations or substitutions, or at most 1 further mutation or substitution, in total across the three CDR sequences, and which mutations or substitutions preferably do not modify the binding affinity to human CD3.

In one embodiment, the present invention relates to a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region), wherein said VH region comprises one of the VH sequences from the group consisting of;

a) a VH sequence as set forth in SEQ ID NO: 13;
b) a VH sequence as set forth in SEQ ID NO: 15;
c) a VH sequence as set forth in SEQ ID NO: 17;
d) a VH sequence as set forth in SEQ ID NO: 19;
e) a VH sequence as set forth in SEQ ID NO: 21;
f) a VH sequence as set forth in SEQ ID NO: 23;
g) a VH sequence as set forth in SEQ ID NO: 25;
h) a VH sequence as set forth in SEQ ID NO: 27;
i) a VH sequence as set forth in SEQ ID NO: 29;
j) a VH sequence as set forth in SEQ ID NO: 31;
k) a VH sequence as set forth in SEQ ID NO: 33;
l) a VH sequence as set forth in SEQ ID NO: 35;
m) a VH sequence as set forth in SEQ ID NO: 37;
n) a VH sequence as set forth in SEQ ID NO: 39;
o) a VH sequence as set forth in SEQ ID NO: 41;
p) a VH sequence as set forth in SEQ ID NO: 43;
q) a VH sequence as set forth in SEQ ID NO: 45;
r) a VH sequence as set forth in SEQ ID NO: 47;
s) a VH sequence as set forth in SEQ ID NO: 49;
t) a VH sequence as set forth in SEQ ID NO: 51;
u) a VH sequence as set forth in SEQ ID NO: 53;
v) a VH sequence as set forth in SEQ ID NO: 55;
w) a VH sequence as set forth in SEQ ID NO: 57;
x) a VH sequence as set forth in SEQ ID NO: 59;
y) a VH sequence as set forth in SEQ ID NO: 61;
z) a VH sequence as set forth in SEQ ID NO: 63;
aa) a VH sequence as set forth in SEQ ID NO: 65;
bb) a VH sequence as set forth in SEQ ID NO: 67;
cc) a VH sequence as set forth in SEQ ID NO: 69;
dd) a VH sequence as set forth in SEQ ID NO: 71;
ee) a VH sequence as set forth in SEQ ID NO: 73;
ff) a VH sequence as set forth in SEQ ID NO: 75;
gg) a VH sequence as set forth in SEQ ID NO: 77;

hh) a VH sequence as set forth in SEQ ID NO: 79;
ii) a VH sequence as set forth in SEQ ID NO: 81;
jj) a VH sequence as set forth in SEQ ID NO: 83;
kk) a VH sequence as set forth in SEQ ID NO: 85;
ll) a VH sequence as set forth in SEQ ID NO: 87;
mm) a VH sequence as set forth in SEQ ID NO: 89;
nn) a VH sequence as set forth in SEQ ID NO: 91;
oo) a VH sequence as set forth in SEQ ID NO: 93;
pp) a VH sequence as set forth in SEQ ID NO: 95;
qq) a VH sequence as set forth in SEQ ID NO: 97;
rr) a VH sequence as set forth in SEQ ID NO: 99;
ss) a VH sequence as set forth in SEQ ID NO: 101;
tt) a VH sequence as set forth in SEQ ID NO: 103;
uu) a VH sequence as set forth in SEQ ID NO: 105;
vv) a VH sequence as set forth in SEQ ID NO: 107;
ww) a VH sequence as set forth in SEQ ID NO: 109;
xx) a VH sequence as set forth in SEQ ID NO: 111;
yy) a VH sequence as set forth in SEQ ID NO: 113;
zz) a VH sequence as set forth in SEQ ID NO: 115;
aaa) a VH sequence as set forth in SEQ ID NO: 117;
bbb) a VH sequence as set forth in SEQ ID NO: 119;
ccc) a VH sequence as set forth in SEQ ID NO: 121;
ddd) a VH sequence as set forth in SEQ ID NO: 123;
eee) a VH sequence as set forth in SEQ ID NO: 125;
fff) a VH sequence as set forth in SEQ ID NO: 127;
ggg) a VH sequence as set forth in SEQ ID NO: 129;
hhh) a VH sequence as set forth in SEQ ID NO: 131;
iii) a VH sequence as set forth in SEQ ID NO: 133;
jjj) a VH sequence as set forth in SEQ ID NO: 135;
kkk) a VH sequence as set forth in SEQ ID NO: 137;
lll) a VH sequence as set forth in SEQ ID NO: 139;
mmm) a VH sequence as set forth in SEQ ID NO: 141;
nnn) a VH sequence as set forth in SEQ ID NO: 143;
ooo) a VH sequence as set forth in SEQ ID NO: 145;
ppp) a VH sequence as set forth in SEQ ID NO: 147;
qqq) a VH sequence as set forth in SEQ ID NO: 149;
rrr) a VH sequence as set forth in SEQ ID NO: 151;

sss) a VH sequence as set forth in SEQ ID NO: 153;

ttt) a VH sequence as set forth in SEQ ID NO: 155;

uuu) a VH sequence as set forth in SEQ ID NO: 157;

vvv) a VH sequence as set forth in SEQ ID NO: 159;

www) a VH sequence as set forth in SEQ ID NO: 161;

xxx) a VH sequence as set forth in SEQ ID NO: 163;

yyy) a VH sequence as set forth in SEQ ID NO: 165;

zzz) a VH sequence as set forth in SEQ ID NO: 167;

aaaa) a VH sequence as set forth in SEQ ID NO: 169;

bbbb) a VH sequence as set forth in SEQ ID NO: 171;

cccc) a VH sequence as set forth in SEQ ID NO: 173;

dddd) a VH sequence as set forth in SEQ ID NO: 175;

eeee) a VH sequence as set forth in SEQ ID NO: 177;

ffff) a VH sequence as set forth in SEQ ID NO: 179;

gggg) a VH sequence as set forth in SEQ ID NO: 181;

hhhh) a VH sequence as set forth in SEQ ID NO: 183;

iiii) a VH sequence as set forth in SEQ ID NO: 185;

jjjj) a VH sequence as set forth in SEQ ID NO: 187;

kkkk) a VH sequence as set forth in SEQ ID NO: 189;

llll) a VH sequence as set forth in SEQ ID NO: 191;

mmmm) a VH sequence as set forth in SEQ ID NO: 193;

nnnn) a VH sequence as set forth in SEQ ID NO: 195;

oooo) a VH sequence as set forth in SEQ ID NO: 197;

pppp) a VH sequence as set forth in SEQ ID NO: 199;

qqqq) a VH sequence as set forth in SEQ ID NO: 201;

rrrr) a VH sequence as set forth in SEQ ID NO: 203;

ssss) a VH sequence as set forth in SEQ ID NO: 205;

tttt) a VH sequence as set forth in SEQ ID NO: 207;

uuuu) a VH sequence as set forth in SEQ ID NO: 209;

vvvv) a VH sequence as set forth in SEQ ID NO: 211;

wwww) a VH sequence as set forth in SEQ ID NO: 213;

xxxx) a VH sequence as set forth in SEQ ID NO: 215;

yyyy) a VH sequence as set forth in SEQ ID NO: 217;

zzzz) a VH sequence as set forth in SEQ ID NO: 219;

aaaaa) a VH sequence as set forth in SEQ ID NO: 221;

bbbbb) a VH sequence as set forth in SEQ ID NO: 223;

ccccc) a VH sequence as set forth in SEQ ID NO: 225;

ddddd) a VH sequence as set forth in SEQ ID NO: 227;
eeeee) a VH sequence as set forth in SEQ ID NO: 229;
fffff) a VH sequence as set forth in SEQ ID NO: 221;
ggggg) a VH sequence as set forth in SEQ ID NO: 223;
hhhhh) a VH sequence as set forth in SEQ ID NO: 225;
iiiii) a VH sequence as set forth in SEQ ID NO: 227;
jjjjj) a VH sequence as set forth in SEQ ID NO: 229;
kkkkk) a VH sequence as set forth in SEQ ID NO: 231;
lllll) a VH sequence as set forth in SEQ ID NO: 233;
mmmmm) a VH sequence as set forth in SEQ ID NO: 235;
nnnnn) a VH sequence as set forth in SEQ ID NO: 237;
ooooo) a VH sequence as set forth in SEQ ID NO: 239;
ppppp) a VH sequence as set forth in SEQ ID NO: 241;
qqqqq) a VH sequence as set forth in SEQ ID NO: 243;
rrrrr) a VH sequence as set forth in SEQ ID NO: 245;
sssss) a VH sequence as set forth in SEQ ID NO: 247;
ttttt) a VH sequence as set forth in SEQ ID NO: 249;
uuuuu) a VH sequence as set forth in SEQ ID NO: 251;
vvvvv) a VH sequence as set forth in SEQ ID NO: 253;
wwwww) a VH sequence as set forth in SEQ ID NO: 255;
xxxxx) a VH sequence as set forth in SEQ ID NO: 257;
yyyyy) a VH sequence as set forth in SEQ ID NO: 259;
zzzzz) a VH sequence as set forth in SEQ ID NO: 261;
aaaaaa) a VH sequence as set forth in SEQ ID NO: 263;
bbbbbb) a VH sequence as set forth in SEQ ID NO: 265;
cccccc) a VH sequence as set forth in SEQ ID NO: 267;
dddddd) a VH sequence as set forth in SEQ ID NO: 269;
eeeeee) a VH sequence as set forth in SEQ ID NO: 271;
ffffff) a VH sequence as set forth in SEQ ID NO: 273;
gggggg) a VH sequence as set forth in SEQ ID NO: 275;
hhhhhh) a VH sequence as set forth in SEQ ID NO: 277;
iiiiii) a VH sequence as set forth in SEQ ID NO: 279;
jjjjjj) a VH sequence as set forth in SEQ ID NO: 281;
kkkkkk) a VH sequence as set forth in SEQ ID NO: 283;
llllll) a VH sequence as set forth in SEQ ID NO: 285;
mmmmmm) a VH sequence as set forth in SEQ ID NO: 287;
nnnnnn) a VH sequence as set forth in SEQ ID NO: 289;

oooooo) a VH sequence as set forth in SEQ ID NO: 291;
pppppp) a VH sequence as set forth in SEQ ID NO: 293;
qqqqqq) a VH sequence as set forth in SEQ ID NO: 295;
rrrrrr) a VH sequence as set forth in SEQ ID NO: 297;
ssssss) a VH sequence as set forth in SEQ ID NO: 299 and
tttttt) a VH sequence as set forth in SEQ ID NO: 301.

In one embodiment, the present invention relates to a humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region, wherein said VH region comprises one of the VH sequences selected from the group consisting of;

a) a VH sequence as set forth in SEQ ID NO: 55[T31M],
b) a VH sequence as set forth in SEQ ID NO: 59 [T31P],
c) a VH sequence as set forth in SEQ ID NO: 107 [N57E]
d) a VH sequence as set forth in SEQ ID NO: 177 [H101G],
e) a VH sequence as set forth in SEQ ID NO: 185 [H101N],
f) a VH sequence as set forth in SEQ ID NO: 221 [G105P],
g) a VH sequence as set forth in SEQ ID NO: 237 [S110A],
h) a VH sequence as set forth in SEQ ID NO: 245 [S110G],
i) a VH sequence as set forth in SEQ ID NO: 285 [Y114M],
j) a VH sequence as set forth in SEQ ID NO: 293 [Y114R], and
k) a VH sequence as set forth in SEQ ID NO: 299 [Y114V].

In one embodiment of the invention the humanized or chimeric antibody comprises a binding region, wherein said binding region comprises a variable light chain (VL) region, wherein said VL region comprises the CDR1, CDR2, and CDR3 having the CDR sequences selected from the group consisting of;

a) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 7;
b) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 302, GTN, 7;
c) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 304, GTN, 7;
d) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 306, GTN, 7;
e) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 308, GTN, 7;
f) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 310, GTN, 7;
g) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 312, GTN, 7;

h) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 314, GTN, 7;
i) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 316, GTN, 7;
j) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 318, GTN, 7;
k) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 320, GTN, 7;
l) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 322, GTN, 7;
m) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 324, GTN, 7;
n) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 326, GTN, 7;
o) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 328, GTN, 7;
p) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 330, GTN, 7;
q) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 332;
r) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 334;
s) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 336;
t) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 338;
u) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 340;
v) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 342;
w) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 344;
x) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 346;
y) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 348;
z) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 350;
aa) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 352;
bb) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 354;
cc) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 356;
dd) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 358;
ee) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 360;
ff) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 362;
gg) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 364;
hh) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 366;
ii) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 368;
jj) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 370;
kk) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 372;
ll) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 374;
mm) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 376;
nn) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 378;
oo) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 380;
pp) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 382;
qq) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 384;
rr) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 386;

ss) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 388;
tt) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 390;
uu) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 392 and
vv) CDR1, CDR2 and CDR3 sequences as set forth in SEQ ID NO: 6, GTN, 394.

In another embodiment of the invention the humanized or chimeric antibody comprises a binding region comprising a variable light chain (VL) region, wherein said VL region comprises one of the VL sequences selected from the group consisting of;

a) a VL sequence as set forth in SEQ ID NO:8; and
b) a VL sequence as set forth in SEQ ID NO:10;

The term "antibody" as used herein is intended to refer to an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative of either thereof, which has the ability to specifically bind to an antigen under typical physiological conditions with a half-life of significant periods of time, such as at least about 30 minutes, at least about 45 minutes, at least about one hour, at least about two hours, at least about four hours, at least about 8 hours, at least about 12 hours, about 24 hours or more, about 48 hours or more, about 3, 4, 5, 6, 7 or more days, etc., or any other relevant functionally-defined period (such as a time sufficient to induce, promote, enhance, and/or modulate a physiological response associated with antibody binding to the antigen and/or time sufficient for the antibody to recruit an effector activity). The binding region (or binding domain which may also be used herein, both terms having the same meaning) which interacts with an antigen, comprises variable regions of both the heavy and light chains of the immunoglobulin molecule. The constant regions of the antibodies (Abs) may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as effector cells and T cells) and components of the complement system such as C1q, the first component in the classical pathway of complement activation. As indicated above, the term antibody as used herein, unless otherwise stated or clearly contradicted by context, includes fragments of an antibody that retain the ability to specifically interact, such as bind, to the antigen. It has been shown that the antigen-binding function of an antibody may be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antibody" include (i) a Fab' or Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains, or a monovalent antibody as described in WO2007059782 (Genmab A/S); (ii) $F(ab')_2$ fragments, bivalent fragments comprising two Fab fragments linked by a

disulfide bridge at the hinge region; (iii) a Fd fragment consisting essentially of the V_H and C_H1 domains; and (iv) a Fv fragment consisting essentially of the V_L and V_H domains of a single arm of an antibody. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see for instance Bird et al., Science 242, 423-426 (1988) and Huston et al., PNAS USA 85, 5879-5883 (1988)). Such single chain antibodies are encompassed within the term antibody unless otherwise noted or clearly indicated by context. Although such fragments are generally included within the meaning of antibody, they collectively and each independently are unique features of the present invention, exhibiting different biological properties and utility. These and other useful antibody fragments in the context of the present invention are discussed further herein. It also should be understood that the term antibody, unless specified otherwise, also includes polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies and humanized antibodies, and antibody fragments retaining the ability to specifically bind to the antigen (antigen-binding fragments) provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques. An antibody as generated can possess any isotype.

The term “immunoglobulin heavy chain”, “heavy chain of an immunoglobulin” or “heavy chain” as used herein is intended to refer to one of the chains of an immunoglobulin. A heavy chain is typically comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region (abbreviated herein as CH) which defines the isotype of the immunoglobulin. The heavy chain constant region typically is comprised of three domains, CH1, CH2, and CH3. The heavy chain constant region may further comprise a hinge region. The term “immunoglobulin” as used herein is intended to refer to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) chains and one pair of heavy (H) chains, all four potentially inter-connected by disulfide bonds. The structure of immunoglobulins has been well characterized (see for instance [14]). Within the structure of the immunoglobulin (e.g. IgG), the two heavy chains are inter-connected via disulfide bonds in the so-called “hinge region”. Equally to the heavy chains each light chain is typically comprised of several regions; a light chain variable region (abbreviated herein as VL) and a light chain constant region (abbreviated herein as CL). The light chain constant region typically is comprised of one domain, CL. Furthermore, the VH and VL regions may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form structurally defined loops), also termed complementarity

determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see [15]). CDR sequences may be determined by use of the method provided by IMGT [16]-[17].

The term "isotype" as used herein, refers to the immunoglobulin (sub)class (for instance IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM) or any allotype thereof, such as IgG1m(za) and IgG1m(f) [SEQ ID NO:407]) that is encoded by heavy chain constant region genes. Thus, in one embodiment, the antibody comprises a heavy chain of an immunoglobulin of the IgG1 class or any allotype thereof. Further, each heavy chain isotype can be combined with either a kappa (κ) or lambda (λ) light chain.

The term "chimeric antibody" as used herein, refers to an antibody wherein the variable region is derived from a non-human species (e.g. derived from rodents) and the constant region is derived from a different species, such as human. Chimeric antibodies may be generated by antibody engineering. "Antibody engineering" is a generic term used for different kinds of modifications of antibodies, and which is a well-known process for the skilled person. In particular, a chimeric antibody may be generated by using standard DNA techniques as described in [18]. Thus, the chimeric antibody may be a genetically engineered recombinant antibody. Some chimeric antibodies may be both genetically or an enzymatically engineered. It is within the knowledge of the skilled person to generate a chimeric antibody, and thus, generation of the chimeric antibody according to the present invention may be performed by other methods than described herein. Chimeric monoclonal antibodies for therapeutic applications are developed to reduce antibody immunogenicity. They may typically contain non-human (e.g. murine) variable regions, which are specific for the antigen of interest, and human constant antibody heavy and light chain domains. The terms "variable region" or "variable domains" as used in the context of chimeric antibodies, refers to a region which comprises the CDRs and framework regions of both the heavy and light chains of the immunoglobulin.

The term "humanized antibody" as used herein, refers to a genetically engineered non-human antibody, which contains human antibody constant domains and non-human variable domains modified to contain a high level of sequence homology to human variable domains. This can be achieved by grafting of the six non-human antibody complementarity-determining regions (CDRs), which together form the antigen binding site, onto a homologous human acceptor framework region (FR) (see [19]-[20]). In order to fully reconstitute the binding affinity and specificity of the parental antibody, the substitution of framework residues from the parental antibody (i.e. the non-human antibody) into the human framework regions (back-mutations) may be required. Structural

homology modeling may help to identify the amino acid residues in the framework regions that are important for the binding properties of the antibody. Thus, a humanized antibody may comprise non-human CDR sequences, primarily human framework regions optionally comprising one or more amino acid back-mutations to the non-human amino acid sequence, and fully human constant regions. Optionally, additional amino acid modifications, which are not necessarily back-mutations, may be applied to obtain a humanized antibody with preferred characteristics, such as affinity and biochemical properties.

The humanized or chimeric antibody according to any aspect or embodiment of the present invention may be termed “humanized or chimeric CD3 antibody”, “humanized or chimeric antibody of the invention”, “CD3 antibody”, or “CD3 antibody of the invention”, which all have the same meaning and purpose unless otherwise contradicted by context.

The amino acid sequence of an antibody of non-human origin is distinct from antibodies of human origin, and therefore a non-human antibody is potentially immunogenic when administered to human patients. However, despite the non-human origin of the antibody, its CDR segments are responsible for the ability of the antibody to bind to its target antigen and humanization aims to maintain the specificity and binding affinity of the antibody. Thus, humanization of non-human therapeutic antibodies is performed to minimize its immunogenicity in man while such humanized antibodies at the same time maintain the specificity and binding affinity of the antibody of non-human origin.

The term “binding region” as used herein, refers to a region of an antibody which is capable of binding to any molecule, such as a polypeptide, e.g. present on a cell, bacterium, or virion.

The term "binding" as used herein, refers to the binding of an antibody to a predetermined antigen or target to which binding typically is with an affinity corresponding to a K_D of about 10^{-6} M or less, e.g. 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, about 10^{-10} M or less, or about 10^{-11} M or even less when determined by for instance surface plasmon resonance (SPR) technology in a BIAcore 3000 instrument using the antigen as the ligand and the antibody as the analyte, and binds to the predetermined antigen with an affinity corresponding to a K_D that is at least ten-fold lower, such as at least 100 fold lower, for instance at least 1,000 fold lower, such as at least 10,000 fold lower, for instance at least 100,000 fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The degree with which the affinity is lower is dependent on the K_D of the antibody, so that when the K_D of the antibody is very low (that is, the antibody is highly

specific), then the degree with which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000 fold. The term "K_D" (M), as used herein, refers to the dissociation equilibrium constant of a particular antibody-antigen interaction.

The term "human CD3" as used herein, refers to the human Cluster of Differentiation 3 protein which is part of the T cell co-receptor protein complex and is composed of four distinct chains. CD3 is also found in other species, and thus, the term "CD3" may be used herein and is not limited to human CD3 unless contradicted by context. In mammals, the complex contains a CD3γ (gamma) chain (human CD3γ chain Swissprot P09693, or cynomolgus monkey CD3γ Swissprot Q95LI7), a CD3δ (delta) chain (human CD3δ Swissprot P04234, or cynomolgus monkey CD3δ Swissprot Q95LI8), two CD3ε (epsilon) chains (human CD3ε Swissprot P07766; or cynomolgus CD3ε Swissprot Q95LI5), rhesus CD3ε (Swissprot G7NCB9), and a CD3ζ-chain (zeta) chain (human CD3ζ Swissprot P20963, cynomolgus monkey CD3ζ Swissprot Q09TK0). These chains associate with a molecule known as the T cell receptor (TCR) and generate an activation signal in T lymphocytes. The TCR and CD3 molecules together comprise the TCR complex.

It is within the knowledge of the skilled person that amino acid sequences referred to as Swissprot numbers include a signal peptide which is removed after translation of the protein. Thus, proteins, such as CD3, present on cell surfaces do not include the signal peptide. In particular, the amino acid sequences listed in Table 1 do not contain such signal peptide. Such proteins as listed in Table 1 may be termed "mature proteins". Thus, SEQ ID NO:398 shows the amino acid sequence of mature human CD3δ (delta), SEQ ID NO:399 shows the amino acid sequence of mature human CD3ε (epsilon), SEQ ID NO:403 shows the amino acid sequence of mature cynomolgus CD3ε, and SEQ ID NO:404 shows the amino acid sequence of mature rhesus CD3ε. Thus, the term "mature" as used herein, refers to a protein which does not comprise any signal or leader sequence.

It is well-known that signal peptide sequence homology, length, and the cleavage site position, varies significantly between different proteins. Signal peptides may be determined by different methods, e.g. SEQ ID NO:399 of the present invention has been determined according to the SignalP application (available on http://www.cbs.dtu.dk/services/SignalP/).

In a particular embodiment, the humanized or chimeric antibody of the present invention binds the epsilon chain of CD3, such as the epsilon chain of human CD3 (SEQ ID NO:399). In yet another particular embodiment, the humanized or chimeric antibody binds an epitope within amino acids 1-27 of the N-terminal part of human CD3ε (epsilon) (SEQ ID NO:402). In such a particular embodiment, the antibody may even further cross-react with other non-human primate species, such as cynomolgus monkeys

(cynomolgus CD3 epsilon SEQ ID NO:403) and/or rhesus monkeys (rhesus CD3 epsilon SEQ ID NO:404).

An antibody according to the present invention comprising the CDR sequences as defined herein, further comprising framework regions may differ in sequence outside the CDR sequences but still retains the full binding ability as compared to the original antibody. Thus, the present invention also relates to antibodies comprising an amino acid sequence of the variable region having a certain sequence identity to any sequence herein described.

The term "sequence identity" as used in the context of the present invention, refers to the percent identity between two sequences as a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The percent identity between two nucleotide or amino acid sequences may e.g. be determined using the algorithm of E. Meyers and W. Miller [21]. In addition, the percent identity between two amino acid sequences may be determined using the Needleman and Wunsch algorithm [22]. Multiple alignments are preferably performed using the Clustal W algorithm [23] (as used e.g., in Vector NTI Advance® software version 11.5; Invitrogen Inc.).

Thus, in one embodiment of the present invention, the antibody comprises a binding region comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1 , CDR2, and CDR3 regions having the three CDR sequences selected from one of the groups consisting of;

a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
b) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3 [T31P];
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];

i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V]; and
l) CDR1, CDR2 and CDR3 sequences having at least 90% or at least 95% amino acid sequence identity, in total across the three CDR sequences, to any one of the three CDR sequences as set forth in a) to k), provided that the CDR1, CDR2 and CDR3 sequences do not have the sequences as set forth in SEQ ID NO: 1, 2, 3.

The VH region, as illustrate in the sequence table 1 of the present document, consists of 125 amino acid sequence. Thus a second VH sequence consisting of 125 amino acids whereof 124 amino acid postions are identical with the one of the first VH sequences listed above has 99,2% sequence identity with said first VH sequence. A second sequence consisting of 125 amino acids whereof 120 amino acid positons are identical with one of the first VH sequences listed above have 96% sequence identity with said first VH sequence. A second sequence consisting of 125 amino acids whereof 115 amino acid postions are identical with one of the first VH sequences listed above have 92% sequence identity with said first VH sequence.

In a particular embodiment thereof, the VH region has at least 96% amino acid sequence identity to at least one of the VH sequences as specified in said group.

In one embodiment of the invention the mutations are located in the frame work regions of the VH region. Hence, in some embodiments the three CDR sequences of the VH region are 100% identical to the antibodies of the present invention, but amino acid variation may occure in the frame work reigon of the VH region. Such amino acid variation in the frame work region may preferably not change the binding affiintiy of the antibody to CD3 compare to the antibody when the CDRs are comprised in the reference frame of SEQ ID NO: 407.

The mutations in the VH sequence causing variations in the sequcence identity may preferabley be conservative, physical or functional amino acids. Substituting amino acids

with similar amino acids may increase the likelihood of keeping the functionality of the parent antibody.

In one embodiment of the invention the antibody is a humanized antibody.

In one embodiment of the invention the antibody is a full-length antibody.

The humanized antibody according to the present invention may be generated by comparison of the heavy and light chain variable region amino acid sequences against a database of human germline variable region sequences in order to identify the heavy and light chain human sequence with the appropriate degree of homology for use as human variable framework regions. A series of humanized heavy and light chain variable regions may be designed by grafting, e.g. the murine, CDRs onto the framework regions (identified as described above) and, if necessary, by back-mutation (mutation of one or more of the human amino acid residues in the framework regions back to the non-human amino acid at the specific position(s)) to the specific murine sequence of residues identified which may be critical to the restoration of the antibody binding efficiency. Variant sequences with the lowest incidence of potential T cell epitopes may then be selected as determined by application of *in silico* technologies; iTope™ and TCED™ ([24], [25], and [26]).

Furthermore, the humanized antibodies according to the present invention may also be "deimmunized". Deimmmunization may be desired, as within a protein sequence, such as a humanized antibody according to the present invention, the presence of human T cell epitopes may increase the immunogenicity risk profile as they have the potential to activate helper T cells. Such activation of helper T cells may be avoided by deimmunization. Deimmunization may be performed by introducing a mutation in the amino acid sequence of the humanized antibody in order to remove the T cell epitopes without significantly reducing the binding affinity of the antibody.

Thus, in one embodiment of the present invention, the humanized antibody may be produced by a method comprising the steps of (i) comparing the non-human full variable heavy chain sequence and/or the full variable light chain sequence to a database of human germline sequences, (ii) selecting the human germline sequence having the highest homology to the non-human sequence to obtain a humanized sequence, (iii) optimizing the humanized sequence by back-mutation(s) if required, and (iv) expressing the sequence in a suitable expression system.

Thus, a full-length antibody according to the present invention may be produced by a method comprising the steps of (i) comparing the non-human variable heavy chain sequence and the variable light chain sequences to a database of human germline sequences, (ii) selecting the human germline sequence having the highest homology to the non-human sequence, (iii) grafting of the non-human CDRs in to the selected human germ-line to obtain a humanized sequence, (iv) optimizing the humanized sequences by back-mutation(s) if required, (v) identifying constant heavy and light chain sequences, and (vi) expressing the complete heavy chain sequences and complete light chain sequences in suitable expression systems. A full-length antibody according to the present invention may, thus, be produced as described in Example 1. It is within the knowledge of the skilled person to produce a full-length antibody when starting out from either CDR sequences or full variable region sequences. Thus, the skilled person would know how to generate a full-length antibody according to the present invention.

The term "complete heavy chain sequences" as used herein, refers to a sequence consisting of variable heavy chain and constant heavy chain sequences.

The term "complete light chain sequences" as used herein, refers to a sequence consisting of variable light chain and constant light chain sequences.

Back-mutation(s) may be introduced by standard DNA mutagenesis. Such standard techniques for DNA mutagenesis are described in [18]. Alternatively, use of commercially available kits such as Quickchange™ Site-Directed Mutagenesis Kit (Stratagene), or the desired back-mutations may be introduced by de novo DNA synthesis.

Thus, in one embodiment, the antibody is a humanized antibody.

Chimeric antibodies may be generated by substituting all constant region sequences of a non-human (such as murine) antibody with constant region sequences of human origin. Thus, fully non-human variable region sequences are maintained in the chimeric antibody. Thus, a chimeric antibody according to the present invention may be produced by a method comprising the step of expressing the non-human variable heavy chain (SEQ ID NO:405), non-human variable light chain sequences (SEQ ID NO:406), human constant heavy chain and human constant light chain sequences in suitable expression systems, and thereby generating a full-length chimeric antibody. Alternative methods may be used. Such methods of producing a chimeric antibody is within the knowledge of the skilled person, and thus, the skilled person would know how to produce a chimeric antibody according to the present invention. Thus to make a chimeric antibody according to the present invention one would introduce the mutations according to the invention in the non-human (such as murine) VH or VL sequence.

Thus, in one embodiment, the antibody is a chimeric antibody.

In one embodiment, the antibody is a full-length antibody. The term "full-length antibody" as used herein, refers to an antibody (e.g., a parent or variant antibody) which contains all heavy and light chain constant and variable domains correspond to those that are normally found in a wild-type antibody of that isotype.

In one embodiment, the antibody comprises an Fc region comprising a first and a second immunoglobulin heavy chain.

The term "Fc region" as used herein, refers to a region comprising, in the direction from the N- to C-terminal, at least a hinge region, a CH2 region and a CH3 region. An Fc region may further comprise a CH1 region at the N-terminal end of the hinge region.

The term "hinge region" as used herein refers to the hinge region of an immunoglobulin heavy chain. Thus, for example the hinge region of a human IgG1 antibody corresponds to amino acids 216-230 according to the Eu numbering as set forth in Kabat.

Unless otherwise stated or contradicted by context, the amino acids of the constant region sequences are herein numbered according to the Eu-index of numbering (described in [27]) and may be termed "according to the Eu numbering as set forth in Kabat", "Eu numbering according to Kabat", or "according to the Eu numbering system".

The term "CH1 region" or "CH1 domain" as used herein, refers to the CH1 region of an immunoglobulin heavy chain. Thus, for example the CH1 region of a human IgG1 antibody corresponds to amino acids 118-215 according to the Eu numbering system. However, the CH1 region may also be any of the other subtypes as described herein.

The term "CH2 region" or "CH2 domain" as used herein, refers to the CH2 region of an immunoglobulin heavy chain. Thus, for example the CH2 region of a human IgG1 antibody corresponds to amino acids 231-340 according to the Eu numbering system. However, the CH2 region may also be any of the other subtypes as described herein.

The term "CH3 region" or "CH3 domain" as used herein, refers to the CH3 region of an immunoglobulin heavy chain. Thus, for example the CH3 region of a human IgG1 antibody corresponds to amino acids 341-447 according to the Eu numbering system. However, the CH3 region may also be any of the other subtypes as described herein.

In one embodiment, the isotype of the immunoglobulin heavy chain is selected from the group consisting of IgG1, IgG2, IgG3, and IgG4. The immunoglobulin heavy chain may be any allotype within each of the immunoglobulin classes, such as IgG1m(f) (SEQ ID NO:407). Thus, in one particular embodiment, the isotype of the

immunoglobulin heavy chains is an IgG1, or any allotype thereof, such as IgG1m(f) (SEQ ID NO:407).

When targeting the antigen CD3 which is part of the T cell Receptor (TCR), the T cell specific mechanisms of cell killing is desirable. Other effector functions, e.g. complement activation, may not be wanted, and therefore, reduction of effector functions is desirable. C1q binding is the first step in the complement cascade, and therefore serves as an indicator for complement-dependent cytotoxicity (CDC) capacity of antibodies. If binding of C1q to the antibody can be avoided, activation of the complement cascade can be avoided as well.

Thus, in one embodiment, the antibody comprises an Fc region which has been modified so that binding of C1q to said antibody is reduced compared to a wild-type antibody by at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 99%, at least 99,9% or 100%, wherein C1q binding is determined by ELISA. In a prefered embodiement the antibody comprises an Fc region, which has been modiefied so that the binding of C1q to said antibody is reduced compared to a wild-type antibody by at least 99% to a 100%, wherein C1q binding is determined by ELISA.

The term "modified" as used herein, refers to the amino acid sequence of an Fc region which is not identical to the amino acid sequence of a wild-type Fc region. I.e. amino acid residues in specific positions of the wild-type Fc region have been substituted, deleted or inserted in order to alter, for example, the binding site for C1q, binding site for other effector molecules or binding to Fc Receptors (FcRs). Such modification(s) of the amino acid sequence may be prepared by substituting one or more amino acids with a conservative amino acid or may be prepared by substituting one or more amino acids with an alternative amino acid which is physically and/or functionally similar to the amino acid present in the wild-type. Substitutions may also be prepared by substituting with a non-conservative amino acid.

In the context of the present invention, amino acids may be described as conservative or non-conservative amino acids, and may therefore be classified accordingly. Amino acid residues may also be divided into classes defined by alternative physical and functional properties. Thus, classes of amino acids may be reflected in one or both of the following tables:

Amino acid residue of conservative class

Acidic Residues	D and E
Basic Residues	K, R, and H
Hydrophilic Uncharged Residues	S, T, N, and Q
Aliphatic Uncharged Residues	G, A, V, L, and I
Non-polar Uncharged Residues	C, M, and P
Aromatic Residues	F, Y, and W

Alternative Physical and Functional Classifications of Amino Acid Residues

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

In the context of the present invention, a substitution in an antibody, such as a humanized or chimeric antibody, is indicated as:

Original amino acid – position – substituted amino acid;

Referring to the well-recognized nomenclature for amino acids, the three letter code, or one letter code, is used, including the codes Xaa and X to indicate any amino acid residue. Accordingly, the notation “L234F” or “Leu234Phe” means, that the antibody comprises a substitution of Leucine with Phenylalanine in amino acid position 234.

Substitution of an amino acid at a given position to any other amino acid is referred to as:

Original amino acid – position; or e.g. “L234”.

For a modification where the original amino acid(s) and/or substituted amino acid(s) may comprise more than one, but not all amino acid(s), the more than one amino acid may be separated by “,” or “/”. E.g. the substitution of Leucine for Phenylalanine, Arginine, Lysine or Tryptophan in position 234 is:

"Leu234Phe,Arg,Lys,Trp" or "Leu234Phe/Arg/Lys/Trp" or "L234F,R,K,W" or "L234F/R/K/W" or "L234 to F, R, K or W"

Such designation may be used interchangeably in the context of the invention but have the same meaning and purpose.

Furthermore, the term "a substitution" embraces a substitution into any one of the other nineteen natural amino acids, or into other amino acids, such as non-natural amino acids. For example, a substitution of amino acid L in position 234 includes each of the following substitutions: 234A, 234C, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234Q, 234R, 234S, 234T, 234V, 234W, 234P, and 234Y. This is, by the way, equivalent to the designation 234X, wherein the X designates any amino acid other than the original amino acid. These substitutions can also be designated L234A, L234C, etc., or L234A,C,etc., or L234A/C/etc. The same applies by analogy to each and every position mentioned herein, to specifically include herein any one of such substitutions.

The antibody according to the invention may also comprise a deletion of an amino acid residue. Such deletion may be denoted "del", and includes, e.g., writing as L234del. Thus, in such embodiments, the Leucine in position 234 has been deleted from the amino acid sequence.

The terms "amino acid" and "amino acid residue" may herein be used interchangeably.

In one embodiment of the invention, the antibody comprises a binindign region comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1 , CDR2, and CDR3 regions having the three CDR sequences selected from one of the groups consisting of;

a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
b) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3 [T31P];
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];

g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V], and
l) CDR1, CDR2 and CDR3 sequences as specified in a) to k) having at most 5 further mutations or substitutions, at most 4 further mutations or substitutions, at most 3 further mutations or substitutions, at most 2 further mutations or substitutions, or at most 1 further mutation or substitution, in total across the three CDR sequences, and which mutations or substitutions preferably do not modify the binding affinity to human CD3.

In one embodiments of the invention the further mutations or substitutions are conservative, physical or functional amino acids.

In some embodiments binding to CD3 may be binding to full length CD3 such as CD3 present on a T cell. In other embodiments binding to CD3 may be binding to a CD3 peptide e.g. as set forth in SEQ ID NO: 402. Bining to the CD3 peptide and whether or not any further mutations may modify binding to CD3 can be deterimined by Bio-Layer Inerferometry as disclosed in Example 7.

In one embodiment, the antibody comprises an Fc region comprising a first and a second immunoglobulin heavy chain.

The term “C1q binding” as used herein, refers to the binding of C1q to an antibody, when said antibody is bound to its antigen. The term “bound to its antigen” as used herein, refers to binding of an antibody to its antigen both *in vivo* and *in vitro*.

The term “reduced” as used herein when referring to C1q binding, refers to the ability of the antibody according to the invention to reduce, minimize or even

completely inhibit the binding of C1q to the antibody when compared to the C1q binding to a wild-type antibody.

The term “reduced” or “reducing” as used herein or any variation thereof when used in relation to binding affinity of an antibody bining to human CD3, referes to a binding affinity that is lower when compared to a reference binding affinity. In this context, the reference binding affinity may be the binding affinity of reference antibody specified by the VH sequence SEQ ID NO:4 and the VL sequence SEQ ID NO:8 when binding to the CD3 peptide as SEQ ID NO: 402 and determined by Bio-Layer Interferomerty as described in example 7.

The term “binding affinity” as used herein referes to the binding of an antibody to a predetermined antigen or target to which binding typically is with an affinity corresponding to a K_D. The term "K_D" (M), as used herein, refers to the dissociation equilibrium constant of a particular antibody-antigen interaction.

The term “wild-type antibody” as used herein, in relation to use in comparison assays of an antibody according to the present invention, refers to an antibody which is identical to the antibody to be tested except for not being inert. In this context, the term “inert” refers to a modified Fc region having reduced or no binding of C1q, i.e. where C1q binding is determined by ELISA; reduced or no Fc-mediated T cell proliferation as determined in a PBMC based functional assay, i.e. T cell proliferation is measured in a peripheral blood mononuclear cell (PBMC)-based functional assay; and/or reduced or no Fc-mediated CD69 expression as determined in a PBMC-based functional assay. Thus, the wild-type antibody comprises the naturally occurring amino acids in the immunoglobulin heavy chains, i.e. an antibody which does not comprise any amino acid modifications which may alter or reduce the ability of the antibody to interact with e.g. C1q, Fc Receptors or the like. Thus, such a wild-type antibody will remain an activating antibody which is able to bind e.g. C1q. A wild-type antibody and an antibody of the present invention may comprise other amino acid modifications than those affecting the antibody’s ability of inducing effector functions, in order to make the antibody a bispecific antibody or the like.

The term “ELISA” as used herein refers to enzyme-linked immunosorbent assay which is a test that uses antibodies and color change to identify a substance. A first specific antibody is attached to the plate surface. Thereby the protein from a sample is added wherein binding to said first specific antibody is tested. A second antibody binding the antibody from the sample is added. The second antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate. The

concept of the ELISA method is well-known within the art and various ways of performing an ELISA are contemplated to be part of a method to evaluate the antibody according to the invention

Specifically, the ability of an antibody according to the present invention to bind C1q may be determined by ELISA comprising the steps of (i) coating said antibody on a 96-well plate, (ii) adding 3% serum, (iii) adding an anti-human C1q antibody, (iv) developing the plate, and (v) measuring OD_{405} nm. Thus, in one embodiment, the antibody comprises an Fc region which has been modified so that binding of C1q to said antibody is reduced compared to a wild-type antibody by at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, or 100%, wherein C1q binding is determined by ELISA comprising the steps of (i) coating said antibodies on a 96-well plate, (ii) adding 3% serum, (iii) adding an anti-human C1q, (iv) developing the plate, and (v) measuring OD_{405} nm.

The terms "Fc Receptor" or "FcR" as used herein, refers to a protein found on the surface of certain cells. FcRs bind to the Fc region of antibodies. There are several different types of FcRs which are classified based on the type of antibody they recognize. E.g. Fcγ (gamma) Receptors bind to antibodies of the IgG class.

The terms "Fcγ Receptor", "Fc gamma Receptor" or "FcγR" as used herein, refers to a group of Fc Receptors belonging to the immunoglobulin superfamily and is the most important Fc receptors for inducing phagocytosis of opsonized (coated) microbes. This family includes several members, FcγRI (CD64), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRIIIa (CD16a), FcγRIIIb (CD16b), which differ in their antibody affinities due to their different molecular structure.

Fc-mediated effector functions form part of the biological activity of human immunoglobulin G (IgG) molecules. Examples of such effector functions include e.g. antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) which are triggered by the binding of various effector molecules to the Fc region. In the context of the present invention, "Fc binding", "Fc Receptor binding", "FcR binding", and "binding of an antibody Fc region to FcR" refers to the binding of the Fc region to an Fc Receptor (FcR) or an effector molecule. The terms "FcγR binding" and "FcγRI binding" refer to binding to or with an Fc region to the Fc gamma Receptor and Fc gamma Receptor I, respectively. When a CD3 antibody binds T cells, the wild-type Fc region of the CD3 antibody binds to FcRs present on other cells, e.g. monocytes, which leads to non-specific, Fc-mediated activation of the T cell. Such non-specific, Fc-mediated activation of T cells may be undesired. T cells may also be activated by targeted, or target-specific, T cell activation. Such targeted T cell activation may be highly desirable for the treatment of a range of indications, such as cancer. The term "targeted T cell

activation" as used herein, refers to directing the T cells to specific cells, such as tumor cells by use of a bispecific antibody comprising a first binding region binding a specific target, such as a tumor target on a tumor cell, and a second binding region binding a T cell specific target, such as CD3. Thus, targeting of T cells to specific cells, e.g. tumor cells, may be facilitated by use of a bispecific antibody, wherein one of the binding regions binds CD3 present on the T cell and the other binding region binds a target specific antigen, e.g. on a tumor cell. Although, non-specific, Fc-mediated T cells activation may still be possible and therefore such undesired non-specific, Fc-mediated T cell activation via Fc-mediated cross-linking should be avoided and may be disabled by making the Fc region inert for such activity. Thereby, interaction between said inert Fc region with Fc Receptors present is prevented.

An antibody according to the present invention may comprise modifications in the Fc region. When an antibody comprises such modifications it may become an inert, or non-activating, antibody. The term "inertness", "inert" or "non-activating" as used herein, refers to an Fc region which is at least not able to bind any Fcγ Receptors, induce Fc-mediated cross-linking via FcRs, or induce FcR-mediated cross-linking of target antigens via the Fc region, or is not able to bind C1q. The inertness of an Fc region of a humanized or chimeric CD3 antibody is advantageously tested using the antibody in a monospecific format although an inert Fc region so identified can be used in bispecific or other humanized or chimeric multispecific CD3 antibodies.

Several variants can be constructed to make the Fc region of an antibody inactive for interactions with Fc gamma Receptors and C1q for therapeutic antibody development. Examples of such variants are described herein.

Thus, in one embodiment, the antibody comprises an Fc region which has been modified so that said antibody mediates reduced Fc-mediated T cell proliferation compared to a wild-type antibody by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 99% or 100%, wherein said T cell proliferation is measured in a peripheral blood mononuclear cell (PBMC)-based functional assay.

The term " reduce" when referring to T cell proliferation, refers to the ability of the antibody according to the invention to reduce, minimize or even completely inhibit the proliferation of T cells when compared to the proliferation of T cells bound by a wild-type antibody. The ability of an antibody to reduce T cell proliferation may be evaluated by a PBMC-based functional assay. In one embodiment the assay is performed with human PBMCs. In another embodiment the assay is performed with cynomolgus PBMCs. In yet another embodiment, the assay is performed with rhesus PBMCs. Since the antibodies according to the present invention are cross-reactive, a PBMC-based assay as herein described may be performed with any species PBMCs to show reduction of T cell

proliferation as long as the species PBMC used are within the cross-reactivity spectra of the antibodies, e.g. human, cynomolgus or rhesus monkeys.

The term "peripheral blood mononuclear cell (PBMC)-based functional assay" as used herein refers to an assay used for evaluating a functional feature of the antibody of the present invention, such as the ability of said antibody to affect T cell proliferation or CD69 expression, wherein the only cells present are peripheral blood mononuclear cells. Thus, in one embodiment, T cell proliferation is measured by a method comprising the steps of incubating PBMCs with antibody in the range of 1-1000 ng/mL at 37°C in a 5% (vol/vol) CO_2 humidified incubator for three days, adding a chemical compound, such as BrdU, which is incorporated into the DNA of proliferating cells, incubating for five hrs., pelleting cells, drying cells, optionally storing the cells at 4°C, coating cells to ELISA plates, incubating with anti-BrdU-peroxidase for 90 min at room temperature, developing for about 30 min with 1 mg/mL 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), adding 100 µL 2% oxalic acid to stop the reaction, and measuring absorbance at 405 nm in a suitable microplate reader.

The term "proliferation" as used herein, refers to cell growth in the context of cell division.

The term "BrdU" as used herein, refers to 5-bromo-2'-deoxyuridine, which is a homologue to thymidine. When BrdU is added to cell culture for a limited period of time (e.g. 4 hours) it will be incorporated into the DNA of proliferating cells. After fixing the cells, detection of incorporated BrdU may be performed in an ELISA using anti-BrdU-peroxidase. BrdU incorporation is therefore a measure for proliferation.

In one embodiment, the antibody comprises an Fc region which has been modified so that said antibody reduces Fc-mediated CD69 expression by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 99% or 100% when compared to a wild-type antibody wherein said Fc-mediated CD69 expression is determined in a PBMC-based functional assay.

In particular, the term "reduce" when referring to expression level of the T cell activation marker CD69, refers to a reduction in expression level of CD69 when compared to expression level of CD69 when the T cell is bound by a wild-type antibody bound to CD3 and interacting with an Fc receptor. An antibody's ability to reduce expression of CD69 may be evaluated by a PBMC-based functional. Thus, in one embodiment, expression of CD69 is measured by a method comprising the steps of incubating PBMCs with an antibody in the range of 1-1000 ng/mL at 37°C in a 5% (vol/vol) CO_2 humidified incubator for 16-24 hrs, washing the cells, staining the cells at 4°C with a mouse anti-human CD28-PE and mouse-anti-human CD69-APC antibody, and determining CD69-expression on CD28 positive cells by flow cytometry.

The term "CD69" as used herein, refers to Cluster of Differentiation 69 which is a human transmembrane C-Type lectin protein encoded by the *CD69* gene. Activation of T lymphocytes and natural killer (NK) cells, both *in vivo* and *in vitro*, induces expression of CD69. CD69 function as a signal transmitting receptor involved in cellular activation events including proliferation, functions as a signal-transmitting receptor in lymphocytes, including natural killer cells and platelets, and the induction of specific genes.

The term "peripheral blood mononuclear cell (PBMC)-based functional assay" as used herein refers to an assay used for evaluating a functional feature of the antibody of the present invention, such as the ability of said antibody to affect T cell proliferation or CD69 expression, wherein the only cells present are peripheral blood mononuclear cells. A PBMC-based functional assay comprises the steps of (i) incubating PBMCs with an antibody at 37°C in a 5% (vol/vol) CO_2 humidified incubator for about 16-24 hrs, (ii) washing the cells, (iii) staining the cells at 4°C with a mouse anti-human CD28-PE and mouse-anti-human CD69-APC antibody, and (iv) determining the CD69 expression on CD28 positive cells by flow cytometry, when CD69 expression is evaluated.

Amino acids in the Fc region that play a dominant role in the interactions with C1q and the Fc Gamma Receptors may be modified. Examples of amino acid positions that may be modified include positions L234, L235 and P331. Combinations thereof, such as L234F/L235E/P331S, can cause a profound decrease in binding to human CD64, CD32A, CD16 and C1q.

Hence, in one embodiment, the amino acid in at least one position corresponding to L234, L235 and P331, may be A, A and S, respectively ([1], [28]). Also, L234F and L235E amino acid substitutions can result in Fc regions with abrogated interactions with Fc Gamma Receptors and C1q ([29]-[30]). Hence, in one embodiment, the amino acids in the positions corresponding to L234 and L235, may be F and E, respectively. A D265A amino acid substitution can decrease binding to all Fc gamma Receptors and prevent ADCC ([31]). Hence, in one embodiment, the amino acid in the position corresponding to D265 may be A. Binding to C1q can be abrogated by mutating positions D270, K322, P329, and P331. Mutating these positions to either D270A or K322A or P329A or P331A can make the antibody deficient in CDC activity ([32]). Hence, in one embodiment, the amino acids in at least one position corresponding to D270, K322, P329 and P331, may be A, A, A, and A, respectively.

An alternative approach to minimize the interaction of the Fc region with Fc gamma Receptors and C1q is by removal of the glycosylation site of an antibody. Mutating position N297 to e.g. Q, A, and E removes a glycosylation site which is critical for IgG-Fc gamma Receptor interactions. Hence, in one embodiment, the amino acid in a position corresponding to N297, may be G, Q, A or E ([33]). Another alternative approach to

minimize interaction of the Fc region with Fc gamma Receptors may be obtained by the following mutations; P238A, A327Q, P329A or E233P/L234V/L235A/G236del ([31]).

Alternatively, human IgG2 and IgG4 subclasses are considered naturally compromised in their interactions with C1q and Fc gamma Receptors although, interactions with Fcγ Receptors (Fc gamma Receptors) were reported ([34]-[35]). Mutations abrogating these residual interactions can be made in both isotypes, resulting in reduction of unwanted side-effects associated with FcR binding. For IgG2, these include L234A and G237A, and for IgG4, L235E. Hence, in one embodiment, the amino acid in a position corresponding to L234 and G237 in a human IgG2 heavy chain, may be A and A, respectively. In one embodiment, the amino acid in a position corresponding to L235 in a human IgG4 heavy chain, may be E.

Other approaches to further minimize the interaction with Fc gamma Receptors and C1q in IgG2 antibodies include those described in [36] and [37].

The hinge region of the antibody can also be of importance with respect to interactions with Fc gamma Receptors and complement ([38]-[39]). Accordingly, mutations in or deletion of the hinge region can influence effector functions of an antibody.

The term “cross-linking” as used herein, refers to the indirect bridging of antibody Fab arm(s) (monovalently or bivalently) bound to the target antigen by FcR-bearing cell through binding to the antibody Fc region. Thus, an antibody which binds its target antigen on target antigen-bearing cells may cross-link with another cell expressing FcRs.

The term “unspecific killing” as used herein, refers to the killing of cells by the cytotoxic function of T cells or other effector cells, through tumor target antigen-independent activation of said cells. Thus, by unspecific killing is meant that the tumor-target bearing cells may be killed by e.g. cytotoxic T cells and not by the antibody binding the tumor target by e.g. induction of CDC.

A non-activating Fc region may be obtained by modifying one or more of at least five specific amino acid positions in the Fc region.

In one embodiment, the antibody comprises an Fc region comprising a first and a second immunoglobulin heavy chain.

Thus, in one embodiment, the antibody comprises a first and a second immunoglobulin heavy chain, wherein in at least one of said first and second immunoglobulin heavy chains one or more amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain, are not L, L, D, N, and P, respectively.

In one embodiment, in both the first and second heavy chains one or more amino acids in the position corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain, are not L, L, D, N, and P, respectively.

In another embodiment, in at least one of the first and second heavy chains one or more amino acids in the positions corresponding to positions L234, L235 and D265 in a human IgG1 heavy chain, are not L, L and D, respectively, and the amino acids in the positions corresponding to N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

The term "amino acid corresponding to positions" as used herein refers to an amino acid position number in a human IgG1 heavy chain. Unless otherwise stated or contradicted by context, the amino acids of the constant region sequences are herein numbered according to the Eu-index of numbering (described in [27]). Thus, an amino acid or segment in one sequence that "corresponds to" an amino acid or segment in another sequence is one that aligns with the other amino acid or segment using a standard sequence alignment program such as ALIGN, ClustalW or similar, typically at default settings and has at least 50%, at least 80%, at least 90%, or at least 95% identity to a human IgG1 heavy chain. It is considered well-known in the art how to align a sequence or segment in a sequence and thereby determine the corresponding position in a sequence to an amino acid position according to the present invention.

In the context of the present invention, the amino acid may be defined as described above.

The term "the amino acid is not" or similar wording when referring to amino acids in a heavy chain is to be understood to mean that the amino acid is any other amino acid than the specific amino acid mentioned. For example, the amino acid in the position corresponding to L234 in a human IgG1 heavy chain is not L, means that the amino acid may be any of the other naturally or non-naturally occurring amino acids than L.

In one embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain, is not D.

In one embodiment, in at least one of the first and second heavy chains the amino acid in the position corresponding to D265 in a human IgG1 heavy chain, is not D, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to position D265 in a human IgG1 heavy chain is hydrophobic or polar amino acids.

The term "hydrophobic" as used herein in relation to an amino acid residue, refers to an amino acid residue selected from the group consisting of; A, C, F, G, H, I, L, M, R, T, V, W, and Y. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group of amino acids consisting of; A, C, F, G, H, I, L, M, R, T, V, W and Y.

The term "polar" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of; C, D, E, H, K, N, Q, R, S, and T. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human heavy chain is selected from the group consisting of; C, E, H, K, N, Q, R, S, and T.

In another embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is an aliphatic uncharged, aromatic or acidic amino acid.

The term "aliphatic uncharged" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of: A, G, I, L, and V. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, G, I, L, and V.

The term "aromatic" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of: F, T, and W. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; F, T, and W.

The term "acidic" as used herein in relation to amino acid residues, refers to any amino acid residue chosen from the group consisting of: D and E. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; D and E.

In a particular embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, E, F, G, I, L, T, V, and W.

In one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain, is not D.

In one embodiment, in both the first and second heavy chains the amino acid in the position corresponding to D265 in a human IgG1 heavy chain, is not D, and the

amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is hydrophobic or polar amino acid.

The term "hydrophobic" as used herein in relation to an amino acid residue, refers to an amino acid residue selected from the group consisting of; A, C, F, G, H, I, L, M, R, T, V, W, and Y. Thus, in one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group of amino acids consisting of; A, C, F, G, H, I, L, M, R, T, V, W and Y.

The term "polar" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of; C, D, E, H, K, N, Q, R, S, and T. Thus, in one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human heavy chain is selected from the group consisting of; C, E, H, K, N, Q, R, S, and T. In one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group of amino acids consisting of; A, C, F, G, H, I, L, M, R, T, V, W and Y.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to position D265 in a human heavy chain is selected from the group consisting of; C, E, H, K, N, Q, R, S, and T.

In another embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is aliphatic uncharged, aromatic or acidic amino acids.

The term "aliphatic uncharged" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of: A, G, I, L, and V. Thus, in one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, G, I, L, and V.

The term "aromatic" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of: F, T, and W. Thus, in one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; F, T, and W.

The term "acidic" as used herein in relation to amino acid residues, refers to any amino acid residue chosen from the group consisting of: D and E. Thus, in one

embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain are selected from the group consisting of; D and E.

In a particular embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, E, F, G, I, L, T, V, and W.

In further embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position N297 in a human IgG1 heavy chain, is not N.

In one embodiment, in at least one of the first and second heavy chains the amino acid in the position corresponding to N297 in a human IgG1 heavy chain, is not N, and the amino acid in the position corresponding to position P331 in a human IgG1 heavy chain, is P.

In one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to positions N297 in a human IgG1 heavy chain, is not N.

In one embodiment, in both the first and second heavy chains the amino acid in the position corresponding to N297 in a human IgG1 heavy chain, is not N, and the amino acid in the position corresponding to position P331 in a human IgG1 heavy chain, is P.

In further embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are not L and L, respectively.

In one embodiment, in at least one of the first and second heavy chains the amino acids in the positions corresponding to L234 and L235 in a human IgG1 heavy chain, are not L and L, respectively, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In one embodiment, in at least one of said first and second heavy chains the amino acids corresponding to positions L234 and L235 in a human IgG1 heavy chain are selected from the group consisting of; A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, Y, V.

In one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are hydrophobic or polar amino acids.

The term “hydrophobic” as used herein in relation to an amino acid residue, refers to an amino acid residue selected from the group consisting of; A, C, F, G, H, I, L, M, R, T, V, W, and Y. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in

a human IgG1 heavy chain are each selected from the group consisting of; A, C, F, G, H, I, M, R, T, V, W, and Y.

The term "polar" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of; C, D, E, H, K, N, Q, R, S, and T. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group of amino acids consisting of; C, D, E, H, K, N, Q, R, S, and T.

In a particular embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, C, D, E, F, G, H, I, K, M, N, Q, R, S, T, V, W, and Y.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are not L and L, respectively.

In one embodiment, in both the first and second heavy chains the amino acids in the positions corresponding to L234 and L235 in a human IgG1 heavy chain, are not L and L, respectively, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to L234 and L235 in a human IgG1 heavy chain are hydrophobic or polar amino acids.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, C, F, G, H, I, M, R, T, V, W, and Y.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group of amino acids consisting of; C, D, E, H, K, N, Q, R, S, and T.

In a particular embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, C, D, E, F, G, H, I, K, M, N, Q, R, S, T, V, W, and Y.

In another embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are aliphatic uncharged, aromatic or acidic amino acids.

The term "aliphatic uncharged" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of: A, G, I, L, and V. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, G, I, and V.

The term "aromatic" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of: F, T, and W. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; F, T, and W.

The term "acidic" as used herein in relation to amino acid residues, refers to any amino acid residue chosen from the group consisting of: D and E. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; D and E.

In a particular embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to L234 and L235 are each selected from the group consisting of; A, D, E, F, G, I, T, V, and W.

In one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are F and E; or A and A, respectively.

In one embodiment, in at least one of the first and second heavy chains the amino acids in the positions corresponding to L234 and L235 in a human IgG1 heavy chain, are F and E; or A and A, respectively, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are F and E; or A and A, respectively.

In one embodiment, in both the first and second heavy chains the amino acids in the positions corresponding to L234 and L235 in a human IgG1 heavy chain, are F and E; or A and A, respectively, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In a particular embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are F and E, respectively.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are F and E, respectively.

In one embodiment, in at least one of said first and second heavy chains at least the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are A and A, respectively.

In one embodiment, in both said first and second heavy chains at least the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are A and A, respectively.

In one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are not L, L, and D, respectively.

In one embodiment, in at least one of the first and second heavy chains the amino acids in the positions corresponding to L234, L235, and D265 in a human IgG1 heavy chain, are not L, L and D, respectively, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In one embodiment, in at least one of said first and second heavy chains the amino acids corresponding to positions L234 and L235 in a human IgG1 heavy chain are selected from the group consisting of; A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, Y, V, and W, and the amino acid corresponding to position D265 is selected from the group consisting of; A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, Y, V, and W.

In one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235 and D265 in a human IgG1 heavy chain are hydrophobic or polar amino acids.

The term “hydrophobic” as used herein in relation to an amino acid residue, refers to an amino acid residue selected from the group consisting of; A, C, F, G, H, I, L, M, R, T, V, W, and Y. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group of amino acids consisting of; A, C, F, G, H, I, L, M, R, T, V, W and Y, and the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, C, F, G, H, I, M, R, T, V, W, and Y.

The term “polar” as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of; C, D, E, H, K, N, Q, R, S, and T. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1

heavy chain are each selected from the group of amino acids consisting of; C, D, E, H, K, N, Q, R, S, and T, the amino acid in the position corresponding to position D265 in a human heavy chain is selected from the group consisting of; C, E, H, K, N, Q, R, S, and T.

In a particular embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, C, D, E, F, G, H, I, K, M, N, Q, R, S, T, V, W, and Y, and the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, C, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, and Y.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to L234, L235, and D265 in a human IgG1 heavy chain are hydrophobic or polar amino acids.

In one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group of amino acids consisting of; A, C, F, G, H, I, L, M, R, T, V, W and Y, and the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, C, F, G, H, I, M, R, T, V, W, and Y.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group of amino acids consisting of; C, D, E, H, K, N, Q, R, S, and T, the amino acid in the position corresponding to position D265 in a human heavy chain is selected from the group consisting of; C, E, H, K, N, Q, R, S, and T.

In a particular embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, C, D, E, F, G, H, I, K, M, N, Q, R, S, T, V, W, and Y, and the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, C, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, and Y.

In another embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235 and D265 in a human IgG1 heavy chain are aliphatic uncharged, aromatic or acidic amino acids.

The term “aliphatic uncharged” as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of: A, G, I, L, and V. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, G, I, L, and V, and the amino acids in the

positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, G, I, and V.

The term "aromatic" as used herein in relation to amino acid residues, refers to any amino acid residue selected from the group consisting of: F, T, and W. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235 and D265 in a human IgG1 heavy chain are each selected from the group consisting of; F, T, and W.

The term "acidic" as used herein in relation to amino acid residues, refers to any amino acid residue chosen from the group consisting of: D and E. Thus, in one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain are each selected from the group consisting of; D and E.

In a particular embodiment, in at least one of said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, E, F, G, I, L, T, V, and W, and the amino acids in the positions corresponding to L234 and L235 are each selected from the group consisting of; A, D, E, F, G, I, T, V, and W.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235 and D265 in a human IgG1 heavy chain, are not L, L, and D, respectively.

In one embodiment, in both the first and second heavy chains the amino acids in the positions corresponding to L234, L235, and D265 in a human IgG1 heavy chain, are not L, L, and D, respectively, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to L234, L235, and D265 in a human IgG1 heavy chain are aliphatic uncharged, aromatic or acidic amino acids.

In one embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is selected from the group consisting of; A, G, I, L, and V, and the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain are each selected from the group consisting of; A, G, I, and V.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain are each selected from the group consisting of; D and E.

In a particular embodiment, in both said first and second heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain is

selected from the group consisting of; A, E, F, G, I, L, T, V, and W, and the amino acids in the positions corresponding to L234 and L235 are each selected from the group consisting of; A, D, E, F, G, I, T, V, and W.

In one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A; or A, A, and A, respectively.

In one embodiment, in at least one of the first and second heavy chains the amino acids in the positions corresponding to L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A; or A, A, and A, respectively, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A; or A, A, and A, respectively.

In one embodiment, in both the first and second heavy chains the amino acids in the positions corresponding to L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A; or A, A, and A, respectively, and the amino acids in the positions corresponding to positions N297 and P331 in a human IgG1 heavy chain, are N and P, respectively.

In a particular embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.

In one embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are A, A, and A, respectively.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are A, A, and A, respectively.

In another embodiment, in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain, are F, E, A, Q, and S, respectively.

In one embodiment, in both said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain, are F, E, A, Q, and S, respectively.

In one embodiment, the antibody according to the invention, comprises a VH sequence as set out in any one of the sequences in the group of: SEQ ID NOs: 107; 59; 245; 299; 285; 55; 185; 179; 237; 177 and 293, a VL sequence as set out in SEQ ID NO:8, and in at least one, or both of the heavy chains the amino acids in positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively. Hereby embodiments are provided of anti CD3 antibodies with reduced affinity to human CD3 epsilon compared to a reference antibody comprising the VH and VL sequences as set out in SEQ ID NO:4 and 8, and where the antibodies further comprises a non-activating Fc region.

In a particular embodiment, the antibody according to the invention, comprises a VH sequence as set out in any one of the sequences set out in SEQ ID NOs: 107; 59; 245; 299; 285; 55; 185; 179; 237; 177 and 293, a VL sequence as set out in SEQ ID NO:10, and in at least one, or both of the heavy chains the amino acids in positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively. Hereby embodiments are provided of anti CD3 antibodies with reduced affinity to human CD3 epsilon compared to a reference antibody comprising the VH and VL sequences as set out in SEQ ID NO:4 and 8, and where the antibodies further comprises a non-activating Fc region and a VL region that allows for enhanced production.

In another embodiment, the antibody according to the invention, comprises a VH sequence as set out in SEQ ID NOs: 221, a VL sequence as set out in SEQ ID NO:8 or 10, and in at least one, or both of the heavy chains the amino acids in positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.

In one embodiment of the present invention the human IgG1 heavy chain has the IgG1m(f) sequence as set out in SEQ ID NO:407. In a further embodiment the amino acids in positons corresponding to postions L234, L235, and D265 in a human IgG1m(f) as set out in SEQ ID NO:407, are F, E, and A, respectively.

In one embodiment of the present invention the human IgG1 heavy chain has the IgG1m(f) sequence as set out in SEQ ID NO:409.

In one aspect, the antibody according to the invention comprises the human IgLC2/IgLC3 constant domain lambda light chain of SEQ ID NO:408.

In one aspect, the antibodies according to the invention may be modified in the light chain (LC) and/or heavy chain (HC) to increase the expression level and/or production yield. In one embodiment, the antibodies according to the invention may be modified in the light chain (LC). Such modifications are known in the art and may be performed according to the methods described in e.g. Zheng, L., Goddard, J.-P., Baumann, U., & Reymond, J.-L. (2004). Expression improvement and mechanistic study of the retro-Diels-Alderase catalytic antibody 10F11 by site-directed mutagenesis. Journal of Molecular Biology, 341(3), 807–14. doi:10.1016/j.jmb.2004.06.014.

In one aspect, the antibodies according to the invention may be modified in the VH region and/or the VL region to modify the affinity of the antibodies, such as to reduce or increase the affinity of the antibodies. This may be advantageous in some settings and lead to increased efficacy. In particular low affinity of the CD3 arm may have an impact on the motility of T cells in circulation and at tumor site thus leading to better engagement of T cells with tumor cells, cf. MølhØj et al, Molecular Immunology 44 (2007). In particular this may be useful in bispecific formats, in which a CD3 antibody is used as one of the binding arms. Modifications that lead to reduced antibody affinity are known in the art, see for example Webster et al. Int J Cancer Suppl. 1988;3:13-6.

Thus in one embodiment the antibody of the present invention comprises a the variable light chain (VL) region comprising the CDR1, CDR2 and CDR3 having the sequences as set forth in SEQ ID NO: 6, GTN, 7 and a variable heavy chain (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 having the CDR sequences selected from one of the groups consisting of;

a) CDR sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
b) CDR1, CDR2 and CDR3 sequence set forth in SEQ ID NO: 58, 2, 3 [T31P];
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];

g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];and
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V].

In another aspect, the present invention provides an antibody binding to human CD3, comprising a binding region comprising a variable light chain (VL) region having the sequence set forth in SEQ ID NO 10 and a variable heavy chain (VH) region the CDR1, CDR2 and CDR3 having the sequences selected from one of the groups consisting of:

a) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
b) CDR1, CDR2 and CDR3 sequence set forth in SEQ ID NO: 58, 2, 3 [T31P];
c) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
d) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
e) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
f) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
g) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
h) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
i) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
j) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R];and
k) CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V].

Hereby embodiments are provided comprising the T41K mutation in the VL region as set forth in SEQ ID NO:10, thereby allowing increased production of said antibodies.

In one aspect, the present invention relates to a multispecific antibody comprising at least a first binding region of an antibody according to any aspect or embodiment herein described, and one or more binding regions which binds one or more different targets than the first binding region. Such a multispecific antibody may be a bispecific antibody.

Thus, in one aspect, the present invention relates to a bispecific antibody comprising a first binding region of an antibody according to any aspect or embodiment herein described, and a second binding region which binds a different target than the first binding region.

The term "multispecific antibody" refers to an antibody having specificities for at least two different, such as at least three, typically non-overlapping, epitopes. Such epitopes may be on the same or different targets. If the epitopes are on different targets, such targets may be on the same cell or different cells or cell types.

The term "bispecific antibody" refers to an antibody having specificities for at least two different, typically non-overlapping, epitopes. Such epitopes may be on the same or different targets. If the epitopes are on different targets, such targets may be on the same cell or different cells or cell types.

In one embodiment, the bispecific antibody comprises a first and a second heavy chain.

The embodiments relating to modification of the Fc region and embodiments relating to specific amino acid substitutions are contemplated to be part of any bispecific antibody according to the invention. Thus, in one embodiment, at least one of the first and second heavy chains comprise one or more amino acids modified as defined in any embodiment herein described, such as those described to in relation to providing an inert Fc region. In one embodiment, both said first and second heavy chains comprise one or more amino acids modified as defined in any embodiment herein described, such as those described to in relation to providing an inert Fc region. Accordingly, the bispecific antibody comprises an Fc region modified according to any aspect or embodiment herein described; or at least one of said first and second heavy chains comprise one or more amino acids modified as defined in any aspect or embodiment herein described.

Examples of bispecific antibody molecules which may be used in the present invention comprise (i) a single antibody that has two arms comprising different antigen-

binding regions, (ii) a single chain antibody that has specificity to two different epitopes, e.g., via two scFvs linked in tandem by an extra peptide linker; (iii) a dual-variable-domain antibody (DVD-Ig™), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage ([40]); (iv) a chemically-linked bispecific (Fab')2 fragment; (v) a TandAb®, which is a fusion of two single chain diabodies resulting in a tetravalent bispecific antibody that has two binding sites for each of the target antigens; (vi) a flexibody, which is a combination of scFvs with a diabody resulting in a multivalent molecule; (vii) a so called "dock and lock" molecule (Dock-and-Lock®), based on the "dimerization and docking domain" in Protein Kinase A, which, when applied to Fabs, can yield a trivalent bispecific binding protein consisting of two identical Fab fragments linked to a different Fab fragment; (viii) a so-called Scorpion molecule, comprising, e.g., two scFvs fused to both termini of a human Fab-arm; and (ix) a diabody.

In one embodiment, the bispecific antibody of the present invention is a diabody, a cross-body, or a bispecific antibody obtained via a controlled Fab arm exchange, e.g. DuoBody® (such as described in [41]) as those described in the present invention.

Examples of different classes of bispecific antibodies include but are not limited to (i) IgG-like molecules with complementary CH3 domains to force heterodimerization; (ii) recombinant IgG-like dual targeting molecules, wherein the two sides of the molecule each contain the Fab fragment or part of the Fab fragment of at least two different antibodies; (iii) IgG fusion molecules, wherein full length IgG antibodies are fused to extra Fab fragment or parts of Fab fragment; (iv) Fc fusion molecules, wherein single chain Fv molecules or stabilized diabodies are fused to heavy-chain constant-domains, Fc-regions or parts thereof; (v) Fab fusion molecules, wherein different Fab-fragments are fused together, fused to heavy-chain constant-domains, Fc-regions or parts thereof; and (vi) ScFv-and diabody-based and heavy chain antibodies (e.g., domain antibodies, Nanobodies®) wherein different single chain Fv molecules or different diabodies or different heavy-chain antibodies (e.g. domain antibodies, Nanobodies®) are fused to each other or to another protein or carrier molecule fused to heavy-chain constant-domains, Fc-regions or parts thereof.

Examples of IgG-like molecules with complementary CH3 domains molecules include but are not limited to the Triomab® (Trion Pharma/Fresenius Biotech, [42]), the Knobs-into-Holes (Genentech, [43]), CrossMAbs (Roche, [44]) and the electrostatically-matched (Amgen, [45]-[46]; Chugai, [47]; Oncomed, [48]), the LUZ-Y (Genentech, Wranik et al. J. Biol. Chem. 2012, 287(52): 43331-9, doi: 10.1074/jbc.M112.397869. Epub 2012 Nov 1), DIG-body and PIG-body (Pharmabcine, WO2010134666, WO2014081202), the Strand Exchange Engineered Domain body (SEEDbody)(EMD

Serono, [49]), the Biclonics (Merus, WO2013157953), FcΔAdp (Regeneron, [50]), bispecific IgG1 and IgG2 (Pfizer/Rinat, [51]), Azymetric scaffold (Zymeworks/Merck, [52]), mAb-Fv (Xencor, [53]), bivalent bispecific antibodies (Roche, WO2009080254) and DuoBody® molecules (Genmab A/S, [41]).

Examples of recombinant IgG-like dual targeting molecules include but are not limited to Dual Targeting (DT)-Ig (GSK/Domantis, WO2009058383), Two-in-one Antibody (Genentech, Bostrom, et al 2009. Science 323, 1610–1614), Cross-linked Mabs (Karmanos Cancer Center), mAb2 (F-Star, [54]), Zybodies™ (Zyngenia, LaFleur et al. MAbs. 2013 Mar-Apr;5(2):208-18), approaches with common light chain (Crucell/Merus, [55]), κλBodies (NovImmune, WO2012023053) and CovX-body® (CovX/Pfizer, Doppalapudi, V.R., et al 2007. Bioorg. Med. Chem. Lett. 17,501–506).

Examples of IgG fusion molecules include but are not limited to Dual Variable Domain (DVD)-Ig™ (Abbott, [56]), Dual domain double head antibodies (Unilever; Sanofi Aventis, [57]), IgG-like Bispecific (ImClone/Eli Lilly, Lewis etal. Nat Biotechnol. 2014 Feb;32(2):191-8), Ts2Ab (MedImmune/AZ, Dimasi et al. J Mol Biol. 2009 Oct 30;393(3):672-92) and BsAb (Zymogenetics, WO2010111625), HERCULES (Biogen Idec, [58]), scFv fusion (Novartis), scFv fusion (Changzhou Adam Biotech Inc, [59]) and TvAb (Roche, [59], [60]).

Examples of Fc fusion molecules include but are not limited to ScFv/Fc Fusions (Academic Institution, Pearce et al Biochem Mol Biol Int. 1997 Sep;42(6):1179-88.), SCORPION (Emergent BioSolutions/Trubion, Blankenship JW, et al. AACR 100 th Annual meeting 2009 (Abstract # 5465); Zymogenetics/BMS, WO2010111625), Dual Affinity Retargeting Technology (Fc-DART™) (MacroGenics, [62], [63]) and Dual(ScFv)2-Fab (National Research Center for Antibody Medicine – China).

Examples of Fab fusion bispecific antibodies include but are not limited to F(ab)2 (Medarex/AMGEN), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock® (DNL) (ImmunoMedics), Bivalent Bispecific (Biotecnol) and Fab-Fv (UCB-Celltech).

Examples of ScFv-, diabody-based and domain antibodies include but are not limited to Bispecific T Cell Engager (BiTE®) (Micromet, Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology (DART™) (MacroGenics), Single-chain Diabody (Academic, Lawrence FEBS Lett. 1998 Apr 3;425(3):479-84), TCR-like Antibodies (AIT, ReceptorLogics), Human Serum Albumin ScFv Fusion (Merrimack, WO2010059315) and COMBODY molecules (Epigen Biotech, Zhu et al. Immunol Cell Biol. 2010 Aug;88(6):667-75), dual targeting nanobodies® (Ablynx, Hmila et al., FASEB J. 2010), dual targeting heavy chain only domain antibodies.

It is further contemplated that any monospecific antibody fulfilling the assay conditions herein described may form the basis of a bispecific antibody. I.e. a bispecific antibody wherein one of the binding regions binds CD3 may originate from any monospecific CD3 antibody tested in the functional assays and fulfilling the requirements stated herein. Such a bispecific antibody may be provided by the methods described in [41], which is hereby incorporated by reference.

In one aspect, the bispecific antibody of the invention comprises a first Fc-region comprising a first CH3 region, and a second Fc-region comprising a second CH3 region, wherein the sequences of the first and second CH3 regions are different and are such that the heterodimeric interaction between said first and second CH3 regions is stronger than each of the homodimeric interactions of said first and second CH3 regions. More details on these interactions and how they can be achieved are provided in WO2011131746 and WO2013060867 (Genmab), which are hereby incorporated by reference.

Thus, in a particular embodiment, each of said first and second heavy chain comprises at least a hinge region, a CH2 and CH3 region, wherein in said first heavy chain at least one of the amino acids in the positions corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, and in said second heavy chain at least one of the amino acids in the positions corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, and wherein said first and said second heavy chains are not substituted in the same positions. In this context the term “substituted”, refers to that the amino acid in a specific amino acid position has been substituted with another naturally or non-naturally occurring amino acid. Thus, a “substituted” amino acid in a position corresponding to the position in a human IgG1 heavy chain means the amino acid at the particular position is different from the naturally occurring amino acid in an IgG1 heavy chain.

In one embodiment, in said first heavy chain the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is not K, L or M, and optionally the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is F, and in said second heavy chain at least one of the amino acids in the positions corresponding to a position selected from the group consisting of; T366, L368, K370, D399, F405, and Y407 in a human IgG1 heavy chain has been substituted.

In one embodiment, in said first heavy chain the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is not K, L or M, and in said second heavy chain the amino acid in the position corresponding to F405 in a human IgG1 heavy

chain is not F and optionally the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is K.

In one embodiment, in said first heavy chain, the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is not F, R, and G, and in said second heavy chain the amino acids in the positions corresponding to a position selected form the group consisting of; T366, L368, K370, D399, Y407, and K409 in a human IgG1 heavy chain has been substituted.

In one embodiment, the amino acid in position corresponding to K409 in a human IgG1 heavy chain is not K, L or M in said first heavy chain, and the amino acid in position corresponding to F405 in a human IgG1 heavy chain is not F.

In a further embodiment, the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in said second heavy chain, or vice versa.

Thus, in one embodiment, the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in the second heavy chain.

In a further embodiment, the humanized or chimeric CD3 antibody of the invention contains in at least one of the first and second heavy chain one or more of the inactivating substitutions as disclosed in any one of the above embodiments, such as L234F, L235E, and D265A; and that the amino acid in the position corresponding to F405 is not F. In one embodiment the humanized or chimeric CD3 antibody of the invention contains in at least one of the first and second heavy chain one or more of the inactivating substitutions as disclosed in any one of the above embodiments, such as L234F, L235E, and D265A; and a further substitution in the K409 position, such as K409R. In particular, in one embodiment, the humanized or chimeric CD3 antibody of the invention contains in both the first and second heavy chain one or more of the inactivating substitutions as disclosed in any one of the above embodiments, such as L234F, L235E, and D265A; and a substitution in the F405 position, such as F405L. In one embodiment the humanized or chimeric CD3 antibody of the invention contains in both the first and second heavy chain one or more of the inactivating substitutions as disclosed in any one of the above embodiments, such as L234F, L235E, and D265A; and a further substitution in the K409 position, such as K409R. Such antibodies are useful for generating a bispecific antibody.

Accordingly, in a further embodiment, in at least one of the first and second heavy chains the amino acids in the positions corresponding to position L234, L235, and D265 in a human IgG1 heavy chain are F, E, and A, respectively, the amino acid in the

position corresponding to F405 in a human IgG1 heavy chain is L in the first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the second heavy chain.

In one embodiment, in at least one of the first and second heavy chains the amino acids in the positions corresponding to L234, L235, D265, N297, and P331 in a human IgG1 heavy chain are F, E, A, N, and P respectively, the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in the first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the second heavy chain.

In an alternative embodiment, in at least one of the first and second heavy chains the amino acids in the positions corresponding to position L234, L235, and D265 in a human IgG1 heavy chain are F, E, and A, respectively, the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in the second heavy chain.

In one embodiment, in at least one of the first and second heavy chains the amino acids in the positions corresponding to L234, L235, D265, N297, and P331 in a human IgG1 heavy chain are F, E, A, N, and P respectively, the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in the second heavy chain.

In another embodiment, in both the first and second heavy chains the amino acids in the positions corresponding to position L234, L235, and D265 in a human IgG1 heavy chain are F, E, and A, respectively, the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in the first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the second heavy chain.

In one embodiment, in both the first and second heavy chains the amino acids in the positions corresponding to L234, L235, D265, N297, and P331 in a human IgG1 heavy chain are F, E, A, N, and P respectively, the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in the first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the second heavy chain.

In an alternative embodiment, in both the first and second heavy chains the amino acids in the positions corresponding to position L234, L235, and D265 in a human IgG1 heavy chain are F, E, and A, respectively, the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the first heavy chain, and the

amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in the second heavy chain.

In one embodiment, in both the first and second heavy chains the amino acids in the positions corresponding to L234, L235, D265, N297, and P331 in a human IgG1 heavy chain are F, E, A, N, and P respectively, the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in the first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in the second heavy chain.

As described herein, T cell recruitment to specific target cells, such as cancer or tumor cells, provides a way of killing the target cells. T cell mediated killing may be obtained by a bispecific antibody targeting CD3 with the first binding region and another target with the second binding region. Thus, in one embodiment, the first binding region is according to any embodiments described herein for the humanized or chimeric CD3 antibody, and the second binding region binds a different target than the first binding region. It is to be understood that when the antibody is a bispecific antibody, at least one half of the antibody, i.e. one of the pair of heavy and light chains of the antibody, is a humanized or chimeric antibody as herein described. Thus, one half of the bispecific antibody is a humanized or chimeric antibody binding CD3 according to the present invention and the other half may be humanized, chimeric, fully non-human or fully human binding a second target. Thus, in one embodiment, the antibody comprises a first and a second heavy chain, a first and second light chain, wherein said first heavy and said first light chains are humanized or chimeric and are connected via disulfide bridges forming a first binding region; and said second heavy and light chains are fully human and are connected via disulfide bridges forming a second binding region, wherein said first binding region is according to any aspect or embodiment herein described, and said second binding region binds a different target. In one embodiment, the antibody comprises a first and a second heavy chain, a first and second light chain, wherein said first heavy and said first light chains are humanized or chimeric and are connected via disulfide bridges forming a first binding region; and said second heavy and light chains are humanized or chimeric and are connected via disulfide bridges forming a second binding region, wherein said first binding region is according to any aspect or embodiment herein described, and said second binding region binds a different epitope of CD3 than said first binding region.

The term “disulfide bridges” as used herein refers to the covalent bond between two Cysteine residues, i.e. said interaction may also be designated a Cys-Cys interaction.

The term “target” as used herein, refers to a molecule to which the binding region of the antibody according to the invention binds. When used in the context of the

binding of an antibody the term includes any antigen towards which the raised antibody is directed.

In one particular embodiment, the first heavy and the first light chains are humanized or chimeric and are connected via disulfide bridges forming a first binding region; and the second heavy and light chains are fully human and are connected via disulfide bridges forming a second binding region, wherein the first binding region is according to any aspect or embodiment herein described, and the second binding region binds a different target; and wherein in at least one of the first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.

In one particular embodiment, the first heavy and the first light chains are humanized or chimeric and are connected via disulfide bridges forming a first binding region; and the second heavy and light chains are fully human and are connected via disulfide bridges forming a second binding region, wherein the first binding region is according to any aspect or embodiment herein described, and the second binding region binds a different epitope of CD3 than the first binding region; and wherein in at least one of the first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.

In one particular embodiment, the first heavy and the first light chains are humanized or chimeric and are connected via disulfide bridges forming a first binding region; and the second heavy and light chains are fully human and are connected via disulfide bridges forming a second binding region, wherein the first binding region is according to any aspect or embodiment herein described, and the second binding region binds a different target; and wherein in both the first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.

In one particular embodiment, the first heavy and the first light chains are humanized or chimeric and are connected via disulfide bridges forming a first binding region; and the second heavy and light chains are fully human and are connected via disulfide bridges forming a second binding region, wherein the first binding region is according to any aspect or embodiment herein described, and the second binding region binds a different epitope of CD3 than the first binding region; and wherein in both the first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.

In another aspect, the present invention relates to a method of reducing the binding affinity of an antibody binding to human CD3 compared to a reference antibody comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 3, which method comprises introducing a mutation in one of the three CDR sequences of the said reference antibody selected from a mutation in one of the positions selected from the group of T31M, T31P, N57, H101, S110 and Y114, wherein the positions are numbered according to the reference sequence of the SEQ ID NO: 4.

The numbering of the amino acids in the VH regions and the positions to be mutated are according to the amino acids in SEQ ID NO: 4. Numbering are accoding to a direct numerical numbering scheme from the first amino acid to number 125 in the direction from N-terminus to the C-terminus. The numerical numbering of positions corresponding to SEQ ID NO:4 is illustrated in figure 2. Further, The CDR regions have been annotated according to the IMGT defnitions.

In one embodiment of the invention the method comprises introducing a T31M or T31P mutation. Position T31 is in accordance to SEQ ID NO:4.

In one embodiment of the invention the method comprises introducing a mutation in the position N57. Position N57 is in accordance to SEQ ID NO:4. In one embodiment the mutation is N57E

In one embodiment of the invention the method comprises introducing a mutation in the position H101. Position H101 is in accordance to SEQ ID NO: 4. In one embodiment the mutation is H101G or H101N.

In one embodiment of the invention the method comprises introducing a mutation in the position Y114. Position Y114 is in accordance to of SEQ ID NO: 4. In one embodiment the mutation is Y114, Y114R or Y114V.

In one embodiment of the invention the method comprises introducing a mutation in the mutation in the VH CDR3 region corresponding to a position selected from the group of H101, S110 and Y114.

In one embodiment of the inventon the method comprises introducing a mutation in the VH CDR3 region is selected from the group consisting of H101G, H101N, S110A, S110G, Y114M, Y114R and Y114V.

In one embodiment of the inventon the method comprises introducing a mutation, wherein antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a K_D value from 1.6 $x10^{-8}$ M to 9.9$x10^{-8}$ M or from 1.0$x10^{-7}$ to 9.9$x10^{-7}$ M as determined by Bio-Layer Interferometry.

In one embodiment of the inventon the method comprises introducing a mutation, wherein the antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a K_D value from 1.4 $x10^{-8}$ to 1.0 $x10^{-8}$ M or from as 9.9$x10^{-9}$ to 1 $x10^{-9}$ M as determined by Bio-Layer Interferometry.

In one embodiments of the present invention the antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a K_D value from 1.6 $x10^{-8}$ M to 9.9$x10^{-8}$ M or from 1.0$x10^{-7}$ to 9.9$x10^{-7}$ M as determined by Bio-Layer Interferometry.

In another aspect, the present invention relates to a method of increasing the binding affinity of an antibody binding to human CD3 compared to a reference antibody comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 3, which method comprises introducing a mutation in the VH CDR3 corresponding to position G105, wherein the position is numbered according to the reference sequence of the SEQ ID NO: 4.

In one embodiment of the invention the method comprises introducing a mutation in the position G105. Position G105 is in accordance to SEQ ID NO: 4. In one embodiment the mutation is G105P.

In one embodiment of the invention the method comprises introducing at most 5 further mutations, at most 4 further mutations, at most 3 further mutations, at most 2 further mutations or at most 1 further mutation into the CDRs of the VH region of the reference antibody as set forth in SEQ ID NO: 1, 2, 3.

In one embodiment of the invention the method of increased or reduced binding affinity comprises a binding region comprising a heavy chain variable (VH) region, wherein said

VH region comprises the CDR1, CDR2, and CDR3 sequences selected from the group consisting of;

a) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 54, 2, 3 [T31M] ;
b) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 58, 2, 3 [T31P];
c) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 106, 3 [N57E];
d) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G];
e) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 184 [H101N];
f) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 220 [G105P];
g) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 236 [S110A];
h) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 244 [S110G];
i) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 284 [Y114M];
j) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 292 [Y114R]; and
k) CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 298 [Y114V].

In another embodiment of the present invention the method comprises introducing a mutation in the VH region CDR2 region corresponding to N57E. In a further embodiment of the present invention the method comprises introducing a mutation in the VH region CDR3 region corresponding to H101G, H101N, G105P, S110A, S110G, Y114M, Y114R or Y114V. In another aspect, the present invention relates to method of reducing or increasing the bining affinity of an antibody to CD3, wherein said antibody comprises a binding region comprising heavy chain variable (VH) region, wherein said VH region comprises a mutation in one of the three CDR sequences of a reference antibody as set forth by CDR1 SEQ ID: 1, CDR2 SEQ ID: 2 and CDR3 SEQ ID: 3, wherein said antibody comprises a mutation in one of the following positions selected from the group of T31M, T31P, N57, H101, G105,

S110 and Y114, wherein the positions are corresponding to the reference sequence of the SEQ ID NO: 4.

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR1 region sequence corresponding to T31M or T31P. In another embodiment of the present invention the method comprises introducing a mutation in the VH region CDR2 region corresponding to N57E. In a further embodiment of the present invention the method comprises introducing a mutation in the VH region CDR3 region corresponding to H101G, H101N, G105P, S110A, S110G, Y114M, Y114R or Y114V.

In further aspect, the present invention relates to a method of reducing the binding affinity of an antibody binding to CD3 compared to a reference antibody comprising a heavy chain variable (VH) region, wherein said VH region comprises CDR1, CDR2, and CDR3 having the CDR sequences set forth in SEQ ID NO: 1, 2 and 3, which method comprises introducing a mutation in one of the VH region CDR1, CDR2 or CDR3 sequences as set forth in SEQ ID NO: 1, 2 or 3.

In one embodiment the present invention the method comprises introducing a mutation in one of the three CDR regions of the VH region corresponding to one of the following positions: T31, N57, H101, S110 or Y114, wherein the positions are corresponding to the reference sequence of the SEQ ID NO: 4.

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR1 sequence corresponding to position T31, wherein the CDR1 sequence is as set forth in SEQ ID NO 1. When the mutation is represented by X the resulting CDR1 sequence may be presented as GFTFNXYA. In one embodiment the three CDR sequences of the VH region may have the following sequences CDR1 GFTFNXYA, CDR2 IRSKYNNYAT and CDR3 VRHGNFGNSYVSWFAY. In one embodiment the mutation in position T31 in in VH region CDR1 is a T31M or T31P mutation.

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR2 sequence corresponding to postion N57, wherein the CDR2 sequence is as set forth in SEQ ID NO 2. When the mutation is represented by X the resulting CDR2 sequence may be presented as IRSKYNXYAT. In one embodiment the three CDR sequences of the VH region may have the following sequences CDR1 GFTFNTYA,

CDR2 IRSKYNXYAT and CDR3 VRHGNFGNSYVSWFAY. In one embodiment the mutation in position N57 in VH region CDR2 is a N57E mutation.

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR3 sequence corresponding to postion H101, wherein the CDR3 sequence is as set forth in SEQ ID NO 3. When the mutation is represented by X the resulting CDR3 sequence may be presented as VRXGNFGNSYVSWFAY. In one embodiment the three CDR sequences of the VH region may have the following sequences CDR1 GFTFNTYA, CDR2 IRSKYNNYAT and CDR3 VRXGNFGNSYVSWFAY. In one embodiment the mutation in position H101 in VH region CDR3 is an H101G or an H101N mutation.

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR3 sequence corresponding to postion S110, wherein the CDR3 sequence is as set forth in SEQ ID NO 3. When the mutation is represented by X the resulting CDR3 sequence may be presented as VRHGNFGNSYVXWFAY. In one embodiment the three CDR sequences of the VH region may have the following sequences CDR1 GFTFNTYA, CDR2 IRSKYNNYAT and CDR3 VRHGNFGNSYVXWFAY. In one embodiment the mutation in position H101 in VH region CDR3 is a S110A or a S110G mutation.

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR3 sequence corresponding to position Y114, wherein the CDR3 sequence is as set forth in SEQ ID NO 3. When the mutation is represented by X the resulting CDR3 sequence may be presented as VRHGNFGNSYVSWFAX. In one embodiment the three CDR sequences of the VH region may have the following sequences CDR1 GFTFNTYA, CDR2 IRSKYNNYAT and CDR3 VRHGNFGNSYVSWFAX. In one embodiment the mutation in position Y114 in VH region CDR3 is a Y114M, Y114R or a Y114V mutation.

In one embodiment of the invention the method comprises introducing at most 3 mutations, at most 2 mutations or at most 1 mutation into the one or more of the three CDRs of the VH region of a reference antibody as set forth in SEQ ID NO: 1, 2, 3.

In one embodiment of the invention the method comprises introducing at most 10 mutations, at most 9 mutations at, at most 8 mutations, at most 7 mutations, at most 6 mutations, at most 5 mutations, at most 4 mutations, at most 3 mutations, at most 2 mutations or at most 1 mutation into variable heavy chain frame work region of an antibod

, wherein said mutations does preferable not alter bining of the antibody to CD3 compared to the same antibody without the mutation(s).

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR1 sequence selected from T31M or T31P. In another embodiment of the present invention the method comprises introducing a mutation in the VH region CDR2 sequence of N57E. In a further embodiment of the present invention the method comprises introducing a mutation in the VH region CDR3 sequence selected from the group of: H101G, H101N, S110A, S110G, Y114M, Y114R and Y114V.

In another aspect, the present invention relates to a method of increasing the binding affinity of an antibody binding to CD3 compared to a reference antibody comprising a heavy chain variable (VH) region, wherein said VH region comprises CDR1, CDR2, and CDR3 having the CDR sequences set forth in SEQ ID NO: 1, 2 and 3, which method comprises introducing a mutation in one of the VH region CDR1, CDR2 or CDR3 sequences as set forth in SEQ ID NO: 1, 2 or 3.

In one embodiment of the present invention the method comprises introducing a mutation in the VH region CDR3 sequence corresponidng to postion G105, wherein the CDR3 sequence is as set forth in SEQ ID NO 3. When the mutation is represented by X the resulting CDR3 sequence may be presented as VRHGNFXNSYVSWFAY. In one embodiment the three CDR sequences of the VH region may have the following sequences CDR1 GFTFNTYA, CDR2 IRSKYNNYAT and CDR3 VRXGNFGNSYVSWFAY. In one embodiment the mutation in position G105 in VH region CDR3 is a G105P mutation.

Nucleic acid constructs, expression vectors, and host cells

In one aspect, the present invention relates to a nucleic acid construct encoding one or more sequences set out in Table 1. Thus, the present invention relates to a nucleic acid construct encoding any one of the sequences set out in SEQ ID NOs: 107; 221; 59; 245; 299; 285; 55; 185; 179; 237; 177 and 293.

In a further aspect, the invention relates to nucleic acid construct encoding a sequence of a humanized or chimeric CD3 antibody according to the present invention, to expression vectors comprising a nucleic acid construct according to the present invention, to host cells comprising such expression vectors, and to methods of producing such an

antibody by culturing such host cells under appropriate conditions whereby the antibody is produced and, optionally, retrieved. Humanized CD3 antibodies may also be denoted as "huCD3".

In one embodiment, the invention provides an expression vector comprising (i) a nucleic acid sequence encoding a heavy chain sequence of a humanized or chimeric antibody according to the invention, (ii) a nucleic acid sequence encoding a light chain sequence of a humanized or chimeric antibody according to the invention, or (iii) both (i) and (ii). Thus, the expression vector comprises one or more nucleic acid constructs or nucleic acid sequences according to any aspect or embodiment herein described.

In one embodiment, the expression vector of the invention comprises a nucleic acid sequence encoding one or more of the heavy chain and light chain CDR sequences wherein the VH CDR sequences are selected from the group consisting of: SEQ ID NOs.: 12, 2, 3; 14, 2, 3; 16, 2, 3; 18, 2, 3; 20, 2, 3; 22, 2, 3; 24, 2, 3; 26, 2, 3; 28, 2, 3; 30, 2, 3; 32, 2, 3; 34, 2, 3; 36, 2, 3; 38, 2, 3;: 40, 2, 3; 42, 2, 3; 44, 2, 3;: 46, 2, 3; 48, 2, 3; 50, 2, 3; 52, 2, 3; 54, 2, 3; 56, 2, 3; 58, 2, 3; 60, 2, 3; 62, 2, 3; 64, 2, 3; 66, 2, 3; 68, 2, 3; 70, 2, 3; 72, 2, 3; 74, 2, 3; 76, 2, 3; 78, 2, 3; 80, 2, 3; 82, 2, 3; 84, 2, 3; 86, 2, 3; 88, 2, 3; 90, 2, 3; 92, 2, 3; 94, 2, 3; 96, 2, 3; 98, 2, 3; 1, 100, 3; 1, 102, 3; 1, 104, 3; 1, 106, 3; 1, 108, 3; 1, 110, 3; 1, 112, 3; 1, 114, 3; 1, 116, 3; 1, 118, 3; 1, 120, 3; 1, 122, 3; 1, 124, 3; 1, 126, 3; 1, 128, 3; 1, 130, 3; 1, 132, 3; 1, 134, 3; 1, 136, 3; 1, 138, 3;; 1, 140, 3; 1, 142, 3; 1, 144, 3; 1, 146, 3; 1, 148, 3; 1, 150, 3; 1, 152, 3; 1, 154, 3; 1, 156, 3; 1, 158, 3; 1: 1, 2, 176; 1, 2, 178; 1, 2, 180; 1, 2, 182; 1, 2, 184; 1, 2, 186; 1, 2, 188; 1, 2, 190;: 1, 2, 192; 1, 2, 194; 1, 2, 196; 1, 2, 198; 1, 2, 200; 1, 2, 202; 1, 2, 204; 1, 2, 206;: 1, 2, 208; 1, 2, 210; 1, 2, 212; 1, 2, 214; 1, 2, 216; 1, 2, 218; 1, 2, 220;: 1, 2, 222; 1, 2, 224; 1, 2, 226; 1, 2, 228; 1, 2, 230; 1, 2, 232; 1, 2, 234; 1, 2, 236; 1, 2, 238; 1, 2, 240; 1, 2, 242; 1, 2, 244; 1, 2, 246; 1, 2, 248; 1, 2, 250; 1, 2, 252; 1, 2, 254; 1, 2, 256; 1, 2, 258; 1, 2, 260;1, 2, 262; 1, 2, 264; 1, 2, 266; 1, 2, 268; 1, 2, 270; 1, 2, 272; 1, 2, 274; 1, 2, 276; 1, 2, 278;: 1, 2, 280; 1, 2, 282; 1, 2, 284; 1, 2, 286;: 1, 2, 288; 1, 2, 290; 1, 2, 292; 1, 2, 294; 1, 2, 296; 1, 2, 298 and 1, 2, 300. and wherein the VL CDR sequences are selected from the group consisting of CDR sequences as set forth in SEQ ID NO: 6, GTN, 7; 302, GTN, 7; 304, GTN, 7; 306, GTN, 7; 308, GTN, 7;: 310, GTN, 7; 312, GTN, 7; 314, GTN, 7; 316, GTN, 7; 318, GTN, 7; 320, GTN, 7; 322, GTN, 7; 324, GTN, 7; 326, GTN, 7; 328, GTN, 7; 330, GTN, 7;: 6, GTN, 332; 6, GTN, 334; 6, GTN, 336; 6, GTN, 338; 6, GTN, 340; 6, GTN, 342; 6, GTN, 344; 6, GTN, 346; 6, GTN, 348; 6, GTN, 350; 6, GTN, 352; 6, GTN, 354; 6, GTN, 356; 6, GTN, 358; 6, GTN, 360; 6, GTN, 362; 6, GTN, 364; 6, GTN, 366; 6, GTN, 368; 6, GTN, 370; 6, GTN, 372; 6, GTN, 374; 6, GTN, 376; 6, GTN, 378; 6, GTN, 380; 6, GTN, 382; 6, GTN, 384; GTN, 386;: 6, GTN, 388; 6, GTN, 390;, GTN, 392; and 6, GTN, 394.

In one embodiment, the expression vector of the invention comprises a nucleic acid sequence encoding one or more of the heavy chain and light chain CDR sequences wherein VL region CDR1, CDR2, CDR3 region CDR sequences comprise the CDR sequences as set forth in SEQ ID NO: 6, GTN, 7 and VH region region CDR1, CDR2, CDR3 region CDR sequences are selected from the group consisting of: CDR1, CDR2, CDR3 as set forth in SEQ ID NOs.: 54, 2, 3; CDR1, CDR2, CDR3 as set forth in SEQ ID NO: 58, 2, 3; CDR1, CDR2, CDR3 as set forth in SEQ ID NO:1, 106, 3; CDR1, CDR2, CDR3 as set forth in SEQ ID NO: 1, 2, 176; CDR1, CDR2, CDR3 as set forth in SEQ ID NO: 1, 2, 184; CDR1, CDR2, CDR3 as set forth in SEQ ID NO 1, 2, 220; 1, 2, CDR1, CDR2, CDR3 as set forth in SEQ ID NO 236; 1, 2, 244; CDR1, CDR2, CDR3 as set forth in SEQ ID NO 1, 2, 284; CDR1, CDR2, CDR3 as set forth in SEQ ID NO 1, 2, 292 and CDR1, CDR2, CDR3 as set forth in SEQ ID NO1, 2, 298.

In a particular embodiment, the expression vector comprises a nucleic acid sequence encoding a variant of one or more of the above amino acid sequences, said variant having at most 25 amino acid modifications, such as at most 20, such as at most 15, 14, 13, 12, or 11 amino acid modifications, such as 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid modifications, such as deletions or insertions, preferably substitutions, such as conservative or non-conservative substitutions, or at least 80% identity to any of said sequences, such as at least 85% identity or 90% identity or 95% identity, such as 96% identity or 97% identity or 98% identity or 99% identity to any of the afore-mentioned amino acid sequences. The present invention also relates to nucleic acid sequences different from the above mentioned nucleic acid sequences but which due to the variance of the genetic code encode the same amino acid sequence as an antibody of the present invention. E.g. the nucleic acid sequence may vary but result in an identical amino acid sequences as any amino acid sequence herein described. It is well-known for the skilled person how to identify such further nucleic acid sequences based on the genetic code.

In a further embodiment, the expression vector further comprises a nucleic acid sequence encoding the constant region of a light chain, a heavy chain or both light and heavy chains of an antibody, e.g. a human antibody.

Such expression vectors as described above may be used for recombinant production of antibodies of the invention.

An expression vector in the context of the present invention may be any suitable vector, including chromosomal, non-chromosomal, and synthetic nucleic acid vectors (a nucleic acid sequence comprising a suitable set of expression control elements). Examples of such vectors include derivatives of SV40, bacterial plasmids, phage DNA,

baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, and viral nucleic acid (RNA or DNA) vectors. In one embodiment, a humanized or chimeric CD3 antibody-encoding nucleic acid is comprised in a naked DNA or RNA vector, including, for example, a linear expression element (as described in for instance [64]), a compacted nucleic acid vector (as described in for instance [65] and/or [66]), a plasmid vector such as pBR322, pUC 19/18, or pUC 118/119, a "midge" minimally-sized nucleic acid vector (as described in for instance [67]), or as a precipitated nucleic acid vector construct, such as a $CaPO_4$-precipitated construct (as described in for instance [68], [69], [70], and [71]). Such nucleic acid vectors and the usage thereof are well known in the art (see for instance [72] and [73]).

In one embodiment, the vector is suitable for expression of the humanized or chimeric CD3 antibody in a bacterial cell. Examples of such vectors include expression vectors such as BlueScript (Stratagene), pIN vectors ([74]), pET vectors (Novagen, Madison WI) and the like.

An expression vector may also or alternatively be a vector suitable for expression in a yeast system. Any vector suitable for expression in a yeast system may be employed. Suitable vectors include, for example, vectors comprising constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH (reviewed in: [75] and [76]).

A nucleic acid construct and/or vector may also comprise a nucleic acid sequence encoding a secretion/localization sequence, which can target a polypeptide, such as a nascent polypeptide chain, to the periplasmic space or into cell culture media. Such sequences are known in the art, and include secretion leader or signal peptides, organelle-targeting sequences (e. g., nuclear localization sequences, ER retention signals, mitochondrial transit sequences, chloroplast transit sequences), membrane localization/anchor sequences (e. g., stop transfer sequences, GPI anchor sequences), and the like which are well-known in the art.

In an expression vector of the invention, humanized or chimeric CD3 antibody-encoding nucleic acids may comprise or be associated with any suitable promoter, enhancer, and other expression-facilitating elements. Examples of such elements include strong expression promoters (e. g., human CMV IE promoter/enhancer as well as RSV, SV40, SL3-3, MMTV, and HIV LTR promoters), effective poly (A) termination sequences, an origin of replication for plasmid product in *E. coli*, an antibiotic resistance gene as selectable marker, and/or a convenient cloning site (e.g., a polylinker). Nucleic acid constructs and/or vectors may also comprise an inducible promoter as opposed to a constitutive promoter such as CMV IE (the skilled person will recognize that

such terms are actually descriptors of a degree of gene expression under certain conditions).

In one embodiment, the humanized or chimeric CD3 antibody-encoding expression vector is positioned in and/or delivered to the host cell or host animal via a viral vector.

Such expression vectors may be used for recombinant production of humanized or chimeric CD3 antibodies.

In one aspect, the invention provides a host cell comprising an expression vector according to the invention.

In one aspect, the humanized or chimeric CD3 antibodies of any aspect or embodiment described herein are provided by use of recombinant eukaryotic, recombinant prokaryotic, or recombinant microbial host cell which produces the antibody. Accordingly, the invention provides a recombinant eukaryotic, recombinant prokaryotic, or recombinant microbial host cell, which produces a humanized or chimeric CD3 antibody or immunoglobulin as defined herein. Examples of host cells include yeast, bacterial and mammalian cells, such as CHO or HEK-293 cells. For example, in one embodiment, the host cell comprises a nucleic acid sequence stably integrated into the cellular genome that comprises a sequence coding for expression of a humanized or chimeric CD3 antibody described herein. In another embodiment, the host cell comprises a non-integrated nucleic acid sequence, such as a plasmid, cosmid, phagemid, or linear expression element, which comprises a sequence coding for expression of a humanized or chimeric CD3 antibody described herein.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which an expression vector or nucleic acid construct or sequence has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell, but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Recombinant host cells include, for example, eukaryotic host cells, such as CHO cells, HEK-293 cells, PER.C6, NS0 cells, and lymphocytic cells, and prokaryotic cells such as *E. coli* and other eukaryotic hosts such as plant cells and fungi.

In a further aspect, the invention relates to a method for producing a humanized or chimeric CD3 antibody of the invention, said method comprising the steps of

a) culturing a host cell of the invention as described herein above, and

b) retrieving and/or purifying the antibody of the invention from the culture media.

In a further aspect, the nucleotide sequence encoding a sequence of a humanized or chimeric CD3 antibody further encodes a second moiety, such as a therapeutic polypeptide. Exemplary therapeutic polypeptides are described elsewhere herein. In one embodiment, the invention relates to a method for producing a humanized or chimeric CD3 antibody fusion protein, said method comprising the steps of

a) culturing a host cell comprising an expression vector comprising such a nucleotide sequence, and

b) retrieving and/or purifying the humanized or chimeric CD3 antibody fusion protein from the culture media.

Compositions

In one aspect, the invention provides a composition comprising the antibody or bispecific antibody according to any aspect and embodiment herein described.

In one aspect, the invention provides a pharmaceutical composition comprising the antibody or bispecific antibody as defined in any one of the aspects and embodiments herein described, and a pharmaceutically acceptable carrier.

The pharmaceutical compositions may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in [77].

The pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients should be suitable for the humanized or chimeric antibody of the present invention and the chosen mode of administration. Suitability for carriers and other components of pharmaceutical compositions is determined based on the lack of significant negative impact on the desired biological properties of the chosen compound or pharmaceutical composition of the present invention (e.g., less than a substantial impact (10% or less relative inhibition, 5% or less relative inhibition, etc.)) on antigen binding.

A pharmaceutical composition of the present invention may also include diluents, fillers, salts, buffers, detergents (e. g., a nonionic detergent, such as Tween-20 or Tween-80), stabilizers (e.g., sugars or protein-free amino acids), preservatives, tissue fixatives, solubilizers, and/or other materials suitable for inclusion in a pharmaceutical composition.

The actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or

the amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

The pharmaceutical composition may be administered by any suitable route and mode. Suitable routes of administering a humanized or chimeric antibody of the present invention *in vivo* and *in vitro* are well known in the art and may be selected by those of ordinary skill in the art.

In one embodiment, a pharmaceutical composition of the present invention is administered parenterally.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and include epidermal, intravenous, intramuscular, intra-arterial, intrathecal, intracapsular, intra-orbital, intracardiac, intradermal, intraperitoneal, intratendinous, transtracheal, subcutaneous, subcuticular, intra-articular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection and infusion.

In one embodiment that pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion.

In a preferred embodiment the pharmaceutical compostion is administered subcutaneous.

Pharmaceutically acceptable carriers include any and all suitable solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonicity agents, antioxidants and absorption-delaying agents, and the like that are physiologically compatible with a humanized or chimeric antibody of the present invention.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the present invention include water, saline, phosphate buffered saline, ethanol, dextrose, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, corn oil, peanut oil, cottonseed oil, and sesame oil, carboxymethyl cellulose colloidal solutions, tragacanth gum and injectable organic esters, such as ethyl oleate, and/or various buffers. Other carriers are well known in the pharmaceutical arts.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of

the present invention is contemplated. When referring to the "active compound" it is contemplated to also refer to the humanized or chimeric antibody according to the present invention.

Proper fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

Pharmaceutical compositions of the present invention may also comprise pharmaceutically acceptable antioxidants for instance (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Pharmaceutical compositions of the present invention may also comprise isotonicity agents, such as sugars, polyalcohols, such as mannitol, sorbitol, glycerol or sodium chloride in the compositions.

The pharmaceutical compositions of the present invention may also contain one or more adjuvants appropriate for the chosen route of administration such as preservatives, wetting agents, emulsifying agents, dispersing agents, preservatives or buffers, which may enhance the shelf life or effectiveness of the pharmaceutical composition. The humanized or chimeric antibody of the present invention may be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and micro-encapsulated delivery systems. Such carriers may include gelatin, glyceryl monostearate, glyceryl distearate, biodegradable, biocompatible polymers such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, poly-orthoesters, and polylactic acid alone or with a wax, or other materials well known in the art. Methods for the preparation of such formulations are generally known to those skilled in the art (see e.g., [78]).

In one embodiment, the humanized or chimeric antibody of the present invention may be formulated to ensure proper distribution *in vivo*. Pharmaceutically acceptable carriers for parenteral administration include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of

the present invention is contemplated. Other active or therapeutic compounds may also be incorporated into the compositions.

Pharmaceutical compositions for injection must typically be sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, micro-emulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier may be an aqueous or a non-aqueous solvent or dispersion medium containing for instance water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as glycerol, mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients e.g. as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients e.g. from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

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

Therapeutic applications

In another aspect, the present invention relates to a humanized or chimeric antibody, or pharmaceutical composition of the invention as defined in any aspect or embodiment herein described, for use as a medicament.

In another aspect, the present invention relates to a humanized or chimeric antibody, or pharmaceutical composition of the invention as defined in any aspect or embodiment herein described, for use in the treatment of a disease.

In one embodiment of the present invention a bispecific antibody, a composition, a pharmaceutical composition, for use in the treatment of a disease.

In one embodiment of the present invention, bispecific antibody, a composition, a pharmaceutical composition is for use for the treatment of a disease, wherein the disease is cancer, an infectious disease, or autoimmune diseases.

The humanized or chimeric antibody or pharmaceutical composition of the invention can be used as in the treatment of any cancer wherein the effector mechanisms of cytotoxic T cells are desired. For example, the humanized or chimeric antibody may be administered to cells in culture, e.g., *in vitro* or *ex vivo*, or to human subjects, e.g. *in vivo*, to treat or prevent disorders such as cancer, inflammatory or autoimmune disorders. As used herein, the term "subject" is typically a human which respond to the humanized or chimeric antibody, or pharmaceutical composition. Subjects may for instance include human patients having disorders that may be corrected or ameliorated by modulating a target function or by leading to killing of the cell, directly or indirectly.

In another aspect, the present invention provides methods for treating or preventing a disorder, such as cancer, wherein recruitment of T cells would contribute to the treatment or prevention, which method comprises administration of a therapeutically effective amount of a humanized or chimeric antibody, or pharmaceutical composition of the present invention to a subject in need thereof. The method typically involves administering to a subject a humanized or chimeric antibody in an amount effective to treat or prevent the disorder.

In one particular aspect, the present invention relates to a method of treatment of cancer comprising administering the humanized or chimeric antibody or pharmaceutical composition of the invention as defined in any aspect and embodiments herein described, to a subject in need thereof.

In another aspect, the present invention relates to the use or the method as defined in any aspect or embodiments herein described wherein the humanized or chimeric antibody is a bispecific antibody specifically binding to both CD3 and a cancer-specific target, or a target that is overexpressed in cancer or associated with cancer, such as HER2, CD19, EpCAM, EGFR, CD66e (or CEA, CEACAM5), CD33, EphA2 or MCSP (or HMW-

MAA), CD20 and wherein the disease is cancer, such as breast cancer, prostate cancer, non-small cell lung cancer, bladder cancer, ovarian cancer, gastric cancer, colorectal cancer, esophageal cancer and squamous cell carcinoma of the head & neck, cervical cancer, pancreatic cancer, testis cancer, malignant melanoma, a soft-tissue cancer (e.g., synovial sarcoma), an indolent or aggressive form of B-cell lymphoma, chronic lymphatic leukemia or acute lymphatic leukemia.

The efficient dosages and dosage regimens for the humanized or chimeric antibody depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

A physician having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician could start doses of the humanized or chimeric antibody employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable dose of a composition of the present invention will be that amount of the humanized or chimeric antibody which is the lowest dose effective to produce a therapeutic effect according to a particular dosage regimen. Such an effective dose will generally depend upon the factors described above.

For example, an "effective amount" for therapeutic use may be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may, for example, be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition may be evaluated by examining the ability of the humanized or chimeric antibody to inhibit cell growth or to induce cytotoxicity by *in vitro* assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound, i.e. a therapeutic humanized or chimeric antibody, or pharmaceutical composition according to the invention, may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

An exemplary, non-limiting range for a therapeutically effective amount of a humanized or chimeric antibody of the invention is about 0.001-30 mg/kg, such as about 0.001-20 mg/kg, such as about 0.001-10 mg/kg, such as about 0.001-5 mg/kg, for example about 0.001-2 mg/kg, such as about 0.001-1 mg/kg, for instance about 0.001, about 0.01, about 0.1, about 1, about 5, about 8, about 10, about 12, about 15, about 18 mg/kg.

Administration may e.g. be intravenous, intramuscular, intraperitoneal, or subcutaneous, and for instance administered proximal to the site of the target.

Dosage regimens in the above methods of treatment and uses are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

In one embodiment, the efficacy of the treatment is monitored during the therapy, e.g. at predefined points in time.

If desired, an effective daily dose of a pharmaceutical composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In another embodiment, the humanized or chimeric antibody, or pharmaceutical composition is administered by slow continuous infusion over a long period, such as more than 24 hours, in order to minimize any unwanted side effects.

While it is possible for a humanized or chimeric antibody of the present invention to be administered alone, it is preferable to administer the humanized or chimeric antibody as a pharmaceutical composition as described above.

An effective dose of a humanized or chimeric antibody of the invention may also be administered using a weekly, biweekly or triweekly dosing period. The dosing period may be restricted to, e.g., 8 weeks, 12 weeks or until clinical progression has been established. Alternatively, an effective dose of a humanized or chimeric antibody of the invention may be administered every second, third or fourth week.

In one embodiment, the humanized or chimeric antibody may be administered by infusion in a weekly dosage of calculated by mg/m^2. Such dosages can, for example, be based on the mg/kg dosages provided above according to the following: dose (mg/kg) x 70: 1.8. Such administration may be repeated, *e.g.*, 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours. In one embodiment, the humanized or chimeric antibody may be administered by slow continuous infusion over a long period, such as more than 24 hours, in order to reduce toxic side effects.

In one embodiment, the humanized or chimeric antibody may be administered in a weekly dosage of calculated as a fixed dose for up to 8 times, such as from 4 to 6 times when given once a week. Such regimen may be repeated one or more times as necessary, for example, after 6 months or 12 months. Such fixed dosages can, for example, be based on the mg/kg dosages provided above, with a body weight estimate of 70 kg. The dosage may be determined or adjusted by measuring the amount of

humanized or chimeric antibody of the present invention in the blood upon administration by for instance taking out a biological sample and using anti-idiotypic antibodies which target the binding region of the humanized or chimeric antibodies of the present invention.

In one embodiment, the humanized or chimeric antibody may be administered by maintenance therapy, such as, *e.g.*, once a week for a period of 6 months or more.

A humanized or chimeric antibody may also be administered prophylactically in order to reduce the risk of developing cancer, delay the onset of the occurrence of an event in cancer progression, and/or reduce the risk of recurrence when a cancer is in remission.

Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

A humanized or chimeric antibody may also be administered prophylactically in order to reduce the risk of developing cancer, delay the onset of the occurrence of an event in cancer progression, and/or reduce the risk of recurrence when a cancer is in remission. This may be especially useful in patients wherein it is difficult to locate a tumor that is known to be present due to other biological factors.

Diagnostic applications

The humanized or chimeric antibody of the invention may also be used for diagnostic purposes, using a composition comprising a humanized or chimeric antibody as described herein. Accordingly, the invention provides diagnostic methods and compositions using the humanized or chimeric antibodies described herein. Such methods and compositions can be used for purely diagnostic purposes, such as detecting or identifying a disease, as well as for monitoring of the progress of therapeutic treatments, monitoring disease progression, assessing status after treatment, monitoring for recurrence of disease, evaluating risk of developing a disease, and the like.

In one aspect, the present invention relates to a method of diagnosing a disease characterized by involvement or accumulation of CD3-expression cells, comprising administering the humanized or chimeric antibody according to the invention, the

composition according to the invention, or the pharmaceutically composition according to the invention to a subject, optionally wherein said humanized or chimeric antibody is labeled with a detectable agent.

In one aspect, the humanized or chimeric antibody of the present invention is used *ex vivo*, such as in diagnosing a disease in which cells expressing a specific target of interest and to which the humanized or chimeric antibody binds, are indicative of disease or involved in the pathogenesis, by detecting levels of the target or levels of cells which express the target of interest on their cell surface in a sample taken from a patient. This may be achieved, for example, by contacting the sample to be tested, optionally along with a control sample, with the humanized or chimeric antibody according to the invention under conditions that allow for binding of the antibody to the target. Complex formation can then be detected (e.g., using an ELISA). When using a control sample along with the test sample, the level of humanized or chimeric antibody or antibody-target complex is analyzed in both samples and a statistically significant higher level of humanized or chimeric antibody or antibody-target complex in the test sample indicates a higher level of the target in the test sample compared with the control sample.

Examples of conventional immunoassays in which humanized or chimeric antibodies of the present invention can be used include, without limitation, ELISA, RIA, FACS assays, plasmon resonance assays, chromatographic assays, tissue immunohistochemistry, Western blot, and/or immunoprecipitation.

Accordingly, in one embodiment, the present invention relates to a method of diagnosing a disease characterized by involvement or accumulation of CD3-expressing cells, comprising administering an antibody, bispecific antibody, composition or pharmaceutical composition according to any aspect or embodiment herein described, to a subject, optionally wherein the antibody is labeled with a detectable label.

In one embodiment, the invention relates to a method for detecting the presence of a target, or a cell expressing the target, in a sample comprising:

- contacting the sample with a humanized or chimeric antibody of the invention under conditions that allow for binding of the humanized or chimeric antibody to the target in the sample; and

- analyzing whether a complex has been formed. Typically, the sample is a biological sample.

In one embodiment, the sample is a tissue sample known or suspected of containing a specific target and/or cells expressing the target. For example, *in situ* detection of the target expression may be accomplished by removing a histological specimen from a patient, and providing the humanized or chimeric antibody of the present invention to such a specimen. The humanized or chimeric antibody may be provided by

applying or by overlaying the humanized or chimeric antibody to the specimen, which is then detected using suitable means. It is then possible to determine not only the presence of the target or target-expressing cells, but also the distribution of the target or target-expressing cells in the examined tissue (e.g., in the context of assessing the spread of cancer cells). Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) may be modified in order to achieve such *in situ* detection.

In the above assays, the humanized or chimeric antibody can be labeled with a detectable substance to allow bound antibody to be detected. Alternatively, bound (primary) specific humanized or chimeric antibody may be detected by an antibody which is labeled with a detectable substance and which binds to the primary specific humanized or chimeric antibody. Furthermore, in the above assays, a diagnostic composition comprising an antibody or bispecific antibody according to any aspect or embodiments herein described may be used. Thus, in one aspect, the present invention relates to a diagnostic composition comprising an antibody or bispecific antibody according to any aspect or embodiment herein described.

The level of target in a sample can also be estimated by a competition immunoassay utilizing target standards labeled with a detectable substance and an unlabeled target-specific humanized or chimeric antibody. In this type of assay, the biological sample, the labeled target standard(s) and the target-specific humanized or chimeric antibody are combined, and the amount of labeled target standard bound to the unlabeled target-specific humanized or chimeric antibody is determined. The amount of target in the biological sample is inversely proportional to the amount of labeled target standard bound to the target-specific humanized or chimeric antibody.

Suitable labels for the target-specific humanized or chimeric antibody, secondary antibody and/or target standard used in *in vitro* diagnostic techniques include, without limitation, various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, and acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, and ^{3}H.

In one aspect, the target-specific humanized or chimeric antibody of the invention is used in the *in vivo* imaging of target-expressing tissues such as tumors. For *in*

vivo methods, antibody fragments such as, e.g., $(Fab')_2$, Fab and Fab' fragments, are particularly advantageous because of their rapid distribution kinetics.

In vivo imaging can be performed by any suitable technique. For example, a target-specific humanized or chimeric antibody (e.g., an antibody or a fragment) labeled with ^{99}Tc, ^{131}I, ^{111}In or other gamma-ray emitting isotope may be used to image target-specific antibody accumulation or distribution in target-expressing tissues such as tumors with a gamma scintillation camera (e.g., an Elscint Apex 409ECT device), typically using low-energy, high resolution collimator or a low-energy all-purpose collimator. Alternatively, labeling with ^{89}Zr, ^{76}Br, ^{18}F or other positron-emitting radionuclide may be used to image target-specific humanized or chimeric antibody, or antibody fragment distribution in tumors using positron emission tomography (PET). The images obtained by the use of such techniques may be used to assess biodistribution of target in a patient, mammal, or tissue, for example in the context of using target as a biomarker for the presence of cancer/tumor cells. Variations on this technique may include the use of magnetic resonance imaging (MRI) to improve imaging over gamma camera techniques. Conventional immunoscintigraphy methods and principles are described in, e.g., [79], [80], and [81]. Moreover, such images may also, or alternatively, serve as the basis for surgical techniques to remove tumors. Furthermore, such *in vivo* imaging techniques may allow for the identification and localization of a tumor in a situation where a patient is identified as having a tumor (due to the presence of other biomarkers, metastases, etc.), but the tumor cannot be identified by traditional analytical techniques. All of these methods are features of the present invention.

The *in vivo* imaging and other diagnostic methods provided by the present invention are particularly useful in the detection of micrometastases in a human patient (e.g., a patient not previously diagnosed with cancer or a patient in a period of recovery/remission from a cancer).

In one embodiment, the present invention provides an *in vivo* imaging method wherein a target-specific humanized or chimeric antibody of the present invention is conjugated to a detection-promoting radio-opaque agent, the conjugated humanized or chimeric antibody is administered to a host, such as by injection into the bloodstream, and the presence and location of the labeled humanized or chimeric antibody in the host is assayed. Through this technique and any other diagnostic method provided herein, the present invention provides a method for screening for the presence of disease-related cells in a human patient or a biological sample taken from a human patient and/or for assessing the distribution of target-specific humanized or chimeric antibody prior to target-specific ADC therapy.

For diagnostic imaging, radioisotopes may be bound to a target-specific humanized or chimeric antibody either directly or indirectly by using an intermediary functional group. Useful intermediary functional groups include chelators, such as ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid (see for instance [82]).

In addition to radioisotopes and radio-opaque agents, diagnostic methods may be performed using target-specific antibodies that are conjugated to dyes (such as with the biotin-streptavidin complex), contrast agents, fluorescent compounds or molecules and enhancing agents (e.g. paramagnetic ions) for magnetic resonance imaging (MRI) (see, e.g., [83], which describes MRI techniques and the preparation of antibodies conjugated to a MRI enhancing agent). Such diagnostic/detection agents may be selected from agents for use in MRI, and fluorescent compounds. In order to load a target-specific humanized or chimeric antibody with radioactive metals or paramagnetic ions, it may be necessary to react it with a reagent having a long tail to which a multiplicity of chelating groups are attached for binding the ions. Such a tail may be a polymer such as a polylysine, polysaccharide, or another derivatized or derivatizable chain having pendant groups to which may be bound chelating groups such as, e.g., porphyrins, polyamines, crown ethers, bisthiosemicarbazones, polyoximes, and like groups known to be useful for this purpose. Chelates may be coupled to target-specific humanized or chimeric antibodies using standard chemistries.

Thus, the present invention provides a diagnostic target-specific humanized or chimeric antibody, wherein the target-specific humanized or chimeric antibody is conjugated to a contrast agent (such as for magnetic resonance imaging, computed tomography, or ultrasound contrast-enhancing agent) or a radionuclide that may be, for example, a gamma-, beta-, alpha-, Auger electron-, or positron-emitting isotope.

In one aspect, the present invention relates to a diagnostic composition comprising an antibody or bispecific antibody according to the invention.

In a further aspect, the invention relates to a kit for detecting the presence of target antigen or a cell expressing the target, in a sample, comprising:

- a target-specific humanized or chimeric antibody of the invention; and
- instructions for use of the kit.

Thus, in one aspect, the present invention provides a kit for detecting the presence of a CD3 antigen, or a cell expressing CD3, in a sample comprising the steps of;

a) contacting the sample with an antibody or bispecific antibody according to the invention, under conditions that allow for formation of a complex between the antibody or bispecific antibody and CD3; and

b) analyzing whether a complex has been formed.

In one embodiment, the present invention provides a kit for diagnosis of cancer comprising a container comprising a target-specific humanized or chimeric antibody, and one or more reagents for detecting binding of the target-specific humanized or chimeric antibody to the target. Reagents may include, for example, fluorescent tags, enzymatic tags, or other detectable tags. The reagents may also include secondary or tertiary antibodies or reagents for enzymatic reactions, wherein the enzymatic reactions produce a product that may be visualized. In one embodiment, the present invention provides a diagnostic kit comprising one or more target-specific humanized or chimeric antibodies of the present invention in labeled or unlabeled form in suitable container(s), reagents for the incubations for an indirect assay, and substrates or derivatizing agents for detection in such an assay, depending on the nature of the label. Control reagent(s) and instructions for use also may be included.

Diagnostic kits may also be supplied for use with a target-specific humanized or chimeric antibody, such as a labeled target-specific antibody, for the detection of the presence of the target in a tissue sample or host. In such diagnostic kits, as well as in kits for therapeutic uses described elsewhere herein, a target-specific humanized or chimeric antibody typically may be provided in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for a target cell or peptide. Typically, a pharmaceutically acceptable carrier (e.g., an inert diluent) and/or components thereof, such as a Tris, phosphate, or carbonate buffer, stabilizers, preservatives, biocides, inert proteins, e.g., serum albumin, or the like, also are included (typically in a separate container for mixing) and additional reagents (also typically in separate container(s)). In certain kits, a secondary antibody capable of binding to the target-specific humanized or chimeric antibody, which typically is present in a separate container, is also included. The second antibody is typically conjugated to a label and formulated in a manner similar to the target-specific humanized or chimeric antibody of the present invention. Using the methods described above and elsewhere herein, target-specific humanized or chimeric antibodies may be used to define subsets of cancer/tumor cells and characterize such cells and related tumor tissues.

Anti-idiotypic antibodies

In a further aspect, the invention relates to an anti-idiotypic antibody which binds to a humanized or chimeric antibody of the invention as described herein.

In one embodiment the invention relates to an anti-idiotypic antibody which binds to an antibody of any one of claims or a bispecific antibody according to the invention.

An anti-idiotypic (Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An anti-Id

antibody may be prepared by immunizing an animal of the same species and genetic type as the source of a CD3 monoclonal antibody with the monoclonal antibody to which an anti-Id is being prepared. The immunized animal typically can recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). Such antibodies are described in for instance US 4,699,880. Such antibodies are further features of the present invention.

An anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. An anti-anti-Id antibody may be epitopically identical to the original monoclonal antibody, which induced the anti-Id antibody. Thus, by using antibodies to the idiotypic determinants of a monoclonal antibody, it is possible to identify other clones expressing antibodies of identical specificity. Anti-Id antibodies may be varied (thereby producing anti-Id antibody variants) and/or derivatized by any suitable technique, such as those described elsewhere herein with respect to CD3-specific antibodies of the present invention. For example, a monoclonal anti-Id antibody may be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize BALB/c mice. Sera from these mice typically will contain anti-anti-Id antibodies that have the binding properties similar, if not identical, to an original/parent CD3 antibody.

Sequences
Table 1

SEQ ID NO:	Clone name	Sequence
SEQ ID NO:1	VH-huCD3-H1 CDR1	GFTFNTYA
SEQ ID NO:2	VH-huCD3-H1 CDR2	IRSKYNNYAT
SEQ ID NO:3	VH-huCD3-H1 CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:4	VH-huCD3-H1	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:5	VH-huCD3-H1	GAAGTGAAGCTGGTGGAATCTGGCGGCGGACTGGTGCAGCCTG GCGGATCTCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCA ACACCTACGCCATGAACTGGGTGCGCCAGGCCCCTGGCAAAGGC CTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGC CACCTACTACGCCGACAGCGTGAAGGACCGGTTCACCATCAGCC GGGACGACAGCAAGAGCAGCCTGTACCTGCAGATGAACAACCTG AAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAA CTTCGGCAACAGCTATGTGTCTTGGTTTGCCTACTGGGGCCAGGG CACCCTCGTGACAGTGTCTAGC
SEQ ID NO:6	VL-huCD3-L1 CDR1	TGAVTTSNY
	VL-huCD3-L1 CDR2	GTN
SEQ ID NO:7	VL-huCD3-L1 CDR3	ALWYSNLWV

SEQ ID NO:8	VL-huCD3-L1	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:9	VL-huCD3-L1	CAGGCCGTCGTGACCCAGGAACCCAGCTTTTCCGTGTCTCCTGGC GGCACCGTGACCCTGACCTGCAGATCTTCTACAGGCGCCGTGACC ACCAGCAACTACGCCAACTGGGTGCAGCAGACACCCGGCCAGGC CTTTAGAGGACTGATCGGCGGCACCAACAAGAGGGCACCTGGCG TGCCAGCCAGATTCAGCGGCAGCCTGATCGGAGATAAGGCCGCC CTGACAATCACTGGCGCCCAGGCTGACGACGAGAGCATCTACTTT TGCGCCCTGTGGTACAGCAACCTGTGGGTGTTCGGCGGAGGCAC CAAGCTGACAGTGCTG
SEQ ID NO:6	VL-huCD3-L1-T41K CDR1	TGAVTTSNY
	VL-huCD3-L1-T41K CDR2	GTN
SEQ ID NO:7	VL-huCD3-L1-T41K CDR3	ALWYSNLWV
SEQ ID NO:10	VL-huCD3-L1-T41K	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:11	VL-huCD3-L1-T41K	CAGGCCGTCGTGACCCAGGAACCCAGCTTTTCCGTGTCTCCTGGC GGCACCGTGACCCTGACCTGCAGATCTTCTACAGGCGCCGTGACC ACCAGCAACTACGCCAACTGGGTGCAGCAGAAGCCCGGCCAGGC CTTTAGAGGACTGATCGGCGGCACCAACAAGAGGGCACCTGGCG TGCCAGCCAGATTCAGCGGCAGCCTGATCGGAGATAAGGCCGCC CTGACAATCACTGGCGCCCAGGCTGACGACGAGAGCATCTACTTT TGCGCCCTGTGGTACAGCAACCTGTGGGTGTTCGGCGGAGGCAC CAAGCTGACCGTCCTA
SEQ ID NO:12	HC_N30A CDR1	GFTFATYA
SEQ ID NO:2	HC_N30A CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30A CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:13	HC_N30A	EVKLVESGGGLVQPGGSLRLSCAASGFTFATYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:14	HC_N30C CDR1	GFTFCTYA
SEQ ID NO:2	HC_N30C CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30C CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:15	HC_N30C	EVKLVESGGGLVQPGGSLRLSCAASGFTFCTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:16	HC_N30D CDR1	GFTFDTYA
SEQ ID NO:2	HC_N30D CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30D CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:17	HC_N30D	EVKLVESGGGLVQPGGSLRLSCAASGFTFDTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:18	HC_N30F CDR1	GFTFFTYA
SEQ ID NO:2	HC_N30F CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30F CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:19	HC_N30F	EVKLVESGGGLVQPGGSLRLSCAASGFTFFTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:20	HC_N30G CDR1	GFTFGTYA
SEQ ID NO:2	HC_N30G CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30G CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:21	HC_N30G	EVKLVESGGGLVQPGGSLRLSCAASGFTFGTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:22	HC_N30H CDR1	GFTFHTYA
SEQ ID NO:2	HC_N30H CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30H CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:23	HC_N30H	EVKLVESGGGLVQPGGSLRLSCAASGFTFHTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:24	HC_N30K CDR1	GFTFKTYA
SEQ ID NO:2	HC_N30K CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30K CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:25	HC_N30K	EVKLVESGGGLVQPGGSLRLSCAASGFTFKTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:26	HC_N30L CDR1	GFTFLTYA
SEQ ID NO:2	HC_N30L CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30L CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:27	HC_N30L	EVKLVESGGGLVQPGGSLRLSCAASGFTFLTYAMNWVRQAPGKGLE WVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTEDT AMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:28	HC_N30P CDR1	GFTFPTYA
SEQ ID NO:2	HC_N30P CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30P CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:29	HC_N30P	EVKLVESGGGLVQPGGSLRLSCAASGFTFPTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:30	HC_N30Q CDR1	GFTFQTYA
SEQ ID NO:2	HC_N30Q CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30Q CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:31	HC_N30Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFQTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:32	HC_N30R CDR1	GFTFRTYA
SEQ ID NO:2	HC_N30R CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30R CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:33	HC_N30R	EVKLVESGGGLVQPGGSLRLSCAASGFTFRTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:34	HC_N30T CDR1	GFTFTTYA
SEQ ID NO:2	HC_N30T CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30T CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:35	HC_N30T	EVKLVESGGGLVQPGGSLRLSCAASGFTFTTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:36	HC_N30V CDR1	GFTFVTYA
SEQ ID NO:2	HC_N30V CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30V CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:37	HC_N30V	EVKLVESGGGLVQPGGSLRLSCAASGFTFVTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:38	HC_N30W CDR1	GFTFWTYA
SEQ ID NO:2	HC_N30W CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_N30W CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:39	HC_N30W	EVKLVESGGGLVQPGGSLRLSCAASGFTFWTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:40	HC_T31A CDR1	GFTFNAYA
SEQ ID NO:2	HC_T31A CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31A CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:41	HC_T31A	EVKLVESGGGLVQPGGSLRLSCAASGFTFNAYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:42	HC_T31C CDR1	GFTFNCYA
SEQ ID NO:2	HC_T31C CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31C CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:43	HC_T31C	EVKLVESGGGLVQPGGSLRLSCAASGFTFNCYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:44	HC_T31D CDR1	GFTFNDYA
SEQ ID NO:2	HC_T31D CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31D CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:45	HC_T31D	EVKLVESGGGLVQPGGSLRLSCAASGFTFNDYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:46	HC_T31E CDR1	GFTFNEYA
SEQ ID NO:2	HC_T31E CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31E CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:47	HC_T31E	EVKLVESGGGLVQPGGSLRLSCAASGFTFNEYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:48	HC_T31F CDR1	GFTFNFYA
SEQ ID NO:2	HC_T31F CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31F CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:49	HC_T31F	EVKLVESGGGLVQPGGSLRLSCAASGFTFNFYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:50	HC_T31H CDR1	GFTFNHYA
SEQ ID NO:2	HC_T31H CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31H CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:51	HC_T31H	EVKLVESGGGLVQPGGSLRLSCAASGFTFNHYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:52	HC_T31L CDR1	GFTFNLYA
SEQ ID NO:2	HC_T31L CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31L CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:53	HC_T31L	EVKLVESGGGLVQPGGSLRLSCAASGFTFNLYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:54	HC_T31M CDR1	GFTFNMYA
SEQ ID NO:2	HC_T31M CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31M CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:55	HC_T31M	EVKLVESGGGLVQPGGSLRLSCAASGFTFNMYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTE DTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:56	HC_T31N CDR1	GFTFNNYA
SEQ ID NO:2	HC_T31N CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31N CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:57	HC_T31N	EVKLVESGGGLVQPGGSLRLSCAASGFTFNNYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:58	HC_T31P CDR1	GFTFNPYA
SEQ ID NO:2	HC_T31P CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31P CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:59	HC_T31P	EVKLVESGGGLVQPGGSLRLSCAASGFTFNPYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:60	HC_T31Q CDR1	GFTFNQYA
SEQ ID NO:2	HC_T31Q CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31Q CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:61	HC_T31Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFNQYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:62	HC_T31W CDR1	GFTFNWYA
SEQ ID NO:2	HC_T31W CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31W CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:63	HC_T31W	EVKLVESGGGLVQPGGSLRLSCAASGFTFNWYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTE DTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:64	HC_T31Y CDR1	GFTFNYYA
SEQ ID NO:2	HC_T31Y CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_T31Y CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:65	HC_T31Y	EVKLVESGGGLVQPGGSLRLSCAASGFTFNYYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:66	HC_Y32A CDR1	GFTFNTAA
SEQ ID NO:2	HC_Y32A CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32A CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:67	HC_Y32A	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTAAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:68	HC_Y32C CDR1	GFTFNTCA
SEQ ID NO:2	HC_Y32C CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32C CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:69	HC_Y32C	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTCAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:70	HC_Y32F CDR1	GFTFNTFA
SEQ ID NO:2	HC_Y32F CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32F CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:71	HC_Y32F	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTFAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:72	HC_Y32G CDR1	GFTFNTGA
SEQ ID NO:2	HC_Y32G CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32G CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:73	HC_Y32G	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTGAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:74	HC_Y32H CDR1	GFTFNTHA
SEQ ID NO:2	HC_Y32H CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32H CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:75	HC_Y32H	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTHAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:76	HC_Y32I CDR1	GFTFNTIA
SEQ ID NO:2	HC_Y32I CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32I CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:77	HC_Y32I	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTIAMNWVRQAPGKGLE WVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTEDT AMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:78	HC_Y32K CDR1	GFTFNTKA
SEQ ID NO:2	HC_Y32K CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32K CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:79	HC_Y32K	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTKAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:80	HC_Y32L CDR1	GFTFNTLA
SEQ ID NO:2	HC_Y32L CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32L CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:81	HC_Y32L	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTLAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:82	HC_Y32M CDR1	GFTFNTMA
SEQ ID NO:2	HC_Y32M CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32M CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:83	HC_Y32M	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTMAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTE DTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:84	HC_Y32N CDR1	GFTFNTNA
SEQ ID NO:2	HC_Y32N CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32N CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:85	HC_Y32N	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTNAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:86	HC_Y32P CDR1	GFTFNTPA
SEQ ID NO:2	HC_Y32P CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32P CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:87	HC_Y32P	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTPAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:88	HC_Y32Q CDR1	GFTFNTQA
SEQ ID NO:2	HC_Y32Q CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32Q CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:89	HC_Y32Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTQAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:90	HC_Y32R CDR1	GFTFNTRA
SEQ ID NO:2	HC_Y32R CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32R CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:91	HC_Y32R	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTRAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:92	HC_Y32S CDR1	GFTFNTSA
SEQ ID NO:2	HC_Y32S CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32S CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:93	HC_Y32S	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTSAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:94	HC_Y32T CDR1	GFTFNTTA
SEQ ID NO:2	HC_Y32T CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32T CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:95	HC_Y32T	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTTAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:96	HC_Y32V CDR1	GFTFNTVA
SEQ ID NO:2	HC_Y32V CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32V CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:97	HC_Y32V	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTVAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:98	HC_Y32W CDR1	GFTFNTWA
SEQ ID NO:2	HC_Y32W CDR2	IRSKYNNYAT
SEQ ID NO:3	HC_Y32W CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:99	HC_Y32W	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTWAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTE DTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57A CDR1	GFTFNTYA
SEQ ID NO:100	HC_N57A CDR2	IRSKYNAYAT
SEQ ID NO:3	HC_N57A CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:101	HC_N57A	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNAYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57C CDR1	GFTFNTYA
SEQ ID NO:102	HC_N57C CDR2	IRSKYNCYAT
SEQ ID NO:3	HC_N57C CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:103	HC_N57C	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNCYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57D CDR1	GFTFNTYA
SEQ ID NO:104	HC_N57D CDR2	IRSKYNDYAT
SEQ ID NO:3	HC_N57D CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:105	HC_N57D	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNDYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57E CDR1	GFTFNTYA
SEQ ID NO:106	HC_N57E CDR2	IRSKYNEYAT
SEQ ID NO:3	HC_N57E CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:107	HC_N57E	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNEYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57F CDR1	GFTFNTYA
SEQ ID NO:108	HC_N57F CDR2	IRSKYNFYAT
SEQ ID NO:3	HC_N57F CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:109	HC_N57F	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNFYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57G CDR1	GFTFNTYA
SEQ ID NO:110	HC_N57G CDR2	IRSKYNGYAT
SEQ ID NO:3	HC_N57G CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:111	HC_N57G	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNGYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57I CDR1	GFTFNTYA
SEQ ID NO:112	HC_N57I CDR2	IRSKYNIYAT
SEQ ID NO:3	HC_N57I CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:113	HC_N57I	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNIYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTEDT AMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57K CDR1	GFTFNTYA
SEQ ID NO:114	HC_N57K CDR2	IRSKYNKYAT
SEQ ID NO:3	HC_N57K CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:115	HC_N57K	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNKYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57L CDR1	GFTFNTYA
SEQ ID NO:116	HC_N57L CDR2	IRSKYNLYAT
SEQ ID NO:3	HC_N57L CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:117	HC_N57L	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNLYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57M CDR1	GFTFNTYA
SEQ ID NO:118	HC_N57M CDR2	IRSKYNMYAT
SEQ ID NO:3	HC_N57M CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:119	HC_N57M	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNMYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57P CDR1	GFTFNTYA
SEQ ID NO:120	HC_N57P CDR2	IRSKYNPYAT
SEQ ID NO:3	HC_N57P CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:121	HC_N57P	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNPYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57Q CDR1	GFTFNTYA
SEQ ID NO:122	HC_N57Q CDR2	IRSKYNQYAT
SEQ ID NO:3	HC_N57Q CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:123	HC_N57Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNQYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57R CDR1	GFTFNTYA
SEQ ID NO:124	HC_N57R CDR2	IRSKYNRYAT
SEQ ID NO:3	HC_N57R CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:125	HC_N57R	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNRYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57T CDR1	GFTFNTYA
SEQ ID NO:126	HC_N57T CDR2	IRSKYNTYAT
SEQ ID NO:3	HC_N57T CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:127	HC_N57T	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNTYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57V CDR1	GFTFNTYA
SEQ ID NO:128	HC_N57V CDR2	IRSKYNVYAT
SEQ ID NO:3	HC_N57V CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:129	HC_N57V	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNVYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57W CDR1	GFTFNTYA
SEQ ID NO:130	HC_N57W CDR2	IRSKYNWYAT
SEQ ID NO:3	HC_N57W CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:131	HC_N57W	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNWYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTE DTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_N57Y CDR1	GFTFNTYA
SEQ ID NO:132	HC_N57Y CDR2	IRSKYNYYAT
SEQ ID NO:3	HC_N57Y CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:133	HC_N57Y	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNYYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59C CDR1	GFTFNTYA
SEQ ID NO:134	HC_A59C CDR2	IRSKYNNYCT
SEQ ID NO:3	HC_A59C CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:135	HC_A59C	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYCTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59D CDR1	GFTFNTYA
SEQ ID NO:136	HC_A59D CDR2	IRSKYNNYDT
SEQ ID NO:3	HC_A59D CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:137	HC_A59D	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYDTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59E CDR1	GFTFNTYA
SEQ ID NO:138	HC_A59E CDR2	IRSKYNNYET
SEQ ID NO:3	HC_A59E CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:139	HC_A59E	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYETYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59F CDR1	GFTFNTYA
SEQ ID NO:140	HC_A59F CDR2	IRSKYNNYFT
SEQ ID NO:3	HC_A59F CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:141	HC_A59F	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYFTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59G CDR1	GFTFNTYA
SEQ ID NO:142	HC_A59G CDR2	IRSKYNNYGT
SEQ ID NO:3	HC_A59G CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:143	HC_A59G	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYGTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59H CDR1	GFTFNTYA
SEQ ID NO:144	HC_A59H CDR2	IRSKYNNYHT
SEQ ID NO:3	HC_A59H CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:145	HC_A59H	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYHTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59I CDR1	GFTFNTYA
SEQ ID NO:146	HC_A59I CDR2	IRSKYNNYIT
SEQ ID NO:3	HC_A59I CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:147	HC_A59I	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYITYYADSVKDRFTISRDDSKSSLYLQMNNLKTEDT AMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59K CDR1	GFTFNTYA
SEQ ID NO:148	HC_A59K CDR2	IRSKYNNYKT
SEQ ID NO:3	HC_A59K CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:149	HC_A59K	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYKTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59L CDR1	GFTFNTYA
SEQ ID NO:150	HC_A59L CDR2	IRSKYNNYLT
SEQ ID NO:3	HC_A59L CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:151	HC_A59L	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYLTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59M CDR1	GFTFNTYA
SEQ ID NO:152	HC_A59M CDR2	IRSKYNNYMT
SEQ ID NO:3	HC_A59M CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:153	HC_A59M	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYMTYYADSVKDRFTISRDDSKSSLYLQMNNLKTE DTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59N CDR1	GFTFNTYA
SEQ ID NO:154	HC_A59N CDR2	IRSKYNNYNT
SEQ ID NO:3	HC_A59N CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:155	HC_A59N	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYNTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59P CDR1	GFTFNTYA
SEQ ID NO:156	HC_A59P CDR2	IRSKYNNYPT
SEQ ID NO:3	HC_A59P CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:157	HC_A59P	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYPTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59Q CDR1	GFTFNTYA
SEQ ID NO:158	HC_A59Q CDR2	IRSKYNNYQT
SEQ ID NO:3	HC_A59Q CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:159	HC_A59Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYQTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59R CDR1	GFTFNTYA
SEQ ID NO:160	HC_A59R CDR2	IRSKYNNYRT
SEQ ID NO:3	HC_A59R CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:161	HC_A59R	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYRTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59S CDR1	GFTFNTYA
SEQ ID NO:162	HC_A59S CDR2	IRSKYNNYST
SEQ ID NO:3	HC_A59S CDR3	VRHGNFGNSYVSWFAY

SEQ ID NO:163	HC_A59S	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYSTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59V CDR1	GFTFNTYA
SEQ ID NO:164	HC_A59V CDR2	IRSKYNNYVT
SEQ ID NO:3	HC_A59V CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:165	HC_A59V	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYVTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59W CDR1	GFTFNTYA
SEQ ID NO:166	HC_A59W CDR2	IRSKYNNYWT
SEQ ID NO:3	HC_A59W CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:167	HC_A59W	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYWTYYADSVKDRFTISRDDSKSSLYLQMNNLKTE DTAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_A59Y CDR1	GFTFNTYA
SEQ ID NO:168	HC_A59Y CDR2	IRSKYNNYYT
SEQ ID NO:3	HC_A59Y CDR3	VRHGNFGNSYVSWFAY
SEQ ID NO:169	HC_A59Y	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYYTYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101A CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101A CDR2	IRSKYNNYAT
SEQ ID NO:170	HC_H101A CDR3	VRAGNFGNSYVSWFAY
SEQ ID NO:171	HC_H101A	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRAGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101C CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101C CDR2	IRSKYNNYAT
SEQ ID NO:172	HC_H101C CDR3	VRCGNFGNSYVSWFAY
SEQ ID NO:173	HC_H101C	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRCGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101F CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101F CDR2	IRSKYNNYAT
SEQ ID NO:174	HC_H101F CDR3	VRFGNFGNSYVSWFAY
SEQ ID NO:175	HC_H101F	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRFGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101G CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101G CDR2	IRSKYNNYAT
SEQ ID NO:176	HC_H101G CDR3	VRGGNFGNSYVSWFAY
SEQ ID NO:177	HC_H101G	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRGGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101I CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101I CDR2	IRSKYNNYAT
SEQ ID NO:178	HC_H101I CDR3	VRIGNFGNSYVSWFAY

SEQ ID NO:179	HC_H101I	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRIGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101K CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101K CDR2	IRSKYNNYAT
SEQ ID NO:180	HC_H101K CDR3	VRKGNFGNSYVSWFAY
SEQ ID NO:181	HC_H101K	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRKGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101L CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101L CDR2	IRSKYNNYAT
SEQ ID NO:182	HC_H101L CDR3	VRLGNFGNSYVSWFAY
SEQ ID NO:183	HC_H101L	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRLGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101N CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101N CDR2	IRSKYNNYAT
SEQ ID NO:184	HC_H101N CDR3	VRNGNFGNSYVSWFAY
SEQ ID NO:185	HC_H101N	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRNGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101P CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101P CDR2	IRSKYNNYAT
SEQ ID NO:186	HC_H101P CDR3	VRPGNFGNSYVSWFAY
SEQ ID NO:187	HC_H101P	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRPGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101Q CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101Q CDR2	IRSKYNNYAT
SEQ ID NO:188	HC_H101Q CDR3	VRQGNFGNSYVSWFAY
SEQ ID NO:189	HC_H101Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRQGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101R CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101R CDR2	IRSKYNNYAT
SEQ ID NO:190	HC_H101R CDR3	VRRGNFGNSYVSWFAY
SEQ ID NO:191	HC_H101R	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRRGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101S CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101S CDR2	IRSKYNNYAT
SEQ ID NO:192	HC_H101S CDR3	VRSGNFGNSYVSWFAY
SEQ ID NO:193	HC_H101S	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRSGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101T CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101T CDR2	IRSKYNNYAT
SEQ ID NO:194	HC_H101T CDR3	VRTGNFGNSYVSWFAY

SEQ ID NO:195	HC_H101T	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRTGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101V CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101V CDR2	IRSKYNNYAT
SEQ ID NO:196	HC_H101V CDR3	VRVGNFGNSYVSWFAY
SEQ ID NO:197	HC_H101V	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRVGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101W CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101W CDR2	IRSKYNNYAT
SEQ ID NO:198	HC_H101W CDR3	VRWGNFGNSYVSWFAY
SEQ ID NO:199	HC_H101W	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRWGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_H101Y CDR1	GFTFNTYA
SEQ ID NO:2	HC_H101Y CDR2	IRSKYNNYAT
SEQ ID NO:200	HC_H101Y CDR3	VRYGNFGNSYVSWFAY
SEQ ID NO:201	HC_H101Y	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRYGNFGNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105A CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105A CDR2	IRSKYNNYAT
SEQ ID NO:202	HC_G105A CDR3	VRHGNFANSYVSWFAY
SEQ ID NO:203	HC_G105A	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFANSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105C CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105C CDR2	IRSKYNNYAT
SEQ ID NO:204	HC_G105C CDR3	VRHGNFCNSYVSWFAY
SEQ ID NO:205	HC_G105C	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFCNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105E CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105E CDR2	IRSKYNNYAT
SEQ ID NO:206	HC_G105E CDR3	VRHGNFENSYVSWFAY
SEQ ID NO:207	HC_G105E	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFENSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105F CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105F CDR2	IRSKYNNYAT
SEQ ID NO:208	HC_G105F CDR3	VRHGNFFNSYVSWFAY
SEQ ID NO:209	HC_G105F	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFFNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105H CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105H CDR2	IRSKYNNYAT
SEQ ID NO:210	HC_G105H CDR3	VRHGNFHNSYVSWFAY

SEQ ID NO:211	HC_G105H	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFHNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105I CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105I CDR2	IRSKYNNYAT
SEQ ID NO:212	HC_G105I CDR3	VRHGNFINSYVSWFAY
SEQ ID NO:213	HC_G105I	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFINSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105L CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105L CDR2	IRSKYNNYAT
SEQ ID NO:214	HC_G105L CDR3	VRHGNFLNSYVSWFAY
SEQ ID NO:215	HC_G105L	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFLNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105M CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105M CDR2	IRSKYNNYAT
SEQ ID NO:216	HC_G105M CDR3	VRHGNFMNSYVSWFAY
SEQ ID NO:217	HC_G105M	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFMNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105N CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105N CDR2	IRSKYNNYAT
SEQ ID NO:218	HC_G105N CDR3	VRHGNFNNSYVSWFAY
SEQ ID NO:219	HC_G105N	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFNNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105P CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105P CDR2	IRSKYNNYAT
SEQ ID NO:220	HC_G105P CDR3	VRHGNFPNSYVSWFAY
SEQ ID NO:221	HC_G105P	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFPNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105Q CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105Q CDR2	IRSKYNNYAT
SEQ ID NO:222	HC_G105Q CDR3	VRHGNFQNSYVSWFAY
SEQ ID NO:223	HC_G105Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFQNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105R CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105R CDR2	IRSKYNNYAT
SEQ ID NO:224	HC_G105R CDR3	VRHGNFRNSYVSWFAY
SEQ ID NO:225	HC_G105R	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFRNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105S CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105S CDR2	IRSKYNNYAT
SEQ ID NO:226	HC_G105S CDR3	VRHGNFSNSYVSWFAY

SEQ ID NO:227	HC_G105S	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFSNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105T CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105T CDR2	IRSKYNNYAT
SEQ ID NO:228	HC_G105T CDR3	VRHGNFTNSYVSWFAY
SEQ ID NO:229	HC_G105T	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFTNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105V CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105V CDR2	IRSKYNNYAT
SEQ ID NO:230	HC_G105V CDR3	VRHGNFVNSYVSWFAY
SEQ ID NO:231	HC_G105V	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFVNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105W CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105W CDR2	IRSKYNNYAT
SEQ ID NO:232	HC_G105W CDR3	VRHGNFWNSYVSWFAY
SEQ ID NO:233	HC_G105W	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFWNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_G105Y CDR1	GFTFNTYA
SEQ ID NO:2	HC_G105Y CDR2	IRSKYNNYAT
SEQ ID NO:234	HC_G105Y CDR3	VRHGNFYNSYVSWFAY
SEQ ID NO:235	HC_G105Y	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFYNSYVSWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110A CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110A CDR2	IRSKYNNYAT
SEQ ID NO:236	HC_S110A CDR3	VRHGNFGNSYVAWFAY
SEQ ID NO:237	HC_S110A	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVAWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110C CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110C CDR2	IRSKYNNYAT
SEQ ID NO:238	HC_S110C CDR3	VRHGNFGNSYVCWFAY
SEQ ID NO:239	HC_S110C	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVCWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110E CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110E CDR2	IRSKYNNYAT
SEQ ID NO:240	HC_S110E CDR3	VRHGNFGNSYVEWFAY
SEQ ID NO:241	HC_S110E	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVEWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110F CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110F CDR2	IRSKYNNYAT
SEQ ID NO:242	HC_S110F CDR3	VRHGNFGNSYVFWFAY

SEQ ID NO:243	HC_S110F	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVFWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110G CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110G CDR2	IRSKYNNYAT
SEQ ID NO:244	HC_S110G CDR3	VRHGNFGNSYVGWFAY
SEQ ID NO:245	HC_S110G	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVGWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110H CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110H CDR2	IRSKYNNYAT
SEQ ID NO:246	HC_S110H CDR3	VRHGNFGNSYVHWFAY
SEQ ID NO:247	HC_S110H	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVHWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110K CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110K CDR2	IRSKYNNYAT
SEQ ID NO:248	HC_S110K CDR3	VRHGNFGNSYVKWFAY
SEQ ID NO:249	HC_S110K	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVKWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110L CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110L CDR2	IRSKYNNYAT
SEQ ID NO:250	HC_S110L CDR3	VRHGNFGNSYVLWFAY
SEQ ID NO:251	HC_S110L	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVLWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110N CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110N CDR2	IRSKYNNYAT
SEQ ID NO:252	HC_S110N CDR3	VRHGNFGNSYVNWFAY
SEQ ID NO:253	HC_S110N	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVNWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110P CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110P CDR2	IRSKYNNYAT
SEQ ID NO:254	HC_S110P CDR3	VRHGNFGNSYVPWFAY
SEQ ID NO:255	HC_S110P	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVPWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110Q CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110Q CDR2	IRSKYNNYAT
SEQ ID NO:256	HC_S110Q CDR3	VRHGNFGNSYVQWFAY
SEQ ID NO:257	HC_S110Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVQWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110R CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110R CDR2	IRSKYNNYAT
SEQ ID NO:258	HC_S110R CDR3	VRHGNFGNSYVRWFAY

SEQ ID NO:259	HC_S110R	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVRWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110T CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110T CDR2	IRSKYNNYAT
SEQ ID NO:260	HC_S110T CDR3	VRHGNFGNSYVTWFAY
SEQ ID NO:261	HC_S110T	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVTWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110W CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110W CDR2	IRSKYNNYAT
SEQ ID NO:262	HC_S110W CDR3	VRHGNFGNSYVWWFAY
SEQ ID NO:263	HC_S110W	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVWWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_S110Y CDR1	GFTFNTYA
SEQ ID NO:2	HC_S110Y CDR2	IRSKYNNYAT
SEQ ID NO:264	HC_S110Y CDR3	VRHGNFGNSYVYWFAY
SEQ ID NO:265	HC_S110Y	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVYWFAYWGQGTLVTVSS
SEQ ID NO:1	HC_Y114A CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114A CDR2	IRSKYNNYAT
SEQ ID NO:266	HC_Y114A CDR3	VRHGNFGNSYVSWFAA
SEQ ID NO:267	HC_Y114A	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAAWGQGTLVTVSS
SEQ ID NO:1	HC_Y114C CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114C CDR2	IRSKYNNYAT
SEQ ID NO:268	HC_Y114C CDR3	VRHGNFGNSYVSWFAC
SEQ ID NO:269	HC_Y114C	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFACWGQGTLVTVSS
SEQ ID NO:1	HC_Y114E CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114E CDR2	IRSKYNNYAT
SEQ ID NO:270	HC_Y114E CDR3	VRHGNFGNSYVSWFAE
SEQ ID NO:271	HC_Y114E	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAEWGQGTLVTVSS
SEQ ID NO:1	HC_Y114F CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114F CDR2	IRSKYNNYAT
SEQ ID NO:272	HC_Y114F CDR3	VRHGNFGNSYVSWFAF
SEQ ID NO:273	HC_Y114F	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAFWGQGTLVTVSS
SEQ ID NO:1	HC_Y114G CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114G CDR2	IRSKYNNYAT
SEQ ID NO:274	HC_Y114G CDR3	VRHGNFGNSYVSWFAG

SEQ ID NO:275	HC_Y114G	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAGWGQGTLVTVSS
SEQ ID NO:1	HC_Y114H CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114H CDR2	IRSKYNNYAT
SEQ ID NO:276	HC_Y114H CDR3	VRHGNFGNSYVSWFAH
SEQ ID NO:277	HC_Y114H	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAHWGQGTLVTVSS
SEQ ID NO:1	HC_Y114I CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114I CDR2	IRSKYNNYAT
SEQ ID NO:278	HC_Y114I CDR3	VRHGNFGNSYVSWFAI
SEQ ID NO:279	HC_Y114I	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAIWGQGTLVTVSS
SEQ ID NO:1	HC_Y114K CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114K CDR2	IRSKYNNYAT
SEQ ID NO:280	HC_Y114K CDR3	VRHGNFGNSYVSWFAK
SEQ ID NO:281	HC_Y114K	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAKWGQGTLVTVSS
SEQ ID NO:1	HC_Y114L CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114L CDR2	IRSKYNNYAT
SEQ ID NO:282	HC_Y114L CDR3	VRHGNFGNSYVSWFAL
SEQ ID NO:283	HC_Y114L	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFALWGQGTLVTVSS
SEQ ID NO:1	HC_Y114M CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114M CDR2	IRSKYNNYAT
SEQ ID NO:284	HC_Y114M CDR3	VRHGNFGNSYVSWFAM
SEQ ID NO:285	HC_Y114M	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAMWGQGTLVTVSS
SEQ ID NO:1	HC_Y114N CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114N CDR2	IRSKYNNYAT
SEQ ID NO:286	HC_Y114N CDR3	VRHGNFGNSYVSWFAN
SEQ ID NO:287	HC_Y114N	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFANWGQGTLVTVSS
SEQ ID NO:1	HC_Y114P CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114P CDR2	IRSKYNNYAT
SEQ ID NO:288	HC_Y114P CDR3	VRHGNFGNSYVSWFAP
SEQ ID NO:289	HC_Y114P	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAPWGQGTLVTVSS
SEQ ID NO:1	HC_Y114Q CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114Q CDR2	IRSKYNNYAT
SEQ ID NO:290	HC_Y114Q CDR3	VRHGNFGNSYVSWFAQ

SEQ ID NO:291	HC_Y114Q	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAQWGQGTLVTVSS
SEQ ID NO:1	HC_Y114R CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114R CDR2	IRSKYNNYAT
SEQ ID NO:292	HC_Y114R CDR3	VRHGNFGNSYVSWFAR
SEQ ID NO:293	HC_Y114R	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFARWGQGTLVTVSS
SEQ ID NO:1	HC_Y114S CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114S CDR2	IRSKYNNYAT
SEQ ID NO:294	HC_Y114S CDR3	VRHGNFGNSYVSWFAS
SEQ ID NO:295	HC_Y114S	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFASWGQGTLVTVSS
SEQ ID NO:1	HC_Y114T CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114T CDR2	IRSKYNNYAT
SEQ ID NO:296	HC_Y114T CDR3	VRHGNFGNSYVSWFAT
SEQ ID NO:297	HC_Y114T	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFATWGQGTLVTVSS
SEQ ID NO:1	HC_Y114V CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114V CDR2	IRSKYNNYAT
SEQ ID NO:298	HC_Y114V CDR3	VRHGNFGNSYVSWFAV
SEQ ID NO:299	HC_Y114V	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAVWGQGTLVTVSS
SEQ ID NO:1	HC_Y114W CDR1	GFTFNTYA
SEQ ID NO:2	HC_Y114W CDR2	IRSKYNNYAT
SEQ ID NO:300	HC_Y114W CDR3	VRHGNFGNSYVSWFAW
SEQ ID NO:301	HC_Y114W	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKSSLYLQMNNLKTED TAMYYCVRHGNFGNSYVSWFAWWGQGTLVTVSS
SEQ ID NO:302	LC_T31A CDR1	TGAVTASNY
	LC_T31A CDR2	GTN
SEQ ID NO:7	LC_T31A CDR3	ALWYSNLWV
SEQ ID NO:303	LC_T31A	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTASNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:304	LC_T31D CDR1	TGAVTDSNY
	LC_T31D CDR2	GTN
SEQ ID NO:7	LC_T31D CDR3	ALWYSNLWV
SEQ ID NO:305	LC_T31D	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTDSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:306	LC_T31E CDR1	TGAVTESNY
	LC_T31E CDR2	GTN
SEQ ID NO:7	LC_T31E CDR3	ALWYSNLWV

SEQ ID NO:307	LC_T31E	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTESNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:308	LC_T31F CDR1	TGAVTFSNY
	LC_T31F CDR2	GTN
SEQ ID NO:7	LC_T31F CDR3	ALWYSNLWV
SEQ ID NO:309	LC_T31F	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTFSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:310	LC_T31G CDR1	TGAVTGSNY
	LC_T31G CDR2	GTN
SEQ ID NO:7	LC_T31G CDR3	ALWYSNLWV
SEQ ID NO:311	LC_T31G	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTGSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:312	LC_T31H CDR1	TGAVTHSNY
	LC_T31H CDR2	GTN
SEQ ID NO:7	LC_T31H CDR3	ALWYSNLWV
SEQ ID NO:313	LC_T31H	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTHSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
	LC_T31I CDR2	GTN
SEQ ID NO:314	LC_T31K CDR1	TGAVTKSNY
	LC_T31K CDR2	GTN
SEQ ID NO:7	LC_T31K CDR3	ALWYSNLWV
SEQ ID NO:315	LC_T31K	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTKSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:316	LC_T31L CDR1	TGAVTLSNY
	LC_T31L CDR2	GTN
SEQ ID NO:7	LC_T31L CDR3	ALWYSNLWV
SEQ ID NO:317	LC_T31L	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTLSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:318	LC_T31M CDR1	TGAVTMSNY
	LC_T31M CDR2	GTN
SEQ ID NO:7	LC_T31M CDR3	ALWYSNLWV
SEQ ID NO:319	LC_T31M	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTMSNYANWVQQKPGQ AFRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAL WYSNLWVFGGGTKLTVL
SEQ ID NO:320	LC_T31N CDR1	TGAVTNSNY
	LC_T31N CDR2	GTN
SEQ ID NO:7	LC_T31N CDR3	ALWYSNLWV
SEQ ID NO:321	LC_T31N	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTNSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:322	LC_T31P CDR1	TGAVTPSNY
	LC_T31P CDR2	GTN

SEQ ID NO:7	LC_T31P CDR3	ALWYSNLWV
SEQ ID NO:323	LC_T31P	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTPSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:324	LC_T31Q CDR1	TGAVTQSNY
	LC_T31Q CDR2	GTN
SEQ ID NO:7	LC_T31Q CDR3	ALWYSNLWV
SEQ ID NO:325	LC_T31Q	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTQSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:326	LC_T31R CDR1	TGAVTRSNY
	LC_T31R CDR2	GTN
SEQ ID NO:7	LC_T31R CDR3	ALWYSNLWV
SEQ ID NO:327	LC_T31R	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTRSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
	LC_T31S CDR2	GTN
SEQ ID NO:328	LC_T31V CDR1	TGAVTVSNY
	LC_T31V CDR2	GTN
SEQ ID NO:7	LC_T31V CDR3	ALWYSNLWV
SEQ ID NO:329	LC_T31V	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTVSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:330	LC_T31Y CDR1	TGAVTYSNY
	LC_T31Y CDR2	GTN
SEQ ID NO:7	LC_T31Y CDR3	ALWYSNLWV
SEQ ID NO:331	LC_T31Y	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTYSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92A CDR1	TGAVTTSNY
	LC_L92A CDR2	GTN
SEQ ID NO:332	LC_L92A CDR3	AAWYSNLWV
SEQ ID NO:333	LC_L92A	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAA WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92C CDR1	TGAVTTSNY
	LC_L92C CDR2	GTN
SEQ ID NO:334	LC_L92C CDR3	ACWYSNLWV
SEQ ID NO:335	LC_L92C	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAC WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92D CDR1	TGAVTTSNY
	LC_L92D CDR2	GTN
SEQ ID NO:336	LC_L92D CDR3	ADWYSNLWV
SEQ ID NO:337	LC_L92D	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAD WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92E CDR1	TGAVTTSNY

	LC_L92E CDR2	GTN
SEQ ID NO:338	LC_L92E CDR3	AEWYSNLWV
SEQ ID NO:339	LC_L92E	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAEW YSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92F CDR1	TGAVTTSNY
	LC_L92F CDR2	GTN
SEQ ID NO:340	LC_L92F CDR3	AFWYSNLWV
SEQ ID NO:341	LC_L92F	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAFW YSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92G CDR1	TGAVTTSNY
	LC_L92G CDR2	GTN
SEQ ID NO:342	LC_L92G CDR3	AGWYSNLWV
SEQ ID NO:343	LC_L92G	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAG WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92I CDR1	TGAVTTSNY
	LC_L92I CDR2	GTN
SEQ ID NO:344	LC_L92I CDR3	AIWYSNLWV
SEQ ID NO:345	LC_L92I	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAIW YSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92K CDR1	TGAVTTSNY
	LC_L92K CDR2	GTN
SEQ ID NO:346	LC_L92K CDR3	AKWYSNLWV
SEQ ID NO:347	LC_L92K	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAK WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92M CDR1	TGAVTTSNY
	LC_L92M CDR2	GTN
SEQ ID NO:348	LC_L92M CDR3	AMWYSNLWV
SEQ ID NO:349	LC_L92M	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAM WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92N CDR1	TGAVTTSNY
	LC_L92N CDR2	GTN
SEQ ID NO:350	LC_L92N CDR3	ANWYSNLWV
SEQ ID NO:351	LC_L92N	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAN WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92P CDR1	TGAVTTSNY
	LC_L92P CDR2	GTN
SEQ ID NO:352	LC_L92P CDR3	APWYSNLWV
SEQ ID NO:353	LC_L92P	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAP WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92R CDR1	TGAVTTSNY

	LC_L92R CDR2	GTN
SEQ ID NO:354	LC_L92R CDR3	ARWYSNLWV
SEQ ID NO:355	LC_L92R	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAR WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92S CDR1	TGAVTTSNY
	LC_L92S CDR2	GTN
SEQ ID NO:356	LC_L92S CDR3	ASWYSNLWV
SEQ ID NO:357	LC_L92S	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCASW YSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92T CDR1	TGAVTTSNY
	LC_L92T CDR2	GTN
SEQ ID NO:358	LC_L92T CDR3	ATWYSNLWV
SEQ ID NO:359	LC_L92T	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCATW YSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92V CDR1	TGAVTTSNY
	LC_L92V CDR2	GTN
SEQ ID NO:360	LC_L92V CDR3	AVWYSNLWV
SEQ ID NO:361	LC_L92V	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAV WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92W CDR1	TGAVTTSNY
	LC_L92W CDR2	GTN
SEQ ID NO:362	LC_L92W CDR3	AWWYSNLWV
SEQ ID NO:363	LC_L92W	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAW WYSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L92Y CDR1	TGAVTTSNY
	LC_L92Y CDR2	GTN
SEQ ID NO:364	LC_L92Y CDR3	AYWYSNLWV
SEQ ID NO:365	LC_L92Y	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCAYW YSNLWVFGGGTKLTVL
SEQ ID NO:6	LC_L97D CDR1	TGAVTTSNY
	LC_L97D CDR2	GTN
SEQ ID NO:366	LC_L97D CDR3	ALWYSNDWV
SEQ ID NO:367	LC_L97D	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNDWVFGGGTKLTVL
SEQ ID NO:6	LC_L97E CDR1	TGAVTTSNY
	LC_L97E CDR2	GTN
SEQ ID NO:368	LC_L97E CDR3	ALWYSNEWV
SEQ ID NO:369	LC_L97E	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNEWVFGGGTKLTVL
SEQ ID NO:6	LC_L97F CDR1	TGAVTTSNY

	LC_L97F CDR2	GTN
SEQ ID NO:370	LC_L97F CDR3	ALWYSNFWV
SEQ ID NO:371	LC_L97F	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNFWVFGGGTKLTVL
SEQ ID NO:6	LC_L97G CDR1	TGAVTTSNY
	LC_L97G CDR2	GTN
SEQ ID NO:372	LC_L97G CDR3	ALWYSNGWV
SEQ ID NO:373	LC_L97G	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNGWVFGGGTKLTVL
SEQ ID NO:6	LC_L97H CDR1	TGAVTTSNY
	LC_L97H CDR2	GTN
SEQ ID NO:374	LC_L97H CDR3	ALWYSNHWV
SEQ ID NO:375	LC_L97H	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNHWVFGGGTKLTVL
SEQ ID NO:6	LC_L97K CDR1	TGAVTTSNY
	LC_L97K CDR2	GTN
SEQ ID NO:376	LC_L97K CDR3	ALWYSNKWV
SEQ ID NO:377	LC_L97K	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNKWVFGGGTKLTVL
SEQ ID NO:6	LC_L97M CDR1	TGAVTTSNY
	LC_L97M CDR2	GTN
SEQ ID NO:378	LC_L97M CDR3	ALWYSNMWV
SEQ ID NO:379	LC_L97M	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNMWVFGGGTKLTVL
SEQ ID NO:6	LC_L97N CDR1	TGAVTTSNY
	LC_L97N CDR2	GTN
SEQ ID NO:380	LC_L97N CDR3	ALWYSNNWV
SEQ ID NO:381	LC_L97N	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNNWVFGGGTKLTVL
SEQ ID NO:6	LC_L97P CDR1	TGAVTTSNY
	LC_L97P CDR2	GTN
SEQ ID NO:382	LC_L97P CDR3	ALWYSNPWV
SEQ ID NO:383	LC_L97P	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNPWVFGGGTKLTVL
SEQ ID NO:6	LC_L97Q CDR1	TGAVTTSNY
	LC_L97Q CDR2	GTN
SEQ ID NO:384	LC_L97Q CDR3	ALWYSNQWV
SEQ ID NO:385	LC_L97Q	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNQWVFGGGTKLTVL
	LC_L97R CDR2	GTN

SEQ ID NO:6	LC_L97S CDR1	TGAVTTSNY
	LC_L97S CDR2	GTN
SEQ ID NO:386	LC_L97S CDR3	ALWYSNSWV
SEQ ID NO:387	LC_L97S	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNSWVFGGGTKLTVL
SEQ ID NO:6	LC_L97T CDR1	TGAVTTSNY
	LC_L97T CDR2	GTN
SEQ ID NO:388	LC_L97T CDR3	ALWYSNTWV
SEQ ID NO:389	LC_L97T	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNTWVFGGGTKLTVL
SEQ ID NO:6	LC_L97V CDR1	TGAVTTSNY
	LC_L97V CDR2	GTN
SEQ ID NO:390	LC_L97V CDR3	ALWYSNVWV
SEQ ID NO:391	LC_L97V	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNVWVFGGGTKLTVL
SEQ ID NO:6	LC_L97W CDR1	TGAVTTSNY
	LC_L97W CDR2	GTN
SEQ ID NO:392	LC_L97W CDR3	ALWYSNWWV
SEQ ID NO:393	LC_L97W	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNWWVFGGGTKLTVL
SEQ ID NO:6	LC_L97Y CDR1	TGAVTTSNY
	LC_L97Y CDR2	GTN
SEQ ID NO:394	LC_L97Y CDR3	ALWYSNYWV
SEQ ID NO:395	LC_L97Y	QAVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA FRGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQADDESIYFCALW YSNYWVFGGGTKLTVL
SEQ ID NO:396	Mature huTRA	KTTQPNSMESNEEEPVHLPCNHSTISGTDYIHWYRQLPSQGPEYVIH GLTSNVNNRMASLAIAEDRKSSTLILHRATLRDAAVYYCILPLAGGTS YGKLTFGQGTILTVHPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQ TNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANA FNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLK VAGFNLLMTLRLWSS
SEQ ID NO:397	Mature huTRB	GVSQSPRYKVAKRGQDVALRCDPISGHVSLFWYQQALGQGPEFLTY FQNEAQLDKSGLPSDRFFAERPEGSVSTLKIQRTQQEDSAVYLCASSL GQAYEQYFGPGTRLTVTEDLNKVFPPEVAVFEPSEAEISHTQKATLVC LATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLS SRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVS AEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLM AMVKRKDF
SEQ ID NO:398	Mature huCD3δ	FKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDPRGIY RCNGTDIYKDKESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLLLA LGVFCFAGHETGRLSGAADTQALLRNDQVYQPLRDRDDAQYSHLG GNWARNK
SEQ ID NO:399	Mature huCD3ε	QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGG DEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRA

		RVCENCMEMDVMSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPVT RGAGAGGRQRGQNKERPPPVPNPDYEPIRKGQRDLYSGLNQRRI
SEQ ID NO:400	Mature huCD3γ	QSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGFLTED KKKWNLGSNAKDPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNA ATISGFLFAEIVSIFVLAVGVYFIAGQDGVRQSRASDKQTLLPNDQLY QPLKDREDDQYSHLQGNQLRRN
SEQ ID NO:401	Mature huCD3ζ	QSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR
SEQ ID NO:402	Mature CD3ε27-GSKa	QDGNEEMGGITQTPYKVSISGTTVILTGGGGSGGGGSGGGGSEIVL TQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDAS NRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPITFGQ GTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGE
SEQ ID NO:403	Mature cyno CD3ε (epsilon)	QDGNEEMGSITQTPYQVSISGTTVILTCSQHLGSEAQWQHNGKNK EDSGDRLFLPEFSEMEQSGYYVCYPRGSNPEDASHHLYLKARVCENC MEMDVMAVATIVIVDICITLGLLLLVYYWSKNRKAKAKPVTRGAGA GGRQRGQNKERPPPVPNPDYEPIRKGQQDLYSGLNQRRI
SEQ ID NO:404	Mature rhesus CD3ε (epsilon)	QDGNEEMGSITQTPYHVSISGTTVILTCSQHLGSEVQWQHNGKNKE DSGDRLFLPEFSEMEQSGYYVCYPRGSNPEDASHHLYLKARVCENC MEMDVMAVATIVIVDICITLGLLLLVYYWSKNRKAKAKPVTRGAGA GGRQRGQNKERPPPVPNPDYEPIRKGQQDLYSGLNQRRI
SEQ ID NO:405	**Parent murine VH**	**EVKLLESGGGLVQPKGSLKLSC AASGFTFNTYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSV KDRFTISRDDSQSILYLQMNNL KTEDTAMYYCVRHGNFGNSYVS WFAYWGQGTLVTVSA**
SEQ ID NO:406	**Parent murine VL**	**QAVVTQESALTTSPGETVTLTC RSSTGAVTTSNYANWVQEKPDH LFTGLIGGTNKRAPGVPARFSG SLIGDKAALTITGAQTEDEAIY FCALWYSNLWVFGGGTKLTVL**
SEQ ID NO:407	**IgG1m(f) heavy chain constant region**	**ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK**
SEQ ID NO:408	**Human IgLC2/IgLC3 constant domain**	**GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADS SPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHE GSTVEKTVAPTECS**
SEQ ID NO:409	IgG1m(f) heavy chain constant region with FEA mutations	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KRVEPKSCDKTHTCPPCPAPE**FE**GGPSVFLFPPKPKDTLMISRTPEVT CVVV**A**VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK

SEQ ID NO:410	Primer CMV P f (MAR5)	GCTTCGCGATGTACGGGCCAGATATAC
SEQ ID NO:411	TK pA r(MAR1)	GGATACCCCCTAGAGCCCCAGCTGCGCAGATCTGCTATGGC

The CDR regions have been annotated according to the IMGT definitions.

Examples

Example 1 - Generation of humanized CD3 antibodies and non-activating antibody variants

Humanization of CD3 antibodies

Humanization of a murine CD3 antibody (US 8,236,308, described herein as IgG1-CD3) was performed by Antitope (Cambridge, UK) using their improved version of the germline humanization (CDR-grafting) technology (EP 0 629 240). Using this technology, 1 different VH chain (SEQ ID NO:4) and 2 different VL chains (SEQ ID NO:8, 10) were designed. By combining these 1 VH with the 2 VL chains, 2 different antibodies were generated. The humanized variants are described herein as huCD3. Thus, humanized variants comprising a VH and a VL according to the invention, are described as, e.g., IgG1-huCD3-H1L1 meaning that said specific variant is of the IgG1 isotype, is a humanized CD3 and comprises the VH amino acid sequence termed "H1" and is defined according to SEQ ID NO:4, and the VL amino acid sequence termed "L1" and is defined according to SEQ ID NO: 8. Thus, H1 refers to the variable heavy chain region VH1, L1 refers to the variable light chain region VL1, and so forth.

In particular, the variants IgG1-huCD3-H1L1 (humanized CD3 comprising the VH1 sequence set forth in SEQ ID NO:4 and the VL1 sequence set forth in SEQ ID NO:8), IgG1-huCD3-L1-T41K (humanized CD3 comprising the VH1 sequence set forth in SEQ ID NO:4 and the VL sequence set forth in SEQ ID NO:10.

b12 antibody

In some of the examples the antibody b12, a HIV-1 gp120 specific antibody (Barbas, CF. J Mol Biol. 1993 Apr 5;230(3):812-23.) was used as a negative control, and is termed "IgG1-b12".

Expression

Antibodies were expressed as IgG1,κ or IgG1,λ with or without the non-activating mutations described below and with a mutation in the CH3 domain enabling the generation of bispecific antibodies by the method described below. Plasmid DNA mixtures

encoding both heavy and light chain of antibodies were transiently transfected to Freestyle HEK293F cells (Invitrogen, US) using 293fectin (Invitrogen, US) essentially as described by the manufacturer.

Purification of antibodies

Culture supernatant was filtered over 0.2 µm dead-end filters, loaded on 5 mL MabSelect SuRe columns (GE Health Care) and eluted with 0.1 M sodium citrate-NaOH, pH 3. The eluate was immediately neutralized with 2M Tris-HCl, pH 9 and dialyzed overnight to 12.6 mM NaH2PO4, 140 mM NaCl, pH 7.4 (B.Braun). Alternatively, subsequent to purification, the eluate was loaded on a HiPrep Desalting column and the antibody was exchanged into 12.6 mM NaH2PO4, 140 mM NaCl, pH 7.4 (B.Braun) buffer. After dialysis or exchange of buffer, samples were sterile filtered over 0.2 µm dead-end filters. Purity was determined by SDS-PAGE and concentration was measured by absorbance at 280 nm. Purified antibodies were stored at 2-8°C.

Example 2: Generation of mutant library

Point mutations were generated by random mutagenesis performed using the Quick change mutagenesis kit (Stratagene, according to the manufacturer's instructions and the HC (p33HGTE-huCD3-H1) and LC (p33L-huCD3-L1-T41K) expression plasmids as templates. The HC plasmid encodes for the monovalent UniBody –TE format as described in WO2011110642. Each selected position was randomized by using primers containing a NNS codon at the selected position (N= G, A, T or C and S = G or C). Mutant libraries were transformed to OneShot DH5alpha (Invitrogen) according to manufacturer's instructions.

Colony picking and LEE PCR

For each mutated position 96 clones were individually picked into 50 µL LEE (linear expression element) PCR buffer (5 µL 10x AccuPrime PCR buffer 1, 44.6 µL water (B.Braun) , 0.1 µL CMV P f (MAR5) and 0.1 µL Tk pA r (MAR1) primers (100 µM stock), 0.2 µL Accuprime Taq (Invitrogen) to amplify the expression cassette from the expression plasmid (promoter up to poly A). LEE PCRs were performed by incubating the mixtures 2' 94 °C, [30'' 94 °C, 30'' 55 0C, 5' 68 °C]35x, 10' 72 °C and storage at 4 °C until further use.

Each library (12) of 96 colonies was sequenced using Sanger sequencing (Beckman Coulter Genomics, UK).

Table 2: Primer sequences for LEE PCR

Primer name	Primer Sequence
CMV P f (MAR5)	GCTTCGCGATGTACGGGCCAGATATAC
TK pA r(MAR1)	GGATACCCCCTAGAGCCCCAGCTGCGCAGATCTGCTATGGC

Example 3: Expression of mutant library and IgG quantification

Of each mutant (12x96 in total) 1.11 µL HC and 1.11 µL LC LEE PCR product were diluted in 2.78 µL water. The 5 µL DNA dilution was used to transfect a single well in a 96 well plate.

Per well 0.4 µL ExpiFectamine™ 293 (Invitrogen, US) and 4.6 µL Opti-MEM (Gibco, US) were mixed and incubated for 5 minutes at room temperature. Next, the Fectin/Opti-MEM mix was added to the 5 µL DNA dilution and incubated for 30 minutes at room temperature. Finally, 8.3 µL of the Fectin/Opti-MEM/DNA mix was added to 117.5 µL Expi293F™ cells. During all procedures, the plates with Expi293F™ cells were shaken to keep the cells in suspension. After transfection, cells were incubated at 37 °C/8% CO2 for 5 days.

Five days post transfection, the supernatant was harvested. Antibody concentration in supernatant was measured by BioLayer Interferometry using the Octet RED (ForteBio, US).

Example 4: Generation of CD3/ TCR-LC13 screening library

Freestyle 293-F cells (Invitrogen, US) were co-transfected with expression constructs encoding the human alpha and beta chains of the TCR (SEQ ID NO: 396 and SEQ ID NO: 397 respectively), human CD3δ (SEQ ID NO: 398), human CD3ε (SEQ ID NO: 399), human CD3γ (SEQ ID NO: 400) and human CD3ζ (SEQ ID NO: 401). The signal peptide sequence is excluded in these sequences. Transfection was performed according to manufacturer's instructions (Invitrogen, US). One day post transfection, cells were frozen until further use.

Example 5: Screening of affinity mutants

Homogeneous assay (Dose Response)

Based on the sequence data, mutants were selected where sequence traces showed high PHRED scores, indicative for the absence of multiple mutations. Per mutation, multiple redundant clones were selected when available.

The binding of recombinantly produced UniBody molecules in cell culture supernatant was determined by homogeneous antigen specific binding assays using

Fluorometric Micro volume Assay Technology (FMAT; Applied Biosystems, Foster City, CA, USA). In the assay test design samples were analyzed in dose response for binding of antibodies or monovalent antibody molecules to CD3/TCR-LC13 (Freestyle 293-F cells transiently expressed human CD3 and human T cell receptor (TCR); produced as described above) and Freestyle 293-F wild-type cells (negative control which does not express human TCR). IgG levels for sample normalization prior to dose response binding were measured using an Octet instrument (Fortebio, Menlo Park, USA).

Dilution series of samples were added to the cells to allow binding to CD3. Subsequently, binding of monovalent antibody molecules was detected using a fluorescent conjugate (Goat anti-Human IgG Fc gamma-Alexa647; Jackson ImmunoResearch). The CD3 specific humanized mouse antibody IgG1-HuM291-F405L (produced in Freestyle 293-F cells) and monovalent anibody UniTE-huCD3-H1L1-LT41K were used as a positive control and ChromPure Human IgG, whole molecule (Jackson ImmunoResearch) was used as negative control. The samples were scanned using an Applied Biosystems 8200 Cellular Detection System (8200 CDS) and total fluorescence over sample concentration was used as read-out. Samples were stated positive when counts were higher than 50 and counts x fluorescence (total florescence) was at least three times higher than the negative control.

Heatmap

From the Homogeneous Dose Response screen, the binding curves were fitted using a 4 parameter sigmoidal model. From the fit, the maximal binding for every mutant was determined. Per mutant, the average maximal binding was calculated, and depicted as a ratio between average maximal over wt binding as shown in Figure 1.

Alignment

Selected HC mutants generated in these libraries are aligned and depicted in Figure 2. The CDR regions have been annotated according to the IMGT definitions. Numbering of the sequence is annotated according to a direct numbering scheme.

Example 6 - Generation of bispecific antibodies by 2-MEA-induced Fab-arm exchange

The bispecific antibodies according to the invention may be generated by use of the methods disclosed in WO2011131746 and WO2013060867 (Genmab).

By way of example a mutation in position F405L may be introduced in one parental antibody of IgG1 isotype, and a mutation in position K409R may be introduced in the other parental antibody of IgG1 isotype.

These two parental antibodies, each at a final concentration of 0.5 mg/mL (equimolar concentration), may be incubated under reducing conditions with 25 mM 2-mercaptoethylamine-HCl (2-MEA) in a total volume of 100 µL Tris-EDTA (TE) at 37°C for 90 min. The reduction reaction is stopped when the reducing agent 2-MEA is removed by using spin columns (Microcon centrifugal filters, 30k, Millipore) according to the manufacturer's protocol.

The bispecific antibodies may be filtered over 0.2 µm dead-end filters and the absorbance at 280 nm (A280) of the bispecific antibodies may be measured to determine the final concentration thereof.

Example 7: Binding data

For all binding assays below a selected panel of heavy chain variants of huCD3-H1L1 were tested in different formats:

Table 3: Selected affinity variants of huCD3-H1L1 in monovalent antibody-TE format

Selected UniTE-huCD3 HC affinity variants
UniTE-huCD3-H1L1-LT41K (WT)
UniTE-huCD3-H1L1-T31M-LT41K
UniTE-huCD3-H1L1-T31P-LT41K
UniTE-huCD3-H1L1-Y32A-LT41K
UniTE-huCD3-H1L1-N57E-LT41K
UniTE-huCD3-H1L1-H101F-LT41K
UniTE-huCD3-H1L1-H101G-LT41K
UniTE-huCD3-H1L1-H101I-LT41K
UniTE-huCD3-H1L1-H101K-LT41K
UniTE-huCD3-H1L1-H101L-LT41K
UniTE-huCD3-H1L1-H101N-LT41K
UniTE-huCD3-H1L1-G105P-LT41K
UniTE-huCD3-H1L1-S110A-LT41K
UniTE-huCD3-H1L1-S110G-LT41K
UniTE-huCD3-H1L1-Y114M-LT41K
UniTE-huCD3-H1L1-Y114R-LT41K
UniTE-huCD3-H1L1-Y114V-LT41K

Octet binding affinity determination of the CD3 affinity mutants in monovalent antibody-TE format

Affinities of a selected panel of affinity VH variants (Table 3) were determined using Bio-Layer Interferometry on a ForteBio Octet HTX. Anti-human Fc Capture (AHC) biosensors (ForteBio, Portsmouth, UK; cat no. 18-5060) were loaded for 600 s with the CD3 affinity mutants in monovalent antibody-TE format (2 µg/mL), aiming at a loading response of 0.4 nm. Antibodies of the UniBody-TE format were used to specifically measure the monovalent interaction affinity between the CD3 affinity mutants and the CD3ε27-GSKa ligand. After a baseline (150 s) the association (1000 s) and dissociation (1000 s) of CD3ε27-GSKa (100 and 1000 nM) was determined. The CD3ε27-GSKa protein consists of the human CD3ε peptide (aa1-27) fused to the N-terminus of a kappa LC (SEQ ID NO: 402). For calculations, the theoretical molecular mass of CD3ε27-GSKa based on the amino acid sequence was used, i.e. 27.1 kDa. Experiments were carried out while shaking at 1000 rpm and at 30°C.

Data was analyzed with ForteBio Data Analysis Software v8.1, using the 1:1 model and a global full fit with 1000 s association time and 200 s dissociation time. Data traces were corrected by subtraction of a reference curve (CD3 affinity mutant without CD3ε27-GSKa), the Y-axis was aligned to the last 5 s of the baseline, and interstep correction as well as Savitzky-Golay filtering was applied.

Table 4: Equilibrium dissociation constant (KD) for selected variants

Antibody ID	KD (M)	1/KD (M^{-1})
UniTE-huCD3-H1L1-N57E-LT41K	$2.4x10^{-8}$	$4.1x10^{7}$
uniTE-huCD3-H1L1-LT41K	$3.4x10^{-8}$	$3.0x10^{7}$
UniTE-huCD3-H1L1-G105P-LT41K	$3.5x10^{-8}$	$2.8x10^{7}$
UniTE-huCD3-H1L1-T31P-LT41K	$4.9x10^{-8}$	$2.0x10^{7}$
UniTE-huCD3-H1L1-S110G-LT41K	$6.1x10^{-8}$	$1.6x10^{7}$
UniTE-huCD3-H1L1-Y114V-LT41K	$7.5x10^{-8}$	$1.3x10^{7}$
UniTE-huCD3-H1L1-Y114M-LT41K	$7.6x10^{-8}$	$1.3x10^{7}$
UniTE-huCD3-H1L1-T31M-LT41K	$2.0x10^{-7}$	$5.1x10^{6}$
UniTE-huCD3-H1L1-H101N-LT41K	$2.0x10^{-7}$	$5.1x10^{6}$
UniTE-huCD3-H1L1-H101I-LT41K	$2.2x10^{-7}$	$4.6x10^{6}$
UniTE-huCD3-H1L1-S110A-LT41K	$3.2x10^{-7}$	$3.1x10^{6}$
UniTE-huCD3-H1L1-H101G-LT41K	$9.7x10^{-7}$	$1.0x10^{6}$
UniTE-huCD3-H1L1-H101L-LT41K	nd	nd
UniTE-huCD3-H1L1-Y32A-LT41K	nd	nd
UniTE-huCD3-H1L1-H101K-LT41K	nd	nd
UniTE-huCD3-H1L1-T31A-LT41K	nd	nd
UniTE-huCD3-H1L1-H101F-LT41K	nd	nd
UniTE-huCD3-H1L1-Y114R-LT41K	nd	nd

nd = not determined

T cell binding of affinity variants of humanized CD3 (UniTE-huCD3-H1L1-LT41K) on flow cytometry (FACS)

T cell binding of purified VH affinity variants of humanized CD3 (IgG1-huCD3-H1L1) antibodies was determined using Fluorescence-Activated Cell Sorting on a FACSCanto 752 (BD Biosciences). T cells were isolated from a buffy coat fraction of anti-coagulated human donor blood samples and resuspended in PBS/0.1% BSA/0.02% azide at 1.8 x 10E6 cells/mL. 50 µL of T cell suspension and 50 µL of the antibody dilutions were combined in a 96 well plate on ice, incubated for 30 min at 4°C and washed twice with PBS/0.1% BSA/0.02% azide. Next, 50 µL of secondary antibody, R-Phycoerythrin (PE)-conjugated goat-anti-human IgG F(ab')2 (109-116-098, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) diluted 1/200 in PBS/0.1% BSA/0.02% azide, was added for staining, the mixture was incubated for 30 min at 4°C and subsequently washed twice with PBS/0.1% BSA/0.02% azide. The cells were resuspended in 120 µL PBS/0.1% BSA/0.02% azide and PE geometric Mean Fluorescence Intensity was measured. Binding curves were analyzed using non-linear regression (sigmoidal dose-response with variable slope) using GraphPad Prism V5.04 software (GraphPad Software, San Diego, CA, USA) and apparent affinity (KD) was derived from the concentration at half-maximal binding. Figure 4 shows binding curves of affinity variants of humanized CD3 (UniTE-huCD3-H1L1-LT41K) and Figure 5 shows the binding curves of low affinity variants of humanized CD3 (UniTE-huCD3-H1L1-LT41K).

Table 5: Summary of binding data of CD3 affinity mutants in monovalent antibody-TE format

antibody ID	Tcell binding average at 5000ng/ml (Geometric MFI)	Max CD3/TCRLC13 (RFU)	KD (M)
UniTE-huCD3-H1L1-LT41K	13206	2030549	$3.4x10^{-8}$
UniTE-huCD3-H1L1-G105P-LT41K	10526	2628228	$3.5x10^{-8}$
UniTE-huCD3-H1L1-T31A-LT41K	10240	1836187	
UniTE-huCD3-H1L1-N57E-LT41K	8492	1788851	$2.4x10^{-8}$
UniTE-huCD3-H1L1-Y114R-LT41K	6197	1599412	
UniTE-huCD3-H1L1-Y114V-LT41K	6025	1807485	$7.5x10^{-8}$
UniTE-huCD3-H1L1-T31P-LT41K	4286	1718317	$4.9x10^{-8}$
UniTE-huCD3-H1L1-Y114M-LT41K	3830	1771714	$7.6x10^{-8}$
UniTE-huCD3-H1L1-T31M-LT41K	1919	1539506	$2.0x10^{-7}$
UniTE-huCD3-H1L1-S110A-LT41K	1660	1411016	$3.2x10^{-7}$
UniTE-huCD3-H1L1-H101K-LT41K	1321	893765	
UniTE-huCD3-H1L1-S110G-	949	385873	$6.1x10^{-8}$

LT41K			
UniTE-huCD3-H1L1-H101N-LT41K	848	1060228	$2.0x10^{-7}$
UniTE-huCD3-H1L1-Y32A-LT41K	719	541497	
UniTE-huCD3-H1L1-H101F-LT41K	641		
UniTE-huCD3-H1L1-H101I-LT41K	511	603683	$2.2x10^{-7}$
UniTE-huCD3-H1L1-H101L-LT41K	509	812768	
UniTE-huCD3-H1L1-H101G-LT41K	492	139712	$9.7x10^{-7}$

For all binding assays below a selected panel of preferred heavy chain variants was tested (see Table 5)

Octet binding affinity determination of IgG1-huCD3-H1L1-FEAL affinity mutants

Affinities of selected CD3 affinity variants in an IgG1-huCD3-H1L1-FEAL format were determined using Bio-Layer Interferometry on a ForteBio Octet HTX (ForteBio, UK) (Table 6). Anti-human Fc capture biosensors (cat: 18-5060, ForteBio, UK) were loaded for 600 s with hIgG (1 µg/mL). After a baseline (200 s) the association (1000 s) and dissociation (2000 s) of CD3E27-GSKa was determined, using a CD3E27-GSKa concentration range of 27.11 µg/mL – 0.04 µg mL (1000 nM – 1.4 nM) with three-fold dilution steps (sample diluent, cat: 18-5028, ForteBio, UK). For calculations, the theoretical molecular mass of CD3E27-GSKa based on the amino acid sequence was used, i.e. 27.11 kDa. Experiments were carried out while shaking at 1000 rpm and at 30 °C. Each antibody was tested in at at least two independent experiments (Table 6).

Data was analyzed with ForteBio Data Analysis Software v8.1, using the 1:1 model and a global full fit with 1000 s association time and 100 s dissociation time. Data traces were corrected by subtraction of a reference curve (antibody without CD3E27-GSKa), the Y-axis was aligned to the last 10 s of the baseline, and interstep correction as well as Savitzky-Golay filtering was applied. Data traces with a response <0.05 nm were excluded from analysis.

Table 6

Average	< KD> (nM)	SDEV	SEM	CV	<Kon>	SDEV	SEM	CV	<Kdis>	SDEV	SEM	CV	pKD	n
IgG1-huCD3-G105P-FEAL	5	2	1	45	4.7E+05	9.7E+04	5.6E+04	21	2.5E-03	1.0E-03	5.8E-04	40	8.3	3
IgG1-huCD3-FEAL	15	6	3	37	2.7E+05	5.1E+04	2.9E+04	19	4.0E-03	1.6E-03	9.1E-04	39	7.8	3
IgG1-huCD3-Y114V-FEAL	29	8	4	26	2.2E+05	3.3E+04	1.9E+04	15	6.3E-03	9.7E-04	5.6E-04	15	7.5	3
IgG1-huCD3-T31P-FEAL	42	9	4	21	1.9E+05	3.8E+04	1.6E+04	20	7.8E-03	1.3E-03	5.3E-04	17	7.4	6
IgG1-huCD3-Y114M-FEAL	42	14	8	33	2.6E+05	6.2E+04	3.6E+04	24	1.0E-02	1.5E-03	8.7E-04	15	7.4	3
IgG1-huCD3-H101N-FEAL	45	13	7	29	4.8E+05	2.2E+05	1.2E+05	45	2.0E-02	3.1E-03	1.8E-03	16	7.3	3
IgG1-huCD3-Y114R-FEAL	46	10	6	22	1.5E+05	4.1E+04	2.4E+04	27	6.8E-03	4.1E-04	2.4E-04	6	7.3	3
IgG1-huCD3-S110A-FEAL	72	15	6	21	1.8E+05	2.5E+04	1.0E+04	14	1.3E-02	1.6E-03	6.4E-04	12	7.1	6
IgG1-huCD3-N57E-FEAL	91	30	17	33	2.1E+05	2.8E+04	1.6E+04	13	1.9E-02	4.0E-03	2.3E-03	21	7.0	3
IgG1-huCD3-T31M-FEAL	99	23	13	23	1.9E+05	2.5E+04	1.5E+04	14	1.8E-02	2.6E-03	1.5E-03	14	7.0	3
IgG1-huCD3-Y32A-FEAL	105	31	22	29	2.2E+05	1.1E+05	7.5E+04	48	2.2E-02	4.4E-03	3.1E-03	20	7.0	2
IgG1-huCD3-H101L-FEAL	107	39	23	37	2.7E+05	4.3E+04	2.5E+04	16	2.8E-02	7.5E-03	4.4E-03	27	7.0	3
IgG1-huCD3-H101K-FEAL	120	94	55	79	2.2E+05	1.9E+05	1.1E+05	84	1.7E-02	9.8E-03	5.7E-03	58	6.9	3
IgG1-huCD3-S110G-FEAL	153	120	70	79	3.8E+05	4.2E+05	2.4E+05	112	2.6E-02	8.3E-03	4.8E-03	32	6.8	3
IgG1-huCD3-H101G-FEAL	683	169	97	25	3.0E+04	9.2E+03	5.3E+03	30	2.0E-02	8.5E-04	4.9E-04	4	6.2	3
IgG1-huCD3-H101I-FEAL	nd													0
IgG1-huCD3-H101F-FEAL	nd													0

T cell binding affinity determination of IgG1-huCD3-H1L1-FEAL affinity mutants

T cells from donor buffy coats (Sanquin, Amsterdam, The Netherlands) were isolated by using RosetteSep human T cell enrichment cocktail (Cat: 15021C.1, Stemcell Technologies, France) according to manufacturer's instructions. Briefly, 50 µL of T cell isolation cocktail was added to 1 mL of buffy coat and incubate at RT for 20 min. Next, the buffy coat was diluted (1:3, v/v) with PBS (cat: 3623140, B.Braun, Germany) and gently transferred to 50 mL falcon tubes (cat: 227261, Greiner bio-one, The Netherlands) filled with 15 mL lymphocyte separation medium (cat: 17-829E, Lonza, Switzerland). Tubes were centrifuged for 20 min at RT 1200xg without brakes. Collect the T cells from the density medium and wash with PBS twice.

2x10E6 T cells/mL were resuspended in FACS buffer and transferred to 50 µL into round bottom 96 well plates (cat: 650101, Greiner bio-one, The Netherlands). 50 µL of the antibody solutions in five-fold dilutions was added starting with 5 µg/mL and incubated for 30 min at 4 °C. The 96 plates were centrifuged at 300xg for 5 min at 4 °C and the supernatant discarded. Cells were washed twice with ice cold FACS buffer on ice and the 1:200 diluted secondary antibody (anti IgG Fcγ-PE (fab)'2, cat: 109-116-098, Jackson Immuno Research, UK) added to 100 µL /well and incubated for 30 min and washed twice with FACS buffer. Fluorescence intensity was measured on FACS Canto and geometric mean calculated by FlowJo V10 software. Graphs were made by GraphPad (V6.04). See Figure 5.

Example 9: In vitro cytotoxicity screening of CD3 affinity mutants

Cytotoxicity of CD3 affinity mutants on solid tumor cell lines (alamar blue assay)

T cells from donor buffy coats (Sanquin, Amsterdam, The Netherlands) were isolated by using RosetteSep human T cell enrichment cocktail (Cat: 15021C.1, Stemcell Technologies, France) according to manufacturer's instructions. NCI-N87 (25.000 cells/well) (Figure 6A), SKOV3 (16.000 cells/well) (Figure 6B) and MDA-MB-231 (16.000 cells/well) (Figure6C) cells were seeded into flat bottom 96 well plates (cat: 655180, Greiner-bio-one, The Netherlands) and adhered for 3-5h at 37 °C. T cells were added to tumor cells in the following ratios: NCI-N87 cell: T cells, 1:3; SKOV3 cell:T cell, 1:4; MDA-MB-231 cell: T cell, 1:8. Subsequently antibody solutions were added in ten-fold dilutions and plates were incubated for 2 days at 37 °C. Next, supernatants were discarded and adhered cells were washed twice

with PBS. 150 µL of 10% alamar blue (cat: DAL1100, Life Technologies, The Netherlands) solution prepared in RPMI-1640 (cat: BE12-115F, Lonza, Switzerland) medium containing 10% donor bovine serum with iron (cat: 10371-029, Life Technologies, The Netherlands) was added to wells and incubated for 3-5h at 37 °C. The absorbance was measured with Envision multilabel plate reader (PerkinElmer, US). Staurosporine (cat: S6942, Sigma-Aldrich, US) treated cells were set as 100% kill and untreated cells were set as 0% kill. Viable cells were calculated by subtracting staurosporine treated cells from all groups and the percentage was plotted against the untreated group. Graphs were made by GraphPad (V6.04). See Figure 6.

Cytotoxicity of CD3 affinity mutants on a hematological cell line (chromium release assay)

5x10E6 Daudi cells/ mL were incubated in complete culture medium with 100 µCi chromium for 1h under shaking conditions at 37°C. Next, cells were washed twice in PBS and resuspended in 5 mL complete cell culture medium (10% donor bovine serum with iron in RPMI 1640). 5.000 Daudi cells were seeded into round bottom 96 well plates. T cells from donor buffy coats (purchased from Sanquin, Amsterdam, The Netherlands) were isolated by using RosetteSep human T cell enrichment cocktail (Cat: 15021C.1, Stemcell Technologies, France) according to manufacturer's instructions. T cells are added in (tumor cell: T cell) 1:10 ratio to Daudi cells followed by the addition of the antibody solutions in two-fold dilutions. Plates were in incubated 24h at 37 °C. After 24h, plates were centrifuges at 300xg for 3 min, supernatant was harvested and measured for radioactivity. See Figure 7.

Tabel 7

		% of kill			
HER2 copy#	Cells	No kill	kill ≤ 50%	kill ≤ wt	kill = wt
HER2 ≥ 1.000.000	NCI-N87	H101I, H101L, H101K, Y32A	H101F, S110G, H101N	N57E, H101G	S110A, G105P, S110A, T31M, T31P, Y114M, Y114R, Y114V
50.000 ≤ HER2 ≤ 180.000	SKOV3	H101I, H101F, H101L, Y32A	H101K	H101G, S110G, H101N, N57E	S110A, G105P, S110A, T31M, T31P, Y114M, Y114R, Y114V
HER2 ≤ 30.000	MDA-MB-231	H101F, H101I, S110G, Y32A, H101L, H101K, H101N, N57E	H101G		S110A, G105P, S110A, T31M, T31P, Y114M, Y114R, Y114V

Example 10: Tumor efficacy of CD3xHER2 bispecific antibodies in a , (human PBMC + NCI-N87 cells) co-engraftment model in NOD-SCID mice

The *in vivo* anti-tumor efficacy of several CD3xHER2 bispecific antibodies was evaluated in a subcutaneous NCI-N87 co-engraftment model (Figure 8). As a CD3-arm of the bispecific antibody, a humanized WT CD3 (huCD3-FEAL) and 4 different CD3 affinity variants (N57E, H101K, S110A, Y114M) were used. The HER2-targeting arm in all cases was the same (Herceptin-FEAR)

BisG1-huCD3-FEALx1014-Herceptin-FEAR

BisG1-huCD3-N57E-FEALx1014-Herceptin-FEAR

BisG1-huCD3-H101K-FEALx1014-Herceptin-FEAR

BisG1-huCD3-S110A-FEALx1014-Herceptin-FEAR

BisG1-huCD3-Y114M-FEALx1014-Herceptin-FEAR

In this model, HLA-A-matched human unstimulated PBMCs, as a source of human T cells, were co-inoculated with NCI-N87 tumor cells at two different dose levels (0.5 and 0.05 mg/kg).

Mice were sorted into treatment groups (n=4 per treatment group. At day 0, a mixture containing HLA-A matched hPBMC (5x10E6, Sanquin) and NCI-N87 (5x10E6) cells in 200 µL PBS/0.1% BSA were inoculated subcutaneously (s.c.) in the right flank of each female NOD-SCID mice (NOD.C.B-17-Prkdc^{scid}/J), age 6-11 weeks old (Charles-River)). Directly after tumor cell injection, single intravenous dosing (150 µL) of five different CD3xHER2 antibodies was performed at two different concentrations (0.5 and 0.05 mg/kg) for all bispecific antibodies. Tumor volumes were determined at least two times per week. Tumor volumes (mm^3) were calculated from caliper (PLEXX) measurements as: 0.52 x (length) x $(width)^2$.

NCI-N87 cells (ATCC# CRL-5822, gastric carcinoma arising from stomach) were thawed, cultured in RPMI 1640 (Lonza, BE12-115F) supplemented with 10% donor bovine serum with iron (Gibco, cat. no. 10371-029), penicillin/streptomycin and 0.45% glucose (Sigma, G8769), sodium pyruvate (Cambrex, BE13-115E) and 0.075% sodium bicarbonate (Cambrex, BE17-613E). Cells were grown in CellSTACK culture chambers and harvested in log-phase and counted by trypan blue exclusion.

For each study, hPBMCs were isolated from human HLA-A matched donors for NCI-N87 (HLA-A-01,23) from a buffy coat (Sanquin) by Ficoll density centrifugation. Isolated cells were frozen in nitrogen and thawed before use. All cells were washed in PBS /0.1% BSA, filtered through a cell strainer and resuspended to a concentration of 50 x 10E6 cells/mL in PBS/0.1% BSA.

The results are shown in Figure 8.Figure 6A-B show average tumor volumes after treatment over time. Figure 6C-D show dot plot representations of average NCI-N87 tumor volume at day 44. Statistical analysis (Mann-Whitney) on the tumor volumes at day 44, last day that all groups were still intact, revealed at a dose of 0.05 mg/kg a significant tumor growth inhibition ($p<0.05$) of BisG1-huCD3-FEALx1014-Herceptin-FEAR, BisG1-huCD3-S110A-FEALx1014-Herceptin-FEAR and BisG1-huCD3-Y114M-FEALx1014-Herceptin-FEAR compared to the control (PBMCs) and not of BisG1-huCD3- N57E-FEALx1014-Herceptin-FEAR and BisG1-huCD3-H101K-FEALx1014-Herceptin-FEAR. At a dose of 0.5 mg/kg BisG1-huCD3-FEALx1014-Herceptin-FEAR and all CD3-arm affinity variants, showed significant tumor growth ($p<0.05$) inhibition compared to the PBS (PBMCs) control group, except BisG1-huCD3-H101K-FEALx1014-Herceptin-FEAR.

BisG1-huCD3-FEALx1014-Herceptin-FEAR, BisG1-huCD3-S110A-FEALx1014-Herceptin-FEAR, and BisG1-huCD3-Y114M-FEALx1014-Herceptin-FEAR significantly ($p<0.05$) reduced NCI-N87 tumor volume at dosages of 0.05 and 0.5 mg/kg. BisG1-huCD3-N57E-FEALx1014-Herceptin-FEAR reduced significantly

($p<0.05$) NCI-N87 tumor volume only at a dosage of 0.5 mg/kg. BisG1-huCD3-H101K-FEALx1014-Herceptin-FEAR did not affect NCI-N87 tumor growth at both tested dosages.

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Claims

1. A humanized or chimeric antibody binding to human CD3, wherein said antibody comprises a binding region comprising a heavy chain variable (VH) region and a variable light chain (VL) region, wherein said VH region comprises the CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 1, 2, 176 [H101G], and wherein said VL region comprises CDR1, CDR2, and CDR3 regions having the CDR sequences as set forth in SEQ ID NO:6, GTN and 7.

2. The antibody according to claim 1, wherein said VL region is selected from the group consisting of;

a) a VL sequence as set forth in SEQ ID NO:8;

b) a VL sequence as set forth in SEQ ID NO:10 or

c) a VL sequence having at least 90%, at least 95%, at least 97% or at least 99% amino acid sequence identity to any one of the sequences as set forth in a) to b).

3. The antibody according to claim 1 or claim 2, wherein human CD3 is human CD3 epsilon as specified in SEQ ID NO: 399.

4. The antibody according to any one of the preceding claims, wherein the antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a K_D value from $1.0x10^{-7}$ to $9.9x10^{-7}$ M as determined by Bio-Layer Interferometry.

5. The antibody according to any one of the preceding claims, wherein the antibody is a humanized antibody.

6. The antibody according to any one of the preceding claims, wherein the antibody is a full-length antibody.

7. The antibody according to any one of the preceding claims, wherein the antibody is of an isotype selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.

8. The antibody according to any one of the preceding claims, wherein the antibody is monovalent, bivalent or multivalent.

9. The antibody according to any one of the preceding claims, wherein said antibody comprises an Fc region comprising a first and a second immunoglobulin heavy chain.

10. The antibody according to any one of the preceding claims, wherein said antibody comprises a first and a second immunoglobulin heavy chain, wherein in at least one of said first and second immunoglobulin heavy chains one or more amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain according to EU numbering, are not L, L, D, N, and P, respectively.

11. The antibody according to claim 10, wherein in at least one of said first and second immunoglobulin heavy chains the amino acid in the position corresponding to position D265 in a human IgG1 heavy chain, is not D.

12. The antibody according to claim 10, wherein in at least one of said first and second heavy chains the amino acid in the position corresponding to position N297 in a human IgG1 heavy chain, is not N.

13. The antibody according to claim 10, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are not L and L, respectively.

14. The antibody according to any one of claims 10 to 13, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are F and E; or A and A, respectively.

15. The antibody according to claim 14, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are F and E, respectively.

16. The antibody according to claim 14, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain, are A and A, respectively.

17. The antibody according to claim 9, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are not L, L, and D, respectively.

18. The antibody according to claim 17, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A; or A, A, and A, respectively.

19. The antibody according to claim 17 or claim 18, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are F, E, and A, respectively.

20. The antibody according to claim 17 or claim 18, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain, are A, A, and A, respectively.

21. The antibody according to claim 10, wherein in at least one of said first and second heavy chains the amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain, are F, E, A, Ct, and S, respectively.

22. A bispecific antibody comprising a first binding region of an antibody according to any one of claims 1 to 21, and a second binding region which binds a different target than said first antigen binding region.

23. The bispecific antibody according to claim 22 wherein said antibody comprises a first and a second heavy chain.

24. The bispecific antibody according to claim 23, wherein each of said first and second heavy chain comprises at least a hinge region, a CH2 and CH3 region, wherein in said first heavy chain at least one of the amino acids in the positions corresponding to a positions selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, and in said second heavy chain at least one of the amino acids in the positions corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, and wherein said first and said second heavy chains are not substituted in the same positions.

25. The bispecific antibody according to claim 24, wherein the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said first heavy chain, and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in said second heavy chain, or vice versa.

26. The bispecific antibody according to any one of claims 22 to 25, wherein said first binding region is according to any one of claims 1 to 9, and said second binding region binds a different target than said first binding region.

27. A method of reducing the binding affinity of an antibody binding to human CD3 compared to a reference antibody comprising a heavy chain variable (VH) region, wherein said VH region comprises the CDR1, CDR2, and CDR3 sequences set forth in SEQ ID NO: 1, 2, 3, which method comprises introducing a mutation in one of the CDR3 sequence of the said reference antibody, wherein said mutation in the VH CDR3 region is H101G, wherein the positions are numbered according to the reference sequence of the SEQ ID NO: 4.

28. The method according to claim 27, wherein antibody has a binding affinity to human CD3 epsilon peptide with SEQ ID NO: 402 corresponding to a K_D value from $1.0x10^{-7}$ to $9.9x10^{-7}$ M as determined by Bio-Layer Interferometry.

29. The method according to claim 27 or claim 28, wherein the human CD3 epsilon is expressed on a T cell.

30. The method according to any one of claims 27 to 29, wherein the human CD3 epsilon is in isolated form, e.g. an isolated human CD3 epsilon-peptide.

31. A nucleic acid construct encoding one or more amino acid sequences of any of claims 1 to 26.

32. An expression vector comprising a nucleic acid sequence encoding a heavy chain sequence and a light chain sequence of a humanized or chimeric antibody according to claim 1.

33. A host cell comprising an expression vector of claim 32.

34. The host cell according to claim 33, wherein said host cell is a recombinant eukaryotic, recombinant prokaryotic, or recombinant microbial host cell.

35. A composition comprising an antibody according to any one of claims 1 to 9 or a bispecific antibody according to claims 10 to 26.

36. A pharmaceutical composition comprising the antibody according to any one of claims 1 to 9, or the bispecific antibody according to any one of claims 10 to 26 and a pharmaceutical acceptable carrier.

37. The antibody according to any one of claims 1 to 26, the composition according to claim 35, or the pharmaceutical composition according to claim 36 for use as a medicament.

38. The antibody according to any one of claims 1 to 9, the bispecific antibody according to claims 10 to 26, the composition according to claim 35, or the pharmaceutical composition according to claim 36, for use in the treatment of a disease.

39. Use of the antibody according to any one of claims 1 to 9, the bispecific antibody according to claims 10 to 26, the composition according to claim 35, or the pharmaceutical composition according to claim 36, in the manufacture of a medicament for the treatment of cancer, an infectious disease, or an autoimmune disease in a subject.

40. The use of claim 39, wherein said antibody is labeled with a detectable agent.

41. A method for producing an antibody according to any one of claims 1 to 9 comprising the steps

a) culturing a host cell according to claim 33 or claim 34; and

b) purifying said antibody from the culture media.

42. A diagnostic composition comprising an antibody according to any one of claims 1 to 9 or a bispecific antibody according to any one of claims 10 to 26.

43. A method for detecting the presence of CD3 antigen, or a cell expressing CD3, in a sample comprising the steps of;

a) contacting the sample with an antibody according to any one of claims 1 to 9 or a bispecific antibody according to any one of claims 10 to 26, under conditions that allow for formation of a complex between said antibody or bispecific antibody and CD3; and

b) analyzing whether a complex has been formed.

44. A kit for detecting the presence of CD3 antigen, or a cell expressing CD3, in a sample comprising

i) an antibody according to any one of claims 1 to 9 or a bispecific antibody according to any one of claims 10 to 26; and

ii) instructions for use of said kit.