IL2Rbeta/Common Gamma Chain Antibodies

Field of the Invention

The present invention relates to antibodies that bind to interleukin 2 receptor β (IL-2Rβ; CD122) and common γ chain (γc; CD132).

Background to the Invention

IL-2 is an essential cytokine that plays a central role in maintaining T cell homeostasis and mediating proper immune responses. Its high potency as an immune stimulator has led to clinical uses to treat a range of conditions, including cancers and AIDS; it is also widely used as an adjuvant for vaccination to stimulate activation and proliferation of various effector cells.

However, the high dose of IL-2 that is required for effective treatment of certain diseases is highly toxic. Major adverse effects of such therapy include vascular leak syndrome (VLS), which results in accumulation of the intravascular fluid in organs such as lung and liver with subsequent pulmonary edema and liver damage. There is no treatment for VLS except withdrawing the therapy.

IL-2 exerts its pleiotropic functions by binding to different combinations of receptor components expressed on different cell types: the alpha chain (IL-2Rα, also known as CD25), the beta chain (IL-2Rβ, or CD122), and the common cytokine receptor gamma chain (IL-2Rγ, γc, or CD132).

Isolated IL-2Rα has been termed the "low affinity" IL-2 receptor (binding affinity K_D ~ 10 nM) and is not involved in signal transduction. A complex of IL-2Rβ and γc binds IL-2 with intermediate affinity (K_D ~ 1 nM), although IL-2Rβ alone has very low affinity (K_D ~ 100 nM) and γc alone has virtually no detectable binding affinity for IL-2. A complex with all three subunits, IL-2Rα, IL-2Rβ, and γc, binds IL-2 with high affinity (K_D ~ 10 pM).

Heterodimerization of IL-2Rβ and γc is necessary and sufficient for effective signal transduction through the interaction of their cytoplasmic domains and subsequent kinase activation of multiple signaling pathways; IL-2Rα plays no role in signal transduction.

High-affinity α-β-γc IL-2Rs are typically found on CD4+ T regulatory cells (Tregs) as well as recently-activated T cells. Intermediate-affinity β-γc IL-2Rs are present at a low level

on naïve CD8+ cells, but are prominent on antigen-experienced (memory) and memory-phenotype (MP) CD8+ T cells as well as natural killer (NK) cells. Both MPCD8+ T cells and NK cells express very high levels of IL-2Rβ and readily respond to IL-2.

Previous studies have indicated that VLS is caused by the release of proinflammatory cytokines from IL-2-activated NK cells. However, a recent study suggested that IL-2-induced pulmonary edema may result from direct binding of IL-2 to lung endothelial cells, which express functional high affinity α-β-γc IL-2Rs. This was evidenced by the observation that interaction of IL-2 with lung endothelial cells was abrogated by blocking anti-IL-2Rα monoclonal antibody (mAb), in IL-2Rα -deficient host mice, or by the use of an IL-2/anti-IL-2 mAb (IL-2/mAb) complex in which the antibody prevents IL-2/ IL-2Rα interaction, thus preventing VLS.

Summary of the Invention

The present invention is concerned with antibodies, or antigen binding fragments, that bind to CD122 and/or common γ chain (γc). Heavy and light chain polypeptides for CD122 and common γ chain (γc) binding antibodies are also disclosed. The antibodies, antigen binding fragments and polypeptides may be provided in isolated and/or purified form and may be formulated into compositions suitable for use in research, therapy and diagnosis.

In a first aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to CD122 and common γ chain (γc).

In some embodiments, the antibody or antigen binding fragment is a bispecific antibody or a bispecific antigen binding fragment.

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to CD122, comprising the amino acid sequences i) to vi):

i)	LC-CDR1:	$TGTSSDIGX_1YDFX_2S$	(SEQ ID NO:85)
		RAGQAISSWLA	(SEQ ID NO:6);
		QASQDIGNYLN	(SEQ ID NO:10); or
		TRSSGSIASNYVQ	(SEQ ID NO:14);
ii)	LC-CDR2:	DX_3NNRX_4S	(SEQ ID NO:86);
		KASNLES	(SEQ ID NO:7);

		DASNLET	(SEQ ID NO:11); or
		DDNQRPT	(SEQ ID NO:15);
iii)	LC-CDR3:	SAYTSSDTX$_5$V	(SEQ ID NO:87);
		QQYQSYPYT	(SEQ ID NO:8);
		LQLYDYPLT	(SEQ ID NO:12); or
		QSSHSTAVV	(SEQ ID NO:16);
iv)	HC-CDR1:	NYYX$_6$H	(SEQ ID NO:88);
		TYAMH	(SEQ ID NO:40);
		SYAMS	(SEQ ID NO:44); or
		GYYWS	(SEQ ID NO:48);
v)	HC-CDR2:	AIMPSRGGTSYPQKFQG	(SEQ ID NO:37);
		WINTGNGNTKYSQNFQG	(SEQ ID NO:41);
		AISGSGGSTYYADSVKG	(SEQ ID NO:45); or
		EINHSGSTNYNPSLKS	(SEQ ID NO:49);
vi)	HC-CDR3:	GEYYYDSSGYYX$_7$	(SEQ ID NO:89);
		DLGQLERLYFW	(SEQ ID NO:42);
		DLGDY	(SEQ ID NO:46); or
		SSSGDAFD	(SEQ ID NO:50);

or a variant thereof in which one or two or three amino acids in one or more of the sequences i) to vi) are replaced with another amino acid, wherein X_1 = H or D; X_2 = V or I; X_3 = I, N or F; X_4 = P or A; X_5 = L or V; X_6 = M or I; and X_7 = Y or N.

In some embodiments, LC-CDR1 is one of TGTSSDIGHYDFVS (SEQ ID NO:2), TGTSSDIGDYDFVS (SEQ ID NO:18), TGTSSDIGHYDFIS (SEQ ID NO:25), RAGQAISSWLA (SEQ ID NO:6), QASQDIGNYLN (SEQ ID NO:10), or TRSSGSIASNYVQ (SEQ ID NO:14).

In some embodiments, LC-CDR2 is one of DINNRPS (SEQ ID NO:3), DNNNRPS (SEQ ID NO:20), DFNNRPS (SEQ ID NO:26), DINNRAS (SEQ ID NO:32), KASNLES (SEQ ID NO:7), DASNLET (SEQ ID NO:11), or DDNQRPT (SEQ ID NO:15).

In some embodiments, LC-CDR3 is one of SAYTSSDTLV (SEQ ID NO:4), SAYTSSDTVV (SEQ ID NO:22), QYQSYPYT (SEQ ID NO:8), LQLYDYPLT (SEQ ID NO:12), or QSSHSTAVV (SEQ ID NO:16).

In some embodiments, HC-CDR1 is one of NYYMH (SEQ ID NO:36), NYYIH (SEQ ID NO:54), TYAMH (SEQ ID NO:40), SYAMS (SEQ ID NO:44), or GYYWS (SEQ ID NO:48).

In some embodiments, HC-CDR2 is one of AIMPSRGGTSYPQKFQG (SEQ ID NO:37), WINTGNGNTKYSQNFQG (SEQ ID NO:41), AISGSGGSTYYADSVKG (SEQ ID NO:45), or EINHSGSTNYNPSLKS (SEQ ID NO:49).

In some embodiments, HC-CDR3 is one of GEYYYDSSGYYY (SEQ ID NO:38), GEYYYDSSGYYN (SEQ ID NO:52), DLGQLERLYFW (SEQ ID NO:42), DLGDY (SEQ ID NO:46), or SSSGDAFD (SEQ ID NO:50).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TGTSSDIGHYDFVS	(SEQ ID NO:2)
LC-CDR2:	DINNRPS	(SEQ ID NO:3)
LC-CDR3:	SAYTSSDTLV	(SEQ ID NO:4).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TGTSSDIGDYDFVS	(SEQ ID NO:18)
LC-CDR2:	DINNRPS	(SEQ ID NO:3)
LC-CDR3:	SAYTSSDTLV	(SEQ ID NO:4).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TGTSSDIGHYDFVS	(SEQ ID NO:2)
LC-CDR2:	DNNNRPS	(SEQ ID NO:20)
LC-CDR3:	SAYTSSDTLV	(SEQ ID NO:4).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TGTSSDIGHYDFVS	(SEQ ID NO:2)
LC-CDR2:	DINNRPS	(SEQ ID NO:3)
LC-CDR3:	SAYTSSDTVV	(SEQ ID NO:22).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TGTSSDIGHYDFIS	(SEQ ID NO:25)
LC-CDR2:	DFNNRPS	(SEQ ID NO:26)
LC-CDR3:	SAYTSSDTLV	(SEQ ID NO:4).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TGTSSDIGHYDFVS	(SEQ ID NO:2)
LC-CDR2:	DNNNRPS	(SEQ ID NO:20)
LC-CDR3:	SAYTSSDTVV	(SEQ ID NO:22).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TGTSSDIGHYDFVS	(SEQ ID NO:2)
LC-CDR2:	DNNNRAS	(SEQ ID NO:32)
LC-CDR3:	SAYTSSDTVV	(SEQ ID NO:22).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TGTSSDIGDYDFVS	(SEQ ID NO:18)
LC-CDR2:	DINNRPS	(SEQ ID NO:3)
LC-CDR3:	SAYTSSDTVV	(SEQ ID NO:22)

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	RAGQAISSWLA	(SEQ ID NO:6)
LC-CDR2:	KASNLES	(SEQ ID NO:7)
LC-CDR3:	QQYQSYPYT	(SEQ ID NO:8).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	QASQDIGNYLN	(SEQ ID NO:10)
LC-CDR2:	DASNLET	(SEQ ID NO:11)
LC-CDR3:	LQLYDYPLT	(SEQ ID NO:12)

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	TRSSGSIASNYVQ	(SEQ ID NO:14)
LC-CDR2:	DDNQRPT	(SEQ ID NO:15)
LC-CDR3:	QSSHSTAVV	(SEQ ID NO:16).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	NYYMH	(SEQ ID NO:36)
HC-CDR2:	AIMPSRGGTSYPQKFQG	(SEQ ID NO:37)
HC-CDR3:	GEYYYDSSGYYY	(SEQ ID NO:38).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	NYYMH	(SEQ ID NO:36)
HC-CDR2:	AIMPSRGGTSYPQKFQG	(SEQ ID NO:37)
HC-CDR3:	GEYYYDSSGYYY	(SEQ ID NO:38).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	NYYMH	(SEQ ID NO:36)
HC-CDR2:	AIMPSRGGTSYPQKFQG	(SEQ ID NO:37)
HC-CDR3:	GEYYYDSSGYYN	(SEQ ID NO:52).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	NYYIH	(SEQ ID NO:54)
HC-CDR2:	AIMPSRGGTSYPQKFQG	(SEQ ID NO:37)
HC-CDR3:	GEYYYDSSGYYY	(SEQ ID NO:38).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	TYAMH	(SEQ ID NO:40)
HC-CDR2:	WINTGNGNTKYSQNFQG	(SEQ ID NO:41)
HC-CDR3:	DLGQLERLYFW	(SEQ ID NO:42).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	SYAMS	(SEQ ID NO:44)
HC-CDR2:	AISGSGGSTYYADSVKG	(SEQ ID NO:45)

HC-CDR3: DLGDY (SEQ ID NO:46).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1: GYYWS (SEQ ID NO:48)
HC-CDR2: EINHSGSTNYNPSLKS (SEQ ID NO:49)
HC-CDR3: SSSGDAFD (SEQ ID NO:50).

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to CD122, comprising a light chain and a heavy chain variable region sequence, wherein:

the light chain comprises a LC-CDR1, LC-CDR2, LC-CDR3, having at least 85% overall sequence identity to LC-CDR1: one of TGTSSDIGX$_1$YDFX$_2$S (SEQ ID NO:85), RAGQAISSWLA (SEQ ID NO:6); QASQDIGNYLN (SEQ ID NO:10); or TRSSGSIASNYVQ (SEQ ID NO:14); LC-CDR2: one of DX$_3$NNRX$_4$S (SEQ ID NO:86); KASNLES (SEQ ID NO:7); DASNLET (SEQ ID NO:11); or DDNQRPT (SEQ ID NO:15); LC-CDR3: one of SAYTSSDTX$_5$V (SEQ ID NO:87); QQYQSYPYT (SEQ ID NO:8); LQLYDYPLT (SEQ ID NO:12); or QSSHSTAVV (SEQ ID NO:16); and

the heavy chain comprises a HC-CDR1, HC-CDR2, HC-CDR3, having at least 85% overall sequence identity to HC-CDR1: one of NYYX$_6$H (SEQ ID NO:88); TYAMH (SEQ ID NO:40); SYAMS (SEQ ID NO:44); or GYYWS (SEQ ID NO:48); HC-CDR2: one of AIMPSRGGTSYPQKFQG (SEQ ID NO:37); WINTGNGNTKYSQNFQG (SEQ ID NO:41); AISGSGGSTYYADSVKG (SEQ ID NO:45); or EINHSGSTNYNPSLKS (SEQ ID NO:49); HC-CDR3: one of GEYYYDSSGYYX$_7$ (SEQ ID NO:89); DLGQLERLYFW (SEQ ID NO:42); DLGDY (SEQ ID NO:46); or SSSGDAFD (SEQ ID NO:50);

wherein X$_1$ = H or D; X$_2$ = V or I; X$_3$ = I, N or F; X$_4$ = P or A; X$_5$ = L or V; X$_6$ = M or I; and X$_7$ = Y or N.

In some embodiments the degree of sequence identity may be one of 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to CD122, comprising a light chain and a heavy chain variable region sequence, wherein:

the light chain sequence has at least 85% sequence identity to the light chain sequence of one of SEQ ID NOs:1, 17, 19, 21, 23, 24, 27, 28, 29, 30, 31, 33, 34, 148, 149, 5, 9, or 13 (Figure 1), and;

the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence of one of SEQ ID NOs:35, 51, 53, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 150, 151, 39, 43, or 47 (Figure 2).

In some embodiments the degree of sequence identity may be one of 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to CD122, which is a bispecific antibody or a bispecific antigen binding fragment comprising (i) an antigen binding fragment according to the present invention, and (ii) an antigen binding fragment capable of binding to common γ chain (γc).

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to common γ chain (γc), comprising the amino acid sequences i) to vi):

i)	LC-CDR1:	RSSQSLLHSNGYNYLD	(SEQ ID NO:68); or
		SGDALPKQFAF	(SEQ ID NO:72);
ii)	LC-CDR2:	LGSNRDS	(SEQ ID NO:69); or
		KDTERPS	(SEQ ID NO:73);
iii)	LC-CDR3:	MQGTHWPWT	(SEQ ID NO:70); or
		QSPDSSGTVEV	(SEQ ID NO:74);
iv)	HC-CDR1:	GYYWS	(SEQ ID NO:48); or
		SSSYYWG	(SEQ ID NO:79);
v)	HC-CDR2:	EINHX_8GSTNYNPSLKS	(SEQ ID NO:90); or
		SIYYSGSTYYNPSLK	(SEQ ID NO:80);
vi)	HC-CDR3:	SPGGYSGGYFQH	(SEQ ID NO:77); or
		DILTGYALDY	(SEQ ID NO:81);

or a variant thereof in which one or two or three amino acids in one or more of the sequences i) to vi) are replaced with another amino acid, wherein X_8 = S or F.

In some embodiments, LC-CDR1 is RSSQSLLHSNGYNYLD (SEQ ID NO:68) or SGDALPKQFAF (SEQ ID NO:72).

In some embodiments, LC-CDR2 is LGSNRDS (SEQ ID NO:69) or KDTERPS (SEQ ID NO:73).

In some embodiments, LC-CDR3 is MQGTHWPWT(SEQ ID NO:70) or QSPDSSGTVEV (SEQ ID NO:74).

In some embodiments, HC-CDR1 is GYYWS (SEQ ID NO:48) or SSSYYWG (SEQ ID NO:79).

In some embodiments, HC-CDR2 is one of EINHSGSTNYNPSLKS (SEQ ID NO:49), EINHFGSTNYNPSLKS (SEQ ID NO:83), or SIYYSGSTYYNPSLK (SEQ ID NO:80).

In some embodiments, HC-CDR3 is SPGGYSGGYFQH (SEQ ID NO:77) or DILTGYALDY (SEQ ID NO:81).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	RSSQSLLHSNGYNYLD	(SEQ ID NO:68)
LC-CDR2:	LGSNRDS	(SEQ ID NO:69)
LC-CDR3:	MQGTHWPWT	(SEQ ID NO:70).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated light chain polypeptide, having at least one light chain variable region incorporating the following CDRs:

LC-CDR1:	SGDALPKQFAF	(SEQ ID NO:72)
LC-CDR2:	KDTERPS	(SEQ ID NO:73)
LC-CDR3:	QSPDSSGTVEV	(SEQ ID NO:74).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	GYYWS	(SEQ ID NO:48)
HC-CDR2:	EINHSGSTNYNPSLKS	(SEQ ID NO:49)
HC-CDR3:	SPGGYSGGYFQH	(SEQ ID NO:77).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	GYYWS	(SEQ ID NO:48)
HC-CDR2:	EINHFGSTNYNPSLKS	(SEQ ID NO:83)
HC-CDR3:	SPGGYSGGYFQH	(SEQ ID NO:77).

In some embodiments in accordance with various aspects, the present invention provides an antibody or fragment, or an isolated heavy chain polypeptide, having at least one heavy chain variable region incorporating the following CDRs:

HC-CDR1:	SSSYYWG	(SEQ ID NO:79)
HC-CDR2:	SIYYSGSTYYNPSLK	(SEQ ID NO:80)
HC-CDR3:	DILTGYALDY	(SEQ ID NO:81).

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated which is capable of binding to common γ chain (γc), comprising a light chain and a heavy chain variable region sequence, wherein:

the light chain comprises a LC-CDR1, LC-CDR2, LC-CDR3, having at least 85% overall sequence identity to LC-CDR1: RSSQSLLHSNGYNYLD (SEQ ID NO:68) or SGDALPKQFAF (SEQ ID NO:72); LC-CDR2: LGSNRDS (SEQ ID NO:69) or KDTERPS (SEQ ID NO:73); LC-CDR3: MQGTHWPWT (SEQ ID NO:70) or QSPDSSGTVEV (SEQ ID NO:74); and

the heavy chain comprises a HC-CDR1, HC-CDR2, HC-CDR3, having at least 85% overall sequence identity to HC-CDR1: GYYWS (SEQ ID NO:48) or SSSYYWG (SEQ ID NO:79); HC-CDR2: EINHX_8GSTNYNPSLKS (SEQ ID NO:90) or SIYYSGSTYYNPSLK (SEQ ID NO:80); HC-CDR3: SPGGYSGGYFQH (SEQ ID NO:77) or DILTGYALDY (SEQ ID NO:81);

wherein X_8 = S or F.

In some embodiments the degree of sequence identity may be one of 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to common γ chain (γc), comprising a light chain and a heavy chain variable region sequence, wherein:

the light chain sequence has at least 85% sequence identity to the light chain sequence of one of SEQ ID NOs:67, 152, 71, or 75 (Figure 3), and;

the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence of one of SEQ ID NOs:76, 153, 78, 82 or 84 (Figure 4).

In some embodiments the degree of sequence identity may be one of 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to common γ chain (γc), which is a bispecific antibody or a bispecific antigen binding fragment comprising (i) an antigen binding fragment according to the present invention, and (ii) an antigen binding fragment capable of binding to CD122.

In another aspect, the present invention provides an antibody or antigen binding fragment, optionally isolated, which is capable of binding to common γ chain (γc) and CD122, comprising:

(i) a γc-binding antigen binding fragment according to the present invention; and

(ii) a CD122- binding antigen binding fragment according to the present invention.

In another aspect, the present invention provides an *in vitro* complex, optionally isolated, comprising an antibody or antigen binding fragment, according to the present invention bound to CD122, optionally wherein the antibody or antigen binding fragment is bound to common γ chain (γc).

In another aspect, the present invention provides an *in vitro* complex, optionally isolated, comprising an antibody or antigen binding fragment, according to the present invention bound to common γ chain (γc), optionally wherein the antibody or antigen binding fragment is bound to CD122.

In some embodiments, the antibody or antigen binding fragment according to the present invention is conjugated to a drug moiety or a detectable moiety.

In another aspect, the present invention provides a chimeric antigen receptor (CAR) comprising an antigen binding fragment, optionally a bispecific antigen binding fragment, according to the present invention.

In another aspect, the present invention provides a cell comprising a chimeric antigen receptor (CAR) according to the present invention.

In another aspect, the present invention provides a composition comprising the antibody, antigen binding fragment, conjugate, chimeric antigen receptor (CAR) or cell according to the present invention, and at least one pharmaceutically-acceptable carrier, excipient, adjuvant or diluent.

In another aspect, the present invention provides an isolated nucleic acid encoding the antibody, antigen binding fragment, conjugate, or chimeric antigen receptor (CAR) according to the present invention. The nucleic acid may have a sequence of one of SEQ ID NOs 130, 131, 132, 133, 134, 135, 136, 137, 138, 19, 140, 141, 142, 143, 144, 145, 146 or 147 (Figures 17 and 18), or a coding sequence which is degenerate as a result of the genetic code, or may have a nucleotide sequence having at least 70% identity thereto, optionally one of 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

In another aspect, the present invention provides a vector comprising the nucleic acid according to the present invention.

In another aspect, the present invention provides a host cell comprising the vector according to the present invention. For example, the host cell may be eukaryotic, or mammalian, e.g. Chinese Hamster Ovary (CHO), or human or may be a prokaryotic cell, e.g. *E. coli.*

In another aspect, the present invention provides a method for making an antibody, antigen binding fragment, conjugate, or chimeric antigen receptor (CAR) according to the present invention, comprising culturing the host cell according to the present invention under conditions suitable for the expression of a vector encoding the antibody, antigen binding fragment, conjugate or CAR, and recovering the antibody, antigen binding fragment, polypeptide, conjugate or CAR.

In another aspect, the present invention provides an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention for use in therapy, or in a method of medical treatment.

In another aspect, the present invention provides an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention for use in the treatment of cancer.

In another aspect, the present invention provides an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention for use in the treatment of an infectious disease.

In another aspect, the present invention provides the use of an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention in the manufacture of a medicament for use in the treatment of cancer.

In another aspect, the present invention provides the use of an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention in the manufacture of a medicament for use in the treatment of an infectious disease.

In another aspect, the present invention provides a method of treating cancer comprising administering an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention to a patient suffering from a cancer.

In another aspect, the present invention provides a method of treating an infectious disease comprising administering an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention to a patient suffering from an infectious disease.

In another aspect, the present invention provides a method comprising contacting, preferably *in vitro*, a sample containing, or suspected to contain, CD122 and/or common γ chain (γc) with an antibody, antigen binding fragment, polypeptide, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention and detecting the formation of a complex of the antibody, antigen binding fragment, conjugate, CAR or cell with CD122 and/or γc.

In another aspect, the present invention provides a method of diagnosing a disease or condition in a subject, the method comprising contacting, preferably *in vitro*, a sample from the subject with an antibody, antigen binding fragment, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention and detecting the formation of a complex of the antibody, antigen binding fragment, conjugate, CAR or cell with CD122 and/or common γ chain (γc).

In another aspect, the present invention provides a method of selecting or stratifying a subject for treatment with an CD122 and/or common γ chain (γc)-targeted agent, the method comprising contacting, preferably *in vitro*, a sample from the subject with an antibody, antigen binding fragment, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention and detecting the formation of a complex of the antibody, antigen binding fragment, conjugate, CAR or cell with CD122 and/or γc.

In another aspect, the present invention provides the use of an antibody, antigen binding fragment, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention for the detection of CD122 and/or common γ chain (γc) *in vitro* or *in vivo*.

In another aspect, the present invention provides the use of an antibody, antigen binding fragment, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention as an *in vitro* or *in vivo* diagnostic or prognostic agent.

In another aspect, the present invention provides a method for expanding a population of T cells and/or NK cells, wherein T cells and/or NK cells are contacted *in vitro*, *in vivo* or *ex vivo* with an antibody, antigen binding fragment, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention.

In another aspect, the present invention provides a method of treating an infectious disease or a cancer in a subject, the method comprising culturing T cells and/or NK cells obtained from a blood sample from a subject in the presence of an antibody, antigen binding fragment, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention so as to expand a T cell and/or NK cell population, collecting expanded T cells and/or NK cells, and administering the expanded T cells and/or NK cells to a subject in need of treatment.

In another aspect, the present invention provides a method of treating an infectious disease or a cancer in a subject, the method comprising administering an antibody, antigen binding fragment, conjugate, chimeric antigen receptor (CAR), cell or composition according to the present invention to the subject so as to expand a T cell and/or NK cell population.

The following numbered paragraphs (paras) describe further aspects of the present invention:

1. An isolated IL-2R bispecific antigen binding protein comprising:
(i) a first IL-2Rβ binding polypeptide:
(a) a binding unit VL1 consisting of an amino acid sequence of SEQ ID NO:1, 5, 9, or 13, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL1;
(b) a binding unit VH1 consisting of an amino acid sequence of SEQ ID NO:35, 39, 43, or 47, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH1;
(ii) a second IL-2Rγ binding polypeptide:
(c) a binding unit VL2 consisting of an amino acid sequence of SEQ ID NO:67 or 71, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL2, and
(d) a binding unit VH2 consisting of an amino acid sequence of SEQ ID NO:76 or 78, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH2.

2. The isolated IL-2R bispecific antigen binding protein as defined in para 1, wherein the said first IL-2Rβ binding polypeptide and said second IL-2Rγ binding polypeptide are linked by a peptide linker.

3. The isolated IL-2R bispecific antigen binding protein as defined in para 2, wherein the said peptide linker is from 5-23 amino acids in length.

4. The isolated IL-2R bispecific antigen binding protein as defined in para 1, wherein said first IL-2Rβ binding polypeptide further comprises an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:92, and wherein said second IL-2Rγ binding polypeptide further comprises an

Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:94.

5. The isolated IL-2R bispecific antigen binding protein as defined in para 4, wherein said first and second polypeptides meet at an engineered interface within the CH3 domains, wherein the first polypeptide comprises at least one engineered protuberance in said interface, said protuberance comprising at least one altered contact residue and the second polypeptide comprises at least one engineered cavity in its said interface, said cavity comprising at least one altered contact residue so as to form a protuberance-into-cavity pairing.

6. The isolated IL-2R bispecific antigen binding protein as defined in para 4 or para 5, wherein the said binding units are linked to said Fc portion by a linker.

7. The isolated IL-2R bispecific antigen binding protein as defined in para 6, wherein said linker is from 5-23 amino acids in length.

8. The isolated, IL-2R bispecific antigen binding protein as defined in any one of paras 4 to 7 comprising:

(i) a first IL-2Rβ binding polypeptide:

(a) a binding unit VL1 consisting of an amino acid sequence of SEQ ID NO:1, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL1;

(b) a binding unit VH1 consisting of an amino acid sequence of SEQ ID NO:35, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH1;

(c) an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:92, and

(ii) a second IL-2Rγ binding polypeptide:

(d) a binding unit VL2 consisting of an amino acid sequence of SEQ ID NO: 67, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL2;

(e) a binding unit VH2 consisting of an amino acid sequence of SEQ ID NO: 76, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH2, and

(f) an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:94.

9. The isolated IL-2R bispecific antigen binding protein as defined in para 8, wherein said binding protein has a dissociation constant (K_D) to human IL-2Rβ of 1.46 x 10^{-7}M and a dissociation constant (K_D) to human IL-2Rγ of 2.09 x 10^{-8}M.

10. The isolated, IL-2R bispecific antigen binding protein as defined in any one of paras 4 to 7 comprising:

(i) a first IL-2Rβ binding polypeptide:

(a) a binding unit VL1 consisting of an amino acid sequence of SEQ ID NO:5, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL1;

(b) a binding unit VH1 consisting of an amino acid sequence of SEQ ID NO:39, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH1;

(c) an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:92, and

(ii) a second IL-2Rγ binding polypeptide:

(d) a binding unit VL2 consisting of an amino acid sequence of SEQ ID NO:71, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL2;

(e) a binding unit VH2 consisting of an amino acid sequence of SEQ ID NO:78, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH2, and

(f) an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:94.

11. The isolated IL-2R bispecific antigen binding protein defined in para 10, wherein said binding protein has a dissociation constant (K_D) to human IL-2Rβ of 1.01 x 10^{-7}M and a dissociation constant (K_D) to human IL-2Rγ of 7.98 x 10^{-8}M.

12. The isolated, IL-2R bispecific antigen binding protein as defined in any one of paras 4 to 7 comprising:

(i) a first IL-2Rβ binding polypeptide:

(a) a binding unit VL1 consisting of an amino acid sequence of SEQ ID NO: 9, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL1;

(b) a binding unit VH1 consisting of an amino acid sequence of SEQ ID NO: 43, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH1 ;

(c) an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID N0:92, and

(ii) a second IL-2Rγ binding polypeptide:

(d) a binding unit VL2 consisting of an amino acid sequence of SEQ ID NO:67, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL2;

(e) a binding unit VH2 consisting of an amino acid sequence of SEQ ID NO:76, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH2, and

(f) an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:94.

13. The isolated IL-2R bispecific antigen binding protein defined in para 12, wherein said binding protein has a dissociation constant (K_D) to human IL-2Rβ of 1.81×10^{-7} M and a dissociation constant (K_D) to human IL-2Rγ of 7.87×10^{-8} M

14. The isolated, IL-2R bispecific antigen binding protein as defined in any one of paras 4 to 7 comprising:

(i) a first IL-2Rβ binding polypeptide:

(a) a binding unit VL1 consisting of an amino acid sequence of SEQ ID NO:13, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL1;

(b) a binding unit VH1 consisting of an amino acid sequence of SEQ ID NO:47, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH 1;

(c) an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:92, and

(ii) a second IL-2Rγ binding polypeptide:

(d) a binding unit VL2 consisting of an amino acid sequence of SEQ ID NO:67, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VL2;

(e) a binding unit VH2 consisting of an amino acid sequence of SEQ ID NO:76, or a variant thereof which contains at least one amino acid substitution, insertion or deletion in the binding unit VH2, and

(f) an Fc portion comprising a CH3 domain, in which the CH3 domain comprises or consists of an amino acid sequence of SEQ ID NO:94.

15. The isolated IL-2R bispecific antigen binding protein as defined in para 14, wherein said binding protein has a dissociation constant (K_D) to human IL-2Rβ of 1.28×10^{-7} M and a dissociation constant (K_D) to human IL-2Rγ of 3.37×10^{-7} M.

16. The isolated IL-2R bispecific antigen binding protein as defined in any one of paras 1 to 15, wherein the IL-2R is human or simian IL-2R.

17. The isolated IL-2R bispecific antigen binding protein as defined in any one of paras 1 to 16, wherein said antigen binding protein is fully human.

18. The isolated IL-2R bispecific antigen binding protein as defined in any one of paras 1 to 17, wherein said binding protein further comprises an agent selected from the group consisting of an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent.

19. The isolated IL-2R bispecific antigen binding protein of para 18, wherein said agent is an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

20. The isolated IL-2R bispecific antigen binding protein of para 18, wherein said agent is a therapeutic or cytotoxic agent selected from the group consisting of an antimetabolite, an alkylating agent, an antibiotic, an antiviral agent, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin and an apoptotic agent.

21. The isolated IL-2R bispecific antigen binding protein as defined in any one of paras 1 to 20, wherein said binding protein is an agonist of IL-2R.

22. A method of treating an infectious disease or cancer in a subject, comprising administering the isolated antigen binding protein as defined in any one of paras 1 to 21 to a subject in need of such treatment.

23. The method as defined in para 22, wherein the cancer is melanoma, renal carcinoma cancer or bladder cancer.

24. Use of the isolated antigen binding protein as defined in any one of paras 1 to 21, in the manufacture of a medicament for treating an infectious disease or cancer.

25. The use as defined in para 24, wherein the cancer is melanoma, renal carcinoma cancer or bladder cancer.

26. A composition comprising the isolated IL-2R bispecific antigen binding protein as defined in any one of paras 1 to 21 and a pharmaceutically acceptable carrier.

27. The composition of para 26, further comprising one or more therapeutic agents.

28. The composition of para 27, wherein said one or more therapeutic agents are selected from antibiotic agents, antiviral agents, antifungal agents, chemotherapeutic agents, small molecule inhibitors, immunotherapy agents, vaccines, adoptive cell therapy agents, immune checkpoint inhibitors or antibody therapeutics.

29. An isolated cell line that is capable of producing the isolated IL-2R bispecific antigen binding protein as defined in any one of paras 1 to 21.

30. A kit comprising the isolated IL-2R bispecific antigen binding protein as defined in any one of paras 1 to 21, together with instructions for use.

Description

The present invention encompasses the nucleotide and amino acid sequences of a bispecific antibody with specificity for interleukin-2 receptor (IL-2R) chains β and γ, able to stimulate cells expressing the medium affinity IL-2R β-γ and not preferentially cells expressing the high affinity IL-2R α-β-γ.

This disclosure describes the design of an IL-2Rα-independent IL-2R agonist in which receptor activation is achieved through heterodimerization of the β-γ_c components by bispecific antibodies (or bi-functional proteins) possessing anti-IL-2Rβ (CD122) and anti-γ_c (CD132) specificities. Use of such molecules can convey desired immune stimulation by activation of immune cells, particularly the T cells, without preferential and high activation of cells expressing the high affinity α-β-γ_c IL-2R. Such molecules would be useful as vaccine adjuvants.

Isolated nucleotide and amino-acid sequences for two fully human monoclonal antibodies binding to IL-2Rβ (CD122) or IL-2Rγ_c (CD132) are described.

Nucleotide and amino-acid sequences of the engineered antibody showing monovalent specificity for IL-2Rβ and IL-2Rγ_c are described.

Bispecific antibody construct containing variable domains derived from the aforementioned isolated monoclonal antibodies and able to bind both IL-2Rβ and IL-2Rγ_c are described.

Bifunctional protein containing IL-2Rβ and IL-2Rγ_c binding domains derived from the sequences of the aforementioned isolated monoclonal antibodies are described.

An antibody or a bifunctional protein binding to the IL-2Rβ/γ_c and triggering phosphorylation of STAT5 and/or Akt is described.

An antibody or a bifunctional protein binding to the IL-2Rβ/γ_c and not binding to the IL-2Rα/β/γ_c (CD25) with higher affinity is described.

An antibody or a bifunctional protein agonistically binding to the IL-2Rβ/γ_c and triggering intracellular signalling, and antagonistically binding to the IL-2Rα/β/γ_c (CD25) without triggering intracellular signalling is described.

The use of any of these molecules in the treatment of cancers or infectious diseases, alone or in combination with anti-cancer or anti-infectious drugs is described.

Antibodies

Antibodies according to the present invention preferably bind to CD122 (interleukin 2 receptor β (IL-2Rβ)) and/or common γ chain (γc). In some embodiments, the antibody/fragment binds to human CD122 and/or human γc. In some embodiments, the antibody/fragment binds to non-human primate CD122 and/or non-human primate γc.

By "antibody" we include a fragment or derivative thereof, or a synthetic antibody or synthetic antibody fragment. Antibodies according to the present invention may be provided in isolated form.

In view of today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The antigen-binding portion may be a part of an antibody (for example a Fab fragment) or a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]). Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques ", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications ", J G R Hurrell (CRC Press, 1982). Chimeric antibodies are discussed by Neuberger et al (1988, 8th International Biotechnology Symposium Part 2, 792-799).

Monoclonal antibodies (mAbs) are useful in the methods of the invention and are a homogenous population of antibodies specifically targeting a single epitope on an antigen.

Antigen binding fragments of antibodies, such as Fab and Fab_2 fragments may also be used/provided as can genetically engineered antibodies and antibody fragments. The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parent antibody (Morrison et al (1984) Proc. Natl. Acad. Sd. USA 81, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better et al (1988) Science 240, 1041); Fv molecules (Skerra

et al (1988) Science 240, 1038); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird et al (1988) Science 242, 423; Huston et al (1988) Proc. Natl. Acad. Sd. USA 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward et al (1989) Nature 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349, 293- 299.

By "ScFv molecules" we mean molecules wherein the V_H and V_L partner domains are covalently linked, e.g. by a flexible oligopeptide.

Fab, Fv, ScFv and dAb antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and $F(ab')_2$ fragments are "bivalent". By "bivalent" we mean that the said antibodies and $F(ab')_2$ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv and dAb fragments are monovalent, having only one antigen combining site.

The present invention provides an antibody or antigen binding fragment which is capable of binding to CD122 and γc. In some embodiments, the antibody/fragment is a bispecific antibody or a bispecific antigen binding fragment. In some embodiments, the bispecific antibody or bispecific antigen binding fragment may be isolated.

In some embodiments, the bispecific antibodies and bispecific antigen binding fragments comprise an antibody/fragment which is capable of binding to CD122, e.g. an antibody/fragment as described herein.

In some embodiments, the bispecific antibodies and bispecific antigen binding fragments comprise an antibody/fragment which is capable of binding to γc, e.g. an antibody/fragment as described herein.

In some embodiments, the bispecific antibodies/fragments comprise an antibody/fragment capable of binding to CD122, and an antibody/fragment capable of binding to another target protein.

In some embodiments, the bispecific antibodies/fragments comprise an antibody/fragment capable of binding to γc, and an antibody/fragment capable of binding to another target protein.

The antigen binding fragment capable of binding to another target protein may be capable of binding to another protein other than CD122 or γc.

In one aspect of the present invention a bispecific antibody is provided, which binds γc but does not bind to CD122.

An antigen-binding fragment of a bispecific antibody/fragment according to the present invention may be any fragment of a polypeptide which is capable of binding to an antigen.

In some embodiments, an antigen binding fragment comprises at least the three light chain complementarity determining regions (CDRs) (i.e. LC-CDR1, LC-CDR2 and LC-CDR3) and three heavy chain CDRs (i.e. HC-CDR1, HC-CDR2 and HC-CDR3) which together define the antigen binding region of an antibody or antigen binding fragment. In some embodiments, an antigen binding fragment may comprise the light chain variable domain and heavy chain variable domain of an antibody or antigen binding fragment. In some embodiments, an antigen binding fragment may comprise the light chain polypeptide and heavy chain polypeptide of an antibody or antigen binding fragment.

Bispecific antibodies and fragments according to the invention may be provided in any suitable format, such as those formats described in Kontermann MAbs 2012, 4(2): 182-197, which is hereby incorporated by reference in its entirety. For example, a bispecific antibody or bispecific antigen binding fragment may be a bispecific antibody conjugate (e.g. an IgG2, $F(ab')_2$ or CovX-Body), a bispecific IgG or IgG-like molecule (e.g. an IgG, $scFv_4$-Ig, IgG-scFv, scFv-IgG, DVD-Ig, IgG-sVD, sVD-IgG, 2 in 1-IgG, mAb^2, or Tandemab common LC), an asymmetric bispecific IgG or IgG-like molecule (e.g. a kih IgG, kih IgG common LC, CrossMab, kih IgG-scFab, mAb-Fv, charge pair or SEED-body), a small bispecific antibody molecule (e.g. a Diabody (Db), dsDb, DART, scDb, tandAbs, tandem scFv (taFv), tandem dAb/VHH, triple body, triple head, Fab-scFv, or $F(ab')_2$-$scFv_2$), a bispecific Fc and C_H3 fusion protein (e.g. a taFv-Fc, Di-diabody, scDb-C_H3, scFv-Fc-scFv, HCAb-VHH, scFv-kih-Fc, or scFv-kih-C_H3), or a bispecific fusion protein (e.g. a $scFv_2$-albumin, scDb-albumin, taFv-toxin, DNL-Fab_3, DNL-Fab_4-IgG, DNL-Fab_4-IgG-$cytokine_2$). See in particular Figure 2 of Kontermann MAbs 2012, 4(2): 182-19.

In some embodiments an scFv dimer format is preferred in which two scFv, each exhibiting specific binding for a different antigen, are connected by a linker, e.g. as illustrated in Figure 25A, right.

A linker may be an amino acid sequence of any desired length, e.g. one of 2 to 50 amino acids, 5 to 50 amino acids, 5 to 40 amino acids, 5 to 30 amino acids, 5 to 20 amino acids, or 5 to 10 amino acids.

The skilled person is able to design and prepare bispecific antibodies and bispecific antigen binding fragments according to the present invention. Methods for producing bispecific antibodies include chemically crosslinking of antibodies or antibody fragments, e.g. with reducible disulphide or non-reducible thioether bonds, for example as described in Segal and Bast, 2001. Production of Bispecific Antibodies. Current Protocols in Immunology. 14:IV:2.13:2.13.1–2.13.16, which is hereby incorporated by reference in its entirety. For example, *N*-succinimidyl-3-(-2-pyridyldithio)-propionate (SPDP) can be used to chemically crosslink e.g. Fab fragments via hinge region SH- groups, to create disulfide-linked bispecific $F(ab)_2$ heterodimers.

Other methods for producing bispecific antibodies include fusing antibody-producing hybridomas e.g. with polyethylene glycol, to produce a quadroma cell capable of secreting bispecific antibody, for example as described in D. M. and Bast, B. J. 2001. Production of Bispecific Antibodies. Current Protocols in Immunology. 14:IV:2.13:2.13.1–2.13.16.

Bispecific antibodies and bispecific antigen binding fragments according to the present invention can also be produced recombinantly, by expression from e.g. a nucleic acid construct encoding polypeptides for the antigen binding molecules, for example as described in Antibody Engineering: Methods and Protocols, Second Edition (Humana Press, 2012), at Chapter 40: Production of Bispecific Antibodies: Diabodies and Tandem scFv (Hornig and Färber-Schwarz), or French, How to make bispecific antibodies, Methods Mol. Med. 2000; 40:333-339, the entire contents of both of which are hereby incorporated by reference.

For example, a DNA construct encoding the light and heavy chain variable domains for the two antigen binding fragments (i.e. the light and heavy chain variable domains for the

antigen binding fragment capable of binding CD122 or γc, and the light and heavy chain variable domains for the antigen binding fragment capable of binding to another target protein), and including sequences encoding a suitable linker or dimerization domain between the antigen binding fragments can be prepared by molecular cloning techniques. Recombinant bispecific antibody can thereafter be produced by expression (e.g. *in vitro*) of the construct in a suitable host cell (e.g. a mammalian host cell), and expressed recombinant bispecific antibody can then optionally be purified.

Antibodies may be produced by a process of affinity maturation in which a modified antibody is generated that has an improvement in the affinity of the antibody for antigen, compared to an unmodified parent antibody. Affinity-matured antibodies may be produced by procedures known in the art, e.g., Marks *et al.,Rio/Technology* 10:779-783 (1992); Barbas *et al. Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al. Gene* 169:147-155 (1995); Yelton *et al. J. Immunol.* 155:1994-2004 (1995); Jackson *et al., J. Immunol.* 154(7):331 0-15 9 (1995); and Hawkins *et al, J. Mol. Biol.* 226:889-896 (1992).

Antibodies according to the present invention may exhibit specific binding to CD122, and/or γc.

An antibody that specifically binds to a target molecule preferably binds the target with greater affinity, and/or with greater duration than it binds to other targets. In some embodiments the present antibodies may bind with greater affinity to CD122 and/or γc than to one or more members of the type I cytokine receptor family. In some embodiments, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, e.g., by ELISA, SPR, Bio-Layer Interferometry or by a radioimmunoassay (RIA). Alternatively, the binding specificity may be reflected in terms of binding affinity where the anti-CD122 and/or γc antibody of the present invention binds to CD122 and/or γc with a K_D that is at least 0.1 order of magnitude (i.e. 0.1 x 10^n, where n is an integer representing the order of magnitude) greater than the K_D of the antibody towards another target molecule. This may optionally be one of at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, or 2.0.

Binding affinity of an antibody for its target is often described in terms of its dissociation constant (K_D). Binding affinity can be measured by methods known in the art, such as by ELISA, Surface Plasmon Resonance (SPR; see e.g. Hearty et al., Methods Mol Biol (2012) 907:411-442), Bio-Layer Interferometry (see e.g. Lad et al., (2015) J Biomol

Screen 20(4): 498-507), or by a radiolabeled antigen binding assay (RIA) performed with the Fab version of the antibody and antigen molecule.

In some embodiments, the antibody according to the present invention has a dissociation constant (K_D) for CD122 of one of ≤1 x 10^{-6} M, ≤7.5 x 10^{-7} M, ≤5 x 10^{-7} M, ≤4.5 x 10^{-7} M, ≤4 x 10^{-7} M, ≤5 x 10^{-7} M, ≤4.5 x 10^{-7} M, ≤3 x 10^{-7} M, ≤3.5 x 10^{-7} M, ≤3 x 10^{-7} M, ≤2.5 x 10^{-7} M, ≤2 x 10^{-7} M, ≤1.9 x 10^{-7} M, ≤1.8 x 10^{-7} M, ≤1.7 x 10^{-7} M, ≤1.6 x 10^{-7} M, ≤1.5 x 10^{-7} M, ≤1.4 x 10^{-7} M, ≤1.3 x 10^{-7} M, ≤1.2 x 10^{-7} M, ≤1.1 x 10^{-7} M, ≤1 x 10^{-7} M, ≤8 x 10^{-8} M, ≤6 x 10^{-8} M, ≤4 x 10^{-8} M, or ≤2 x 10^{-8} M.

In some embodiments, the antibody according to the present invention has a K_D for γc of one of ≤10 x 10^{-7} M, ≤7.5 x 10^{-7} M, ≤5 x 10^{-7} M, ≤2.5 x 10^{-7} M, ≤1 x 10^{-7} M, ≤9.5 x 10^{-8} M, ≤9 x 10^{-8} M, ≤8.5 x 10^{-8} M, ≤8 x 10^{-8} M, ≤7.5 x 10^{-8} M, ≤7 x 10^{-8} M, ≤6.5 x 10^{-8} M, ≤6 x 10^{-8} M, ≤5.5 x 10^{-8} M, ≤5 x 10^{-8} M, ≤4.5 x 10^{-8} M, ≤4 x 10^{-8} M, ≤3.5 x 10^{-8} M, ≤3 x 10^{-8} M, ≤2.5 x 10^{-8} M, ≤2 x 10^{-8} M, ≤1.5 x 10^{-8} M, ≤1 x 10^{-8} M, ≤8 x 10^{-9} M, ≤6 x 10^{-9} M, ≤4 x 10^{-9} M or ≤2 x 10^{-9} M.

In some embodiments, the antibody according to the present invention has a K_D for CD122 of one of ≤1 x 10^{-6} M, ≤7.5 x 10^{-7} M, ≤5 x 10^{-7} M, ≤4.5 x 10^{-7} M, ≤4 x 10^{-7} M, ≤5 x 10^{-7} M, ≤4.5 x 10^{-7} M, ≤3 x 10^{-7} M, ≤3.5 x 10^{-7} M, ≤3 x 10^{-7} M, ≤2.5 x 10^{-7} M, ≤2 x 10^{-7} M, ≤1.9 x 10^{-7} M, ≤1.8 x 10^{-7} M, ≤1.7 x 10^{-7} M, ≤1.6 x 10^{-7} M, ≤1.5 x 10^{-7} M, ≤1.4 x 10^{-7} M, ≤1.3 x 10^{-7} M, ≤1.2 x 10^{-7} M, ≤1.1 x 10^{-7} M, ≤1 x 10^{-7} M, ≤8 x 10^{-8} M, ≤6 x 10^{-8} M, ≤4 x 10^{-8} M, or ≤2 x 10^{-8} M and a K_D for γc of one of ≤10 x 10^{-7} M, ≤7.5 x 10^{-7} M, ≤5 x 10^{-7} M, ≤2.5 x 10^{-7} M, ≤1 x 10^{-7} M, ≤9.5 x 10^{-8} M, ≤9 x 10^{-8} M, ≤8.5 x 10^{-8} M, ≤8 x 10^{-8} M, ≤7.5 x 10^{-8} M, ≤7 x 10^{-8} M, ≤6.5 x 10^{-8} M, ≤6 x 10^{-8} M, ≤5.5 x 10^{-8} M, ≤5 x 10^{-8} M, ≤4.5 x 10^{-8} M, ≤4 x 10^{-8} M, ≤3.5 x 10^{-8} M, ≤3 x 10^{-8} M, ≤2.5 x 10^{-8} M, ≤2 x 10^{-8} M, ≤1.5 x 10^{-8} M, ≤1 x 10^{-8} M, ≤8 x 10^{-9} M, ≤6 x 10^{-9} M, ≤4 x 10^{-9} M or ≤2 x 10^{-9} M.

In some embodiments, the antibody according to the present invention binds to CD122 and/or γc expressed at the cell surface of a cell expressing CD122 and/or γc. Such binding can be analysed by analysis of binding of the antibody to PBMCs incubated with the antibody, or cells transfected with constructs expressing CD122 and/or γc, and subsequent analysis of antibody binding, e.g. by flow cytometry.

In some embodiments, the antibody according to the present invention is an agonist of one or more signalling pathways which are activated by signal transduction through

receptors comprising CD122 and/or γc, e.g. IL-2 receptor and/or IL-15 receptor. In some embodiments, the antibody is capable of stimulating signalling through one or more immune receptor complexes comprising CD122 and/or γc, e.g. IL-2 receptor and/or IL-15 receptor.

In some embodiments, the antibody is an IL-2 receptor agonist. Accordingly in some embodiments, the antibody is capable of activating IL-2/IL-2 receptor-mediated signalling and associated functions. For example, the in some embodiments, the antibody is capable of promoting cell division/proliferation/survival of a cell expressing the IL-2 receptor.

In some embodiments, the antibody is an IL-15 receptor agonist. Accordingly in some embodiments, the antibody is capable of activating IL-15/IL-15 receptor-mediated signalling and associated functions. For example, the in some embodiments, the antibody is capable of promoting cell division/proliferation/survival of a cell expressing the IL-15 receptor.

In some embodiments bispecific antibodies according to the present invention may preferentially bind (e.g. with greater affinity and/or specificity) receptors comprising or consisting of CD122 and γc compared to receptors that further comprise CD25. Bispecific antibodies may preferentially stimulate receptors (or cells expressing receptors) comprising or consisting of CD122 and γc, but not further comprising CD25.

In some embodiments bispecific antibodies according to the present invention that bind CD122 and γc preferably do not exhibit significant inhibition of ligand binding to receptors having the common gamma chain and not containing CD122, e.g. receptors other than IL-2R and IL-15R. For example, bispecific antibodies according to the present invention may be specific for receptors comprising both CD122 and γc over receptors that comprise γc but do not comprise CD122.

In some embodiments, the antibody according to the present invention is capable of stimulating CD122:γc mediated signalling through one of more of the following intracellular signalling pathways: STAT5, Akt, ERK. Signalling through STAT5, Akt, ERK intracellular signalling pathways can be analysed by methods well known to the skilled person, such as e.g. by detection of phosphorylated STAT5, phosphorylated Akt and

optionally phosphorylated ERK, respectively following stimulation of cells expressing CD122 and/or γc with the antibody.

In some embodiments, the antibody according to the present invention is a less potent stimulator of intracellular signalling in Treg cells than IL-2. For example, treatment with the antibody may result in less phosphorylation of STAT5 as compared to treatment with IL-2.

In some embodiments, the antibody is capable of stimulating proliferation of immune cells *in vitro* and or *in vivo*. Stimulation of proliferation results in an increase in the number of the cell type whose proliferation is stimulated, effective to achieve expansion of the cell population. For example, the antibody is useful in stimulating proliferation and/or expansion of leukocytes *in vitro* or *in vivo*, preferably exhibiting reduced toxicity when compared to the effects of a corresponding amount of IL-2.

In some embodiments, the antibody is capable of stimulating proliferation of IL-2 dependent cells. In some embodiments, the antibody is capable of stimulating proliferation of CD3+ T cells (e.g. CD4+ T cells, CD8+ T cells) and/or NK cells.

Whether an antibody is capable of stimulating proliferation of cells can be analysed *in vitro* by methods well known in the art, such as by analysis of the number of a given cell type before and after stimulation, and/or by another assay, such as thymidine incorporation, CFSE dilution (e.g. as described in Anthony et al., 2012 Cells 1:127-140), AlamarBlue signal etc.

In some embodiments, the antibody according to the present invention is capable of binding to non-human primate (e.g. cynomolgous macaque) CD122 and/or γc. The antibody may be cross-reactive for human and non-human primate CD122 and/or γc. Accordingly, in some embodiments, the antibody is capable of stimulating IL-2/IL-2 receptor- and/or IL-15/IL-15 receptor-mediated mediated signalling in cells expressing non-human primate (e.g. cynomolgous macaque) CD122 and/or γc.

In some embodiments, the antibody of the present invention is capable of stimulating proliferation of CD3+ T cells. In some embodiments, the antibody of the present invention is capable of stimulating proliferation of CD4+ T cells. In some embodiments, the antibody of the present invention is capable of stimulating proliferation of CD8+ T cells.

In some embodiments, the antibody of the present invention is capable of increasing the ratio of CD8+ T cells to CD4+ T cells. That is, in some embodiments, following stimulation (e.g. *in vitro* or *in vivo*) of a population of cells comprising CD8+ T cells and CD4+ T cells, the ratio of CD8+ T cells to CD4+ T cells may be higher following stimulation as compared to before stimulation.

In some embodiments, the antibody of the present invention is not a potent stimulator of proliferation of CD4+FoxP3+ cells (i.e. Tregs). In some embodiments, the antibody does not stimulate proliferation of Tregs.

In some embodiments, the antibody of the invention stimulates proliferation of Tregs to a lesser extent than IL-2. That is, in some embodiments, the antibody of the invention is a less potent agonist of proliferation of Tregs than IL-2. In some embodiments, the antibody of the invention stimulates proliferation of Tregs to an extent which is ≤1 times, ≤0.8 times, ≤0.6 times, ≤0.4 times, ≤0.2 times, ≤0.1 times the level of proliferation of Tregs stimulated by treatment with IL-2 at a comparable concentration, in a given assay.

In some embodiments, the antibody of the present invention preferentially stimulates proliferation of the effector memory subset of CD8+ T cells over the central memory CD8+ T cell and naïve CD8+ T cell populations.

In some embodiments, the antibody of the present invention stimulates proliferation of effector memory CD8+ T cells to a greater extent than IL-2 at a comparable concentration, in a given assay.

In some embodiments, the antibody of the present invention is capable of stimulating proliferation of antigen-specific T cells, e.g. antigen-specific CD8+ T cells and/or antigen-specific CD4+ T cells. Stimulation may occur *in vitro* or *in vivo*. In some embodiments, the antibody of the present invention is capable of stimulating proliferation of antigen-specific CD8+ cytotoxic T lymphocytes (CTLs). Ability of cells to stimulate proliferation of antigen-specific T cells can be evaluated e.g. by analysis of proliferation of T cells incubated with cells presenting an antigen of interest in the presence of the antibody, e.g. as described in Examples 8 and 11 herein.

In some embodiments, the antibody of the present invention is capable of stimulating proliferation of antigen-specific T cells to a greater extent than IL-2 at a comparable concentration, in a given assay.

In some embodiments, the antibody of the present invention is capable of stimulating proliferation of antigen-specific CD8+ T cells to a greater extent than IL-2 at a comparable concentration, in a given assay.

In some embodiments, the antibody of the present invention is capable of increasing the ratio of antigen-specific CD8+ T cells to CD4+ T cells. This may occur *in vitro* or *in vivo*. That is, in some embodiments, following stimulation (e.g. *in vitro*) of a population of cells comprising antigen-specific CD8+ T cells and CD4+ T cells, the ratio of CD8+ T cells to CD4+ T cells is higher following stimulation as compared to before stimulation. In some embodiments, the antibody of the invention is capable of increasing the ratio of antigen-specific CD8+ T cells to CD4+ T cells to a greater extent than IL-2 at a comparable concentration, in a given assay.

In some embodiments, the antibody of the present invention is capable of stimulating proliferation of CD8+ PD1+ T cells (e.g. antigen-specific CD8+PD1+ T cells). In some embodiments, the antibody of the present invention is capable of stimulating proliferation of CD8+ PD1+ T cells to a greater extent than IL-2 at a comparable concentration, in a given assay.

In some embodiments, the antibody of the present invention is capable of reducing the proportion of Tregs as a proportion of CD4+ T cells. That is, in some embodiments, following treatment of a population of CD4+ T cells with the antibody, the percentage of the CD4+ T cells which are Tregs is reduced. In some embodiments, the antibody of the present invention is capable of reducing the proportion of Tregs as a proportion of CD4+ T cells to a greater extent than IL-2 at a comparable concentration, in a given assay.

In some embodiments, the antibody of the present invention is capable of stimulating CTL cytotoxicity. Ability of an antibody to stimulate CTL cytotoxicity can be measured by methods known to the skilled person. Cytotoxicity of a T cell to a given target cell can be investigated, for example, using any of the methods reviewed in Zaritskaya et al. Expert Rev Vaccines (2011), 9(6):601-616, hereby incorporated by reference in its entirety.

In some embodiments, the antibody is capable of stimulating the proliferation of immune cells *in vivo*. In some embodiments, the antibody is capable of stimulating the proliferation of CD3+ cells *in vivo*. In some embodiments, the antibody is capable of stimulating the proliferation of CD8+ T cells *in vivo*. In some embodiments, the antibody is capable of stimulating the proliferation of CD4+ T cells *in vivo*. In some embodiments, the antibody is capable of stimulating the proliferation of NK cells *in vivo*.

In aspects and embodiments of the present invention stimulation or expansion of cells having desired characteristics may occur *in vitro* or *in vivo*. *In vitro* stimulation or expansion may provide a population of cells enriched for desired characteristics that may be collected and used for a desired purpose, which may include administration to a subject. *In vivo* stimulation or expansion may enrich for a population of cells that are beneficial to the subject, e.g. in treating or preventing a disease. *In vivo* stimulation or expansion may have an adjuvant effect or action.

In some aspects, the antibody of the present invention comprises the antibody/fragment of a CD122-binding antibody clone. In some aspects, the antibody comprises the antibody/fragment of clone P2C4 or a variant of P2C4. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_A4 or a variant of P2C4_A4. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_B1 or a variant of P2C4_B1. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_B5 or a variant of P2C4_B5. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_C1 or a variant of P2C4_C1. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_C4 or a variant of P2C4_C4. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_C7 or a variant of P2C4_C7. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_D10 or a variant of P2C4_D10. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_E6 or a variant of P2C4_E6. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_E7 or a variant of P2C4_E7. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_F8 or a variant of P2C4_F8. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_C1D10 or a variant of P2C4_C1D10. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_FW2 or a variant of P2C4_FW2. In some aspects, the antibody comprises the antibody/fragment of clone P2H7 or a variant of P2H7. In some aspects, the antibody comprises the antibody/fragment of clone P2D12 or a variant of P2D12. In some aspects, the antibody comprises the antibody/fragment of

clone P1G11 or a variant of P1G11. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_A9 or a variant of P2C4_A9. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_B6 or a variant of P2C4_B6. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_E9 or a variant of P2C4_E9. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_B8 or a variant of P2C4_B8. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_B12 or a variant of P2C4_B12. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_C12 or a variant of P2C4_C12. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_E2 or a variant of P2C4_E2. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_E3 or a variant of P2C4_E3. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_E8 or a variant of P2C4_E8. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_F11 or a variant of P2C4_F11. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_G2 or a variant of P2C4_G2. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_G11 or a variant of P2C4_G11. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_H1 or a variant of P2C4_H1. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_H2 or a variant of P2C4_H2. In some aspects, the antibody comprises the antibody/fragment of clone P2C4_H3 or a variant of P2C4_H3.

The amino acid sequences of the VL domains of the CD122-binding antibody clones of the preceding paragraph are shown in Figure 1, as are the CDRs defined according to the Kabat system. The amino acid sequences of the VH domains of the CD122-binding antibody clones of the preceding paragraph are shown in Figure 2, as are the CDRs defined according to the Kabat system. The full amino acid sequences of the antibody constructs (including linkers) are shown in Figure 15, and the encoding nucleotide sequences are shown in Figure 17.

Antibodies according to the present invention may comprise the CDRs of P2C4, P2C4_A4, P2C4_B1, P2C4_B5, P2C4_C1, P2C4_C4, P2C4_C7, 2C4_D10, P2C4_E6, P2C4_E7, P2C4_F8, P2C4_C1D10, P2C4_FW2, P2H7, P2D12, P1G11, P2C4_A9, P2C4_B6, P2C4_E9, P2C4_B8, P2C4_B12, P2C4_C12, P2C4_E2, P2C4_E3, P2C4_E8, P2C4_F11, P2C4_G2, P2C4_G11, P2C4_H1, P2C4_H2, or P2C4_H3. In an antibody according to the present invention one or two or three or four of the six CDR sequences

may vary. A variant may have one or two amino acid substitutions in one or two of the six CDR sequences.

Antibodies according to the present invention may comprise VL and/or VH chains comprising an amino acid sequence that has a high percentage sequence identity to one or more of the VL and/or VH amino acid sequences shown in Figures 1 and 2, respectively. For example, antibodies according to the present invention include antibodies that bind CD122 and have a VL chain that comprises an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the VL chain amino acid sequence of one of SEQ ID NOs:1, 17, 19, 21, 23, 24, 27, 28, 29, 30, 31, 33, 34, 148, 149, 5, 9, or 13 shown in Figure 1. Antibodies according to the present invention include antibodies that bind CD122 and have VH chain that comprises an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the VH chain amino acid sequence of one of SEQ ID NOs:35, 51, 53, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 150, 151, 39, 43, or 47 shown in Figure 2.

Antibodies according to the present invention include an antibody or CD122-binding fragment thereof having the amino acid sequence encoded by the nucleotide sequence of any one of SEQ ID NOs:130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, or 141 shown in Figure 17. Antibodies of the present invention include an antibody comprising the VL and/or VH domain sequence of an amino acid sequence encoded by the nucleotide sequence of any one of SEQ ID NOs:130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, or 141 shown in Figure 17.

In some aspects, the antibody of the present invention comprises the antibody/fragment of a γc-binding antibody clone. In some aspects, the antibody comprises the antibody/fragment of clone P1A3 or a variant of P1A3. In some aspects, the antibody comprises the antibody/fragment of clone P1A3_B3 or a variant of P1A3_B3. In some aspects, the antibody comprises the antibody/fragment of clone P1A3_E8 or a variant of P1A3_E8. In some aspects, the antibody comprises the antibody/fragment of clone P1A3_E9 or a variant of P1A3_E9. In some aspects, the antibody comprises the antibody/fragment of clone P1A3_B4 or a variant of P1A3_B4. In some aspects, the antibody comprises the antibody/fragment of clone P1A3_FW2 or a variant of

P1A3_FW2. In some aspects, the antibody comprises the antibody/fragment of clone P2B9 or a variant of P2B9.

The amino acid sequences of the VL domains of the γc-binding antibody clones of the preceding paragraph are shown in Figure 3, as are the CDRs defined according to the Kabat system. The amino acid sequences of the VH domains of the γc-binding antibody clones of the preceding paragraph are shown in Figure 4, as are the CDRs defined according to the Kabat system. The full amino acid sequences of the antibody constructs (including linkers) are shown in Figure 16, and the encoding nucleotide sequences are shown in Figure 18.

Antibodies according to the present invention may comprise the CDRs of P1A3, P1A3_B3 P1A3_E8, P1A3_E9, P1A3_B4, P1A3_FW2, or P2B9. In an antibody according to the present invention one or two or three or four of the six CDR sequences may vary. A variant may have one or two amino acid substitutions in one or two of the six CDR sequences.

Antibodies according to the present invention may comprise VL and/or VH chains comprising an amino acid sequence that has a high percentage sequence identity to one or more of the VL and/or VH amino acid sequences shown in Figures 3 and 4, respectively. For example, antibodies according to the present invention include antibodies that bind γc and have VL chain that comprises an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the VL chain amino acid sequence of one of SEQ ID NOs:67, 152, 71, or 75 shown in Figure 3. Antibodies according to the present invention include antibodies that bind γc and have VH chain that comprises an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the VH chain amino acid sequence of one of SEQ ID NOs:76, 153, 78, 82 or 84 shown in Figure 4.

Antibodies according to the present invention include an antibody or γc-binding fragment thereof having the amino acid sequence encoded by the nucleotide sequence of any one of SEQ ID NOs:142, 143, 144, 145, 146, or 147 shown in Figure 18. Antibodies of the present invention include an antibody comprising the VL and/or VH domain sequence of

an amino acid sequence encoded by the nucleotide sequence of any one of SEQ ID NOs:142, 143, 144, 145, 146, or 147 shown in Figure 18.

In some aspects, the antibody of the present invention comprises the antibody/fragment of a CD122-binding antibody clone, e.g. a CD122-binding antibody clone as described, and also comprises a γc-binding clone, e.g. a γc-binding clone as described herein.

The light and heavy chain CDRs disclosed herein may also be particularly useful in conjunction with a number of different framework regions. Accordingly, light and/or heavy chains having LC-CDR1-3 or HC-CDR1-3 may possess an alternative framework region. Suitable framework regions are well known in the art and are described for example in M. Lefranc & G. Le:franc (2001) "The Immunoglobulin FactsBook", Academic Press, incorporated herein by reference.

Antibodies according to the present invention may be detectably labelled or, at least, capable of detection. For example, the antibody may be labelled with a radioactive atom or a coloured molecule or a fluorescent molecule or a molecule which can be readily detected in any other way. Suitable detectable molecules include fluorescent proteins, luciferase, enzyme substrates, and radiolabels. The binding moiety may be directly labelled with a detectable label or it may be indirectly labelled. For example, the binding moiety may be an unlabelled antibody which can be detected by another antibody which is itself labelled. Alternatively, the second antibody may have bound to it biotin and binding of labelled streptavidin to the biotin is used to indirectly label the first antibody.

Chimeric antigen receptors

The present invention provides a chimeric antigen receptor (CAR) capable of binding to CD122 and/or γc. The CAR comprises one or more antigen binding fragments or polypeptides according to the present invention.

Chimeric Antigen Receptors (CARs) are recombinant receptors that provide both antigen-binding and T cell activating functions. CAR structure and engineering is reviewed, for example, in Dotti et al., Immunol Rev (2014) 257(1), hereby incorporated by reference in its entirety.

Antigen-binding fragments according to the present invention are provided herein as the antigen-binding domain of a chimeric antigen receptor (CAR). In some embodiments, the

CAR comprises a VL domain and a VH domain according to any embodiment of an antibody, antigen binding fragment or polypeptide described herein. Accordingly, the antigen bound by the CAR according to the present invention is CD122 and/or γc.

CARs may be combined with costimulatory ligands, chimeric costimulatory receptors or cytokines to further enhance T cell potency, specificity and safety (Sadelain et al., The basic principles of chimeric antigen receptor (CAR) design. Cancer Discov. 2013 April; 3(4): 388–398. doi:10.1158/2159-8290.CD-12-0548, specifically incorporated herein by reference).

The present invention also provides a cell comprising a CAR according to the invention. The CAR according to the present invention may be used to generate T cells targeted to cells expressing CD122 and/or γc.

Engineering of CARs into T cells may be performed during culture, *in vitro*, for transduction and expansion, such as happens during expansion of T cells for adoptive T cell therapy. The transduction may utilize a variety of methods, but stable gene transfer is required to enable sustained CAR expression in clonally expanding and persisting T cells.

A CAR typically combines an antigen binding domain with an intracellular domain of the CD3- zeta chain or FcγRI protein in a single chimeric protein. The structural features of a CAR are described by Sjouke et al., (The pharmacology of second-generation chimeric antigen receptors. Nature Reviews Drug Discovery, 14, 499 509 (2015) doi:10.1038/nrd4597). A CAR typically has an extracellular antigen-binding domain linked to a transmembrane domain and endodomain. An optional hinge or spacer domain may provide separation between the binding moiety and transmembrane domain and may act as a flexible linker.

In accordance with the present invention, the antigen recognition domain of the CAR is, or is derived from, an antibody, antigen binding fragment or polypeptide which is capable of binding to CD122 and/or γc, as described herein.

Hinge or spacer regions of the CAR may be flexible domains allowing the binding moiety to orient in different directions. Hinge or spacer regions may be derived from IgG1 or the CH2CH3 region of immunoglobulin.

Transmembrane domains may be hydrophobic alpha helix that spans the cell membrane. The transmembrane domain associated with the endodomain is commonly used.

The endodomain is responsible for receptor clustering/dimerization after antigen binding and for initiation of signal transduction to the cell. One commonly used transmembrane domain is the CD3-zeta transmembrane and endodomain. Intracellular domains from one or more co-stimulatory protein receptors, such as CD28 4-1BB, OX40, ICOS, may optionally be incorporated into the cytoplasmic tail of the CAR to provide additional co-stimulatory signaling, which may be beneficial in terms of anti-tumor activity.

In one embodiment, a CAR comprises an extracellular domain having an antigen recognition domain, a transmembrane domain, and a cytoplasmic domain. A transmembrane domain that is naturally associated with one of the domains in the CAR may be used or the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

The cytoplasmic domain may be designed to comprise the CD28 and/or 4-1BB signaling domain by itself or be combined with any other desired cytoplasmic domain(s). The cytoplasmic domain may be designed to further comprise the signaling domain of CD3-zeta. For example, the cytoplasmic domain of the CAR can include but is not limited to CD3-zeta, 4-1BB and CD28 signaling modules and combinations thereof.

The present invention also provides CAR T cells comprising as a CAR an antigen binding fragment capable of binding to CD122 and/or γc, according to the present invention.

CAR T cells of the invention can be generated by introducing a lentiviral vector *in vitro* comprising a desired CAR, for example a CAR comprising anti-IL2Rβ/γc, CD8a hinge and transmembrane domain, and human 4-1BB and CD3zeta signaling domains, into the cells. The CAR T cells of the invention are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control.

In one embodiment the invention relates to administering a genetically modified T cell expressing a CAR capable of binding to CD122 and/or γc for the treatment of a patient having cancer or at risk of having cancer or an infectious disease using lymphocyte

infusion. Preferably, autologous lymphocyte infusion is used in the treatment. Autologous PBMCs are collected from a patient in need of treatment and T cells are activated and expanded using the methods described herein and known in the art and then infused back into the patient.

Methods of detection

Antibodies, or antigen binding fragments, described herein may be used in methods that involve the binding of the antibody or antigen binding fragment to CD122 and/or γc. Such methods may involve detection of the bound complex of antibody, or antigen binding fragment, and CD122 and/or γc. As such, in one embodiment a method is provided, the method comprising contacting a sample containing, or suspected to contain, CD122 and/or γc with an antibody or antigen binding fragment as described herein and detecting the formation of a complex of antibody, or antigen binding fragment, and CD122 and/or γc.

Suitable method formats are well known in the art, including immunoassays such as sandwich assays, e.g. ELISA. The method may involve labelling the antibody, or antigen binding fragment, or CD122 and/or γc, or both, with a detectable label, e.g. fluorescent, luminescent or radio- label. CD122 and/or γc expression may be measured by immunohistochemistry (IHC), for example of a tissue sample obtained by biopsy.

Methods of this kind may provide the basis of a method of diagnosis of a disease or condition requiring detection and or quantitation of CD122 and/or γc. Such methods may be performed *in vitro* on a patient sample, or following processing of a patient sample. Once the sample is collected, the patient is not required to be present for the *in vitro* method of diagnosis to be performed and therefore the method may be one which is not practised on the human or animal body.

Such methods may involve determining the amount of CD122 and/or γc present in a patient sample. The method may further comprise comparing the determined amount against a standard or reference value as part of the process of reaching a diagnosis. Other diagnostic tests may be used in conjunction with those described here to enhance the accuracy of the diagnosis or prognosis or to confirm a result obtained by using the tests described here.

The level of CD122 and/or γc present in a patient sample may be indicative that a patient may respond to treatment with an anti-CD122 and/or anti-γc antibody. The presence of a high level of CD122 and/or γc in a sample may be used to select a patient for treatment with an anti-CD122 and/or anti-γc antibody. The antibodies of the present invention may therefore be used to select a patient for treatment with anti-CD122 and/or anti-γc antibody therapy.

Detection in a sample of anti-CD122 and/or anti-γc antibody may be used for the purpose of diagnosis of an infectious disease, autoimmune disorder or a cancerous condition in the patient, diagnosis of a predisposition to an infectious disease, autoimmune disorder or a cancerous condition or for providing a prognosis (prognosticating) of an infectious disease, autoimmune disorder or a cancerous condition. The diagnosis or prognosis may relate to an existing (previously diagnosed) infectious disease, autoimmune disorder or cancerous condition.

A sample may be taken from any tissue or bodily fluid. The sample may comprise or may be derived from: a quantity of blood; a quantity of serum derived from the individual's blood which may comprise the fluid portion of the blood obtained after removal of the fibrin clot and blood cells; a tissue sample or biopsy; or cells isolated from said individual.

Methods according to the present invention may preferably performed *in vitro*. The term "*in vitro*" is intended to encompass experiments with cells in culture whereas the term "*in vivo*" is intended to encompass experiments with and/or treatment of intact multi-cellular organisms.

Therapeutic applications

Antibodies, antigen binding fragments and polypeptides according to the present invention and compositions comprising such agents may be provided for use in methods of medical treatment. Treatment may be provided to subjects having a disease or condition in need of treatment.

Methods of medical treatment may involve treatment of cancer by a method of ameliorating, treating, or preventing a malignancy in a human subject wherein the steps of the method assist or boost the immune system in eradicating cancerous cells. Such methods may include the administration of cells, antibodies, proteins, or nucleic acids according to the present invention that invoke an active (or achieve a passive) immune

response to destroy cancerous cells. Methods of treatment may optionally include the co-administration of biological adjuvants (e.g., interleukins, cytokines, Bacillus Comette-Guerin, monophosphoryl lipid A, etc.) in combination with conventional therapies for treating cancer such as chemotherapy, radiation, or surgery. Methods of treatment may involve administering a composition according to the present invention as a vaccine that works by activating the immune system to prevent or destroy cancer cell growth. Methods of medical treatment may also involve *in vivo*, *ex vivo*, and adoptive immunotherapies, including those using autologous and/or heterologous cells or immortalized cell lines.

The disease or condition may be one of an infectious disease, an autoimmune disorder (e.g. e.g. Crohn's disease, Multiple Sclerosis), a cancer, an inflammatory disease (e.g. arthritis), a disease/disorder associated with deficient IL-2-mediated signalling and/or IL-15-mediated signalling, deficient T cell proliferation or T cell dysfunction.

In some embodiments, the treatment is of a disease or disorder for which increased IL-2-mediated signalling and/or IL-15-mediated signalling is therapeutic.

In some embodiments, the treatment is of a disease or disorder associated with a deficient T cell response, e.g. a deficient CD8+ T cell response.

The treatment may be aimed at preventing or treating a disease/disorder by one of more of: increasing the number of CD3+ T cells, increasing the number of CD4+ T cells, increasing the number of CD8+ T cells, increasing the number of CD8+ effector T cells (e.g. CTLs), increasing the number NK cells, increasing the ratio of CD8+ T cells to CD4+ T cells, or decreasing the proportion of Tregs.

The T-cell dysfunctional disorder may be manifest as an infection, or inability to mount an effective immune response against an infection. The infection may be chronic, persistent, latent or slow, and may be the result of bacterial, viral, fungal or parasitic infection. As such, treatment may be provided to patients having a bacterial, viral or fungal infection. Examples of bacterial infections include infection with *Helicobacter pylori*. Examples of viral infections include infection with EBV, HIV, hepatitis B or hepatitis C.

The T-cell dysfunctional disorder may be associated with a cancer, such as tumor immune escape. Many human tumors express tumor-associated antigens recognised by T cells and capable of inducing an immune response.

Cancers may also be treated where there is no indication of a T-cell dysfunctional disorder but the use of an antibody, antigen binding fragment or polypeptide according to the present invention promotes an effective immune response.

The treatment may be aimed at prevention of a disease/disorder associated with deficient/reduced IL-2-mediated signalling and/or IL-15-mediated signalling. As such, the antibodies, antigen binding fragments and polypeptides may be used to formulate pharmaceutical compositions or medicaments and subjects may be prophylactically treated against development of a disease state. This may take place before the onset of symptoms of the disease state, and/or may be given to subjects considered to be at greater risk of the disease or disorder.

Treatment may comprise co-therapy with a vaccine, e.g. T-cell vaccine, which may involve simultaneous, separate or sequential therapy, or combined administration of vaccine and antibody, antigen binding fragment or polypeptide in a single composition. In this context, the antibody, antigen binding fragment or polypeptide may be provided as an adjuvant to the vaccine.

Administration of an antibody, antigen binding fragment or polypeptide is preferably in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

The CARs and cells comprising the CARs (i.e. CAR-T cells) of the present invention find use to treat autoimmune disorders, e.g. Crohn's disease, Multiple Sclerosis. In such treatments, the CAR-T cells are effective to kill autoimmune aggressor cells (e.g. autoreactive T cells) expressing CD122 and/or γc.

Formulating pharmaceutically useful compositions and medicaments

Antibodies, antigen binding fragments, and polypeptides, CARs and cells according to the present invention may be formulated as pharmaceutical compositions or medicaments for clinical use and may comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

In accordance with the present invention methods are also provided for the production of pharmaceutically useful compositions, such methods of production may comprise one or more steps selected from: isolating an antibody, antigen binding fragment, polypeptide, CAR or cell as described herein; and/or mixing an isolated antibody, antigen binding fragment or polypeptide as described herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

For example, a further aspect of the present invention relates to a method of formulating or producing a medicament or pharmaceutical composition for use in a method of medical treatment, method comprising formulating a pharmaceutical composition or medicament by mixing an antibody, antigen binding fragment, polypeptide, CAR or cell as described herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

Infection

An infection may be any infection or infectious disease, e.g. bacterial, viral, fungal, or parasitic infection. In some embodiments it may be particularly desirable to treat chronic/persistent infections, e.g. where such infections are associated with T cell dysfunction or T cell exhaustion.

It is well established that T cell exhaustion is a state of T cell dysfunction that arises during many chronic infections (including viral, bacterial and parasitic), as well as in cancer (Wherry *Nature Immunology* Vol.12, No.6, p492-499, June 2011).

Examples of bacterial infections that may be treated include infection by *Bacillus spp., Bordetella pertussis, Clostridium spp., Corynebacterium spp., Vibrio chloerae, Staphylococcus spp., Streptococcus spp. Escherichia, Klebsiella, Proteus, Yersinia, Erwina, Salmonella, Listeria* sp, *Helicobacter pylori*, mycobacteria (e.g. *Mycobacterium tuberculosis*) and *Pseudomonas aeruginosa.* For example, the bacterial infection may be sepsis or tuberculosis.

Examples of viral infections that may be treated include infection by Epstein-Barr virus, influenza virus, measles virus, hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), lymphocytic choriomeningitis virus (LCMV), Herpes simplex virus and human papilloma virus.

Examples of fungal infections that may be treated include infection by *Alternaria sp, Aspergillus sp, Candida sp* and *Histoplasma sp.* The fungal infection may be fungal sepsis or histoplasmosis. Examples of parasitic infections that may be treated include infection by *Plasmodium* species (e.g. *Plasmodium falciparum, Plasmodium yoeli, Plasmodium ovale, Plasmodium* vivax, or *Plasmodium chabaudi chabaudi*). The parasitic infection may be a disease such as malaria, leishmaniasis and toxoplasmosis.

Cancer

A cancer may be any unwanted cell proliferation (or any disease manifesting itself by unwanted cell proliferation), neoplasm or tumor or increased risk of or predisposition to the unwanted cell proliferation, neoplasm or tumor. The cancer may be benign or malignant and may be primary or secondary (metastatic). A neoplasm or tumor may be any abnormal growth or proliferation of cells and may be located in any tissue. Examples of tissues include the adrenal gland, adrenal medulla, anus, appendix, bladder, blood, bone, bone marrow, brain, breast, cecum, central nervous system (including or excluding the brain) cerebellum, cervix, colon, duodenum, endometrium, epithelial cells (e.g. renal epithelia), gallbladder, oesophagus, glial cells, heart, ileum, jejunum, kidney, lacrimal glad, larynx, liver, lung, lymph, lymph node, lymphoblast, maxilla, mediastinum, mesentery, myometrium, nasopharynx, omentum, oral cavity, ovary, pancreas, parotid gland, peripheral nervous system, peritoneum, pleura, prostate, salivary gland, sigmoid colon, skin, small intestine, soft tissues, spleen, stomach, testis, thymus, thyroid gland, tongue, tonsil, trachea, uterus, vulva, white blood cells.

Tumors to be treated may be nervous or non-nervous system tumors. Nervous system tumors may originate either in the central or peripheral nervous system, e.g. glioma, medulloblastoma, meningioma, neurofibroma, ependymoma, Schwannoma, neurofibrosarcoma, astrocytoma and oligodendroglioma. Non-nervous system cancers/tumors may originate in any other non-nervous tissue, examples include melanoma, mesothelioma, lymphoma, myeloma, leukemia, Non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), cutaneous T-cell lymphoma (CTCL),

chronic lymphocytic leukemia (CLL), hepatoma, epidermoid carcinoma, prostate carcinoma, breast cancer, lung cancer , colon cancer, ovarian cancer, pancreatic cancer, thymic carcinoma, NSCLC, haematologic cancer and sarcoma.

In particular, treatment of melanoma, kidney cancer (e.g. renal carcinoma) or bladder cancer is contemplated.

In some embodiments the cancer is an EBV or HPV positive cancer.

Adoptive cell transfer therapy

Adoptive cell transfer therapy generally refers to a process in which white blood cells are removed from a subject, typically by drawing a blood sample from which white blood cells are separated, expanded in vitro or ex vivo and returned either to the same subject or to a different subject. The treatment is typically aimed at increasing the amount/concentration of an active form of the required cell population in the subject.

The antibodies/fragments of the present invention provide a means of expanding the number and/or enhancing the activity of cells expressing CD122 and/or γc. In some embodiments, the cells are T cells and/or NK cells.

Accordingly, in a further aspect of the present invention a method is provided for expanding a population of cells, wherein cells are contacted *in vitro*, *in vivo* or *ex vivo* with an antibody, antigen binding fragment, polypeptide or composition according to the present invention. Also provided is a method for expanding a population of cells in a subject, comprising an administering an antibody/fragment an antibody, antigen binding fragment, polypeptide or composition according to the present invention to a subject.

In some embodiments, the antibodies/fragments of the present invention are capable of delivering a survival signal to cells expressing CD122 and/or γc. In some embodiments, the antibodies/fragments are useful to enhance/promote the survival of a cell or a population of cells (e.g. T cells (e.g. CD8+ T cells (e.g. CTLs), CD4+ T cells) and/or NK cells), e.g. *in vitro*, *in vivo* or *ex vivo*.

Accordingly, in a further aspect of the present invention a method is provided for enhancing/promoting the survival of a cell or a population of cells, wherein cells are contacted *in vitro*, *in vivo* or *ex vivo* with an antibody, antigen binding fragment,

polypeptide or composition according to the present invention. Also provided is a method for enhancing/promoting the survival of a cell or a population of cells in a subject, comprising an administering an antibody/fragment an antibody, antigen binding fragment, polypeptide or composition according to the present invention to the subject.

The methods may optionally comprise one or more of the following steps: taking a blood sample from a subject; isolating cells (e.g. one of PBMCs, T cells, NK cells etc.) from the blood sample; culturing the cells in *in vitro* or *ex vivo* cell culture (where they may be contacted with the antibody, antigen binding fragment or polypeptide), collecting an expanded population of cells; mixing the cells with an adjuvant, diluent, or carrier; administering the expanded cells to a subject.

Accordingly, in some aspects of the present invention a method of treatment of a subject, e.g. a subject having a T-cell dysfunctional disorder, is provided, the method comprising obtaining a blood sample from a subject in need of treatment, culturing T cells obtained from the blood sample in the presence of an antibody, antigen binding fragment, polypeptide or composition according to the present invention so as to expand the T cell population, collecting expanded T cells, and administering the expanded T cells to a subject in need of treatment.

The T cells may be obtained from a subject requiring treatment, and may be isolated and/or purified. They may be a $CD4^+$ and/or $CD8^+$ T-cell population. They may be a CD122+ and/or γc+ population.

During culture, T cells may be contacted with the antibody, antigen binding fragment, polypeptide or composition under conditions and for a period of time suitable to allow expansion of the T cells to a desired number of cells. After a suitable period of time the T cells may be harvested, optionally concentrated, and may be mixed with a suitable carrier, adjuvant or diluent and returned to the subject's body. A subject may undergo one or more rounds of such therapy.

Methods of T cell expansion are well known in the art, such as those described in Kalamasz et al., *J Immunother* 2004 Sep-Oct; 27(5):405-18; Montes et al., *Clin Exp Immunol* 2005 Nov;142(2):292-302; Wölfl and Greenburg *Nature Protocols* 9 p950-966 27 March 2014; Trickett and Kwan *Journal of Immunological Methods* Vol. 275, Issues 1-2, 1 April 2003, p251-255; Butler et al *PLoSONE* 7(1) 12 Jan 2012.

Simultaneous or Sequential Administration

Compositions may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

In this specification an antibody, antigen binding fragment, polypeptide, CAR, cell or composition of the present invention and an anti-infective agent or chemotherapeutic agent (therapeutic agent) may be administered simultaneously or sequentially.

In some embodiments, treatment with an antibody, antigen binding fragment or polypeptide of the present invention may be accompanied by chemotherapy.

Simultaneous administration refers to administration of the antibody, antigen binding fragment or polypeptide and therapeutic agent together, for example as a pharmaceutical composition containing both agents (combined preparation), or immediately after each other and optionally via the same route of administration, e.g. to the same artery, vein or other blood vessel.

Sequential administration refers to administration of one of the antibody, antigen binding fragment or polypeptide or therapeutic agent followed after a given time interval by separate administration of the other agent. It is not required that the two agents are administered by the same route, although this is the case in some embodiments. The time interval may be any time interval.

In some embodiments, the antibody, antigen binding fragment, polypeptide, or composition of the present invention may be administered to a patient undergoing treatment by adoptive cell transfer. The administration may be aimed at stimulating proliferation of the adoptively transferred cells in the patient *in vivo*.

Anti-infective agents

In treating infection, an antibody, antigen binding fragment or polypeptide of the present invention may be administered in combination with an anti-infective agent, as described above. The anti-infective agent may be an agent known to have action against the microorganism or virus responsible for the infection.

Suitable anti-infective agents include antibiotics (such as penicillins, cephalosporins, rifamycins, lipiarmycins, quinolones, sulfonamides, macrolides, lincosamides, tetracyclines, cyclic lipopeptides, glycylcyclines, oxazolidinones, and lipiarmycins), anti-viral agents (such as reverse transcriptase inhibitors, integrase inhibitors, transcription factor inhibitors, antisense and siRNA agents and protease inhibitors), anti-fungal agents (such as polyenes, imidiazoles, triazoles, thiazoles, allylamines, and echinocandins) and anti-parasitic agents (such as antinematode agents, anticestode agents, antitrematode agents, antiamoebic agents and antiprotozoal agents).

Chemotherapy

Chemotherapy and radiotherapy respectively refer to treatment of a cancer with a drug or with ionising radiation (e.g. radiotherapy using X-rays or γ-rays).

The drug may be a chemical entity, e.g. small molecule pharmaceutical, antibiotic, DNA intercalator, protein inhibitor (e.g. kinase inhibitor), or a biological agent, e.g. antibody, antibody fragment, nucleic acid or peptide aptamer, nucleic acid (e.g. DNA, RNA), peptide, polypeptide, or protein. The drug may be formulated as a pharmaceutical composition or medicament. The formulation may comprise one or more drugs (e.g. one or more active agents) together with one or more pharmaceutically acceptable diluents, excipients or carriers.

A treatment may involve administration of more than one drug. A drug may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. For example, the chemotherapy may be a co-therapy involving administration of two drugs, one or more of which may be intended to treat the cancer.

The chemotherapy may be administered by one or more routes of administration, e.g. parenteral, intravenous injection, oral, subcutaneous, intradermal or intratumoral.

The chemotherapy may be administered according to a treatment regime. The treatment regime may be a pre-determined timetable, plan, scheme or schedule of chemotherapy administration which may be prepared by a physician or medical practitioner and may be tailored to suit the patient requiring treatment.

The treatment regime may indicate one or more of: the type of chemotherapy to administer to the patient; the dose of each drug or radiation; the time interval between administrations; the length of each treatment; the number and nature of any treatment holidays, if any etc. For a co-therapy a single treatment regime may be provided which indicates how each drug is to be administered.

Chemotherapeutic drugs and biologics may be selected from: alkylating agents such as cisplatin, carboplatin, mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide; purine or pyrimidine anti-metabolites such as azathiopurine or mercaptopurine; alkaloids and terpenoids, such as vinca alkaloids (e.g. vincristine, vinblastine, vinorelbine, vindesine), podophyllotoxin, etoposide, teniposide, taxanes such as paclitaxel (Taxol™), docetaxel; topoisomerase inhibitors such as the type I topoisomerase inhibitors camptothecins irinotecan and topotecan, or the type II topoisomerase inhibitors amsacrine, etoposide, etoposide phosphate, teniposide; antitumor antibiotics (e.g. anthracyline antibiotics) such as dactinomycin, doxorubicin (Adriamycin™), epirubicin, bleomycin, rapamycin; antibody based agents, such as anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-TIM-3 antibodies, anti-CTLA-4, anti-4-1BB, anti-GITR, anti-CD27, anti-BLTA, anti-OX43, anti-VEGF, anti-TNFα, anti-IL-2, antiGpIIb/IIIa, anti-CD-52, anti-CD20, anti-RSV, anti-HER2/neu(erbB2), anti-TNF receptor, anti-EGFR antibodies, monoclonal antibodies or antibody fragments, examples include: cetuximab, panitumumab, infliximab, basiliximab, bevacizumab (Avastin®), abciximab, daclizumab, gemtuzumab, alemtuzumab, rituximab (Mabthera®), palivizumab, trastuzumab, etanercept, adalimumab, nimotuzumab; EGFR inihibitors such as erlotinib, cetuximab and gefitinib; anti-angiogenic agents such as bevacizumab (Avastin®); cancer vaccines such as Sipuleucel-T (Provenge®).

In one embodiment the chemotherapeutic agent is an anti-PD-1 antibody, anti-PD-L1 antibody, anti-TIM-3 antibody, anti-LAG-3, anti-CTLA-4, anti-41BB, anti-GITR, anti-CD27, anti-BLTA, anti-OX43, anti-VEGF, anti-TNFα, anti-IL2, anti-GpIIb/IIIa, anti-CD-52, anti-CD20, anti-RSV, anti-HER2/neu(erbB2), anti-TNF receptor, anti-EGFR or other antibody. In some embodiments, the chemotherapeutic agent is an immune checkpoint inhibitor or costimulation molecule.

Further chemotherapeutic drugs may be selected from: 13-cis-Retinoic Acid, 2-Chlorodeoxyadenosine, 5-Azacitidine 5-Fluorouracil, 6-Mercaptopurine, 6-Thioguanine, Abraxane, Accutane®, Actinomycin-D Adriamycin®, Adrucil®, Afinitor®, Agrylin®, Ala-

Cort®, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ®, Alkeran®, All-transretinoic Acid, Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron®, Anastrozole, Arabinosylcytosine, Aranesp®, Aredia®, Arimidex®, Aromasin®, Arranon®, Arsenic Trioxide, Asparaginase, ATRA Avastin®, Azacitidine, BCG, BCNU, Bendamustine, Bevacizumab, Bexarotene, BEXXAR®, Bicalutamide, BiCNU, Blenoxane®, Bleomycin, Bortezomib, Busulfan, Busulfex®, Calcium Leucovorin, Campath®, Camptosar®, Camptothecin-11, Capecitabine, Carac™, Carboplatin, Carmustine, Casodex®, CC-5013, CCI-779, CCNU, CDDP, CeeNU, Cerubidine®, Cetuximab, Chlorambucil, Cisplatin, Citrovorum Factor, Cladribine, Cortisone, Cosmegen®, CPT-11, Cyclophosphamide, Cytadren®, Cytarabine Cytosar-U®, Cytoxan®, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib, Daunomycin, Daunorubicin, Daunorubicin Hydrochloride, Daunorubicin Liposomal, DaunoXome®, Decadron, Decitabine, Delta-Cortef®, Deltasone®, Denileukin, Diftitox, DepoCyt™, Dexamethasone, Dexamethasone Acetate, Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex, Docetaxel, Doxil®, Doxorubicin, Doxorubicin Liposomal, Droxia™, DTIC, DTIC-Dome®, Duralone®, Eligard™, Ellence™, Eloxatin™, Elspar®, Emcyt®, Epirubicin, Epoetin Alfa, Erbitux, Erlotinib, Erwinia L-asparaginase, Estramustine, Ethyol Etopophos®, Etoposide, Etoposide Phosphate, Eulexin®, Everolimus, Evista®, Exemestane, Faslodex®, Femara®, Filgrastim, Floxuridine, Fludara®, Fludarabine, Fluoroplex®, Fluorouracil, Fluoxymesterone, Flutamide, Folinic Acid, FUDR®, Fulvestrant, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gleevec™, Gliadel® Wafer, Goserelin, Granulocyte - Colony Stimulating Factor, Granulocyte Macrophage Colony Stimulating Factor, Herceptin ®, Hexadrol, Hexalen®, Hexamethylmelamine, HMM, Hycamtin®, Hydrea®, Hydrocort Acetate®, Hydrocortisone, Hydrocortisone Sodium Phosphate, Hydrocortisone Sodium Succinate, Hydrocortone Phosphate, Hydroxyurea, Ibritumomab, Ibritumomab Tiuxetan, Idamycin®, Idarubicin, Ifex®, IFN-alpha, Ifosfamide, IL-11, IL-2, Imatinib mesylate, Imidazole Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG Conjugate), Interleukin - 2, Interleukin-11, Intron A® (interferon alfa-2b), Iressa®, Irinotecan, Isotretinoin, Ixabepilone, Ixempra™, Kidrolase, Lanacort®, Lapatinib, L-asparaginase, LCR, Lenalidomide, Letrozole, Leucovorin, Leukeran, Leukine™, Leuprolide, Leurocristine, Leustatin™, Liposomal Ara-C, Liquid Pred®, Lomustine, L-PAM, L-Sarcolysin, Lupron®, Lupron Depot®, Matulane®, Maxidex, Mechlorethamine, Mechlorethamine Hydrochloride, Medralone®, Medrol®, Megace®, Megestrol, Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex™, Methotrexate, Methotrexate Sodium, Methylprednisolone, Meticorten®, Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol®,

MTC, MTX, Mustargen®, Mustine, Mutamycin®, Myleran®, Mylocel™, Mylotarg®, Navelbine®, Nelarabine, Neosar®, Neulasta™, Neumega®, Neupogen®, Nexavar®, Nilandron®, Nilutamide, Nipent®, Nitrogen Mustard, Novaldex®, Novantrone®, Octreotide, Octreotide acetate, Oncospar®, Oncovin®, Ontak®, Onxal™, Oprevelkin, Orapred®, Orasone®, Oxaliplatin, Paclitaxel, Paclitaxel Protein-bound, Pamidronate, Panitumumab, Panretin®, Paraplatin®, Pediapred®, PEG Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON™, PEG-L-asparaginase, PEMETREXED, Pentostatin, Phenylalanine Mustard, Platinol®, Platinol-AQ®, Prednisolone, Prednisone, Prelone®, Procarbazine, PROCRIT®, Proleukin®, Prolifeprospan 20 with Carmustine Implant Purinethol®, Raloxifene, Revlimid®, Rheumatrex®, Rituxan®, Rituximab, Roferon-A® (Interferon Alfa-2a), Rubex®, Rubidomycin hydrochloride, Sandostatin® Sandostatin LAR®, Sargramostim, Solu-Cortef®, Solu-Medrol®, Sorafenib, SPRYCEL™, STI-571, Streptozocin, SU11248, Sunitinib, Sutent®, Tamoxifen, Tarceva®, Targretin®, Taxol®, Taxotere®, Temodar®, Temozolomide, Temsirolimus, Teniposide, TESPA, Thalidomide, Thalomid®, TheraCys®, Thioguanine, Thioguanine Tabloid®, Thiophosphoamide, Thioplex®, Thiotepa, TICE®, Toposar®, Topotecan, Toremifene, Torisel®, Tositumomab, Trastuzumab, Treanda®, Tretinoin, Trexall™, Trisenox®, TSPA, TYKERB®, VCR, Vectibix™, Velban®, Velcade®, VePesid®, Vesanoid®, Viadur™, Vidaza®, Vinblastine, Vinblastine Sulfate, Vincasar Pfs®, Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VM-26, Vorinostat, VP-16, Vumon®, Xeloda®, Zanosar®, Zevalin™, Zinecard®, Zoladex®, Zoledronic acid, Zolinza, Zometa®.

Routes of administration

Antibodies, antigen binding fragments, polypeptides and other therapeutic agents, medicaments and pharmaceutical compositions according to aspects of the present invention may be formulated for administration by a number of routes, including but not limited to, parenteral, intravenous, intra-arterial, intramuscular, subcutaneous, intradermal, intratumoral and oral. Antibodies, antigen binding fragments, polypeptides and other therapeutic agents, may be formulated in fluid or solid form. Fluid formulations may be formulated for administration by injection to a selected region of the human or animal body.

Dosage regime

Multiple doses of the antibody, antigen binding fragment, polypeptide, CAR, cell, or composition of the invention may be provided. One or more, or each, of the doses may be accompanied by simultaneous or sequential administration of another therapeutic agent.

Multiple doses may be separated by a predetermined time interval, which may be selected to be one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days, or 1, 2, 3, 4, 5, or 6 months. By way of example, doses may be given once every 7, 14, 21 or 28 days (plus or minus 3, 2, or 1 days).

The antibody of the present invention has favourable pharmacokinetics as compared to IL-2 as a therapeutic agent. An advantage of the antibody/fragment of the present invention is the improved half-life in vivo, e.g. in the blood or serum, as compared to IL-2, which means that administration of the antibody/fragment can be less frequent, and/or of a lower amount of the agent.

Kits

In some aspects of the present invention a kit of parts is provided. In some embodiments the kit may have at least one container having a predetermined quantity of the antibody, fragment, polypeptide, CAR, cell or composition. The kit may provide the antibody, fragment, polypeptide, CAR, or cell in the form of a medicament or pharmaceutical composition, and may be provided together with instructions for administration to a patient in order to treat a specified disease or condition. The antibody, fragment, polypeptide, CAR, cell or composition may be formulated so as to be suitable for injection or infusion to a tumor or to the blood.

In some embodiments the kit may further comprise at least one container having a predetermined quantity of another therapeutic agent (e.g. anti-infective agent or chemotherapy agent). In such embodiments, the kit may also comprise a second medicament or pharmaceutical composition such that the two medicaments or pharmaceutical compositions may be administered simultaneously or separately such that they provide a combined treatment for the specific disease or condition. The therapeutic agent may also be formulated so as to be suitable for injection or infusion to a tumor or to the blood.

Subjects

The subject to be treated may be any animal or human. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but is more preferably human. The subject may be male or female. The subject may be a

patient. A subject may have been diagnosed with a disease or condition requiring treatment, or be suspected of having such a disease or condition.

Protein Expression

Molecular biology techniques suitable for producing the antibody, fragment, polypeptide, or CAR according to the invention in cells are well known in the art, such as those set out in Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989

The polypeptide may be expressed from a nucleotide sequence. The nucleotide sequence may be contained in a vector present in a cell, or may be incorporated into the genome of the cell.

A "vector" as used herein is an oligonucleotide molecule (DNA or RNA) used as a vehicle to transfer exogenous genetic material into a cell. The vector may be an expression vector for expression of the genetic material in the cell. Such vectors may include a promoter sequence operably linked to the nucleotide sequence encoding the gene sequence to be expressed. A vector may also include a termination codon and expression enhancers. Any suitable vectors, promoters, enhancers and termination codons known in the art may be used to express polypeptides from a vector according to the invention. Suitable vectors include plasmids, binary vectors, viral vectors and artificial chromosomes (e.g. yeast artificial chromosomes).

In this specification the term "operably linked" may include the situation where a selected nucleotide sequence and regulatory nucleotide sequence (e.g. promoter and/or enhancer) are covalently linked in such a way as to place the expression of the nucleotide sequence under the influence or control of the regulatory sequence (thereby forming an expression cassette). Thus a regulatory sequence is operably linked to the selected nucleotide sequence if the regulatory sequence is capable of effecting transcription of the nucleotide sequence. Where appropriate, the resulting transcript may then be translated into a desired protein or polypeptide.

Any cell suitable for the expression of polypeptides may be used for producing peptides according to the invention. The cell may be a prokaryote or eukaryote. Suitable prokaryotic cells include *E.coli*. Examples of eukaryotic cells include a yeast cell, a plant cell, insect cell or a mammalian cell. In some cases the cell is not a prokaryotic cell

because some prokaryotic cells do not allow for the same post-translational modifications as eukaryotes. In addition, very high expression levels are possible in eukaryotes and proteins can be easier to purify from eukaryotes using appropriate tags. Specific plasmids may also be utilised which enhance secretion of the protein into the media.

Methods of producing a polypeptide of interest may involve culture or fermentation of a cell modified to express the polypeptide. The culture or fermentation may be performed in a bioreactor provided with an appropriate supply of nutrients, air/oxygen and/or growth factors. Secreted proteins can be collected by partitioning culture media/fermentation broth from the cells, extracting the protein content, and separating individual proteins to isolate secreted polypeptide. Culture, fermentation and separation techniques are well known to those of skill in the art.

Bioreactors include one or more vessels in which cells may be cultured. Culture in the bioreactor may occur continuously, with a continuous flow of reactants into, and a continuous flow of cultured cells from, the reactor. Alternatively, the culture may occur in batches. The bioreactor monitors and controls environmental conditions such as pH, oxygen, flow rates into and out of, and agitation within the vessel such that optimum conditions are provided for the cells being cultured.

Following culture of cells that express the polypeptide of interest, that polypeptide is preferably isolated. Any suitable method for separating polypeptides/proteins from cell culture known in the art may be used. In order to isolate a polypeptide/protein of interest from a culture, it may be necessary to first separate the cultured cells from media containing the polypeptide/protein of interest. If the polypeptide/protein of interest is secreted from the cells, the cells may be separated from the culture media that contains the secreted polypeptide/protein by centrifugation. If the polypeptide/protein of interest collects within the cell, it will be necessary to disrupt the cells prior to centrifugation, for example using sonification, rapid freeze-thaw or osmotic lysis. Centrifugation will produce a pellet containing the cultured cells, or cell debris of the cultured cells, and a supernatant containing culture medium and the polypeptide/protein of interest.

It may then be desirable to isolate the polypeptide/protein of interest from the supernatant or culture medium, which may contain other protein and non-protein components. A common approach to separating polypeptide/protein components from a supernatant or culture medium is by precipitation. Polypeptides/proteins of different solubility are

precipitated at different concentrations of precipitating agent such as ammonium sulfate. For example, at low concentrations of precipitating agent, water soluble proteins are extracted. Thus, by adding increasing concentrations of precipitating agent, proteins of different solubility may be distinguished. Dialysis may be subsequently used to remove ammonium sulfate from the separated proteins.

Other methods for distinguishing different polypeptides/proteins are known in the art, for example ion exchange chromatography and size chromatography. These may be used as an alternative to precipitation, or may be performed subsequently to precipitation.

Once the polypeptide/protein of interest has been isolated from culture it may be necessary to concentrate the protein. A number of methods for concentrating a protein of interest are known in the art, such as ultrafiltration or lyophilisation.

Sequence Identity

Alignment for purposes of determining percent amino acid or nucleotide sequence identity can be achieved in various ways known to a person of skill in the art, for instance, using publicly available computer software such as ClustalW 1.82. T-coffee or Megalign (DNASTAR) software. When using such software, the default parameters, e.g. for gap penalty and extension penalty, are preferably used. The default parameters of ClustalW 1.82 are: Protein Gap Open Penalty = 10.0, Protein Gap Extension Penalty = 0.2, Protein matrix = Gonnet, Protein/DNA ENDGAP = -1, Protein/DNA GAPDIST = 4.

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The invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word "comprise," and variations such as "comprises" and "comprising," will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent "about," it will be understood that the particular value forms another embodiment.

Brief Description of the Figures

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures, in which:

Figure 1. Light chain variable domain sequences for anti-IL-2Rβ antibody clones. CDRs are underlined and shown separately.

Figure 2. Heavy chain variable domain sequences for anti-IL-2Rβ antibody clones. CDRs are underlined and shown separately.

Figure 3. Light chain variable domain sequences for anti-γc antibody clones. CDRs are underlined and shown separately.

Figure 4. Heavy chain variable domain sequences for anti-γc antibody clones. CDRs are underlined and shown separately.

Figure 5. Table showing light chain CDR sequences for anti-IL-2Rβ antibody clones.

Figure 6. Table showing heavy chain CDR sequences for anti-IL-2Rβ antibody clones.

Figure 7. Table showing light chain CDR sequences for anti-γc antibody clones.

Figure 8. Table showing heavy chain CDR sequences for anti-γc antibody clones.

Figure 9. Table showing light chain CDR sequences for P2C4-derived anti-IL-2Rβ antibody clones.

Figure 10. Table showing heavy chain CDR sequences for P2C4-derived anti-IL-2Rβ antibody clones.

Figure 11. Table showing light chain CDR sequences for P1A3-derived anti-γc antibody clones.

Figure 12. Table showing heavy chain CDR sequences for P1A3-derived anti-γc antibody clones.

Figure 13. CH2 and CH3 domain sequences for anti-IL-2Rβ antibody clone P2C4.

Figure 14. CH2 and CH3 domain sequences for anti-γc antibody clone P1A3.

Figure 15. Amino acid sequences for anti-IL-2Rβ antibody clones. V_H domains are shown underlined. $(GGGS)_3$ linkers (and variants thereof) are shown in **bold**. V_L domains are shown double underlined. Short linkers are in ***italics and bold***. Hinges are shown in *italics*. CH2 domains are shown dotted underline. CH3 domains are shown dashed underlined.

Figure 16. Amino acid sequences for anti-γc antibody clones. V_H domains are shown underlined. $(GGGS)_3$ linkers (and variants thereof) are shown in **bold**. V_L domains are shown double underlined. Short linkers are in ***italics and bold***. Hinges are shown in *italics*. CH2 domains are shown dotted underline. CH3 domains are shown dashed underlined.

Figure 17. Nucleotide sequences for anti-IL-2Rβ antibody clones.

Figure 18. Nucleotide sequences for anti-γc antibody clones.

Figure 19. Schematic representation of the bispecific anti-IL-2Rβ/γc antibody.

Figure 20. Sensorgrams showing binding of the bispecific anti-IL-2Rβ/γc antibody to (**A**) human IL-2Rβ and (**B**) human γc as determined by surface plasmon resonance analysis.

Figure 21. Histograms showing binding of bispecific anti-IL-2Rβ/γc antibody (**A**) to cells expressing (and control cells not expressing) IL-2Rβ/γc, and (**B**) to PBMCs, as determined by flow cytometry.

Figure 22. Histograms showing induction of STAT5, Akt and ERK mediated signalling by treatment of NK92 cells with IL-2 or bispecific anti-IL-2Rβ/γc antibody *in vitro*, as determined by flow cytometry.

Figure 23. Graph showing percent phosphorylation of STAT5 in response to treatment with IL-2 or bispecific anti-IL-2Rβ/γc antibody *in vitro* for different immune cell subsets, as determined by flow cytometry.

Figure 24. Graph showing proliferation of IL-2 dependent NK92 cells in response to treatment with bispecific anti-IL-2Rβ/γc antibody or control antibodies exhibiting specificity for only IL-2Rβ or γc.

Figure 25. Graphs and schematic showing results of analysis of linker length on binding by anti-IL-2Rβ/γc antibody. (**A**) Schematic representation of antibody and scFv formats, and linkers (linkers shown in *italics*). (**B**) Graph showing proliferation of NK92 cells in response to treatment with bispecific anti-IL-2Rβ/γc antibody comprising linkers of different length. (**C**) Graph showing proliferation of NK92 cells in response to treatment with bispecific anti-IL-2Rβ/γc in bis-scFv format, comprising linkers of different length.

Figure 26. Histograms showing induction of STAT5 signalling in cynomolgus macaque splenocytes by treatment with IL-2 or bispecific anti-IL-2Rβ/γc antibody *in vitro*, as determined by flow cytometry.

Figure 27. Graphs showing T cell numbers and ratios following culture of PBMCs for 1 week in the presence of recombinant human IL-2 or the indicated amount of bispecific anti-IL-2Rβ/γc antibody (Mega2). (**A**) CD3+ cells, (**B**) CD4+ cells, (**C**) CD8+ cells, and (**D**) the ratio of CD8+ to CD4+ cells.

Figure 28. Graph showing the percentage of Tregs following culture of PBMCs for 1 week in the presence of recombinant human IL-2 or the indicated amount of bispecific anti-IL-2Rβ/γc antibody (Mega2).

Figure 29. Graph showing CD8+ T cell subsets as a percentage of CD8+ cells following culture of PBMCs for 1 week in the presence of recombinant human IL-2 or the indicated amount of bispecific anti-IL-2Rβ/γc antibody (Mega2). For each subset, from left to right, the data points are: IL-2 200 ng/ml, Mega2 3ug/ml, Mega2 1ug/ml, Mega2 0.3ug/ml, Mega2 0.3ug/ml and CD3/28.

Figure 30. Graphs showing T cell numbers and ratios following culture of PBMCs from a EBV-seropositive donor in the presence of EBV-LCLs and recombinant human IL-2 or the indicated amount of bispecific anti-IL-2Rβ/γc antibody (Mega2). (**A**) CD3+ cells, (**B**) CD4+ cells, (**C**) CD8+ cells, and (**D**) the ratio of CD8+ to CD4+ cells.

Figure 31. Graphs showing T cell subsets following culture of PBMCs from a EBV-seropositive donor in the presence of EBV-LCLs and recombinant human IL-2 or the indicated amount of bispecific anti-IL-2Rβ/γc antibody (Mega2). (**A**) CD8+ T cell subsets as a percentage of CD8+ cells. For each subset, from left to right, the data points are: IL-2 200 ng/ml, Mega2 3ug/ml, Mega2 1ug/ml, Mega2 0.3ug/ml, Mega2 0.3ug/ml and CD3/28. (**B**) CD8+PD1+ cells as a percentage of CD8+ cells, and (**C**) Tregs as a percentage of CD4+ cells.

Figure 32. Graph showing CTL cytotoxicity following culture of PBMCs from a EBV-seropositive donor in the presence of EBV-LCLs and recombinant human IL-2 or the indicated amount of bispecific anti-IL-2Rβ/γc antibody (Mega2).

Figure 33. Bar charts showing thermostability of P1A3 family clones. Binding of P1A3 and the mutated clones (**A**) B4 and E9 and (**B**) B3 and E8 to γc before and after heat treatment. Mean±SD on duplicates is shown.

Figure 34. Bar charts showing thermostability of P2C4 family clones. Binding of P2C4 and the mutated clones (**A**) A9, B1, B5, B6, B8, C4, C7, C12, E2, E3, E7, E8, E9, G2, G11, H1, H2, and H3, and (**B**) A4, B12, C1, D10, E6, F8, F11 and C1D10 to IL2-Rβ before and after heat treatment. Mean±SD on duplicates is shown.

Figure 35. Graphs showing binding of (**A**) P2C4_FW2 single chain antibody to IL-2Rβ, and (**B**) P1A3_FW2 single chain antibody to γc.

Figure 36. Graphs showing binding of the bispecific antibody clone P2C4/P1A3 to (**A**) IL-2Rβ, and (**B**) γc, for antibodies having the NSGAGTAAA (SEQ ID NO:157) or GGGGSAAA (SEQ ID NO:158) short linkers.

Figure 37. Graphs showing binding of bispecific engineered antibody clones to (**A**) IL-2Rβ, and (**B**) γc.

Figure 38. Graphs showing *in vitro* response of antigen-specific CD8+T cells to bispecific anti-IL-2Rβ/γc antibody exposure, as measured by flow cytometry. (**A**) Bispecific anti-IL-2Rβ/γc antibody-dependent expansion of CD8+ T cells. (**B**) CD8:CD4+ T cell ratio following exposure to the antibody relative to IL-2, following autologous LCL co-culture. *p value <0.05.

Figure 39. Graphs showing *in vitro* response of Treg cells to bispecific anti-IL-2Rβ/γc antibody exposure, as measured by flow cytometry, in (**A**) an antigen-specific, or (**B**) non-specific setting. *p value <0.05.

Figure 40. Bar charts showing levels of cytokines as measured by Luminex analysis (**A**) IFNγ, (**B**) IL-15, (**C**) IL-1β, (**D**) IL-6, and (**E**) TNFα in the plasma of non-human primates, before and after administration of anti-IL-2Rβ/γc antibody.

Figure 41. Bar charts showing *in vivo* response of T cell subsets to bispecific anti-IL-2Rβ/γc antibody injection, as measured by flow cytometry. (**A**) T cells as a proportion of the total leukocyte population, (**B**) Ki-67+ positive CD8+ cells as a proportion of the total CD8+ T cell population. (**C**) Ki-67+ positive CD4+ cells as a proportion of the total CD4+ T cell population. Bispecific antibody dependent expansion is indicated by the increase in T cells relative to the total leukocyte population.

Figure 42. Bar charts showing *in vivo* response of NK cells to bispecific anti-IL-2Rβ/γc antibody injection, as measured by flow cytometry. (**A**) NK cells as a proportion of the pre-dose total leukocyte population, (**B**) Ki-67+ positive NK cells as a proportion of the total NK cell population.

Examples

In the following Examples, the inventors describe the isolation of anti-IL-2Rβ and anti-γc antibodies, construction, engineering and *in vitro* and *in vivo* functional characterisation of bispecific anti-IL-2Rβ/γc antibodies.

Example 1: Isolation of anti-human IL-2Rβ and anti-human γc antibodies

Anti-IL-2Rβ and anti-γc antibodies were isolated from a human antibody phage display library via *in vitro* selection. Specific Fab antibodies were originally identified by ELISA using recombinant IL-2Rβ and γc proteins as antigens.

Example 2: Construction of a bispecific antibody targeting the medium affinity IL-2Rβ-γc

Clones showing a strong binding in ELISA (Example 1) were selected and used to construct a "knob-in-hole" monovalent, bispecific human antibody based on a single chain variable fragment (scFv) linked to a IgG1 backbone Fc region as schematised in Figure 19.

The "knob-in-hole" format prevents homodimerisation and formation of bivalent, monospecific antibodies.

A LALA mutation (substitution of leucine residues 234 and 235 in wild type heavy chain constant domain 2 by alanine) was introduced in the Fc portion of the antibody to abrogate binding to Fc receptor.

The size of the linker between the scFv domain and the Fc domain has no effect on the function of the construct (see Example 6.2 and Figure 25).

Bispecific scFv (bis-scFv) format:

P1A3 and P2C4 scFv were tied with a linker to form a bispecific antibody composed of two single chain variable domains connected by a linker (Figure 25A, right). Different linker sizes were tested (Figure 25C) and activity was tested by measuring NK92 cell growth.

The bis-scFv was effective in maintaining proliferation of NK92 cells in the absence of IL-2. The linker size between the two single chain fragments did not affect the bispecific compound activity (Figure 25C).

Example 3: Analysis of binding to IL-2R chains

The binding of the bispecific antibody to either IL-2Rβ or γc was analysed by flow cytometry.

Antibodies were incubated with HEK-293.6E cells that had previously been transfected with constructs encoding either IL-2Rβ or γc.

Binding to the cells was detected using a fluorescent-conjugated secondary antibody. An isotype IgG1 was used as a negative control. Bispecific constructs with specificity for either IL-2Rβ or γc and for an irrelevant target were also tested.

The anti-IL-2Rβ/γc antibody was shown to bind to cells expressing its targets (Figure 21A).

Example 4: Analysis of affinity for IL-2R chains

Association/dissociation of the anti-IL-2Rβ/γc bispecific antibody to/from the receptor chains was measured in Surface Plasmon Resonance using recombinant IL-2Rβ or γc chains immobilised on a chip, and flowing various concentrations of the antibody over the surface.

The antibody showed a very high affinity for IL-2Rβ or γc chains, with a very rapid binding and a very slow dissociation (Figure 20).

Affinity was measured for bispecific anti-IL-2Rβ/γc antibody P2C4/P1A3, and other bispecific antibodies shown in Table 1.

Table 1:

Bispecific Ab		**K_D (M)**	
anti-IL-2Rβ clone	**anti-γc clone**	**for IL-2Rβ**	**for γc**
P2C4	P1A3	1.43×10^{-7}	2.09×10^{-8}
P2H7	P2B9	1.01×10^{-7}	7.98×10^{-8}
P2D12	P1A3	1.81×10^{-7}	7.87×10^{-8}
P1G11	P1A3	1.28×10^{-7}	3.37×10^{-7}

Example 5: Binding to PBMC subsets

The bispecific IgG anti-IL-2Rβ/γc antibody was tested on PBMCs isolated from healthy donors to check which cell subsets it binds to. Antibody or isotype IgG control were added to PBMCs and detected with a fluorescently-conjugated secondary anti-human IgG antibody in flow cytometry assays.

The anti-IL-2Rβ/γc bispecific antibody did not show high binding for CD4+ or CD8+ T cells. However, the antibody bound efficiently to CD56+ NK cells, CD19+ B cells and CD14+/CD16+ monocytes (Figure 21B).

Example 6: Activity/IL-2 agonistic effects of anti-IL-2Rβ/γc bispecific antibody

6.1 Signalling pathway phosphorylation

IL-2 is known to trigger intracellular signalling via STAT5, ERK and Akt pathways. The anti-IL-2Rβ/γc bispecific antibody was tested for its ability to induce signalling through these pathways.

IL-2-sensitive NK92 cells were deprived from serum and then stimulated either with IL-2 (100 U/ml, i.e. ~0.5 nM^1) or the anti-IL-2Rβ/γc antibody (10μg/ml, i.e. ~95nM^2) for 30 minutes, and phosphorylation of STAT5, Akt and ERK was detected using fluorescent antibodies in flow cytometry assays.

The anti-IL-2Rβ/γc antibody induced STAT5 and Akt phosphorylation, although in a milder way than IL-2 (Figure 22). In this assay, the IL-2Rβ/γc antibody did not trigger phosphorylation of ERK (Figure 22).

One of the biggest obstacles to therapeutic use of IL-2 is the preferential stimulation of cells expressing the high affinity heterotrimeric receptor CD25, e.g. regulatory T cells (Tregs), activated T cells, activated B cells, some myeloid precursor cells, and epithelial cells.

Phosphorylation of STAT5 in the presence of IL-2 or anti-IL-2Rβ/γc antibody was measured by flow cytometry in Tregs, CD8+ T cells and NK cells obtained from healthy donors.

Small amounts of IL-2 were sufficient to activate NK or T cells, but even at low levels of IL-2 Tregs were preferentially and strongly activated. At concentrations giving less than 20% activation of the STAT5 signaling pathway in NK or CD8+ T cells, Tregs already showed 100% activation (Figure 23).

By contrast, the bispecific antibody showed a different activation profile with a lower preferential activation of Tregs. At concentrations resulting in a 20% activation of NK and CD8+ T cells, Tregs showed between 39 and 49% STAT5 phosphorylation. At concentrations giving 50% of activation in NK and CD8+ T cells, the Treg population was still not completely activated, with 73-78% STAT5 phosphorylation (Figure 23).

6.2 Proliferation of IL-2-dependent cells

Viability and growth of NK92 cells was measured with Alamar blue dye in the absence of IL-2.

The anti-IL-2Rβ/γc antibody was able to maintain proliferation of NK92 cells in the absence of IL-2, whilst the antibody constructs binding to only one chain of the IL-2 receptor did not show any effect (Figure 24).

To assess whether the length of the linker had an effect on the functionality of the antibody, the same assay was conducted using antibodies with different linker sizes.

Growth of NK92 cells was not affected by linker size (Figure 25B). The data in Figure 25B were obtained using the shortest and the longest linkers (between 5 and 23 amino-acids).

Linkers of different length were analysed in the bispecific antibody format, or bispecific scFv format, as represented schematically in Figure 25A. Briefly, P1A3 and P2C4 scFv were linked with linkers of different size, and the activity was tested by measuring NK92 cell growth.

The results are shown in Figures 25B and 25C. The bis-scFv is effective in maintaining proliferation of NK92 cells in the absence of IL-2, and the size of the linker between the two scFv fragments does not affect activity.

6.3 Cross-reactivity with cynomolgus monkey cells

The anti-IL-2Rβ/γc antibody was also tested on Non-Human Primate cells. Briefly, Cynomolgus splenocytes were incubated in the presence of human IL-2 or the bispecific antibody and STAT5 phosphorylation was measured. The antibody was found to be cross-reactive with Cynomolgus IL-2R, and triggered phosphorylation of STAT5 as efficiently as human IL-2 (Figure 26).

6.4 Conclusion

Taken together, these data show that the anti-IL-2Rβ/γc bispecific antibody has some agonist effects to IL-2, and that these effects are not highly preferentially directed towards CD25-expressing cells.

Example 7: Modulation of the immune response: control of T cell expansion in a non-specific stimulation setting

Peripheral blood mononuclear cells (PBMCs) were isolated from a volunteer donor and cultured for 1 week in the presence of recombinant human IL-2 (200 ng/ml), the anti-IL-2Rβ/γc bispecific antibody (3, 1, 0.3, 0.1, or 0.03μg/ml) or anti-CD3/CD28 beads as a positive control. After 1 week, cell expansion was assessed by measuring absolute cell counts; cell subset proportions were measured by FACS.

7.1 Expansion of T cells

At comparable concentrations (IL-2 200ng/ml ≈ 12nM; Bispecific anti-IL-2Rβ/γc antibody 3μg/ml ≈ 20nM), the antibody triggers T cell proliferation to a lesser extent than IL-2 (Figure 27A to 27C). The bispecific antibody shows a dose-dependent effect on T cell proliferation (Figure 27A to 27D). In a non-specific stimulation setting, the CD8:CD4 cell ratio was not significantly different in the presence of the anti-IL-2Rβ/γc antibody as compared to when cells were cultured with IL-2 (Figure 27D).

7.2 Stimulation of regulatory T cells

Regulatory T cells (Tregs) express the high affinity IL-2 receptor sub-chain IL-2Rα. In a non-specific stimulation setting, IL-2 preferentially stimulates regulatory T cells amongst CD3+CD4+ T cells; such Treg expansion was not triggered by the bispecific anti-IL-2Rβ/γc antibody (Figure 28).

7.3 Stimulation of effector vs memory cells

With respect to memory CD8+ lymphocytes, the bispecific anti-IL-2Rβ/γc antibody triggers greater expansion of the effector memory CD8+ T cell subset, whilst triggering less expansion of the central memory and naïve CD8+ T cell subsets as compared to expansion in response to stimulation with IL-2 (Figure 29).

Example 8: Modulation of the immune response: control of T cell expansion in a specific stimulation setting

PBMCs from an Epstein-Barr virus (EBV) seropositive volunteer donor were infected with EBV to make lymphoblastoid cell lines (LCLs). LCLs were sorted and γ-irradiated in order to inhibit their subsequent proliferation. Irradiated LCLs were co-cultured at a density of 1 x 10^5 cells/ml with 2 x 10^6 autologous PBMCs/ml for 2 weeks, in the presence of IL-2, the anti-IL-2Rβ/γc bispecific antibody or anti-CD3/CD28 beads (positive control). Cells were then analysed for proliferation and the proportions of different cell subsets.

A cytotoxic killing assay was performed using a fluorescent peptide substrate for granzyme B and capsase 8. Expanded T-cells were co-incubated with live LCLs at a ratio of 2:1 for one hour. Killing was measured by analysis of peptide-fluorescent positive cells by flow cytometry, which indicated that cells were undergoing CTL-induced programmed cell death.

8.1 Expansion of T cells

The bispecific antibody triggers expansion of T cells, even at low concentrations. Anti-IL-2Rβ/γc bispecific antibody-mediated T cell expansion is slightly greater than expansion observed following stimulation with IL-2 (Figure 30A). Whilst the antibody does not significantly influence the number of CD4+ T cells (Figure 30B), the antibody elicits an increase in the number of CD8+ T cells to a greater extent than IL-2 (Figure 30C), and hence increased the CD8:CD4 cell ratio (Figure 30D).

8.2 Effects on T cell subsets

At the highest concentration (1 μg/ml), the anti-IL-2Rβ/γc antibody favours the expansion of effector CD8+ T cells over CD8+ memory cells as compared to stimulation with IL-2 (Figure 31A). Compared to IL-2 stimulation, the anti-IL-2Rβ/γc antibody also triggers an increase in the CD8+ PD-1+ subset (Figure 31B), whilst decreasing the proportion of Tregs (Figure 31C).

8.3 Cytotoxic T lymphocyte-mediated killing

The anti-IL-2Rβ/γc bispecific antibody is able to elicit CTL cytotoxicity. At comparable molarity (12nM (200ng/ml) for IL-2 vs. 7nM (1 µg/mL) for the antibody), the antibody-mediated cytotoxicity is lower than cytotoxicity triggered by IL-2 (Figure 32).

8.4 Conclusion

Taken together, the data suggest that the bispecific anti-IL-2Rβ/γc antibody triggers a different mechanism of action than that of IL-2. The antibody preferentially elicits expansion of effector CD8+ T cells. The antibody allows stimulation of cytotoxic T cells but does not preferentially stimulate Tregs as IL-2 does.

Example 9: Sequence engineering to improve stability

One of the greatest challenges whilst constructing bispecific antibodies is the stability of the heterogenic construct. Unlike monospecific IgGs, the present bispecific anti-IL-2Rβ/γc antibody is an artificial assembling of two different pairs of light/heavy chains.

In order to improve the general stability of constructs, original antibody clones P2C4 and P1A3 were engineered to increase their thermostability.

9.1 Thermostable clones

Libraries of randomly mutagenised clones were built from the parent clones P2C4 and P1A3 and mutants were screened for binding to the respective targets in a two-round panning followed by ELISA. Binders were then subjected to heating to 55°C. The mutants still binding after heating were sequenced, and unique clones were identified.

Thermostability of the clones was assessed after heating for 4 hours between 45°C and 65°C, by measuring binding to their respective target, either γc (Figure 33A and 33B) or IL-2Rβ (Figure 34A and 34B), in ELISA. The mutated clones showed higher thermal stability than the parent clones.

9.1 Engraftment of highly stable framework

In order to further increase stability of the antibody, clones were engrafted in frameworks of antibodies that were known to be highly stable.

P2C4 and P1A3 were engrafted into frameworks of antibodies known to have high stability. ELISA experiments were conducted to ensure that the new clones retained the ability to bind to IL-2Rβ and γc.

Both P2C4_FW2 and P1A3_FW2 showed a dose-dependent binding profile to IL-2Rβ and γc respectively (Figure 35A and 35B).

Example 10: Binding of new bispecific constructs to IL-2Rβ/γc

10.1 Short linker between variable and constant domains

Bispecific antibody constructs were prepared including one of the following short linkers between the scFv and constant domain (antibody format: VH domain – linker – VL domain – short linker – hinge – CH2 domain (+ LALA) – CH3 domain (+ knob/hole + cys)): NSGAGTAAA (SEQ ID NO:157) or GGGGSAAA (SEQ ID NO:158).

Bispecific constructs with NSGAGTAAA (SEQ ID NO:157) or GGGGSAAA (SEQ ID NO:158) short linkers were generated and tested for binding to IL-2Rβ and γc by ELISA.

The bispecific antibodies were found to bind to with similar affinity irrespective of the identity of the short linker (Figures 36A and 36B).

Bispecific antibodies were constructed with the new sequences and binding was assessed by ELISA on either IL-2Rβ or IL-2Rγc. The constructs were found to bind similar or better affinity than the parent bispecific antibodies to IL-2R (Figures 37A and 37B).

Example 11: Effects on T cell expansion and polarisation

Assays using T cells were performed in order to measure the effect of the anti-IL-2Rβ/γc bispecific antibody on T cell expansion *in vitro* and its impact on antigen specific and non-specific qualitative polarisation and subset specificity. Peripheral blood from EBV-positive individuals was used to generate both EBV-transformed lymphoblastoid B-cell lines (LCLs) and EBV-specific CTL lines.

Briefly, to generate LCLs, PBMCs were cultured for 1 week in the presence of cyclosporine and EBV, and for 2 additional weeks in refreshed media with cyclosporine

but without EBV. After culture, cells were transferred to a G-Rex column and growth was monitored. For the generation of CTLs, LCLs were irradiated to act as an antigen source for CTLs. PBMCs were co-cultured with LCLs at an effector to stimulator (E:S) ratio of 40:1. Cells were stimulated by addition of IL-2, the anti-IL-2Rβ/γc bispecific antibody, or CD3/28 beads.

After 7 days, cells underwent a media change and additional stimulations. At day 10, cells were analysed for lymphocyte expansion and phenotype by flow cytometry.

Addition of the bispecific antibody was found to result in a significant increase in antigen-specific CD8+ T cell expansion as compared to expansion in response to stimulation with IL-2 (Figure 38A). Furthermore, *in vitro* cultures showed improved CD8:CD4 ratios following antibody stimulation (Figure 38B).

The impact of the bispecific antibody on the expansion of regulatory T cells (Tregs) was then measured and compared to expansion of Tregs in response to stimulation with IL-2, in the antigen specific (autologous LCL co-culture) and non-specific (anti-CD3/CD28 microbead) settings. Addition of the bispecific antibody results in significantly reduced expansion of Tregs as compared to Treg expansion in response to IL-2, in both the non-specific (Figure 39A) and antigen-specific stimulation settings (Figure 39B).

Example 12: *In vivo* data in Non-Human Primates

A dose escalating experiment was established in Cynomolgus macaques in order to measure the effects of intravenous (iv) injection of the anti-IL-2Rβ/γc bispecific antibody, its ability to drive proliferation of T cells and NK cells, and its potential toxicity through "cytokine storm".

Three macaques were administered a single dose of the anti-IL-2Rβ/γc antibody, intravenously through the femoral artery; macaque A received 1 mg/kg, macaque B received 5 mg/kg, and macaque C received 10mg/kg. Blood was collected before antibody injection and at 1 h, 24 h, 72 h and 120 h post-injection.

Vital signs and physical examinations were performed throughout the study and then for a further 3 weeks. PBMCs were isolated at all time points, leukocyte subsets were analysed by immune-staining and flow cytometry, and cell expansion was assessed by analysis of Ki-67 expression. Plasma cytokine levels were measured by Luminex® at all time points.

Veterinary physical examination indicated no abnormalities in general appearance, mucosal membranes, cardiovascular, respiratory, integumentary, alimentary, musculoskeletal, nervous, urogenital, auditory, or ocular systems. Animals displayed no clinical findings of febrile illness or depression. One animal (macaque B) showed mild weight loss from which he recovered during the course of the study. Animals showed no overt signs of toxicity commonly associated with IL-2 administration (PMID: 1418698 and 8454416).

Consistent with these observations, cytokine analysis demonstrated only mild increases in inflammatory mediators post injection (Figures 40A to 40E). Flow cytometric analysis indicated a marked proliferation of CD4+ and CD8+ T cell populations (Figures 41A to 41C).

NK cell proliferation was also observed in response to the antibody treatment (Figures 42A and 42B). It should be noted that this expansion was observed after a single dose of antibody, compared to continuous infusion or repeated doses required for IL-2, suggesting that anti-IL-2Rβ/γc bispecific antibody has a longer half-life than IL-2.

Claims:

1. A bispecific antibody or bispecific antigen binding fragment, optionally isolated, which is capable of binding to CD122 and common γ chain (γc), wherein the bispecific antibody or bispecific antigen binding fragment is an IL-2 receptor agonist, and comprises:

(a) an antigen-binding fragment which binds to CD122, comprising:

(i) a light chain variable region comprising the following CDRs:

LC-CDR1:	TGTSSDIGHYDFVS	(SEQ ID NO:2)
LC-CDR2:	DINNRPS	(SEQ ID NO:3)
LC-CDR3:	SAYTSSDTLV	(SEQ ID NO:4); and

(ii) a heavy chain variable region comprising the following CDRs:

HC-CDR1:	NYYMH	(SEQ ID NO:36)
HC-CDR2:	AIMPSRGGTSYPQKFQG	(SEQ ID NO:37)
HC-CDR3:	GEYYYDSSGYYY	(SEQ ID NO:38); and

(b) an antigen-binding fragment which binds to common γ chain.

2. The bispecific antibody or bispecific antigen binding fragment according to claim 1, wherein the antigen-binding fragment which binds to CD122 comprises a light chain variable region sequence and a heavy chain variable region sequence, wherein:

the light chain variable region sequence has at least 70% sequence identity to the light chain variable region sequence of SEQ ID NO:1, and

the heavy chain variable region sequence has at least 70% sequence identity to the heavy chain variable region sequence of SEQ ID NO:35.

3. The bispecific antibody or bispecific antigen binding fragment according to claim 1 or claim 2, wherein the antigen-binding fragment which binds to common γ chain comprises a light chain variable region sequence and a heavy chain variable region sequence, wherein the light chain variable region sequence has at least 70% sequence identity to the light chain variable region sequence of SEQ ID NO:67.

4. The bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 3, wherein the antigen-binding fragment which binds to common γ chain comprises:

(i) a light chain variable region comprising the following CDRs:

LC-CDR1:	RSSQSLLHSNGYNYLD	(SEQ ID NO:68)
LC-CDR2:	LGSNRDS	(SEQ ID NO:69)

LC-CDR3: MQGTHWPWT (SEQ ID NO:70); and

(ii) a heavy chain variable region comprising the following CDRs:

HC-CDR1: GYYWS (SEQ ID NO:48)

HC-CDR2: EINHSGSTNYNPSLKS (SEQ ID NO:49)

HC-CDR3: SPGGYSGGYFQH (SEQ ID NO:77).

5. The bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 4, wherein the antigen-binding fragment which binds to common γ chain comprises a light chain variable region sequence and a heavy chain variable region sequence, wherein:

the light chain variable region sequence has at least 70% sequence identity to the light chain variable region sequence of SEQ ID NO:67, and

the heavy chain variable region sequence has at least 70% sequence identity to the heavy chain variable region sequence of SEQ ID NO:76.

6. The bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 5, conjugated to a drug moiety or a detectable moiety.

7. A chimeric antigen receptor (CAR) comprising a bispecific antigen binding fragment according to any one of claims 1 to 5.

8. A cell comprising a chimeric antigen receptor (CAR) according to claim 7.

9. A composition comprising the bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 6, the chimeric antigen receptor (CAR) according to claim 7, or the cell according to claim 8, and at least one pharmaceutically-acceptable carrier.

10. An isolated nucleic acid encoding the bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 6, or the chimeric antigen receptor (CAR) according to claim 7.

11. A vector comprising the isolated nucleic acid of claim 10.

12. A host cell comprising the vector of claim 11.

13. A method for making a bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 6, comprising culturing the host cell of claim 12 under conditions suitable for the expression of the vector encoding the bispecific antibody or bispecific antigen binding fragment.

14. A bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 6, a chimeric antigen receptor (CAR) according to claim 7, a cell according to claim 8, or a composition according to claim 9, for use in therapy, or in a method of medical treatment.

15. A bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 6, a chimeric antigen receptor (CAR) according to claim 7, a cell according to claim 8, or a composition according to claim 9, for use in the treatment of cancer or an infectious disease.

16. A method comprising contacting, *in vitro*, a sample containing, or suspected to contain, CD122 and/or common γ chain (γc) with a bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 6, a chimeric antigen receptor (CAR) according to claim 7, a cell according to claim 8, or a composition according to claim 9, and detecting the formation of a complex of the bispecific antibody or bispecific antigen binding fragment, CAR or cell with CD122 and/or γc.

17. Use of a bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 6, a chimeric antigen receptor (CAR) according to claim 7, a cell according to claim 8, or a composition according to claim 9, for the detection of CD122 and/or common γ chain (γc) *in vitro* .

18. A method for expanding a population of T cells and/or NK cells expressing CD122 and common γ chain (γc), wherein the T cells and/or NK cells are contacted *in vitro* or *ex vivo* with a bispecific antibody or bispecific antigen binding fragment according to any one of claims 1 to 6, a chimeric antigen receptor (CAR) according to claim 7, a cell according to claim 8, or a composition according to claim 9.