C-KIT ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of GB Patent Application No. 1806468.3, filed on April 20, 2018, and GB Patent Application No. 1802201.2, filed on February 9, 2018, the disclosure of each of which is hereby incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: UHFL_001_01WO_SeqList_ST25.txt, date recorded: February 8, 2019, file size 101,274 bytes).

FIELD OF THE INVENTION

The invention relates to antibody molecules binding specifically to C-KIT (also known as KIT, Cluster of Differentiation 117 (CD117), PBT, SCFR, KIT proto-oncogene receptor tyrosine kinase) and medical uses therefor.

BACKGROUND OF THE INVENTION

C-KIT (also known as KIT, Cluster of Differentiation 117 (CD117), PBT, SCFR, KIT proto-oncogene receptor tyrosine kinase) is a transmembrane protein that belongs to the immunoglobulin superfamily and binds to the soluble factor SCF (stem cell factor). C-KIT is a receptor tyrosine kinase type III that is highly expressed by hematopoietic stem cells as well as multiple other cell types, such as mature Mast Cells, where SCF signalling acts as a cytokine. On binding to SCF, this receptor dimerises, activating its tyrosine kinase activity. This kinase activation leads to further downstream activation of signal transduction molecules that play known roles in cell survival, proliferation, and differentiation.

Altered forms of C-KIT, such as constitutively active mutants, are strongly associated with the progression of several important types of cancer, such as Gastrointestinal Stromal Tumours (GIST), Acute Myeloid Leukaemia (AML), Mast Cell tumours and Melanoma. Preclinical and clinical evidence suggests that blocking C-KIT-SCF signalling can have clear therapeutic benefit in multiple cancers, but this has predominantly been achieved using small molecule inhibitors of C-KIT kinase function. Resistance mutations commonly develop after treatment, causing the therapeutic efficacy of the small molecule kinase inhibitor to be lost. Therapeutic antibodies that antagonise KIT signalling by blocking the ability of the receptors to dimerise have the potential to overcome kinase inhibitor resistance and mediate anti-

tumour effects, via two mechanisms: 1. Potent inhibition of the KIT signalling pathway by locking the receptors into a non-activating monomeric form. 2. Antibody effector-function mediated engagement of immune cells. Importantly, it has recently been recognised in preclinical studies that C-KIT-SCF signalling in mast cells found in the tumour microenvironment (via SCF produced by stromal cells), can promote downstream cytokine signalling that recruits myeloid cells such as Myeloid-Derived Suppressor Cells (MDSCs). As MDSCs are believed to be a key cell population that suppress immune responses against tumours, the indirect inhibition of their tumour infiltration via KIT signalling antagonism may be an attractive therapeutic strategy.

The majority of currently approved antibody therapeutics are derived from immunized rodents. Many of those antibodies have undergone a process known as “humanization”, via the “grafting” of murine Complementarity-Determining Regions (CDRs) into human v-gene framework sequences (see Nelson *et al.*, 2010, Nat Rev Drug Discov 9: 767-774). This process is often inaccurate and leads to a reduction in target binding affinity of the resulting antibody. To return the binding affinity of the original antibody, murine residues are usually introduced at key positions in the variable domain frameworks of the grafted v-domains (also known as “back-mutations”).

While antibodies humanized via CDR grafting and back mutations have been shown to induce lower immune response rates in the clinic in comparison to those with fully murine v-domains, antibodies humanized using this basic grafting method still carry significant clinical development risks due to the potential physical instability and immunogenicity motifs still housed in the grafted CDR loops. As animal testing of protein immunogenicity is often non-predictive of immune responses in man, antibody engineering for therapeutic use focuses on minimizing predicted human T-cell epitope content, non-human germline amino acid content and aggregation potential in the purified protein.

The ideal humanized antagonistic anti-C-KIT antibody would therefore have as many residues as possible in the v-domains that are identical to those found in both the frameworks and CDRs of well-characterized human germline sequences. This high level of identity to high-stability germlines that are highly expressed in the maximum number of potential patients minimises the risk of a therapeutic antibody having unwanted immunogenicity in the clinic, or unusually high ‘cost of goods’ in manufacturing.

Townsend *et al.* (2015; PNAS 112: 15354-15359) describe a method for generating antibodies in which CDRs derived from rat, rabbit and mouse antibodies were grafted into

preferred human frameworks and then subject to a human germ-lining approach termed "Augmented Binary Substitution". Although the approach demonstrated a fundamental plasticity in the original antibody paratopes, in the absence of highly accurate antibody-antigen co-crystal structural data, it is still not possible to reliably predict which individual residues in the CDR loops of any given antibody can be converted to human germline, and in what combination. Additionally, the Townsend et al. study did not address the addition of mutagenesis beyond the residues found in the human germline at positions where the removal of development risk motifs might be beneficial. This is a technological limitation which renders the process inherently unsatisfactory as it allows the retention of development liability motifs in CDRs.

CDR germ-lining is thus a complex, multifactorial problem, as multiple functional properties of the molecule should preferably be maintained, including in this instance: target binding specificity, affinity to C-KIT from both human and animal test species (e.g. cynomolgus monkey, also known as the crab-eating macaque, i.e. *Macaca fascicularis*), v-domain biophysical stability and/or IgG yield from protein expression platforms used in research, clinical and commercial supply. Antibody engineering studies have shown that mutation of even single residue positions in key CDRs can have dramatic effects on all of these desired molecular properties.

WO2014018625A1 describes an antagonistic murine anti-C-KIT IgG molecule termed "37M", and also the preparation of humanized forms of 37M. Those humanized forms of 37M were produced using classical humanization techniques, i.e. by grafting of Kabat-defined murine CDRs into human heavy and light chain framework sequences, with some of the human framework residues being potentially back-mutated to the correspondingly positioned 37M murine residues. For reasons noted above, such humanized forms of 37M described in WO2014018625A1 are not ideal.

The present invention provides a number of optimized anti-C-KIT antibodies and medical uses thereof.

SUMMARY OF THE INVENTION

According to one aspect of the invention, there is provided an antibody molecule which specifically binds to human C-KIT, and optionally also to cynomolgus monkey C-KIT, or an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:

an HCDR1 having amino acids in sequence in the following order: G-Y-T-F-T-D or a conservative substitution of D-Y or a conservative substitution of Y-Y-M or a conservative substitution of M-N (SEQ ID NO: 1);
an HCDR2 having amino acids in sequence in the following order: M or a conservative substitution of M (for example, I)-G or a conservative substitution of G (for example, A)-R-I-Y-P-G or a conservative substitution of G (for example, A)-S or a conservative substitution of S (for example, A or T)-G-N-T-Y-Y-A-Q-K-F-Q-G (SEQ ID NO: 2); and
an HCDR3 having amino acids in sequence in the following order: G-V-Y or any amino acid (for example, W or F)-Y or any amino acid (for example, E or H)-F or any amino acid (for example, Y, Q or L)-D or any amino acid (for example, G, N or S)-Y or any amino acid (for example, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V) (SEQ ID NO: 3).

In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GYTFTDYYIN (SEQ ID NO: 4; 37M murine/humanized antibody HCDR1 disclosed in WO2014018625A1), the HCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence IARIYPGSGNTYYNEKFKG (SEQ ID NO: 5; 37M murine/humanized antibody HCDR2 disclosed in WO2014018625A1), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence GVYYFDY (SEQ ID NO: 6; 37M murine/humanized antibody HCDR3 disclosed in WO2014018625A1).

The antibody molecule or antigen-binding portion may further comprise a light chain variable region with:
an LCDR1 having amino acids in sequence in the following order: R-A-S-Q-G-I or a conservative substitution of I-R or a conservative substitution of R-T or any amino acid (such as N)-N or any amino acid (for example, Y)-L-A (SEQ ID NO: 7);
an LCDR2 having amino acids in sequence in the following order: A or any amino acid (for example, S, Y)-A-S-S or any amino acid (for example, Y)-L or any amino acid (for example, R)-Q or any amino acid (for example, Y)-S (SEQ ID NO: 8); and
an LCDR3 having amino acids in sequence in the following order: Q-Q-Y-N or any amino acid (for example, A)-S or any amino acid (for example, N or D)-Y-P-R-T (SEQ ID NO: 9).

In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence KASQNVRTNVA (SEQ ID NO: 10; 37M murine/humanized antibody LCDR1 disclosed in WO2014018625A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence SASYRYS (SEQ ID NO: 11; 37M murine/humanized antibody LCDR2 disclosed in WO2014018625A1). In some embodiments, the LCDR1 of the antibody molecule or antigen-binding portion may exclude

the sequence KASQNVRTNVA (SEQ ID NO: 10; 37M murine/humanized antibody LCDR1 disclosed in WO2014018625A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence SASYRYS (SEQ ID NO: 11; 37M murine/humanized antibody LCDR2 disclosed in WO2014018625A1) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence QQYNSYPRT (SEQ ID NO: 12; 37M murine/humanized antibody LCDR3 disclosed in WO2014018625A1).

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12);

(b) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYANYPRT (SEQ ID NO: 25);

(c) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of SASSLQS (SEQ ID NO: 17) and LCDR3 of QQYANYPRT (SEQ ID NO: 25);

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of SASSLQS (SEQ ID NO: 17) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12);

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYLDY (SEQ ID NO: 18); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNVA (SEQ ID NO: 19), LCDR2 of SASYRQS (SEQ ID NO: 20) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12);

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of

GVYYFDE (SEQ ID NO: 21); and the VL region amino acid sequence comprises LCDR1 of RASQGVRTNLA (SEQ ID NO: 22), LCDR2 of AASSRQS (SEQ ID NO: 23) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12); or

(g) the VH region amino acid sequence comprises HCDR1 of GYTFTDFYMN (SEQ ID NO: 180), HCDR2 of MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26) and HCDR3 of GVWYYDY (SEQ ID NO: 27); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYANYPRT (SEQ ID NO: 25).

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

the VH region amino acid sequence comprises:

(a) HCDR1 of SEQ ID NO: 13;

(b) HCDR2 of SEQ ID NO: 14; and

(c) HCDR3 of SEQ ID NO: 15, SEQ ID NO: 18 or SEQ ID NO: 21; and

the VL region amino acid sequence comprises:

(a') LCDR1 of SEQ ID NO: 16, SEQ ID NO: 19 or SEQ ID NO: 22;

(b') LCDR2 of SEQ ID NO: 24, SEQ ID NO: 17, SEQ ID NO: 20 or SEQ ID NO: 23 and

(c') LCDR3 of SEQ ID NO: 12 or SEQ ID NO: 25.

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises SEQ ID NO:185 and the VL region amino acid sequence comprises SEQ ID NO:186;

(b) the VH region amino acid sequence comprises SEQ ID NO:187 and the VL region amino acid sequence comprises SEQ ID NO:188;

(c) the VH region amino acid sequence comprises SEQ ID NO:189 and the VL region amino acid sequence comprises SEQ ID NO:190;

(d) the VH region amino acid sequence comprises SEQ ID NO:191 and the VL region amino acid sequence comprises SEQ ID NO:192;

(e) the VH region amino acid sequence comprises SEQ ID NO:193 and the VL region amino acid sequence comprises SEQ ID NO:194; or

(f) the VH region amino acid sequence comprises SEQ ID NO:195 and the VL region amino acid sequence comprises SEQ ID NO:196.

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the HCDR1 comprises the amino acid sequence G-Y-T-F-T-X_1-X_2-Y-X_3-N, wherein X_1 is D or a conservative substitution of D, X_2 is Y or a conservative substitution of Y and X_3 is M or a conservative substitution of M (SEQ ID NO: 1);

(b) the HCDR2 comprises X_1-X_2-R-I-Y-P-X_3-X_4-G-N-T-Y-Y-A-Q-K-F-Q-G, wherein X_1 is M or a conservative substitution of M, X_2 is G or a conservative substitution of G, X_3 is G or a conservative substitution of G and X_4 is S or a conservative substitution of S (SEQ ID NO: 2);

(c) the HCDR3 comprises G-V-X_1-X_2-X_3-X_4-X_5, wherein X_1 is Y or any other amino acid, X_2 is Y or any other amino acid, X_3 is F or any other amino acid, X_4 is D or any other amino acid and X_5 is Y or any other amino acid (SEQ ID NO: 3);

(d) the LCDR1 comprises R-A-S-Q-G-X_1-X_2-X_3-X_4-L-A, wherein X_1 is I or a conservative substitution of I, X_2 is R or a conservative substitution of R, X_3 is T or any other amino acid and X_4 is N or any other amino acid (SEQ ID NO: 7);

(e) the LCDR2 comprises X_1-A-S-X_2-X_3-X_4-S, wherein X_1 is A or any other amino acid, X_2 is S or any other amino acid, X_3 is L or any other amino acid and X_4 is Q or any other amino acid (SEQ ID NO: 8); and

(f) the LCDR3 comprises Q-Q-Y-X_1-X_2-Y-P-R-T, wherein X_1 is N or any other amino acid and X_2 is S or any other amino acid (SEQ ID NO: 9).

Also provided according to the invention is an immunoconjugate comprising the antibody molecule or antigen-binding portion thereof as defined herein linked to a therapeutic agent.

In another aspect the invention provides nucleic acid molecule encoding the antibody molecule or antigen-binding portion thereof as defined herein.

Further provided is a vector comprising the nucleic acid molecule of the invention.

Also provided is a host cell comprising the nucleic acid molecule or the vector of the invention as defined herein.

In a further aspect there is provided a method of producing an anti-C-KIT antibody and/or an antigen-binding portion thereof, comprising culturing the host cell of the invention under conditions that result in expression and/or production of the antibody and/or the antigen-

binding portion thereof, and isolating the antibody and/or the antigen-binding portion thereof from the host cell or culture.

In another aspect of the invention there is provided a pharmaceutical composition comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein.

Further provided is a method for enhancing an immune response in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

In a further aspect there is provided a method for treating or preventing cancer in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

The invention also provides an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein, for use in the treatment of cancer.

In another aspect the invention provides the antibody molecule, or antigen-binding portion thereof, or the immunoconjugate, or the nucleic acid molecule, or the vector for use, or the method of treatment of the invention as defined herein, for separate, sequential or simultaneous use in a combination with a second therapeutic agent, for example an anti-cancer agent.

In a further aspect there is provided the use of an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or an immunoconjugate of the invention as defined herein, or a nucleic acid molecule of the invention as defined herein, or a vector

of the invention as defined herein, or a pharmaceutical composition of the invention as defined herein, in the manufacture of a medicament for the treatment of cancer.

The invention also provides a method for treating or preventing an autoimmune disease or an inflammatory disease in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

The autoimmune disease or inflammatory disease may be selected in all aspects from the group consisting of: arthritis, asthma, multiple sclerosis, psoriasis, Crohn's disease, inflammatory bowel disease, lupus, Grave's disease and Hashimoto's thyroiditis, and ankylosing spondylitis.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of an autoimmune disease or an inflammatory disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of an autoimmune disease or an inflammatory disease.

The invention also provides a method for treating or preventing a cardiovascular disease or a fibrotic disease in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

Further provided herein is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use as a medicament. Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical

composition as defined herein, for use in the treatment of a cardiovascular disease or a fibrotic disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of an autoimmune disease, an inflammatory disease or a fibrotic disease.

The cardiovascular disease in any aspect of the invention may for example be coronary heart disease or atherosclerosis.

The fibrotic disease in any aspect of the invention may be selected from the group consisting of myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, cystic fibrosis, bronchitis and asthma.

The invention also provides a method of producing an antibody molecule which specifically binds to human C-KIT and optionally also to cynomolgus monkey C-KIT, or an antigen-binding portion thereof, comprising the steps of:

(1) grafting anti-C-KIT CDRs from a non-human source into a human v-domain framework to produce a humanized anti-C-KIT antibody molecule or antigen-binding portion thereof;

(2) generating a phage library of clones of the humanized anti-C-KIT antibody molecule or antigen-binding portion thereof comprising one or more mutations in the CDRs;

(3) screening the phage library for binding to human C-KIT and optionally also to cynomolgus monkey C-KIT;

(4) selecting clones from the screening step (3) having binding specificity to human C-KIT and optionally also to cynomolgus monkey C-KIT; and

(5) producing an antibody molecule which specifically binds to human C-KIT and optionally also to cynomolgus monkey C-KIT, or an antigen-binding portion thereof from clones selected from step (4).

The method may comprise a further step of producing additional clones based on the clones selected in step (4), for example based on further exploratory mutagenesis at specific positions in the CDRs of the clones selected in step (4), to enhance humanization and/or minimise human T cell epitope content and/or improve manufacturing properties in the antibody molecule or antigen-binding portion thereof produced in step (5).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A – FIG. 1B. Direct binding ELISA and HTRF competition screening of library-derived anti-C-KIT scFvs against human and cyno C-KIT-Fc proteins. Clones were derived from separate phage selection branches on biotinylated human and/or cynomolgus monkey C-KIT-Fc proteins in each round. After multiple rounds of selection, library-derived clones (black circles) were screened for binding against both human (FIG. 1A) and cyno (FIG. 1B) C-KIT-Fc and epitope blocking of h37M IgG1 against both orthologs. Negative control (non-C-KIT-binding) and positive control h37M scFvs are represented in grey triangles and squares, respectively.

FIG. 2A – FIG. 2B. Analysis of CDR residue tolerance for mutation to germline. A plot of murine amino acid retention frequencies in the CDRs (SEQ ID NOs: 4, 5 and 6) of the ELISA-positive population of 219 unique scFv clones is shown for V_H (FIG. 2A) and V_L (FIG. 2B) domains, respectively. Only those residues targeted for human/murine residue mutagenesis are plotted, other than in the HCDR3. CDR residues noted in parentheses on the X-axes were identical to those found in the human germlines used for grafting (IGKV1-16 and IGHV1-46). Those residues in the HCDR2 that are not in parentheses, but whose values are set at 0, were mutated to human germline during the grafting process. In both plots the dashed line in grey at 75% represents the cut off for tolerance of murine residue replacement by human germline.

FIG. 3A – FIG. 3B. Direct titration ELISA for IgG binding to human and cyno C-KIT-Fc proteins. Chimeric anti-C-KIT (m37M), humanized h37M, library-derived and designer clones in human IgG1 format were titrated (in μg/ml) in a direct binding ELISA against human (FIG. 3A) and cyno (FIG. 3B) C-KIT-Fc proteins. The 37M, library-derived clones and designer clone MH demonstrated binding activity against both orthologs of C-KIT. All library derived and several designer clones retained approximately equivalent or improved human and cyno C-KIT binding, while all TTP clones and MH clones 4, 6, 7, 9, 13, 14 and 15 all exhibited reduced binding signal on one or both orthologs.

FIG. 4A – FIG. 4E. HTRF-based solution-phase, high-sensitivity, C-KIT epitope competition assay. HTRF binding signal for the h37M IgG to human or cyno C-KIT was examined in the presence of titrated competitor IgGs including library-derived and designer leads, plus Isotype IgG1 as a negative control and unlabelled h37M IgG1 as a positive control. All library-derived and multiple designer IgGs exhibited full concentration-dependent inhibition of h37M binding to human (FIG. 4A) and cyno (FIG. 4C, FIG. 4D) c-KIT, similar to unlabelled h37M IgG, suggesting maintenance of a shared epitope and binding affinity. All TTP clones, plus MH clones 4, 6, 7 and 9 were found to have reduced ability to inhibit h37M binding to either human (FIG. 4B) or cyno (FIG. 4E) C-KIT.

FIG. 5A – FIG. 5F. Flow cytometric binding to human and cyno C-KIT+ CHO-K1 cells

for library-derived and primary designer leads. Anti-C-KIT controls m37M and h37M, library-derived and designer leads in IgG1 format were examined for specific binding on human C-KIT-transfected CHO-K1 cells (FIG. 5A, FIG. 5B), cyno C-KIT-transfected CHO-K1 cells (FIG. 5C, FIG. 5D), and wild type (WT, i.e. untransfected) CHO-K1 cells (FIG. 5E, FIG. 5F). IgGs were tested at concentrations ranging from 0.02-100 μg/ml. Concentration-dependent binding was observed against both human and cyno cell lines for all C-KIT-specific antibodies but not isotype controls. No binding signals above background were observed against wild type CHO-K1 cells.

FIG. 6A – FIG. 6B. Direct titration ELISA for second-generation designer IgGs binding to human and cyno C-KIT-Fc proteins. Anti-C-KIT h37M, MH5 and second-generation designer clones in human IgG1 format were titrated (in μg/ml) in a direct binding ELISA against human (FIG. 6A) and cyno (FIG. 6B) C-KIT-Fc proteins. The h37M and MH5 clones demonstrated binding activity against both orthologs of C-KIT. All designer clones other than MH5.33 retained human and cyno C-KIT binding, but only clones MH5.1, 5.2, 5.3, 5.22, 5.23, 5.24, 5.34 and 5.35 exhibited binding comparable to clone h37M on both orthologs.

FIG. 7A – FIG. 7B. HTRF-based C-KIT epitope competition assay for key second-generation designer leads. HTRF binding signal for the h37M IgG to human or cyno C-KIT was examined in the presence of titrated competitor second-generation designer leads in IgG1 format, plus isotype IgG1 as a negative control and unlabelled h37M IgG1 as a positive control. All IgGs (other than the Isotype control IgG1) exhibited full concentration-dependent inhibition of h37M binding to human (FIG. 7A) and cyno (FIG. 7B) C-KIT, similar to unlabelled h37M IgG.

FIG. 8A – FIG. 8C. Flow cytometric binding to human and cyno C-KIT+ CHO-K1 cells for second-generation designer leads. Anti-C-KIT library-derived and designer leads in IgG1 format were examined for specific binding on human C-KIT-transfected CHO-K1 cells (FIG. 8A), cyno C-KIT-transfected CHO-K1 cells (FIG. 8B), and wild type (wt, i.e. untransfected) CHO-K1 cells (FIG. 8C). IgGs were tested at concentrations ranging from 24-100,000 ng/ml. Concentration-dependent binding was observed against both human and cyno cell lines for all C-KIT-specific antibodies but not Isotype controls. No binding signals above background were observed against wild type CHO-K1 cells.

FIG. 9A – FIG. 9F. Binding specificity analyses for prioritized lead clones. Off-target homologue binding risk for h37M and multiple lead antibodies in IgG1 format at 1 μg/ml was examined by direct ELISA on C-KIT-Fc orthologs and a panel of 14 human immunoglobulin superfamily proteins. For all IgGs, binding was observed to human and cyno C-KIT-Fc alone. No binding above background was observed for any other human or ortholog protein.

FIG. 10A – FIG. 10C. Development risk ELISAs. This assay showed that the E-C7, E-C3, MH1, MH5, MH5.22 and h37M antibodies in IgG1 form exhibit equivalent or lower binding to

the negatively charged biomolecules Insulin (FIG. 10A), double-stranded DNA (dsDNA) (FIG. 10B) and single-stranded DNA (ssDNA) (FIG. 10C) than the negative control IgG1 Ustekinumab analogue. Strong off-target binding to these molecules, as observed for Bococizumab and Briakinumab analogues has been shown to be a high-risk indicator of poor pharmacokinetics of therapeutic antibodies.

FIG. 11A – FIG. 11H. T cell epitope peptide content in lead antibody v-domains. The v-domains of h37M (FIG. 11A), E-C7 (FIG. 11B), F-C5 (FIG. 11C), E-C3 (FIG. 11D), MH1 (FIG. 11E), MH5 (FIG. 11F), MH5.22 (FIG. 11G) and MH5-DI (FIG. 11H) antibodies were examined for the presence of Germline (GE), High Affinity Foreign (HAF), Low Affinity Foreign (LAF) and TCED+ T cell receptor epitopes. Both the VH and VL domains of h37M were found to contain multiple high-risk human T cell epitopes and few germline epitopes. In all lead clones, the high-risk epitope content was significantly reduced and germline epitope content improved.

FIG. 12A – FIG. 12C. Affinity analyses for IgG1 Fc engineered variants on human FcγRIIb. Purified anti-KIT IgGs were analysed for binding affinity to FcγRIIb in a 'steady state' affinity measurement on a BIACORE® T200 instrument. Clones h37M-IgG1 (FIG. 12A), h37M-IgG1-3M (FIG. 12B) and MH1-IgG-4M (FIG. 12C) all exhibited measurable binding to FcγRIIb, with MH1-IgG-4M showing the lowest overall binding signal across the full concentration range. In A-C, the raw data for chip interaction is shown in each upper panel and the plotted curves for Response Units (RU) per concentration are shown in the lower panel.

FIG. 13A – FIG. 13C. Human proteome re-array analyses. After a full screen of 5528 unique proteins, analyses of binding specificity were performed on chips in which plasmids encoding potentially relevant targets were arrayed and used to transfect HEK293 cells. Transfection of all plasmids was confirmed by screening for the co-encoded marker ZS green (FIG. 13A). Separate chips were then probed using Rituximab analog and secondary labelled antibody only (FIG. 13A), h37M at 0.5 and 2 μg/ml (FIG. 13B) and MH1 at 0.5 and 2 μg/ml (FIG. 13C). These analyses confirmed that h37M and MH1 both exhibited highly specific binding to C-KIT. Binding signals to MMP7, CRIM1 and F13A1 on all chips were found to be an artefact of the secondary antibody (FIG. 13A).

FIG. 14A – FIG. 14C. Anti-C-KIT cell killing assays. Internalisation and toxin delivery was examined on CHO cells transfected with human (FIG. 14A) and cyno (FIG. 14B) C-KIT, and the human erythroleukaemia cell line TF-1 (FIG. 14C), in the presence of FabZAP reagent. Clones h37M, MH1 and MH5-DI in IgG1-3M format all drove highly similar, high-potency cell killing.

FIG. 15A – FIG. 15B. HTRF-based C-KIT epitope competition assay for h37M, MH1, MH5. HTRF binding signal for the h37M IgG to human or cyno C-KIT was examined in the

presence of titrated competitor MH1 and MH5 in IgG1-3M format, plus isotype IgG1 as a negative control and unlabelled h37M IgG1-3M as a positive control. All IgGs (other than the Isotype control IgG1) exhibited full concentration-dependent inhibition of h37M binding to human (FIG. 15A) and cyno (FIG. 15B) C-KIT, overlapping with unlabelled h37M.

FIG. 16. TF-1 cell-based assay for inhibition of C-KIT/SCF-driven cellular proliferation. Human TF-1 cell line was cultured in the presence of SCF, which promotes cell proliferation through C-KIT. Antibodies were applied for 72 hours and proliferation measured via resazurin fluorescence. h37M was found to be unexpectedly significantly more potent than any other antibody tested.

DETAILED DESCRIPTION OF THE INVENTION

According to a first aspect of the invention, there is provided an antibody molecule which specifically binds to human C-KIT and optionally also to cynomolgus monkey C-KIT, or an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:

an HCDR1 (heavy chain complementarity determining region 1) having amino acids in sequence in the following order: G-Y-T-F-T-D or a conservative substitution of D-Y or a conservative substitution of Y-Y-M or a conservative substitution of M-N (SEQ ID NO: 1);

an HCDR2 (heavy chain complementarity determining region 2) having amino acids in sequence in the following order: M or a conservative substitution of M (for example, I)-G or a conservative substitution of G (for example, A)-R-I-Y-P-G or a conservative substitution of G (for example, A)-S or a conservative substitution of S (for example, A or T)-G-N-T-Y-Y-A-Q-K-F-Q-G (SEQ ID NO: 2); and

an HCDR3 (heavy chain complementarity determining region 3) having amino acids in sequence in the following order: G-V-Y or any amino acid (for example, W or F)-Y or any amino acid (for example, E or H)-F or any amino acid (for example, Y, Q or L)-D or any amino acid (for example, G, N or S)-Y or any amino acid (for example, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V) (SEQ ID NO: 3).

In some aspects an anti-C-KIT antibody or antigen-binding portion provided herein specifically binds to a C-KIT protein comprising or consisting of SEQ ID NO:208. In some aspects an anti-C-KIT antibody or antigen-binding portion provided herein specifically binds to a C-KIT protein having an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:208. In some aspects an anti-C-KIT antibody or antigen-binding portion provided

herein specifically binds to a C-KIT protein encoded by a nucleic acid molecule comprising or consisting of SEQ ID NO:209 or SEQ ID NO:210.

In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GYTFTDYYIN (SEQ ID NO: 4; 37M murine/humanized antibody HCDR1 disclosed in WO2014018625A1), the HCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence IARIYPGSGNTYYNEKFKG (SEQ ID NO: 5; 37M murine/humanized antibody HCDR2 disclosed in WO2014018625A1), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence GVYYFDY (SEQ ID NO: 6; 37M murine/humanized antibody HCDR3 disclosed in WO2014018625A1).

The antibody molecule or antigen-binding portion may further comprise a light chain variable region with:

an LCDR1 (light chain complementarity determining region 1) having amino acids in sequence in the following order: R-A-S-Q-G-I or a conservative substitution of I-R or a conservative substitution of R-T or any amino acid (such as N)-N or any amino acid (for example, Y)-L-A (SEQ ID NO: 7);

an LCDR2 (light chain complementarity determining region 2) having amino acids in sequence in the following order: A or any amino acid (for example, S, Y)-A-S-S or any amino acid (for example, Y)-L or any amino acid (for example, R)-Q or any amino acid (for example, Y)-S (SEQ ID NO: 8); and

an LCDR3 (light chain complementarity determining region 3) having amino acids in sequence in the following order: Q-Q-Y-N or any amino acid (for example, A)-S or any amino acid (for example, N or D)-Y-P-R-T (SEQ ID NO: 9).

In some aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence KASQNVRTNVA (SEQ ID NO: 10; 37M murine/humanized antibody LCDR1 disclosed in WO2014018625A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence SASYRYS (SEQ ID NO: 11; 37M murine/humanized antibody LCDR2 disclosed in WO2014018625A1). In some embodiments, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence KASQNVRTNVA (SEQ ID NO: 10; 37M murine/humanized antibody LCDR1 disclosed in WO2014018625A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence SASYRYS (SEQ ID NO: 11; 37M murine/humanized antibody LCDR2 disclosed in WO2014018625A1) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence QQYNSYPRT (SEQ ID NO: 12; 37M murine/humanized antibody LCDR3 disclosed in WO2014018625A1).

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the HCDR1 comprises the amino acid sequence G-Y-T-F-T-X_1-X_2-Y-X_3-N, wherein X_1 is D or a conservative substitution of D, X_2 is Y or a conservative substitution of Y and X_3 is M or a conservative substitution of M (SEQ ID NO: 1);

(b) the HCDR2 comprises X_1-X_2-R-I-Y-P-X_3-X_4-G-N-T-Y-Y-A-Q-K-F-Q-G, wherein X_1 is M or a conservative substitution of M (for example, I), X_2 is G or a conservative substitution of G (for example, A), X_3 is G or a conservative substitution of G (for example, A) and X_4 is S or a conservative substitution of S (for example, A or T) (SEQ ID NO: 2);

(c) the HCDR3 comprises G-V-X_1-X_2-X_3-X_4-X_5, wherein X_1 is Y or any other amino acid (for example, W or F), X_2 is Y or any other amino acid (for example, E or H), X_3 is F or any other amino acid (for example, Y, Q or L), X_4 is D or any other amino acid (for example, G, N or S) and X_5 is Y or any other amino acid (for example, A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T or V) (SEQ ID NO: 3);

(d) the LCDR1 comprises R-A-S-Q-G-X_1-X_2-X_3-X_4-L-A, wherein X_1 is I or a conservative substitution of I, X_2 is R or a conservative substitution of R, X_3 is T or any other amino acid (for example, N) and X_4 is N or any other amino acid (for example, Y) (SEQ ID NO: 7);

(e) the LCDR2 comprises X_1-A-S-X_2-X_3-X_4-S, wherein X_1 is A or any other amino acid (for example, S or Y), X_2 is S or any other amino acid (for example, Y), X_3 is L or any other amino acid (for example, R) and X_4 is Q or any other amino acid (for example, Y) (SEQ ID NO: 8); and

(f) the LCDR3 comprises Q-Q-Y-X_1-X_2-Y-P-R-T, wherein X_1 is N or any other amino acid (for example, A) and X_2 is S or any other amino acid (for example, N or D) (SEQ ID NO: 9).

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising, in amino-terminal to carboxyl-terminal order, FR1-HCDR1-FR2-HCDR2-FR3-HCDR3-FR4 and a light chain variable (VL) region comprising, in amino-terminal to carboxyl-terminal order, FR1-LCDR1-FR2-LCDR2-FR3-LCDR3-FR4, wherein the HCDR1 is SEQ ID NO:1, the HCDR2 is SEQ ID NO:2, the HCDR3 is SEQ ID NO:3, the LCDR1 is SEQ ID NO:7, the LCDR2 is SEQ ID NO:8 and the LCDR3 is SEQ ID NO:9, wherein the heavy chain FR1, FR2, FR3 and FR4 amino acid sequences are the heavy chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO: 89 (see Table 2) and wherein the light chain FR1,

FR2, FR3 and FR4 amino acid sequences are the light chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO: 92 (see Table 2).

As elaborated herein, the present inventors have succeeded for the first time in generating a number of optimized anti-C-KIT antibody molecules using CDR sequences derived from the murine anti-C-KIT antibody 37M disclosed in WO2014018625A1. In embodiments of the present invention, these antibody molecules have been selected to have binding specificity to both human C-KIT as well as cynomolgus monkey C-KIT (to facilitate *in vivo* studies in an appropriate animal test species). Further refining of the optimized antibody molecules as described herein has provided improved variable domain stability, higher expression yields, and/or reduced immunogenicity.

Preferred optimized anti-C-KIT antibody molecules of the present invention do not necessarily have the maximum number of human germline substitutions at corresponding murine CDR or other (such as framework) amino acid positions. As elaborated in the experimental section below, we have found that “maximally humanized” antibody molecules are not necessary “maximally optimized” in terms of anti-C-KIT binding characteristics and/or other desirable features.

The present invention encompasses modifications to the amino acid sequence of the antibody molecule or antigen-binding portion thereof as defined herein. For example, the invention includes antibody molecules and corresponding antigen-binding portions thereof comprising functionally equivalent variable regions and CDRs which do not significantly affect their properties as well as variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to C-KIT. Insertions which include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues, are envisaged. Examples of terminal insertions include an antibody molecule with an N-terminal methionyl residue or the antibody molecule fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

The antibody molecule or antigen-binding portion of the invention may include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. The antibody molecule or antigen-binding portion of the invention may be

mutated to alter such post-translational modifications, for example by adding, removing or replacing one or more amino acid residues to form or remove a glycosylation site.

The antibody molecule or antigen-binding portion of the invention may be modified for example by amino acid substitution to remove potential proteolytic sites in the antibody.

In the antibody molecule or antigen-binding portion thereof, the HCDR1 may have the amino acid sequence: G-Y-T-F-T-D/N-Y/H/F-Y-M/I-N (SEQ ID NO: 28); the HCDR2 may have the amino acid sequence: M/I-G/A-R-I-Y-P-G/A-S/T/A-G-N-T-Y-Y-A-Q-K-F-Q-G (SEQ ID NO: 29); and the HCDR3 may have the amino acid sequence: G-V-Y/W/F-Y/E/H-F/Y/Q/L--D/G/N/S-Y/A/E/F/GH/I/K/L/M/N/P/Q/R/S/T/V (SEQ ID NO: 30).

For example, the HCDR1 may have the amino acid sequence: G-Y-T-F-T-D/N-Y/F-Y-M-N (SEQ ID NO: 31); the HCDR2 may have the amino acid sequence: M/I-G-R-I-Y-P-G/A-S-G-N-T-Y-Y-A-Q-K-F-Q-G (SEQ ID NO: 32); and the HCDR3 may have the amino acid sequence: G-V-Y/W-Y/E/H-Y/Q/L--D-Y/S/T/E (SEQ ID NO: 33).

In the antibody molecule or antigen-binding portion thereof, the LCDR1 may have the amino acid sequence: R-A-S-Q-G-I/V-R-T/N-N-L-A (SEQ ID NO: 34); the LCDR2 may have the amino acid sequence: A/S/Y-A-S-S-L-Q-S (SEQ ID NO: 35); and the LCDR3 may have the amino acid sequence: Q-Q-Y-N/A/S/E/T-S/A/N/D-Y-P-R-T (SEQ ID NO: 36).

For example, the LCDR1 may have the amino acid sequence: R-A-S-Q-G-I-R-T/N-N-L-A (SEQ ID NO: 37); the LCDR2 may have the amino acid sequence: A-A-S-S-L-Q-S (SEQ ID NO: 24); and the LCDR3 may have the amino acid sequence: Q-Q-Y-N/A-S/N-Y-P-R-T (SEQ ID NO: 38).

In specific embodiments of the invention, the antibody molecule or antigen-binding portion may comprise:

(a) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVWYFDY (HCDR3; SEQ ID NO: 40), RASQGVRTNVA (LCDR1; SEQ ID NO: 39), SASSLQS (LCDR2; SEQ ID NO: 17), QQYNSYPRT (LCDR3; SEQ ID NO: 12), [Clone E-E10]; or

(b) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYQDY (HCDR3; SEQ ID NO: 41), RASQGVRTNVA (LCDR1; SEQ ID NO: 39), AASSRQS (LCDR2; SEQ ID NO: 23) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone F-F2];

(c) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYEFDY (HCDR3; SEQ ID NO: 42), RASQGVRNNLA (LCDR1; SEQ ID NO: 43), AASYRQS (LCDR2; SEQ ID NO: 44) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone C-B12];

(d) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), IGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 45), GVYYFDS (HCDR3; SEQ ID NO: 46), RASQGVRNNVA (LCDR1; SEQ ID NO: 47), YASSLQS (LCDR2; SEQ ID NO: 48) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone C-A7];

(e) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYHFDY (HCDR3; SEQ ID NO: 49), RASQGVRNNVA (LCDR1; SEQ ID NO: 47), AASYLQS (LCDR2; SEQ ID NO: 50) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone C-A5];

(f) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYFDT (HCDR3; SEQ ID NO: 51), RASQGVRTNLA (LCDR1; SEQ ID NO: 22), AASSRQS (LCDR2; SEQ ID NO: 23) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone D-A10];

(g) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone E-C7];

(h) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGVRTNLA (LCDR1; SEQ ID NO: 22), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone D-D5];

(i) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYLDY (HCDR3; SEQ ID NO: 18), RASQGIRTNVA (LCDR1; SEQ ID NO: 19), SASYRQS (LCDR2; SEQ ID NO: 20) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone E-C2];

(j) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYLDY (HCDR3; SEQ ID NO: 18), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASYRQS (LCDR2; SEQ ID NO: 44) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone F-B11];

(k) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYLDY (HCDR3; SEQ ID NO: 18), RASQGVRTNVA (LCDR1; SEQ ID NO: 39), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone D-D9];

(l) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYFDE (HCDR3; SEQ ID NO: 21), RASQGVRTNVA (LCDR1; SEQ ID NO: 39), SASSRQS (LCDR2; SEQ ID NO: 52) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone E-G7];

(m) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYFDE (HCDR3; SEQ ID NO: 21), RASQGVRTNLA (LCDR1; SEQ ID NO: 22), AASSRQS (LCDR2; SEQ ID NO: 23) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone F-C5];

(n) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone MH1];

(o) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYASYPRT (LCDR3; SEQ ID NO: 53) [Clone MH2];

(p) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYNAYPRT (LCDR3; SEQ ID NO: 54) [Clone MH3];

(q) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYNDYPRT (LCDR3; SEQ ID NO: 55) [Clone MH4];

(r) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5];

(s) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYNSYPKT (LCDR3; SEQ ID NO: 56) [Clone MH6];

(t) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYNSYPHT (LCDR3; SEQ ID NO: 57) [Clone MH7];

(u) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYSSYPRT (LCDR3; SEQ ID NO: 58) [Clone MH8];

(v) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYESYPRT (LCDR3; SEQ ID NO: 59) [Clone MH9];

(w) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), SASSLQS (LCDR2; SEQ ID NO: 17) and QQYTSYPRT (LCDR3; SEQ ID NO: 60) [Clone MH10];

(x) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYNAYPRT (LCDR3; SEQ ID NO: 54) [Clone MH11];

(y) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGVRTNLA (LCDR1; SEQ ID NO: 22), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone MH12];

(z) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGVRTNLA (LCDR1; SEQ ID NO: 22), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYNAYPRT (LCDR3; SEQ ID NO: 54) [Clone MH13];

(aa) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGVRTNLA (LCDR1; SEQ ID NO: 22), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYNSYPRT (LCDR3; SEQ ID NO: 12) [Clone MH14];

(bb) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGVRTNLA (LCDR1; SEQ ID NO: 22), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYNAYPRT (LCDR3; SEQ ID NO: 54) [Clone MH15];

(cc) the amino acid sequences GYTFTNYYMN (HCDR1; SEQ ID NO: 181), MGRIYPGTGNTYYAQKFQG (HCDR2; SEQ ID NO: 61), GVWYYDY (HCDR3; SEQ ID NO: 27), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5.1];

(dd) the amino acid sequences GYTFTDFYMN (HCDR1; SEQ ID NO: 180), MGRIYPGTGNTYYAQKFQG (HCDR2; SEQ ID NO: 61), GVWYYDY (HCDR3; SEQ ID NO: 27), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5.2];

(ee) the amino acid sequences GYTFTDFYMN (HCDR1; SEQ ID NO: 180), MGRIYPASGNTYYAQKFQG (HCDR2; SEQ ID NO: 26), GVWYYDY (HCDR3; SEQ ID NO: 27), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5.22];

(ff) the amino acid sequences GYTFTDFYMN (HCDR1; SEQ ID NO: 180), MGRIYPASGNTYYAQKFQG (HCDR2; SEQ ID NO: 26), GVWYFDS (HCDR3; SEQ ID NO: 62), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5.23];

(gg) the amino acid sequences GYTFTDFYMN (HCDR1; SEQ ID NO: 180), MGRIYPASGNTYYAQKFQG (HCDR2; SEQ ID NO: 26), GVWYFDT (HCDR3; SEQ ID NO: 63), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5.24];

(hh) the amino acid sequences GYTFTNYYMN (HCDR1; SEQ ID NO: 181), MGRIYPASGNTYYAQKFQG (HCDR2; SEQ ID NO: 26), GVWYFDS (HCDR3; SEQ ID NO: 62), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5.34];

(ii) the amino acid sequences GYTFTNYYMN (HCDR1; SEQ ID NO: 181), MGRIYPASGNTYYAQKFQG (HCDR2; SEQ ID NO: 26), GVWYFDT (HCDR3; SEQ ID NO: 63), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5.35];

(jj) the amino acid sequences GYTFTDYYMN (HCDR1; SEQ ID NO: 13), MGRIYPGSGNTYYAQKFQG (HCDR2; SEQ ID NO: 14), GVYYYDY (HCDR3; SEQ ID NO: 15), RASQGIRTNLA (LCDR1; SEQ ID NO: 16), AASSLQS (LCDR2; SEQ ID NO: 24) and QQYANYPRT (LCDR3; SEQ ID NO: 25) [Clone MH5-DI].

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12);

(b) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYANYPRT (SEQ ID NO: 25);

(c) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of SASSLQS (SEQ ID NO: 17) and LCDR3 of QQYANYPRT (SEQ ID NO: 25);

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of SASSLQS (SEQ ID NO: 17) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12);

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYLDY (SEQ ID NO: 18); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNVA (SEQ ID NO: 19), LCDR2 of SASYRQS (SEQ ID NO: 20) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12);

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYFDE (SEQ ID NO: 21); and the VL region amino acid sequence comprises LCDR1 of RASQGVRTNLA (SEQ ID NO: 22), LCDR2 of AASSRQS (SEQ ID NO: 23) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12); or

(g) the VH region amino acid sequence comprises HCDR1 of GYTFTDFYMN (SEQ ID NO: 180), HCDR2 of MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26) and HCDR3 of GVWYYDY (SEQ ID NO: 27); and the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYANYPRT (SEQ ID NO: 25).

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region comprises any one of the VH region amino acid sequences in Table 12 and the VL region comprises any one of the VL region amino acid sequences in Table 12.

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises or consists of SEQ ID NO:185 and the VL region amino acid sequence comprises or consists of SEQ ID NO:186;

(b) the VH region amino acid sequence comprises or consists of SEQ ID NO:187 and the VL region amino acid sequence comprises or consists of SEQ ID NO:188;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:189 and the VL region amino acid sequence comprises or consists of SEQ ID NO:190;

(d) the VH region amino acid sequence comprises or consists of SEQ ID NO:191 and the VL region amino acid sequence comprises or consists of SEQ ID NO:192;

(e) the VH region amino acid sequence comprises or consists of SEQ ID NO:193 and the VL region amino acid sequence comprises or consists of SEQ ID NO:194; or

(f) the VH region amino acid sequence comprises or consists of SEQ ID NO:195 and the VL region amino acid sequence comprises or consists of SEQ ID NO:196.

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:185 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:186;

(b) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:187 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:188;

(c) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:189 and the VL region amino acid sequence is at least about 90%, at least about

91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:190;

(d) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:191 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:192;

(e) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:193 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:194; or

(f) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:195 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:196. In some aspects, the CDR amino acid sequences of an anti-C-KIT antibody are 100% identical to the CDR amino acid sequences in the recited sequences while the FR amino acid sequences are less than 100% identical to the FR amino acid sequences in the recited sequences.

In some aspects, the antibody or antigen-binding portion as defined herein may be isolated.

The antibody molecule or antigen-binding portion as defined herein may cross-compete for binding to C-KIT with an antibody or antigen-binding portion thereof comprising the sets of CDRs disclosed herein. In some embodiments, the invention provides an isolated anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody or antigen-binding portion cross-competes for binding to C-KIT with the antibody or antigen-binding portion comprising the sets of CDRs disclosed herein and (a) comprises fully germline human framework amino acid sequences; and/or (b) does not comprise a deamidation site in the

LCDR3; and/or (c) comprises a human germline peptide sequence with high MHC class II binding affinity in HCDR1 and/or LCDR2; and/or (d) comprises a reduced number of immunogenic peptides compared to an anti-C-KIT antibody comprising the variable domain sequences of antibody h37M (Table 2); and/or (e) exhibits reduced immunogenicity compared to an anti-C-KIT antibody comprising the variable domain sequences of antibody h37M (Table 2); and/or (f) exhibits similar potency in receptor internalization to an anti-C-KIT antibody comprising the variable domain sequences of antibody h37M (Table 2) and exhibits reduced potency in C-KIT signalling blockade to an anti-C-KIT antibody comprising the variable domain sequences of antibody h37M (Table 2); and/or (g) is immune effector null.

In some embodiments, an anti-C-KIT antibody or antigen-binding portion has low immunogenicity. In certain cases, an antibody or antigen-binding portion exhibits reduced immunogenicity compared to an anti-C-KIT antibody comprising HCDR1 of GYTFTDYYIN (SEQ ID NO: 4), HCDR2 of IARIYPGSGNTYYNEKFKG (SEQ ID NO: 5), HCDR3 of GVYYFDY (SEQ ID NO: 6), LCDR1 of KASQNVRTNVA (SEQ ID NO: 10), and LCDR2 of SASYRYS (SEQ ID NO: 11). In some examples, immunogenicity risk of an antibody or antigen-binding portion may be determined *in silico* by identifying the location of T cell epitopes in the antibody or portion (e.g., in the variable regions of the antibody or portion).

For example, T cell epitopes in an antibody or antigen-binding portion may be identified by using iTope™. iTope™ can used to analyse VL and VH region sequences for peptides with promiscuous high affinity binding to human MHC class II. Promiscuous high affinity MHC class II binding peptides are thought to correlate with the presence of T cell epitopes that are high risk indicators for clinical immunogenicity of drug proteins. The iTope™ software predicts favourable interactions between amino acid side chains of a peptide and specific binding pockets (in particular pocket positions; p1, p4, p6, p7 and p9) within the open-ended binding grooves of 34 human MHC class II alleles. These alleles represent the most common HLA-DR alleles found world-wide with no weighting attributed to those found most prevalently in any particular ethnic population. Twenty of the alleles contain the ‘open’ p1 configuration and 14 contain the ‘closed’ configuration where glycine at position 83 is replaced by a valine. The location of key binding residues is achieved by the *in silico* generation of 9mer peptides that overlap by eight amino acids spanning the test protein sequence. This process successfully discriminates with high accuracy between peptides that either bind or do not bind MHC class II molecules.

T cell epitopes in an antibody or antigen-binding portion may be identified by analysing VL and VH region sequences using TCED™ (T Cell Epitope Database™) to search for matches to T cell epitopes previously identified by *in vitro* human T cell epitope mapping analyses of other protein sequences. The TCED™ is used to search any test sequence against a large (>10,000 peptides) database of peptides derived from unrelated protein and antibody sequences.

In some embodiments, an anti-C-KIT antibody or antigen-binding portion may exhibit a low immunogenicity because the antibody or portion has a low number of one or more of the following peptides in its sequences: High Affinity Foreign ('HAF' – high immunogenicity risk), Low Affinity Foreign ('LAF' – lower immunogenicity risk), and/or TCED+ (previously identified epitope in TCED™ database).

In some embodiments, an anti-C-KIT antibody or antigen-binding portion may have high Germline Epitope (GE) content in its sequence. In some examples, an anti-C-KIT antibody or antigen-binding portion has 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 (or greater than 20) germline epitopes in its sequence. Germline Epitope may be defined as a human germline peptide sequence with high MHC Class II binding affinity. Germline Epitope 9mer peptides are unlikely to have immunogenic potential due to T cell tolerance, as validated by previous studies with a wide range of germline peptides. Importantly, such germline v-domain epitopes (aided further by similar sequences in the human antibody constant regions) also compete for MHC Class II occupancy at the membrane of antigen presenting cells, reducing the risk of foreign peptide presentation being sufficient to achieve the 'activation threshold' required for T cell stimulation. High GE content is therefore a beneficial quality in clinical development of an antibody therapeutic and can provide low immunogenicity. In some examples, an anti-C-KIT antibody or antigen-binding portion comprises a human germline peptide sequence with high MHC class II binding affinity (*e.g.*, germline epitope) in the HCDR1 and/or LCDR2.

In certain embodiments, an anti-C-KIT antibody or antigen-binding portion may have a reduced number of HAF, LAF and/or TCED+ epitopes found in the frameworks of both the heavy and light chain variable regions compared to an anti-C-KIT antibody comprising the variable domain sequences of antibody h37M (Table 2). For example, a TCED+ and HAF peptide 'VTITCKASQ' (SEQ ID NO: 64) found in the LCDR-1 of h37M may be eliminated in an anti-C-KIT antibody or antigen-binding portion by the mutation K>R at position 6, converting this sequence to the light chain GE 'VTITCRASQ' (SEQ ID NO: 65; Fig 11B-H). Similarly, one or both of the HAF peptides 'LIYSASSLQ' (SEQ ID NO: 66) and

'IYSASSLQS' (SEQ ID NO: 67) may be converted to GE sequences by mutation of the LCDR2 sequence to the fully germline sequence 'AASSLQS' (SEQ ID NO: 24).

In one embodiment, an anti-C-KIT antibody or antigen-binding portion comprises the HCDR1 germlining mutation I>M at position 3 of the TCED+ and HAF peptide sequence 'YYINWVRQA' (SEQ ID NO: 68; spanning the HCDR-1 and FW2).

In some embodiments, an anti-C-KIT antibody or antigen-binding portion comprises the mutation Y>W in the HCDR3 that eliminates two LAF peptide sequences found in antibody h37M.

The terms "cross-compete", "cross-competition", "cross-block", "cross-blocked" and "cross-blocking" are used interchangeably herein to mean the ability of an antibody or portion thereof to interfere with the binding directly or indirectly through allosteric modulation of the anti-C-KIT antibodies of the invention to the target C-KIT (e.g., human C-KIT). The extent to which an antibody or portion thereof is able to interfere with the binding of another to the target, and therefore whether it can be said to cross-block or cross-compete according to the invention, can be determined using competition binding assays. One example of a binding competition assay is Homogeneous Time Resolved Fluorescence (HTRF). One particularly suitable quantitative cross-competition assay uses a FACS- or an AlphaScreen-based approach to measure competition between the labelled (e.g. His tagged, biotinylated or radioactive labelled) antibody or portion thereof and the other antibody or portion thereof in terms of their binding to the target. In general, a cross-competing antibody or portion thereof is, for example, one which will bind to the target in the cross-competition assay such that, during the assay and in the presence of a second antibody or portion thereof, the recorded displacement of the immunoglobulin single variable domain or polypeptide according to the invention is up to 100% (e.g. in a FACS based competition assay) of the maximum theoretical displacement (e.g. displacement by cold (e.g. unlabeled) antibody or fragment thereof that needs to be cross-blocked) by the potentially cross-blocking antibody or fragment thereof that is present in a given amount. Preferably, cross-competing antibodies or portions thereof have a recorded displacement that is between 10% and 100%, or between 50% and 100%.

The antibody molecule or antigen-binding portion as defined herein may comprise one or more substitutions, deletions and/or insertions which remove a post-translational modification (PTM) site, for example a glycosylation site (N-linked or O-linked), a deamination site, a phosphorylation site or an isomerisation/fragmentation site.

More than 350 types of PTM are known. Key forms of PTM include phosphorylation, glycosylation (*N*- and *O*-linked), sumoylation, palmitoylation, acetylation, sulfation, myristoylation, prenylation and methylation (of K and R residues). Statistical methods to identify putative amino acid sites responsible for specific PTMs are well known in the art (see Zhou *et al.*, 2016, Nature Protocols 1: 1318-1321). Removal of such a site for example by substitution, deletion and/or insertion and then optionally testing (experimentally and/or theoretically) for (a) binding activity and/or (b) loss of the PTM is contemplated.

For example, the 37M murine LCDR3 (as defined herein, i.e. the amino acid sequence QQYNSYPRT (SEQ ID NO: 12) has been identified to have a putative deamidation site at residue 4 (N). Removal this site at equivalent positions in an LCDR3 of the invention, for example by substitution (such as to A, S, E or T), is envisaged (as for example in clone MH5 and mutant derivatives of MH5, as in Tables 4 and 6).

The antibody molecule or antigen-binding portion thereof may be human, humanized or chimeric.

The antibody molecule or antigen-binding portion thereof may comprise one or more human variable domain framework scaffolds into which the CDRs have been inserted. For example, the VH region, the VL region, or both the VH and the VL region may comprise one or more human framework region (FR) amino acid sequences.

The antibody molecule or antigen-binding portion thereof may comprise an IGHV1-46 human germline scaffold into which the corresponding HCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV1-46 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted.

The antibody molecule or antigen-binding portion thereof may comprise an IGKV1-16 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VL region that comprises an IGKV1-16 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted.

The antibody molecule or antigen-binding portion thereof may comprise an IGHV1-46 human germline scaffold into which the corresponding HCDR sequences have been inserted and

an IGKV1-16 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV1-46 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted and a VL region that comprises an IGKV1-16 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted. The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences may be the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences of any one of the clones in Table 4 or Table 6 (with all six CDR sequences being from the same clone).

In some aspects, the antibody molecule or antigen-binding portion thereof may comprise an immunoglobulin constant region. In some aspects, the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2. In additional aspects, the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2. The antibody molecule or antigen-binding portion thereof may comprise an immunologically inert constant region. In some aspects, an anti-C-KIT antibody or antigen-binding portion thereof may comprise an immunoglobulin constant region comprising a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S. In some aspects, an anti-C-KIT antibody may comprise an immunoglobulin constant region comprising any one of the amino acid sequences in Table 13. The Fc region sequences in Table 13 begin at the CH1 domain. In some aspects, an anti-C-KIT antibody may comprise an immunoglobulin constant region comprising an amino acid sequence of an Fc region of human IgG1, human IgG1-3M or human IgG1-4M. For example, the human IgG1-3M Fc region comprises the following substitutions: L234A, L235A and G237A, while the human IgG1-4M Fc region comprises the following substitutions: L234A, L235A, G237A and P331S. In some aspects, a position of an amino acid residue in a constant region of an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94). In some aspects, an immunoglobulin constant region may comprise an RDELT (SEQ ID NO:203) motif or an REEM (SEQ ID NO:204) motif (underlined in Table 13). The REEM (SEQ ID NO:204) allotype is found in a smaller human population than the RDELT (SEQ ID NO:203) allotype. In some aspects, an anti-C-KIT antibody may comprise an immunoglobulin constant region comprising any one of SEQ ID NOs:197-202. In some aspects, an anti-C-KIT antibody may comprise the six CDR amino acid sequences of any one of the clones in Table 4 or 6 and any one of the Fc region amino acid sequences in Table 13. In some aspects, an anti-C-KIT antibody may comprise an immunoglobulin

heavy chain constant region comprising any one of the Fc region amino acid sequences in Table 13 and an immunoglobulin light chain constant region that is a kappa light chain constant region or a lambda light chain constant region.

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12); and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(b) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYANYPRT (SEQ ID NO: 25); and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(c) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of SASSLQS (SEQ ID NO: 17) and LCDR3 of QQYANYPRT (SEQ ID NO: 25); and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(d) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYYDY (SEQ ID NO: 15); the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of SASSLQS (SEQ ID NO: 17) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12); and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(e) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of

GVYYLDY (SEQ ID NO: 18); the VL region amino acid sequence comprises LCDR1 of RASQGIRTNVA (SEQ ID NO: 19), LCDR2 of SASYRQS (SEQ ID NO: 20) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12); and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(f) the VH region amino acid sequence comprises HCDR1 of GYTFTDYYMN (SEQ ID NO: 13), HCDR2 of MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14) and HCDR3 of GVYYFDE (SEQ ID NO: 21); the VL region amino acid sequence comprises LCDR1 of RASQGVRTNLA (SEQ ID NO: 22), LCDR2 of AASSRQS (SEQ ID NO: 23) and LCDR3 of QQYNSYPRT (SEQ ID NO: 12); and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202; or

(g) the VH region amino acid sequence comprises HCDR1 of GYTFTDFYMN (SEQ ID NO: 180), HCDR2 of MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26) and HCDR3 of GVWYYDY (SEQ ID NO: 27); the VL region amino acid sequence comprises LCDR1 of RASQGIRTNLA (SEQ ID NO: 16), LCDR2 of AASSLQS (SEQ ID NO: 24) and LCDR3 of QQYANYPRT (SEQ ID NO: 25); and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202.

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

(a) the VH region amino acid sequence comprises or consists of SEQ ID NO:185, the VL region amino acid sequence comprises or consists of SEQ ID NO:186 and the heavy chain constant region comprises a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S;

(b) the VH region amino acid sequence comprises or consists of SEQ ID NO:187, the VL region amino acid sequence comprises or consists of SEQ ID NO:188 and the heavy chain constant region comprises a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:189, the VL region amino acid sequence comprises or consists of SEQ ID NO:190 and the

heavy chain constant region comprises a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S;

(d) the VH region amino acid sequence comprises or consists of SEQ ID NO:191, the VL region amino acid sequence comprises or consists of SEQ ID NO:192 and the heavy chain constant region comprises a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S;

(e) the VH region amino acid sequence comprises or consists of SEQ ID NO:193, the VL region amino acid sequence comprises or consists of SEQ ID NO:194 and the heavy chain constant region comprises a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S; or

(f) the VH region amino acid sequence comprises or consists of SEQ ID NO:195, the VL region amino acid sequence comprises or consists of SEQ ID NO:196 and the heavy chain constant region comprises a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A, G237A and P331S.

In some aspects, disclosed herein is an anti-C-KIT antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

(a) the VH region amino acid sequence comprises or consists of SEQ ID NO:185, the VL region amino acid sequence comprises or consists of SEQ ID NO:186 and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(b) the VH region amino acid sequence comprises or consists of SEQ ID NO:187, the VL region amino acid sequence comprises or consists of SEQ ID NO:188 and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:189, the VL region amino acid sequence comprises or consists of SEQ ID NO:190 and the

heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(d) the VH region amino acid sequence comprises or consists of SEQ ID NO:191, the VL region amino acid sequence comprises or consists of SEQ ID NO:192 and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202;

(e) the VH region amino acid sequence comprises or consists of SEQ ID NO:193, the VL region amino acid sequence comprises or consists of SEQ ID NO:194 and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202; or

(f) the VH region amino acid sequence comprises or consists of SEQ ID NO:195, the VL region amino acid sequence comprises or consists of SEQ ID NO:196 and the heavy chain constant region comprises SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201 or SEQ ID NO:202.

In some aspects, an anti-C-KIT antibody may be immune effector null. In some aspects, an anti-C-KIT antibody or an antigen-binding portion thereof does not induce immune effector function and, optionally, suppresses immune effector function. In some aspects, an anti-C-KIT antibody may lack measurable binding to human FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb receptors but maintain binding to human FcγRIIb receptor and optionally maintain binding to human FcRn receptor. FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb are examples of activating receptors. FcγRIIb is an example of an inhibitory receptor. FcRn is an example of a recycling receptor. In some aspects, binding affinity of an anti-C-KIT antibody or an antigen-binding portion thereof for human Fc receptors may be measured by BIACORE® analysis. In some aspects, Homogeneous Time Resolved Fluorescence (HTRF) can be used to study binding of an anti-C-KIT antibody to human Fc receptors. In one example of HTRF, human IgG1 (wild type) is labelled, as is the full suite of Fc gamma receptors and then antibodies with engineered Fc fragments are used in titration competition. In some aspects, KIT-positive cells may be mixed with human white blood cells and anti-C-KIT antibodies, and cell killing by CDC, ADCC and/or ADCP may be measured. In some aspects, an anti-C-KIT antibody may comprise an amino acid sequence of an Fc region of human IgG1-3M or human IgG1-4M (see Table 13) is effector null. In some aspects, an anti-C-KIT antibody may comprise an amino acid sequence of an Fc region of human IgG1-3M or human IgG1-4M (see Table 13) is not effector null.

The antibody molecule or antigen-binding portion thereof may be a Fab fragment, a $F(ab)_2$ fragment, an Fv fragment, a tetrameric antibody, a tetravalent antibody, a multispecific

antibody (for example, a bispecific antibody), a domain-specific antibody, a single domain antibody, a monoclonal antibody or a fusion protein. In one embodiment, an antibody may be a bispecific antibody that binds specifically to a first antigen and a second antigen, wherein the first antigen is C-KIT and the second antigen is not C-KIT. Antibody molecules and methods for their construction and use are described, in for example Holliger & Hudson (2005, Nature Biotechnol. 23(9): 1126-1136).

In another aspect of the invention, there is provided an immunoconjugate comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein linked to a therapeutic agent.

Examples of suitable therapeutic agents include cytotoxins, radioisotopes, chemotherapeutic agents, immunomodulatory agents, anti-angiogenic agents, antiproliferative agents, pro-apoptotic agents, and cytostatic and cytolytic enzymes (for example RNAses). Further therapeutic agents include a therapeutic nucleic acid, such as a gene encoding an immunomodulatory agent, an anti-angiogenic agent, an anti-proliferative agent, or a pro-apoptotic agent. These drug descriptors are not mutually exclusive, and thus a therapeutic agent may be described using one or more of the above terms.

Examples of suitable therapeutic agents for use in immunoconjugates include the taxanes, maytansines, CC-1065 and the duocarmycins, the calicheamicins and other enediynes, and the auristatins. Other examples include the anti-folates, vinca alkaloids, and the anthracyclines. Plant toxins, other bioactive proteins, enzymes (i.e., ADEPT), radioisotopes, photosensitizers may also be used in immunoconjugates. In addition, conjugates can be made using secondary carriers as the cytotoxic agent, such as liposomes or polymers, Suitable cytotoxins include an agent that inhibits or prevents the function of cells and/or results in destruction of cells. Representative cytotoxins include antibiotics, inhibitors of tubulin polymerization, alkylating agents that bind to and disrupt DNA, and agents that disrupt protein synthesis or the function of essential cellular proteins such as protein kinases, phosphatases, topoisomerases, enzymes, and cyclins.

Representative cytotoxins include, but are not limited to, doxorubicin, daunorubicin, idarubicin, aclarubicin, zorubicin, mitoxantrone, epirubicin, carubicin, nogalamycin, menogaril, pitarubicin, valrubicin, cytarabine, gemcitabine, trifluridine, ancitabine, enocitabine, azacitidine, doxifluhdine, pentostatin, broxuhdine, capecitabine, cladhbine, decitabine, floxuhdine, fludarabine, gougerotin, puromycin, tegafur, tiazofuhn, adhamycin, cisplatin, carboplatin, cyclophosphamide, dacarbazine, vinblastine, vincristine, mitoxantrone,

bleomycin, mechlorethamine, prednisone, procarbazine, methotrexate, flurouracils, etoposide, taxol, taxol analogs, platins such as cis-platin and carbo-platin, mitomycin, thiotepa, taxanes, vincristine, daunorubicin, epirubicin, actinomycin, authramycin, azaserines, bleomycins, tamoxifen, idarubicin, dolastatins/auristatins, hemiasterlins, esperamicins and maytansinoids.

Suitable immunomodulatory agents include anti-hormones that block hormone action on tumors and immunosuppressive agents that suppress cytokine production, down-regulate self-antigen expression, or mask MHC antigens.

Also provided is a nucleic acid molecule encoding the antibody molecule or antigen-binding portion thereof of the invention as defined herein. An isolated nucleic acid molecule may encode (a) the VH region amino acid sequence; (b) the VL region amino acid sequence; or (c) both the VH and the VL region amino acid sequences of an anti-C-KIT antibody or an antigen-binding portion thereof described herein.

Further provided is a vector comprising the nucleic acid molecule of the invention as defined herein. The vector may be an expression vector. The vector may further comprise one or more regulatory sequences (e.g., a promoter and/or an enhancer).

Also provided is a host cell comprising the nucleic acid molecule or the vector of the invention as defined herein. The host cell may be a recombinant host cell.

In a further aspect there is provided a method of producing an anti-C-KIT antibody and/or an antigen-binding portion thereof, comprising culturing the host cell of the invention under conditions that result in expression and/or production of the antibody and/or the antigen-binding portion thereof, and isolating the antibody and/or the antigen-binding portion thereof from the host cell or culture.

In another aspect of the invention there is provided a pharmaceutical composition comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein.

Further provided is a method for enhancing an immune response in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein,

or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

In a further aspect there is provided a method for treating or preventing cancer in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

The cancer may for example be selected from the group consisting of: Gastrointestinal Stromal cancer (GIST), pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, and cancer of hematological tissues.

The invention also provides an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein, for use in the treatment of cancer.

In another aspect the invention provides the antibody molecule, or antigen-binding portion thereof, or the immunoconjugate, or the nucleic acid molecule, or the vector for use, or the method of treatment of the invention as defined herein, for separate, sequential or simultaneous use in a combination combined with a second therapeutic agent, for example an anti-cancer agent.

In a further aspect there is provided the use of an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or an immunoconjugate of the invention as defined herein, or a nucleic acid molecule of the invention as defined herein, or a vector of the invention as defined herein, or a pharmaceutical composition of the invention as defined herein, in the manufacture of a medicament for the treatment of cancer.

The invention also provides a method for treating or preventing an autoimmune disease or an inflammatory disease in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

The autoimmune disease or inflammatory disease may be selected from the group consisting of: arthritis, asthma, multiple sclerosis, psoriasis, Crohn's disease, inflammatory bowel disease, lupus, Grave's disease and Hashimoto's thyroiditis, and ankylosing spondylitis.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of an autoimmune disease or an inflammatory disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of an autoimmune disease or an inflammatory disease.

The invention also provides a method for treating or preventing a cardiovascular disease or a fibrotic disease in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

Also provided is an antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of a cardiovascular disease or a fibrotic disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of a cardiovascular disease or a fibrotic disease.

The cardiovascular disease in any aspect of the invention may for example be coronary heart disease or atherosclerosis.

The fibrotic disease in any aspect of the invention may be selected from the group consisting of myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, asthma, cystic fibrosis and bronchitis.

In one embodiment, the invention provides an anti-C-KIT antibody or an antigen-binding portion thereof comprising the amino acid sequences disclosed herein for use in therapy.

The pharmaceutical composition of the invention may comprise a pharmaceutically acceptable excipient, carrier or diluent. A pharmaceutically acceptable excipient may be a compound or a combination of compounds entering into a pharmaceutical composition which does not provoke secondary reactions and which allows, for example, facilitation of the administration of the anti-C-KIT antibody molecule, an increase in its lifespan and/or in its efficacy in the body or an increase in its solubility in solution. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the mode of administration of the anti-C-KIT antibody molecule.

In some embodiments, the anti-C-KIT antibody molecule may be provided in a lyophilised form for reconstitution prior to administration. For example, lyophilised antibody molecules may be re-constituted in sterile water and mixed with saline prior to administration to an individual.

The anti-C-KIT antibody molecules will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the antibody molecule. Thus pharmaceutical compositions may comprise, in addition to the anti-C-KIT antibody molecule, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the anti-C-KIT antibody molecule. The precise nature of the carrier or other material will depend on the route of administration, which may be by bolus, infusion, injection or any other suitable route, as discussed below.

For parenteral, for example sub-cutaneous or intra-venous administration, e.g. by injection, the pharmaceutical composition comprising the anti-C-KIT antibody molecule may be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable

pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride Injection, Ringe's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be employed as required including buffers such as phosphate, citrate and other organic acids; antioxidants, such as ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3'-pentanol; and m-cresol); low molecular weight polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagines, histidine, arginine, or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose or dextrins; chelating agents, such as EDTA; sugars, such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions, such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants, such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

A pharmaceutical composition comprising an anti-C-KIT antibody molecule may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

An anti-C-KIT antibody molecule as described herein may be used in a method of treatment of the human or animal body, including prophylactic or preventative treatment (e.g. treatment before the onset of a condition in an individual to reduce the risk of the condition occurring in the individual; delay its onset; or reduce its severity after onset). The method of treatment may comprise administering the anti-C-KIT antibody molecule to an individual in need thereof.

Administration is normally in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the composition, the method of administration, the scheduling of administration and other factors known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of antibody molecules are well known in the art (Ledermann J.A. *et al.*, 1991, Int. J.

Cancer 47: 659-664; Bagshawe K.D. *et al.*, 1991, Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922). Specific dosages may be indicated herein or in the Physician's Desk Reference (2003) as appropriate for the type of medicament being administered may be used. A therapeutically effective amount or suitable dose of an antibody molecule may be determined by comparing its *in vitro* activity and *in vivo* activity in an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the antibody is for prevention or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment) and the nature of any detectable label or other molecule attached to the antibody.

A typical antibody dose will be in the range 100 µg to 1 g for systemic applications, and 1 µg to 1 mg for topical applications. An initial higher loading dose, followed by one or more lower doses, may be administered. Typically, the antibody will be a whole antibody, e.g. the IgG1 or IgG4 isotype. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician. The treatment schedule for an individual may be dependent on the pharmocokinetic and pharmacodynamic properties of the antibody composition, the route of administration and the nature of the condition being treated.

Treatment may be periodic, and the period between administrations may be about two weeks or more, e.g. about three weeks or more, about four weeks or more, about once a month or more, about five weeks or more, or about six weeks or more. For example, treatment may be every two to four weeks or every four to eight weeks. Treatment may be given before, and/or after surgery, and/or may be administered or applied directly at the anatomical site of surgical treatment or invasive procedure. Suitable formulations and routes of administration are described above.

In some embodiments, anti-C-KIT antibody molecules as described herein may be administered as sub-cutaneous injections. Sub-cutaneous injections may be administered using an auto-injector, for example for long or short-term prophylaxis/treatment.

In some preferred embodiments, the therapeutic effect of the anti-C-KIT antibody molecule may persist for several multiples of the antibody half-life in serum, depending on the dose. For example, the therapeutic effect of a single dose of the anti-C-KIT antibody molecule may

persist in an individual for 1 month or more, 2 months or more, 3 months or more, 4 months or more, 5 months or more, or 6 months or more.

The invention also provides a method of producing an antibody molecule which specifically binds to human C-KIT and optionally also to cynomolgus monkey C-KIT or an antigen-binding portion thereof, comprising the steps of:
(1) grafting anti-C-KIT CDRs from a non-human source into a human v-domain framework to produce a humanized anti-C-KIT antibody molecule or antigen-binding portion thereof;
(2) generating a phage library of clones of the humanized anti-C-KIT antibody molecule or antigen-binding portion thereof comprising one or more mutations in the CDRs;
(3) selecting the phage library for binding to human C-KIT and optionally also to cynomolgus monkey C-KIT;
(4) screening clones from the selection step (3) having binding specificity to human C-KIT and optionally also to cynomolgus monkey C-KIT; and
(5) producing an antibody molecule which specifically binds to human C-KIT and optionally also to cynomolgus monkey C-KIT, or an antigen-binding portion thereof from clones selected from step (4).

The method may comprise a further step of producing additional clones based on the clones selected in step (4), for example based on further exploratory mutagenesis at specific positions in the CDRs of the clones selected in step (4), to enhance humanization and/or minimise human T cell epitope content and/or improve manufacturing properties in the antibody molecule or antigen-binding portion thereof produced in step (5).

Refinements applicable to the above method are as described in Example 1 below.

As used herein, the term “C-KIT” refers to CD117 (Cluster of Differentiation 117) and variants thereof that retain at least part of the biological activity of C-KIT. This protein is also known as KIT, PBT, SCFR and KIT proto-oncogene receptor tyrosine kinase. As used herein, C-KIT includes all mammalian species of native sequence C-KIT, including human, rat, mouse and chicken. The term “C-KIT” is used to include variants, isoforms and species homologs of human C-KIT. Examples of human C-KIT sequences are provided in Table 14. Antibodies of the invention may cross-react with C-KIT from species other than human, in particular C-KIT from cynomolgus monkey (*Macaca fascicularis*). In certain embodiments, the antibodies may be completely specific for human C-KIT and do not exhibit non-human cross-reactivity.

As used herein, an “antagonist” as used in the context of the antibody of the invention or an “anti-C-KIT antagonist antibody” (interchangeably termed “anti-C-KIT antibody”) refers to an antibody which is able to bind to C-KIT and inhibit C-KIT biological activity and/or downstream pathway(s) mediated by C-KIT signalling. An anti-C-KIT antagonist antibody encompasses antibodies that can block, antagonize, suppress or reduce (including significantly) C-KIT biological activity, including downstream pathways mediated by C-KIT signalling, such as receptor binding and/or elicitation of a cellular response to C-KIT. For the purposes of the present invention, it will be explicitly understood that the term “anti-C-KIT antagonist antibody” encompass all the terms, titles, and functional states and characteristics whereby C-KIT itself, and C-KIT biological activity (including but not limited to its ability to enhance the activation of phagocytosis by cells of the myeloid lineage), or the consequences of the activity or biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree.

The antibody “specifically binds” “specifically interacts”, “preferentially binds”, “binds” or “interacts” with C-KIT if it binds with greater affinity, avidity, more readily and/or with greater duration than it binds to other receptors.

An “antibody molecule” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody molecule” encompasses not only intact polyclonal or monoclonal antibodies, but also any antigen-binding fragment (for example, an “antigen-binding portion”) or single chain thereof, fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site including, for example without limitation, scFv, single domain antibodies (for example, shark and camelid antibodies), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv.

An “antibody molecule” encompasses an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta,

epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term “antigen-binding portion” of an antibody molecule, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to C-KIT. Antigen-binding functions of an antibody molecule can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term “antigen binding-portion” of an antibody molecule include Fab; Fab'; $F(ab')_2$; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment, and an isolated complementarity determining region (CDR).

The term “Fc region” is used to define a C-terminal region of an immunoglobulin heavy chain. The “Fc region” may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3. As is known in the art, an Fc region can be present in dimer or monomeric form.

A “variable region” or “v-domain” of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, and contribute to the formation of the antigen binding site of antibodies. When choosing FRs to flank CDRs, for example when humanizing or optimizing an antibody, FRs from antibodies which contain CDR sequences in the same canonical class are preferred.

The CDR definitions used in the present application combine the domains used in the many disparate, often conflicting schemes that have been created in the field, which are based on the combination of immunoglobulin repertoire analyses and structural analyses of antibodies in isolation and in their co-crystals with antigens (see review by Swindells *et al.*, 2016, abYsis: Integrated Antibody Sequence and Structure-Management, Analysis, and Prediction. J Mol Biol. [PMID: 27561707; Epub 22 August 2016]). The CDR definition used herein (a “Unified” definition) incorporates the lessons of all such prior insights and includes all appropriate loop

positions required to sample the full residue landscape that potentially mediates target-binding complementarity.

Table 1 shows the amino acid sequences of the 37M murine anti-C-KIT antibody CDRs as defined herein (a “Unified” scheme), in comparison to well-known alternative systems for defining the same CDRs.

As used herein the term “conservative substitution” refers to replacement of an amino acid with another amino acid which does not significantly deleteriously change the functional activity. A preferred example of a “conservative substitution” is the replacement of one amino acid with another amino acid which has a value ≥ 0 in the following BLOSUM 62 substitution matrix (see Henikoff & Henikoff, 1992, PNAS 89: 10915-10919):

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4.

As used herein, “sequence identity” refers to a relationship between two or more polynucleotide sequences or between two or more polypeptide sequences. When a position in one sequence is occupied by the same nucleic acid base or amino acid residue in the corresponding position of the comparator sequence, the sequences are said to be “identical” at that position. The percentage sequence identity is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of identical positions. The number of identical positions is then divided by the total number of positions in the comparison window and multiplied by 100 to yield the percentage of sequence identity. Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window. The

comparison window for polynucleotide sequences can be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 or more nucleic acids in length. The comparison window for polypeptide sequences can be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300 or more amino acids in length. In order to optimally align sequences for comparison, the portion of a polynucleotide or polypeptide sequence in the comparison window can comprise additions or deletions termed gaps while the reference sequence is kept constant. An optimal alignment is that alignment which, even with gaps, produces the greatest possible number of "identical" positions between the reference and comparator sequences. Percentage "sequence identity" between two sequences can be determined using the version of the program "BLAST 2 Sequences" which was available from the National Center for Biotechnology Information as of September 1, 2004, which program incorporates the programs BLASTN (for nucleotide sequence comparison) and BLASTP (for polypeptide sequence comparison), which programs are based on the algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90(12):5873-5877, 1993). When utilizing "BLAST 2 Sequences," parameters that were default parameters as of September 1, 2004, can be used for word size (3), open gap penalty (11), extension gap penalty (1), gap dropoff (50), expect value (10) and any other required parameter including but not limited to matrix option. Two nucleotide or amino acid sequences are considered to have "substantially similar sequence identity" or "substantial sequence identity" if the two sequences have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity relative to each other.

The term "monoclonal antibody" (Mab) refers to an antibody, or antigen-binding portion thereof, that is derived from a single copy or clone, including for example any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Preferably, a monoclonal antibody of the invention exists in a homogeneous or substantially homogeneous population.

A "humanized" antibody molecule refers to a form of non-human (for example, murine) antibody molecules, or antigen-binding portion thereof, that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding sub-sequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies may be human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity.

"Human antibody or fully human antibody" refers to an antibody molecule, or antigen-binding portion thereof, derived from transgenic mice carrying human antibody genes or from human cells.

The term "chimeric antibody" is intended to refer to an antibody molecule, or antigen-binding portion thereof, in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody molecule in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

"Antibody-drug conjugate" and "immunoconjugate" refer to an antibody molecule, or antigen-binding portion thereof, including antibody derivatives that binds to C-KIT, which is conjugated to cytotoxic, cytostatic and/or therapeutic agents.

Antibody molecules of the invention, or antigen-binding portion thereof, can be produced using techniques well known in the art, for example recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art.

The term "isolated molecule" (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody molecule, or antigen-binding portion thereof, at one or more of the

antibody molecule's antigen-binding regions. Epitopes can consist of defined regions of primary secondary or tertiary protein structure and includes combinations of secondary structural units or structural domains of the target recognised by the antigen-binding regions of the antibody, or antigen-binding portion thereof. Epitopes can likewise consist of a defined chemically active surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. The term “antigenic epitope” as used herein, is defined as a portion of a polypeptide to which an antibody molecule can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays, antibody competitive binding assays or by x-ray crystallography or related structural determination methods (for example NMR).

The term “binding affinity” or “KD” refers to the dissociation rate of a particular antigen-antibody interaction. The KD is the ratio of the rate of dissociation, also called the “off-rate (k_{off})”, to the association rate, or “on-rate (k_{on})”. Thus, K_D equals k_{off} / k_{on} and is expressed as a molar concentration (M). It follows that the smaller the K_D, the stronger the affinity of binding. Therefore, a K_D of 1 μM indicates weak binding affinity compared to a K_D of 1 nM. KD values for antibodies can be determined using methods well established in the art. One method for determining the KD of an antibody is by using surface plasmon resonance (SPR), typically using a biosensor system such as a BIACORE® system.

The term “potency” is a measurement of biological activity and may be designated as IC_{50}, or effective concentration of an antibody or antibody drug conjugate to the antigen C-KIT to inhibit 50% of activity measured in a C-KIT activity assay as described herein.

The phrase “effective amount” or “therapeutically effective amount” as used herein refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount is at least the minimal amount, but less than a toxic amount, of an active agent which is necessary to impart therapeutic benefit to a subject.

The term “inhibit” or “neutralize” as used herein with respect to bioactivity of an antibody molecule of the invention means the ability of the antibody to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse for example progression or severity of that which is being inhibited including, but not limited to, a biological activity or binding interaction of the antibody molecule to C-KIT.

A “host cell” includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

As used herein, “vector” means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

The term “treating”, as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, delaying the progression of, delaying the onset of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term “treatment”, as used herein, unless otherwise indicated, refers to the act of treating as defined above. The term “treating” also includes adjuvant and neoadjuvant treatment of a subject. For the avoidance of doubt, reference herein to “treatment” includes reference to curative, palliative and prophylactic treatment. For the avoidance of doubt, references herein to “treatment” also include references to curative, palliative and prophylactic treatment.

It is understood that wherever embodiments are described herein with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided.

Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention

belongs. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word “comprise,” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Any example(s) following the term “e.g.” or “for example” is not meant to be exhaustive or limiting.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art.

Particular non-limiting embodiments of the present invention will now be described with reference to accompanying drawings.

EXAMPLE 1. Generation of optimized anti-C-KIT therapeutic antibodies

Introduction

In this example, we successfully generated a panel of antagonistic, optimized anti-C-KIT antibodies. These anti-C-KIT antibodies were well expressed, biophysically stable, highly soluble and of maximized amino acid sequence identity to preferred human germlines.

Materials and methods

IgG cloning, transient expression, purification

Antibody v-domain encoding DNA sequences were cloned via restriction-ligation cloning into separate IgG heavy and light-chain expression cassettes in separate plasmid vectors. Antibodies were expressed in two human IgG1 formats: IgG1 and IgG1null – IgG1 with the lower hinge mutations L234A/L235A/G237A, which minimise Fcγ receptor-driven effector functions. IgGs were expressed in HEK-293expi cells after transient transfection with endotoxin-free IgG expression plasmid preparations, per manufacturer's protocols. IgGs were purified using a single-step protocol: Conditioned media were loaded (neat) onto a 1 ml ProA sepharose column, pre-equilibrated in PBS pH7.4. The column was washed with 5 column volumes of PBS pH7.4, before the protein was eluted with 100mM glycine, pH 2.7 and subjected to dialysis in PBS pH 7.4 using 30 kDa cutoff dialysis membrane.

IgG titration binding ELISAs

To coat Greiner Bio-One High bind ELISA plates, target proteins were diluted to 1 μg/ml in carbonate buffer and added at 100 μl per well, at 4°C, o/n. Coated plates were washed 3x with PBS pH7.4, blocked with 1% BSA in PBS (380 μl/ well) for 1 hr at RT, then washed 3x with PBS-Tween 20 (PBST). C-KIT antibodies (100 μl/well; diluted in PBST) were then added and then incubated 1 hr at RT. Plates were then washed 3x with PBST and goat anti-human kappa chain-HRP added (100 μl/well) at RT, for 1 hr. Plates were then washed 3x with PBST and twice with PBS before the addition of 100 μl TMB per well. Reactions were stopped by adding 100 μl 2M H_2SO_4/well and OD was read on a plate reader at 450nm.

Anti-C-KIT antibodies were tested for polyreactivity by ELISA. Purified, recombinant, target and non-target antigens were coated in 96-well Nunc maxisorp plates at 100 ng per well in carbonate buffer, at 4°C overnight. Plates were then washed 3x with PBS, blocked with 1% BSA in PBS, then washed 3x with PBS-Tween20. A dilution series of primary antibodies was then applied, plates were washed 3x with PBS-Tween20 followed by application of goat anti-human kappa chain-HRP 1:4,000 secondary antibody. Wells were then washed 3x with PBS-Tween20 and 2x with PBS, 100 μl TMB peroxidase substrate was added per well, the reaction was stopped by adding 100 μl 2M H_2SO_4 and absorbances were read at 450 nm.

IgG binding analysis via ELISA on negatively charged biomolecular surfaces were performed as previously described (see Mouquet *et al.*, 2010, Nature 467: 591-595).

C-KIT library generation and selection

The C-KIT scFv repertoire was assembled by mass oligonucleotide synthesis and PCR. The amplified scFv repertoire was then cloned via restriction-ligation into a phagemid vector, transformed into *E.coli* TG-1 cells, and the phage repertoire rescued essentially as previously described in detail (Finlay *et al.*, 2011, Methods Mol Biol 681: 383-401).

Phage selections were performed by coating streptavidin magnetic microbeads with C-KIT-Fc protein (either human or cyno), washing the beads thrice with PBS and resuspending in PBS pH7.4 plus 5% skim milk protein (MPBS). These beads were coated at 200 nM target protein in round 1 of selection, followed by 100, 50 and 10 nM in subsequent rounds.

HTRF binding competition assay

A competition homogeneous time resolved fluorescence (HTRF) assay was established to examine epitope competition against h37M IgG by grafted and library-derived clones. The purified h37M IgG1 was labelled with terbium using a labelling kit (CisBio) per the manufacturer's instructions. The final reaction mix contained biotinylated human C-KIT–Fc, SA-XL665 (CisBio), terbium-labelled parental h37M, and competitor IgG of interest, prepared as described above, in a total reaction volume of 20 µl in 1× assay buffer [50 mM sodium phosphate, pH 7.5, 400 mM potassium fluoride, and 0.1% BSA (w/v)]. Reagents were added sequentially into 384-well low-volume black plates (Nunc). Reactions proceeded for 1 h at room temperature, and plates were subsequently read on a plate reader with excitation at 340 nm and two emission readings at 615 nm (measuring input donor fluorescence from h37M-terbium) and 665 nm (measuring output acceptor fluorescence from SAXL665). Readings were expressed as 665 nm/615 nm ratios.

Antibody v-domain T cell epitope content: *in silico* analyses

In silico technologies (Abzena, Ltd.), which are based on identifying the location of T cell epitopes in therapeutic antibodies and proteins, were used for assessing potential immunogenicity in antibody v-domains. iTope™ was used to analyse the VL and VH sequences of key leads for peptides with promiscuous high affinity binding to human MHC class II. Promiscuous high affinity MHC class II binding peptides are thought to correlate with the presence of T cell epitopes that are high risk indicators for clinical immunogenicity of drug proteins. The iTope™ software predicts favourable interactions between amino acid side chains of a peptide and specific binding pockets (in particular pocket positions; p1, p4, p6, p7 and p9) within the open-ended binding grooves of 34 human MHC class II alleles.

These alleles represent the most common HLA-DR alleles found world-wide with no weighting attributed to those found most prevalently in any particular ethnic population. Twenty of the alleles contain the 'open' p1 configuration and 14 contain the 'closed' configuration where glycine at position 83 is replaced by a valine. The location of key binding residues is achieved by the *in silico* generation of 9mer peptides that overlap by eight amino acids spanning the test protein sequence. This process successfully discriminates with high accuracy between peptides that either bind or do not bind MHC class II molecules.

In addition, the sequences were analysed using TCED™ (T Cell Epitope Database™) search for matches to T cell epitopes previously identified by *in vitro* human T cell epitope mapping analyses of other protein sequences. The TCED™ is used to search any test sequence against a large (>10,000 peptides) database of peptides derived from unrelated protein and antibody sequences.

BIACORE® analyses of IgG affinity for human Fc receptors

Interaction affinities for IgGs were determined by surface plasmon resonance using a BIACORE® T200 instrument. For most analyses, His6-tagged FcγRI, FcγRIIa (167R and 167H variants), FcγRIIb, FcγRIIIa (176F and 176 V variants), and FcγRIIIb receptors (all Sino Biological) were captured on a CM5 sensor chip coated with an anti-HIS antibody by standard amine coupling. Receptor-specific formats of analyses were then applied, as below.

FcγRI is a high-affinity receptor for IgG1 monomers, so 1:1 kinetic analysis was performed under the following conditions: 'single cycle' analysis using flow rate 30 µl/min, receptor protein loaded to ~30 RU at 10 µl/min (diluted 0.25 µg/ml in HBS-P+), 5 point three-fold dilution of purified antibodies titrated 0.411 nM to 33.33 nM applied with an association time of 200 s, dissociation time of 300 s. Regeneration with 2x injections glycine pH 1.5 and analysis using 1:1 fit.

The interactions between monomeric IgG and FcγRII and FcγRIII receptors are relatively low affinity interactions, so 'steady state' affinity analyses were performed under the following conditions: flow rate 30 µl/min, receptor protein loaded to ~60 RU at 10 µl/min (diluted 0.25 µg/ml in HBS-P+), 5 point three-fold dilution series of purified antibodies titrated between 33 nM and 24000 nM applied with an association time of 30 s, dissociation time of 25 s. Regeneration with 2x injections glycine pH 1.5 and analysis using steady state affinity calculation.

The interactions between monomeric IgG and FcRn are relatively low affinity and pH-sensitive interactions, so 'steady state' affinity analyses were performed under the following conditions: A CM5 chip was directly coupled with hFcRn in sodium acetate pH 5.5 using standard amine chemistry. Running buffer was PBS 0.05% P20 + 150 mM NaCl (pH 6.0 or pH 7.4), flow rate 30 µl/min, 5 point three-fold dilutions of purified antibodies from 3000 nM to 37.0 nM applied with an association time of 18s, dissociation time 100 s. Regeneration with 0.1 M Tris pH 8.0 and analysis using steady state affinity calculation.

Antibody v-domain specificity testing: human receptor array analyses

Human cell membrane receptor proteome arrays were performed at Retrogenix Ltd. Primary screens: IgG1-h37M and IgG1-MH1 antibodies were screened for binding against fixed HEK293 cells/slides expressing 5528 human plasma membrane and cell surface-tethered secreted proteins individually (n=2 slides per slide set). All transfection efficiencies exceeded the minimum threshold. Antibody binding was detected using AF647 fluorescent secondary anti-human IgG1 antibody. Primary hits (duplicate spots) were identified by analysing fluorescence (AF647 and ZsGreen1) on ImageQuant. Vectors encoding all hits were sequenced to confirm their correct identities. Confirmation/specificity screens: Vectors encoding all hits, plus control vectors encoding MS4A1 (CD20), EGFR and other proteins, were spotted in duplicate on new slides, and used to reverse transfect human HEK293 cells as before. All transfection efficiencies exceeded the minimum threshold. Identical fixed slides were treated with 0.5 and 2 µg/ml of each test antibody, 1 µg/ml of the negative control antibody, 1 µg/ml Rituximab biosimilar (positive control), or no test molecule (secondary only; negative control) (n=1 slide per treatment). Slides were analysed as above.

Analyses of internalisation and cell-killing potential in armed antibody formats

CHO cells stably expressing human or cyno c-KIT, grown in Ham's F12 media containing 20% Fetal Bovine Serum, 1 mM L-Glutamine and 1 µg/ml G418 were seeded into 384-well black clear bottomed tissue culture treated assay plates (500 cells in 30 µl per well) and incubated overnight in a CO_2 incubator at 37˚C. Purified antibodies were serially diluted in media then an equal volume of 120 nM Fab-ZAP added (Advanced Targeting Systems, IT-51). The antibody/Fab-ZAP mixtures were incubated for 30 minutes at 37˚C before being added (10 µl per well) to the cell assay plates. The cells were incubated for 72 hours in CO_2 incubator at 37˚C. On each plate, background controls (media only) and Fab-ZAP control wells (cells incubated with FAB-ZAP reagent but no c-KIT antibody) were included for the purposes of data normalisation. At the end of the 72-hour incubation, cell viability was determined using the CellTiter-Glo® Cell Viability assay (Promega G7571) according to the manufacturer's instructions. The relative luminescent signal (RLU) for each well was

measured using the BMG FLUOstar Omega plate reader. The data was blank corrected by subtraction of the RLU signal of the media only wells and expressed as a % of the blank corrected signal of the Fab-ZAP control wells.

TF-1 cells in RPMI media containing 10% Fetal Bovine Serum, 2 mM L-Glutamine, 10 mM HEPES, 4.5 g/l D-Glucose, penicillin, streptomycin and 2 ng/ml GM-CSF were seeded into 96-well black clear bottomed tissue culture treated assay plates (2500 cells in 75 µl per well). Purified antibodies were serially diluted in media then an equal volume of 40 nM Fab-ZAP added. The antibody/Fab-ZAP mixtures were incubated for 30 minutes at 37˚C before being added (25 µl per well) to the cell assay plates. The cells were incubated for 72 hours in CO_2 incubator at 37˚C. On each plate, background controls (media only) and Fab-ZAP control wells (cells incubated with FAB-ZAP reagent but no c-KIT antibody) were included for the purposes of data normalisation. At the end of the 72-hour incubation, cell viability was determined using the CellTiter-Glo® Cell Viability assay (Promega G7571) according to the manufacturer's instructions. The relative luminescent signal (RLU) for each well was measured using the BMG FLUOstar Omega plate reader. The data was blank corrected by subtraction of the RLU signal of the media only wells and expressed as a % of the blank corrected signal of the Fab-ZAP control wells.

Analyses of antibody potency in C-KIT receptor activity neutralisation

TF-1 cells in RPMI media containing 10% Fetal Bovine Serum, 2 mM L-Glutamine, 10 mM HEPES, 4.5 g/l D-Glucose, penicillin, streptomycin and 2 ng/ml GM-CSF were seeded into 96-well clear bottomed tissue culture treated assay plates. Purified antibodies were serially diluted in media then added to the cell assay plates and incubated for 60 minutes at 37˚C. Recombinant h-SCF was then added at 50 ng/ml and the cells were incubated for 72 hours in CO_2 incubator at 37˚C. On each plate, background controls (media only) were included for the purposes of data normalisation. At the end of the 72-hour incubation, cell viability was determined using the resazurin fluorescence viability assay according to the manufacturer's instructions.

Results and Discussion

CDR grafting onto preferred human germline v-genes

The CDRs of an antagonistic murine anti-C-KIT IgG 37M (37M; see WO2014018625A1 and Table 2) were initially introduced to human germline immunoglobulin v-domain framework sequence scaffolds using CDR grafting. To bias our engineering efforts towards final lead therapeutic IgG compounds with optimal drug-like properties, we chose to graft the CDRs of

the parental antibody onto "preferred" germline scaffolds IGHV1-46 and IGKV1-16, which are known to have good solubility, high physical stability and are used at high frequency in the expressed human antibody repertoire.

Those scaffolds and grafted CDR definitions are outlined in Table 2. The heavy and light chain sequences for chimeric anti-C-KIT antibody m37M and humanized h37M are also shown in Table 2. While this process of CDR grafting is well known, it is still problematic to predict whether a given set of human v-domain sequences will act as suitable acceptor frameworks for non-human CDR grafting. The use of unsuitable frameworks can lead to the loss of target binding function, protein stability issues or even impaired expression of the final IgG. Indeed, the inclusion of multiple murine residues in the framework regions of h37M are indicative that full target binding affinity could not be maintained in the direct CDR graft into germline frameworks. The IGHV1-46/IGKV1-16 graft was therefore taken forward as the template for CDR mutagenesis and selection of improved clones.

Library generation and screening

The CDR-grafted IGKV1-16/IGHV1-46 v-domain sequences were combined into a VL-VH scFv format and a mutagenesis library cassette was generated by mass oligonucleotide synthesis and assembly. The final scFv library was ligated into a phage display vector and transformed into *E. coli* via electroporation to generate 1.0 x 10^9 independent clones. Library build quality was verified by sequencing 96 clones. This sequencing data showed that the positions encoding either the murine or human germline residue at each position of variance had been effectively sampled at a frequency of approximately 50%. Libraries were rescued using helper phage M13 and selections performed on biotinylated human and cynomolgus monkey C-KIT-Fc proteins in multiple separate branches.

Post-selection screening (as shown in Fig. 1) and DNA sequencing revealed the presence of 219 unique, human and mouse C-KIT-binding scFv clones that retained epitope binding competition with h37M IgG1 and contained significantly increased human content within the CDRs, while the framework sequences remained fully germline. Amongst these 219 clones, germ-lining mutations were observed in all CDRs (Table 3). Lead clones were ranked based on the level of CDR germ-lining versus ELISA and HTRF signals for both human and cyno C-KIT-Fc (Fig. 1). The v-domains of the 13 top clones from this ranking were then sub-cloned into IgG expression vectors for further testing as below (Table 4).

While germ-lining mutations were observed in all CDRs for the lead clones derived directly from library selections, it remained possible that sequence analyses might allow further

clones to be designed to have maximal humanization. The 219 sequence-unique hits with binding signals against human and mouse protein were therefore used to analyse the retention frequency for murine amino acids in the CDRs of this functionally characterized population. Positional amino acid retention frequency was expressed as a percentage found in the V_H and V_L domains (Fig. 2A, 2B). Murine residues with RF < 75% were regarded as positions that are possibly not essential to the target-binding paratope and are likely to be open to germ-lining, in a series of combinatorial designs.

Fifteen designs containing principally those murine residues with RF > 75%, in a number of combinations, were designated "MH1-MH15" (MH = Maximally Humanized). As the LCDR3 of h37M contained a potential deamidation site ('NS' at positions 3 and 4 of the CDR), multiple 'MH' clones also sampled possible substitutions such as N/A/S/T/E and S/A/N/D, which might remove this development risk motif while maintaining acceptable target binding function. The sole non-human germline residue found in the LCDR3 ('R' at position 8 in the CDR) was also sampled for the tolerance of homologous substitutions R/K/H, to establish if stability or immunogenicity-enhancing mutations might be identified. Another six designer clones "TTP1-6" ('TTP' = Total Theoretically Possible) were also created that combined the most humanized CDRs observed in the high-functioning population of scFv sequences, plus deamidation motif-disrupting mutations (Table 4). The MH and TTP clones were generated by gene synthesis (along with the 13 library-derived clones outlined above, positive controls h37M and m37M, and negative control non-C-KIT-reactive isotype v-domains), then cloned into expression vectors for production as human IgG1. All IgGs were readily expressed and purified from transient transfections of HEK-293 cells.

Lead IgG specificity and potency characteristics

The purified IgGs described above were then tested for binding to human and cyno C-KIT-Fc in direct titration ELISA format (Fig. 3). This analysis demonstrated that while all library derived clones and MH1-3, 5, 10, 11 and 12 designer clones retained binding affinity for human and cyno C-KIT that was comparable to the h37M IgG1, all of the other MH and TTP clones showed reduced binding to both orthologues (Fig. 3).

As direct ELISA binding signal is influenced by avidity and does not prove the maintenance a specific epitope, all IgGs were then examined in a solution-phase HTRF competition assay (Fig. 4). All library-derived and multiple designer IgGs exhibited full concentration-dependent inhibition of h37M binding to human (Fig. 4A) and cyno (Fig. 4C, D) c-KIT, with key leads showing highly similar IC50 values to that observed for unlabelled h37M IgG (Table 5). This demonstrated maintenance of a shared epitope and binding affinity in these clones. All TTP

clones, plus MH clones 4, 6, 7 and 9 were found not to fully inhibit h37M binding to either human (Fig. 4B) or cyno (Fig. 4E) C-KIT.

Flow cytometric analyses of lead IgG binding specificity at the cell membrane

Antibodies to C-KIT were analysed for concentration-dependent binding at the cell surface via flow cytometry. CHO-K1 cells were stably transfected with either human or cyno C-KIT full-length cDNAs. Anti-C-KIT IgGs and an isotype control IgG1 were then all tested in IgG1 format, over a concentration range of 100-0.02 μg/ml for binding to human (Fig. 5A, 5B), cyno (Fig. 5C, 5D) or wild type control ('wt', i.e. untransfected) CHO-K1 (Fig. 5E, 5F). All IgGs other than the isotype control showed concentration-dependent binding to human and cyno C-KIT+ cells, with a maximum MFI in each case being >50-fold higher than observed background signals for binding to untransfected CHO-K1. Anti-C-KIT antibodies exhibited no measurable background binding on untransfected CHO-K1 cells, comparable to the Isotype control IgG1 (Fig. 5E, 5F).

Analyses of designer IgGs based on the lead clone MH5

As described above, clone MH5 had proven to have highly specific binding to human and cyno C-KIT, low off-target binding potential, reduced deamidation potential in the CDRs, fully germline framework regions and multiple human germlining mutations in the CDRs. As a first-generation designer clone, however, the MH5 sequence had been targeted for improved human germline content and development qualities in the light chain, but not the heavy chain. The VH domain still retained a number of non-germline (mouse-derived) residues in the CDRs that were suggested to be potentially modifiable by the data found in Fig. 2A and 2B and observed functional mutant sequences outlined in Table 3. In an attempt to sample further mutations in the VH that might improve expression, stability, immunogenicity or functional characteristics, 36 'second generation' mutants were generated as outlined in Table 6. These clones were expressed and purified in IgG1 format and examined for target binding by ELISA on human and cyno C-KIT orthologs (Fig. 6A and 6B, respectively) and neutralisation of h37M/C-KIT interaction for both orthologs by HTRF. In this phase, it was found that strong ELISA binding signals for several clones suggested multiple mutations could be tolerated in the CDRs of the majority of the IgGs, but these changes could not always be combined without some changes in potency. In the HTRF analyses (Fig. 7A, 7B) it was found that key second generation leads MH5.1, 5.2, 5.22, 5.23, 5.24, 5.33 and 5.34, in particular, were capable of fully neutralising h37M/C-KIT interaction, albeit with reduced potency by IC50 calculation (Table 7).

Finally, the second-generation clones MH5.1, 5.2, 5.22, 5.23, 5.24, 5.33 and 5.34 were examined for their binding to human (Fig. 8A), cyno (Fig. 8B) or wild type control ('wt', i.e. untransfected) CHO-K1 (Fig. 8C) cells by flow cytometry. These analyses confirmed that each of these 7 prioritised second-generation clones still exhibit strong, concentration-dependent binding to human or cyno KIT+ CHO-K1 cells that is near identical to the signals generated by h37M and MH5, but also show no observable binding to untransfected CHO-K1.

Lead IgG analyses in 'Developability' ELISA assays

To ensure that high-affinity lead clones had not suffered from loss of target specificity during the mutation and reselection process; clones E-C2, E-C7, MH1, MH5, MH5.22 and h37M were tested for binding to a panel of 14 purified human proteins from the immunoglobulin superfamily (Fig. 9A-F). All six IgGs exhibited strong binding signals at 1 μg/ml to C-KIT-Fc (human OD450 nm > 1.8, cyno > 2.0), and no detectable binding (OD450 nm <0.1) against any other protein, including the closely-related murine and rat C-KIT-Fc proteins, which demonstrated that target binding specificity had been maintained in these clones.

It is known in the art that the binding of IgGs intended for therapeutic use to several indicative biological substrates is an indicator of high risk for poor performance in patients due to poor bioavailability and short *in vivo* half-life. Three such biological substrates are Insulin, dsDNA and ssDNA. These three substrates were therefore used to coat ELISA plates and examine the binding of the IgG1null versions of the optimised lead antibodies. Binding signals for these human IgG-based antibodies was compared to 'positive control' human IgG antibodies that have been found to have polyreactivity and poor performance, which stopped their progress in clinical trials (Bococizumab and Briakinumab human IgG1 analogues). For a negative control human IgG1 antibody, an IgG1 Ustekinumab analogue was used as it reacts with the same therapeutic target as Briakinumab, but has longer pK and was successfully approved as a therapeutic product. In the ELISA analyses shown in Fig. 10A, B and C, the positive control antibodies exhibited the expected strong reactivity to all 3 substrates, while the negative control Ustekinumab showed low reactivity. Importantly, all of the IgG1 lead clones tested showed binding ≤ the negative control against all 3 substrates. This finding underlined the maintenance of highly specific, target-driven binding in the optimised clones E-C2, E-C7, MH1, MH5 and MH5.22.

Antibody v-domain T cell epitope analyses

In silico technologies (Abzena, Ltd.), which are based on identifying the location of T cell epitopes in therapeutic antibodies and proteins, were used for assessing the immunogenicity

of both the h37M and lead antibody v-domains. Analysis of the v-domain sequences was performed with overlapping 9mer peptides (with each overlapping the last peptide by 8 residues) which were tested against each of the 34 MHC class II allotypes. Each 9mer was scored based on the potential 'fit' and interactions with the MHC class II molecules. The peptide scores calculated by the software lie between 0 and 1. Peptides that produced a high mean binding score (>0.55 in the iTope™ scoring function) were highlighted and, if >50% of the MHC class II binding peptides (i.e. 17 out of 34 alleles) had a high binding affinity (score >0.6), such peptides were defined as 'high affinity' MHC class II binding peptides which are considered a high risk for containing CD4+ T cell epitopes. Low affinity MHC class II binding peptides bind a high number of alleles (>50%) with a binding score >0.55 (but without a majority >0.6). Further analysis of the sequences was performed using the TCED™. The sequences were used to interrogate the TCED™ by BLAST search in order to identify any high sequence homology between peptides (T cell epitopes) from unrelated proteins/antibodies that stimulated T cell responses in previous *in vitro* T cell epitope mapping studies performed at Abzena Ltd.

Peptides were grouped into four classes: High Affinity Foreign ('HAF' – high immunogenicity risk), Low Affinity Foreign ('LAF' – lower immunogenicity risk), TCED+ (previously identified epitope in TCED™ database), and Germline Epitope ('GE' - human germline peptide sequence with high MHC Class II binding affinity). Germline Epitope 9mer peptides are unlikely to have immunogenic potential due to T cell tolerance, as validated by previous studies with a wide range of germline peptides. Importantly, such germline v-domain epitopes (aided further by similar sequences in the human antibody constant regions) also compete for MHC Class II occupancy at the membrane of antigen presenting cells, reducing the risk of foreign peptide presentation being sufficient to achieve the 'activation threshold' required for T cell stimulation. High GE content is therefore a beneficial quality in clinical development of an antibody therapeutic.

As shown in Figure 11 and Table 8, key lead v-domains exhibited significant beneficial changes in peptide epitope content in comparison to h37M. As the v-domain engineering process undertaken here had successfully selected for antibodies that maintained anti-KIT potency without the need for the murine residues included in the frameworks of h37M (Table 2), multiple HAF and LAF epitopes found in the frameworks of both the heavy and light chain v-domains of h37M (Fig. 11A) were absent in all library-derived and designer leads (Table 8). GE epitope content was also found to be significantly increased (from 4 to ≥ 10 in all leads), particularly in the VH regions of lead clones (Fig. 11B-11H), and TCED+ epitopes were reduced or eliminated in all leads (Table 8). Importantly, however, multiple foreign

epitopes were also eliminated by germlining mutations found in the CDRs of lead clones. For example, a TCED+ and HAF peptide 'VTITCKASQ' (SEQ ID NO: 64) found in the LCDR-1 of h37M was eliminated in all lead clones by the mutation K>R at position 6, converting this sequence to the light chain GE 'VTITCRASQ' (SEQ ID NO: 65; Fig 11B-11H). Similarly, for clones MH1 (Fig. 11E) and MH5.22 (Fig. 11G), the HAF peptides 'LIYSASSLQ' (SEQ ID NO: 66) and 'IYSASSLQS' (SEQ ID NO: 67) were both converted to GE sequences by mutation of the LCDR-2 sequence to the fully germline sequence 'AASSLQS' (SEQ ID NO: 24). The stabilising mutations found in the LCDR-3 of clones MH5 and MH5.22 (Tables 4 and 6), which are mutations away from the IGKV1-16 germline, were not found to generate any epitopes.

In the VH region of h37M, the peptide sequence 'YYINWVRQA' (SEQ ID NO: 68; spanning the HCDR-1 and FW2) was found to be both a TCED+ and HAF. The HCDR-1 germlining mutation I>M at position 3, found in all leads other than MH5.22 (Table 6), eliminated this risk and converted the peptide sequence into a GE. For clone MH5.22 however, the mutation Y>W in the HCDR-3 compensated by eliminating two LAF peptide sequences (Fig. 11G, Table 8). The above findings allowed the generation of the fully sequence-optimised clone MH5-DI (by changing the LCDR-2 sequence of MH5 from SASSLQS (SEQ ID NO: 17) to AASSLQS (SEQ ID NO: 24)), which had minimised chemical stability and immunogenicity risk characteristics in its primary sequence (Table 8, Fig. 11H).

BIACORE® analyses of affinity of IgG variants for human Fc receptors

Antibodies targeting the KIT receptor have been shown in human clinical studies and *in vitro* human immunological analyses to have a high risk of injection reactions in patients due to Fc receptor cross-linking during KIT engagement on mast cells (L'Italien et al., Clin Cancer Res 24(14):3465-3474, 2018). In order to examine receptor-engagement potential of Fc-engineered variants of the anti-KIT antibodies, h37M was expressed in IgG1 and IgG1-3M (L234A/L235A/G237A) formats, while clone MH1 was expressed in the novel IgG1-4M format (L234A/L235A/G237A/P331S). Each purified IgG1 variant was then examined for binding affinity to all human Fc receptors via surface plasmon resonance analyses. These analyses demonstrated that both the isotype control human IgG1 and h37M-IgG1 exhibited strong binding affinity for all human Fcγ receptors (Table 9) and normal affinity for FcRn at pH 6.0 but not pH 7.4 (Table 10). In contrast, while h37M-IgG1-3M and MH1 IgG1-4M also demonstrated normal affinity for FcRn at pH 6.0 but not pH 7.4 (Table 10), they both exhibited no measurable binding affinity for any human Fcγ receptors other than the inhibitory receptor FcγRIIb (Table 9). The lowest signals on FcγRIIb were observed for the MH1 IgG-4M (Figure 12). As demonstrated by the isotype control human

IgG4 antibody, use of this isotype reduces the affinity of the antibody to most Fcγ receptors, but is not sufficient to fully ablate activating receptor interaction (Table 9).

Lack of binding to activating FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb, but maintained binding to inhibitory receptor FcγRIIb and recycling receptor FcRn is an ideal combination of characteristics for an anti-KIT therapeutic antibody in either standard IgG form or as an armed antibody (used in antibody drug conjugate form, or in targeting another immune-activating mechanism such as CD3 ligation, CD16A ligation or CD47 blockade), as this potentially maximises the half-life of the molecule in circulation and simultaneously minimises the risk of mast cell activation-related toxicity in man.

Human extracellular proteome array analyses of antibody specificity

The exquisite specificity of antibody target recognition is one of the primary reasons they are chosen as drug candidates. This specificity is not guaranteed however, and impaired specificity after in vitro engineering of antibodies is a risk. To examine this possibility in the antibodies generated here, *in vitro* technologies (Retrogenix, Ltd.), which are based on using high-density arrays of cells expressing 5528 unique human plasma membrane and cell surface-tethered secreted proteins, were used to screen for off-target binding specificities in IgG1-h37M and IgG1-MH1. This receptor array binding screen identified that both antibodies exhibited strong binding to membrane-expressed C-KIT, but also had 3 potential off-target binding specificities: MMP7, CRIM1 and F13A1. Analysis of these interactions on re-array slides showed that these interactions were in fact artefactual and were mediated by the secondary antibody (Figure 13A). For IgG1-h37M and IgG1-MH1 (Figure 13B, 13C), they therefore showed extreme specificity, both binding only to the 2 known isoforms of C-KIT: accession numbers NM_000222.2 (canonical isoform; SEQ ID NO:209) and NM_001093772 (short isoform; SEQ ID NO:210). Repeat analyses of clones E-C2, E-C7, F-C5 and MH5 also exhibited highly specific binding to both C-KIT isoforms.

Cell-killing potential in armed antibody formats and comparison with C-KIT/SCF neutralisation potency

A key characteristic of antibody drug conjugates is the ability to internalise into cells expressing the antibody's cognate target. Internalisation of anti-c-KIT antibodies was assessed using a goat polyclonal heavy and light chain specific Fab conjugated to saporin toxin. The Fab-ZAP reagent binds to the human IgG1 and if that IgG1 binds to a protein on a cell surface and is internalised, then the Fab-ZAP reagent is also internalised into endosomes. Once in the endosome, the saporin toxin is released from the complex and inactivates ribosomes, eventually resulting in cell death. This method therefore allows direct

comparison of the potential potency of IgG1 antibodies as antibody drug conjugates. In these internalisation experiments, all antibodies h37M, MH1 and MH5-DI in IgG1-3M form drove highly potent internalisation and killing of CHO cells expressing human C-KIT (Fig. 14A), cyno C-KIT (Fig. 14B), and the TF-1 human erythroleukaemia line (Fig. 14C), while isotype control human IgG1 did not drive any internalisation or killing of any of the 3 cell types. Importantly, all 3 clones also generated highly similar potencies, as measured in IC50, with all 3 IC50 values being within ~2-fold on KIT+ CHO cells and within ~4-fold on TF-1 cells (Table 11). These highly similar values for potency in cell-killing experiments were expected, as clones MH1 and MH5 exhibited fully overlapping curves with h37M (and therefore indistinguishable affinities for C-KIT) in high-sensitivity, solution-phase HTRF epitope competition for binding to human C-KIT (Fig. 15A) and cyno C-KIT (Fig. 15B), as above.

As the erythroleukaemia line TF-1 constitutively expresses C-KIT, it is driven to proliferate when the natural ligand for C-KIT, SCF, is added to culture media. This provided an ideal experimental context in which to examine the potency of the anti-C-KIT IgGs in the neutralisation of C-KIT/SCF signalling. TF-1 cells were cultured in the presence of SCF and multiple antibodies tested for their ability to inhibit cellular proliferation. This analysis demonstrated that all antibodies tested were capable of inhibiting cellular proliferation being driven by SCF, but that h37M was significantly more potent than any other clone (Fig. 16). Indeed, when IC50 values were generated, it was found that only h37M exhibited potency in the pM range (0.11 nM), while MH1 and MH5-DI were 26.5-fold and 243.6-fold less potent, respectively (Table 11).

This potency differential in C-KIT/SCF inhibition versus internalisation and toxin delivery is a significant unexpected benefit for clones such as MH1 and MH5-DI. C-KIT is highly expressed on haematopoietic stem cells in the bone marrow, so anti-C-KIT antibody drug conjugates for the treatment of cancer exhibit improved therapeutic index when they efficiently deliver toxins in the pM concentration range but are incapable of blocking C-KIT/SCF signalling when dosed at such low concentrations. This leads to improved tumor targeting, but reduced bone marrow toxicity (L'Italien et al., Clin Cancer Res 24(14):3465-3474, 2018). Reduced potency in C-KIT/SCF signalling inhibition, but retention of high affinity binding to the C-KIT ectodomain may also prove to be beneficial for improving the therapeutic index of other forms of armed anti-C-KIT antibody, for example in immune targeting via CD3 ligation, CD16A ligation, or CD47 blockade. Clones described here, such as MH1 and MH5-DI in IgG1-4M format may therefore have the ideal set of improved characteristics over h37M-IgG1 for development as armed anti-C-KIT antibodies: low immunogenicity, high

affinity/high specificity targeting of C-KIT, high potency delivery of toxins via internalisation, lower potency C-KIT/SCF signalling inhibition and no interaction with human Fcγ receptors.

Although the present invention has been described with reference to preferred or exemplary embodiments, those skilled in the art will recognize that various modifications and variations to the same can be accomplished without departing from the spirit and scope of the present invention and that such modifications are clearly contemplated herein. No limitation with respect to the specific embodiments disclosed herein and set forth in the appended claims is intended nor should any be inferred.

All documents cited herein are incorporated by reference in their entirety.

Table 1. Amino acid sequences murine anti-C-KIT CDRs as defined here ("Unified" scheme) in comparison to alternative definitions.

Scheme	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
Unified	GYTFTDYYIN (SEQ ID NO: 4)	IARIYPGSGNTYYNEKFKG (SEQ ID NO: 5)	GVYYFDY (SEQ ID NO: 6)	KASQNVRTNVA (SEQ ID NO: 10)	SASYRYS (SEQ ID NO: 11)	QQYNSYPRT (SEQ ID NO: 12)
Kabat	DYYIN (SEQ ID NO: 69)	RIYPGSGNTYYNEKFKG (SEQ ID NO: 73)	GVYYFDY (SEQ ID NO: 6)	KASQNVRTNVA (SEQ ID NO: 10)	SASYRYS (SEQ ID NO: 11)	QQYNSYPRT (SEQ ID NO: 12)
Chothia	GYTFTDY (SEQ ID NO: 70)	YPGSGN (SEQ ID NO: 74)	GVYYFDY (SEQ ID NO: 6)	KASQNVRTNVA (SEQ ID NO: 10)	SASYRYS (SEQ ID NO: 11)	QQYNSYPRT (SEQ ID NO: 12)
IMGT	GYTFTDYY (SEQ ID NO: 71)	IYPGSGNT (SEQ ID NO: 75)	ARGVYYFDY (SEQ ID NO: 79)	QNVRTN (SEQ ID NO: 82)	SAS	QQYNSYPRT (SEQ ID NO: 12)
AHo	GYTFTDYYIN (SEQ ID NO: 4)	IYPGSGNTYYNEKFKG (SEQ ID NO: 76)	GVYYFD (SEQ ID NO: 80)	ASQNVRTN (SEQ ID NO: 83)	SASYRYS (SEQ ID NO: 11)	YNSYPR (SEQ ID NO: 86)
AbM	GYTFTDYYIN (SEQ ID NO: 4)	RIYPGSGNTY (SEQ ID NO: 77)	GVYYFDY (SEQ ID NO: 6)	KASQNVRTNVA (SEQ ID NO: 10)	SASYRYS (SEQ ID NO: 11)	QQYNSYPRT (SEQ ID NO: 12)
Contact	TDYYIN (SEQ ID NO: 72)	IARIYPGSGNTY (SEQ ID NO: 78)	ARGVYYFD (SEQ ID NO: 81)	VRTNVAWY (SEQ ID NO: 84)	ALIYSASYRY (SEQ ID NO: 85)	QQYNSYPR (SEQ ID NO: 207)

Table 2. Amino acid sequence of 37M murine anti-C-KIT v-domains (m37M) and human germline CDR grafts (h37M).

V DOMAIN	Human germline[1]	Amino acid sequence[2]
m37M-VH	n/a	QVQLKQSGAELVRPGASVKLSCKAS**GYTFTDYYIN**WVKQRPGQGLEW**IARIYPGSGNTYYNEKFKG**KATLTAEKSSSTAYMQLSSLTSEDSAVYFCAR**GVYYFDY**WGQGTTLTVSS (SEQ ID NO: 87)
h37M-VH	IGHV1-46[3]	QVQLVQSGAEVKKPGASVK***L***SCKAS**GYTFTDYYIN**WVRQAPG***K***GLEW**IARIYPGSGNTYYNEKFKG**R***A***T***L***T***A***D***K***STST***A***YM***Q***LSSLRSEDTAVY***F***CAR**GVYYFDY**WGQGTTVTVSS (SEQ ID NO: 88)
VH graft	IGHV1-46[4]	QVQLVQSGAEVKKPGASVKVSCKAS**GYTFTDYYIN**WVRQAPGQGLEW**IARIYPGSGNTYYAQKFQG**RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR**GVYYFDY**WGQGTLVTVSS (SEQ ID NO: 89)
m37M-VL	n/a	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVRTNVA**WYQQKPGQSPKALIY**SASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLADYFC**QQYNSYPRT**FGGGTKLEIKR (SEQ ID NO: 90)
h37M-VL	IGKV1-16[3]	DI***V***MTQSPSSLSASVGDRVTITC**KASQNVRTNVA**W***Y***QQKPGKAPK***A***LIY**SASYRYS**GVP***D***RF***T***GSGSGTDFTLTISSLQPEDFA***D***YFC**QQYNSYPRT**FGGGTKVEIK (SEQ ID NO: 91)
VL graft	IGKV1-16[4]	DIQMTQSPSSLSASVGDRVTITC**RASQGVRTNVA**WFQQKPGKAPKSLIY**SASYRYS**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQYNSYPRT**FGGGTKVEIK (SEQ ID NO: 92)

[1]Human germline definitions used for grafting, based on IMGT system. [2]CDR residues are in bold and underlined. As noted above, the "Unified" CDR definitions used in this manuscript are an expanded definition in comparison to the classical Kabat definition. Each sequence above shows the framework regions (FRs) and the CDRs in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. [3]h37M domains contain several murine residues in the framework regions, shown here ***italicized***. [4]Grafts are fully germline in the framework regions, used as the template for CDR mutant library construction.

Table 3. Amino acid sequences of unique CDRs from 219 unique anti-C-KIT v-domains.

LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
RASQGIRNNLA (SEQ ID NO: 93)	AASSLQS (SEQ ID NO: 24)	QQYASYPLT (SEQ ID NO: 119)	GYTFTDYYMH (SEQ ID NO: 123)	IAIIYPGSGNTYYAQKFQG (SEQ ID NO: 126)	GVYYFDA (SEQ ID NO: 151)
RASQGIRNNVA (SEQ ID NO: 94)	AASSLYS (SEQ ID NO: 111)	QQYASYPRT (SEQ ID NO: 53)	GYTFTDYYMN (SEQ ID NO: 13)	IARINPGSGNTSYAQKFQG (SEQ ID NO: 127)	GVYYFDD (SEQ ID NO: 152)
RASQGIRNYVA (SEQ ID NO: 95)	AASSRQS (SEQ ID NO: 23)	QQYNSYPLT (SEQ ID NO: 120)	GYTFTSYYIN (SEQ ID NO: 124)	IARINPGSGNTYYAQKFQG (SEQ ID NO: 128)	GVYYFDE (SEQ ID NO: 21)
RASQGIRTNLA (SEQ ID NO: 16)	AASSRYS (SEQ ID NO: 112)	QQYQSYPLT (SEQ ID NO: 121)	GYTFTSYYMN (SEQ ID NO: 125)	IARIYPGSGNTSYAQKFQG (SEQ ID NO: 129)	GVYYFDF (SEQ ID NO: 153)
RASQGIRTNVA (SEQ ID NO: 19)	AASYLQS (SEQ ID NO: 50)	QQYQSYPRT (SEQ ID NO: 122)		IARIYPGSGNTYYAQKFQG (SEQ ID NO: 206)	GVYYFDG (SEQ ID NO: 154)
RASQGISNYLA (SEQ ID NO: 96)	AASYLYS (SEQ ID NO: 113)			IARIYPSSGNTSYAQKFQG (SEQ ID NO: 130)	GVYYFDH (SEQ ID NO: 155)
RASQGISNYVA (SEQ ID NO: 97)	AASYRQS (SEQ ID NO: 44)			IARIYPSSGNTYYAQKFQG (SEQ ID NO: 131)	GVYYFDI (SEQ ID NO: 156)
RASQGISTNLA (SEQ ID NO: 98)	AASYRYS (SEQ ID NO: 114)			IGIIYPGSGNTYYAQKFQG (SEQ ID NO: 132)	GVYYFDK (SEQ ID NO: 157)
RASQGISTNVA (SEQ ID NO: 99)	SASSLQS (SEQ ID NO: 17)			IGRINPGSGNTSYAQKFQG (SEQ ID NO: 133)	GVYYFDL (SEQ ID NO: 205)
RASQGISTYLA (SEQ ID NO: 100)	SASSLYS (SEQ ID NO: 115)			IGRINPGSGNTYYAQKFQG (SEQ ID NO: 134)	GVYYFDM (SEQ ID NO: 158)
RASQGISTYVA (SEQ ID NO: 101)	SASSRQS (SEQ ID NO: 52)			IGRIYPGSGNTSYAQKFQG (SEQ ID NO: 135)	GVYYFDN (SEQ ID NO: 159)
RASQGVRNNLA (SEQ ID NO:43)	SASSRYS (SEQ ID NO: 116)			IGRIYPGSGNTYYAQKFQG (SEQ ID NO: 45)	GVYYFDP (SEQ ID NO: 160)
RASQGVRNNVA (SEQ ID NO: 47)	SASYLQS (SEQ ID NO: 117)			IGRIYPSSGNTSYAQKFQG (SEQ ID NO: 136)	GVYYFDQ (SEQ ID NO: 161)
RASQGVRNYVA (SEQ ID NO: 102)	SASYLYS (SEQ ID NO: 118)			IGRIYPSSGNTYYAQKFQG (SEQ ID NO: 137)	GVYYFDR (SEQ ID NO: 162)
RASQGVRTNLA (SEQ ID NO: 22)	SASYRQS (SEQ ID NO: 20)			MAIIYPGSGNTYYAQKFQG (SEQ ID NO: 138)	GVYYFDS (SEQ ID NO: 46)
RASQGVRTNVA (SEQ ID NO: 39)	YASSLQS (SEQ ID NO: 48)			MARINPGSGNTSYAQKFQG (SEQ ID NO: 139)	GVYYFDT (SEQ ID NO: 51)
RASQGVSNNLA (SEQ ID NO: 103)				MARINPGSGNTYYAQKFQG (SEQ ID NO: 140)	GVYYFDV (SEQ ID NO: 163)
RASQGVSNNVA (SEQ ID NO: 104)				MARIYPGSGNTSYAQKFQG (SEQ ID NO: 141)	GVYYFDW (SEQ ID NO: 164)
RASQGVSNYLA (SEQ ID NO: 105)				MARIYPGSGNTYYAQKFQG (SEQ ID NO: 142)	GVYYADY (SEQ ID NO: 165)
RASQGVSNYVA (SEQ ID NO: 106)				MARIYPSSGNTSYAQKFQG (SEQ ID NO: 143)	GVYYGDY (SEQ ID NO: 166)
RASQGVSTNLA (SEQ ID NO: 107)				MARIYPSSGNTYYAQKFQG (SEQ ID NO: 144)	GVYYHDY (SEQ ID NO: 167)
RASQGVSTNVA (SEQ ID NO: 108)				MGIIYPGSGNTYYAQKFQG (SEQ ID NO: 145)	GVYYQDY (SEQ ID NO: 41)
RASQGVSTYLA				MGRINPGSGNTSYAQKFQG (SEQ ID NO: 146)	GVYYLDY

(SEQ ID NO: 109)					(SEQ ID NO: 18)
RASQGVSTYVA (SEQ ID NO: 110)				MGRINPGSGNTYYAQKFQG (SEQ ID NO: 147)	GVYYKDY (SEQ ID NO: 168)
				MGRIYPGSGNTSYAQKFQG (SEQ ID NO: 148)	GVYYNDY (SEQ ID NO: 169)
				MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYMDY (SEQ ID NO: 170)
				MGRIYPSSGNTSYAQKFQG (SEQ ID NO: 149)	GVYYYDY (SEQ ID NO: 15)
				MGRIYPSSGNTYYAQKFQG (SEQ ID NO: 150)	GVYYWDY (SEQ ID NO: 171)
					GVYAFDY (SEQ ID NO: 172)
					GVYQFDY (SEQ ID NO: 173)
					GVYHFDY (SEQ ID NO: 49)
					GVYWFDY (SEQ ID NO: 174)
					GVYNFDY (SEQ ID NO: 175)
					GVYEFDY (SEQ ID NO: 42)
					GVYTFDY (SEQ ID NO: 176)
					GVYDFDY (SEQ ID NO: 177)
					GVWYFDY (SEQ ID NO: 40)

Table 4. Amino acid sequences of CDRs of unique, library-derived and designer, human/cyno cross-reactive anti-C-KIT antibodies.

Clone Name	LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
E-E10	RASQGVRTNVA (SEQ ID NO: 39)	SASSLQS (SEQ ID NO: 17)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVWYFDY (SEQ ID NO: 40)
F-F2	RASQGVRTNVA (SEQ ID NO: 39)	AASSRQS (SEQ ID NO: 23)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYQDY (SEQ ID NO: 41)
C-B12	RASQGVRNNLA (SEQ ID NO:43)	AASYRQS (SEQ ID NO: 44)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYEFDY (SEQ ID NO: 42)
C-A7	RASQGVRNNVA (SEQ ID NO: 47)	YASSLQS (SEQ ID NO: 48)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	IGRIYPGSGNTYYAQKFQG (SEQ ID NO: 45)	GVYYFDS (SEQ ID NO: 46)
C-A5	RASQGVRNNVA (SEQ ID NO: 47)	AASYLQS (SEQ ID NO: 50)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYHFDY (SEQ ID NO: 49)
D-A10	RASQGVRTNLA (SEQ ID NO: 22)	AASSRQS (SEQ ID NO: 23)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYFDT (SEQ ID NO: 51)
E-C7	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
D-D5	RASQGVRTNLA (SEQ ID NO: 22)	SASSLQS (SEQ ID NO: 17)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
E-C2	RASQGIRTNVA (SEQ ID NO: 19)	SASYRQS (SEQ ID NO: 20)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYLDY (SEQ ID NO: 18)
F-B11	RASQGIRTNLA (SEQ ID NO: 16)	AASYRQS (SEQ ID NO: 44)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYLDY (SEQ ID NO: 18)
D-D9	RASQGVRTNVA (SEQ ID NO: 39)	SASSLQS (SEQ ID NO: 17)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYLDY (SEQ ID NO: 18)
E-G7	RASQGVRTNVA (SEQ ID NO: 39)	SASSRQS (SEQ ID NO: 52)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYFDE (SEQ ID NO: 21)
F-C5	RASQGVRTNLA (SEQ ID NO: 22)	AASSRQS (SEQ ID NO: 23)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYFDE (SEQ ID NO: 21)
MH1	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH2	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYASYPRT (SEQ ID NO: 53)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH3	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYNAYPRT (SEQ ID NO: 54)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH4	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYNDYPRT (SEQ ID NO: 55)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH5	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH6	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYNSYPKT (SEQ ID NO: 56)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH7	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYNSYPHT (SEQ ID NO: 57)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH8	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYSSYPRT (SEQ ID NO: 58)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH9	RASQGIRTNLA	SASSLQS	QQYESYPRT	GYTFTDYYMN	MGRIYPGSGNTYYAQKFQG	GVYYYDY

	(SEQ ID NO: 16)	(SEQ ID NO: 17)	(SEQ ID NO: 59)	(SEQ ID NO: 13)	(SEQ ID NO: 14)	(SEQ ID NO: 15)
MH10	RASQGIRTNLA (SEQ ID NO: 16)	SASSLQS (SEQ ID NO: 17)	QQYTSYPRT (SEQ ID NO: 60)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH11	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYNAYPRT (SEQ ID NO: 54)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH12	RASQGVRTNLA (SEQ ID NO: 22)	AASSLQS (SEQ ID NO: 24)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH13	RASQGVRTNLA (SEQ ID NO: 22)	AASSLQS (SEQ ID NO: 24)	QQYNAYPRT (SEQ ID NO: 54)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
MH14	RASQGVRTNLA (SEQ ID NO: 22)	AASSLQS (SEQ ID NO: 24)	QQYNSYPRT (SEQ ID NO: 12)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYLDY (SEQ ID NO: 18)
MH15	RASQGVRTNLA (SEQ ID NO: 22)	AASSLQS (SEQ ID NO: 24)	QQYNAYPRT (SEQ ID NO: 54)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYLDY (SEQ ID NO: 18)
TTP1	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYNSYPKT (SEQ ID NO: 56)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYLDY (SEQ ID NO: 18)
TTP2	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYNSYPHT (SEQ ID NO: 57)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
TTP3	RASQGVRTNLA (SEQ ID NO: 22)	SASSLQS (SEQ ID NO: 17)	QQYNAYPKT (SEQ ID NO: 178)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYLDY (SEQ ID NO: 18)
TTP4	RASQGVRTNLA (SEQ ID NO: 22)	SASSLQS (SEQ ID NO: 17)	QQYNAYPHT (SEQ ID NO: 179)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)
TTP5	RASQGVRTNLA (SEQ ID NO: 22)	AASSLQS (SEQ ID NO: 24)	QQYNAYPKT (SEQ ID NO: 178)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYLDY (SEQ ID NO: 18)
TTP6	RASQGVRTNLA (SEQ ID NO: 22)	AASSLQS (SEQ ID NO: 24)	QQYNAYPHT (SEQ ID NO: 179)	GYTFTDYYMN (SEQ ID NO: 13)	MGRIYPGSGNTYYAQKFQG (SEQ ID NO: 14)	GVYYYDY (SEQ ID NO: 15)

Table 5. HTRF epitope competition IC50 values for key library derived and designer lead anti-C-KIT antibodies in IgG1 format.

Clone Name	Human IC50 (µg/ml)	Cyno IC50 (µg/ml)
C-KIT-h37M	0.025	0.035
C-KIT-D-A10	0.027	0.059
C-KIT-E-C7	0.028	0.051
C-KIT-E-E10	0.031	0.097
C-KIT-F-C5	0.031	0.079
C-KIT-C-A7	0.037	0.081
C-KIT-F-F2	0.046	0.121
C-KIT-MH11	0.051	0.101
C-KIT-MH5	0.063	0.036
C-KIT-C-B12	0.066	0.166

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Table 6. Amino acid sequences of CDRs of second generation, designer, human/cyno cross-reactive anti-C-KIT antibodies.

Clone Name	LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
MH5.1	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYYDY (SEQ ID NO: 27)
MH5.2	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYYDY (SEQ ID NO: 27)
MH5.3	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYYDY (SEQ ID NO: 27)
MH5.4	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDS (SEQ ID NO: 62)
MH5.5	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDT (SEQ ID NO: 63)
MH5.6	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDE (SEQ ID NO: 184)
MH5.7	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYYDY (SEQ ID NO: 27)
MH5.8	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDS (SEQ ID NO: 62)
MH5.9	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDT (SEQ ID NO: 63)
MH5.10	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDE (SEQ ID NO: 184)
MH5.11	RASQGIRTNLA	AASSLQS	QQYANYPRT	GYTFTDHYMN	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYYDY

	(SEQ ID NO: 16)	(SEQ ID NO: 24)	(SEQ ID NO: 25)	(SEQ ID NO: 182)		(SEQ ID NO: 27)
MH5.12	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDS (SEQ ID NO: 62)
MH5.13	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDT (SEQ ID NO: 63)
MH5.14	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDHYMN (SEQ ID NO: 182)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDE (SEQ ID NO: 184)
MH5.15	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDS (SEQ ID NO: 62)
MH5.16	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDT (SEQ ID NO: 63)
MH5.17	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDE (SEQ ID NO: 184)
MH5.18	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYYDY (SEQ ID NO: 27)
MH5.19	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDS (SEQ ID NO: 62)
MH5.20	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDT (SEQ ID NO: 63)
MH5.21	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDE (SEQ ID NO: 184)
MH5.22	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYYDY (SEQ ID NO: 27)
MH5.23	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDS (SEQ ID NO: 62)
MH5.24	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDT (SEQ ID NO: 63)
MH5.25	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTDFYMN (SEQ ID NO: 180)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDE (SEQ ID NO: 184)
MH5.26	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDS (SEQ ID NO: 62)
MH5.27	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDT (SEQ ID NO: 63)
MH5.28	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPGTGNTYYAQKFQG (SEQ ID NO: 61)	GVWYFDE (SEQ ID NO: 184)
MH5.29	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYYDY (SEQ ID NO: 27)
MH5.30	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDS (SEQ ID NO: 62)
MH5.31	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDT (SEQ ID NO: 63)
MH5.32	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPGAGNTYYAQKFQG (SEQ ID NO: 183)	GVWYFDE (SEQ ID NO: 184)
MH5.33	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYYDY (SEQ ID NO: 27)
MH5.34	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDS (SEQ ID NO: 62)

MH5.35	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDT (SEQ ID NO: 63)
MH5.36	RASQGIRTNLA (SEQ ID NO: 16)	AASSLQS (SEQ ID NO: 24)	QQYANYPRT (SEQ ID NO: 25)	GYTFTNYYMN (SEQ ID NO: 181)	MGRIYPASGNTYYAQKFQG (SEQ ID NO: 26)	GVWYFDE (SEQ ID NO: 184)

Table 7. HTRF epitope competition IC50 values for second generation designer anti-C-KIT antibodies in IgG1 format.

Clone Name	Human IC50 (µg/ml)	Cyno IC50 (µg/ml)
C-KIT-h37M	0.016	0.036
C-KIT-MH5	0.067	0.058
C-KIT-MH5.1	0.684	0.633
C-KIT-MH5.2	0.272	0.254
C-KIT-MH5.22	0.134	0.164
C-KIT-MH5.23	2.024	3.256
C-KIT-MH5.24	2.201	2.743
C-KIT-MH5.34	0.839	0.532
C-KIT-MH5.35	2.047	0.785

Table 8. Human T cell epitope content in v-domains predicted by iTOPE™ and TCED™.

Clone Name	Germline epitopes	High Affinity Foreign	Low Affinity Foreign	TCED+
C-KIT-h37M	4	7	6	3
C-KIT-E-C7	10	3	4	0
C-KIT-F-C5	10	3	4	1
C-KIT-E-C2	10	2	4	0
C-KIT-MH1	12	1	4	0
C-KIT-MH5	10	3	4	0
C-KIT-MH5.22	11	3	1	1
C-KIT-MH5-DI	12	1	2	0

Table 9. BIACORE® analyses of affinity of IgG variants for human Fc receptors.

Receptor	Antibody	KD (M)	Chi2
FcG RI	Isotype IgG1	3.44 E-09	0.0289
FcG RI	Isotype IgG4	9.25 E-09	0.016
FcG RI	h37M-IgG1	1.06 E-09	0.573
FcG RI	h37M-IgG1-3M	NS	NS
FcG RI	MH1-IgG1-4M	NS	NS
FcG RIIa (167R)	Isotype IgG1	3.71 E-06	3.57
FcG RIIa (167R)	Isotype IgG4	Low	Low
FcG RIIa (167R)	h37M-IgG1	4.47 E-07	23.7
FcG RIIa (167R)	h37M-IgG1-3M	NS	NS
FcG RIIa (167R)	MH1-IgG1-4M	NS	NS
FcG RIIa (167H)	Isotype IgG1	6.14 E-06	2.09
FcG RIIa (167H)	Isotype IgG4	Low	Low
FcG RIIa (167H)	h37M-IgG1	5.12 E-07	4.16
FcG RIIa (167H)	h37M-IgG1-3M	NS	NS
FcG RIIa (167H)	MH1-IgG1-4M	NS	NS
FcG RIIb	Isotype IgG1	1.72 E-05	1.55
FcG RIIb	Isotype IgG4	2.31 E-05	0.431
FcG RIIb	h37M-IgG1	1.44 E-06	2.58
FcG RIIb	h37M-IgG1-3M	Low	Low
FcG RIIb	MH1-IgG1-4M	Low	Low
FcG RIIIa (176F)	Isotype IgG1	3.53 E-06	0.0343
FcG RIIIa (176F)	Isotype IgG4	NS	NS
FcG RIIIa (176F)	h37M-IgG1	8.20 E-08	0.205
FcG RIIIa (176F)	h37M-IgG1-3M	NS	NS
FcG RIIIa (176F)	MH1-IgG1-4M	NS	NS
FcG RIIIa (176V)	Isotype IgG1	1.40 E-06	0.448
FcG RIIIa (176V)	Isotype IgG4	Low	Low
FcG RIIIa (176V)	h37M-IgG1	4.11 E-08	3.36
FcG RIIIa (176V)	h37M-IgG1-3M	NS	NS
FcG RIIIa (176V)	MH1-IgG1-4M	NS	NS
FcG RIIIb	Isotype IgG1	9.9 E-06	1.85
FcG RIIIb	Isotype IgG4	NS	NS
FcG RIIIb	h37M-IgG1	2.49 E-07	5.6
FcG RIIIb	h37M-IgG1-3M	NS	NS
FcG RIIIb	MH1-IgG1-4M	NS	NS

NS = No Signal observed at any concentration of IgG

Low = Signal observed at high IgG concentrations

Table 10. BIACORE® analyses of affinity of IgG variants for human FcRn.

pH	Antibody	KD (M)	Chi2
6	Isotype IgG1	1.18 E-06	0.756
7.4	Isotype IgG1	NS	NS
6	Isotype IgG4	2.6 E-06	0.213
7.4	Isotype IgG4	NS	NS
6	h37M-IgG1	1.24 E-06	8.75
7.4	h37M-IgG1	NS	NS
6	h37M-IgG1-3M	1.32 E-06	6.58
7.4	h37M-IgG1-3M	NS	NS
6	MH1-IgG1-4M	1.59 E-06	3
7.4	MH1-IgG1-4M	NS	NS

NS = No Signal observed at any concentration of IgG

Table 11. Potency values for anti-C-KIT antibodies in IgG format in cell-based assays.

Clone Name	TF-1 proliferation IC50 (nM)	TF-1 FabZAP IC50 (pM)	Human C-KIT CHO FabZAP IC50 (pM)	Cyno C-KIT CHO FabZAP IC50 (pM)
C-KIT-h37M	0.11	12.76	5.7	7.823
C-KIT-MH1	2.86	51.09	8.7	13.96
C-KIT-MH5-DI	26.31	53.68	12.6	13.54

Table 12. Examples of antibody variable region amino acid sequences.

Antibody MH1 heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMGRIYPGSGNT
YYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGVYYYDYWGQGTLVTVSS
(SEQ ID NO: 185)

Antibody MH1 VL light chain variable (VL) region
DIQMTQSPSSLSASVGDRVTITCRASQGIRTNLAWFQQKPGKAPKSLIYAASSLQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPRTFGGGTKVEIK (SEQ ID NO: 186)

Antibody MH5-DI heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMGRIYPGSGNT
YYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGVYYYDYWGQGTLVTVSS
(SEQ ID NO: 187)

Antibody MH5-DI light chain variable (VL) region
DIQMTQSPSSLSASVGDRVTITCRASQGIRTNLAWFQQKPGKAPKSLIYAASSLQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYANYPRTFGGGTKVEIK (SEQ ID NO: 188)

Antibody MH5 heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMGRIYPGSGNT
YYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGVYYYDYWGQGTLVTVSS
(SEQ ID NO: 189)

Antibody MH5 light chain variable (VL) region
DIQMTQSPSSLSASVGDRVTITCRASQGIRTNLAWFQQKPGKAPKSLIYSASSLQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYANYPRTFGGGTKVEIK (SEQ ID NO: 190)

Antibody EC7 heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMGRIYPGSGNT
YYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGVYYYDYWGQGTLVTVSS
(SEQ ID NO: 191)

Antibody EC7 light chain variable (VL) region
DIQMTQSPSSLSASVGDRVTITCRASQGIRTNLAWFQQKPGKAPKSLIYSASSLQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPRTFGGGTKVEIK (SEQ ID NO: 192)

Antibody EC2 heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMGRIYPGSGNT
YYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGVYYLDYWGQGTLVTVSS
(SEQ ID NO: 193)

Antibody EC2 light chain variable (VL) region
DIQMTQSPSSLSASVGDRVTITCRASQGIRTNVAWLQQKPGKAPKSLIYSASYRQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPRTFGGGTKVEIK (SEQ ID NO: 194)

Antibody F-C5 heavy chain variable (VH) region
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMGRIYPGSGNT
YYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGVYYFDEWGQGTLVTVSS
(SEQ ID NO: 195)

Antibody F-C5 light chain variable (VL) region
DIQMTQSPSSLSASVGDRVTITCRASQGVRTNLAWFQQKPGKAPKSLIYAASSRQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPRTFGGGTKVEIK
(SEQ ID NO: 196)

Table 13. Examples of antibody Fc region amino acid sequences.

Human IgG1 wild type
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 197)

Human IgG1-3M
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
AAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 198)

Human IgG1-4M
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
AAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 199)

Human IgG1 wild type “REEM” allotype
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 200)

Human IgG1-3M “REEM” allotype
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
AAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 201)

Human IgG1-4M "REEM" allotype
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
AAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 202)

Table 14. Examples of human C-KIT amino acid and nucleotide sequences.

Human C-KIT protein
MRGARGAWDFLCVLLLLLRVQTGSSQPSVSPGEPSPPSIHPGKSDLIVRVGDEIRLLCTDPGFVKWTFEI
LDETNENKQNEWITEKAEATNTGKYTCTNKHGLSNSIYVFVRDPAKLFLVDRSLYGKEDNDTLVRCPLTD
PEVTNYSLKGCQGKPLPKDLRFIPDPKAGIMIKSVKRAYHRLCLHCSVDQEGKSVLSEKFILKVRPAFKA
VPVVSVSKASYLLREGEEFTVTCTIKDVSSSVYSTWKRENSQTKLQEKYNSWHHGDFNYERQATLTISSA
RVNDSGVFMCYANNTFGSANVTTTLEVVDKGFINIFPMINTTVFVNDGENVDLIVEYEAFPKPEHQQWIY
MNRTFTDKWEDYPKSENESNIRYVSELHLTRLKGTEGGTYTFLVSNSDVNAAIAFNVYVNTKPEILTYDR
LVNGMLQCVAAGFPEPTIDWYFCPGTEQRCSASVLPVDVQTLNSSGPPFGKLVVQSSIDSSAFKHNGTVE
CKAYNDVGKTSAYFNFAFKGNNKEQIHPHTLFTPLLIGFVIVAGMMCIIVMILTYKYLQKPMYEVQWKVV
EEINGNNYVYIDPTQLPYDHKWEFPRNRLSFGKTLGAGAFGKVVEATAYGLIKSDAAMTVAVKMLKPSAH
LTEREALMSELKVLSYLGNHMNIVNLLGACTIGGPTLVITEYCCYGDLLNFLRRKRDSFICSKQEDHAEA
ALYKNLLHSKESSCSDSTNEYMDMKPGVSYVVPTKADKRRSVRIGSYIERDVTPAIMEDDELAIDLEDLL
SFSYQVAKGMAFLASKNCIHRDLAARNILLTHGRITKICDFGLARDIKNDSNYVVKGNARLPVKWMAHES
IFNCVYTFESDVWSYGIFLWELFSLGSSPYPGMPVDSKFYKMIKEGFRMLSPEHAPAEMYDIMKTCWDAD
PLKRPTFKQIVQLIEKQISESTNHIYSNLANCSPNRQKPVVDHSVRINSVGSTASSSQPLLVHDDV
(SEQ ID NO: 208)

Human C-KIT protein, transcript variant 1
TCTGGGGGCTCGGCTTTGCCGCGCTCGCTGCACTTGGGCGAGAGCTGGAACGTGGACCAGAGCTCGGATC
CCATCGCAGCTACCGCGATGAGAGGCGCTCGCGGCGCCTGGGATTTTCTCTGCGTTCTGCTCCTACTGCT
TCGCGTCCAGACAGGCTCTTCTCAACCATCTGTGAGTCCAGGGGAACCGTCTCCACCATCCATCCATCCA
GGAAAATCAGACTTAATAGTCCGCGTGGGCGACGAGATTAGGCTGTTATGCACTGATCCGGGCTTTGTCA
AATGGACTTTTGAGATCCTGGATGAAACGAATGAGAATAAGCAGAATGAATGGATCACGGAAAAGGCAGA
AGCCACCAACACCGGCAAATACACGTGCACCAACAAACACGGCTTAAGCAATTCCATTTATGTGTTTGTT
AGAGATCCTGCCAAGCTTTTCCTTGTTGACCGCTCCTTGTATGGGAAAGAAGACAACGACACGCTGGTCC
GCTGTCCTCTCACAGACCCAGAAGTGACCAATTATTCCCTCAAGGGGTGCCAGGGGAAGCCTCTTCCCAA
GGACTTGAGGTTTATTCCTGACCCCAAGGCGGGCATCATGATCAAAAGTGTGAAACGCGCCTACCATCGG
CTCTGTCTGCATTGTTCTGTGGACCAGGAGGGCAAGTCAGTGCTGTCGGAAAAATTCATCCTGAAAGTGA
GGCCAGCCTTCAAAGCTGTGCCTGTTGTGTCTGTGTCCAAAGCAAGCTATCTTCTTAGGGAAGGGGAAGA
ATTCACAGTGACGTGCACAATAAAAGATGTGTCTAGTTCTGTGTACTCAACGTGGAAAAGAGAAAACAGT
CAGACTAAACTACAGGAGAAATATAATAGCTGGCATCACGGTGACTTCAATTATGAACGTCAGGCAACGT
TGACTATCAGTTCAGCGAGAGTTAATGATTCTGGAGTGTTCATGTGTTATGCCAATAATACTTTTGGATC
AGCAAATGTCACAACAACCTTGGAAGTAGTAGATAAAGGATTCATTAATATCTTCCCCATGATAAACACT
ACAGTATTTGTAAACGATGGAGAAAATGTAGATTTGATTGTTGAATATGAAGCATTCCCCAAACCTGAAC
ACCAGCAGTGGATCTATATGAACAGAACCTTCACTGATAAATGGGAAGATTATCCCAAGTCTGAGAATGA

AAGTAATATCAGATACGTAAGTGAACTTCATCTAACGAGATTAAAAGGCACCGAAGGAGGCACTTACACA
TTCCTAGTGTCCAATTCTGACGTCAATGCTGCCATAGCATTTAATGTTTATGTGAATACAAAACCAGAAA
TCCTGACTTACGACAGGCTCGTGAATGGCATGCTCCAATGTGTGGCAGCAGGATTCCCAGAGCCCACAAT
AGATTGGTATTTTTGTCCAGGAACTGAGCAGAGATGCTCTGCTTCTGTACTGCCAGTGGATGTGCAGACA
CTAAACTCATCTGGGCCACCGTTTGGAAAGCTAGTGGTTCAGAGTTCTATAGATTCTAGTGCATTCAAGC
ACAATGGCACGGTTGAATGTAAGGCTTACAACGATGTGGGCAAGACTTCTGCCTATTTTAACTTTGCATT
TAAAGGTAACAACAAAGAGCAAATCCATCCCCACACCCTGTTCACTCCTTTGCTGATTGGTTTCGTAATC
GTAGCTGGCATGATGTGCATTATTGTGATGATTCTGACCTACAAATATTTACAGAAACCCATGTATGAAG
TACAGTGGAAGGTTGTTGAGGAGATAAATGGAAACAATTATGTTTACATAGACCCAACACAACTTCCTTA
TGATCACAAATGGGAGTTTCCCAGAAACAGGCTGAGTTTTGGGAAAACCCTGGGTGCTGGAGCTTTCGGG
AAGGTTGTTGAGGCAACTGCTTATGGCTTAATTAAGTCAGATGCGGCCATGACTGTCGCTGTAAAGATGC
TCAAGCCGAGTGCCCATTTGACAGAACGGGAAGCCCTCATGTCTGAACTCAAAGTCCTGAGTTACCTTGG
TAATCACATGAATATTGTGAATCTACTTGGAGCCTGCACCATTGGAGGGCCCACCCTGGTCATTACAGAA
TATTGTTGCTATGGTGATCTTTTGAATTTTTTGAGAAGAAAACGTGATTCATTTATTTGTTCAAAGCAGG
AAGATCATGCAGAAGCTGCACTTTATAAGAATCTTCTGCATTCAAAGGAGTCTTCCTGCAGCGATAGTAC
TAATGAGTACATGGACATGAAACCTGGAGTTTCTTATGTTGTCCCAACCAAGGCCGACAAAAGGAGATCT
GTGAGAATAGGCTCATACATAGAAAGAGATGTGACTCCCGCCATCATGGAGGATGACGAGTTGGCCCTAG
ACTTAGAAGACTTGCTGAGCTTTTCTTACCAGGTGGCAAAGGGCATGGCTTTCCTCGCCTCCAAGAATTG
TATTCACAGAGACTTGGCAGCCAGAAATATCCTCCTTACTCATGGTCGGATCACAAAGATTTGTGATTTT
GGTCTAGCCAGAGACATCAAGAATGATTCTAATTATGTGGTTAAAGGAAACGCTCGACTACCTGTGAAGT
GGATGGCACCTGAAAGCATTTTCAACTGTGTATACACGTTTGAAAGTGACGTCTGGTCCTATGGGATTTT
TCTTTGGGAGCTGTTCTCTTTAGGAAGCAGCCCCTATCCTGGAATGCCGGTCGATTCTAAGTTCTACAAG
ATGATCAAGGAAGGCTTCCGGATGCTCAGCCCTGAACACGCACCTGCTGAAATGTATGACATAATGAAGA
CTTGCTGGGATGCAGATCCCCTAAAAAGACCAACATTCAAGCAAATTGTTCAGCTAATTGAGAAGCAGAT
TTCAGAGAGCACCAATCATATTTACTCCAACTTAGCAAACTGCAGCCCCAACCGACAGAAGCCCGTGGTA
GACCATTCTGTGCGGATCAATTCTGTCGGCAGCACCGCTTCCTCCTCCCAGCCTCTGCTTGTGCACGACG
ATGTCTGAGCAGAATCAGTGTTTGGGTCACCCCTCCAGGAATGATCTCTTCTTTTGGCTTCCATGATGGT
TATTTTCTTTTCTTTCAACTTGCATCCAACTCCAGGATAGTGGGCACCCCACTGCAATCCTGTCTTTCTG
AGCACACTTTAGTGGCCGATGATTTTTGTCATCAGCCACCATCCTATTGCAAAGGTTCCAACTGTATATA
TTCCCAATAGCAACGTAGCTTCTACCATGAACAGAAAACATTCTGATTTGGAAAAAGAGAGGGAGGTATG
GACTGGGGGCCAGAGTCCTTTCCAAGGCTTCTCCAATTCTGCCCAAAAATATGGTTGATAGTTTACCTGA
ATAAATGGTAGTAATCACAGTTGGCCTTCAGAACCATCCATAGTAGTATGATGATACAAGATTAGAAGCT
GAAAACCTAAGTCCTTTATGTGGAAAACAGAACATCATTAGAACAAAGGACAGAGTATGAACACCTGGGC
TTAAGAAATCTAGTATTTCATGCTGGGAATGAGACATAGGCCATGAAAAAAATGATCCCCAAGTGTGAAC
AAAAGATGCTCTTCTGTGGACCACTGCATGAGCTTTTATACTACCGACCTGGTTTTTAAATAGAGTTTGC
TATTAGAGCATTGAATTGGAGAGAAGGCCTCCCTAGCCAGCACTTGTATATACGCATCTATAAATTGTCC
GTGTTCATACATTTGAGGGGAAAACACCATAAGGTTTCGTTTCTGTATACAACCCTGGCATTATGTCCAC
TGTGTATAGAAGTAGATTAAGAGCCATATAAGTTTGAAGGAAACAGTTAATACCATTTTTTAAGGAAACA
ATATAACCACAAAGCACAGTTTGAACAAAATCTCCTCTTTTAGCTGATGAACTTATTCTGTAGATTCTGT
GGAACAAGCCTATCAGCTTCAGAATGGCATTGTACTCAATGGATTTGATGCTGTTTGACAAAGTTACTGA
TTCACTGCATGGCTCCCACAGGAGTGGGAAAACACTGCCATCTTAGTTTGGATTCTTATGTAGCAGGAAA
TAAAGTATAGGTTTAGCCTCCTTCGCAGGCATGTCCTGGACACCGGGCCAGTATCTATATATGTGTATGT

ACGTTTGTATGTGTGTAGACAAATATTTGGAGGGGTATTTTTGCCCTGAGTCCAAGAGGGTCCTTTAGTA
CCTGAAAAGTAACTTGGCTTTCATTATTAGTACTGCTCTTGTTTCTTTTCACATAGCTGTCTAGAGTAGC
TTACCAGAAGCTTCCATAGTGGTGCAGAGGAAGTGGAAGGCATCAGTCCCTATGTATTTGCAGTTCACCT
GCACTTAAGGCACTCTGTTATTTAGACTCATCTTACTGTACCTGTTCCTTAGACCTTCCATAATGCTACT
GTCTCACTGAAACATTTAAATTTTACCCTTTAGACTGTAGCCTGGATATTATTCTTGTAGTTTACCTCTT
TAAAAACAAAACAAAACAAAACAAAAAACTCCCCTTCCTCACTGCCCAATATAAAAGGCAAATGTGTACA
TGGCAGAGTTTGTGTGTTGTCTTGAAAGATTCAGGTATGTTGCCTTTATGGTTTCCCCCTTCTACATTTC
TTAGACTACATTTAGAGAACTGTGGCCGTTATCTGGAAGTAACCATTTGCACTGGAGTTCTATGCTCTCG
CACCTTTCCAAAGTTAACAGATTTTGGGGTTGTGTTGTCACCCAAGAGATTGTTGTTTGCCATACTTTGT
CTGAAAAATTCCTTTGTGTTTCTATTGACTTCAATGATAGTAAGAAAAGTGGTTGTTAGTTATAGATGTC
TAGGTACTTCAGGGGCACTTCATTGAGAGTTTTGTCTTGGATATTCTTGAAAGTTTATATTTTTATAATT
TTTTCTTACATCAGATGTTTCTTTGCAGTGGCTTAATGTTTGAAATTATTTTGTGGCTTTTTTTGTAAAT
ATTGAAATGTAGCAATAATGTCTTTTGAATATTCCCAAGCCCATGAGTCCTTGAAAATATTTTTTATATA
TACAGTAACTTTATGTGTAAATACATAAGCGGCGTAAGTTTAAAGGATGTTGGTGTTCCACGTGTTTTAT
TCCTGTATGTTGTCCAATTGTTGACAGTTCTGAAGAATTCTAATAAAATGTACATATATAAATCAAAAAA
AAAAAAAAAA (SEQ ID NO: 209)

Human C-KIT protein, transcript variant 2

TCTGGGGGCTCGGCTTTGCCGCGCTCGCTGCACTTGGGCGAGAGCTGGAACGTGGACCAGAGCTCGGATC
CCATCGCAGCTACCGCGATGAGAGGCGCTCGCGGCGCCTGGGATTTTCTCTGCGTTCTGCTCCTACTGCT
TCGCGTCCAGACAGGCTCTTCTCAACCATCTGTGAGTCCAGGGGAACCGTCTCCACCATCCATCCATCCA
GGAAAATCAGACTTAATAGTCCGCGTGGGCGACGAGATTAGGCTGTTATGCACTGATCCGGGCTTTGTCA
AATGGACTTTTGAGATCCTGGATGAAACGAATGAGAATAAGCAGAATGAATGGATCACGGAAAAGGCAGA
AGCCACCAACACCGGCAAATACACGTGCACCAACAAACACGGCTTAAGCAATTCCATTTATGTGTTTGTT
AGAGATCCTGCCAAGCTTTTCCTTGTTGACCGCTCCTTGTATGGGAAAGAAGACAACGACACGCTGGTCC
GCTGTCCTCTCACAGACCCAGAAGTGACCAATTATTCCCTCAAGGGGTGCCAGGGGAAGCCTCTTCCCAA
GGACTTGAGGTTTATTCCTGACCCCAAGGCGGGCATCATGATCAAAAGTGTGAAACGCGCCTACCATCGG
CTCTGTCTGCATTGTTCTGTGGACCAGGAGGGCAAGTCAGTGCTGTCGGAAAAATTCATCCTGAAAGTGA
GGCCAGCCTTCAAAGCTGTGCCTGTTGTGTCTGTGTCCAAAGCAAGCTATCTTCTTAGGGAAGGGGAAGA
ATTCACAGTGACGTGCACAATAAAAGATGTGTCTAGTTCTGTGTACTCAACGTGGAAAAGAGAAAACAGT
CAGACTAAACTACAGGAGAAATATAATAGCTGGCATCACGGTGACTTCAATTATGAACGTCAGGCAACGT
TGACTATCAGTTCAGCGAGAGTTAATGATTCTGGAGTGTTCATGTGTTATGCCAATAATACTTTTGGATC
AGCAAATGTCACAACAACCTTGGAAGTAGTAGATAAAGGATTCATTAATATCTTCCCCATGATAAACACT
ACAGTATTTGTAAACGATGGAGAAAATGTAGATTTGATTGTTGAATATGAAGCATTCCCCAAACCTGAAC
ACCAGCAGTGGATCTATATGAACAGAACCTTCACTGATAAATGGGAAGATTATCCCAAGTCTGAGAATGA
AAGTAATATCAGATACGTAAGTGAACTTCATCTAACGAGATTAAAAGGCACCGAAGGAGGCACTTACACA
TTCCTAGTGTCCAATTCTGACGTCAATGCTGCCATAGCATTTAATGTTTATGTGAATACAAAACCAGAAA
TCCTGACTTACGACAGGCTCGTGAATGGCATGCTCCAATGTGTGGCAGCAGGATTCCCAGAGCCCACAAT
AGATTGGTATTTTTGTCCAGGAACTGAGCAGAGATGCTCTGCTTCTGTACTGCCAGTGGATGTGCAGACA
CTAAACTCATCTGGGCCACCGTTTGGAAAGCTAGTGGTTCAGAGTTCTATAGATTCTAGTGCATTCAAGC
ACAATGGCACGGTTGAATGTAAGGCTTACAACGATGTGGGCAAGACTTCTGCCTATTTTAACTTTGCATT
TAAAGAGCAAATCCATCCCCACACCCTGTTCACTCCTTTGCTGATTGGTTTCGTAATCGTAGCTGGCATG
ATGTGCATTATTGTGATGATTCTGACCTACAAATATTTACAGAAACCCATGTATGAAGTACAGTGGAAGG

TTGTTGAGGAGATAAATGGAAACAATTATGTTTACATAGACCCAACACAACTTCCTTATGATCACAAATG
GGAGTTTCCCAGAAACAGGCTGAGTTTTGGGAAAACCCTGGGTGCTGGAGCTTTCGGGAAGGTTGTTGAG
GCAACTGCTTATGGCTTAATTAAGTCAGATGCGGCCATGACTGTCGCTGTAAAGATGCTCAAGCCGAGTG
CCCATTTGACAGAACGGGAAGCCCTCATGTCTGAACTCAAAGTCCTGAGTTACCTTGGTAATCACATGAA
TATTGTGAATCTACTTGGAGCCTGCACCATTGGAGGGCCCACCCTGGTCATTACAGAATATTGTTGCTAT
GGTGATCTTTTGAATTTTTTGAGAAGAAAACGTGATTCATTTATTTGTTCAAAGCAGGAAGATCATGCAG
AAGCTGCACTTTATAAGAATCTTCTGCATTCAAAGGAGTCTTCCTGCAGCGATAGTACTAATGAGTACAT
GGACATGAAACCTGGAGTTTCTTATGTTGTCCCAACCAAGGCCGACAAAAGGAGATCTGTGAGAATAGGC
TCATACATAGAAAGAGATGTGACTCCCGCCATCATGGAGGATGACGAGTTGGCCCTAGACTTAGAAGACT
TGCTGAGCTTTTCTTACCAGGTGGCAAAGGGCATGGCTTTCCTCGCCTCCAAGAATTGTATTCACAGAGA
CTTGGCAGCCAGAAATATCCTCCTTACTCATGGTCGGATCACAAAGATTTGTGATTTTGGTCTAGCCAGA
GACATCAAGAATGATTCTAATTATGTGGTTAAAGGAAACGCTCGACTACCTGTGAAGTGGATGGCACCTG
AAAGCATTTTCAACTGTGTATACACGTTTGAAAGTGACGTCTGGTCCTATGGGATTTTTCTTTGGGAGCT
GTTCTCTTTAGGAAGCAGCCCCTATCCTGGAATGCCGGTCGATTCTAAGTTCTACAAGATGATCAAGGAA
GGCTTCCGGATGCTCAGCCCTGAACACGCACCTGCTGAAATGTATGACATAATGAAGACTTGCTGGGATG
CAGATCCCCTAAAAAGACCAACATTCAAGCAAATTGTTCAGCTAATTGAGAAGCAGATTTCAGAGAGCAC
CAATCATATTTACTCCAACTTAGCAAACTGCAGCCCCAACCGACAGAAGCCCGTGGTAGACCATTCTGTG
CGGATCAATTCTGTCGGCAGCACCGCTTCCTCCTCCCAGCCTCTGCTTGTGCACGACGATGTCTGAGCAG
AATCAGTGTTTGGGTCACCCCTCCAGGAATGATCTCTTCTTTTGGCTTCCATGATGGTTATTTTCTTTTC
TTTCAACTTGCATCCAACTCCAGGATAGTGGGCACCCCACTGCAATCCTGTCTTTCTGAGCACACTTTAG
TGGCCGATGATTTTTGTCATCAGCCACCATCCTATTGCAAAGGTTCCAACTGTATATATTCCCAATAGCA
ACGTAGCTTCTACCATGAACAGAAAACATTCTGATTTGGAAAAAGAGAGGGAGGTATGGACTGGGGGCCA
GAGTCCTTTCCAAGGCTTCTCCAATTCTGCCCAAAAATATGGTTGATAGTTTACCTGAATAAATGGTAGT
AATCACAGTTGGCCTTCAGAACCATCCATAGTAGTATGATGATACAAGATTAGAAGCTGAAAACCTAAGT
CCTTTATGTGGAAAACAGAACATCATTAGAACAAAGGACAGAGTATGAACACCTGGGCTTAAGAAATCTA
GTATTTCATGCTGGGAATGAGACATAGGCCATGAAAAAAATGATCCCCAAGTGTGAACAAAAGATGCTCT
TCTGTGGACCACTGCATGAGCTTTTATACTACCGACCTGGTTTTTAAATAGAGTTTGCTATTAGAGCATT
GAATTGGAGAGAAGGCCTCCCTAGCCAGCACTTGTATATACGCATCTATAAATTGTCCGTGTTCATACAT
TTGAGGGGAAAACACCATAAGGTTTCGTTTCTGTATACAACCCTGGCATTATGTCCACTGTGTATAGAAG
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(SEQ ID NO: 210)