ANTI-5T4 ANTIBODIES AND ANTIBODY-DRUG CONJUGATES

FIELD OF THE INVENTION

The present invention relates to antibodies against the 5T4 (oncofoetal) antigen and corresponding antibody-drug conjugates (ADCs).

BACKGROUND OF THE PRESENT INVENTION

The 5T4 oncofoetal antigen is a 72 kDa glycoprotein defined by a monoclonal antibody raised against wheat germ agglutinin isolated glycoproteins from human placental syncytiotrophoblast microvillus membrane. This monoclonal antibody (mAb) was named 5T4 in WO89/07947. Whereas the 5T4 antigen has a limited expression in normal tissue, it is (over)expressed by various types of cancer cells. This renders the 5T4 antigen a specific cancer target and a potential and promising therapeutic target. However, although the target was discovered in the late eighties, approved therapeutic antibodies are still not available.

WO2006/031653 discloses the original mAb 5T4, i.e., H8 and its humanized version, both of which are not cross-reactive towards various non-human animal species typically used in *in vivo* preclinical (toxicity) studies. These preclinical studies aim to identify an initial safe dose for subsequent dose escalation schemes in humans; to identify healthy tissues or organs that are potential targets of reversible or irreversible toxic effects; and to identify safety parameters for clinical monitoring. For biopharmaceuticals, such as monoclonal antibodies, regulatory guidelines require testing in at least one relevant species (e.g. ICH S6 regulatory guideline). The species is not relevant in case the monoclonal antibody is not cross-reactive for the species and therefore insufficiently potent in said species. In such cases an alternative animal model may be used, e.g. a genetically modified species. However, this will require

extensive effort not only to develop the model, but also to be able to provide an acceptable scientific justification to the regulatory authorities.

WO2007/106744 discloses the anti-5T4 antibodies A1, A2 and A3, but although these three antibodies exhibit some cross-reactivity towards non-human animal species, e.g. cynomolgus monkey, their affinities for human 5T4 are much lower than the affinity of H8 for human 5T4.

Anti-5T4 antibodies H8, A1, A2 and A3 linked via 4-(4'-acetylphenoxy)-butanoic acid to calicheamicin were disclosed in WO2007/106744 on p. 73. WO2012/131527 discloses A1 linked to maleimidocapronic-monomethylauristatin F (A1-mc-MMAF).

Only a few therapies targeting the 5T4 antigen have reached the clinical trial stage. The vaccine of modified vaccinia virus Ankara (MVA) vector encoding the 5T4 antigen induces an endogenous antibody response against the 5T4 antigen, but its phase III study on metastatic renal cancer failed to meet its primary end point of increased survival. Another example is naptumomab estafenatox, which is the Fab fragment of mAb 5T4 conjugated to a modified Staphylococcal enterotoxin E. This conjugate is thought to activate a T-cell response in the proximity of the tumour. However, a randomized phase II/III study of naptumomab estafenatox plus IFN-α *versus* IFN-α in advanced renal cell carcinoma did not meet its primary endpoint of prolonged survival. Recently as well, clinical development evaluating the A1-mc-MMAF ADC was discontinued. Currently, no active clinical trials are listed in the US and EU clinical trials registers.

WO 2015/155345 discloses new anti-5T4 antibodies and corresponding ADCs wherein an anti-5T4 antibody is (site-specifically) linked to a pyrrolobenzodiazepine (PDB) dimer or to a tubulysin. However, no clinical data is yet available.

The above leads to the conclusion that the clinically tested 5T4 targeted therapies, i.e. antibodies, antibody-drug conjugates and vaccines, do not measure up to the requirements of

a cancer therapeutic. Therefore, there is a need for new antibodies against the 5T4 antigen and for corresponding antibody-drug conjugates for cancer therapy. To determine the suitability of these antibodies and corresponding ADCs in a preclinical setting, such antibodies should be cross-reactive for the 5T4 antigen of non-human animal species relevant for the preclinical development of a drug candidate.

SUMMARY

The present disclosure describes antibodies against the human 5T4 oncofoetal antigen and corresponding ADCs that are suitable for testing in clinical trials. Suitable antibodies and corresponding ADCs in a preclinical setting should be cross-reactive for the 5T4 antigen of non-human animal species relevant for preclinical development of a drug candidate.

Preferably, the antibodies are cross-reactive for humans and cynomolgus monkeys and exhibit an affinity for human 5T4 antigen (hu 5T4) which is in the same order of magnitude as their affinity for cynomolgus monkey 5T4 antigen (cyno 5T4).

The disclosure further describes the use of the antibodies and corresponding ADCs in the treatment of solid tumours and haematological malignancies.

In one aspect, the present invention refers to an anti-5T4 antibody comprising heavy chain (HC) and light chain (LC) variable region (VR) complementarity determining regions (CDRs) selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and

CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12;

wherein the antibody comprises at least one engineered cysteine at one or more positions of said antibody selected from heavy chain framework region positions 40, 41 and 89 according to Kabat numbering and light chain framework region positions 40 and 41 according to Kabat numbering.

In another aspect, the present invention refers to an antibody-drug conjugate comprising the antibody as disclosed herein.

In yet another aspect, the present invention refers to a pharmaceutical composition comprising the antibody as disclosed herein or the antibody-drug conjugate as disclosed herein and one or more pharmaceutically acceptable excipients, preferably in the form of a lyophilized powder.

In a further aspect, the present invention refers to the antibody as disclosed herein, the antibody-drug conjugate as disclosed herein or the pharmaceutical composition as disclosed herein for use as a medicament.

In another aspect, the present invention refers to use of the antibody, the antibody-drug conjugate or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating human solid tumours and haematological malignancies.

In a further aspect, the present invention refers to use of a combination of the antibody as disclosed herein, the antibody-drug conjugate as disclosed herein or the pharmaceutical composition as disclosed herein with a therapeutic antibody, a chemotherapeutic agent and/or an ADC against a cancer-related target other than the 5T4 antigen in the manufacture of a medicament for treating human solid tumours and haematological malignancies.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the binding of chimeric mAbs, humanized mAbs with HC-41C mutation and corresponding anti-5T4 ADCs to hu 5T4-expressing CHO cells (CHOZN) *versus* that of H8 and A1 (Figure 1A clone 789, Figure 1B clone 833, Figure 1C clone 825).

Figure 2 shows the binding of chimeric mAbs, humanized mAbs with HC-41C mutation and corresponding anti-5T4 ADCs to cyno 5T4-expressing CHO cells (CHOZN) *versus* that of H8 and A1 (Figure 2A clone 789, Figure 2B clone 833, Figure 2C clone 825).

Figure 3 shows the *in vivo* efficacy of the anti-5T4 ADCs 833a-, 833b-, 833c-, 833d-, 825a-, 825c-vc-*seco*-DUBA *versus* H8-vc-*seco*-DUBA and non-binding control rituximab-vc-*seco*-DUBA in the 5T4-positive BT474 cell line xenograft in immunodeficient mice.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

Whereas 5T4 oncofoetal antigen has a limited expression in normal tissues, it is (over)expressed by various cancer cells, thus rendering the 5T4 antigen a specific cancer target and a potential and promising therapeutic target. However, although the target was discovered in the late eighties, there currently are no approved therapeutics directed towards this target.

The present invention relates to antibodies against the 5T4 antigen and corresponding antibody-drug conjugates (ADCs), which are cross-reactive for cyno 5T4 and also exhibit excellent affinity for hu 5T4 antigen. The anti-5T4 antibodies and ADCs according to the invention show an affinity for cyno 5T4 antigen in the same order of magnitude as their affinity for hu 5T4. The term "same order of magnitude" means that the affinities for hu and cyno 5T4 antigen differ less than a factor ten from each other. The anti-5T4 antibodies of the invention have an improved affinity for hu 5T4 antigen compared to the prior art anti-5T4 antibodies A1 and A3, and an improved affinity for cyno 5T4 antigen compared to the prior art anti-5T4 antibody H8. Affinity is preferably measured as EC_{50} in μg/ml in a cell-based assay using cells expressing hu or cyno 5T4 antigen. The present inventors measured the EC_{50} on various cells, such as MDA-MB-468, PA-1 and Chinese Hamster Ovary (CHO) cells engineered to express hu 5T4 or cyno 5T4. The antibodies of the invention typically exhibit an EC_{50} lower than 0.8 μg/ml measured using cells expressing hu 5T4 or cyno 5T4 antigen after incubation of the cells with the antibodies for 30 minutes at 4°C.

Prior art anti-5T4 antibody A1 is characterised by a heavy chain (HC) variable region (VR) of the mouse A1 amino acid sequence from US8044178, SEQ ID NO:2, positions 20 – 138 and a light chain (LC) VR of the mouse A1 amino acid sequence from US8044178, SEQ ID NO:4, positions 21 - 127. Prior art anti-5T4 antibody A3 is characterised by an HCVR of the mouse A3 amino acid sequence from US8044178, SEQ ID NO:10, positions 20 – 141 and an LCVR of the mouse A3 amino acid sequence from US8044178, SEQ ID NO:12, positions 21 - 127. Prior art anti-5T4 antibody H8 is characterised by the HCVR of SEQ ID NO:52 and the LCVR of SEQ ID NO:53.

The term "antibody" as used throughout the present specification refers to a monoclonal antibody (mAb) comprising two heavy chains and two light chains or an antigen binding fragment thereof, e.g. a Fab, Fab' or $F(ab')_2$ fragment, a single chain (sc) antibody, a scFv, a single domain (sd) antibody, a diabody, or a minibody. Antibodies may be of any isotype such as IgG, IgA or IgM antibodies. Preferably, the antibody is an IgG antibody, more preferably an IgG1 or IgG2 antibody. The antibodies may be chimeric, humanized or human. Preferably, the antibodies of the invention are humanized. Even more preferably, the antibody is a humanized or human IgG antibody, most preferably a humanized or human IgG1 mAb. The antibody may have κ (kappa) or λ (lambda) light chains, preferably κ (kappa) light chains, i.e., a humanized or human IgG1-κ antibody.

In humanized antibodies, the antigen-binding complementarity determining regions (CDRs) in the variable regions of the HC and LC are derived from antibodies from a non-human species, commonly mouse, rat or rabbit. These non-human CDRs may be placed within a human framework (FR1, FR2, FR3 and FR4) of the variable regions of the HC and LC. Selected amino acids in the human FRs may be exchanged for the corresponding original non-human species amino acids to improve binding affinity, while retaining low immunogenicity. Alternatively, selected amino acids of the original non-human species FRs

are exchanged for their corresponding human amino acids to reduce immunogenicity, while retaining the antibody's binding affinity. The thus humanized variable regions are combined with human constant regions.

The present invention particularly relates to an anti-5T4 antibody comprising HCVR and LCVR CDRs selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:3 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:4;

c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6;

d. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:7 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:8;

e. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:9 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:10;

f. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12;

g. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:13 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:14;

h. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:15 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:16;

i. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:17 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:18;

j. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:19 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:20;

k. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:21 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:22;

l. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:23 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:24;

m. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:25 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:26;

n. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:27 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:28;

o. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:29 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:30;

p. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:31 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:32; and

q. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:33 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:34.

For clarity, the CDR1, CDR2 and CDR3 sequences of the HCVR and the CDR1, CDR2 and CDR3 sequences of the LCVR of the antibodies listed under a to q hereinabove are underlined in the sequence listings given at the end of the present description.

In one embodiment, the anti-5T4 antibody of the invention comprises HCVR and LCVR CDRs selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6;

c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12;

d. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:13 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:14;

e. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:17 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:18; and

f. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:25 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:26.

In a preferred embodiment, the anti-5T4 antibody of the invention comprises HCVR and LCVR CDRs selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and

c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12.

In a more preferred embodiment, the anti-5T4 antibody of the invention comprises HCVR and LCVR CDRs selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12.

In another embodiment, the invention relates to a humanized anti-5T4 antibody comprising a HCVR and a LCVR selected from the group consisting of:

a. HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:45;

b. HCVR amino acid sequence of SEQ ID NO:36 and LCVR amino acid sequence of SEQ ID NO:45;

c. HCVR amino acid sequence of SEQ ID NO:37 and LCVR amino acid sequence of SEQ ID NO:44;

d. HCVR amino acid sequence of SEQ ID NO:37 and LCVR amino acid sequence of SEQ ID NO:46;

e. HCVR amino acid sequence of SEQ ID NO:40 and LCVR amino acid sequence of SEQ ID NO:51;

f. HCVR amino acid sequence of SEQ ID NO:41 and LCVR amino acid sequence of SEQ ID NO:51;

g. HCVR amino acid sequence of SEQ ID NO:42 and LCVR amino acid sequence of SEQ ID NO:49;

h. HCVR amino acid sequence of SEQ ID NO:43 and LCVR amino acid sequence of SEQ ID NO:50;

i. HCVR amino acid sequence of SEQ ID NO:38 and LCVR amino acid sequence of SEQ ID NO:47;

j. HCVR amino acid sequence of SEQ ID NO:39 and LCVR amino acid sequence of SEQ ID NO:47; and

k. HCVR amino acid sequence of SEQ ID NO:39 and LCVR amino acid sequence of SEQ ID NO:48.

In a first embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:45.

In a second embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:36 and LCVR amino acid sequence of SEQ ID NO:45.

In a third embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:37 and LCVR amino acid sequence of SEQ ID NO:44.

In a fourth embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:37 and LCVR amino acid sequence of SEQ ID NO:46.

In a fifth, preferred embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:40 and LCVR amino acid sequence of SEQ ID NO:51.

In a sixth, preferred embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:41 and LCVR amino acid sequence of SEQ ID NO:51.

In a seventh, preferred embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:42 and LCVR amino acid sequence of SEQ ID NO:49.

In an eighth, preferred embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:43 and LCVR amino acid sequence of SEQ ID NO:50.

In a ninth, preferred embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:38 and LCVR amino acid sequence of SEQ ID NO:47.

In a tenth embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:39 and LCVR amino acid sequence of SEQ ID NO:47.

In an eleventh, preferred embodiment of the invention the humanized anti-5T4 antibody comprises HCVR amino acid sequence of SEQ ID NO:39 and LCVR amino acid sequence of SEQ ID NO:48.

The present invention additionally relates to an ADC, wherein a linker drug is conjugated to an anti-5T4 antibody according to the invention.

In one embodiment, the present invention relates to an ADC wherein a linker drug is randomly conjugated to an anti-5T4 antibody according to the invention through a native cysteine liberated through reduction of the interchain disulfide bonds.

In another embodiment, the present invention relates to an ADC wherein a linker drug is site-specifically conjugated to an anti-5T4 antibody according to the invention through an engineered cysteine (site-specific ADC).

The anti-5T4 antibodies comprising at least one engineered cysteine in the HC or LC have several advantages. Antibodies comprising engineered cysteines provide the opportunity to prepare site-specific ADCs, can provide conjugation positions that show good reactivity with the linker drug, and at the same time have a reduced risk of forming additional disulfide bonds between antibodies (leading to aggregation) or disturbing the antibody structure. An additional advantage of having cysteines at specific positions in the HC or LC is the effect of decreased hydrophobicity of the resulting ADCs.

Multiple suitable conjugation positions for linker drug attachment have been identified in and in close proximity to cavities which are present in all antibody structures, i.e., with good accessibility of engineered cysteines at these locations as disclosed and claimed in WO2015/177360.

When linker drugs are conjugated at the specific positions of the anti-5T4 antibodies as claimed herein, said linker drug fits into the Fab cavity that is formed by the constant heavy chain 1 (CH1), variable heavy chain (VH), variable light chain (VL) and constant light chain (CL) regions of the antibody. As a result, the linker drug (most toxins/linker drugs are hydrophobic) is shielded from the aqueous environment surrounding the antibody and the ADC as such is less hydrophobic as compared to ADCs wherein the linker drug is conjugated through native interchain disulfide bond cysteines of the antibody and is much less hydrophobic as compared to ADCs wherein the linker drug is site-specifically conjugated at different positions where the linker drug is forced to the outside of the antibody, i.e., more exposed to the hydrophilic aqueous environment.

In a preferred embodiment of the present invention, the anti-5T4 antibody according to the invention comprises at least one engineered cysteine at a position in a HC variable region FR or a LC variable region FR. The term "engineered cysteine" as used throughout the present specification means replacing a non-cysteine amino acid in the HC or LC of an antibody by a cysteine. As is known by the person skilled in the art, this can be done either at the amino acid level or at the DNA level, e.g. by using site-directed mutagenesis. Preferably, such engineered cysteine is introduced by specific point mutations, replacing an existing amino acid in the original/parent antibody.

Preferably, the at least one engineered cysteine is present at one or more positions of the anti-5T4 antibodies according to the invention selected from HC 40, 41 and 89 (according to Kabat numbering); and LC 40 and 41 (according to Kabat numbering). The expression "Kabat numbering" refers to the numbering system commonly used for HC variable regions or LC variable regions of the compilation of antibodies in Kabat, E.A. *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991). Using this numbering system, the actual linear amino acid sequence

may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable region. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

In addition to the anti-5T4 antibodies comprising the HCVR and LCVR CDRs and the humanized antibodies comprising HCVRs and LCVRs as disclosed hereinabove, the present invention also relates to said antibodies comprising in the HC and/or LC at least one engineered cysteine at a position selected from HC 40, 41 and 89, and LC 40 and 41.

In a preferred embodiment, the anti-5T4 antibody of the invention comprises at least one engineered cysteine at a position selected from HC 40, 41 and 89, and LC 40 and 41 and comprises HCVR and HCVR CDRs selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and

c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12.

In another preferred embodiment, the invention relates to a humanized anti-5T4 antibody comprising at least one engineered cysteine at a position selected from HC 40, 41 and 89, and LC 40 and 41 and comprising HCVR and LCVR selected from the group consisting of:

a. HC amino acid sequence of SEQ ID NO:40 and LC amino acid sequence of SEQ ID NO:51;

b. HC amino acid sequence of SEQ ID NO:41 and LC amino acid sequence of SEQ ID NO:51;

c. HC amino acid sequence of SEQ ID NO:42 and LC amino acid sequence of SEQ ID NO:49;

d. HC amino acid sequence of SEQ ID NO:43 and LC amino acid sequence of SEQ ID NO:50;

e. HC amino acid sequence of SEQ ID NO:38 and LC amino acid sequence of SEQ ID NO:47; and

f. HC amino acid sequence of SEQ ID NO:39 and LC amino acid sequence of SEQ ID NO:48.

Positions HC 40, 41 and 89 and LC 40 and 41 are located in the variable region FRs of the antibody as well as in the Fab part of the antibody.

In a preferred embodiment, the present invention relates to an ADC wherein a linker drug is site-specifically conjugated to an anti-5T4 antibody according to the invention through an engineered cysteine at one or more positions of said anti-5T4 antibody selected from HC 40, 41 and 89 (according to Kabat numbering) and LC 40 and 41 (according to Kabat numbering).

The present inventors surprisingly have found that the site-specifically conjugated ADCs of the present invention show improved physicochemical, pharmacological and/or pharmacokinetic properties, as compared to conventional ADCs in which the linker drug is conjugated through native interchain disulfide bond cysteines of the anti-5T4 antibody.

Modification of the variable part of an antibody is generally avoided as it can lead to partial or complete loss of antigen binding affinities. However, contrary to the general expectations, it was found that specific residues in the FRs of the HC and LC of the antibody are both suitable for conjugation and do not lead to (significant) reduction of antigen binding after conjugation of the linker drug. In a particularly preferred embodiment, the present invention relates to an ADC wherein said engineered cysteine is at one or more positions of

said anti-5T4 antibody selected from HC 40 and 41, and LC 40 and 41 (in the Fab part of said antibody). Preferably, said engineered cysteine is at position HC 41 or LC 40 or 41, more preferably at HC 41.

As it is known from the literature that tumour-associated proteases in the tumour microenvironment can partially cleave the Fc constant domains, under the hinge region, conjugation in the Fab part is preferred over conjugation in the Fc part. Cleavage of the Fc constant domains would result in loss of Fc-conjugated linker drugs, which in turn could lead to a decreased activity of the ADC *in vivo*. (Fan *et al.* Breast Cancer Res. 2012; 14: R116 and Brezsky *et al.* PNAS 2009; 106: 17864-17869). Moreover, conjugation to these positions in the Fab part also enables the use of antigen binding fragments of the anti-5T4 antibodies disclosed herein.

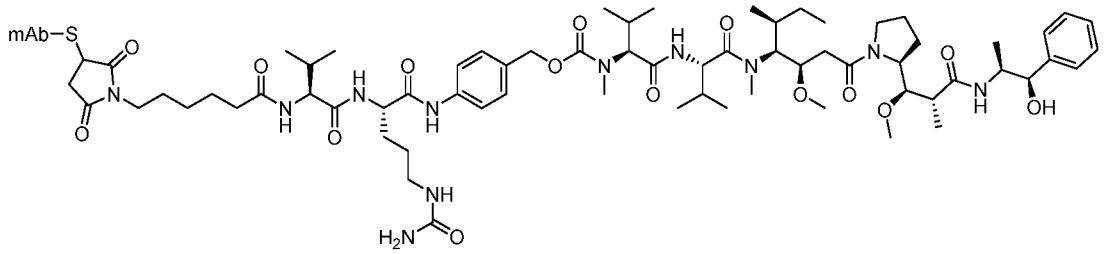
The (site-specific) ADCs in accordance with the present invention have binding affinities similar to the naked antibodies and excellent *in vitro* potency, and have an improved *in vivo* profile over the 5T4-targeting ADCs known from the prior art. It is expected that the site-specific ADCs in accordance with the present invention will exhibit low non-specific toxicity *in vivo* in comparison with the 5T4-targeting ADCs of the prior art. In the anti-5T4 ADCs of the invention the linker drug is shielded (rendering the ADCs less susceptible to cleavage by extracellular proteases) and thus the drug is less likely to be released prematurely.

In accordance with the present invention, any linker drug known in the art of ADCs can be used for (site-specific) conjugation to the antibodies according to the present invention, provided it has a chemical group which can react with the thiol group of a native or an engineered cysteine, typically a maleimide or haloacetyl group. Suitable linker drugs may comprise a duocarmycin, calicheamicin, pyrrolobenzodiazepine (PBD) dimer, maytansinoid or auristatin derivative as a cytotoxic drug. Either a cleavable or a non-cleavable linker may be used in accordance with the present invention. Preferably, the cytotoxic drug is a

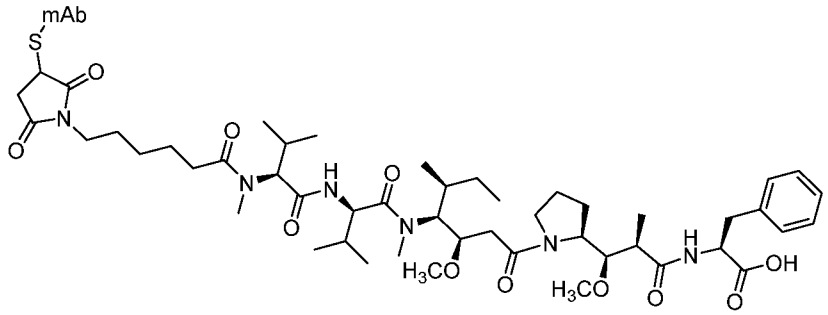
duocarmycin, a maytansinoid or an auristatin derivative. Suitable examples of maytansinoid drugs include DM1 and DM4. Suitable examples of auristatin drugs include MMAE and MMAF.

These abbreviations are well-known to the skilled artisan. Examples of suitable linker drugs known to the person skilled in the art include mc-vc-PAB-MMAE (also abbreviated as mc-vc-MMAE and vc-MMAE), mc-MMAF, and mc-vc-MMAF. Preferably, the linker used is a cleavable linker comprising valine-citrulline (vc) or valine-alanine (va).

The generic molecular structures of a (site-specific) vc-MMAE ADC and mc-MMAF ADC in accordance with the present invention are depicted below.



Molecular structure of vc-MMAE linked to a mAb



Molecular structure of mc-MMAF linked to a mAb

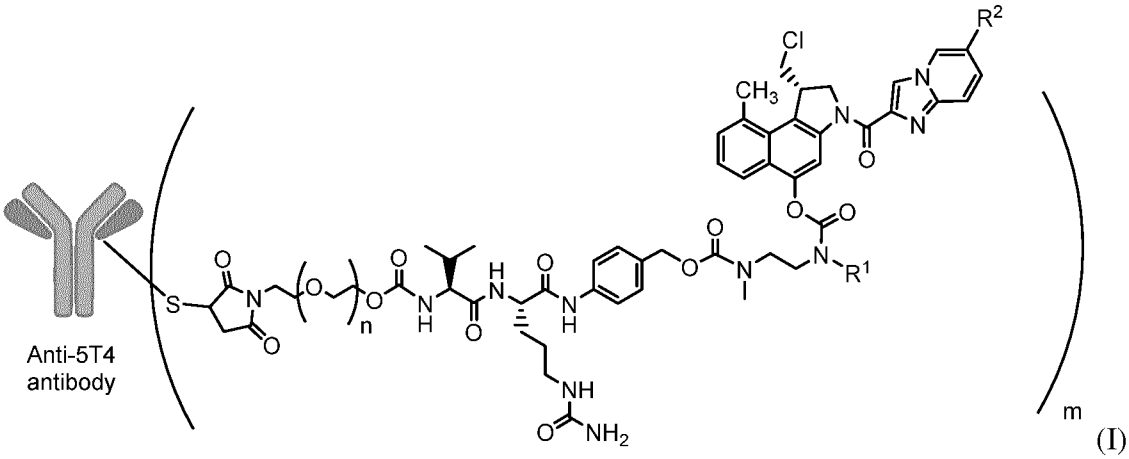
In one embodiment, the present invention relates to an ADC wherein the linker drug comprises a duocarmycin derivative.

Duocarmycins, first isolated from a culture broth of *Streptomyces* species, are members of a family of antitumour antibiotics that include duocarmycin A, duocarmycin SA, and

CC-1065. Duocarmycins bind to the minor groove of DNA and subsequently cause irreversible alkylation of DNA. This disrupts the nucleic acid architecture, which eventually leads to tumour cell death.

WO2011/133039 discloses a series of linker drugs comprising a duocarmycin derivative of CC-1065. Suitable linker-duocarmycin derivatives to be used in accordance with the present invention are disclosed on pages 182-197. The chemical synthesis of a number of these linker drugs is described in Examples 1-12 of WO2011/133039.

In one embodiment, the present invention relates to an ADC of formula (I)



(I)

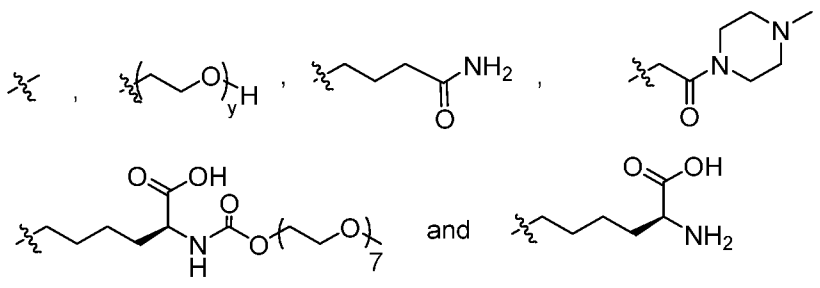
wherein

"Anti-5T4 antibody" is an anti-5T4 antibody according to the present invention either without or with at least one engineered cysteine in the HC or LC as disclosed herein,

n is 0-3, preferably 0-1,

m represents an average DAR of from 1 to 6, preferably of from 1 to 4,

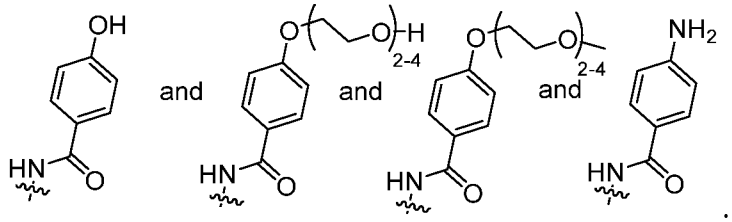
R^1 is selected from



,

y is 1-16, and

R^2 is selected from



In a preferred embodiment, the ADC of formula (I) comprises an anti-5T4 antibody according to the present invention comprising at least one engineered cysteine in the HC or LC, wherein the linker drug is site-specifically conjugated to the anti-5T4 antibody through the engineered cysteine. Preferably, the engineered cysteine is at position HC 40, 41 or 89 or LC 40 or 41, more preferably at HC 41 or LC 40 or 41, most preferably at HC 41.

In the structural formulae shown in the present specification, n represents an integer from 0 to 3, while m represents an average drug-to-antibody ratio (DAR) of from 1 to 6. As is well-known in the art, the DAR and drug load distribution can be determined, for example, by using hydrophobic interaction chromatography (HIC) or reversed phase high-performance liquid chromatography (RP-HPLC). HIC is particularly suitable for determining the average DAR.

ADCs of formula (I) in accordance with the present invention can be obtained according to methods and procedures that are well known to a person skilled in the art.

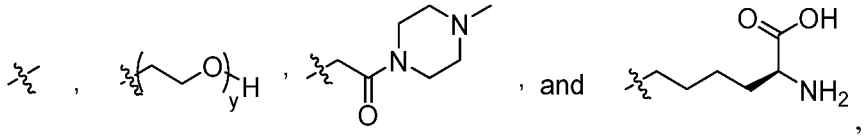
A suitable method for the aspecific (random) conjugation of duocarmycin linker drugs, i.e., conjugation to a native cysteine, is disclosed in Example 15 of WO2011/133039, whereas Doronina *et al.* Bioconjugate Chem. 17 (2006): 114-124 describes aspecific conjugation with mc-MMAF.

Suitable methods for site-specifically conjugating linker drugs can for example be found in Examples 7 and 8 of WO2005/084390, which describe complete reduction strategies for (partial) loading of antibodies with the linker drug vc-MMAE, and in Examples 11 and 12 of

WO2006/034488, which describe the site-specific conjugation of a maytansinoid (DM1)-comprising linker drug.

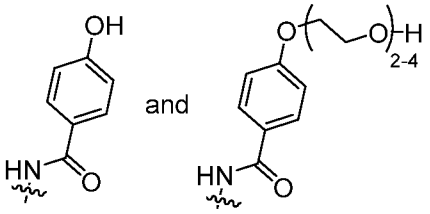
In a particular embodiment, the present invention relates to an ADC of formula (I) as disclosed hereinabove, wherein n is 0-1, m represents an average DAR of from 1 to 6, preferably of from 1 to 4, more preferably of from 1 to 2, even more preferably of from 1.5 to 2, most preferably of from 1.8 to 2,

R^1 is selected from

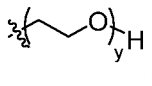


y is 1-16, preferably 1-4, and

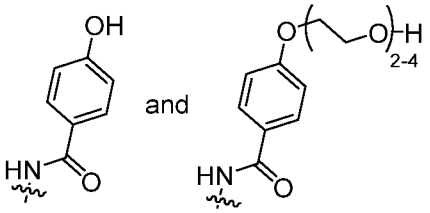
R^2 is selected from



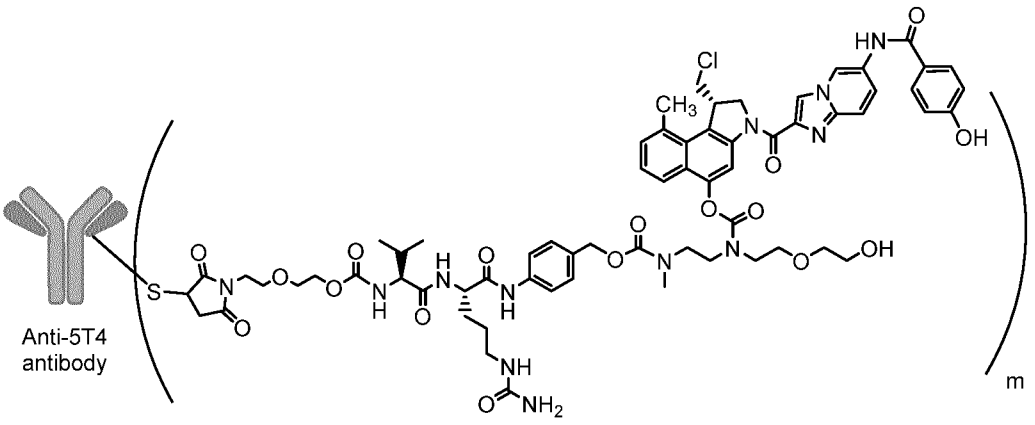
In a specific embodiment, the present invention relates to an ADC of structural formula (I) as disclosed hereinabove, wherein n is 0-1, m represents an average DAR of from 1.5 to 2, preferably of from 1.8 to 2, R^1 is



y is 1-4, and R^2 is selected from



In a particularly preferred embodiment, the present invention relates to an ADC of formula (II)



(II),

wherein "Anti-5T4 antibody" is an anti-5T4 antibody according to the present invention either without or with at least one engineered cysteine in the HC or LC as disclosed herein and m represents an average DAR of from 1.5 to 2, preferably of from 1.8 to 2.

In a preferred embodiment, the ADC of formula (II) comprises an anti-5T4 antibody according to the present invention comprising at least one engineered cysteine in the HC or LC, wherein the linker drug is site-specifically conjugated to the antibody through the engineered cysteine. Preferably, said engineered cysteine is at position HC 40, 41 or 89 or LC 40 or 41, more preferably at HC 41 or LC 40 or 41, most preferably at HC 41.

In a preferred embodiment, the present invention relates to an ADC of formula (II) comprising an anti-5T4 antibody comprising HCVR and LCVR CDRs selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and

c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12.

In a more preferred embodiment, the present invention relates to an ADC of formula (II) comprising a humanized anti-5T4 antibody comprising HCVR and LCVR selected from the group consisting of:

a. HC amino acid sequence of SEQ ID NO:40 and LC amino acid sequence of SEQ ID NO:51;

b. HC amino acid sequence of SEQ ID NO:41 and LC amino acid sequence of SEQ ID NO:51;

c. HC amino acid sequence of SEQ ID NO:42 and LC amino acid sequence of SEQ ID NO:49;

d. HC amino acid sequence of SEQ ID NO:43 and LC amino acid sequence of SEQ ID NO:50;

e. HC amino acid sequence of SEQ ID NO:38 and LC amino acid sequence of SEQ ID NO:47; and

f. HC amino acid sequence of SEQ ID NO:39 and LC amino acid sequence of SEQ ID NO:48.

In an even more preferred embodiment, the present invention relates to an ADC of formula (II) comprising an anti-5T4 antibody according to the present invention comprising at least one engineered cysteine at one or more positions selected from HC 40 and 41, and LC 40 and 41, wherein the linker drug is site-specifically conjugated to the anti-5T4 antibody through the engineered cysteine. Preferably, said engineered cysteine is at position HC 41 or LC 40 or 41, most preferably at HC 41.

In a most preferred embodiment, the present invention relates to an ADC of formula (II) comprising a humanized anti-5T4 antibody comprising HCVR and LCVR selected from the group consisting of:

a. HCVR amino acid sequence of SEQ ID NO:61 and LCVR amino acid sequence of SEQ ID NO:51;

b. HCVR amino acid sequence of SEQ ID NO:62 and LCVR amino acid sequence of SEQ ID NO:51;

c. HCVR amino acid sequence of SEQ ID NO:63 and LCVR amino acid sequence of SEQ ID NO:49;

d. HCVR amino acid sequence of SEQ ID NO:64 and LCVR amino acid sequence of SEQ ID NO:50;

e. HCVR amino acid sequence of SEQ ID NO:59 and LCVR amino acid sequence of SEQ ID NO:47; and

f. HCVR amino acid sequence of SEQ ID NO:60 and LCVR amino acid sequence of SEQ ID NO:48;

wherein the linker drug is site-specifically conjugated to the anti-5T4 antibody through the engineered cysteine at position HC 41.

The present invention further relates to a pharmaceutical composition comprising an anti-5T4 antibody or an anti-5T4 ADC as described hereinabove and one or more pharmaceutically acceptable excipients. Typical pharmaceutical formulations of therapeutic proteins such as mAbs and (monoclonal) ADCs take the form of lyophilized cakes (lyophilized powders), which require (aqueous) dissolution (i.e., reconstitution) before intravenous infusion, or frozen (aqueous) solutions, which require thawing before use.

Typically, the pharmaceutical composition is provided in the form of a lyophilized cake. Suitable pharmaceutically acceptable excipients for inclusion into the pharmaceutical composition (before freeze-drying) in accordance with the present invention include buffer solutions (e.g. citrate, histidine or succinate containing salts in water), lyoprotectants (e.g. sucrose, trehalose), tonicity modifiers (e.g. sodium chloride), surfactants (e.g. polysorbate),

and bulking agents (e.g. mannitol, glycine). Excipients used for freeze-dried protein formulations are selected for their ability to prevent protein denaturation during the freeze-drying process as well as during storage. As an example, the sterile, lyophilized powder single-use formulation of Kadcyla™ (Roche) contains - upon reconstitution with Bacteriostatic or Sterile Water for Injection (BWFI or SWFI) - 20 mg/mL ado-trastuzumab emtansine, 0.02% w/v polysorbate 20, 10 mM sodium succinate, and 6% w/v sucrose with a pH of 5.0.

The present invention further relates to an anti-5T4 antibody, ADC or pharmaceutical composition as described hereinabove for use as a medicament.

In one embodiment, the present invention relates to an anti-5T4 antibody, ADC or pharmaceutical composition as described hereinabove for use in the treatment of human solid tumours and haematological malignancies, preferably human solid tumours.

In a preferred embodiment, the present invention relates to an anti-5T4 antibody, an ADC or a pharmaceutical composition as described hereinabove, particularly an ADC comprising a duocarmycin derivative linker drug, for use in the treatment of human solid tumours selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, ovarian cancer, lung cancer (especially non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC)), and (malignant pleural) mesothelioma.

In a further embodiment, the present invention relates to an anti-5T4 antibody, ADC or pharmaceutical composition as described hereinabove, particularly an ADC comprising a duocarmycin derivative linker drug, for use in the treatment of human haematological malignancies, particularly leukaemia, selected from the group consisting of acute lymphoblastic and myeloid leukaemia (ALL and AML, respectively).

The present invention further relates to the use of a sequentially or simultaneously administered combination of an anti-5T4 antibody, an anti-5T4 ADC or a pharmaceutical

composition as described hereinabove with a therapeutic antibody, a chemotherapeutic agent, and/or an ADC against a cancer-related target other than the 5T4 antigen for the treatment of human solid tumours and haematological malignancies as described hereinabove.

In one embodiment of the present invention, particularly in case of an anti-5T4 ADC comprising a duocarmycin derivative linker drug, the therapeutic antibody is bevacizumab, cetuximab, nivolumab, or ramucirumab and the chemotherapeutic agent is an alkylating agent, particularly cyclophosphamide, ifosfamide or a triazine, particularly temozolomide, or a platinum drug, more particularly cisplatin or carboplatin, an anti-metabolite, particularly gemcitabine or pemetrexed, a topoisomerease II inhibitor, particularly etoposide, a mitotic inhibitor, particularly a taxane, more particularly paclitaxel or docetaxel, or a vinca alkaloid, more particularly vinblastine or vinorelbine, or a signalling cascade inhibitor, particularly a tyrosine kinase inhibitor, more particularly imatinib, erlotinib, ceritinib, crizotinib or afatinib.

In a further embodiment of the present invention, particularly in case of an anti-5T4 ADC comprising a duocarmycin derivative linker drug, the therapeutic antibody is bevacizumab and the chemotherapeutic agent is an alkylating agent, particularly a nitrogen mustard, particularly ifosfamide or cyclophosphamide, a platinum drug, particularly cisplatin or carboplatin, or a triazine, particularly temozolomide, an anti-tumour antibiotic, particularly doxorubicin, an anti-metabolite, particularly gemcitabine, a topoisomerease I or II inhibitor, particularly topotecan, irinotecan or etoposide, or a mitotic inhibitor, particularly a taxane, more particularly paclitaxel or docetaxel, or a vinca alkaloid, more particularly vincristine or vinorelbine.

In yet a further embodiment of the present invention, particularly in case of an anti-5T4 ADC comprising a duocarmycin derivative linker drug, the therapeutic antibody is amatuximab and the chemotherapeutic agent is an alkylating agent, particularly a platinum

drug, more particularly cisplatin or carboplatin, an anti-metabolite, particularly gemcitabine or pemetrexed, or a mitotic inhibitor, particularly a vinca alkaloid, more particularly vinorelbine.

A therapeutically effective amount of the anti-5T4 antibody or ADC in accordance with the present invention lies in the range of about 0.01 to about 15 mg/kg body weight, particularly in the range of about 0.1 to about 10 mg/kg body weight, more particularly in the range of about 0.3 to about 10 mg/kg body weight. This latter range corresponds roughly to a flat dose in the range of 20 to 800 mg of the antibody or ADC. The compound of the present invention may be administered weekly, bi-weekly, three-weekly, monthly or six-weekly. Suitable treatment regimens are depending upon the severity of the disease, the age of the patient, the compound being administered, and such other factors as would be considered by the treating physician.

EXAMPLES

Immunization protocol and selection

Rabbits were repeatedly immunized with a mixture of hu 5T4/cyno5T4 protein (2 rabbits) and MDA-MB-468 cells (2 rabbits). About 20 ml blood was collected at different time points. Single B-cells were deposited into single wells of microtiter plates. These B-cells were cultivated for several days in the presence of conditioned medium and feeder cells. During this time they produced and released monoclonal antibodies into the cultivation medium (B-cell supernatants). The supernatants of these single B-cells were analyzed for IgG production, subsequently specific binding of the hu and cyno 5T4 antigen and binding to 5T4 expressing MDA-MB-468 cells was determined. 160 B-cell supernatants were selected and sequenced, as these antibodies bound to both human and cyno 5T4 antigen as well as to the MDA-MB-468 cells. 131 unique variable regions of antibody heavy and light chains were obtained, gene synthesized and cloned on human immunoglobulin constant parts of the IgG1

subclass. HEK 293 cells were transiently transfected with the immunoglobulin sequence containing plasmids using an automated procedure on a Tecan Freedom Evo platform. Immunoglobulins were purified from the cell supernatant using affinity purification (Protein A) on a Dionex Ultimate 3000 HPLC system with a plate autosampler. 4 samples with very low productivity were excluded, resulting in a total number of 127 antibodies for retesting. Antibodies were selected based upon their specific binding of the human and cyno 5T4 antigen and for binding to human 5T4 expressing MDA-MB-468 cells.

Binding to hu and cyno 5T4 antigen was determined by the following procedure. Hu or cyno 5T4 antigen was coated on a 384-format microtiter plate. A reference antibody, B-cell supernatants or a recombinantly produced antibody was added and the binding was detected via an anti-rabbit or human-POD antibody.

For binding to MDA-MB-468 cells, the cells were seeded on black cell culture microtiter plates (Corning). Specific antibodies originating from B-cell supernatants or the reference antibodies were allowed to interact with the cells. Binding was detected using an Alexa Fluor 488-labeled antibody. The fluorescence was read using a CellInsight (Thermo Fischer) device.

17 antibodies were selected and affinity to 5T4 antigen was measured using MDA-MB-468 cells, PA-1 cells and Chinese Hamster Ovary (CHO) mammalian cells expressing human or cyno 5T4 antigen (Table 2). In the current application the utilized CHO cells are referred to as CHOZN as these CHO cells expressing human or cyno 5T4 antigen were obtained using the CHOZN® Platform of Sigma-Aldrich. CHOZN cells (SAFC) were transiently transfected, using an Amaxa nucleofector device (Lonza) according to the manufacturer's instructions. Standard, commercially available mammalian expression vectors (SAFC, Life Technologies) were used, which contained the full length human and cyno 5T4 antigen coding sequence (according to accession number NP_006661.1 and Q4R8Y9 respectively), preceded by a

human CMV promoter. Transiently transfected CHOZN cells were cultured according the manufacturer's instructions, before being used in antibody binding studies. The 17 selected antibodies are characterised by the amino acid sequences according to the following table (Table 1).

Table 1. Chimeric anti-5T4 monoclonal antibodies

Antibody	**HCVR**	**LCVR**
789	SEQ ID NO:1	SEQ ID NO:2
811	SEQ ID NO:3	SEQ ID NO:4
825	SEQ ID NO:5	SEQ ID NO:6
828	SEQ ID NO:7	SEQ ID NO:8
829	SEQ ID NO:9	SEQ ID NO:10
833	SEQ ID NO:11	SEQ ID NO:12
834	SEQ ID NO:13	SEQ ID NO:14
835	SEQ ID NO:15	SEQ ID NO:16
841	SEQ ID NO:17	SEQ ID NO:18
843	SEQ ID NO:19	SEQ ID NO:20
845	SEQ ID NO:21	SEQ ID NO:22
847	SEQ ID NO:23	SEQ ID NO:24
848	SEQ ID NO:25	SEQ ID NO:26
849	SEQ ID NO:27	SEQ ID NO:28
868	SEQ ID NO:29	SEQ ID NO:30
899	SEQ ID NO:31	SEQ ID NO:32
908	SEQ ID NO:33	SEQ ID NO:34

In vitro affinity protocol

MDA-MB-468 cells, PA-1 cells or CHOZN cells expressing human or cyno 5T4 antigen (100,000 cells/well in a 96-well plate) were washed three times with ice-cold FACS buffer (1x PBS (Lonza) containing 0.2% v/w BSA (Sigma-Aldrich, St. Louis, MO)) and 0.02% v/w NaN_3 (Sigma-Aldrich), followed by the addition of a concentration range of each primary mAb (50 µl/well) diluted in ice-cold FACS buffer. After an incubation of 30 minutes at 4°C, cells were washed three times with ice-cold FACS buffer and 50 µl/well secondary mAb (AffiniPure $F(ab')_2$ fragment Goat-anti-human IgG-APC, 1:6,000 dilution, Jackson Immuno Research) was added. After 30 minutes at 4°C, cells were washed twice and resuspended in 150 µl FACS buffer. Fluorescence intensities were determined by flow cytometry (BD FACSVerse, Fanklin Lakes, NJ) and indicated as the median fluorescence intensity (MFI). Curves were fitted by nonlinear regression using the sigmoidal dose-response equation with variable slope (four parameters) in GraphPad Prism (version 5.01/6.01 for Windows, GraphPad, San Diego, CA). EC_{50} values were calculated as the concentration in µg/ml that gives a response half way between bottom and top of the curve, when using a 4 parameter logistic fit.

The affinity of the chimeric antibodies measured on human 5T4 antigen (hu 5T4)-expressing MDA-MB-468 cells ranges from an EC_{50} of 0.040 µg/ml to 0.730 µg/ml, comparable to the EC_{50} value of H8, which is 0.19 µg/ml. However, the A1 antibody exhibits binding to hu 5T4 with a lower affinity (the EC_{50} value is 4.72 µg/ml) as measured on MDA-MB-468 cells. The affinity of the chimeric antibodies for hu 5T4 was also measured on PA-1 cells and on CHOZN cells expressing hu 5T4. The EC_{50} values of the chimeric antibodies were again in the same range as the EC_{50} values of H8, whereas A1 showed at least a 3-fold lower binding affinity for hu 5T4 (Table 2). The EC_{50} values of A3 on the three cell types

expressing hu 5T4 were lower than the EC_{50} values of the chimeric antibodies on the corresponding cell types.

The chimeric anti-5T4 antibodies have similar affinity for hu 5T4 and for cyno 5T4 when measured using CHOZN expressing hu 5T4 or CHOZN cells expressing cyno 5T4 as is shown in Table 2. Compared to the binding of H8 to cyno 5T4, the binding shows a 32-fold improvement for most chimeric antibodies, except for 846 (10-fold) and 828 (7-fold).

Table 2. Affinity of chimeric mAbs measured on hu 5T4- and cyno 5T4 expressing cells

	Affinity (hu 5T4)			**Affinity (cyno 5T4)**
mAb	**MDA-MB-468 EC_{50} (µg/ml)**	**PA-1 EC_{50} (µg/ml)**	**CHOZN EC_{50} (µg/ml)**	**CHOZN EC_{50} (µg/ml)**
833	0.04	0.02	0.14	0.08
825	0.07	0.03	0.13	0.14
843	0.08	0.04	0.21	0.21
835	0.09	0.08	0.17	0.18
828	0.10	0.10	0.19	0.82
789	0.10	0.03	0.27	0.15
848	0.09	0.05	0.17	0.12
868	0.14	0.04	0.22	0.17
899	0.09	0.05	0.17	0.18
841	0.10	0.02	0.24	0.14
845	0.11	0.05	0.20	0.17
834	0.13	0.05	0.19	0.13
829	0.17	0.07	0.24	0.22
847	0.20	0.22	0.22	0.20

	Affinity (hu 5T4)			Affinity (cyno 5T4)
mAb	**MDA-MB-468 EC_{50} (µg/ml)**	**PA-1 EC_{50} (µg/ml)**	**CHOZN EC_{50} (µg/ml)**	**CHOZN EC_{50} (µg/ml)**
908	0.21	0.04	0.23	0.24
846	0.42	0.33	0.57	1.11
811	0.73	0.40	0.34	0.25
Reference mAb				
H8	0.19	0.07	0.21	8.11
A1	4.72	1.34	3.00	3.23
A3	0.99	0.62	0.82	0.57

Humanization

Humanized antibodies were prepared by CDR grafting as described below.

The CDRs of the clones 789, 825 and 833 were identified using the CDR-definitions from the numbering system IMGT (LEFRANC, MP, The IMGT unique numbering for immunoglobulins, T cell receptors and Ig-like domains. The Immunologist, 7 (1999), pp. 132–136) and Kabat.

Online public databases of human IgG sequences were searched using the rabbit VH domain using BLAST search algorithms, and candidate human variable domains were selected from the top 200 BLAST results. Five candidates were selected based on criteria such as framework homology, maintaining key framework residues, canonical loop structure and immunogenicity. The same procedure was repeated for the VL domain of the antibody. All humanized VH variants were combined with all humanized VL variants resulting in 25 humanized variants for each antibody.

The humanized variants comprising a HC-41C mutation were synthesized according to the procedure below and their affinity for human and cyno 5T4 was measured using CHOZN cells expressing either human or cyno 5T4. 11 variants were selected for further evaluation.

Transient expression of antibodies

a) Preparation of cDNA constructs and expression vectors

The HCVR of the mouse A1 amino acid sequence from US8044178, SEQ ID NO:2, positions 20 – 138, the HCVR of the mouse A3 amino acid sequence from US8044178, SEQ ID NO:10, positions 20 – 141, and the HCVR of H8 humanized variant 1 amino acid sequence from SEQ ID NO:52 were each joined at the N-terminus to a HAVT20 leader sequence (SEQ ID NO:54), and at the C-terminus to the constant domain of a human IgG1 HC according to SEQ ID NO:55. The resulting chimeric amino acid sequences were back-translated into a cDNA sequence codon-optimized for expression in human cells (Homo sapiens).

Similarly, the chimeric cDNA sequence for the LC of the construct was obtained by joining the sequences of a suitable secretion signal (also the HAVT20 leader sequence), the LCVR of the mouse A1 amino acid sequence from US8044178, SEQ ID NO:4, positions 21 - 127, the LCVR of the mouse A3 amino acid sequence from US8044178, SEQ ID NO:12, positions 21 - 127, or the LCVR of the H8 humanized variant 1 amino acid sequence SEQ ID NO:53, and a human IgG κ light chain constant region (SEQ ID NO:56), and back-translating the obtained amino acid sequences into a cDNA sequence codon-optimized for expression in human cells (Homo sapiens).

The cDNA sequences for the LC and HC of the humanized variants with the HC-41C mutation were obtained using a similar procedure, however, in this case the HC and LC sequences were joined at the N terminus to rabbit leader sequences (SEQ ID NO:57 and 58,

respectively), and at the C-terminus to the constant domain of the human IgG1 HC according to SEQ ID NO:55. The sequences according to the following table were used, having a cysteine at position 41 of the HCVR according to Kabat numbering (Table 3).

Table 3. HCVR and LCVR of HC-41C humanized variants

Humanized variant	**HCVR**	**LCVR**
789a	SEQ ID NO:35	SEQ ID NO:45
789b	SEQ ID NO:36	SEQ ID NO:45
789c	SEQ ID NO:37	SEQ ID NO:44
789d	SEQ ID NO:37	SEQ ID NO:46
833a	SEQ ID NO:61	SEQ ID NO:51
833b	SEQ ID NO:62	SEQ ID NO:51
833c	SEQ ID NO:63	SEQ ID NO:49
833d	SEQ ID NO:64	SEQ ID NO:50
825a	SEQ ID NO:59	SEQ ID NO:47
825b	SEQ ID NO:60	SEQ ID NO:47
825c	SEQ ID NO:60	SEQ ID NO:48

b) Vector construction and cloning strategy

For expression of the antibody chains a derivative of the commercially available (Thermo Fisher) mammalian expression vector pcDNA3.3 was used, which contains a CMV:BGHpA expression cassette. This vector was slightly adapted by changing the multiple cloning site downstream of the CMV promoter to contain AscI and NheI restriction sites, giving rise to expression vector 0080pcDNA3.3-SYN.

The cDNAs for the HC and the LC of the construct were ligated directly into the 0080pcDNA3.3-SYN vector, using AscI and NheI restriction sites. The final vectors containing either the HC or the LC expression cassette (CMV:HC:BGHpA and CMV:LC-BGHpA, respectively) were transferred to and expanded in E. coli NEB 5-alpha cells. Large-scale production of the final expression vectors for transfection was performed using Maxi- or Megaprep kits (Qiagen).

c) Transient expression in mammalian cells

Commercially available Expi293F cells (Thermo Fisher) were transfected with the expression vectors using the ExpiFectamine transfection agent according to the manufacturer's instructions as follows: $75x10^7$ cells were seeded in 300 mL FortiCHO medium, 300 µg of the expression vector was combined with 800 µl of ExpiFectamine transfection agent and added to the cells. One day after transfection, 1.5 ml Enhancer 1 and 15 ml Enhancer 2 were added to the culture. Six days post transfection, the cell culture supernatant was harvested by centrifugation at 4,000 g for 15 minutes and filtering the clarified harvest over PES bottle filters/ MF 75 filters (Nalgene).

Affinity measurements using a cell based assay

The humanized anti-5T4 antibodies have similar affinity for hu 5T4 and cyno 5T4 as measured on CHOZN cells expressing either hu 5T4 or cyno 5T4, except for the 825a, 825b and 825c humanized anti-5T4 antibodies, which show 2- to 3-fold lower binding to cyno 5T4 as compared to hu 5T4 (Table 4). Compared to the binding of H8 to cyno 5T4, the binding is 4- to 17-fold improved for the humanized anti-5T4 antibodies. Compared to A1, the binding is similarly improved. The affinity of the humanized anti-5T4 antibodies for hu 5T4 expressing CHOZN cells is comparable to H8.

Table 4. The affinity of humanized mAbs with HC-41C mutation and one chimeric mAb with HC-41C mutation measured on hu 5T4- and cyno 5T4-expressing CHOZN cells

mAb	**CHOZN - hu 5T4**		**CHOZN - cyno 5T4**	
	Avg EC_{50} (μg/ml)	**95% CI[1] (μg/ml)**	**Avg EC_{50} (μg/ml)**	**95% CI[1] (μg/ml)**
789a	0.20	0.14 to 0.28	0.33	0.24 to 0.48
789b	0.15	0.10 to 0.20	0.24	0.16 to 0.30
789c	0.20	0.11 to 0.29	0.21	0.16 to 0.26
789d	0.12	0.08 to 0.18	0.15	0.08 to 0.27
833a	0.22	0.11 to 0.30	0.12	0.084 to 0.17
833b	0.15	0.08 to 0.24	0.18	0.12 to 0.26
833c	0.18	0.11 to 0.26	0.17	0.12 to 0.20
833d	0.18	0.11 to 0.25	0.16	0.11 to 0.23
825a	0.15	0.09 to 0.21	0.53	0.33 to 0.80
825b	0.16	0.11 to 0.23	0.46	0.30 to 0.58
825c	0.21	0.13 to 0.31	0.42	0.34 to 0.49
chimeric 899	0.14	0.08 to 0.26	0.18	0.11 to 0.27
Reference mAbs				
H8	0.22	0.15 to 0.32	1.99	1.19 to 2.52
A1	4.42	0.53 to 19.19	1.58	1.09 to 2.27

[1] 95% CI is 95% confidence interval

General site-specific conjugation protocol

To a solution of cysteine engineered anti-5T4 antibody (5-10 mg/ml in 4.2 mM histidine, 50 mM trehalose, pH 6) EDTA (25 mM in water, 4% v/v) was added. The pH was

adjusted to ~7.4 using TRIS (1 M in water, pH 8) after which TCEP (10 mM in water, 20 equivalents) was added and the resulting mixture was incubated at room temperature for 1-3 hrs. The excess TCEP was removed by either a PD-10 desalting column or a Vivaspin centrifugal concentrator (30 kDa cut-off, PES) using 4.2 mM histidine, 50 mM trehalose, pH 6. The pH of the resulting antibody solution was raised to ~7.4 using TRIS (1 M in water, pH 8) after which dehydroascorbic acid (10 mM in water, 20 equivalents) was added and the resulting mixture was incubated at room temperature for 1-2 hrs. DMA was added followed by a solution of linker drug (10 mM in DMA). The final concentration of DMA was 5-10%. The resulting mixture was incubated at room temperature in the absence of light for 1-16 hrs. In order to remove the excess of linker drug, activated charcoal was added and the mixture was incubated at room temperature for 1 hr. The coal was removed using a 0.2 μm PES filter and the resulting ADC was formulated in 4.2 mM histidine, 50 mM trehalose, pH 6 using a Vivaspin centrifugal concentrator (30 kDa cut-off, PES). Finally, the ADC solution was sterile filtered using a 0.22 μm PES filter.

General (random) conjugation protocol for conjugation via partially reduced native interchain disulfide bond cysteines (wild-type or wt conjugation)

To a solution of anti-5T4 antibody (5-10 mg/ml in 4.2 mM histidine, 50 mM trehalose, pH 6) EDTA (25 mM in water, 4% v/v) was added. The pH was adjusted to ~7.4 using TRIS (1 M in water, pH 8) after which TCEP (10 mM in water, 1-3 equivalents depending on the antibody and the desired DAR) was added and the resulting mixture was incubated at room temperature for 1-3 hrs. DMA was added followed by a solution of linker drug (10 mM in DMA). The final concentration of DMA was 5-10%. The resulting mixture was incubated at room temperature in the absence of light for 1-16 hrs. In order to remove the excess of linker drug, activated charcoal was added and the mixture was incubated at room temperature for

1 hr. The coal was removed using a 0.2 µm PES filter and the resulting ADC was formulated in 4.2 mM histidine, 50 mM trehalose, pH 6 using a Vivaspin centrifugal concentrator (30 kDa cut-off, PES). Finally, the ADC solution was sterile filtered using a 0.22 µm PES filter.

Using the above general procedures, cysteine engineered and wild-type ADCs based on vc-*seco*-DUBA (SYD980; i.e., compound **18b**, n=1 in Example 10 on page 209 of WO2011/133039), were synthesized and characterized using analytical Hydrophobic Interaction Chromatography (HIC), Size Exclusion Chromatography (SEC), Shielded Hydrophobic Phase Chromatography (SHPC), RP-HPLC and LAL endotoxin-testing.

For analytical HIC, 5-10 µL of sample (1 mg/ml) was injected onto a TSKgel Butyl-NPR column (4.6 mm ID x 3.5 cm L, Tosoh Bioscience, cat. nr. 14947). The elution method consisted of a linear gradient from 100% Buffer A (25 mM sodium phosphate, 1.5 M ammonium sulphate, pH 6.95) to 100% of Buffer B (25 mM sodium phosphate, pH 6.95, 20% isopropanol) at 0.4 ml/min over 20 minutes. A Waters Acquity H-Class UPLC system equipped with PDA-detector and Empower software was used. Absorbance was measured at 214 nm and the retention time of ADCs was determined.

As made apparent by analytical HIC, there were differences in the retention times (RTs) for the DAR2 species of the different cysteine engineered ADCs. As well, the RT of the DAR2 species of most of the engineered ADCs was lower than the RT of the wt H8 conjugate (Table 5) and the increase in retention time (RT_{DAR2}-RT_{DAR0}) upon conjugation of two linker drugs is lower for the HC-41C engineered humanized ADCs compared to the increase for the wt H8-vc-*seco*-DUBA. All the engineered humanized ADCs have a RT_{DAR2}-RT_{DAR0} value of between 2.1-3.1, which is lower than the RT_{DAR2}-RT_{DAR0} value of wt H8-vc-*seco*-DUBA of 3.5, indicating that the engineered humanized ADCs exhibit decreased hydrophobicity.

Table 5. The hydrophobicity of vc-*seco*-DUBA ADCs by analytical HIC

ADC	DAR	RT_{DAR2}	RT_{DAR0}	RT_{DAR2}-RT_{DAR0}
H8-wt[1]	2.0	9.9	6.4	3.5
789a	1.8	9.4	6.3	3.1
789b	1.7	9.1	6.4	2.7
789c	1.7	9.1	6.3	2.8
789d	1.7	9.2	6.4	2.8
833a	1.7	9.4	6.9	2.5
833b	1.7	9.5	6.9	2.6
833c	1.7	9.0	6.9	2.1
833d	1.5	9.9	7.1	2.8
825a	1.7	8.5	6.4	2.1
825b	1.7	8.6	6.4	2.2
825c	1.7	8.2	6.0	2.2
899 chimeric	0.9	10.3	6.5	3.8

[1] Random (non-site specific) attachment

***In vitro* cytotoxicity**

The antigen binding affinities of the (site-specific) anti-5T4 ADCs were unaffected by the attached duocarmycin derivative linker drug as measured on CHOZN cells expressing either hu 5T4 or cyno 5T4 (Figures 1 and 2). As expected, the non-binding control ADC (rituximab-vc-*seco*-DUBA) had an effect on the growth of the 5T4-expressing tumour cells only at high concentrations. All humanized anti-5T4 ADCs were inactive (IC_{50} > 10 nM) on SK-MEL-30, a 5T4-negative human tumour cell line (about 400 5T4 antigen binding sites per

cell).

The potencies of the engineered humanized anti-5T4 ADCs were comparable to the potency of the conventionally conjugated H8-wt ADC on hu 5T4-expressing MDA-MB-468 cells and PA-1 cells (Table 6). However, the 833-ADC series were unable to decrease the PA-1 cell viability completely (efficacy 65 to 72%). Furthermore, the engineered humanized anti-5T4 ADCs were over 2.5 times more potent than the A3-vc-*seco*-DUBA and more than 14 times more potent than the A1-vc-*seco*-DUBA.

Table 6. *In vitro* cytotoxicity of the vc-*seco*-DUBA ADCs in human tumour cells expressing 5T4

ADC	**MDA-MB-468**			**PA-1**		
HC-41C	**Avg IC_{50} (nM)**	**Efficacy (%)**	**95% CI[1] (nM)**	**Avg IC_{50} (nM)**	**Efficacy (%)**	**95% CI[1] (nM)**
789a	0.16	99	0.18 to 0.22	0.13	96	0.09 to 0.16
789 b	0.14	99	0.15 to 0.19	0.11	97	0.07 to 0.14
789 c	0.14	99	0.15 to 0.20	0.11	91	0.07 to 0.13
789d	0.10	99	0.10 to 0.14	0.08	89	0.05 to 0.10
833a	0.11	99	0.13 to 0.17	0.13	67	0.07 to 0.18
833b	0.10	99	0.12 to 0.15	0.13	72	0.06 to 0.20
833c	0.13	98	0.15 to 0.21	0.13	66	0.07 to 0.17
833d	0.12	99	0.11 to 0.14	0.18	65	0.08 to 0.26
825a	0.14	99	0.15 to 0.19	0.11	95	0.08 to 0.13
825b	0.14	99	0.15 to 0.18	0.12	97	0.08 to 0.13
825c	0.12	99	0.15 to 0.18	0.09	97	0.06 to 0.10

ADC	MDA-MB-468			PA-1		
HC-41C	**Avg IC_{50} (nM)**	**Efficacy (%)**	**95% CI[1] (nM)**	**Avg IC_{50} (nM)**	**Efficacy (%)**	**95% CI[1] (nM)**
Reference ADC						
H8-vc-*seco*-DUBA	0.11	99	0.10 to 0.12	0.09	93	0.04 to 0.09
A1-vc-*seco*-DUBA	2.25	95	1.95 to 2.59	-	-	-
A3-vc-seco-DUBA	0.45	97	0.40 to 0.51	-	-	-
Rituximab-vc-*seco*-DUBA	32.92	91	26.86 to 47.15	8.32	N/A	N/A
Duocarmycin toxin	0.05	99	0.04 to 0.04	0.14	99	0.10 to 0.16

[1] 95% CI is 95% confidence interval

[2] N/A is not applicable

***In vivo* efficacy study**

The *in vivo* efficacy of anti-5T4 ADCs was evaluated in a BT474 (invasive ductal breast carcinoma from a 60-year old Caucasian female patient) cell-line xenograft model in B6;D2-Ces1c^{e} Foxn1^{nu}/J mice. Immunohistochemical staining confirmed presence of hu 5T4 on the cellular membrane of the BT474 cell line.

Tumours were induced subcutaneously by injecting $2x10^{7}$ BT-474 cells in 200 µL RPMI 1640 medium containing matrigel (50:50, v:v) into the right flank of 110 female Ces1c^{e} nude mice, 24 to 72 hrs after a whole body irradiation with a γ-source (2 Gy, ^{60}Co, BioMep, Dijon, France). When the tumours had reached a mean volume of 200-300 mm^{3}, the mice were dosed with a single injection of 3 mg/kg H8-, 833a-, 833b-, 833c-, 833d-, 825a- or 825c-vc-*seco*-DUBA ADC. Vehicle and the non-binding rituximab-vc-*seco*-DUBA ADC were used as controls. Mice having Ces1c activity in plasma were excluded from analysis.

All site-specifically conjugated anti-5T4 ADCs reduced the tumour volume more than the prior art H8-vc-*seco*-DUBA (Figure 3), indicating improved *in vivo* efficacy.

***In vivo* pharmacokinetics**

A pharmacokinetic study was performed with anti-5T4 ADCs in the B6(Cg)-Ces1c$^{tm1.1Loc}$/J mouse strain. These mice lack exon 5 of the Ces1c gene leading to the abolishment of the function of the enzyme. Mice were dosed with the anti-5T4 ADCs (3 mg/kg, i.v. in the tail vein) and plasma was collected at 0.25, 1, 6, 24, 48, 96, 168, 336, and 504 hrs post dosing. ELISA-based assays were used to quantify total antibody and conjugated antibody. The conjugated antibody assay captures ADC species that contain at least one linker drug. The results presented in Table 7 show that the site-specific ADCs are very stable, are cleared slower and have a longer half life than the prior art H8-vc-*seco*-DUBA ADC.

Table 7 Pharmacokinetics of anti-5T4 ADCs in Ces1c KO mice

Total antibody	Approximate $t_{1/2}$ (hrs)	C_{max} (µg/mL)	AUC_{last} (hrs*µg/mL)
H8-vc-*seco*-DUBA	nd	nd	nd
833a-vc-seco-DUBA	247	50.3	6484
833b-vc-seco-DUBA	388	46.6	8702
833c-vc-seco-DUBA	459	64.7	13177
833d-vc-seco-DUBA	nd	nd	nd
825a-vc-seco-DUBA	582	47.7	12596
825c-vc-seco-DUBA	2471	74.9	20901

Conjugated antibody	Approximate $t_{1/2}$ (hrs)	C_{max} (µg/mL)	AUC_{last} (hrs*µg/mL)
H8-vc-*seco*-DUBA	113	54.5	3979
833a-vc-seco-DUBA	172	61.0	4991
833b-vc-seco-DUBA	255	56.3	8116
833c-vc-seco-DUBA	327	52.5	8348
833d-vc-seco-DUBA	nd	nd	nd
825a-vc-seco-DUBA	398	67.7	11934
825c-vc-seco-DUBA	1049	67.2	15558

nd is not determined

Sequence listings with underlined CDR1, CDR2 and CDR3 amino acid sequences in HCVR and LCVR amino acid sequences

SEQ ID NO:1 (HCVR of clone/well 789 - rabbit)

```
  1   QSVEESGGRL VTPGTPLTLT CTASGFSLSS YWMSWVRQAP GKGLEWIGII
 51   AGRGSTYYAS WAKGRCTISK TSTTVDLKIT SPTTEDTAAY FCARVSSIYY
101   TFNLWGQGTL VTVSS
```

SEQ ID NO:2 (LCVR of clone/well 789 - rabbit)

```
  1   AQVLTQTPSS VSAAVGGTVT INCQSSQSVY SNNELSWYQQ KPGQPPKLLI
 51   YYASTLASGV PSRFKGSGSG TQFTLTISGV QSDDAATYYC QGSYYSGSGW
101   YYAFGGGTEVVVK
```

SEQ ID NO:3 (HCVR of clone/well 811 - rabbit)

```
  1   QSVEESGGRL VTPGTSLTLT CTASGFSLST YAMIWVRQAP GKGLEWIGII
 51   NSSGYTYYAN WAKGRFTISK TSTTVDLKIT SPTTEDTATY FCARGNAGIS
101   YDVSFNLWGQ GTLVTVSS
```

SEQ ID NO:4 (LCVR of clone/well 811 - rabbit)

```
  1   AYDMTQTPAS VEAGVGGTVT INCQASESIS SWLAWYQQKP GQPPNLLIYE
 51   ASKLASGVPS RFSGSGSGTE FTLTISGVES ADAATYYCQQ GWTSSNIDNA
101   FGGGTEVVVK
```

SEQ ID NO:5 (HCVR of clone/well 825 - rabbit)

```
  1   QSVEESGGRL VTPGTPLTLT CTVSGIDLSS YGMGWVRQAP GKGLEYIGII
 51   SRNSVTYYAT WAKGRFTISK TSTTVDLKMT SPTTEDTATY FCARRATYSG
101   ALGYFDIWGP GTLVTVSF
```

SEQ ID NO:6 (LCVR of clone/well 825 - rabbit)

```
  1    GYDMTQTPAS VSAAVGGTVT INCQASENIY STLAWYQQKP GQPPKVLIYD
 51    AFDLASGVPS RFKGSGSGTE YTLTISGVQS DDAATYYCQQ GYSGTNVDNA
104    FGGGTEVVVK
```

SEQ ID NO:7 (HCVR of clone/well 828 - rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTVSEIDLST YAMSWVRQAP GKGLEWIGII
 51    SAGGSAYYAS WAKGRFTISR TSTTVDLKMT SLTTEDTATY FCARGAAYAG
101    YTYGFSFFDI WGPGTLVTVS L
```

SEQ ID NO:8 (LCVR of clone/well 828 - rabbit)

```
  1    DIVMTQTPSS VSAAVGGTVT INCQASQNIY SNLAWYQQKP GQRPKLLIYG
 51    ASNLESGVPS RFKGSGSGTE YTLTISDLES DDAATYYCQS IDYGNNYLGS
101    FGGGTEVVVK
```

SEQ ID NO:9 (HCVR of clone/well 829 - rabbit)

```
  1    QSLEESGGRL VTPGTPLTLT CTASGFSLSS YDMSWVRQAP GKGLEYIGWI
 51    NSDDGAYYAN WAKGRFTISR TSTTVDLKIT SPTTEDTATY FCARDAGSTY
101    LYGLDPWGPG TLVTVSS
```

SEQ ID NO:10 (LCVR of clone/well 829 - rabbit)

```
  1    DVVMTQTPSS VSAGVGGTVT IKCQASQSIS SYLAWYQQKP GQRPKLLIYA
 51    ASTLASGVSS RFKGSGSGTE YTLTINDLES ADAATYYCQC TYYGGTYNTF
101    GGGTEVVVK
```

SEQ ID NO:11 (HCVR of clone/well 833 - rabbit)

```
  1    QSLEESGGGL VTPGGSLTLT CTGSGIDLSH YVVGWVRQAP GKGLEWIGII
 51    YGSGRTYYAN WAKGRFTISK TSTTVDLRIA RPTAEDTATY FCARDASVSV
101    YYWGYFDLWG QGTLVTVSS
```

SEQ ID NO:12 (LCVR of clone/well 833 - rabbit)

```
  1    AYDMTQTPVS VEVAVGGTVT IKCQASQSIG SELAWYQQKP GQPPKLLIYR
 51    ASTLESGVPS RFSGSGSGTE FTLTISGVES ADAATYYCQQ GYTYSEIDNA
101    FGGGTEVVVK
```

SEQ ID NO: 13 (HCVR of clone/well 834 - rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTVSGFSLST YSMSWVRQAP GKGLEWIGVI
 51    SRGGSAYYAS WAKGRFTISK TSTTVDLKVT SPTTEDTATY FCARGAISSG
101    YYVYDGMDLW GPGTLVTVSS
```

SEQ ID NO:14 (LCVR of clone/well 834 - rabbit)

```
  1    DIVMTQTPGS VEAAVGGTVT IKCQASESIS SYLAWYQQKP GQPPKFLIYS
 51    ASTLASGVPS RFKGSGSGTE FTLTISDLES ADAATYYCQC TDYGSDYMGA
101    FGGGTGVVVK
```

SEQ ID NO:15 (HCVR of clone/well 835 - rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTVSGIDLSS GAMGWVRQAP GKGLEWIGLI
 51    SSSPITYYAN WARGRFTISK TSTTVDLKIT SPTTADTATY FCARGYDDYG
101    EIWFNIWGPG TLVTVSL
```

SEQ ID NO:16 (LCVR of clone/well 835 - rabbit)

```
  1    AIEMTQSPPS LSASVGETVR IRCLAGEDIY SSISWYQQKP GKPPTLLIYG
 51    ASNLESGVPP RFSGSGSGTD YTLTIGGVQA EDAATYYCLG GWSYSSSLTF
101    GAGTKVEIK
```

SEQ ID NO:17 (HCVR of clone/well 841 - rabbit)

```
  1    QSLEESGGRL VTPGTPLTLT CKASGFSLS TYWMSWVRQA PGKGLEWIGI
 51    MLSYGNTVYA NWAKGRFTIS KTSSTTVDLK ITSPTTEDTA TYFCARGLYG
101    GYPNYGVYDL WGQGTLVTVS S
```

SEQ ID NO:18 (LCVR of clone/well 841 - rabbit)

```
  1    DVVMTQTPAS VEAAVGGTVT IKCQASQSIS SYLSWYQQKP GQPPKLLIYA
 51    ASNLASGVSS RFKGSRSGTE YTLTISDLES ADAATYYCQC TDYGSNYVGA
101    FGGGTEVVVK
```

SEQ ID NO:19 (HCVR of clone/well 843 - rabbit)

```
  1    QSLEESGGRL VTPGTPLTLT CTASGFSLNN AYMNWVRQAP GKGLEWIGII
 51    NTYGSTYFAT WAKGRFTFSK TSTTVDLKIT SPTTEDTATY FCARAYAPFS
101    TYSHYYGMDL WGPGTLVTVS S
```

SEQ ID NO:20 (LCVR of clone/well 843 - rabbit)

```
  1    DVVMTQTPSS VSAAVGGTVT IKCQASESIG SWLSWYQQKP GQPPKLLIYE
 51    ASKLISGVPS RFKGSGSGTE YTLTISDLES ADAATYYCQY TDYGSNYLGT
101    FGGGTEVVVK
```

SEQ ID NO:21 (HCVR of clone/well 845 - rabbit)

```
  1    QSLEESGGRL VTPGTPLTLT CTVSGIDLSS YTMNWVRQAP GKGLEWIGVI
 51    TSHNTYYASW AKGRFTISKT STTVDLKITS PTTEDTATYF CARSNYGSTI
101    YYMGGMDPWG PGTLVTVSS
```

SEQ ID NO:22 (LCVR of clone/well 845 - rabbit)

```
  1    DVVMTQTPAS VSAAVGGTVT INCQASQSIG SYLAWYQHQP GQPPKLLIYS
 51    ASTLESGVSS RFEGSRSGTE YTLTISDLDS ADAATYYCQC TDYGASYLGA
101    FGGGTEVVVK
```

SEQ ID NO:23 (HCVR of clone/well 847 - rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTVSGFSLSS YDMSWVRQAP GKGLEYIGYI
 51    NSDGSAYYAS WAKGRFTISK TSSTTVDLKI TSPTTEDTAT YFCARDAGST
101    YLYGMDPWGP GTLVTVSS
```

SEQ ID NO:24 (LCVR of clone/well 847 - rabbit)

```
  1    DVVMTQTPAS VSEPVGGTVT INCQASQSIY SYLAWYQQKP GQRPKLLIYA
 51    ASTLASGVSS RFKGSGSGTQ FTLTISDLES ADAATYYCQC TYYGGTFNTF
101    GGGTEVVVK
```

SEQ ID NO:25 (HCVR of clone/well 848 – rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTVSGFSLSS YTMSWVRQAP GKGLEWIGII
 51    SSIGSIWYAS WAKGRFTISK TSTTVDLKMT SLTTEDTATY FCARDGTGSK
101    YYTWDRLDLW GQGTLVTVSS
```

SEQ ID NO:26 (LCVR of clone/well 848 - rabbit)

```
  1    NIVMTQTPSP VSGAVGGTVT INCQASQSIY NELSWYQQKP GQPPKLLIYY
 51    TSTLASGVSS RFKGSGSGTQ FTLTISGVES VDAATYYCQQ GYSSSDVDNVF
101    GGGTEVVVK
```

SEQ ID NO:27 (HCVR of clone/well 849 - rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTVSGFSLSR YDMSWVRQAP GKGLEYIGYI
 51    NRDGSAYYAN WAKGRFTISK TSTTVDLKIT SPTTDDTATY FCARHAGSTY
101    LYGMDPWGPG TLVTVSS
```

SEQ ID NO:28 (LCVR of clone/well 849 - rabbit)

```
  1    DVVMTQTPSS VSAAVGGTVT IKCQASQSIS NYLAWYQQKP GQPPKLLIYA
 51    ASTLASGVSS RFKGSGSGTE FTLTISDLES ADAATYYCQC TYFGDTYNVF
101    GGGTEVVVK
```

SEQ ID NO:29 (HCVR of clone/well 868 - rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTASGFSLSD YTMGWVRQAP GKGLEWIGII
 51    NGYGSTYYAN WAKGRFAISK TSTTVDLKIT SPATEDTATY FCARGDTGRT
101    YDMHFNLWGQ GTLVTVSS
```

SEQ ID NO:30 (LCVR of clone/well 868 - rabbit)

```
  1    AYDMTQTPAS VSAAVGGTVT IKCQASESIR SWLAWYQQKP GQPPKLLIYS
 51    ASTLASGVSS RFKGSGSGTQ FTLTIGDLES ADAATYYCQQ GYTSSNLDNA
101    FGGGTEVLVK
```

SEQ ID NO:31 (HCVR of clone/well 899 - rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTVSGFSLSS YTMSWVRQAP GKGLEYIGII
 51    SSSDGTWYAN WVKGRFTISK TSTTVDLKMT SLTTEDTATY FCARDGTGNK
101    YYTWDRLDLW GQGTLVTVSS
```

SEQ ID NO:32 (LCVR of clone/well 899 - rabbit)

```
  1    NIVMTQTPSP VSGAVGGTVT INCQASQSIY NELSWYQQKP GQPPKLLIYY
 51    ASTLASGVSS RFKGSGSGTQ FTLTISGVES VDAATYYCQQ GYSSSNVDNV
101    FGGGTEVVVK
```

SEQ ID NO:33 (HCVR of clone/well 908 - rabbit)

```
  1    QSVEESGGRL VTPGTPLTLT CTVSGFSLSN FAMSWVRQAP GKGLEWIGII
 51    NGYGSIYYAT WAKGRFTISK TSTTVDLKIT SPTTEDTATY FCARGDAGRT
101    YNHYFNIWGP GTLVTVSL
```

SEQ ID NO:34 (LCVR of clone/well 908 - rabbit)

```
  1    DVVMTQTPAS VEAAVGGTVT IKCQASQSIS SWLSWYQQKP GQRPKLLIYA
 51    ASNLASGVPS RFKGSGSGTQ FTLTISDLES DDAATYYCQQ GYTSYNVDNA
101    FGGGTEVVVK
```

Humanized HCVR amino acid sequences with preferred positions for cysteine mutation 40, 41 and 89 underlined

SEQ ID NO:35 (HCVR 789 humanized version 1)

```
  1    EVKVEESGGG LVQPGGSLRL SCAASGFSLS SYWMSWVRQA PGKGLEWVSI
 51    IAGRGSTYYA SWAKGRFTIS KDNSEGMVYL QMNSLRAEDT AVYYCARVSS
101    IYYTFNLWGQ GTTVTVSS
```

SEQ ID NO:36 (HCVR 789 humanized version 2)

```
  1    EVQLLESGGS LVLPGGSLRL SCAASGFSLS SYWMSWVRQA PGKGLEWVSI
 51    IAGRGSTYYA SWAKGRFTIS RDNSKNTLYM QMNSLRAEDT ALYFCARVSS
101    IYYTFNLWGQ GTLVTVSS
```

SEQ ID NO:37 (HCVR 789 humanized version 3)

```
  1    QSVEESGGGL VQPGGSLRLS CAASGFSLSS YWMSWVRQAP GKGLEWIGII
 51    AGRGSTYYAS WAKGRFTISR DNSKNTLYLQ MNSLRAEDTA VYYCARVSSI
101    YYTFNLWGQG TLVTVSS
```

SEQ ID NO:38 (HCVR 825 humanized version 1)

```
  1    EVQLVESGGD LAQPGGSLRL SCAVSGIDLS SYGMGWVRQA PGKGLEWVSI
 51    ISRNSVTYYA TWAKGRFTIS RDNSKNTVYL QMTSLRAEDT ALYFCARRAT
101    YSGALGYFDI WGQGTLVTVS S
```

SEQ ID NO:39 (HCVR 825 humanized version 2)

```
  1    EVQLEESGGG LVKPGGSLRL SCAASGIDLS SYGMGWVRQA PGKGLEWVSI
 51    ISRNSVTYYA TWAKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARRAT
101    YSGALGYFDI WGRGTLVTVS S
```

SEQ ID NO:40 (HCVR 833 humanized version 1)

```
  1    EVQLVESGGG LIQPGGSLRL SCAASGIDLS HYVVGWVRQA PGKGLEWVSI
 51    IYGSGRTYYA NWAKGRFTIS KDNSKNTLYV RMNSLRAEDT AVYYCARDAS
101    VSVYYWGYFD LWGQGTLVTV SS
```

SEQ ID NO:41 (HCVR 833 humanized version 2)

```
  1    EVQLVESGGG LVQPGGSLRL SCAASGIDLS HYVVGWVRQA PGKGLEWVSI
 51    IYGSGRTYYA NWAKGRFTIS RDNSKNTLFL RMNSLRVEDT AVYFCARDAS
101    VSVYYWGYFD LWGQGTLVTV SS
```

SEQ ID NO:42 (HCVR 833 humanized version 3)

```
  1    EVQLEESGGG LVKPGGSLRL SCAASGIDLS HYVVGWVRQA PGKGLEWVSI
 51    IYGSGRTYYA NWAKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARDAS
101    VSVYYWGYFD LWGRGTLVTV SS
```

SEQ ID NO:43 (HCVR region 833 humanized version 4)

```
  1    QSLEESGGGL VQPGGSLRLS CAASGIDLSH YVVGWVRQAP GKGLEWIGII
 51    YGSGRTYYAN WAKGRFTISR HNSKNTLYLQ MNSLRAEDTA VYYCARDASV
101    SVYYWGYFDL WGQGTLVTV SS
```

Humanized LCVR amino acid sequences with preferred positions for cysteine mutation 40 and 41 underlined

SEQ ID NO:44 (LCVR 789 humanized version 1)

```
  1    DIVMTQSPDS LAVSLGERAT INCQSSQSVY SNNELSWYQQ KPGQPPKLLI
 51    YYASTLASGV PDRFSGSGSG TDFTLTISSL QAEDVAVYYC QGSYYSGSGW
101    YYAFGGGTKL EIK
```

SEQ ID NO:45 (LCVR 789 humanized version 2)

```
  1    EIVLTQSPSS LSASVGDRVT ITCQSSQSVY SNNELSWYQQ KPGKAPKLLI
 51    YYASTLASGV PSRFSGSGSG TDFTLTISSL QPEDFATYYC QGSYYSGSGW
101    YYAFGQGTKL EIK
```

SEQ ID NO:46 (LCVR 789 humanized version 3)

```
  1    AQVLTQSPSS LSASVGDRVT ITCQSSQSVY SNNELSWYQQ KPGKAPKLLI
 51    YYASTLASGV PSRFSGSGSG TDFTLTISSL QPEDFATYYC QGSYYSGSGW
101    YYAFGGGTKV EIK
```

SEQ ID NO:47 (LCVR 825 humanized version 1)

```
  1    EIVMTQSPSS LSASVGDRVT ITCQASENIY STLAWYQQKP GKAPKLLIYD
 51    AFDLASGVPS RFSGSGSGTD YTLTISSLQP EDFATYYCQQ GYSGTNVDNA
101    FGQGTKLEIK
```

SEQ ID NO:48 (LCVR 825 humanized version 2)

```
  1    GYDMTQSPSS VSASVGDRVT ITCQASENIY STLAWYQQKP GKAPKLLIYD
 51    AFDLASGVPS RFKGSGSGTE YTLTISSLQP EDFATYYCQQ GYSGTNVDNA
101    FGGGTKVEIK
```

SEQ ID NO:49 (LCVR 833 humanized version 1)

```
  1    DIQMTQSPST LSASVGDRVT ITCQASQSIG SELAWYQQKP GKAPKLLIYR
 51    ASTLESGVPS RFSGSGSGTE FTLTISSLQP DDFATYYCQQ GYTYSEIDNA
101    FGQGTKVEIK
```

SEQ ID NO:50 (LCVR 833 humanized version 2)

```
  1    DIQMTQSPSS LSASVGDRVT ITCQASQSIG SELAWYQQKP GQAPKLLIYR
 51    ASTLESGVPS RFSGSGSGTE FTFTISSLQP EDLATYYCQQ GYTYSEIDNA
101    FGQGTKLEIK
```

SEQ ID NO:51 (LCVR 833 humanized version 3)

```
  1    AYDMTQSPSS VSASVGDRVT ITCQASQSIG SELAWYQQKP GKAPKLLIYR
 51    ASTLESGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ GYTYSEIDNA
101    FGGGTKVEIK
```

SEQ ID NO:52 (H8 HC)

```
  1    QVQLVQSGAE VKKPGASVKV SCKASGYSFT GYYMHWVKQS PGQGLEWIGR
 51    INPNNGVTLY NQKFKDRVTM TRDTSISTAY MELSRLRSDD TAVYYCARST
101    MITNYVMDYW GQGTLVTVSS
```

SEQ ID NO:53 (H8 LC)

```
  1    DIVMTQSPDS LAVSLGERAT INCKASQSVS NDVAWYQQKP GQSPKLLISY
 51    TSSRYAGVPD RFSGSGSGTD FTLTISSLQA EDVAVYFCQQ DYNSPPTFGG
101    GTKLEIK
```

SEQ ID NO:54 (HAVT20 leader sequence)

```
  1    MACPGFLWAL VISTCLEFSM A
```

SEQ ID NO:55 (human IgG1 antibody HC constant region)

```
  1   ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
 51   HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
101   KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
151   HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
201   EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC
251   LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
301   QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
```

SEQ ID NO:56 (human IgG antibody LC κ constant region)

```
  1   RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
 51   NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
101   SFNRGEC
```

SEQ ID NO:57 (HC rabbit leader sequence)

```
  1   MGWTLVFLFL LSVTAGVHS
```

SEQ ID NO:58 (LC rabbit leader sequence)

```
  1   MVSSAQFLGL LLLCFQGTRC
```

Humanized HCVR amino acid sequences with cysteine mutation at position 41 according to the numbering system of Kabat

SEQ ID NO:59 (HCVR 825 humanized version 1 41C)

```
  1    EVQLVESGGD LAQPGGSLRL SCAVSGIDLS SYGMGWVRQA CGKGLEWVSI
 51    ISRNSVTYYA TWAKGRFTIS RDNSKNTVYL QMTSLRAEDT ALYFCARRAT
101    YSGALGYFDI WGQGTLVTVS S
```

SEQ ID NO:60 (HCVR 825 humanized version 2 41C)

```
  1    EVQLEESGGG LVKPGGSLRL SCAASGIDLS SYGMGWVRQA CGKGLEWVSI
 51    ISRNSVTYYA TWAKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARRAT
101    YSGALGYFDI WGRGTLVTVS S
```

SEQ ID NO:61 (HCVR 833 humanized version 1 41C)

```
  1    EVQLVESGGG LIQPGGSLRL SCAASGIDLS HYVVGWVRQA CGKGLEWVSI
 51    IYGSGRTYYA NWAKGRFTIS KDNSKNTLYV RMNSLRAEDT AVYYCARDAS
101    VSVYYWGYFD LWGQGTLVTV SS
```

SEQ ID NO:62 (HCVR 833 humanized version 2 41C)

```
  1    EVQLVESGGG LVQPGGSLRL SCAASGIDLS HYVVGWVRQA CGKGLEWVSI
 51    IYGSGRTYYA NWAKGRFTIS RDNSKNTLFL RMNSLRVEDT AVYFCARDAS
101    VSVYYWGYFD LWGQGTLVTV SS
```

SEQ ID NO:63 (HCVR 833 humanized version 3 41C)

```
  1    EVQLEESGGG LVKPGGSLRL SCAASGIDLS HYVVGWVRQA CGKGLEWVSI
 51    IYGSGRTYYA NWAKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARDAS
101    VSVYYWGYFD LWGRGTLVTV SS
```

SEQ ID NO:64 (HCVR 833 humanized version 4 41C)

```
  1    QSLEESGGGL VQPGGSLRLS CAASGIDLSH YVVGWVRQAC GKGLEWIGII
 51    YGSGRTYYAN WAKGRFTISR HNSKNTLYLQ MNSLRAEDTA VYYCARDASV
101    SVYYWGYFDL WGQGTLVTV SS
```

CLAIMS

1. A humanized anti-5T4 antibody comprising heavy chain (HC) and light chain (LC) variable region (VR) complementarity determining regions (CDRs) selected from the group consisting of:

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and
CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12;

wherein the antibody comprises at least one engineered cysteine at one or more positions of said antibody selected from heavy chain framework region positions 40, 41 and 89 according to Kabat numbering and light chain framework region positions 40 and 41 according to Kabat numbering.

2. The humanized antibody according to claim 1, comprising

a. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2 and HCVR amino acid sequence of SEQ ID NO:35 and LCVR amino acid sequence of SEQ ID NO:45;

b. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2 and HCVR amino acid sequence of SEQ ID NO:36 and LCVR amino acid sequence of SEQ ID NO:45;

c. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2 and HCVR amino acid sequence of SEQ ID NO:37 and LCVR amino acid sequence of SEQ ID NO:44;

d. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:1 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:2 and HCVR amino acid sequence of SEQ ID NO:37 and LCVR amino acid sequence of SEQ ID NO:46;

e. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12; and HCVR amino acid sequence of SEQ ID NO:40 and LCVR amino acid sequence of SEQ ID NO:51;

f. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12; and HCVR amino acid sequence of SEQ ID NO:41 and LCVR amino acid sequence of SEQ ID NO:51;

g. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12; and HCVR amino acid sequence of SEQ ID NO:42 and LCVR amino acid sequence of SEQ ID NO:49;

h. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:11 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:12; and HCVR amino acid sequence of SEQ ID NO:43 and LCVR amino acid sequence of SEQ ID NO:50;

i. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and HCVR amino acid sequence of SEQ ID NO:38 and LCVR amino acid sequence of SEQ ID NO:47;

j. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and HCVR amino acid

sequence of SEQ ID NO:39 and LCVR amino acid sequence of SEQ ID NO:47; and

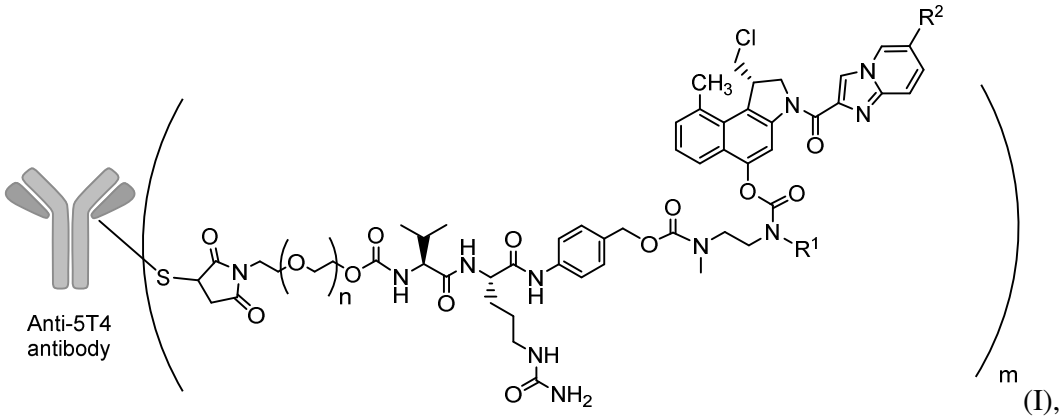
k. CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:5 and CDR1, CDR2 and CDR3 amino acid sequences of SEQ ID NO:6; and HCVR amino acid sequence of SEQ ID NO:39 and LCVR amino acid sequence of SEQ ID NO:48;

wherein the HCVR amino acid sequence comprises at least one engineered cysteine at one or more positions selected from heavy chain framework region positions 40, 41 and 89 according to Kabat numbering and/or the LCVR amino acid sequence comprises at least one engineered cysteine at one or more positions selected from light chain framework region positions 40 and 41 according to Kabat numbering.

3. The antibody according to claim 1 or claim 2 wherein the at least one engineered cysteine is present at one or more positions of said antibody selected from heavy chain framework region positions 40 and 41, and light chain framework region positions 40 and 41, preferably selected from heavy chain framework region position 41 and light chain framework region positions 40 and 41, more preferably the at least one engineered cysteine is present at heavy chain framework region position 41.

4. An antibody-drug conjugate comprising the antibody according to any one of claims 1 to 3, wherein a linker drug is site-specifically conjugated to the antibody through the at least one engineered cysteine.

5. The antibody-drug conjugate according to claim 4 of formula (I)



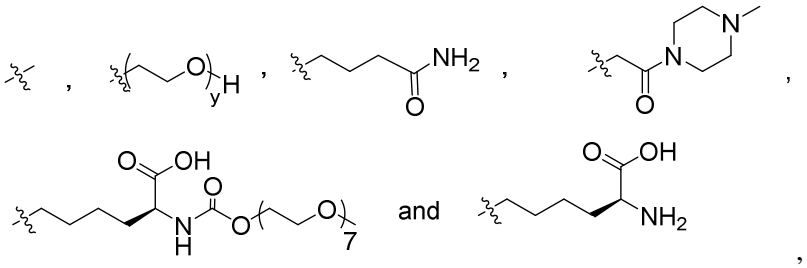
(I),

wherein

n is 0-3,

m represents an average DAR of from 1 to 6,

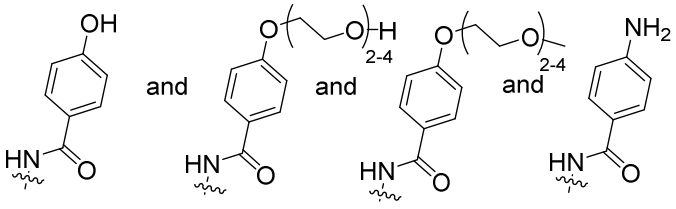
R^1 is selected from



,

y is 1-16, and

R^2 is selected from



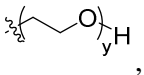
.

6. The antibody-drug conjugate according to claim 5, wherein

n is 0-1,

m represents an average DAR of from 1.5 to 2,

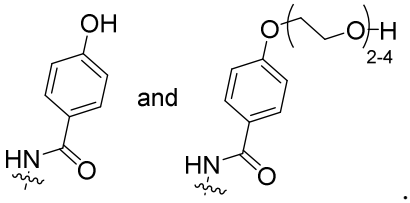
R^1 is



,

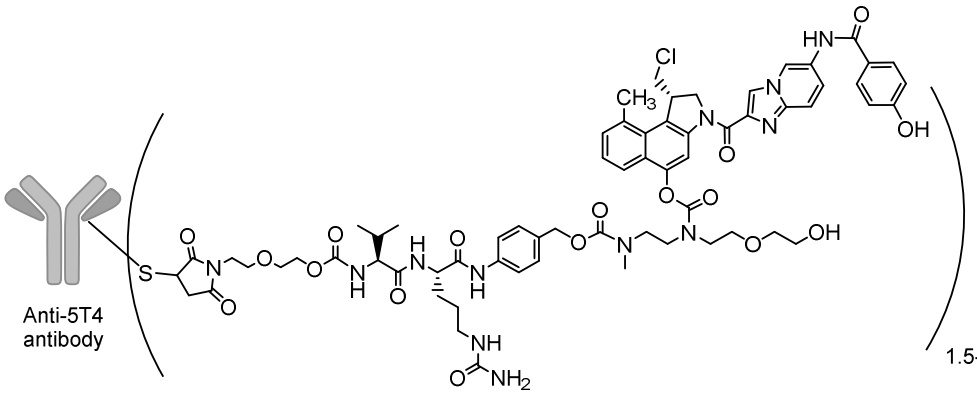
y is 1-4, and

R^2 is selected from



.

7. The antibody-drug conjugate according to any one of claims 4 to 6 of formula (II)



(II).

8. The antibody-drug conjugate according to any one of claims 4 to 7, wherein the antibody comprises HCVR and LCVR selected from the group consisting of:

a. HCVR amino acid sequence of SEQ ID NO:61 and LCVR amino acid sequence of SEQ ID NO:51;

b. HCVR amino acid sequence of SEQ ID NO:62 and LCVR amino acid sequence of SEQ ID NO:51;

c. HCVR amino acid sequence of SEQ ID NO:63 and LCVR amino acid sequence of SEQ ID NO:49;

d. HCVR amino acid sequence of SEQ ID NO:64 and LCVR amino acid sequence of SEQ ID NO:50;

e. HCVR amino acid sequence of SEQ ID NO:59 and LCVR amino acid sequence of SEQ ID NO:47; and

f. HCVR amino acid sequence of SEQ ID NO:60 and LCVR amino acid sequence of SEQ ID NO:48;

wherein the linker drug is site-specifically conjugated to the antibody through the engineered cysteine at heavy chain framework region position 41.

9. A pharmaceutical composition comprising the antibody according to any one of claims 1 to 3 or the antibody-drug conjugate according to any one of claims 4 to 8 and one or more pharmaceutically acceptable excipients, preferably in the form of a lyophilized powder.

10. The antibody according to any one of claims 1 to 3, the antibody-drug conjugate according to any one of claims 4 to 8 or the pharmaceutical composition according to claim 9 for use as a medicament.

11. Use of the antibody according to any one of claims 1 to 3, the antibody-drug conjugate according to any one of claims 4 to 8 or the pharmaceutical composition according to claim 9 in the manufacture of a medicament for treating human solid tumours and haematological malignancies.

12. The use according to claim 11, wherein the human solid tumours are selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, ovarian cancer, lung cancer, and mesothelioma.