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HUMANIZED ANTI-CD7 ANTIBODY

The present invention relates to an antibody binding to CD7, wherein the antibody comprises the V_H region determined by the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence being at least 90%, preferably at least 95% identical thereto; and the V_L region determined by the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence being at least 90%, preferably at least 95% identical thereto, provided that the three CDRs of the V_H region are determined by the amino acid sequences of SEQ ID NOs 3 to 5, and the three CDRs of the V_L region are determined by the amino acid sequences of SEQ ID NOs 6 to 8 (SEQ ID NO: 7 "AAS").

In this specification, a number of documents including patent applications and manufacturer's manuals are cited. The disclosure of these documents, while not considered relevant for the patentability of this invention, is herewith incorporated by reference in its entirety. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

For the development of antibody-based immunotherapy approaches in T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoma the CD7 antigen represents an attractive target antigen.

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One very promising candidate of a CD7-specific antibody is the murine TH69 antibody as described in WO 2003/051926. The murine TH69 antibody demonstrated strong anti-tumor activity in mouse models of T-ALL and was used as the basis for the design of different immunotherapeutic approaches including immunotoxins and CAR T-cells.

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However, TH69 is a murine antibody. It is well-known that the use of murine antibodies in order to fight several diseases (e. g. cancer, rejection after transplantation, rheumatic diseases and autoimmune diseases) did not deliver the expected therapeutic success. The immune response initiated by murine antibodies is one of the main problems. The human immune system recognizes murine antibodies from mice or rats as foreign substances and starts the production of antibodies against these. This leads on the one hand to ineffectiveness of the applied murine antibodies and on the other hand to unpleasant side effects like an anaphylactic shock or serum sickness.

Hence, for further clinical development humanized antibody variants are desired to reduce the risk of immunogenicity and formation of anti-drug antibodies when applied to patients. The humanization of murine antibodies which results in antibodies being similar to those of humans is a well-established procedure in order to avoid an unfavorable immune response. Humanization of murine monoclonal antibodies has vastly improved their *in vivo* tolerability. Humanization is the replacement of the mouse

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constant regions and V framework regions for human sequences and results in a significantly less immunogenic product (Harding et al. (2010), MAbs. 2010 May-Jun; 2(3): 256–265).

However, it is known and also turned out in connection with the attempt of humanizing TH69 mAb that it is challenging to obtain a humanized version of a monoclonal murine antibody which fully retains the high affinity in the nanomolar range and the thermal stability of the mouse TH69 antibody. Importantly, it was found to be challenging to obtain humanized version of TH69 with no unfavorable characteristics or even favorable characteristics. This challenging task is solved by the antibody of the present invention.

Accordingly the present invention relates in a first aspect to an antibody binding to CD7, wherein the antibody comprises the V_H region determined by the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence being at least 90%, preferably at least 95% identical thereto; and the V_L region determined by the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence being at least 90%, preferably at least 95% identical thereto, provided that the three CDRs of the V_H region are determined by the amino acid sequences of SEQ ID NOs 3 to 5, and the three CDRs of the V_L region are determined by the amino acid sequences of SEQ ID NOs 6 to 8 (SEQ ID NO: 7 "AAS").

The term "antibody" as used in accordance with the present invention comprises, for example, polyclonal or monoclonal antibodies. Furthermore, also derivatives or fragments thereof, which still retain the binding specificity to the target are comprised in the term "antibody". Antibody fragments (in particular (antigen) binding fragments) or derivatives comprise, inter alia, Fab or Fab' fragments, Fd, F(ab')₂, Fv or scFv fragments, single domain V_H or V-like domains, such as VHH or V-NAR-domains, as well as multimeric formats such as minibodies, diabodies, tribodies or triplebodies, tetrabodies or chemically conjugated Fab'-multimers (see, for example, Harlow and Lane "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 198; Harlow and Lane "Using Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, 1999; Altshuler EP, Serebryanaya DV, Katrukha AG. 2010, Biochemistry (Mosc)., vol. 75(13), 1584; Holliger P, Hudson PJ. 2005, Nat Biotechnol., vol. 23(9), 1126). The multimeric formats in particular comprise bispecific antibodies that can simultaneously bind to two different types of antigens and trispecific antibodies that can simultaneously bind to three different types of antigens. The first antigen can be found on the protein in accordance with the invention. A second and/or third antigen may, for example, be a tumor marker that is specifically expressed on cancer cells or a certain type of cancer cells. Non-limiting examples of bispecific antibodies formats are Biclonics (bispecific, full length human IgG antibodies), DART (Dual-affinity Re-targeting Antibody), DuoBodies (Genmab) and BiTE (consisting of two single-chain variable fragments (scFvs) of different antibodies) molecules (Kontermann and Brinkmann (2015), Drug Discovery Today, 20(7):838-847). The antibody to be coupled to the antibody of the invention is generally distinct from the antibody of the invention. By the second antibody a second binding specificity to an antigen other than CD7 can be coupled to the CD7 antibody of the invention, thereby generating a bispecific construct.

The term "antibody" also encompasses an antibody or binding fragment thereof being fused to Fc parts (thereby generating a Fc fusion protein). The coupling of an Fc part of an antibody to the antibody of the invention is a further means for extending the *in vivo* half-life of the antibody of the invention.

- 5 The term "antibody" according to the invention is a humanized antibody; i.e. a human antibody with the exception of non-human CDRs of the antibody.

Various techniques for the production of antibodies are well known in the art. Examples for such techniques are described, e.g. in Harlow E and Lane D, Cold Spring Harbor Laboratory Press, 1988; 10 Harlow E and Lane D, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999. The parent TH69 mAb is a murine antibody that was prepared by immunizing mice with CD7-positive T-ALL cells. Furthermore, recombinant antibodies may be obtained from monoclonal antibodies or can be prepared *de novo* using various display methods such as phage, ribosomal, mRNA, or cell display. A suitable system for the expression of the recombinant (humanized) antibodies may be 15 selected from, for example, bacteria, yeast, insects, mammalian cell lines or transgenic animals or plants (see, e.g., US patent 6,080,560; Holliger P, Hudson PJ. 2005, Nat Biotechnol., vol. 23(9), 11265). Further, techniques described for the production of single chain antibodies (see, inter alia, US Patent 4,946,778) can be adapted to produce single chain antibodies specific for an epitope of CD7. Surface plasmon resonance as employed in the BIAcore system can be used to measure the affinity of 20 antibodies.

CD7 (Cluster of Differentiation 7) is a protein that in humans is encoded by the CD7 gene. The CD7 antigen is a cell surface glycoprotein found on thymocytes and mature T cells. It is one of the earliest antigens to appear on the surface of cells of the T-lymphocyte lineage and the most reliable clinical 25 marker of T-cell acute lymphocytic leukemia. CD7 is an attractive therapeutic target for T-lymphoblastic leukemia/lymphoma (T-ALL/LBL).

The antibody of the invention specifically binds to CD7.

- 30 The amino acid sequences of SEQ ID NOs 3 and 4 are identical to the first and second CDRs of the V_H region of the murine monoclonal antibody TH69. The amino acid sequence of SEQ ID NO 5 is similar to the third CDR of the V_H region of the murine monoclonal antibody TH69 with one amino acid exchange in position 2. SEQ ID NOs 6 to 8 (SEQ ID NO: 7 "AAS") are the three CDRs of the fully-human V_L region and not derived from the murine monoclonal antibody TH69. The corresponding nucleotide sequences 35 of the six CDRs are shown in SEQ ID NOs 11 to 16 (SEQ ID NO: 15: GCTGCATCC).

The amino acid sequences of SEQ ID NOs 1 and 2 are the V_H region and the V_L region of the exemplified humanized TH69 antibody (huTH69; see examples section herein below) and the corresponding nucleotide sequences are SEQ ID NOs 9 and 10.

In accordance with the first aspect of the invention also amino acid sequences being at least 90%, preferably at least 95% identical to SEQ ID NO: 1 and SEQ ID NO: 2 are envisioned, provided that the six CDRs of SEQ ID NOs 3 to 8 (SEQ ID NO: 7 "AAS") remain unchanged. Since such amino acid sequences retain a high sequence identity with SEQ ID NO: 1 and SEQ ID NO: 2, it can safely be assumed that such antibodies retain the favorable properties of huTH69 that will be discussed herein below.

In accordance with the present invention, the term "percent (%) sequence identity" describes the number of matches ("hits") of identical nucleotides/amino acids of two or more aligned nucleic acid or amino acid sequences as compared to the number of nucleotides or amino acid residues making up the overall length of the template nucleic acid or amino acid sequences. In other terms, using an alignment for two or more sequences or subsequences the percentage of amino acid residues or nucleotides that are the same (e.g. 90% or 95% identity) may be determined, when the (sub)sequences are compared and aligned for maximum correspondence over a window of comparison, or over a designated region as measured using a sequence comparison algorithm as known in the art, or when manually aligned and visually inspected. This definition also applies to the complement of any sequence to be aligned.

Nucleotide and amino acid sequence analysis and alignment in connection with the present invention are preferably carried out using the NCBI BLAST algorithm (Stephen F. Altschul, Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), Nucleic Acids Res. 25:3389-3402). BLAST can be used for nucleotide sequences (nucleotide BLAST) and amino acid sequences (protein BLAST). The skilled person is aware of additional suitable programs to align nucleic acid and amino acid sequences.

The antibody of the invention binds CD7 with increased preference with a K_D of 200 nM or lower, 100 nM or lower, 50 nM or lower, 10 nM or lower, 5 nM or lower and 2 nM or lower. It is known in the art that the specific K_D value also depends on the particular antibody format; e.g. Fab or full-length antibody; see examples herein below.

The term " K_D " refers to the equilibrium dissociation constant (the reciprocal of the equilibrium binding or affinity constant) and is used herein according to the definitions provided in the art.

The K_D value with which the antibody of the invention binds CD7 can be determined by well-known methods including, without being limiting, surface plasmon resonance (SPR) spectroscopy, fluorescence titration, competition ELISA, calorimetric methods, such as isothermal titration calorimetry (ITC), flow cytometric titration analysis (FC titration), radioligand binding assays and surface plasmon resonance spectroscopy (BIAcore). Such methods are well known in the art and have been described e.g. in De Jong, L.A.A. et al. [2005] J. Chromatogr. B 829(1-2):1-25; Heinrich, L. et al. [2010] J. Immunol. Methods 352(1-2):13-22.

Preferably, surface plasmon resonance (SPR) spectroscopy is employed to determine the K_D . Even more preferably, the K_D is determined by ELISA. The use of SPR spectroscopy is illustrated by the appended examples (see section "Surface plasmon resonance spectroscopy") and the SPR spectroscopy is preferably carried out as in the examples.

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The antibody (huTH69-DE) of the invention displays with increased preference a thermal stability as a purified protein of at least 40°C, at least 45°C, at least 47.5°C, and at least 50°C, and at least 50.3°C. The antibody (huTH69-wt) of the invention displays with increased preference a thermal stability as a purified protein of at least 60°C, at least 65°C, at least 70°C., at least 72°C, and at least 72.3°C. The

10 thermal stability is preferably determined by a thermal shift assay.

The use of a thermal shift assay is illustrated by the appended examples (see section "Thermal shift assay") and the thermal shift assay is preferably carried out as in the examples.

15 A humanized version of the murine CD7 antibody TH69 (mTH69) was generated by combining CDR-grafting of the VH in a human framework and guided selection of a fully-human VL by phage display. The humanized TH69 (huTH69) retains a high affinity in the nanomolar range comparable to the chimeric TH69 (chimTH69) with fully-mouse V regions and an equilibrium dissociation constant (K_D) of 1.79 nM analyzed by surface plasmon resonance (SPR) spectroscopy as shown in the appended
20 examples. The huTH69 showed a thermal stability comparable to the chimTH69 antibody and clinical approved antibodies such as cetuximab. It displays a melting temperature of 50.3°C as determined by a thermal shift assay for the huTH69 with a DE-modification in the Fc part (huTH69-DE) and a melting temperature of 72.3°C for huTH69 without the DE-modification (huTH69-wt). It is of note that the DE-modification in the Fc part (huTH69-DE vs. huTH69-wt) lowers melting temperatures (Amersdorffer J.,
25 et al., U.S. Patent 20140227277. 2012.). Importantly, no unfavorable biochemical and biophysical properties potentially negatively impacting its ability for clinical development were found in the variable regions of the humanized antibody (e.g., unpaired cysteines, N-glycosylation sites, asparagine deamidation, aspartate isomerization).

30 As outlined in the appended examples, the humanized version (huTH69) of the murine CD7 antibody TH69 (TH69 or mTH69) was generated by combining CDR-grafting of the VH in a human framework and guided selection of a fully-human VL by phage display from a human κ light-chain library. The described unique candidate huTH69 is the result of a complex and inventive selection process in terms of the chosen humanization strategy and the used screening process. It was in particular difficult to
35 obtain the light chain of huTH69. The attempt to obtain a humanized light chain of TH69 via CDR-grafting failed. As an alternative approach for the humanization of the light chain a fully-human VL using guided-selection was carried out. A scFv-antibody library was generated from the CDR-grafted humanized VH of TH69 and the VL fragments (κ and λ light chains) were amplified from the RNA of B cells from eight healthy human donors. The huTH69 light chain was isolated from a pool of candidates (1×10^8 κ light
40 chains and 1×10^8 λ light chains) by phage display performing two rounds of screening (panning) on the CD7-positive cell line CEM. Finally, only one CD7-specific light chain was enriched by phage display

out of 1 x10⁸ κ light chains and 1 x10⁸ λ light chains. As explained, only this huTH69 antibody, with humanized CDR-grafted heavy chain and the fully-human light chain, showed the same CD7 binding specificity as TH69.

5 In accordance with a preferred embodiment of the first aspect of the invention the antibody comprises a V_H region differing by no more than 5, preferably by no more than 3 amino acid substitutions from SEQ ID NO: 1 and most preferably V_H region comprising SEQ ID NO: 1, and/or a V_L region differing by no more than 5, preferably by no more than 3 amino acid substitutions from SEQ ID NO: 2 and most preferably V_L region comprising SEQ ID NO: 2.

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In accordance with this preferred embodiment the framework regions within the V_H region and the V_L region of SEQ ID NO: 1 and SEQ ID NO: 2 may only differ from the exact sequences of SEQ ID NO.1 and SEQ ID NO: 2, each independently by no more than 5 and preferably by no more than 3 amino acid substitutions. The no more than 3 amino acid substitutions are preferably 2 amino acid substitutions and
15 most preferably 1 amino acid substitution.

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Since the antibody of this preferred embodiment only allows for very few amino acid replacements as compared to SEQ ID NO: 1 and SEQ ID NO: 2, it can safely be assumed that such antibodies retain the above-discussed favorable properties of huTH69.

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In accordance with a more preferred embodiment of the first aspect of the invention the amino acid substitutions are conservative amino acid substitutions.

It is well known that particular conservative amino acid substitutions in the framework regions of an
25 antibody essentially do not affect the properties of the antibody.

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The term “conservative amino acid substitution” designates the replacement of an amino acid by another amino acid having a side chain with similar biochemical properties. The naturally occurring amino acids can be classified as shown in the following table:

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Class	Amino acids	1-letter code
Aliphatic	Alanine, Valine, Leucine, Isoleucine	A, V, L, I
S/Se-containing	Cysteine, Selenocysteine, Methionine	C, U, M
Cyclic	Proline	P
35 Non-chiral	Glycine	G
Aromatic	Phenylalanine, Tyrosine, Tryptophan	F, Y, W
Basic	Histidine, Lysine, Arginine	H, K, R
Acidic	Aspartate, Glutamate,	D, E
Hydrophilic	Serine, Threonine, Asparagine, Glutamine	S, T, N, Q

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It is preferred that a conservative amino acid substitution is the replacement of (i) an aliphatic amino acid (G, A, V, L, I) by another aliphatic amino acid, (ii) a S/Se-containing amino acid (C, U, M) by another S/Se-containing amino acid, (iii) an aromatic amino acid (F, Y, W) by another aromatic amino acid, (iv) a basic amino acid (H, K, R) by another basic amino acid, (v) an acidic amino acid (D, E) by another
5 acidic amino acid, or (vi) a hydrophilic amino acid (S, T, N, Q) by another hydrophilic amino acid.

In accordance with a further preferred embodiment of the first aspect of the invention CD7 is determined by the amino acid sequence of SEQ ID NO: 17.

10 SEQ ID NO: 17 shows the amino acid sequence of the human CD7 protein. The human CD7-positive T-ALL cell line (HSB-2) was used to generate and characterize the mTH69. Recombinant purified CD7-Fc fusion protein was used for surface plasmon resonance (SPR) spectroscopy to characterize chimTH69 and huTH69 antibodies.

15 In accordance with another preferred embodiment of the first aspect of the invention, the antibody comprises an Fc domain of SEQ ID NO: 18.

As discussed herein above, huTH69 was also produced with a variant Fc part carrying the so-called DE-modification (huTH69-DE). The DE-modification is known to improve Fc-mediated effector functions by
20 enhancing FcγR binding and refers to the amino acid exchanges S239D and I332E in the CH2 domain of the Fc part. SEQ ID NO: 18 is census sequence taking into account the unmodified Fc domain and the exchanges S239D and/or I332E. All four options covered by SEQ ID NO: 18 are suitable Fc domains unmodified Fc domain, Fc domain with S239D, Fc domain with I332E and Fc domain with S239D and I332E, wherein the first and the last option are preferred and the last option is most preferred.

25 The data in the appended examples show that huTH69 potently triggered antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). These Fcγ receptor (FcγR)-mediated effector functions were successfully enhanced by amino acid exchanges S239D and I332E in the CH2 domain of the Fc part.

30 Furthermore, the data in the appended examples show that huTH69 as wildtype IgG1 unexpectedly triggers complement dependent cytotoxicity more efficiently than the parental chimTH69 antibody and selected published humanized CD7 antibodies (Heinrich G, et al. J Immunol. 1989 Dec 1;143(11):3589-97, WO 2022/095803, WO 2020/212710 and WO 2022/257835). huTH69 was especially more active
35 when target cells expressed lower levels of the CD7 antigen.

In accordance with a further preferred embodiment of the first aspect of the invention, the antibody is coupled to (a) a labelling group, (b) a toxin, (c) a drug, (d) a radionucleotide, (e) a cytokine, (f) a chemokine, (g) an enzyme, (h) a component modulating serum half-life, (i) an antibody or an Fc part
40 thereof, or (j) an antibody mimetic.

The labelling group is a group that allows the detection of the antibody, preferably *in vivo* in a subject. The labelling group is preferably a fluorescent dye. The fluorescent dye is preferably a component selected from Alexa Fluor dyes, (BODIPY) dye, Cy dyes, Dy dyes, IRDye dyes, HiLyte Fluor dyes, Oregon dyes, TRITC, Rhodamine, and Fluorescein. and derivatives thereof including, but not limited to, NHS esters, maleimides, phosphines, and free acids. Non-limiting further examples of fluorescent proteins are green fluorescent protein (GFP), yellow fluorescent protein (YFP), red fluorescent protein (RFP), cyan fluorescent protein (CFP) and infrared fluorescent protein (IFP). The labelling group is also preferably a contrast agent. A contrast agent as used herein is a substance used to enhance the contrast of structures or fluids within the body in medical imaging. Common contrast agents work based on X-ray attenuation or magnetic resonance signal enhancement.

The antibody of the invention can be used for photodynamic therapy by the attachment of suitable photosensitizing functional group comprising, but not limited to, porphyrins, chlorins, bacteriochlorins, phthalocyanines (e.g., IR700DX), phenothiazinium salts, benzophenothiazinium salts, squaraine, phenalenone, BODIPY dyes, ruthenium, rhodium, and iridium complexes, hypericin, flavins, and genetically encoded proteins such as KillerRed protein.

The toxin is preferably a small organic compound or a polypeptide, more preferably a toxic compound selected from the group consisting of, but not limited to, calicheamicin, maytansinoid, neocarzinostatin, esperamicin, dynemicin, kedarcidin, maduropeptin, doxorubicin, daunorubicin, auristatin, Ricin-A chain, modeccin, truncated *Pseudomonas* exotoxin A, diphtheria toxin and gelonin.

The drug is a compound that is capable to treat or prevent a disease. The disease is preferably a tumor and most preferably T-lymphoblastic leukemia/lymphoma (T-ALL/LBL). As discussed above, CD7 is a therapeutic target for tumor treatment. CD7 could also be a target for clinical settings in which T cells (or NK cells) contribute to the pathogenic manifestation e.g graft versus host disease (GVHD).

The radionuclide is preferably either selected from the group of gamma-emitting isotopes, more preferably ^{99m}Tc , ^{123}I , ^{125}I , or ^{111}In , and/or from the group of positron emitters, more preferably ^{18}F , ^{60}Cu , ^{62}Cu , ^{64}Cu , ^{68}Ga , ^{86}Y , ^{89}Zr , or ^{124}I , and/or from the group of beta-emitters, more preferably ^{131}I , ^{90}Y , ^{177}Lu , or ^{67}Cu , and/or from the group of alpha-emitters, preferably ^{213}Bi , ^{212}Bi , ^{227}Th , ^{212}Ph , ^{223}Ra , ^{225}Ac , or ^{211}At . The radionuclide is more preferably a positron emitter since they are particularly suitable for diagnostics, e.g. via positron emission tomography imaging or single-photon emission computed tomography (SPECT). For therapeutic applications the radionuclide is more preferably an alpha-emitter or a beta-emitter.

The cytokine is preferably selected from the group consisting of IL-2, IL-12, TNF-alpha, IFN alpha, IFN beta, IFN gamma, IL-10, IL-15, IL-24, GM-CSF, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-13, IL-18, IL21, LIF, CD80, B70, TNF beta, LT-beta, CD-40 ligand, Fas-ligand, TGF-beta, IL-1alpha and IL-1beta. As it is well known in the art, cytokines may favor a pro-inflammatory or an anti-inflammatory response of the

immune system. Thus, depending on the disease to be treated either fusion constructs with a pro-inflammatory or an anti-inflammatory cytokine may be favored. For example, for the treatment of cancer, in particular prostate cancer in general fusion constructs comprising pro-inflammatory cytokines are preferred. Anti-inflammatory cytokines may be used, for example, to avoid autoimmune reactions.

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The chemokine is preferably selected from the group consisting of IL-8, GRO alpha, GRO beta, GRO gamma, ENA-78, LDGF-PBP, GCP-2, PF4, Mig, IP-10, SDF-1alpha/beta, BUNZO/STRC33, I-TAC, BLC/BCA-1, MIP-1alpha, MIP-1 beta, MDC, TECK, TARC, RANTES, HCC-1, HCC-4, DC-CK1, MIP-3 alpha, MIP-3 beta, MCP-1-5, eotaxin, Eotaxin-2, I-309, MPIF-1, 6Ckine, CTACK, MEC, lymphotactin and fractalkine.

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An enzyme is a protein that catalyzes a particular chemical or biochemical reaction. Antibody-enzyme fusion proteins have been used, for example, to target tumors for cancer therapy in two ways. In one system, an antibody-enzyme is pretargeted to the tumor followed by administration of an inactive prodrug that is converted to its active form by the pretargeted enzyme. This system has been described as antibody-directed enzyme prodrug therapy (ADEPT). Suitable enzymes for prodrug activation will be further discussed herein below in the section on enzymes. The other system uses antibody-enzyme fusion proteins as direct therapeutics, where the enzyme is toxic by itself. The key feature in this approach is that the antibody is used to target and subsequently internalize the toxic enzyme into the tumor cell, which activates cell-death processes. This antibody-enzyme system has been largely applied to deliver ribonucleases.

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Enzymes may also be used for imaging in diagnostics. Conjugation partners in this regard include enzymes capable of catalyzing chromogenic, chemiluminescent or fluorescent reactions, such as e.g. horseradish peroxidase (HRP), luciferase, alpha-galactosidase and alkaline phosphatase (AP). For example, the conjugation partner can also be an enzyme capable of liberating or activating cytotoxic agents that have been brought into the vicinity of the targeted tissue, for example an enzyme for prodrug activation, such as e.g. an enzyme selected from the group consisting of carboxypeptidases, glucuronidases and glucosidases (Bagshawe, K.D. [2009] Curr. Drug Targets 10:152-157; Chen, K.-C. [2011] Bioconjugate Chem. 22:938-948). For certain applications a truncated version of an enzyme is preferred, for example by omitting a binding domain, provided that the truncated version retains or essentially retains the enzymatic activity of the full-length enzyme. Thus, with respect to the truncated version of the enzyme it is to be understood that they retain or essentially retain the enzymatic activity of the full-length enzyme.

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Several compounds are available to extend the half-life of therapeutics, including antibodies. Two of the most common and preferred options are PEGylation and fusion with human serum albumin. Other options are the chemical coupling of polymers and carbohydrates, post-translational modifications such as N -glycosylation, and fusion to recombinant polymer mimetics.

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In accordance with a more preferred embodiment of the first aspect of the invention the antibody mimetic is selected from affibodies, adnectins, anticalins, DARPin, avimers, nanofitins, affilins, Kunitz domain peptides, Fynomers®, and trispecific binding molecules and probodies.

- 5 As used herein, the term “antibody mimetics” refers to compounds or proteins which, like antibodies, can specifically bind antigens, but which are not structurally related to antibodies. Antibody mimetics are usually artificial peptides or proteins with a molar mass of about 3 to 30 kDa. The antibody mimetic is preferably selected from the group consisting of an Anticalin, Affibody, Adnectin, DARPin, Avimer, Nanofitin, Affilin, β -Wrapin, ADAPT, Monobody, RasIn, FingR, Pronectin, Centyrin, Affilin, Affimer, 10 Adhiron, Affitin, α Rep, Repebody, i-body, Fynomer or Kunitz domain protein.

- Lipocalin-derived binding proteins, also referred to as anticalins, represent a class of non-immunoglobulin binding proteins based on the human lipocalin scaffold. Lipocalins comprise a diverse family of small (20 kDa) extracellular proteins that occur in many species ranging from bacteria to 15 humans and serve for the transport or scavenging of physiological compounds. Despite mutually low sequence homology, the three-dimensional fold of lipocalins is highly conserved (Rothe and Skerra (2018), *BioDrugs*. 2018; 32(3): 233–243.). Anticalins constitute an emerging class of artificial binding proteins obtained by combinatorial design based on the compact and robust human lipocalin scaffold. Due to their human origin, anticalins have low immunogenic potential, and in several clinical trials 20 anticalins with different target specificities have demonstrated safety. The lipocalin-derived binding protein is preferably a lipocalin 2 (Lcn2)-derived binding protein. Lipocalin-2 (Lcn2), also known as neutrophil gelatinase-associated lipocalin (NGAL), is a protein that in humans is encoded by the LCN2 gene. Human LCN2 mRNA is, for example, represented by the NCBI Reference Sequence: NM_005564.5 (as available on March 12, 2019) and the amino acid sequence of human Lcn2 protein 25 including the signal peptide is, for example, represented by the UniProt ID P80188-2 (as available on November 1, 1995).

- “Affibodies”, in accordance with the present invention, are a family of antibody mimetics derived from the Z-domain of staphylococcal protein A. Affibodies are structurally based on a three-helix bundle 30 domain. An affibody has a molecular mass of around 6 kDa and is stable at high temperatures and under acidic or alkaline conditions. Target specificity is obtained by randomization of amino acids located in two alpha-helices involved in the binding activity of the parent protein domain (Feldwisch, J & Tolmachev, V. [2012] *Methods Mol. Biol.* 899:103-126).

- 35 “Adnectins” and also “Monobodies”, in accordance with the present invention, are based on the 10th extracellular domain of human fibronectin III (10Fn3), which adopts an Ig-like sandwich fold with 2 to 3 exposed loops, but lacks the central disulphide bridge (Gebauer, M. & Skerra, A. [2009] *Curr. Opin. Chem. Biol.* 13:245-255 and Koide et al. 1998, *J. Mol. Biol.* 284:1141–51). Adnectins and Monobodies with the desired target specificity can be genetically engineered by introducing modifications into specific 40 loops or other surface areas of the protein.

"DARPin", in accordance with the present invention, are designed ankyrin repeat domains that provide a rigid interface arising from typically three repeats corresponding to an artificial consensus sequence, whereby six positions per repeat are randomized. Consequently, DARPins lack structural flexibility (Gebauer, M. & Skerra, A. [2009] *Curr. Opin. Chem. Biol.* 13:245-255).

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The term "Avimer", as used herein, refers to a class of antibody mimetics which consist of two or more peptide sequences of 30 to 35 amino acids each, which are derived from A-domains of various membrane receptors and which are connected by linker peptides. Binding of target molecules occurs via the A-domain and domains with desired binding specificity can be selected, for example, by phage display techniques. The target specificity of the different A-domains contained in an avimer may, but do not have to be identical (Weidle UH, et al., (2013), *Cancer Genomics Proteomics*; 10(4):155-68).

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"Nanofitins" and also an "Affitins" are antibody mimetic proteins that are derived from the DNA binding protein Sac7d of *Sulfolobus acidocaldarius*. Nanofitins and Affitins usually have a molecular weight of around 7kDa and are designed to specifically bind a target molecule by randomizing the amino acids on the binding surface (Mouratou B, Béhar G, Paillard-Laurance L, Colinet S, Pecorari F., (2012) *Methods Mol Biol.*; 805:315-31).

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The term "Affilin", as used herein, refers to antibody mimetics that are developed by using either gamma-B crystalline or ubiquitin as a scaffold and modifying amino acids on the surface of these proteins by random mutagenesis. Selection of affilins with the desired target specificity is effected, for example, by phage display or ribosome display techniques. Depending on the scaffold, affilins have a molecular weight of approximately 10 or 20kDa. As used herein, the term affilin also refers to di- or multimerized forms of affilins (Weidle UH, et al., (2013), *Cancer Genomics Proteomics*; 10(4):155-68).

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As used herein, the term " β -Wrapins" designates affibody protein homodimers with a disulfide bond between the pair of Cys28 residues connecting the two identical monomer subunits, referred to as subunits 1 and 2. The scaffold used in engineering β -wrapins is ZA β_3 , an A β -binding affibody protein that not only prohibits the initial aggregation of A β monomers into toxic forms, but also dissociates pre-formed oligomeric aggregates by sequestering and stabilizing a β -hairpin conformation of A β monomers (Orr et al. (2018), *Computers & Chemical Engineering*, 116(4):322-332).

As used herein, the term "ABD-Derived Affinity Proteins (ADAPT)" refers to a class of antibody mimetics that has been created using the albumin-binding domain (ABD) of streptococcal protein G as a stable protein scaffold (Garousi et al (2015), *Cancer Res.*; 75(20):4364-71). By diversifying a surface of the domain that is not directly involved in albumin binding, molecules can be selected to bind a novel target and still retain their ability to bind albumin. This strategy has been used to select binders to a number of proteins, for example, the cancer-related epidermal growth factor receptor 3.

As used herein "RasIns" are 10FnIII-based antibody mimetics. Hence, they use the 10th domain of human fibronectin as their scaffold. RasIns are disulfide-free intrabodies. They were shown to be stable

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inside cells and also when fused with a fluorescent protein label (Cetin et al. (2017), J Mol Biol.; 429(4):562–573).

As used herein, the term “FingRs (Fibronectin intrabodies generated with mRNA display)” designates
5 recombinant antibody-like proteins also being based on the 10FnIII scaffold (Gross et al. (2013),
Neuron.; 78(6): 971–985.).

As used herein, the term “Pronectins” designates recombinant antibody-like proteins being based on
the fourteenth type-III scaffold of human fibronectin (14Fn3). The well-characterized fibronectin protein
10 is prevalent throughout the human body. Human fibronectin, an extracellular protein, is naturally
abundant in human serum. Intelligent loop-diversity has been designed to closely mimic the natural
human repertoire and avoid sequence immunogenicity. The intrinsic properties of a Pronectin align with
the pharmacological properties needed to make it a successful drug, including high potency, specificity,
stability, favorable small size, and high-yield production in *E. coli* and yeast
15 (http://www.protelica.com/pronectin_tech.html).

As used herein, the term “Centyrins” designates recombinant antibody-like proteins being based on the
consensus tenascin FN3 framework (Tencon) (Diem et al. (2014), Protein Eng., Des. and Sel. 27, 419–
429). Centyrins against different targets, e.g. human c-MET, rTNF α and mIL-17A, were generated.
20

As used herein, “Affimers” refer to small proteins that bind to target molecules with similar specificity
and affinity to that of antibodies. These engineered non-antibody binding proteins are designed to mimic
the molecular recognition characteristics of monoclonal antibodies in different applications. In addition,
these affinity reagents have been optimized to increase their stability, make them tolerant to a range of
25 temperatures and pH, reduce their size, and to increase their expression in *E. coli* and mammalian cells.
Derived from the cysteine protease inhibitor family of cystatins, which function in nature as cysteine
protease inhibitors, these 12–14 kDa proteins share the common tertiary structure of an α -helix lying on
top of an anti-parallel β -sheet (Tiede et al. (2017), eLife.; 6: e24903).

30 The class of recombinant antibody-like proteins designated as “Adhirons” herein is based on a
phytolectin consensus sequence as the scaffold (Tiede et al. (2014) Protein Eng. Des. Sel. 27, 145–
55).

The class of recombinant antibody-like proteins designated as “ α Rep” herein is derived from alpha-
35 helicoidal HEAT-like repeat protein scaffolds. In more detail, the α Rep proteins are derived from a
natural family of modular proteins comprising alpha-helical repeats, related to HEAT repeats, named
after Huntingtin, the elongation factor 3 (EF3), the protein phosphatase 2A (PP2A), and the yeast kinase
TOR. The association of several HEAT repeats forms alpha-solenoids of various lengths, which are
naturally found in a number of cellular proteins involved in intracellular transport and protein-protein
40 interaction (Hadpech et al. (2017), Scientific Reports; 7:Article number16335).

- As used herein, the term "Repebodies" designates recombinant antibody-like proteins which are composed of leucine-rich repeat (LRR) modules. In more detail, the binding scaffold of Repebodies is based on variable lymphocyte receptors, which are non-immunoglobulin antibodies composed of LRR modules in jawless vertebrates. A template scaffold was first constructed by joining consensus repeat
- 5 modules between the N- and C-capping motifs of variable lymphocyte receptors. The N-terminal domain of the template scaffold was redesigned based on the internalin-B cap by analyzing the modular similarity between the respective repeat units using a computational approach (Lee et al. (2012), Proc Natl Acad Sci; 109(9): 3299-3304).
- 10 As used herein, the term "i-bodies" refers to recombinant antibody-like proteins built on the scaffold of a human protein and engineered with two loops that mimic the shape of shark antibodies. These loops are responsible for binding or interacting with a particular target (in circulation or on a cell) that is causing disease. The i-body is a human analogue of the antigen binding domain of the shark antibody, which combines the advantages of monoclonal antibodies (high target specificity and affinity) with the
- 15 beneficial stability features of small molecules (<https://www.ibodies.eu/>).

- As used herein, the term "Fynomer" refers to a non-immunoglobulin-derived binding polypeptide derived from the human Fyn SH3 domain. Fyn SH3-derived polypeptides are well-known in the art and have been described e.g. in Grabulovski et al. (2007) JBC, 282, p. 3196-3204, WO 2008/022759,
- 20 Bertschinger et al (2007) Protein Eng Des Sel 20(2):57-68, Gebauer and Skerra (2009) Curr Opinion in Chemical Biology 13:245-255, or Schlatter et al. (2012), MAbs 4:4, 1-12).

- A "Kunitz domain peptide" is derived from the Kunitz domain of a Kunitz-type protease inhibitor such as bovine pancreatic trypsin inhibitor (BPTI), amyloid precursor protein (APP) or tissue factor pathway
- 25 inhibitor (TFPI). Kunitz domains have a molecular weight of approximately 6kDa and domains with the required target specificity can be selected by display techniques such as phage display (Weidle et al., (2013), Cancer Genomics Proteomics; 10(4):155-68).

- In accordance with a more preferred embodiment of the first aspect of the invention the drug is selected
- 30 from erlotinib (TARCEVA; Genentech/OSI Pharm.), bortezomib (VELCADE; MilleniumPharm.), fulvestrant (FASLODEX; AstraZeneca), sunitinib (SU11248; Pfizer), letrozole (FEMARA; Novartis), imatinib mesylate (GLEEVEC; Novartis), PTK787/ZK 222584 (Novartis), oxaliplatin (Eloxatin; Sanofi), 5-fluorouracil (5-FU, leucovorin, rapamycin (Sirolimus, RAPAMUNE; Wyeth), lapatinib (TYKERB, GSK572016; GlaxoSmithKline), lonafarnib (SCH 66336), sorafenib (BAY43-9006; Bayer Labs.), gefitinib
- 35 (IRESSA; AstraZeneca), AG1478, AG1571 (SU 5271; Sugen), alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimine and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially, bullatacin and
- 40 bullatacinone); camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly

- cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrousureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamycin, especially calicheamycin gamma1 I and calicheamycin omega1 (see, e.g., Agnew, Chem Intl ed Engl., 33: 183-186 (1994)) and dynemicin, including dynemicin A; bisphosphonate such as clodronate; esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, antrmycin, azaserine, bleomycins, cactinomycin, carabacin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRLIMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, liposomal doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites such as 5-fluorouracil(5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thiguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanane, and testolactone; anti-adrenals such as aminoglutethimide, mitotane, and trilostane; folic acid replenisher such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguanzone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethane; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ('Ara-C'); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N. J.) ABRAXANETM cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumber, Ill.), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin, carboplatin; vinblastine; platinum; etoposide, ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DFMO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, solvates and acids thereof.
- The above more preferred embodiment is directed to preferred antibody-drug conjugates (ADCs). ADCs are one of the fastest growing anticancer drugs. This approach comprises a mAb conjugated to the

cytotoxic payload via a chemical linker directed toward a target antigen expressed on the tumor cell surface, reducing systemic exposure and therefore toxicity. TH69 binds CD7 which directs the antibody into the tumor cells, wherein the drug can battle the tumor.

- 5 In accordance with a further more preferred embodiment of the first aspect of the invention the toxin is selected from auristatin (preferably monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF), domains II and HI of Pseudomonas exotoxin A, diphtheria toxin, ricin A, pokeweed antiviral protein, human pancreatic RNase, geldanamycin, maytansinoids, calicheamycin, daunomycin, doxorubicin, methotrexate, vindesine, SG2285, dolastatin, dolastatin analogue's auristatin, cryptophycin, camptothecin, rhizoxin derivatives, CC-1065, duocarmycin, enediyne antibiotics, 10 esperamicin, epothilone, an amatoxin (such alpha-amanitin), deruxtecan, exatecan analogue's (including exatecan), a pyrrolobenzodiazepin (PBD) dimer and toxoids.

- The principle of the antibody-toxin conjugates is similar to the ADCs. TH69 binds CD7 which directs the toxin into the tumor cells, whereby the toxin can exert the toxic effect in the tumor cells. The mechanistic 15 proof of concept that huTH69 is capable to deliver a truncated version of Pseudomonas exotoxin A is described in the appended Example 5.

- The present invention relates in a second aspect to a nucleic acid or a set of two nucleic acid molecules, 20 wherein the nucleic acid molecule encoding the antibody of the first aspect, and the set of two nucleic acid molecules, wherein the first nucleic acid molecule encodes the V_H region of the antibody of the first aspect and the second nucleic acid molecule encodes the V_L region of the antibody of the first aspect.

- As far as the nucleic acid molecule(s) refer to an antibody being coupled to one of the compounds as 25 defined in the above items (a) to (j) it is to be understood that these compounds can also be encoded by the nucleic acid molecule(s) as long as the compound is a proteinaceous compound.

- The term "nucleic acid molecule" in accordance with the present invention includes DNA, such as cDNA or double or single stranded genomic DNA and RNA. In this regard, "DNA" (deoxyribonucleic acid) 30 means any chain or sequence of the chemical building blocks adenine (A), guanine (G), cytosine (C) and thymine (T), called nucleotide bases, that are linked together on a deoxyribose sugar backbone. DNA can have one strand of nucleotide bases, or two complementary strands which may form a double helix structure. "RNA" (ribonucleic acid) means any chain or sequence of the chemical building blocks adenine (A), guanine (G), cytosine (C) and uracil (U), called nucleotide bases, that are linked together 35 on a ribose sugar backbone. RNA typically has one strand of nucleotide bases, such as mRNA. Included are also single- and double-stranded hybrids molecules, i.e., DNA-DNA, DNA-RNA and RNA-RNA. The nucleic acid molecule may also be modified by many means known in the art. Non-limiting examples of such modifications include methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, and internucleotide modifications such as, for example, those with 40 uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoroamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.). Nucleic acid

molecules, in the following also referred as polynucleotides, may contain one or more additional covalently linked moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), intercalators (e.g., acridine, psoralen, etc.), chelators (e.g., metals, radioactive metals, iron, oxidative metals, etc.), and alkylators. The polynucleotides may be derivatized by formation of a methyl or ethyl phosphotriester or an alkyl phosphoramidate linkage. Further included are nucleic acid mimicking molecules known in the art such as synthetic or semi-synthetic derivatives of DNA or RNA and mixed polymers. Such nucleic acid mimicking molecules or nucleic acid derivatives according to the invention include phosphorothioate nucleic acid, phosphoramidate nucleic acid, 2'-O-methoxyethyl ribonucleic acid, morpholino nucleic acid, hexitol nucleic acid (HNA), peptide nucleic acid (PNA) and locked nucleic acid (LNA) (see Braasch and Corey, Chem Biol 2001, 8: 1). LNA is an RNA derivative in which the ribose ring is constrained by a methylene linkage between the 2'-oxygen and the 4'-carbon. Also included are nucleic acids containing modified bases, for example thio-uracil, thio-guanine and fluoro-uracil. A nucleic acid molecule typically carries genetic information, including the information used by cellular machinery to make proteins and/or polypeptides. The nucleic acid molecule of the invention may comprise promoters, enhancers, response elements, signal sequences, polyadenylation sequences, introns, 5'- and 3'- non-coding regions, and the like.

The nucleic acid molecule according to the invention encodes the antibody of the invention. The antibody of the invention may also be encoded by a set of two nucleic acid molecules. This is because an antibody (a full-length antibody or fragments, such as scFv or Fab) comprises heavy and light chain sequences which, for example, upon expression in a cell, self-assemble into an antibody. The heavy and light chain sequences can be encoded by a set of two different nucleic acid molecules.

The present invention relates in a third aspect to a vector comprising the nucleic acid molecule of the second aspect in an expressible form or a vector or a set of two vectors comprising the set of two nucleic acid molecules of the second aspect in an expressible form.

The vector or set of two vectors can optionally also comprise nucleic acid molecule encoding a proteinaceous compound as defined in any one of items (a) to (j) in an expressible form.

The term "vector" in accordance with the invention means preferably a plasmid, cosmid, virus, bacteriophage or another vector used e.g. conventionally in genetic engineering which encoding the antibody of the invention in expressible form. For the same reasons as discussed in connection with the set of nucleic acid molecules of the invention, the antibody of the invention may also be encoded by a set of vectors, preferably by a set of two vectors.

The nucleic acid molecule(s) encoding the antibody of the invention may, for example, be inserted into several commercially available vectors. Non-limiting examples include prokaryotic plasmid vectors, such as of the pUC-series, pBluescript (Stratagene), the pET-series of expression vectors (Novagen) or pCRTOPO (Invitrogen) and vectors compatible with an expression in mammalian cells like pREP (Invitrogen), pcDNA3 (Invitrogen), pCEP4 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene),

pSG5 (Stratagene), EBO-pSV2neo, pBPV-1, pdBPVMMTneo, pRSVgpt, pRSVneo, pSV2-dhfr, pLZD35, pLXIN, pSIR (Clontech), pIRES-EGFP (Clontech), pEAK-10 (Edge Biosystems) pTriEx-Hygro (Novagen) and pCINeo (Promega). Examples for plasmid vectors suitable for *Pichia pastoris* comprise e.g. the plasmids pAO815, pPIC9K and pPIC3.5K (all Invitrogen).

5

The nucleic acid molecules inserted into the vector can e.g. be synthesized by standard methods or isolated from natural sources. Ligation of the coding sequences to transcriptional regulatory elements and/or to other amino acid encoding sequences can also be carried out using established methods. Transcriptional regulatory elements (parts of an expression cassette) ensuring expression in
10 prokaryotes or eukaryotic cells are well known to those skilled in the art. These elements comprise regulatory sequences ensuring the initiation of transcription (e. g., translation initiation codon, promoters, such as naturally-associated or heterologous promoters and/or insulators; see above), internal ribosomal entry sites (IRES) (Owens, Proc. Natl. Acad. Sci. USA 98 (2001), 1471-1476) and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional
15 regulatory elements may include transcriptional as well as translational enhancers. Preferably, the polynucleotide(s) encoding the antibody of the invention is operatively linked to such expression control sequences allowing expression in prokaryotes or eukaryotic cells. The vector may further comprise nucleic acid sequences encoding secretion signals as further regulatory elements. Such sequences are well known to the person skilled in the art. Furthermore, depending on the expression system used,
20 leader sequences capable of directing the expressed polypeptide to a cellular compartment may be added to the coding sequence of the polynucleotide of the invention. Such leader sequences are well known in the art.

Furthermore, it is preferred that the vector comprises a selectable marker. Examples of selectable
25 markers include genes encoding resistance to neomycin, ampicillin, hygromycin, and kanamycin. Specifically designed vectors allow the shuttling of DNA between different hosts, such as bacteria-fungal cells or bacteria-animal cells (e. g. the Gateway system available at Invitrogen). An expression vector according to this invention is capable of directing the replication, and the expression, of the polynucleotide and an encoded peptide or a fusion protein of this invention. Apart from introduction via
30 vectors such as phage vectors or viral vectors (e.g. adenoviral, retroviral), the nucleic acid molecules as described herein above may be designed for direct introduction or for introduction via liposomes into a cell. Additionally, baculoviral systems or systems based on vaccinia virus or Semliki Forest virus can be used as eukaryotic expression systems for the nucleic acid molecules of the invention.

35 The present invention relates in a fourth aspect to a host cell, comprising the nucleic acid molecule or set of two nucleic acid molecules of the second aspect or the vector of the set of two vectors of the third aspect, wherein the host cell is preferably an anti-tumor leucocyte and wherein the anti-tumor leucocyte is preferably a chimeric antigen receptor T-cell (CAR T-cell), T-cell-receptor-engineered T-cell (TCR T-cell), chimeric antigen receptor NK-cell (CAR NK-cell), NK cell receptor-engineered NK cell (NCR NK-cell), TCR/CAR hybrid T-cell, NCR/CAR hybrid NK-cells, tumor-infiltrating lymphocytes (TIL) or CAR
40 macrophage.

The host cell is preferably a non-human host cell or human host cell.

5 The term "host cell" means any cell of any organism that is selected, modified, transformed, grown, or used or manipulated in any way, for the production of the antibody of the invention by the cell and optionally a proteinaceous compound as defined in any one of items (a) to (j). The host cell is therefore generally an *ex vivo* or *in vitro* cell and/or an isolated cell or may be part of a non-human transgenic animal.

10 The host cell of the invention is typically produced by introducing the nucleic acid molecule(s) or vector(s) of the invention into the host cell which upon its/their presence mediates the expression of the nucleic acid molecule(s) of the invention encoding the antibody of the invention. The host from which the host cell is derived or isolated may be any prokaryote or eukaryotic cell or organism, preferably with the exception of human embryonic stem cells that have been derived directly by destruction of a human
15 embryo.

Suitable prokaryotes (bacteria) useful as hosts for the invention are, for example, those generally used for cloning and/or expression like *E. coli* (e.g., *E. coli* strains BL21, HB101, DH5a, XL1 Blue, Y1090 and JM101), *Salmonella typhimurium*, *Serratia marcescens*, *Burkholderia glumae*, *Pseudomonas putida*,
20 *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, *Streptomyces lividans*, *Lactococcus lactis*, *Mycobacterium smegmatis*, *Streptomyces coelicolor* or *Bacillus subtilis*. Appropriate culture mediums and conditions for the above-described host cells are well known in the art.

A suitable eukaryotic host cell may be a vertebrate cell, an insect cell, a fungal/yeast cell, a nematode
25 cell or a plant cell. The fungal/yeast cell may be a *Saccharomyces cerevisiae* cell, *Pichia pastoris* cell, *Kluyveromyces lactis* cell, or an *Aspergillus* cell. Preferred examples of a host cell to be genetically engineered with the nucleic acid molecule or the vector(s) of the invention is a cell of yeast, *E. coli* and/or a species of the genus *Bacillus* (e.g., *B. subtilis*). In one preferred embodiment the host cell is a yeast cell (e.g. *S. cerevisiae*, *K. lactis*, or *P. pastoris*).

30 In a different preferred embodiment the host cell is a mammalian host cell, such as a Chinese Hamster Ovary (CHO) cell, mouse myeloma lymphoblastoid, human embryonic kidney cell (HEK-293), human embryonic retinal cell (Crucell's Per.C6), or human amniocyte cell (Glycotope and CEVEC). These cells are frequently used in the art to produce recombinant proteins. CHO cells are the most commonly used
35 mammalian host cells for industrial production of recombinant protein therapeutics for humans.

Anti-tumor leucocytes are also preferred as host cells since they are useful as therapeutic cells in particular in tumor immune cell therapies. Furthermore, the humanized variable regions as described herein may be used as basis for the development of different immunotherapeutic strategies, including
40 CAR T / CAR NK cells / CAR macrophages.

An anti-tumor leucocyte (or an anti-tumor effector leucocyte) is a leucocyte capable of eliciting a cytolytic response that can cause tumor cell death. These leucocytes are specializing in and equipped for tumor cell elimination. The first category encompasses clonally expanded T lymphocytes expressing a unique T cell receptor (TCR) and recognizing tumor epitopes in the context of the major histocompatibility complex (MHC) molecules. These T cells, optionally together with B cells producing tumor-specific antibodies and dendritic cells (DC) processing and presenting tumor epitopes, can mediate an adaptive immunity against tumors. The second category of effector cells includes natural killer (NK) cells, NK-T cells, and macrophages (M). These cells are not restricted by the MHC molecules in their interactions with tumor targets, and they mediate innate immunity. Each type of effector cells, whether specific or nonspecific, contains subsets of cells at different stages of differentiation and activation. This means that each type of effector cell is potentially able to target tumor cells within a heterogeneous mix of cells with distinct functional capabilities, depending on their stage of differentiation, maturation, and/or activation (Holland, Frei; Cancer Medicine; 6th edition. chapter "Antitumor Effector Cells in Humans"). All the above-described types of anti-tumor leucocytes are applicable in accordance with the present invention.

The anti-tumor leucocytes as describe herein are preferably T-cells, NK cells or macrophages.

T-cells or T-lymphocytes can be distinguished from other lymphocytes by the presence of a T-cell receptor (TCR) on their cell surface. One of the functions of T-cells is mediating immune-mediated cell death, and it is carried out by two major subtypes: CD8+ "killer" and CD4+ "helper" T-cells. CD8+ T cells are cytotoxic which means that they are able to directly kill selected cells. These selected cells are in accordance with the invention tumor cells (such as cancer cells). CD4+ cells function as "helper cells". Unlike CD8+ killer T-cells, these CD4+ helper T-cells function by further activating memory B cells and cytotoxic T-cells, which leads to a larger immune response which is in accordance with the invention directed against tumor cells. The specific adaptive immune response regulated by the T-helper cell depends on its subtype, which is distinguished by the types of cytokines they secrete. The T-cells are preferably a CD8+ killer T-cells or a mixture of CD8+ killer and CD4+ helper T-cells.

A natural killer (NK) cell is a type of cytotoxic lymphocyte being critical to the innate immune system that belong to the rapidly expanding family of the innate lymphoid cells (ILC) and represent 5–20% of all circulating lymphocytes in humans. The role of NK cells in the innate immune system is analogous to that of cytotoxic T-cells in the vertebrate adaptive immune response. NK cells provide rapid responses to virus-infected cells and other intracellular pathogens acting at around 3 days after infection and respond to tumor formation. Typically, immune cells detect the major histocompatibility complex (MHC) presented on infected cell surfaces, triggering cytokine release, causing the death of the infected cell by lysis or apoptosis. NK cells are unique, however, as they have the ability to recognize and kill stressed cells in the absence of antibodies and MHC, allowing for a much faster immune reaction. They were named "natural killers" because they do not require activation to kill cells that are missing "self" markers of MHC class I. This role is especially important because harmful cells that are missing MHC I markers cannot be detected and destroyed by other immune cells, such as T-cells.

The anti-tumor leucocytes are also preferably chimeric antigen receptor T-cells (CAR T-cells), T-cell-receptor-engineered T-cells (TCR T-cells), chimeric antigen receptor NK-cells (CAR NK-cells), NK cell receptor-engineered NK cells (NCR NK-cells), TCR/CAR hybrid T-cells, NCR/CAR hybrid NK-cells,
5 chimeric antigen receptor macrophages (CAR-macrophages) or tumor-infiltrating lymphocytes (TILs).

Chimeric antigen receptor (CAR) T-cells are T-cells that have been genetically engineered to produce a chimeric T cell receptor (CAR) for use in immunotherapy. The receptors are chimeric because they combine both antigen-binding and T-cell activating functions into a single receptor. CAR-T cell therapy
10 uses T-cells engineered with CARs for tumor (such as cancer) therapy. The premise of CAR-T immunotherapy is to modify T-cells to recognize tumor cells in order to more effectively target and destroy them. In order to generate CAR T-cells, T-cells are harvested from a subject, genetically altered, and then infused into patients to attack a tumor in the subject. CAR T-cells can be both CD4+ and/or CD8+ cells. A 1-to-1 ratio of both cell types is preferred since it provides synergistic antitumor effects.

15 CAR T-cells are engineered to transfer arbitrary specificity onto an immune effector cell, like a T cell, which specifically eliminates antigen-bearing tumor cells. The CAR may comprise a scFv being derived from an antibody, a CD3 ζ and a transmembrane domain (so-called first-generation CARs). In this way, the engineered CAR is able to recognize specific tumor-associated antigens. Therefore, the CAR has
20 the ability to bind unprocessed tumor surface antigens without MHC processing while TCRs engage with both tumor intracellular and surface antigenic peptides embedded in MHC molecules.

In contrast, TCRs are α/β heterodimers that bind to the MHC-bound antigens. As discussed above, CARs recognize tumor antigen which leads to T-cell activation with different functions compared with
25 TCRs. CAR-T cell therapy has certain disadvantages like off-tumor toxicities when targeting tumor-specific antigen. Compared with CARs, TCRs have several structural advantages in T cell-based therapy, such as more subunits in their receptor structure (ten subunits vs one subunit), more immunoreceptor tyrosine-based activation motif (ITAMs) (ten vs three), less dependence on antigens (one vs 100), and more co-stimulate receptors (CD3, CD4, CD28, etc.) (Zhao et al. (2021) Front.
30 Immunol., | <https://doi.org/10.3389/fimmu.2021.658753>).

CAR NK-cells are distinguished from CAR T-cells in that the chimeric antigen receptor is introduced into NK cells instead of T-cells. Just as CAR T-cells, CAR-NK cells can be engineered to target diverse antigens, enhance proliferation and persistence *in vivo*, increase infiltration into solid tumors, overcome
35 resistant tumor microenvironment, and ultimately achieve an effective anti-tumor response.

CAR macrophages are distinguished from CAR T- / CAR NK-cells in that the chimeric antigen receptor is introduced into macrophages. Just as CAR T-cells / CAR-NK CAR macrophages can be engineered to target diverse antigens and enhance persistence *in vivo*, increase infiltration into solid tumors,
40 overcome resistant tumor microenvironment, and ultimately achieve an effective anti-tumor response.

- Natural cytotoxicity receptors (NCR) NK cells are NK-cell that have been genetically engineered to express a NCR. The NCRs have been proposed to bind to many cellular ligands which are implicated in NK cell surveillance of tumor cells. Many of these interactions have been shown to evoke the cytotoxic and cytokine-secreting functions of NK cells. However, it is also possible that the NCRs regulate other anti-tumor pathways. NCRs and their ligands can be successfully targeted for tumor (such as cancer) immunotherapy. NCRs have been classically defined as activating receptors delivering potent signals to NK cells in order to lyse harmful cells and to produce inflammatory cytokines.
- TCR/CAR hybrid T-cells are T-cells that have been genetically engineered to express a TCR and CAR. Similarly, NCR/CAR hybrid NK-cells are T-cells that have been genetically engineered to express a NCR and CAR.
- Tumor-infiltrating lymphocytes (TILs) are white blood cells that have left the bloodstream and migrate towards a tumor. TILs are implicated in killing tumor cells. The presence of lymphocytes in tumors is often associated with better clinical outcomes.
- The tumor-infiltrating lymphocytes are preferably tumor-infiltrating T-cells or tumor-infiltrating NK cells.
- In adoptive T-cell transfer therapy, TILs are expanded *ex vivo* from surgically resected tumors that have been cut into small fragments or from single cell suspensions isolated from the tumor fragments. Multiple individual cultures are established, grown separately and assayed for specific tumor recognition. TILs are typically expanded over the course of a few weeks with a high dose of IL-2 in 24-well plates. Selected TIL lines that presented best tumor reactivity are then further expanded in a "rapid expansion protocol" (REP), which uses anti-CD3 activation for a typical period of two weeks. The final post-REP TIL is infused back into a patient in order to treat a tumor of the patient. This *applies mutatis mutandis* to adoptive NK-cell transfer with TILs.
- In addition, the anti-tumor lymphocytes are preferably autologous anti-tumor lymphocytes.
- In an anti-tumor therapy with autologous lymphocytes the lymphocytes are taken from a subject having a tumor and are genetically engineered (e.g. to produce CAR T-cells) and/or selected and/or expanded (e.g. to produce TILs) *ex vivo* and then transferred back into the same subject. These autologous therapies are subject-specific because the therapeutic cells are created from a subject's own cells.
- The present invention also relates to a transgenic animal, preferably a non-human transgenic animal comprising the vector or the set of two vectors of the invention.
- Transgenic animals can be used for the production of antibodies as is reviewed, for example, in Brüggemann (2014), Arch Immunol Ther Exp (Warsz). 2015; 63(2): 101–108. The transgenic animal is preferably a mammal other than human. The antibodies may also be produced such that the antibodies

can be obtained from the milk of transgenic mammals. The mammal is therefore preferably a goat, sheep or cow.

The present invention relates in a fifth aspect to a method for producing an antibody of the first aspect, comprising (a) culturing the host of the fourth aspect under conditions that allow synthesis of said antibody; and (b) recovering said antibody from said culture.

When in the following the term "antibody" is used in connection with the discussion of the various embodiments of the invention, it is to be understood that this optionally also includes the proteinaceous compound as defined in any one of items (a) to (j) and, where applicable, any non-proteinaceous compound as defined in any one of items (a) to (j).

The term "culturing" specifies the process by which host cells are grown under controlled conditions. These conditions may vary dependent on the host cell used. The skilled person is well-aware of methods for establishing optimized culturing conditions. Moreover, methods for establishing, maintaining and manipulating a cell culture have been extensively described in the state of the art.

Methods of isolation of the antibody of the invention are well-known in the art and comprise without limitation method steps such as ion exchange chromatography, gel filtration chromatography (size exclusion chromatography), affinity chromatography, high pressure liquid chromatography (HPLC), reversed phase HPLC, disc gel electrophoresis or immunoprecipitation, see, for example, Antibody Purification Handbook, GE Healthcare, 18-1037-46.

The term "recovering said antibody from said culture" in accordance with the invention refers to the obtention of a product of a process implying that in the host cell a process can be induced by which information from nucleic acid molecule(s) encoding the antibody of the invention is/are used in the synthesis of the antibody of the invention. Several steps in this process may be modulated, including the transcription, RNA splicing, translation, and post-translational modification of the antibody of the invention by methods known in the art. Accordingly, such modulation may allow for control of the timing, location, and amount of antibody produced.

The present invention relates in a sixth aspect to a diagnostic composition or a pharmaceutical composition comprising the antibody, the nucleic acid molecule or set of two nucleic acid molecules, the vector or set of two vectors or the host cell of the invention (or combination thereof).

In accordance with the present invention, the term "pharmaceutical composition" relates to compositions for administration to a subject, preferably a human subject. In connection with the pharmaceutical composition the subject may be a patient, i.e. subject having a disease. In accordance with the present invention, also a "diagnostic composition" relates to a composition for administration to a subject, preferably a human subject. A "diagnostic composition" may furthermore relate to a composition to be contacted *in vitro* or *ex vivo* with a sample from a subject, preferably a human subject.

The diagnostic or pharmaceutical composition of the invention comprises the compounds recited above. It may, optionally, comprise further molecules capable of altering the characteristics of the compounds of the invention thereby, for example, stabilizing, modulating and/or activating their function. The composition may be in solid or liquid form or any other appropriate form and may be, *inter alia*, in the form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). The composition may, optionally and additionally, comprise a pharmaceutically acceptable carrier. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, organic solvents including DMSO etc. Compositions comprising such carriers can be formulated by well-known conventional methods.

While the diagnostic composition is to diagnose a disease within a subject or *ex vivo* or *in vitro* based on a sample from the subject (and in this regard, e.g. the presence, location and /or severity of the disease) the pharmaceutical composition is to treat or prevent the development of a disease in a subject.

The pharmaceutical compositions can be administered to the subject at a suitable dose in order to achieve a curative or disease preventive effect. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. The therapeutically effective amount for a given situation will readily be determined by routine experimentation and is within the skills and judgement of the ordinary clinician or physician. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 to 50 mg antibody / kg bodyweight every 1, 2, 3 or 4 weeks. However, a more preferred dosage might be in the range of 2 to 25 mg/kg every 1, 2, 3 or 4 weeks, even more preferably 3 to 20 mg/kg every 1, 2, 3 or 4 weeks. The length of treatment needed to observe changes and the interval following treatment for responses to occur vary depending on the desired effect. The particular amounts may be determined by conventional tests which are well known to the person skilled in the art.

The present invention relates in a seventh aspect to the antibody, the nucleic acid molecule or set of two nucleic acid molecules, the vector or set of two vectors or the host cell of the invention for use in a method of treating, inhibiting or diagnosing *in vivo* a tumor, wherein the tumor is preferably a T-cell neoplasia, lymphoma or leukemia, and is most preferably a T-cell lymphoma or acute lymphocytic leukemia.

The tumor can be a benign or a malignant tumor and is preferably a malignant tumor. A malignant tumor is also referred to herein as cancer. The tumor is also preferably a solid tumor and the cancer preferably a solid cancer.

Alternatively, the tumor is preferably a T-cell neoplasia, lymphoma or leukemia, and is most preferably a T-cell lymphoma or acute lymphocytic leukemia. As discussed herein above targeting CD7 is in particular useful in the diagnosis and treatment of these kinds of tumors.

- 5 Regarding the embodiments characterized in this specification, in particular in the claims, it is intended that each embodiment mentioned in a dependent claim is combined with each embodiment of each claim (independent or dependent) said dependent claim depends from. For example, in case of an independent claim 1 reciting 3 alternatives A, B and C, a dependent claim 2 reciting 3 alternatives D, E and F and a claim 3 depending from claims 1 and 2 and reciting 3 alternatives G, H and I, it is to be
- 10 understood that the specification unambiguously discloses embodiments corresponding to combinations A, D, G; A, D, H; A, D, I; A, E, G; A, E, H; A, E, I; A, F, G; A, F, H; A, F, I; B, D, G; B, D, H; B, D, I; B, E, G; B, E, H; B, E, I; B, F, G; B, F, H; B, F, I; C, D, G; C, D, H; C, D, I; C, E, G; C, E, H; C, E, I; C, F, G; C, F, H; C, F, I, unless specifically mentioned otherwise.
- 15 Similarly, and also in those cases where independent and/or dependent claims do not recite alternatives, it is understood that if dependent claims refer back to a plurality of preceding claims, any combination of subject-matter covered thereby is considered to be explicitly disclosed. For example, in case of an independent claim 1, a dependent claim 2 referring back to claim 1, and a dependent claim 3 referring back to both claims 2 and 1, it follows that the combination of the subject-matter of claims 3 and 1 is
- 20 clearly and unambiguously disclosed as is the combination of the subject-matter of claims 3, 2 and 1. In case a further dependent claim 4 is present which refers to any one of claims 1 to 3, it follows that the combination of the subject-matter of claims 4 and 1, of claims 4, 2 and 1, of claims 4, 3 and 1, as well as of claims 4, 3, 2 and 1 is clearly and unambiguously disclosed.
- 25 The above considerations apply *mutatis mutandis* to all appended claims.

The figures show.

- Figure 1. Generation and identification of humanized TH69.** (a) A scFv antibody library was
- 30 generated from the VH of murine TH96 (mTH69), humanized by CDR-grafting, and the VL fragments amplified from the RNA of B cells from eight human donors. The humanized TH69 (huTH69) was isolated from the library by phage display performing two panning rounds on the CD7-positive cell line CEM. (b) VH and VL amino acid sequences of TH69 before (mVH/mVL; SEQ ID NO: 21 and 22) and after humanization (huVH/huVL; SEQ ID NO: 1 and 2) by CDR-grafting or guided selection, respectively.
- 35 CDR are labelled according to IMGT (bold/line). Backmutations in VH indicated in red. CDR: complementary determining region, VH: variable domain of heavy chain, VL: variable domain of light chain.

- Figure 2: The humanized scFv huTH69 specifically binds CD7 in cell ELISA.** 1×10^6 CEM cells
- 40 without (black) and with (grey) pre-incubation with the parental mTH69 and 1×10^6 CEM cells with CD7 knock-out (CD7-KO-CEM, white) were incubated with 1×10^{10} colony-forming-units (CFU) phages

displaying the huTH69 or the mTH69 as scFvs. HER2-specific phage (4D5-scFv) was used as negative control. Mean values $\pm$ SEM on n=3 independent experiments, * $p \leq 0.05$, Two-way ANOVA with Bonferroni post-test.

5 **Figure 3. Purification and binding characteristics of the optimized humanized TH69. (a)** Structural representation of the chimeric and humanized TH69 antibody. The schematic IgG1 model structure is based on the pdb file provided by Dr. Mike Clark modified using Chimera X and MODELLER software (Clark, M.R. Chem Immunol, 1997. **65**: p. 88-110.; Goddard, T.D., et al., Protein Sci, 2018. **27**(1): p. 14-25.; Sali, A. and T.L. Blundell, J Mol Biol, 1993. **234**(3): p. 779-815. huTH69-DE, red: CDRs according to IMGT and four backmutations to mouse V gene between CDR2 and CDR3 **(b)** Purity of huTH69-DE and chimTH69-DE was analyzed by size exclusion chromatography. **(c)** SDS-PAGE under reducing and non-reducing conditions with Coomassie blue staining and western blot analysis with immunodetection of the heavy and light chains (HC and LC) validated the purity and molecular mass of huTH69-DE compared to chimTH69-DE. **(d)** Concentration dependent binding of huTH69-DE compared to chimTH69-DE was tested on CD7-positive cell line CEM via flow cytometry. Mean values $\pm$ SEM of three independent experiments.

Figure 4. Stability characteristics of the Fc-engineered humanized TH69 antibody. Stability of wildtype and Fc-optimized TH69 variants in comparison to the approved human IgG1 antibody cetuximab and a tafasitamab-like antibody (CD19-DE) was tested in a thermal shift assay. Mean values $\pm$ SEM of three independent experiments.

Figure 5. The humanized and Fc-optimized antibody huTH69-DE is able to trigger efficient Fc γ R-mediated effector functions of different T-ALL cell lines comparable to chimTH69-DE. (a) ADCC of CD7-positive tumor cell lines (CEM, HSB-2 and MOLT-16) was performed in standard chromium release assays for 4h with increasing concentrations of the respective antibodies and peripheral mononuclear cells (PBMCs) of healthy donors at an Effector:Target (E:T) ratio of 40:1. The antibody mediated tumor cell lysis was calculated for huTH69-DE, chimTH69-DE, chimTH69-wt and the control antibody 4D5-DE. Mean values $\pm$ SEM on n=3 independent experiments, * $p \leq 0.05$, n.s not significant, Two-way ANOVA with Bonferroni post-test (black *: huTH69-DE vs. chimTH69-DE; color *: DE vs. wt). **(b)** High-throughput fluorescence microscopy was performed for 4h to analyze antibody-dependent cell-mediated phagocytosis (ADCP). CEM, HSB-2 and MOLT-16 cells were labelled with a pH-sensitive red-fluorescent dye and incubated at an E:T ratio of 1:1 with polarized M0 macrophages and 10 μ g/ml of the indicated antibodies. Phagocytosis is depicted as red object counts per image. Mean values $\pm$ SEM on n=3 independent experiments, * $p \leq 0.05$, n.s not significant, Two-way ANOVA with Bonferroni post-test.

Figure 6. The Fc-optimized huTH69-DE shows similar internalization kinetics in CD7-positive cell lines in comparison to chimTH69-DE. Internalization of chimTH69-DE and huTH69-DE (1 μ g/ml) in CD7-positive cell lines (CEM, HSB-2, MOLT-16) was performed in high-throughput fluorescence microscopy for 24h. Antibodies were labelled with pH-sensitive red-fluorescent dye. CD19-DE antibody

and untreated cells served as negative controls. The internalization is depicted as total red object area (μm^2) per image. Mean values $\pm$ SEM on $n=3$ independent experiments, * $p \leq 0.05$, n.s not significant, Two-way ANOVA with Bonferroni post-test.

5 **Figure 7. The Fc-optimized ADC huTH69-DE-MMAE mediated similar direct anti-proliferative effects as the chimeric ADC chimTH69-DE-MMAE against a cell line expressing high levels of CD7. (A)** CD7-positive cell line HSB-2 was treated with increasing concentrations of huTH69-DE-MMAE, chimTH69-DE-MMAE, huTH69-DE or chimTH69-DE for 96 h and the cell viability was tested by MTT-assay. Mean values $\pm$ SEM of $n=3$ independent experiments. **(B)** As a control for antigen-specific effects,
10 cells were pretreated with a 10-fold molar excess of the parental murine TH69 antibody for 30 minutes to mask the CD7 epitope (blockade) and were incubated afterwards with huTH69-DE-MMAE (2.08 nM) for 96h. The cell viability was tested by MTT-assay. Mean values $\pm$ SEM on $n=3$ independent experiments.

15 **Figure 8. The Fc-optimized ADC huTH69-DE-MMAE mediated increased direct anti-proliferative effects compared to the chimeric ADC chimTH69-DE-MMAE against CD7-positive cell lines expressing lower levels of CD7. (A-B)** CD7-positive cell lines CEM **(A)** and Karpas-45 **(B)** with medium to low levels of CD7, respectively, were treated with increasing concentrations of huTH69-DE-MMAE, chimTH69-DE-MMAE, huTH69-DE or chimTH69-DE for 96h and the cell viability was tested by
20 MTT-assay. Mean values $\pm$ SEM of $n=3$ independent experiments, * $p \leq 0.05$ for huTH69-DE-MMAE vs. chimTH69-DE-MMAE, Two-way ANOVA with Tukey's multiple comparison test. **(C)** As control, the CD7-knockout cell line CEM-CD7KO was treated with increasing concentrations of huTH69-DE-MMAE, chimTH69-DE-MMAE, huTH69-DE or chimTH69-DE for 96h and the cell viability was determined by MTT-assay. Mean values $\pm$ SEM of $n=2$ independent experiments are presented.

25

Figure 9: Applied strategies for the humanization of TH69. (A) In the first humanization strategy, both the VH and VL of the murine TH69 (mTH69) were humanized by CDR-grafting (CDR-huTH69). **(B)** In the second humanization strategy, a scFv-antibody library was generated from the VH of murine TH69, humanized by CDR-grafting, and the VL fragments (κ and λ light chains) amplified from the RNA
30 of B cells from eight human donors. As outlined in the above examples, our final candidate (huTH69) was isolated from the pool of candidates by phage display performing two rounds of screening (panning) on the CD7-positive cell line CEM.

Figure 10: Purification of huTH69 generated by CDR-grafting of VH and VL. (A) Structural representation of the CDR-grafting humanized TH69 antibody (CDR-huTH69) and a hybrid TH69
35 antibody composed of the CDR-grafted heavy chain and the chimeric light chain (hybTH69-huVH-chimVL). **(B)** Purity of CDR-huTH69 and hybTH69-huVH-chimVL in comparison to chimTH69 was analyzed by size exclusion chromatography. **(C)** SDS-PAGE under reducing and non-reducing conditions with Coomassie blue staining was performed to verify the purity and molecular mass of CDR-
40 huTH69 and hybrid antibodies compared to control IgG1.

Figure 11: Characterization of huTH69 generated by CDR-grafting of VH and VL. (A) Structural representation of the CDR-grafting humanized TH69 antibody (CDR-huTH69). (B) Concentration dependent binding of CDR-huTH69 compared to chimTH69 was tested on CD7-positive cell line CEM via flow cytometry. Depicted is one representative experiment. (C) ADCC of CD7-positive tumor cell lines (CEM) was performed in standard chromium release assays for 4h with increasing concentrations of the respective antibodies and peripheral mononuclear cells (PBMCs) of healthy donors at an Effector:Target (E:T) ratio of 40:1. The tumor cell lysis was calculated for CDR-huTH69-wt, chimTH69-wt and the control antibody 4D5-wt. Mean values $\pm$ SEM of three independent experiments. * $p \leq 0.05$, n.s. not significant, Two-way ANOVA with Bonferroni post-test. (D) Structural representation of the hybrid TH69 antibody composed of the CDR-grafted heavy chain and the chimeric light chain (hybTH69-huVH-chimVL). (E) Concentration dependent binding of hybTH69-huVH-chimVL compared to chimTH69 was tested on CD7-positive cell line CEM via flow cytometry. Mean values $\pm$ SEM of three independent experiments. * $p \leq 0.05$, n.s. not significant, Two-way ANOVA with Bonferroni post-test. (F) ADCC of CD7-positive tumor cell lines (CEM) was performed in standard chromium release assays for 4h with increasing concentrations of the respective antibodies and peripheral mononuclear cells (PBMCs) of healthy donors at an Effector:Target (E:T) ratio of 40:1. The tumor cell lysis was calculated for hybTH69-huVH-chimVL, chimTH69-wt and the control antibody 4D5-DE. Mean values $\pm$ SEM of three independent experiments. * $p \leq 0.05$, n.s. not significant, Two-way ANOVA with Bonferroni post-test.

Figure 12: Binding analyses of monoclonal phage antibodies. (A+B) 1×10^6 CEM cells (CD7-positive; black) and 1×10^6 SKBR3 cells (CD7-; white) were incubated with phages displaying huTH69-scFv candidates (CDR-grafted VH and κ light chains (A) or λ light chains (B)) or the mTH69-scFv and tested in ELISA experiments. Cell surface bound phages were detected using anti-M13-HRP antibody and absorbance at 405 nm was measured after incubation with ABTS substrate. (C) 1×10^6 CEM cells (CD7-positive; black) and 1×10^6 CEM cells with CD7 knock-out (CD7KO-CEM; CD7-; white) and 1×10^6 CEM cells with (grey) pre-incubation with the parental mTH69 were incubated with 1×10^{10} colony-forming-units (CFU) phages displaying different huTH69-scFvs (κ : kappa light chain; λ : lambda light chain) or the mTH69-scFv and tested in ELISA experiments. HER2-specific phage (4D5-scFv) was used as negative control. Cell surface bound phages were detected using anti-M13-HRP antibody and absorbance at 405 nm was measured after incubation with ABTS substrate.

Figure 13: Characterization of mertansine (DM1), deruxtecan (Dx) or monomethyl auristatin E (MMAE) conjugated to huTH69-DE. (A) Absorbance spectrums of the generated ADCs were measured in the range between 230 nm and 440 nm in comparison to an equimolar amount of unconjugated huTH69-DE. Characteristic absorbance peaks of the used drugs are indicated by arrows (DM1: 255nm, Dx: 365nm, MMAE: 243 nm). (B) Concentration dependent binding of huTH69-DE ADCs was tested on the CD7-positive cell line CEM using flow cytometry.

Figure 14: The Fc-engineered huTH69-DE mediates direct anti-proliferative effects against target antigen expressing cell line after conjugation to mertansine (DM1), deruxtecan (Dx) or

monomethyl auristatin E (MMAE). (A-C) The CD7-positive cell line CEM and CD7-knockout cell line CEM-CD7KO were treated with increasing concentrations of huTH69-DE-DM1 (A), huTH69-DE-Dx (B) and huTH69-DE-MMAE (C) for 96h and the cell viability was determined by MTT-assay. The antibody huTH69-DE without conjugated drug was used as control. Mean values $\pm$ SEM of two independent assays.

Figure 15: The humanized antibody huTH69 mediates direct anti-proliferative effects against target antigen expressing cell line after non-covalent linking to truncated *Pseudomonas* exotoxin A (ETA') using α -kappa-ETA' fusion protein. The CD7-positive cell line CEM was treated with increasing concentration of huTH69 for 72h in the presence (black) or absence (grey) of 1 μ g/ml α -kappa-ETA' fusion protein. At a concentration of 1 μ g/ml, α -kappa-ETA' fusion protein alone generated a neglectable reduction of cell viability (dashed line). Mean values $\pm$ SEM of three independent assays are presented.

Figure 16: Comparison of the humanness of huTH69, the parental antibody mTH69 and various published CD7 antibodies by calculation of the humanness score. (A-B) The OASis identity (A) and OASis percentile (B) were calculated for huTH69 (black), the parental antibody mTH69 (white) and a panel of published CD7 antibodies (grey) to compare the level of humanness of variable regions (VH and VL).

Figure 17: Comparison of the liability to post-translational modifications / degradation for huTH69 and various published CD7 antibodies by sequence-based analysis of liability motifs. The liability to post-translational modifications / degradation was analyzed for huTH69 and a panel of published CD7 antibodies. Methionine (M), aspartate isomerization motifs (D(G/S/D/N/R/Y)) and asparagine deamidation motifs (N(G/H/S/N/T/Q/F/W/Y)) in the CDR of the VH and VL were rated as highly critical for modifications / degradation of the antibody and counted for comparison.

Figure 18: Comparison of the effector cell-mediated lysis of leukemia cells by huTH69 and various published CD7 antibodies using chromium release assay. The capacity of huTH69 in comparison to other published CD7 antibodies (antibody backbone: human IgG1) to trigger NK cell-mediated lysis of leukemia cells was analyzed in chromium release assays using PBMC as effector cells. (A) Dose-dependent lysis of CEM cells was evaluated in 4h chromium release assays at an E:T ratio of 40:1. Data are presented as mean values $\pm$ SEM from three experiments using different effector cell donors (B) Differences in the capacity to trigger NK cell-mediated lysis was observed at subsaturating concentrations as exemplified at an antibody concentration of 0.016 μ g/ml. (C) From the dose-response curves, EC50 values were calculated and the fold-change of alternative CD7 antibodies was calculated compared to huTH69 which was set to the factor 1.

Figure 19: Comparison of the complement dependent cytotoxicity (CDC) of huTH69, chimTH69 and various published CD7 antibodies using chromium release assay. (A-B) CDC of CD7-positive tumor cell lines CEM (A) and HSB-2 (B) was measured in standard chromium release assays for 4h

with increasing concentrations of huTH69 and chimTH69 (IgG1 wildtype backbone) and serum/plasma of different healthy donors at a concentration of 25%. Mean values $\pm$ SEM of n=3 independent experiments for CEM and n=2 independent experiments for HSB-2, * p $\leq$ 0.05 for huTH69 vs. chimTH69, Two-way ANOVA with Bonferroni's multiple comparisons test. **(C-D)** CDC of CD7-positive tumor cell lines CEM **(C)** and HSB-2 **(D)** was measured in standard chromium release assays for 4h with increasing concentrations of huTH69 and respective CD7 antibodies (IgG1 wildtype backbone) and serum/plasma of different healthy donors at a concentration of 25%. Mean values $\pm$ SEM of n=3 independent experiments for CEM and n=2 independent experiments for HSB-2, * p $\leq$ 0.05 for huTH69 vs. all of the antibodies of the CD7-antibody panel, Two-way ANOVA with Dunnett's multiple comparisons test.

Figure 20: Location of the huTH69 epitope on the CD7 extracellular region by single and grouped amino acid exchange screening. (A) The extracellular part of CD7 can be divided in a disordered region and a well-structured Ig-like domain. **(B)** A panel of CHO-S cells was generated expressing the human CD7 extracellular region with selected single and grouped amino acid exchanges arranged across the Ig-like domain. **(C)** Binding of the humanized antibody huTH69, the chimeric antibody chimTH69 and a panel of published CD7 antibodies to the wildtype CD7 molecule and mutated variants was measured by flow cytometry. MFI: mean fluorescence intensity

The examples illustrate the claimed invention.

Example 1 - The humanized huTH69 antibody retains binding properties of the parental mTH69 antibody

To generate a humanized CD7 antibody based on mTH69 a combined strategy of CDR grafting and guided selection was applied (Fig. 1a). The VH of mTH69 was humanized by CDR-grafting. Human V and J genes IGHV3-23 and IGHJ6 were identified as similar to mouse genes of mTH69 and used as acceptor framework for CDRs (according to IMGT definition). Furthermore, four backmutations were inserted between CDR2 and CDR3. Thereby, the percentage of identity to the closest human V gene increased from 82.7 % to 92.9 % (**Table 1, Figure 1b**) (Ehrenmann, F. and M.P. Lefranc, Cold Spring Harb Protoc, 2011. 2011(6): p. 737-49.).

Table 1. Humanization scores.

	VH humanization		VL humanization	
	before	after	before	after
V gene % identity	82.7 % (chimeric)	92.9 % (humanized)	76.8 % (chimeric)	92.6 % (humanized)
T20 score	79.40 (not human-like)	88.59 (human-like)	76.96 (not human-like)	91.12 (human-like)
OASis identity	55 % (chimeric)	75 % (humanized)	49 % (chimeric)	100 % (human)

V gene % identity (Ehrenmann, F. and M.P. Lefranc, Cold Spring Harb Protoc, 2011. 2011(6): p. 737-49.; Jones, T.D., et al., MAb, 2016. 8(1): p. 1-9.); T20 score (Gao, S.H., et al., BMC Biotechnol, 2013. 13: p. 55.); OASis identity (Prihoda, D., et al., MAb, 2022. 14(1): p. 2020203.)

- 5 According to the definition of the World Health Organization from 2014 and the American Medical Association, the generated VH can be rated as humanized (≥ 85 % identity) (Jones, T.D., et al., MAb, 2016. 8(1): p. 1-9.)]. Furthermore, two different scoring systems were used to analyze the humanness: the T20 score that is based on multiple-alignments with human antibody repertoire sequences increased from 79.40 to 88.58 and therefore can be rated as human-like (> 80) (Gao, S.H., et al., BMC Biotechnol, 10 2013. 13: p. 55.). The OASis identity that is based on comparison of 9-mer peptides with human antibody peptide databases (medium threshold) increased from 55 % and 75 % identity which is in line with other approved humanized antibodies (**Table 1**) (Prihoda, D., et al., MAb, 2022. 14(1): p. 2020203.). For both scoring systems, an increased humanness was reported to correlate with a decreasing clinical immunogenicity and, therefore, can be assumed for our humanized VH (Gao, S.H., et al., BMC 15 Biotechnol, 2013. 13: p. 55.; Prihoda, D., et al., MAb, 2022. 14(1): p. 2020203.).

For humanization of the light chain (LC) an alternative approach was followed by replacing the VL of mTH69 by a fully-human VL using guided-selection (Fig. 1a). To achieve this, a human VL library was prepared from total RNA isolated from B cells of 8 healthy donors (**Figure 1a**). The integrity of RNA was 20 verified. From pooled total RNA, cDNA was synthesized and VL of the κ LCs were amplified using degenerated primer mixes. In parallel, the VH was amplified using the humanized heavy chain (HC) of TH69 as template. To generate scFvs, VH and VL were assembled by PCR and the produced fragment of approximately 800 bp was inserted in the pJB12 phagemid and transformed in XL1 Blue *E. coli* leading to 1.4×10^8 colony-forming units (CFU) with an insertion rate of 100 % estimated by colony-screening 25 PCR. Panning was performed using the CD7-positive T-ALL cell line CEM. After the 1st panning and 2nd panning 24,990 and 1×10^7 phages were eluted from the cells, respectively, leading to an enrichment by the factor 36 (**Table 2**).

Table 2. Enrichment of phages by panning.

CFU = colony-forming units, ratio = output phages / input phages, enrichment = 2nd ratio / 1st ratio

	input phages (CFU)	output phages (CFU)	ratio	enrichment
1 st panning	1.6×10^{11}	24,990	1.6×10^{-7}	-
2 nd panning	1.8×10^{12}	10×10^6	5.7×10^{-6}	36

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After the 2nd panning, phagemids from 18 bacterial colonies were prepared and analyzed by Sanger sequencing. 17 candidates had the identical scFv nucleotide sequence that was termed huTH69-scFv and showed specific binding to CEM cells in an initial whole-cell phage ELISA, whereas the remaining candidate showed no binding (data not shown). The V and J gene of the isolated human VL of huTH69-scFv was assigned to IGKV1-39 and IGKJ4 (Fig. 1b). The calculated percentage of identity between VL 35 and IGKV1-39 was 92.9 %. Therefore, the full antibody huTH69 can be rated as humanized (**Table 1**).

Furthermore, the VL can be rated as human-like or human according to the calculated T20 score (91.12) and the OASis identity (100 %) (**Table 1**).

- To exclude a potential strong epitope drift, binding properties of phages displaying huTH69-scFv or mTH69-scFv were compared in whole-cell ELISA. Binding of both huTH69-scFv and mTH69-scFv could be blocked significantly by pre-incubation of CEM cells with the parental mTH69 antibody and no binding to CEM cells with CD7 knock-out (CD7-KO-CEM) was shown, demonstrating the same specificity of huTH69-scFv for CD7 as mTH69 (**Figure 2**).
- For further biochemical and functional characterization of the novel humanized V regions, huTH69-scFv and mTH69-scFv were converted into human IgG1 antibodies with κ LCs (**Figure 3a**). For improved Fc-mediated effector functions the amino acid exchanges S239D and I332E (DE-modification) were introduced in the CH2 domain of the Fc part to enhance Fc γ R binding Lazar, G.A., et al., Proc Natl Acad Sci U S A, 2006. 103(11): p. 4005-10.). The humanized (huTH69-DE) and chimeric (chimTH69-DE) Fc-engineered antibody variants were produced in Chinese hamster ovary cells and purified by affinity chromatography and size exclusion chromatography (SEC). Purity and molecular masses were confirmed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie staining or immunoblotting (**Figure 3c**). The huTH69-DE antibody showed the calculated molecular mass of approx. 150 kDa under non-reducing conditions and the expected protein bands of the HC and LC at molecular masses of approx. 50 kDa and 25 kDa using reducing conditions, similar to the chimeric antibody variant chimTH69-DE (**Figure 3c**). Analysis by SEC validated the purity of the antibody through a single protein peak (**Figure 3b**). No higher molecular weight peaks were detected indicating that humanization did not result in a tendency to aggregate. To determine the affinity of the chimeric and humanized TH69, Fab fragments were prepared from IgG and binding to a CD7-Fc fusion protein was analyzed by surface plasmon resonance (SPR) spectroscopy. Equilibrium dissociation constants (K_D) of 1.79 nM and 0.265 nM were measured for humanized and chimeric TH69, respectively, showing a moderate reduction of affinity after humanization. In the bivalent IgG format, huTH69-DE and chimTH69-DE showed an almost identical concentration dependent binding activity on CD7-positive CEM cells in flow cytometry and similar EC_{50} -values in the nanomolar range (huTH69-DE: 10.11 nM and chimTH69-DE: 13.67 nM) (**Figure 3d**). These data indicate that the loss in affinity could probably be compensated by avidity effects in the IgG format.

Example 2 - The Fc-engineered huTH69 shows high thermal stability and favorable characteristics for developability

- Thermal stability of the novel antibody was tested by thermal shift assay. The Fc-engineered chimeric or humanized TH69 antibodies showed similar melting curves and melting temperatures of 50 °C and 50.3 °C, respectively (**Figure 4**). Due to the DE-modification in the Fc part lower melting temperatures were observed for the Fc-engineered antibody variants compared to unmodified human IgG1 antibodies as already described for the clinically approved CD19 antibody tafasitamab (MOR208, 47 °C melting temperature (Amersdorffer J., et al., U.S. Patent 20140227277. 2012.); 49.8 °C melting temperature of

tafasitamab-like antibody CD19-DE). In order to exclude that the DE-modification is masking a negative effect of the humanization on the thermal stability, thermal shift assays were repeated with Fc-wildtype antibodies (chimTH69-wt and huTH69-wt). Again, similar melting curves and melting temperatures were observed for the chimeric and humanized TH69 antibodies (chimTH69-wt: 70.6 °C; huTH69-wt: 72.3 °C) in line with melting temperatures of approved IgG1 antibodies (e.g., 69.9 °C for cetuximab).

Further extended biochemical / biophysical characterizations of the novel humanized antibody huTH69-DE were performed to get first insights whether post-translational modifications or degradation known to potentially negatively impact clinical development could be excluded. First, the amino acid sequence was successfully confirmed by peptide mapping with liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). Subsequently, common post-translational modification sites or degradations in the V regions of huTH69-DE were predicted by sequence analyses and further experimentally investigated by using LC-ESI-MS data. Sequence analyses revealed that no N-glycosylation sites or additional – and therefore unpaired - cysteines appear in the V region of the huTH69-DE HC and LC (**Table 3**).

Table 3. Predicted and detected post-translational modifications / degradations in VH and VL of huTH69-DE.

n.d. = not detected; X₁ = any amino acid excluding proline, X₂ = any amino acid; bold mutation = appears in CDR

	amino acid / motif / site	VH of huTH69-DE		VL of huTH69-DE	
		prediction	detection	prediction	detection
Asn glycosylation	N X ₁ (S/T/C)	-	-	-	-
Cys unpaired	C	-	-	-	-
Met oxidation	M	M53 M101	n.d. 2.4 %	M24	1.3 %
Asp isomerization	D(G/S/D/N/R/Y)	D80 D91 D108	n.d. n.d. n.d.	D37	n.d.
Asn deamidation	N(G/H/S/N/T/Q/F/W/Y)	N102	n.d.	N54 N112	n.d. n.d.
Lys glycation	K D K X ₂ (D/K/E)	K116	n.d.	-	-

Three methionine amino acids are present in the V regions and are prone for oxidation, two in the VH and one in the VL. Only a minor proportion of 1.3 % (relative amount) at position M24 in the VL and 2.4 % (relative amount) at position M101 in the VH underwent oxidation while M53 in the VH was not oxidized (**Table 3**). Furthermore, these low frequent oxidations are located outside of CDRs and probably do not compromise the activity of the humanized antibody. Asparagine deamidation, aspartate isomerization and lysin glycation are associated with liability motifs that can be used to predict a possible degradation / modification of the antibody sequence (Lu, X., et al., MAbs, 2019. 11(1): p. 45-57.; Jacobitz, A.W., et al., J Pharm Sci, 2020. 109(1): p. 293-300). Four asparagine deamidation, three aspartate isomerization and one lysin (in CDR) glycation motif were found in the V regions of huTH69-DE. However, none of these modifications / degradations could be detected in the LC-ESI-MS analysis

(Table 3). Further post-translational modifications or degradation were neither predicted nor observed in the V regions of huTH69-DE according to the LC-ESI-MS data.

Charge variants of a monoclonal antibody describe the sum of modifications and degradations that can occur to the molecule during production process. Analysis of the isoelectric point (pI) and relative charge variants by capillary isoelectric focusing (cIEF) revealed that huTH69-DE displayed a pI of 8.47 (acidic peaks: 52.51 %, main peaks: 38.04 % and basic peaks: 9.46 %). Published analyses of 23 approved mAbs (Goyon, A., et al., J Chromatogr B Analyt Technol Biomed Life Sci, 2017. 1065-1066: p. 119-128.) demonstrate pI-values between 6.1 to 9.4 (acidic peaks: 18-60 %, main peaks: 20-70 %, basic peaks: 1-40 %), confirming that our novel humanized antibody is already in the range of successfully developed monoclonal antibodies approved in the clinic. An additional reduction of charge variants can be realized by customized optimization of the production process for huTH69-DE. Together, these data show that no characteristics were found that may prevent further development towards clinical application.

Example 3 - The Fc-engineered huTH69-DE induces natural killer cell-mediated killing and macrophage-mediated phagocytosis of leukemia cells and shows similar internalization kinetics in CD7-positive cell lines

To investigate if the humanized and Fc-optimized TH69 antibody was able to trigger Fc-mediated effector functions similar effective as the chimeric TH69-DE antibody, ADCC and ADCP assays were performed with the T-ALL cell lines CEM, MOLT-16 and HSB-2. A comparable ADCC activity towards different CD7-positive T-ALL cell lines was observed. Tumor cell lysis occurred in a strict dose dependent manner by huTH69-DE and chimTH69-DE (**Figure 5a**). No significant differences in the tumor cell lysis of MOLT-16 and HSB-2 were detectable between the humanized and chimeric Fc-optimized antibody variants. Only for CEM cells designated concentrations of the antibodies showed minor but significant differences in the tumor cell lysis (black *). Nevertheless, the EC_{50} -values determined for the huTH69-DE antibody (CEM: 12.75 pM MOLT-16: 9.7 pM HSB-2: 2.62 pM) were comparable for all three T-ALL cell lines with the EC_{50} -values of the chimTH69-DE antibody (CEM: 8.83 pM MOLT-16: 6.93 pM HSB-2: 2.94 pM). The Fc-optimized antibody variants displayed a significantly more efficient tumor cell lysis of T-ALL cell lines in comparison to the chimeric wildtype antibody chimTH69-wt. (**Figure 5a**). The phagocytic activity of the TH69 antibody variants were compared using CEM, MOLT-16 and HSB-2 cells as target cell lines and monocyte-derived macrophages from healthy donors as effector cells (**Figure 5b**). HuTH69-DE antibody was able to trigger significant ADCP of different T-ALL cell lines comparable to the chimeric antibody variants chimTH69-DE and chimTH69-wt (**Figure 5b**). In conclusion, the humanized Fc-engineered antibody huTH69-DE was able to mediate ADCC and ADCP of different T-ALL cell lines to a similar extent as the chimeric Fc-optimized antibody chimTH69-DE, which suggests that the humanization of the antibody has no negative impact on the Fc-mediated effector functions of the antibody.

CD7 is described as a rapidly internalizing target antigen after antibody binding and therefore represents an appropriate target antigen for immunotoxins or antibody drug conjugates (ADC). To analyze the

internalization kinetics of chimTH69-DE and huTH69-DE in T-ALL cell lines, the antibodies were labelled with a pH-sensitive red-fluorescent dye and the uptake was measured by high-throughput fluorescence microscopy for 24h. The Fc-optimized antibody variants huTH69-DE and chimTH69-DE showed comparable internalization in CD7-positive cell lines CEM, HSB-2 and MOLT-16 (**Figure 6**). Hence, the humanized Fc-engineered antibody huTH69-DE was expected to achieve a similar potent efficacy as an ADC, as already seen for the chimeric antibody (Gehlert *et al.* unpublished). Therefore, the huTH69-DE antibody was conjugated to the cytotoxic compound monomethyl auristatin E (MMAE) via a cathepsin B cleavable linker (mc-vc-PABC), resulting in the ADC huTH69-DE-MMAE. The drug to antibody ratio (DAR) analyses revealed a DAR of 3.2 MMAE-molecules per antibody. The direct cytotoxic efficacy of huTH69-DE-MMAE against CD7-positive T-ALL cell line HSB-2 in comparison to chimTH69-DE-MMAE was characterized. HuTh69-DE-MMAE showed a significant concentration dependent reduction of the cell viability similar to the chimeric ADC with IC₅₀ values at low nanomolar concentrations (**Figure 7 A**; ~0.13 nM;). As an additional proof of antigen-specific activity of huTH69-DE-MMAE a blocking experiment was performed. HSB-2 cells were pretreated with an excess of the parental murine TH69 to mask the CD7 epitope prior to incubation with huTH69-DE-MMAE. As expected, no reduction in the cell viability was detected after blocking CD7 which proofs a target antigen specific cytotoxic effect of huTH69-DE-MMAE (**Figure 7 B**). Interestingly, for the cell lines CEM and Karpas-45 huTh69-DE-MMAE showed a significantly stronger reduction of cell viability than the ADC based on the non-humanized chimeric antibody (chimTH69-DE-MMAE). The measured IC₅₀ value for chimeric and humanized ADC was reduced by a factor of 6.45 for Karpas-45 (IC₅₀ huTH69-DE-MMAE: 1.79 nM vs. IC₅₀ chimTH69-DE-MMAE: 11.55 nM) and factor 2.21 for CEM (IC₅₀ huTH69-DE-MMAE: 0.71 nM vs. IC₅₀ chimTH69-DE-MMAE: 1.57 nM). Furthermore, the maximal inhibition increased by 19% and 11% for CEM and Karpas-45, respectively, after humanization of TH69 (**Figure 8 A-B**). To further demonstrate the specificity of huTH69-DE-MMAE, the CD7-knockout cell line CEM-CD7KO was incubated with increasing concentrations of huTH69-DE-MMAE and chimTH69-DE-MMAE. As expected, no significant reduction in the cell viability was detected for the CD7-negative cells proofing a target antigen specific cytotoxic effect of the used ADCs (**Figure 8 C**). For all used cell lines, the unconjugated antibodies huTH69-DE and chimTH69-DE did not show any growth inhibitory effects (**Figure 7 and Figure 8**).

The cell line HSB-2 has the highest number of CD7 molecules on the cell surface and is highly sensitive to the MMAE ADCs. CEM and Karpas-45 show a medium to low expression level. However, the humanization increased the efficiency of the derived CD7-targeting ADC. These data are largely unexpected since the humanized antibody variant displays a reduced binding affinity. Therefore, it might be speculated, that during the humanization process by altering the affinity / avidity and potentially by slightly changing the fine specificity / epitope the mechanism of action has been unexpectedly improved. These parameters e.g. could have changed the mode of action by influencing the intracellular processing of the ADC after internalization (e.g., intracellular routing) or the fate of the bound CD7 molecule after internalization (e.g., recycling of CD7 back to the cell surface).

Taken together, the Fc-engineered huTH69-DE induces ADCC, ADCP and similar internalization kinetics in CD7-positive cell lines. Unexpectedly, huTH69 turned out to be an exceptionally superior antibody variant compared to the chimTH69 antibody when used in ADCs, especially for targeting cells

that express lower levels of CD7 and which have been previously rated as less sensitive to the CD7-targeting ADC based on the chimeric antibody.

Example 4 – Further details on the election of the humanized antibody huTH69

As outlined in the above examples, the huTH69 of mTH69 was generated by combining CDR-grafting of the VH in a human framework and guided selection of a fully-human VL by phage display from a human κ light-chain library. The described unique candidate huTH69 is the result of a selection process in terms of the chosen humanization strategy and the used screening process.

Humanization strategies applied for TH69

Today, probably the most widely used method for humanization of antibodies is grafting of CDRs of both the variable domain of heavy chain (VH) and the variable domain of light chain (VL) from the non-human antibody in human framework sequences that serve as acceptors. This approach had been performed for the antibody TH69. The CDRs of the VH were grafted on the human genes IGHV3-23 and IGHJ6. In previous unsuccessful attempts the CDRs of the VL were grafted on a human framework too (human genes IGKV1-33 and IGKJ2) (**Figure 9 A**). The resulting humanized antibody “CDR-huTH69” was produced (both with wildtype Fc and DE-modification) in Chinese hamster ovary cells and purified by affinity chromatography and size exclusion chromatography (SEC). Purity and molecular masses were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie staining (**Figure 10 C**). Analysis by SEC showed the purity of the antibody through a single protein peak (**Figure 10 B**). However, compared to chimTH69 antibody the CDR-huTH69 antibody showed a lower molecular weight peak in the SEC analysis and SDS-PAGE. The CDR-huTH69 antibody showed lower molecular mass of approx. 100 kDa compared to calculated molecular mass of approx. 150 kDa of IgG1-antibodies under non-reducing conditions (**Figure 10 C**). Under reducing conditions, the CDR-huTH69 antibody showed only one protein band at molecular mass of 50 kDa and not the expected protein bands of the HC and LC at molecular masses of approx. 50 kDa and 25 kDa as the control IgG1 antibody (**Figure 10 C**). These results indicated that either CDR-huTH69 was incorrectly assembled and purified without (or a very low content) of humanized light chain or the CDR-grafted light chain of CDR-huTH69 disintegrated after production.

After these observations, it was planned to validate the characteristic and functionality of our CDR-grafted heavy chain by producing a hybrid TH69 antibody that composed of the CDR-grafted heavy chain and the chimeric light chain with the variable region of the initial mouse antibody (hybTH69-huVH-chimVL; **Figure 10 A**). Analysis by SEC validated the purity of the antibody through a single protein peak similar to the protein peak of the chimeric TH69 antibody (chimTH69) (**Figure 10 B**). The hybTH69-huVH-chimVL antibody showed the calculated molecular mass of approx. 150 kDa under non-reducing conditions and the expected protein bands of the HC and LC at molecular masses of approx. 50 kDa and 25 kDa using reducing conditions, similar to control IgG1 antibody (**Figure 10 C**). These results revealed a correct production and purification of a fully IgG antibody containing our CDR-grafted heavy chain. Furthermore, a hybrid antibody that composed of the chimeric heavy chain with the variable

region of the initial mouse antibody and the CDR-grafted light chain (hybTH69-chimVH-CDRhuVL; **Figure 10 C**) showed only one protein band at molecular mass of 50 kDa under reducing conditions what was already observed for the fully-CDR-grafted antibody CDR-huTH69-wt or CDR-huTH69-DE (**Figure 10 C**) indicating a light chain driven problem.

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Binding analysis and functional characterization of the CDR-huTH69 antibody (**Figure 11 A-C**) in comparison to the chimeric TH69 antibody, display lower binding avidity against CD7-positive CEM cells (**Figure 11 B**) and significant lower ADCC efficacy against T-ALL cell line CEM (**Figure 11 C**). In comparison the hybTH69-huVH-chimVL antibody (**Figure 11 D**) showed no differences in concentration dependent binding on CEM cells (**Figure 11 E**) and in induction of tumor cells lysis via ADCC (**Figure 11 F**) in comparison to the chimeric antibody. These results confirmed the functionality of the humanized heavy chain.

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Generation of a fully-human κ light chain via guided selection

After problems by the generation of a humanized light chain of TH69 via CDR-grafting, an alternative approach for humanization of the light chain was followed by replacing the VL of mTH69 by a fully-human VL using guided-selection, as outlined in Example 1. A scFv-antibody library was generated from the CDR-grafted humanized VH of TH69 and the VL fragments (κ and λ light chains) were amplified from the RNA of B cells from eight healthy human donors (**Figure 9 B**). The CDR-grafted humanized VH of TH69, that showed no problems during production and characterization, was kept to prevent potential epitope drift and to receive CD7 specificity and functional characteristics of mTH69.

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Our final unique candidate huTH69 was isolated from the pool of candidates (1×10^8 κ light chains and 1×10^8 λ light chains) by phage display performing two rounds of screening (panning) on the CD7-positive cell line CEM. After the 2nd panning, phagemids from 18 bacterial colonies of κ and λ light chains were prepared and specific binding to CD7-positive CEM cells and CD7⁻ SKBR3 control cells was analysed in an initial whole cell phage ELISA (**Figure 12 A+B**). 17 candidates of κ light chains showed specific binding to CD7-positive CEM cells and no binding to CD7⁻ control cell line, whereas the remaining candidate (K7) showed no binding to CEM cells (**Figure 12 A**). In comparison only 9 candidates of λ light chains showed binding to CD7-positive CEM cells and no/lower binding to CD7⁻ SKBR3 cells, whereas the remaining 9 candidates showed no or unspecific binding (**Figure 12 B**). Analysis by Sanger sequencing revealed that the 17 CEM cell binding κ light chain candidates had the identical scFv nucleotide sequence, whereas λ light chain candidates had different scFv nucleotide sequences (data not shown). To exclude a potential strong epitope drift or CD7-independent binding, binding properties of selected phages displaying huTH69-scFv (κ and λ light chains with different nucleotide sequence) or mTH69-scFv were further compared by whole-cell ELISA (**Figure 12 C**). Binding of huTH69-K1-scFv (K1: κ light chain candidate sequence is used for generation of final huTH69 antibody) and mTH69-scFv could be blocked significantly by pre-incubation of CEM cells with the parental mTH69 antibody and no binding to CEM cells with CD7 knock-out (CD7KO-CEM) was observed, demonstrating the same specificity of huTH69-K1-scFv for CD7 as mTH69 (**Figure 12 C**).

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The different λ light chain candidates demonstrated no or CD7-independent binding to CEM and CD7KO-CEM cells (Figure 12 C).

Only one CD7 specific light chain was enriched by phage display out of 1×10^8 κ light chains and 1×10^8 λ light chains. As outlined in Example 1, the huTH69-IgG1 antibody, with humanized CDR-grafted heavy chain and the fully-human light chain, showed the same CD7 binding specificity as the chimeric TH69 antibody and was able to induce efficient Fc-mediated effector functions.

Analysis of humanization scores of the previous CDR-grafted light chain and the fully-human κ light chain (after guided-selection) further depicted benefits of this chosen humanization strategy. The calculated percentage of V gene identity for VL after guided-selection was 92.9 %, whereas the percentage of V gene identity after CDR-grafting was only 89.5 % (Table 4).

Table 4: Humanization scores.

	VL of TH69		
	mouse	after CDR-grafting	after guided-selection
V gene % identity	76.8 % (chimeric)	89.5 % (humanized)	92.6 % (humanized)
T20 score	76.96 (not human-like)	88.3 (human-like)	91.12 (human-like)
OASis identity	49 % (chimeric)	83 % (humanized/human)	100 % (human)

More information about humanization scoring can be found in Example 1.
V gene % identity (Ehrenmann, F. and M.P. Lefranc, Cold Spring Harb Protoc, 2011. 2011(6): p. 737-49.; Jones, T.D., et al., MAbs, 2016. 8(1): p. 1-9.); T20 score (Gao, S.H., et al., BMC Biotechnol, 2013. 13: p. 55.); OASis identity (Prihoda, D., et al., MAbs, 2022. 14(1): p. 2020203.)

As it can be expected for a fully-human light chain after guided-selection, the VL can be rated as human-like or human according to the calculated T20 score (91.12) and the OASis identity (100 %) (Table 4). In contrast, the calculated T20 score (88.3) and the OASis identity (83 %) of the CDR-grafted VL assign lower humanization scores (Table 4). An increased humanness was reported to correlate with a decreasing clinical immunogenicity and, therefore, can be assumed for our fully-human κ light chain after guided-selection.

Example 5 - The humanized antibody huTH69 specifically reduces cell viability of leukemia cells after conjugation to mertansine (DM1), deruxtecan (Dx) or monomethyl auristatin E (MMAE) or non-covalent linking to truncated *Pseudomonas* exotoxin A (ETA')

The identified CD7 antibody huTH69 is efficiently internalized after binding to the membrane protein CD7 on the cell surface. To test, whether huTH69 can be used as carrier antibody for a broader panel

of drugs, huTH69-DE was conjugated to the exatecan-derivative topoisomerase I inhibitor deruxtecan (Dx) and the tubulin inhibitors mertansine (DM1) and monomethyl auristatin E (MMAE). DM1 was conjugated via SMCC (succinimidyl trans-4-(maleimidylmethyl)cyclohexane-1-carboxylate) linker to surface amines whereas Dx and MMAE were linked via enzymatic-cleavable peptide linker to thiol by reduction of the antibody. After generation, the three ADCs showed the characteristic absorbance of the used drugs in the UV-Vis region indicating the successful conjugation (**Figure 13 A**). The binding of huTH69-DE ADCs to the CD7-positive cell line CEM persisted with EC50 values in the nanomolar range (**Figure 13 B**), indicating the intact overall structure of the ADCs. The three huTH69-DE-based ADCs were further tested in cell-viability assays using the CD7-positive cell line CEM and the CD7-knockout cell line CEM-CD7KO. As shown in **Figure 14**, the three ADCs reduced the cell viability of the leukemia cells in a concentration dependent manner with huTH69-DE-MMAE showing the strongest reduction at low concentrations compared to huTH69-DE-DM1 and huTH69-DE-Dx. None of the generated ADCs reduced the viability of the CD7-negative CEM-CD7KO cells verifying the antigen-restricted cytotoxic effect.

Beside small organic compounds, antibodies can also be linked to polypeptide toxins, like *Pseudomonas* exotoxin A or diphtheria toxin. After internalization of the antibody, the toxin will be released in the endosomal system of the cell and mediates its toxic effect. To test this type of payload, CD7-positive CEM cells were incubated with a fusion protein of an anti-human kappa light chain specific domain antibody (α -kappa) and a truncated *Pseudomonas* exotoxin A (ETA') (Kellner C, et al., J Immunol Methods. 2011 Aug 31;371(1-2):122-33). At the indicated concentration, the fusion protein itself is not reducing the cell viability. However, by addition of huTH69, a strong reduction of the cell viability can be observed in a concentration dependent manner (**Figure 15**). Due to the internalization of huTH69 after binding CD7 on the cell surface, the non-covalently bound ETA' can enter the endosomal system together with the antibody and mediate its toxic effect.

Taken together, huTH69 can be used in combination with different small organic compounds in ADCs or in combination with polypeptide toxins, demonstrating its broad applicability for the generation of antibody conjugates.

Example 6 - The chosen humanization strategy for huTH69 led to outstanding high

humanization scores compared to various published CD7 antibodies

The general aim of humanization is to reduce the risk that the patient's immune system develops an immune reaction against the non-human variable regions of an animal-derived therapeutic antibody. This immune reaction could lead to severe side-effects and/or could reduce the effective dose of the therapeutic antibody or antibody derivate (Baert F, et al. N Engl J Med. 2003 Feb 13;348(7):601-8). However, humanization remains a challenging task: the fraction of non-human regions of the antibody needs to be reduced while the binding properties need to be retained. To quantify the success of a humanization, humanness scoring has been established. One state-of-the-art method is the OASis scoring system (Prihoda D, et al. MAbs 2022 Jan-Dec;14(1):2020203). The output are two values: (a) OASis identity compares 9-mer peptides of the entered antibody sequence with human antibody database and replies its fraction of "human" peptides. (b) OASis percentile compares the entered

antibody sequence with therapeutic antibodies in the clinic. 100% percentile corresponds to the most human therapeutic antibody in the clinic. Taken together, both OASis identity and OASis percentile should be as small as possible to verify a high degree of humanness and a low risk of immunogenicity in humans. For comparison huTH69 was analyzed together with the humanized CD7 antibodies h189-1 and 189-4 of the parental antibody m189 (WO 2022/095803; leading candidate: h189-4), the CD7 antibodies G09 and F05 from a humanized mouse (WO 2020/212710; leading candidate G09), the humanized antibody huCD7scFv (WO 2022/257835) and the human single domain antibody #53 (VH only) (CN 115 991 776 A). Furthermore, the murine antibody RFT2 (Heinrich G, et al. J Immunol. 1989 Dec 1;143(11):3589-97) and the parental antibody mTH69 (Peipp M, et al. Cancer Res. 2002 May 15;62(10):2848-55; WO 2003/051926) were added to the panel.

For both scorings, huTH69 showed the highest score (**Figure 16**) compared to other humanized/human CD7 antibodies as described in WO 2020/095503. Interestingly, the antibody huCD7scFv described as humanized can be rated as non-humanized and surprisingly matches with the parental mTH69 antibody. Taken together, a high degree of humanization of huTH69 could be realized by the chosen combination of CDR-grafting and guided-selection by phage display.

Example 7 - The selected VL and designed VH of huTH69 show an outstanding low liability to post-translational modifications / degradation compared to various published CD7 antibodies

For development of therapeutic antibodies or antibody derivatives it is critical to choose a leading candidate with high resistance to post-translational modifications and degradation. Low stability of the antibody can lead to problems during both manufacturing and administration (Jarasch, A. et al., J Pharm Sci. 2015 Jun;104(6):1885-1898). Three common problems that can appear are methionine oxidation, aspartate isomerization and asparagine deamidation. Especially surface exposed residues in the CDRs are susceptible and modifications / degradation in this region can lead to aggregation or interfere with the binding properties of the therapeutic antibody. Aspartate isomerization and asparagine deamidation appear in amino acid sequence motifs that are a strong hint for liability to this modification / degradation (Vatsa, S. mAbs 2022, 14 (1); Lu, X. et al. mAbs 2018, 11 (1): 45-57). In **Figure 17**, the number of methionine, aspartate isomerization motifs and asparagine deamidation motifs in the CDRs of the VH and VL are shown for huTH69 and published CD7 antibodies (h189-1 and 189-4 from WO 2022/095803; G09 and F05 from WO 2020/212710; single domain antibody #53 (VH only) from CN 115991776; RFT2 from Heinrich G, et al. J Immunol. 1989 Dec 1;143(11):3589-97). The CD7 antibody huTH69 demonstrates a low liability with only one potential asparagine deamidation site. For comparison, e.g. the prior art CD7 antibody G09 (WO 2020/212710) could be affected by all three degradation / modification pathways. Taken together, huTH69 has a high developability potential, as further outlined in the above Example 2.

Example 8 - The humanized CD7 antibody huTH69 induces effector cell- and complement-mediated killing of leukemia cells with a higher efficiency compared to various published CD7 antibodies

- The ability of monoclonal antibodies to trigger Fc-mediated effector functions such as ADCC and CDC is affected by different parameters. Besides specific characteristics of the target antigen and antigen density on the target cell (Derer, S. et al., J Immunol. 2012 Dec 1;189(11):5230-9), the epitope bound by the antibody and the affinity / avidity (Tang, Y. et al. J Immunol. 2007 Sep 1;179(5):2815-23) of the antibody have been demonstrated to affect ADCC and CDC activity (Oostindie, S.C. et al. Nat Rev Drug Discov. 2022 Oct;21(10):715-735).
- The ability of the humanized huTH69 antibody to mediate ADCC via wildtype Fc was compared with selected published CD7-specific antibodies in classical chromium release assays using CEM cells as targets and PBMC as effector cells. All antibodies triggered target cell lysis in a dose-dependent fashion. Importantly, huTH69 triggered half-maximal lysis at lower antibody concentrations and demonstrated a higher capacity at subsaturating concentration (e.g. 0.016 µg/ml) compared to antibodies RFT2 (Heinrich G, et al. J Immunol. 1989 Dec 1;143(11):3589-97), G09 (WO 2020/212710), F05 (WO 2020/212710), h189-1 (WO 2022/095803) and h189-4 (WO 2022/095803) (**Figure 18**).
- The ability of the humanized huTH69 antibody to mediate CDC via wildtype Fc was compared with chimeric antibody chimTH69 and the panel of selected CD7-specific antibodies in classical chromium release assays using CEM and HSB-2 cells as targets and serum/plasma of healthy donors as source for complement. The antibody huTH69 unexpectedly mediated CDC against CEM and HSB-2 with a significantly higher efficiency than the chimeric antibody chimTH69 (**Figure 19 A-B**). Furthermore, huTH69 mediated CDC against the cell lines CEM and HSB-2 whereas previously described CD7-antibodies triggered a lower activation or even no activation of the complement against the CD7-positive cell line when used in a human IgG1 background with wildtype Fc (**Figure 19 C-D**).
- These data demonstrate that huTH69 has unique technically and biologically advantageous characteristics differentiating the antibody from other previously described human or humanized CD7 antibodies.

Example 9 - The humanized CD7 antibody huTH69 binds a unique epitope on the extracellular part of CD7 compared to various published CD7 antibodies

- The epitope on the corresponding antigen is an important property of a therapeutic antibody. On the one hand, the target cell expressing the antigen will be influenced depending on the epitope (e.g. internalization or induction of cell death after cross-linking). On the other hand, the epitope of an antibody influences the orientation and distance of the constant part of the antibody on the target cell and therefore impacts the effector functions mediated by the patient's immune system (e.g. ADCC or CDC) (Dechant, M. et al. Cancer Res. 2008 Jul 1;68(13):4998-5003). The epitope of an antibody can be determined by screening of antigens with mutations on the protein surface that may affect the binding properties of the antibody. The extracellular part of the human CD7 membrane protein consists of a disordered region and a N-terminal Ig-like domain (Aruffo, A. et al. EMBO J. 1987 Nov;6(11):3313-6; UniProt: P09564; **Figure 20 A**). The disordered region has various post-translational modifications and

is not strictly structured. In contrast, the Ig-like domain is well-structured and the likely binding region of CD7 antibodies.

Various amino acid exchanges were inserted in the CD7 Ig-like domain scattered across the protein surface. The mutated CD7 variants were expressed in CHO-S cells and could be detected on the cell surface by flow cytometry (**Figure 20 B**, gray scale). The binding of huTH69, chimTH69 and a panel of CD7 antibodies (described in Example 8) to the mutated CD7 variants was measured by flow cytometry. Interestingly, binding of huTH69 and chimTH69 was undetectable when the amino acids G53, R55, I117 and V120 were changed (**Figure 20 C**). These amino acids are involved in the formation of two N-terminal located and aligned loops (UniProt: AF-P09564-F1 (AlphaFold)). These observations were not made for other CD7 antibodies of the tested panel. Furthermore, differences in binding behavior were observed for chimTH69 compared to huTH69 regarding the impact of the amino acid D68 on the measured fluorescence signal. Therefore - in comparison to the selected prior art CD7 antibodies - huTH69 shows a unique epitope on the Ig-like domain of human CD7. The significant differences in binding compared to other CD7 antibodies and also the difference in binding compared to chimTH69 may also explain the unique and unexpected properties of huTH69 in terms of target cell killing.

Example 10 - Discussion / Conclusion

Herein the generation of a novel humanized version of the murine TH69 hybridoma antibody by a combination of CDR-grafting and guided selection is described. The huTH69 antibody showed favorable biophysical characteristics for further clinical development such as thermal stability. In addition, the antibody epitope, the extend to trigger selected effector functions and the level of humanization clearly differentiated huTH69 in an advantageous manner from a panel of previously published CD7 antibodies as well as from the parental murine / chimeric TH69 antibody. The unique humanized V regions are well suited as a starting point for the development of various antibody-based immunotherapeutic agents for the treatment of T-ALL and other CD7-positive malignancies, including but not limited to Fc-optimized antibodies, bispecific antibodies, multifunctional fusion proteins, immunotoxins, antibody-drug conjugates (ADCs) and CAR T / CAR NK cells.

Example 11 - Material and Methods

Cell separation

Peripheral blood mononuclear cells (PBMC) from healthy donors were isolated by density gradient centrifugation and B cells were purified from PBMC via magnetic-activated cell sorting (MACS) using the B Cell Isolation Kit II (130-091-151) from Miltenyi Biotec following the manufacturer's recommendations. Monocytes were generated through adherence of PBMC in monocyte attachment medium (PromoCell) for 30 minutes at 37 °C. Monocytes were differentiated into macrophages in serum-free X-vivo medium (Lonza) containing, 50 U/mL penicillin and 50 µg/mL streptomycin and 50 ng/mL recombinant macrophage colony stimulating factor (MCSF; PeproTech) for 11 to 14 days.

Culture of eukaryotic cells

CCRF-CEM, MOLT-16 and HSB-2 cells were purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ no.: ACC 240, ACC 29, ACC 435) and cultured in RPMI 1640 GlutaMAX medium containing 10 % FCS, 1 % penicillin and streptomycin (all Thermo Fisher Scientific; cat. no. 72400-021, 10270-106, 15140-122). CCRF-CEM CD7-knockout cells were generated via CRISPR/Cas9 technology. Briefly, 2.5×10^6 CEM cells were transfected with 1.5 μ M Alt-R® S.p. Cas9 Nuclease V3 (Integrated DNA Technologies) and 4.4 μ M guide RNA (gRNA according to a published sequence (Gomes-Silva, D., et al., Blood, 2017. 130(3): p. 285-296.) was synthesized by Synthego) via the MaxCyte (STX) Scalable Transfection System, following the manufacturer's recommendations. The CD7-negative cell fraction was isolated by cell-sorting on a FACSaria (BD) automated cell sorter.

Humanization of VH by CDR-grafting and humanness scoring

To identify human V and J genes as acceptor framework for CDR-grafting, the mouse VH sequence was analyzed by IMGT/DomainGapAlign setting the species to *Homo sapiens* (Ehrenmann, F. and M.P. Lefranc, Cold Spring Harb Protoc, 2011. 2011(6): p. 737-49.; Ehrenmann, et al., Nucleic Acids Res, 2010. 38(Database issue): p. D301-7.). Humanness was scored by three different methods: V gene % identity was calculated using IMGT/DomainGapAlign setting the species to *Homo sapiens* (Ehrenmann, F. and M.P. Lefranc, Cold Spring Harb Protoc, 2011. 2011(6): p. 737-49.). T20 scores were calculated according to Gao *et al.* with full-length sequences (Gao, S.H., et al., BMC Biotechnol, 2013. 13: p. 55). OASis identity was determined using BioPhi platform with medium threshold (50% prevalence) (Prihoda, D., et al., MAbs, 2022. 14(1): p. 2020203.).

Generation of scFv antibody library

Total RNA from 2 - 14 million B cells from 8 healthy donors was prepared using TRIzol (Thermo Fisher Scientific; cat. no. 15596026) and quality was controlled by agarose-formaldehyde gel electrophoresis with 2 μ g pooled total RNA. 20 μ g of pooled total RNA was used for cDNA synthesis using a oligo(dT)₁₅ primer and reverse transcriptase SuperScript IV (Thermo Fisher Scientific; cat. no. 18090010) according to manufacturer's instructions. VL regions of the κ LCs were amplified by PCR using degenerated primer mixes binding the V genes and CL region. For PCR reactions 2 μ l cDNA, 35.5 μ l RNase and DNase-free water, 10 μ l 5x reaction buffer, 1 μ l dNTP mix (each 10 mM; Thermo Fisher Scientific; cat. no. 18427013), 0.5 μ l forward primer mix (100 μ M), 0.5 μ l reverse primer mix (100 μ M) and 0.5 μ l Phusion Plus polymerase (Thermo Fisher Scientific; cat. no. F630S) were used. After initial denaturation at 98 °C for 30 sec, 30 cycles (10 sec at 98 °C, 10 sec 60 °C, 15 sec at 72 °C) and final elongation at 72 °C for 5 min were performed. The approximately 400 bp VL fragments were separated by agarose gel electrophoresis and purified by gel extraction using the QIAquick kit from Qiagen (cat. no. 28706) according to manufacturer's introductions. To add the linker sequence to huTH69 VH, 10 ng of plasmid (pSec-huTH69-HC) were used as template for PCR using primers binding the leader region and J gene. PCR and purification were performed as described above. VH and VL were assembled to scFv by nested-PCR. The forward primer binds the V region of the huTH69 VH whereas the reverse primer mix

binds the J genes of the LC. For PCR reactions 60 ng purified huTH69 VH PCR product and 60 ng purified VL PCR product were added to 10 µl 5x reaction puffer, 1 µl dNTP mix (each 10 mM; Thermo Fisher Scientific; cat. no. 18427013), 0.5 µl forward primer (100 µM), 0.5 µl reverse primer mix (100 µM) and 0.5 µl Phusion Plus polymerase (Thermo Fisher Scientific; cat. no. F630S) in a final volume of 50 µl. The approximately 800 bp scFv was separated by agarose gel electrophoresis and purified by gel extraction using the QIAquick kit according to manufacturer's introductions. The purified PCR product and pJB12 phagemid (Burmester, J. and A. Plückthun, Antibody Engineering, 2001, Springer Berlin Heidelberg p. 19-40.) were digested with 1 U *Sfi*I restriction enzyme (NEB; cat. no. R0123L), purified by agarose gel electrophoresis and assembled using T4 ligase from NEB (cat. no. M0202T) according to manufacturer's introductions. The reaction was treated with 10 volumes n-butanol (Carl Roth; cat. no. 7724.1) and centrifuged (25,000 g, 5 min). The precipitate was washed two times with 70 % ethanol and dissolved in distilled water. XL1 Blue *E. coli* (Agilent; cat. no. 200228) were transformed with de-salted ligation reaction using a MicroPulser electroporator (Bio-Rad; cat. no. 1652100) according to manufacturer's introductions and bacteria were plated by using standard procedures. Insertion rate was estimated by colony-screening PCR with insert-flanking primers on the phagemid (forward: CGTATGTTGTGTGGAATTGTGAGCGG; (SEQ ID NO: 19), reverse: CATAGCCCCCTTATTAGCGTTTGCC; SEQ ID NO: 20) and samples of separated single bacterial colonies as templates. Samples with approximately 1,000 bp fragments were rated as positive for insertion. Bacteria were suspended in 2YT medium containing 20% glycerol, snap frozen as aliquots and stored at -80°C.

Phage display using cellular panning

Five hundred ml medium (SB medium containing 20 g/l yeast extract, 30 g/l tryptone, 10 g/l MOPS (pH 7.0) supplemented with 30 µg/ml chloramphenicol, 10 µg/ml tetracycline and 1% glucose; all Carl Roth; cat. no. 8952.2, 2363.2, 6979.2, X997.2, 3886.3, 0237.3) were inoculated with *E. coli* containing the pJB12-scFv library and phages were prepared and titrated by determining CFU as described (Peipp, M., et al., J Immunol Methods, 2001. 251(1-2): p. 161-76.; Kay, B.K., et al., 1996: Elsevier Science.). 10^{12} phages were added to 2×10^6 CEM cells in 2 ml PBS supplemented with 4% BSA (Carl Roth; cat. no. 8076.3) and incubated on a roller incubator at 4 °C for 30 min. Cells were washed five times using ice-cold PBS supplemented with 2 % BSA and two times using ice-cold PBS. For elution, cells were incubated with trypsin (1 mg/ml in PBS; Sigma-Aldrich; cat. no. T1426) for 10 min at room temperature and centrifuged (18,000 x g, 10 min). The supernatant was added to 10 ml XL1 Blue *E. coli* (OD₆₀₀ = 0.5, 10 µg/ml tetracycline) and incubated for 30 min at 37 °C. Bacteria were plated on 2xYT agar plates containing 30 µg/ml chloramphenicol, 10 µg/ml tetracycline and 1 % glucose to prepare phages for second panning round.

Sanger sequencing

After the second panning round, 5 ml SB medium (supplemented with 30 µg/ml chloramphenicol, 10 µg/ml tetracycline and 1 % glucose) were inoculated with separate bacterial colonies. Phagemids were isolated from *E. coli* using the NucleoBond Mini Kit (Machery-Nagel; cat. no. 740588.250) according to

manufacturer's introduction. Sanger sequencing was performed at the Institute of Clinical Molecular Biology (IKMB) in Kiel.

Whole cell ELISA

- 5 One hundred ml SB medium (supplemented with 30 µg/ml chloramphenicol, 10 µg/ml tetracycline and 1 % glucose) were inoculated with a separated bacterial colony and phages were prepared and titrated as described above. 96-well-plates were blocked overnight and 1×10^6 cells per well were blocked for 30 min on ice with PBS supplemented with 4 % BSA. 1×10^{10} CFU phages were mixed with the cells in a total volume of 100 µl and incubated for 1 h at 4 °C. Plates were washed 3-times with cold PBS
- 10 (supplemented with 0.1% BSA) and subsequently incubated for 1 h at 4°C with anti-M13-HRP antibody (GE Healthcare; cat. no. 27-9421-01). After washing, 100 µl ABTS solution/well (Roche; cat. no. 11112422001) were added and absorbance was measured at 405 nm with Sunrise absorbance microplate reader after 15 min (Tecan; reference wavelength 492 nm).

Generation of chimeric and humanized IgG1

- 15 Murine and humanized VH were fused to the constant part of human IgG1 (with and without the S239D/I332E amino acid exchanges) and inserted into expression vectors. Mouse and human VL were fused to the constant part of human κ LC and inserted into expression vectors. Endotoxin-free plasmid DNA was purified by Nucleo Bond 2000 EF (Macherey-Nagel) and correct sequences were confirmed by Sanger sequencing (IKMB Kiel). For expression, Chinese hamster ovary cells (CHO-S) were
- 20 electroporated with corresponding HC and LC expression vectors via MaxCyte (STX) large scale electroporation system, according to the manufacturer's recommendations. Antibodies were purified from cell supernatant with Capture Select IgG-CH1-XL affinity matrix (ThermoFisher), followed by size exclusion chromatography (ÄKTA pure, GE Healthcare/Cytiva). The antibody variants based on G09 (WO 2020/212710), F05 (WO 2020/212710), h189-1 (WO 2022/095803), h189-4 (WO 2022/095803)
- 25 and RFT2 (Heinrich G, et al. J Immunol. 1989 Dec 1;143(11):3589-97) were generated in a human IgG1 background applying identical production procedures.

SDS-PAGE and Western blot analysis

- 2 µg of the purified antibodies were loaded on 12 % Tris–acrylamide gels under reducing or on 4-15 %
- 30 precast polyacrylamide gels (Mini-PROTEAN® TGX™, BioRad) under non-reducing conditions and were directly stained with Coomassie brilliant blue staining solution (Carl Roth GmbH) or blotted to PVDF membranes according to standard procedures. The membranes were blocked using 5 % BSA or skim milk powder in tris-buffered saline (TBS) for 1 h at RT. For detection of the HC anti-IgG-POX antibody (Sigma-Aldrich) in a final dilution of 1:5000 and for detection of the LC anti-human κ-LC antibody (Sigma-
- 35 Aldrich) in a final dilution of 1:10000 were incubated overnight at 4 °C. Blots were washed with TBST-buffer and as the secondary antibody the HRP-conjugated goat-anti-mouse-IgG (Invitrogen) was added to a final dilution of 1:5000 and incubated for 1 h at RT. Blots were finally analyzed using the

chemoluminescent substrate (Pierce, Thermo Fisher Scientific) and a ChemiDoc imaging system (Biorad).

Surface plasmon resonance spectroscopy

For expression of the CD7-Fc fusion protein, CHO-S were electroporated with a CD7-Fc expression vector via MaxCyte (STX) large scale electroporation system. The fusion protein was purified from cell supernatant with Capture Select IgG-Fc (multispecies) affinity matrix (ThermoFisher), followed by size exclusion chromatography (ÄKTA pure, GE Healthcare/Cytiva). Fab fragments were prepared from huTH69-DE and chimTH69-DE using Pierce Fab Preparation Kit (ThermoFisher), according to manufacturer's instructions. To analyze the binding kinetics of the antibody fragments with the CD7-Fc fusion protein, dilution series of the fragments were prepared in the surface plasmon resonance (SPR) running buffer (12 mM phosphate, 137 mM NaCl, 2.7 mM KCl, 0.5 mM EDTA, 0.005 % polysorbate 20, pH 7.4). CD7-Fc fusion protein was immobilized on a CMD50L hydrogel biosensor chip (Xantec) via amine coupling and the diluted samples were injected into the system (2SPR, Reichert) for 60 s. Dissociation of the antigen Fab fragment complex was allowed for 320 s and regeneration of the biosensor chip was achieved by injecting the regeneration buffer (10 mM glycine HCl, pH 1.5) for 60 s. All samples were subjected to double referencing (via blank samples and a reference channel without the immobilized antigen). The resulting SPR sensorgrams were analyzed using the TraceDrawer software (Reichert). To determine interaction kinetic constants k_a , k_d , and K_D , the data were evaluated using the 1:1 binding model.

Flow cytometric analyses

To analyze the concentration dependent binding of the antibodies 3×10^5 cells were washed in PBS containing 1 % BSA and 0.1 % sodium azide (PBA buffer) and were incubated on ice for 60 min with increasing concentrations of the indicated antibodies. After three times washing with 1 ml PBA buffer cells were stained with a secondary anti-human- κ -FITC antibody (SouthernBiotech) or anti-human-IgG-Fc-FITC antibody (Jackson ImmunoResearch) on ice for 30 min. Flow cytometry analysis was performed on a Navios flow cytometer (Beckman Coulter) and analyzed with Kaluza Analysis software (Beckman Coulter).

Thermal shift assay

Thermal shift assay was performed using SYPRO Orange (Thermo Fisher Scientific; cat. no. S6650). 1 μ l 500x SYPRO Orange was added to 20 μ l of 1 mg/ml antibody diluted with PBS and provided on a white 96-well thin-wall PCR plate. Plate was sealed and heated in a LightCycler 480 (Roche) from 20 °C to 99 °C with Ramp Rate of 0.06 °C/s. Fluorescence was recorded simultaneously using 483 nm as excitation and 568 nm as emission wavelengths.

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Sequence verification by LC-ESI-MS

huTH69-DE was rebuffed by ultrafiltration (MWCO 10 kDa) against 50 mM phosphate buffer, pH 5.8. Detergents were removed by detergent removal spin columns. The sample was reduced and denatured

with DTT in the presence of urea or GuHCl, alkylated with IAA and enzymatically digested at enzyme specific conditions with trypsin and chymotrypsin. The samples were acidified in approx. 0.5 % TFA and separated on a UPLC-system (Waters Acquity Premier H-class) using a reversed phase column (AcquityPremier CSH130 C18 Peptide, 2.1 x 100 mm, 1.7 μ m, Waters). Eluents were 0.1 % FA in water and 0.1 % FA in acetonitrile. The mass spectrometric analysis was performed with a Compact QTOF mass spectrometer (Bruker Daltonik). The recorded LC-ESI-MS and -MS/MS spectra were processed, annotated and searched against a customized sequence database using Mascot (Matrix Science).

Analysis of modifications and degradation in V regions

huTH69-DE was rebuffed by ultrafiltration (MWCO 10 kDa) against 50 mM phosphate buffer, pH 5.8 (low artifact workflow) or 50 mM NH_4HCO_3 , pH 8.0 (stress sample). Detergents were removed by detergent removal spin columns. The sample was reduced and denatured with DTT in the presence of urea, alkylated with IAA and enzymatically digested at enzyme specific conditions with trypsin. In order to identify and quantify further potentially deamidated or oxidized peptides, the sample was rebuffed to pH 8.0 and incubated for 30 min at 70 °C and analyzed in parallel. Incubation at high pH and temperature leads to increased deamidation/oxidation of the protein and allows better identification of potentially modified sites. The samples were acidified in approx. 0.5 % TFA and separated on a UPLC-system (Waters Acquity Premier H-class) using a reversed phase column (AcquityPremier CSH130 C18 Peptide, 2.1 x 100 mm, 1.7 μ m, Waters). Eluents were 0.1 % FA in water and 0.1 % FA in acetonitrile. The mass spectrometric analysis was performed with a Compact QTOF mass spectrometer (Bruker Daltonik). The recorded LC-ESI-MS and -MS/MS spectra were processed, annotated and searched against a customized sequence database using Mascot (Matrix Science). Modified peptides were identified by their exact mass and retention time and quantified by their mass spectrometric signal intensity.

Capillary isoelectric focusing

Samples were desalted against 1:50 diluted PBS pH 7.4 using Amicon Ultra-0.5 centrifugal filter devices (Millipore). Protein concentration was determined by UV measurement at 280 nm. Capillary isoelectric focusing (cIEF) was performed on a CESI 8000 PLUS system (Sciex) using an eCAP neutral capillary with length to detector 20 cm (ID: 50 μ m). Samples were detected using a UV detector set at 280 nm. The samples were mixed with 3.75 M urea cIEF gel, Pharmalyte 3-10, pI peptide markers (pI 10.0, pI 9.5, pI 7, pI 5.5 and pI 4.1, Sciex) and cathodic and anodic stabilizers. The cIEF separation consists of the two steps focusing and mobilization. Focusing of proteins was performed at 25.0 kV using phosphoric acid (anolyte) and sodium hydroxide solution (catholyte). Chemical mobilization using phosphoric acid (anolyte) and acetic acid solution (catholyte) was carried out at 30.0 kV. The recorded electropherograms were integrated and analyzed using the 32 KaratTM software (Beckmann Coulter / Sciex).

Chromium release assay

The capacity of the novel antibodies to trigger ADCC and CDC was measured by chromium release assay after 4 h incubation as previously described (Gehlert, C.L., et al., Front Immunol, 2022. 13: p. 957874.). Briefly, tumor cells were labelled with radioactive $^{51}\text{CrO}_4^{2-}$ and incubated with either healthy

donors' PBMC at an effector to target (E:T) cell ratio of 40:1 or plasma/serum at a concentration of 25% in the presence of the indicated antibodies. Refludan (Bayer HealthCare Pharmaceuticals) was added to plasma as anticoagulant at a concentration of 10 µg/ml. Percentage of lysis was calculated from counts per minute (cpm) as follows: % lysis = (experimental cpm x basal cpm)/(maximal cpm x basal cpm) x 100.

Phagocytosis assay

For ADCP analysis, target cells were labelled with a pH-sensitive red fluorescence dye pHrodo (Thermo Fisher Scientific) following the manufacturer's protocols. 10⁴ macrophages were seeded in a 96-well flat-bottom plate and allowed to adhere for 1h at RT. Labelled target cells were added to the macrophages, resulting in an E:T ratio of 1:1 and antibodies were used at a final concentration of 10 µg/mL. The assay was incubated under physiological conditions in the IncuCyte high-throughput fluorescence microscope system (Satorius) and fluorescence pictures of each well were created every 20 minutes for 4 h. Phagocytosis was defined as red object counts per image (displayed phagocytosed T-ALL cells) over the time (Baumann, N., et al., Cancer Sci, 2021. 112(8): p. 3029-3040.).

Internalization assay

For analysis of antibody internalization, 6 x 10⁴ antigen positive cells were seeded in a 96-well flat-bottom plate. Antibodies were labelled with a pH-sensitive red fluorescence dye (Human Fabfluor-pH Antibody Labeling Dye; Thermo Fisher Scientific) following the manufacturer's protocols. Labelled antibodies were added to the cells, resulting in a final concentration of 1 µg/ml. The assay was incubated under physiological conditions in the IncuCyte high-throughput fluorescence microscope system (Satorius) and fluorescence pictures of each well were created every 20 minutes for 24 h. Internalization was defined as total red object area (µm²) per image over the time.

Cell viability assay

Direct growth inhibitory effects were analyzed by 3-(4,5-Dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) assay (Cell Proliferation Kit I, Roche). 1 x 10⁴ cells per well were seeded in 100 µL medium in flat-bottom 96-well culture plates and treated for 96h with serial dilutions of the indicated antibodies. Following the manufacturer's recommendations MTT reagent and solubilization-solution was added and the cell viability was quantified as the percentage of growth inhibition compared to untreated control cells.

Preparation and characterization of mertansine, deruxtecan and monomethyl auristatin E antibody drug conjugates

Human IgG1 antibody was conjugated to mertansine (DM1) via SMCC (succinimidyl trans-4-(maleimidylmethyl)cyclohexane-1-carboxylate) linker and deruxtecan (Dx) via enzymatically cleavable peptide linker GGFG with a maleimide active group using PerKits™ antibody conjugation kits (CellMosaic). Monomethyl auristatin E (MMAE) was conjugated via an enzymatic-cleavable mc-vc-PABC linker making use of contract manufacturing (Cfm Oskar Tropitzsch GmbH). The generated ADCs

were analyzed spectroscopically (NanoDrop One, Thermo Fischer Scientific) and further analyzed by flow cytometry as outlined above.

Expression and purification of α -kappa-ETA' fusion protein

- 5 The fusion protein of anti-human kappa light chain specific domain antibody (α -kappa) and truncated *Pseudomonas* exotoxin A (ETA') was expressed and purified as previously published (Kellner C, et al., J Immunol Methods. 2011 Aug 31;371(1-2):122-33)

Generation of CD7-expressing CHO-S cells for binding analysis

- 10 Nucleotide sequences of human CD7 (NCBI Reference Sequence: NM_006137) with or without referred amino acid exchanges were inserted into expression vectors (pcDNA3.1(+)). Endotoxin-free plasmid DNA was purified and Chinese hamster ovary cells (CHO-S) were electroporated via MaxCyte (STX) large scale electroporation system, according to the manufacturer's recommendations. After 24 h, expression of CD7 with or without referred amino acid exchange mutations was verified by flow
15 cytometry. Binding of selected CD7 antibodies was measured by flow cytometric analysis as outlined above.

Data processing and statistical analyses

Data were analyzed with GraphPad Prism 9 (GraphPad Software Inc.). Data are shown as mean $\pm$ SEM.

- 20 Differences between groups were analyzed by two-tailed t-test or two-way ANOVA with Bonferroni post-test if not otherwise stated in the corresponding figure descriptions. Significance was accepted with $p < 0.05$. Curves were fitted using a nonlinear regression model with a sigmoidal dose response (variable slope).

CLAIMS

1. An antibody binding to CD7, wherein the antibody comprises
 - 5 the V_H region determined by the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence being at least 90%, preferably at least 95% identical thereto; and
 - the V_L region determined by the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence being at least 90%, preferably at least 95% identical thereto,
 - provided that
 - 10 the three CDRs of the V_H region are determined by the amino acid sequences of SEQ ID NOs 3 to 5, and
 - the three CDRs of the V_L region are determined by the amino acid sequences of SEQ ID NOs 6 to 8 (SEQ ID NO: 7 "AAS").
- 15 2. The antibody of claim 1, wherein the antibody comprises
 - a V_H region differing by no more than 5, preferably by no more than 3 amino acid substitutions from SEQ ID NO: 1 and most preferably V_H region comprising SEQ ID NO: 1, and/or
 - a V_L region differing by no more than 5, preferably by no more than 3 amino acid substitutions from SEQ ID NO: 2 and most preferably V_L region comprising SEQ ID NO: 2.
- 20 3. The antibody of claim 2, wherein the amino acid substitutions are conservative amino acid substitutions.
4. The antibody according to any one of claims 1 to 3, wherein the CD7 is determined by the amino
 - 25 acid sequence of SEQ ID NO: 17.
5. The antibody according to any one of claims 1 to 4, wherein the antibody comprises an Fc domain of SEQ ID NO: 18.
- 30 6. The antibody according to any one of claims 1 to 5, wherein said antibody is coupled to
 - (a) a labelling group,
 - (b) a toxin,
 - (c) a drug,
 - (d) a radionucleotide,
 - 35 (e) a cytokine,
 - (f) a chemokine,
 - (g) an enzyme,
 - (h) a component modulating serum half-life,
 - (i) an antibody, or
 - 40 (j) an antibody mimetic.

7. The antibody according to claim 6, wherein the antibody mimetic is selected from affibodies, adnectins, anticalins, DARPins, avimers, nanofitins, affilins, Kunitz domain peptides, Fynomers®, and trispecific binding molecules and probodies.
- 5 8. The antibody according to claim 6, wherein the drug is selected from erlotinib (TARCEVA; Genentech/OSI Pharm.), bortezomib (VELCADE; MilleniumPharm.), fulvestrant (FASLODEX; AstraZeneca), sutent (SU11248; Pfizer), letrozole (FEMARA; Novartis), imatinib mesylate (GLEEVEC; Novartis), PTK787/ZK 222584 (Novartis), oxaliplatin (Eloxatin; Sanofi), 5-fluorouracil (5-FU, leucovorin, rapamycin (Sirolimus, RAPAMUNE; Wyeth), lapatinib (TYKERB, GSK572016; GlaxoSmithKline), lonafarnib (SCH 66336), sorafenib (BAY43-9006; Bayer Labs.), gefitinib (IRESSA; AstraZeneca), AG1478, AG1571 (SU 5271; Sugen), alkylating agents such as thiotepea and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimine and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine; acetogenins (especially, bullatacin and bullatacinone); camptothecin (including the synthetic analogue topotecan); bryostatins; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamycin, especially calicheamycin gamma1 I and calicheamycin omega1 (see, e.g., Agnew, Chem Intl ed Engl., 33: 183-186 (1994)) and dynemicin, including dynemicin A; bisphosphonate such as clodronate; esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, antrmycin, azaserine, bleomycins, cactinomycin, carabycin, carinomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRLIMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, liposomal doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites such as 5-fluorouracil(5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thiguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens such as calusterone, dromostanolone propionate, epitioestanol, mepitiostane, and testolactone; anti-adrenals such as aminoglutethimide, mitotane, and trilostane; folic acid replenisher such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine;

- demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethane; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ('Ara-C'); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N. J.) ABRAXANETM cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumber, Ill.), and TAXOTERE® doxorubicin (Rhone-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin, carboplatin; vinblastine; platinum; etoposide, ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DFMO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, solvates and acids thereof.
9. The antibody according to claim 6, wherein the toxin is selected from auristatin (preferably monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF), domains II and III of Pseudomonas exotoxin A, diphtheria toxin, ricin A, pokeweed antiviral protein, human pancreatic RNase, geldanamycin, maytansinoids, calicheamicin, daunomycin, doxorubicin, methotrexate, vindesine, SG2285, dolastatin, dolastatin analogue's auristatin, cryptophycin, camptothecin, rhizoxin derivatives, CC-1065, duocarmycin, enediyne antibiotics, esperamicin, epothilone, an amatoxin (such alpha-amanitin), deruxtecan, exatecan analogue's, a pyrrolbenzodiazepin (PBD) dimer and toxoids.
10. A nucleic acid molecule or a set of two nucleic acid molecules, wherein the nucleic acid molecule encoding the antibody according to any one of claims 1 to 9, and the set of two nucleic acid molecules, wherein the first nucleic acid molecule encodes the V_H region of the antibody according to any one of claims 1 to 9 and the second nucleic acid molecule encodes the V_L region of the antibody according to any one of claims 1 to 9.
11. A vector comprising the nucleic acid molecule of claim 10 in an expressible form or a vector of a set of two vectors comprising the set of two nucleic acid molecules of claim 10 in an expressible form.
12. A host cell, comprising the nucleic acid molecule or set of two nucleic acid molecules of claim 10 or the vector of the set of two vectors of claim 11, wherein the host cell is preferably an anti-tumor leucocyte and wherein the anti-tumor leucocyte is preferably a chimeric antigen receptor T-cell (CAR T-cell), T-cell-receptor-engineered T-cells (TCR T-cell), chimeric antigen receptor NK-cells

(CAR NK-cell), NK cell receptor-engineered NK cell (NCR NK-cell), TCR/CAR hybrid T-cell, NCR/CAR hybrid NK-cell, tumor-infiltrating lymphocytes (TIL), or CAR macrophage.

13. A method for producing an antibody of any one of claims 1 to 12, comprising
- 5 (a) culturing the host of claim 12 under conditions that allow synthesis of said antibody; and
- (b) recovering said antibody from said culture.
14. A diagnostic composition or a pharmaceutical composition comprising the antibody of any one of
- 10 claims 1 to 9, the nucleic acid molecule or set of two nucleic acid molecules of claim 10, the vector or set of two vectors of claim 11 or the host cell of claim 12.
15. The antibody of any one of claims 1 to 9, the nucleic acid molecule or set of two nucleic acid
- 15 molecules of claim 10, the vector or set of two vectors of claim 11 or the host cell of claim 12 for use in a method of treating, inhibiting or diagnosing *in vivo* a tumor, wherein the tumor is preferably a T-cell neoplasia, lymphoma or leukemia, and is most preferably a T-cell lymphoma or acute lymphocytic leukemia.