Novel pre-targeting antibodies and uses thereof

FIELD OF THE INVENTION

The present invention relates to antibodies which bind to antigens on target cells and which target radionuclides to said cells, and to methods of using the same.

BACKGROUND

The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumour cells, while leaving healthy cells and tissues intact and undamaged.

In this regard, bispecific antibodies have been designed which bind with one "arm" to a surface antigen on target cells, and with the second "arm" to an effector moiety such as a drug. A large variety of bispecific formats have been developed, but the task of developing bispecific antibodies is by no means trivial.

In pre-targeted radioimmunotherapy (PRIT), use is made of an antibody construct which has affinity for the tumour-associated antigen on the one hand and for a radiolabelled compound on the other. In a first step, the antibody is administered and localises to tumour. Subsequently, the radiolabelled compound is administered. Because the radiolabelled compound is small, it can be delivered quickly to the tumour and non-bound compound is fast-clearing, which reduces radiation exposure outside of the tumour (Goldenberg et al Theranostics 2012, 2(5), 523-540). A similar procedure can also be used for imaging. Pre-targeting can make use of a bispecific antibody or systems using avidin-biotin, although the latter has the disadvantage that avidin/streptavidin is immunogenic.

Methods of pre-targeted radioimmunotherapy or imaging commonly make use of a clearing or blocking agent, which is administered between the step of administering the antibody and the step of administering the radiolabelled compound. The purpose is to clear antibody from the blood and/or to block the binding site of the circulating antibody for the radiolabelled compound (see for instance Karacay et al, Bioconj. Chem., 13(5), 1054-1070 (2002)). The use of a clearing or

blocking agent allows for sufficient levels of radioactivity to be administered for an efficient treatment while limiting adverse toxicity, but the timing and dosage must be chosen with care, and there is the possibility of the clearing agent introducing risks of adverse effects such as immune reactions. Thus, the use of a clearing phase is a complicating aspect in pre-targeting methods. Such methods are, for example, disclosed in WO2019/201959 and WO2019/202399.

More recent methods of pre-targeted radioimmunotherapy therefore aimed at avoiding the use of a clearing agent. WO2021/009047, WO2022/152656 and WO2022/008688 disclose such methods. However, there remains a need to provide sets of antibodies for use in such methods with improved properties allowing for large scale manufacturing while at the same time providing the desired anti-tumour effect and acceptable safety profiles.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1: Experimental design of protocol 1819

Fig. 2: Biodistribution of pretargeted ^{212}Pb-DOTAM in SCID mice carrying SC BxPC3 tumors 24 hours after injection of ^{212}Pb-DOTAM pretargeted by SPLIT antibodies (cycle 1–3). The radioactive content in organs and tissues is expressed as average % IA/g ± standard deviation (SD; n = 3 or 5).

Fig. 3: Average tumor volume for groups A–D in the SC BxPC3 model ± standard deviation (SD; n = 10). Dotted vertical lines indicate ^{212}Pb-DOTAM administration (20 µCi) for all PRIT-treated groups, according to the study design.

Fig. 4: Tumor growth curves for individual mice in groups A–D in the SC BxPC3 model (n=10). Dotted vertical lines indicate ^{212}Pb-DOTAM administration (20 µCi) for all PRIT-treated groups, according to the study design.

Fig. 5: Kaplan-Meier curves showing the survival in groups A–D in the SC BxPC3 model (n=10). Dotted vertical lines indicate ^{212}Pb-DOTAM (20 µCi) administration for all PRIT-treated groups, according to the study design.

Fig. 6: Average change in BW after the various treatments, expressed as percentage of initial BW ± SD. Dotted vertical lines indicate ^{212}Pb-DOTAM (20 µCi) administration for all PRIT-treated groups, according to the study design.

Fig. 7: Experimental design of protocol 1917

Fig. 8: Distribution of ^{212}Pb in tumor-bearing SCID mice 24 hours after injection of ^{212}Pb-DOTAM pretargeted by SPLIT antibodies (cycle 1–3). The radioactive content in organs and tissues is expressed as average % IA/g ± standard deviation (SD; n = 3).

Fig. 9: Average tumor volume for groups A and B in the SC BxPC3 model ± standard deviation (SD; n = 10). Dotted vertical lines indicate ^{212}Pb-DOTAM administration (20 µCi) for the PRIT-treated group, according to the study design.

Fig. 10: Tumor growth curves for individual mice in groups A and B in the SC BxPC3 model (n=10). Dotted vertical lines indicate ^{212}Pb-DOTAM administration (20 µCi) for the PRIT-treated group, according to the study design

Fig. 11: Kaplan-Meier curves showing the survival in groups A and B in the SC BxPC3 model (n=10). Dotted vertical lines indicate ^{212}Pb-DOTAM (20 µCi) administration for the PRIT-treated group, according to the study design.

Fig. 12: Average change in BW after the various treatments, expressed as percentage of initial BW ± SD. Dotted vertical lines indicate ^{212}Pb-DOTAM (20 µCi) administration for the PRIT-treated group, according to the study design.

Fig. 13A/B: Schematic drawings of the antibody formats and designations used herein. Designations for the full length antibodies ("Protein"), start with "P1A" (herein also "Protein ID"), whereas designations of the different antibody sub-sections, such as heavy – and light chains (HC, LC), start with "D1A". For example, the antibody of the present invention with the Protein ID "P1AI0446" consists of a first heavy chain called D1AC4023, a second heavy chain called D1AK6882 and a light chain called D1AD3421. The terms (e.g. "target", "effector") used in this figure have the meanings as defined in the specification. Especially the term "effector" encompasses ^{212}Pb chelated by DOTAM.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a set of antibodies comprising:

i) a first antibody that binds to an antigen expressed on the surface of a target cell, and which further comprises a VH domain of an antigen binding site for a radiolabeled compound, but which does not comprise a VL domain of an antigen binding site for the radiolabeled compound, or comprises a VL domain which is not effective to form, together with the VH domain, an antigen binding site for said radiolabeled compound; and

ii) a second antibody that binds to said antigen expressed on the surface of the target cell, and which further comprises a VL domain of an antigen binding site for the radiolabeled compound, but which does not comprise a VH domain of the antigen binding site for the radiolabeled compound, or comprises a VH domain which is not effective to form, together with the VL domain, an antigen binding site for said radiolabeled compound,

wherein said VH domain of the first antibody and said VL domain of the second antibody are together capable of forming a functional antigen binding site for the radiolabeled compound, and wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by a peptide linker selected from GGSGGGGSGGGGSGGGGSGG (SEQ ID NO: 35) or GGGGSGGGGSGGGGSGGSGG (SEQ ID NO: 36).

I. DEFINITIONS

Terms are used herein as generally used in the art, unless otherwise defined in the following.

The term “an antibody that binds to an antigen expressed on the surface of a target cell” refers to an antibody that is capable of binding said antigen with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting said antigen. In one aspect, the extent of binding of the antibody to an unrelated, non antigen protein is less than about 10% of the binding of the antibody to the antigen as measured, e.g., by surface plasmon resonance (SPR). In certain aspects, an antibody that binds to an antigen expressed on the surface

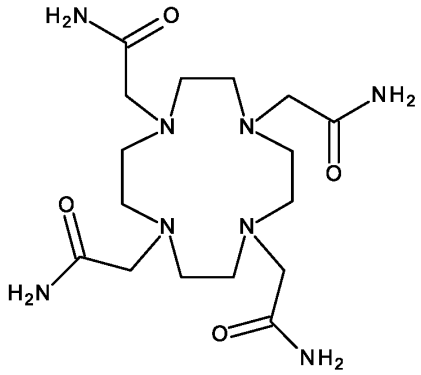
of a target cell has a dissociation constant (K_D) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^{-8} M or less, e.g., from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). An antibody is said to "specifically bind" to an antigen expressed on the surface of a target cell when the antibody has a K_D of 1μM or less. In certain aspects, the antibody binds to an epitope of said antigen that is conserved among said antigen from different species.

In some aspects the "antigen expressed on the surface of a target cell" (or in brief "target") is carcinoembryonic antigen-related cell adhesion molecule 5 ("CEACAM5", sometimes also just referred to as carcinoembryonic antigen or "CEA") and refers to any native CEACAM5 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed CEACAM5 as well as any form of CEACAM5 that results from processing in the cell. The term also encompasses naturally occurring variants of CEACAM5, e.g., splice variants or allelic variants. In particular aspects, CEACAM5 is membrane-bound CEACAM5. In further particular aspects, CEACAM5 is human CEACAM5. See for the human protein UniProt (www.uniprot.org) accession no. P06731 (version 195), or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP_004354.2. In preferred aspects, the antibody binds to human CEACAM5. In further preferred aspects, the antibody binds to membrane-bound CEACAM5.

The terms "an antigen binding site for a radiolabelled compound" or "a functional antigen binding site for a radiolabelled compound" refer to an antigen binding site comprising VH and a VL domain, capable of binding to the radiolabelled compound with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent to associate the radiolabelled compound with the antibody. In one aspect, the extent of binding of the antigen binding site to an unrelated, non antigen -compound is less than about 10% of the binding of the antibody to the radiolabelled compound as measured, e.g., by surface plasmon resonance (SPR). In certain aspects, an antigen binding site that binds to a radiolabelled compound has a dissociation constant (KD) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^{-8} M or less, e.g., from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). It may be preferred that it has a KD of 100pM, 50pM, 20pM, 10pM, 5pM, 1pM or less, e.g, 0.9pM or less, 0.8pM or less, 0.7pM or less, 0.6pM or less or 0.5pM or less. For instance, the functional binding site may bind

the radiolabelled compound with a Kd of about 1pM-1nM, e.g., about 1-10 pM, 1-100pM, 5-50 pM, 100-500 pM or 500pM-1 nM. An antigen binding site is said to "specifically bind" to a radiolabelled compound when the antigen binding site has a KD of 1μM or less.

The term "effector" or "effector agent" means a radiolabelled compound. The term "radiolabelled compound" means a chelated radionuclide (or radioisotope), preferably an emitter of alpha- or beta radiation such as ^{212}Pb, ^{212}Bi, ^{213}Bi, ^{90}Y, ^{177}Lu, ^{225}Ac, ^{211}At, ^{227}Th, ^{223}Ra. In one aspect the chelated radioisotope is chelated ^{212}Pb. In another aspect the chelated radioisotope means ^{212}Pb chelated by DOTAM. The term "DOTAM" means 1,4,7,10-Tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane, which is a compound of the following formula:



The term "antibody" encompasses various antibody structures exhibiting the desired antigen-binding activity, including but not limited to monoclonal antibodies, polyclonal antibodies, and antibody fragments. Sometimes, antibodies of the present invention are designated as "SPLIT constructs" or "SPLIT antibodies" or "CEA-Split-DOTAM VH/VL antibodies". The term SPLIT as used herein means SeParated v-domains LInkage Technology. In some aspects, the present antibodies are monoclonal antibodies (mAbs). In some aspects the present antibodies belong to the class of IgG antibodies, more particularly to the IgG_1, IgG_2, IgG_3 or IgG_4 subclasses (isotypes). In some aspects, the present antibodies are human or humanized monoclonal antibodies. In some aspects, the present antibodies are human or humanized monoclonal IgG_1 antibodies.

An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab’-SH, $F(ab')_2$, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv and scFab), single-domain antibodies, and multispecific antibodies formed from antibody fragments. For a review of certain antibody fragments, see Hollinger and Hudson, Nature Biotechnology 23:1126-1136 (2005). The terms “full-length antibody”, “intact antibody,” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure.

The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprised in the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, monoclonal antibodies may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

An “isolated” antibody is one which has been separated from a component of its natural environment. In some aspects, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC, affinity chromatography, size exclusion chromatography) methods. For review of methods for assessment

of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007). In some aspects, the antibodies provided by the present invention are isolated antibodies.

A "humanized" antibody refers to an antibody comprising amino acid residues from non-human CDRs and amino acid residues from human FRs. In certain aspects, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human antibody (the "parental" antibody), and all or substantially all of the FRs correspond to those of a human antibody. Such variable domains are referred to herein as "humanized variable region". A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. In some aspects, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived, i.e. the parental antibody), e.g., to restore or improve antibody specificity or affinity. The term "humanized" antibody also encompasses antibodies comprising certain mutations (e.g. amino acid substitutions) in the CDRs as compared to the CDRs of the parental antibody. In certain aspects, a humanized antibody comprises up to six (i.e. none, one, two, three, four, five or six) amino acid substitutions in one or more of its CDRs as compared to the parental antibody. In certain aspects, a humanized antibody comprises at least one CDR which is identical (i.e. does not comprise any amino acid substitutions as compared to the parental antibody) to the corresponding CDR of the parental antibody. In certain aspects, a humanized antibody comprises at least three CDRs (particularly at least one heavy chain CDR – more particularly at least HCDR3 – and at least one light chain CDR) which are identical to the corresponding CDRs of the parental antibody. A "humanized form" of an antibody, e.g. of a non-human antibody, refers to an antibody that has undergone humanization.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. In certain aspects, a human antibody is derived from a non-human transgenic mammal, for example a mouse, a rat, or a rabbit. In certain aspects, a human antibody is derived

from a hybridoma cell line. Antibodies or antibody fragments isolated from human antibody libraries are also considered human antibodies or human antibody fragments herein.

The term "antigen binding domain" refers to the part of an antibody that comprises the area which binds to and is complementary to part or all of an antigen. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). In preferred aspects, an antigen binding domain comprises an antibody light chain variable domain (VL) and an antibody heavy chain variable domain (VH).

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and complementarity determining regions (CDRs). See, e.g., Kindt et al., *Kuby Immunology*, 6th ed., W.H. Freeman & Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol. 150*:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

Glutamine or glutamate residues at the N-terminus of antibody heavy or light chains may be converted to pyro-glutamate spontaneously (see e.g. Liu et al., Journal of Pharmaceutical Sciences 97, 2426-2447 (2008), Rehder et al., Journal of Chromatography A 1102, 164-175 (2006), Chelius et al., Anal Chem 78, 2370-2376 (2006)). Hence, variable regions or variable domains disclosed herein which comprise either a glutamine (Q) or a glutamate (E) amino acid residue at the N-terminus of an the antibody heavy or light chain, may comprise an N- terminal pyro-glutamate (pyroE) residue instead of the N-terminal Q or E residue. Likewise, antibody heavy chains or light chains disclosed herein which comprise either a glutamine (Q) or a glutamate (E) amino acid residue at the N-terminus, may comprise an N terminal pyro-glutamate (pyroE) residue instead of the N-terminal Q or E residue. Accordingly, for each antibody heavy chain, light chain, or variable domain or region sequence disclosed herein that contains an N-terminal Q or E residue, the corresponding sequence with an N-terminal pyroE residue is also encompassed.

The term "complementarity determining region" or "CDR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence and which determine antigen binding specificity. Generally, antibodies comprise six CDRs: three in the VH (CDR-H1, CDR-H2, CDR-H3), and three in the VL (CDR-L1, CDR-L2, CDR-L3). CDRs are defined by a variety of methods/systems by those skilled in the art. These systems and/or definitions have been developed and refined over a number of years and include Kabat, Chothia, IMGT, AbM, and Contact.

"Framework" or "FR" refers to variable domain residues other than complementary determining regions (CDRs). The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the CDR and FR sequences generally appear in the following sequence in VH (or VL): FR1-CDR-H1(CDR-L1)-FR2- CDR-H2(CDR-L2)-FR3- CDR-H3(CDR-L3)-FR4.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one aspect, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain. Therefore an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-length heavy chain may include the full-length heavy chain, or it may include a cleaved variant of the full-length heavy chain. This may be the case in particular where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, Kabat EU numbering). Therefore, the C-terminal lysine (Lys447), or the C-terminal glycine (Gly446) and lysine (Lys447), of the Fc region may or may not be present.

Unless otherwise indicated, CDR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, Clustal W, Megalign (DNASTAR) software or the FASTA program package. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Alternatively, the percent identity values can be generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087 and is described in WO 2001/007611. Unless otherwise indicated, for purposes herein, % amino acid sequence identity values are generated using the ggsearch program of the FASTA package version 36.3.8c or later with a BLOSUM50 comparison matrix. The FASTA program package was authored by W. R. Pearson and D. J. Lipman ("Improved Tools for Biological Sequence Analysis", PNAS 85 (1988) 2444-2448), W. R. Pearson ("Effective protein sequence comparison" Meth. Enzymol. 266 (1996) 227- 258), and Pearson et. al. (Genomics 46 (1997) 24-36) and is publicly available from www.fasta.bioch.virginia.edu/fasta_www2/fasta_down.shtml or www.ebi.ac.uk/Tools/sss/fasta. Alternatively, a public server accessible at fasta.bioch.virginia.edu/fasta_www2/index.cgi can be used to compare the sequences, using the ggsearch (global protein:protein) program and default options (BLOSUM50; open: -10; ext: -2; Ktup = 2) to ensure a global, rather than local, alignment is performed. Percent amino acid identity is given in the output alignment header.

An "antigen binding site" refers to the site, i.e. one or more amino acid residues, of an antigen binding molecule which provides interaction with the antigen. For example, the antigen binding site of an antibody comprises amino acid residues from the complementarity determining regions (CDRs). A "functional antigen binding site" is a binding site that can exercise its desired function. A binding site can become non-functional (or not effective) e.g. by modifications in its

amino acid residues. A non-functional (or not effective) binding site can be used, in accordance with the present invention, to protect one moiety of a functional binding site (see e.g. Fig.13B)

The term "polynucleotide" or "nucleic acid molecule" includes any compound and/or substance that comprises a polymer of nucleotides. Each nucleotide is composed of a base, specifically a purine- or pyrimidine base (i.e. cytosine (C), guanine (G), adenine (A), thymine (T) or uracil (U)), a sugar (i.e. deoxyribose or ribose), and a phosphate group. Often, the nucleic acid molecule is described by the sequence of bases, whereby said bases represent the primary structure (linear structure) of a nucleic acid molecule. The sequence of bases is typically represented from 5' to 3'. Herein, the term nucleic acid molecule encompasses deoxyribonucleic acid (DNA) including e.g., complementary DNA (cDNA) and genomic DNA, ribonucleic acid (RNA), in particular messenger RNA (mRNA), synthetic forms of DNA or RNA, and mixed polymers comprising two or more of these molecules. The nucleic acid molecule may be linear or circular. In addition, the term nucleic acid molecule includes both, sense and antisense strands, as well as single stranded and double stranded forms. Moreover, the herein described nucleic acid molecule can contain naturally occurring or non-naturally occurring nucleotides. Examples of non-naturally occurring nucleotides include modified nucleotide bases with derivatized sugars or phosphate backbone linkages or chemically modified residues. Nucleic acid molecules also encompass DNA and RNA molecules which are suitable as a vector for direct expression of an antibody of the invention *in vitro* and/or *in vivo*, e.g., in a host or patient. Such DNA (e.g., cDNA) or RNA (e.g., mRNA) vectors, can be unmodified or modified. For example, mRNA can be chemically modified to enhance the stability of the RNA vector and/or expression of the encoded molecule so that mRNA can be injected into a subject to generate the antibody *in vivo* (see e.g., Stadler et al. (2017) Nature Medicine 23:815-817, or EP 2101823 B1).

An "isolated" nucleic acid molecule refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated polynucleotide (or nucleic acid) encoding an antibody" refers to one or more polynucleotide molecules encoding antibody heavy and light chains (or fragments thereof), including such polynucleotide molecule(s) in a single vector or separate vectors, and such polynucleotide molecule(s) present at one or more locations in a host cell.

The term "vector", as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors". The terms "host cell", "host cell line", and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells", which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate the antibodies of the present invention. Host cells include cultured cells, *e.g.* mammalian cultured cells, such as HEK cells, CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one aspect, the host cell of the invention is a eukaryotic cell, particularly a mammalian cell. In one aspect, the host cell is an isolated host cell. In one aspect, the host cell is not a cell within a human body.

The term "pharmaceutical composition" or "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the composition would be administered.

A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical composition or formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some aspects, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

An “individual” or “subject” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g. cows, sheep, cats, dogs, and horses), primates (e.g. humans and non-human primates such as monkeys), rabbits, and rodents (e.g. mice and rats). In certain aspects, the individual or subject is a human.

An “effective amount” of an agent, e.g., a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term “cancer” includes hematological – or solid tumors, preferably solid tumors. Solid tumors include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer.

The terms “Pb” or “lead” as used herein include ions thereof, e.g., Pb(II). References to other metals also include ions thereof. Thus, the skilled reader understands that, for example, the terms lead, Pb, ^{212}Pb or ^{203}Pb are intended to encompass ionic forms of the element, in particular, Pb(II).

The term "PRIT" as used herein means Pretargeted radioimmunotherapy.

II. COMPOSITIONS AND METHODS

The invention is based on a set of antibodies comprising a first and a second antibody, wherein each antibody can bind to an antigen on a target cell, but wherein a functional antigen binding site for an effector agent is formed only when the first and second antibodies are associated with each other. More particularly, the present invention provides antibodies that bind to CEA (CEACAM5) and, when combined, to a radiolabeled compound as defined herein, such as for example, an alpha emitter chelated by DOTAM. A schematic view as well as designations used herein for the antibodies, or antibody pairs, is shown in Figure 13A and 13B.

The present inventors found that modifications to the sequence of the CEA – and/or DOTAM binders improve properties of the present antibodies which lead to a better developability or manufacturability. In one aspect such modifications are in the acceptor frameworks of the CEA – and/or DOTAM binders and are reflected in the specific sequences disclosed herein. Improvements in developability or manufacturability of the present antibodies can generally be characterized by improving their yield and/or stability. In one aspect such improvements can be characterized by a higher aggregation temperature (T_{agg}), less percentage of high molecular weight ("HMW") products or an improved hydrophilicity of the present antibodies, or a combination of several or all of these parameters. In one aspect such modifications provide a more hydrophilic DOTAM binder and/or an improved CEA binder (T84.66v, or "new" T84.66). Therefore, the antibodies of the invention show improved developability and/or manufacturability, combined with other favorable properties for therapeutic application, e.g. with respect to immunogenicity, affinity, specificity, efficacy and safety/tolerability. More specifically, in one aspect, any improvements of the present antibodies is with respect to antibodies disclosed in the art, for example, in WO2019/201959 and WO2021/009047.

In one aspect, the invention provides a set of antibodies comprising:

i) a first antibody that binds to an antigen expressed on the surface of a target cell, and which further comprises a VH domain of an antigen binding site for a radiolabeled compound, but which does not comprise a VL domain of an antigen binding site for the radiolabeled compound, or comprises a VL domain which is not effective to form, together with the VH domain, an antigen binding site for said radiolabeled compound; and

ii) a second antibody that binds to said antigen expressed on the surface of the target cell, and which further comprises a VL domain of an antigen binding site for the radiolabeled compound, but which does not comprise a VH domain of the antigen binding site for the radiolabeled compound, or comprises a VH domain which is not effective to form, together with the VL domain, an antigen binding site for said radiolabeled compound,

wherein said VH domain of the first antibody and said VL domain of the second antibody are together capable of forming a functional antigen binding site for the radiolabeled compound, and wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by a peptide linker selected from GGSGGGGSGGGGSGGGGSGG (SEQ ID NO: 35) or GGGGSGGGGSGGGGSGGSGG (SEQ ID NO: 36).

In another aspect, the present invention provides the set of antibodies as defined herein, wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by the peptide linker GGSGGGGSGGGGSGGGGSGG (SEQ ID NO: 35)

In another aspect, the present invention provides the set of antibodies as defined herein, wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by the peptide linker GGGGSGGGGSGGGGSGGSGG (SEQ ID NO: 36).

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the radiolabeled compound comprises Pb-DOTAM (or ^{212}Pb-DOTAM).

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the functional binding site for Pb-DOTAM binds with a Kd value of the binding affinity of

100pM, 50pM, 20pM, 10pM, 5pM, 1pM or less, e.g, 0.9pM or less, 0.8pM or less, 0.7pM or less, 0.6pM or less or 0.5pM or less.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the functional binding site for Pb-DOTAM binds to Pb-DOTAM and to Bi-DOTAM.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises:

a) heavy chain CDR2 of the amino acid sequence FIGSRGDTYYASWAKG (SEQ ID NO: 42), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 42, wherein these substitutions do not include Phe50, Asp56 and/or Tyr58, and optionally also do not include Gly52 and/or Arg54; or wherein the variant is the amino acid sequence AIGSRGDTAYASWAKG (SEQ ID NO: 47).

b) heavy chain CDR3 of the amino acid sequence ERDPYGGGAYPPHL (SEQ ID NO:43), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 43, wherein these substitutions do not include Glu95, Arg96, Asp97, Pro98, and optionally also do not include Ala100C, Tyr100D, and/or Pro100E and/or optionally also do not include Tyr99; and

c) a heavy chain CDR1 of the amino acid sequence TYSMS (SEQ ID NO:41);

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises (a) VH CDR1 comprising the amino acid sequence of TYSMS (SEQ ID NO: 41); (b) VH CDR2 comprising the amino acid sequence of FIGSRGDTYYASWAKG (SEQ ID NO: 42) and (c) VH CDR3 comprising the amino acid sequence of ERDPYGGGAYPPHL (SEQ ID NO: 43).

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 44 and SEQ ID NO 48, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 44 or SEQ ID NO: 48.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the VH domain of the antigen binding site for the radiolabeled compound consists of an amino acid sequence selected from SEQ ID NO: 44 or SEQ ID NO 48.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein residues A or AST are added to the C-terminus of the VH domain of the antigen binding site for the radiolabeled compound.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises

a) light chain CDR1 comprising the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO: 37) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 37, wherein these substitutions do not include Tyr28 and Asp32; or the amino acid sequence QSSHSVASDNRLA (SEQ ID NO: 45)

b) light chain CDR3 comprising the amino acid sequence LGGYDDESDTYG (SEQ ID NO: 39) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 39, wherein these substitutions do not include Gly91, Tyr92, Asp93, Thr95c and Tyr96, and

c) light chain CDR 2 comprising the amino acid sequence QASKLAS (SEQ ID NO: 38) or a variant thereof having at least 1, 2 or 3 substitutions in SEQ ID NO: 5, optionally not including Gln50.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises (a) LC CDR1 of the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO: 37); (b) LC CDR2 of the amino acid sequence QASKLAS (SEQ ID NO: 38); and (c) LC CDR3 of the amino acid sequence LGGYDDESDTYG (SEQ ID NO: 39).

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises an amino acid sequence of SEQ ID NO: 40 or 46, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 40 or 46.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the VL domain of the antigen binding site for the radiolabeled compound consists of the amino acid sequence of SEQ ID NO: 40 or 46.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the first and second antibody bind to the same epitope of the antigen expressed on the surface of a target cell.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the first antibody binds to a different epitope of the antigen from the second antibody.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen expressed on the surface of a target cell is a tumor-associated antigen.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen expressed on the surface of a target cell is selected from the group consisting of carcinoembryonic antigen (CEA), CD20, HER2, EGP-1 (epithelial glycoprotein-1, also known as trophoblast-2), colon-specific antigen-p (CSAp), a pancreatic mucin MUC1, GPRC5D and FAP.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen expressed on the surface of a target cell is carcinoembryonic antigen (CEA).

In another aspect, the present invention provides the set of antibodies as defined herein, wherein one or both of the first and second antibody comprises an antigen-binding site which binds to CEA, comprising

a light chain variable region comprising (a) LC CDR1 comprising the amino acid sequence KASAAVGTYVA (SEQ ID NO:1); (b) LC CDR2 comprising the amino acid sequence SASYRKR (SEQ ID NO:2); and (c) LC CDR3 comprising the amino acid sequence HQYYTYPLFT (SEQ ID NO:3); and

a heavy chain variable region comprising (d) HC CDR1 comprising the amino acid sequence EFGMN (SEQ ID NO:6); (e) HC CDR2 comprising the amino acid sequence WINTKTGEATYVEEFKG (SEQ ID NO:7); and (f) HC CDR3 comprising the amino acid sequence WDFAYYVEAMDY (SEQ ID NO:8).

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen-binding site for CEA comprises a VH sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 9, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 9.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen-binding site for CEA comprises the VH sequence having the amino acid sequence of SEQ ID NO: 9.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen-binding site for CEA comprises a VL sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 4.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen-binding site for CEA comprises the VL sequence having the amino acid sequence of SEQ ID NO: 4.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein one or both of the first and second antibody comprises an antigen-binding site which binds to CEA, comprising

a light chain variable region comprising (a) LC CDR1 comprising the amino acid sequence RAGESVDIFGVGFLH (SEQ ID NO:11); (b) LC CDR2 comprising the amino acid sequence RGSNRAT (SEQ ID NO:12); and (c) LC CDR3 comprising the amino acid sequence QQTSEYPYT (SEQ ID NO:13); and

a heavy chain variable region comprising (d) HC CDR1 comprising the amino acid sequence DTYMH (SEQ ID NO:16); (e) HC CDR2 comprising the amino acid sequence RIDPANGNSKYADSVKG (SEQ ID NO:17); and (f) HC CDR3 comprising the amino acid sequence FGYYVSDYAMAY (SEQ ID NO:18).

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen-binding site for CEA comprises a VH sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19, or a variant thereof comprising an amino

acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 19.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen-binding site for CEA comprises the VH sequence having the amino acid sequence of SEQ ID NO: 19.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen-binding site for CEA comprises a VL sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 14, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 14.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein the antigen-binding site for CEA comprises the VL sequence having the amino acid sequence of SEQ ID NO: 14.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein

the first antibody ("P1AI0446") comprises or consists of a first heavy chain of SEQ ID NO: 21, a second heavy chain of SEQ ID NO: 23, and a light chain of SEQ ID NO: 5; and

the second antibody ("P1AI0084") comprises or consists of a first heavy chain of SEQ ID NO: 22, a second heavy chain of SEQ ID NO: 24, and a light chain of SEQ ID NO: 5.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein

the first antibody ("P1AJ6059") comprises or consists of a first heavy chain of SEQ ID NO: 25, a second heavy chain of SEQ ID NO: 26, and a light chain of SEQ ID NO: 15; and

the second antibody ("P1AJ6062") comprises or consists of a first heavy chain of SEQ ID NO: 27, a second heavy chain of SEQ ID NO: 28, and a light chain of SEQ ID NO: 15.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein

the first antibody ("P1AI0034") comprises or consists of a first heavy chain of SEQ ID NO: 29, a second heavy chain of SEQ ID NO: 23, and a light chain of SEQ ID NO: 5; and

the second antibody ("P1AI1622") comprises or consists of a first heavy chain of SEQ ID NO: 30, a second heavy chain of SEQ ID NO: 31, and a light chain of SEQ ID NO: 5.

In another aspect, the present invention provides the set of antibodies as defined herein, wherein

the first antibody ("P1AJ6063") comprises or consists of a first heavy chain of SEQ ID NO: 32, a second heavy chain of SEQ ID NO: 26, and a light chain of SEQ ID NO: 15; and

the second antibody ("P1AJ6064") comprises or consists of a first heavy chain of SEQ ID NO: 33, a second heavy chain of SEQ ID NO: 34, and a light chain of SEQ ID NO: 15.

In certain aspects, an antibody provided herein is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which the CDRs (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some aspects, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature

(somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

Polynucleotides and Recombinant Methods

The invention further provides a set of nucleic acids expressing the set of antibodies as defined herein.

In one aspect the invention provides an isolated polynucleotide encoding an antibody of the invention. Said isolated polynucleotide may be a single polynucleotide or a plurality of polynucleotides.

The polynucleotides encoding antibodies of the invention may be expressed as a single polynucleotide that encodes the entire antibody or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional antibody. For example, the light chain portion of an antibody may be encoded by a separate polynucleotide from the portion of the antibody comprising the heavy chain of the antibody. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the antibody. In another example, the portion of the antibody comprising one of the two Fc domain subunits and optionally (part of) one or more Fab molecules could be encoded by a separate polynucleotide from the portion of the antibody comprising the other of the two Fc domain subunits and optionally (part of) a Fab molecule. When co-expressed, the Fc domain subunits will associate to form the Fc domain.

In some aspects, the isolated polynucleotide encodes the entire antibody molecule according to the invention as described herein. In other aspects, the isolated polynucleotide encodes a polypeptide comprised in the antibody according to the invention as described herein.

In certain aspects, the polynucleotide or nucleic acid is DNA. In other aspects, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). RNA of the present invention may be single stranded or double stranded.

In yet another aspect, the present invention provides an expression vector or set of expression vectors comprising the set of nucleic acids as described above. In yet another aspect, the present invention provides a host cell or set of host cells comprising the expression vector or set of expression.

For recombinant production one or more polynucleotide encoding the antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In some aspects a vector, particularly an expression vector, comprising the polynucleotide (i.a. a single polynucleotide or a plurality of polynucleotides) of the invention is provided. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of an antibody along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. (1989); and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y (1989). The expression vector can be part of a plasmid, virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide encoding the antibody (i.e. the coding region) is cloned in operable association with a promoter and/or other transcription or translation control elements. As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the antibody of the invention, or variant or

derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and retroviruses (such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit β-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoters inducible by tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the antibody is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding an antibody of the invention or a fragment thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated polypeptide to produce a secreted or "mature" form of the polypeptide. In certain aspects, the native signal peptide, *e.g.* an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β-glucuronidase.

DNA encoding a short protein sequence that could be used to facilitate later purification (e.g. a histidine tag) or assist in labeling the antibody may be included within or at the ends of the antibody (fragment) encoding polynucleotide.

In a further aspect, a host cell comprising a polynucleotide (i.e. a single polynucleotide or a plurality of polynucleotides) of the invention is provided. In certain aspects a host cell comprising a vector of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combination, described herein in relation to polynucleotides and vectors, respectively. In some such aspects, a host cell comprises (e.g. has been transformed or transfected with) one or more vector comprising one or more polynucleotide that encodes (part of) an antibody of the invention. As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to generate the antibody of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of antibodies are well known in the art. Such cells may be transfected or transduced as appropriate with the particular expression vector and large quantities of vector containing cells can be grown for seeding large scale

fermenters to obtain sufficient quantities of the antibody for clinical applications. Suitable host cells include prokaryotic microorganisms, such as *E. coli*, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), insect cells, or the like. For example, polypeptides may be produced in bacteria in particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized", resulting in the production of a polypeptide with a partially or fully human glycosylation pattern. See Gerngross, Nat Biotech 22, 1409-1414 (2004), and Li et al., Nat Biotech 24, 210-215 (2006). Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts. See e.g. US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., J Gen Virol 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described, e.g., in Mather, Biol Reprod 23, 243-251 (1980)), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TRI cells (as described, e.g., in Mather et al., Annals N.Y. Acad Sci 383, 44-68 (1982)), MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including dhfr$^-$ CHO cells (Urlaub et al., Proc Natl Acad Sci USA 77, 4216 (1980)); and myeloma cell lines such as YO, NS0, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003). Host cells include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a

transgenic animal, transgenic plant or cultured plant or animal tissue. In some aspects, the host cell is a eukaryotic cell, particularly a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., Y0, NS0, Sp20 cell). In some aspects, the host cell is an isolated host cell. In some aspects, the host cell is not a cell within a human body.

Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an antigen binding domain such as an antibody, may be engineered so as to also express the other of the antibody chains such that the expressed product is an antibody that has both a heavy and a light chain.

In one aspect, a method of producing an antibody according to the invention is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the antibody, as provided herein, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

The components of the (multispecific) antibody of the invention may be genetically fused to each other. The (multispecific) antibody can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Examples of linker sequences between different components of (multispecific) antibodies are provided herein. Additional sequences may also be included to incorporate a cleavage site to separate the individual components of the fusion if desired, for example an endopeptidase recognition sequence.

Antibodies prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those having skill in the art. For affinity chromatography purification, an antibody, ligand, receptor or antigen can be used to which the antibody binds. For example, for affinity chromatography purification of antibodies of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate an antibody essentially

as described in the Examples. The purity of the antibody can be determined by any of a variety of well-known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like.

Compositions, Formulations, and Routes of Administration

In one aspect, the present invention provides a pharmaceutical composition comprising the set of antibodies as defined herein together with pharmaceutically acceptable excipients.

In another aspect, the present invention provides a pharmaceutical composition comprising a first or a second antibody as defined herein together with pharmaceutically acceptable excipients.

In another aspect, the present invention provides a pharmaceutical product for use in the combined, simultaneous or sequential, administration to a patient with cancer, such product comprising A) as a first component a pharmaceutical composition comprising the first antibody; and B) as a second component a pharmaceutical composition comprising the second antibody, whereby the antibodies are as defined herein.

In another aspect, the present invention provides a pharmaceutical product for use in the combined, simultaneous or sequential, administration to a patient with cancer, such product comprising A) as a first component a pharmaceutical composition comprising the first antibody as defined herein; and B) as a second component a pharmaceutical composition comprising the second antibody as defined herein.

Pharmaceutical compositions of the present invention comprise an effective amount of antibody dissolved or dispersed in a pharmaceutically acceptable carrier. The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e. do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains an antibody and optionally an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general

safety and purity standards as required by FDA Office of Biological Standards or corresponding authorities in other countries. Preferred compositions are lyophilized formulations or aqueous solutions. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.* antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, antioxidants, proteins, drugs, drug stabilizers, polymers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the pharmaceutical compositions is contemplated.

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral administration includes intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

Parenteral compositions include those designed for administration by injection, e.g. subcutaneous, intradermal, intralesional, intravenous, intraarterial intramuscular, intrathecal or intraperitoneal injection. For injection, the antibodies of the invention may be formulated in aqueous solutions, particularly in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the antibodies may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the antibodies of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated below, as required. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-

drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Suitable pharmaceutically acceptable carriers include, but are not limited to: buffers such as histidine, phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Aqueous injection suspensions may contain compounds which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, dextran, or the like. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl cleats or triglycerides, or liposomes.

Pharmaceutical compositions comprising the antibodies of the invention may be manufactured by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

The antibodies may be formulated into a composition in a free acid or base, neutral or salt form. Pharmaceutically acceptable salts are salts that substantially retain the biological activity of the free acid or base. These include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

Therapeutic Methods and Uses

Any of the antibodies, or sets – or pairs of antibodies, provided herein may be used in therapeutic methods. Antibodies of the invention may be used as radiotherapeutic agents, for example in the treatment of cancers, in particular cancers characterized by the expression of CEACAM5, such as colorectal cancer.

Thus, in one aspect the present invention provides a method of pretargeting radioimmunotherapy, comprising

i) administering to a subject the set of antibodies according to any one of claims 1 to 38, wherein the first and second antibodies are administered simultaneously or sequentially, in either order, wherein the antibodies bind to the target antigen and localize to the surface of a cell expressing the target antigen; and wherein association of the first and second antibody forms a functional binding site for the radiolabeled compound; and

ii) subsequently administering a radiolabeled compound, wherein the radiolabeled compound binds to functional binding site for the radiolabeled compound.

In another aspect the present invention provides a method as described above, wherein the radiolabeled compound is led-DOTAM (Pb-DOTAM, or ^{212}Pb-DOTAM).

For use in therapeutic methods, antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this

context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

In some aspects, antibodies of the invention for use as a medicament are provided. In further aspects, antibodies of the invention for use in treating a disease are provided. In certain aspects, antibodies of the invention for use in a method of treatment are provided. In some aspects, the invention provides an antibody of the invention for use in the treatment of a disease in an individual in need thereof. In certain aspects, the invention provides an antibody for use in a method of treating an individual having a disease comprising administering to the individual an effective amount of the antibody. In certain aspects, the disease is a proliferative disorder. In certain aspects, the disease is cancer, particularly a CEACAM5-expressing cancer, for example colorectal cancer. In certain aspects, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An “individual” according to any of the above aspects may be a mammal, preferably a human.

In a further aspect, the invention provides for the use of an antibody of the invention in the manufacture or preparation of a medicament. In some aspects, the medicament is for the treatment of a disease in an individual in need thereof. In a further aspect, the medicament is for use in a method of treating a disease comprising administering to an individual having the disease an effective amount of the medicament. In certain aspects, the disease is a proliferative disorder. In certain aspects, the disease is cancer, particularly a CEACAM5-expressing cancer, for example colorectal cancer. In some aspects, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An “individual” according to any of the above aspects may be a mammal, preferably a human.

In a further aspect, the invention provides a medicament (adapted) for the treatment of a disease, comprising the antibody of the invention. In some aspects the medicament is (adapted) for the treatment of a disease in an individual in need thereof. In further aspects, the medicament is (adapted) for use in a method of treating a disease comprising administering to an individual

having the disease an effective amount of the medicament. In certain aspects, the disease is a proliferative disorder. In certain aspects, the disease is cancer, particularly a CEACAM5-expressing cancer, for example colorectal cancer. In some aspects, the treatment or method of treating further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An "individual" according to any of the above aspects may be a mammal, preferably a human.

In a further aspect, the invention provides a method for treating a disease. In some aspects, the method comprises administering to an individual having such disease an effective amount of an antibody of the invention. In some aspects, a composition is administered to said individual, comprising the antibody of the invention in a pharmaceutically acceptable form. In certain aspects, the disease is a proliferative disorder. In certain aspects, the disease is cancer, particularly a CEACAM5-expressing cancer, for example colorectal cancer. In certain aspects, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An "individual" according to any of the above aspects may be a mammal, preferably a human.

In certain aspects, the disease to be treated is a proliferative disorder, such as cancer, preferably a solid tumor. Non-limiting examples of cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer. Other cell proliferation disorders include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases. In certain aspects, the cancer is a cancer expressing CEACAM5. In some aspects, the cancer is selected from the group consisting of colorectal cancer, gastric cancer, pancreatic cancer lung cancer (e.g. non-small cell lung cancer (NSCLC)) and breast cancer. A skilled artisan readily recognizes that in many cases the antibody may not provide a cure but may only provide partial benefit. In some aspects, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some aspects, an amount of antibody that provides

a physiological change is considered an "effective amount". The subject, patient, or individual in need of treatment is typically a mammal, more specifically a human.

In some aspects, an effective amount of an antibody of the invention is administered to an individual for the treatment of disease.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the route of administration, the body weight of the patient, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous or concurrent therapeutic interventions, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg – 10 mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. The progress of this therapy is easily monitored by conventional techniques and assays.

The antibodies of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the antibodies of the invention, or pharmaceutical compositions thereof, are administered or applied in an effective amount.

For systemic administration, an effective dose can be estimated initially from *in vitro* assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating

concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from *in vivo* data, *e.g.,* animal models, using techniques that are well known in the art.

Dosage amount and interval may be adjusted individually to provide plasma levels of the antibodies which are sufficient to maintain therapeutic effect. Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

An effective dose of the antibodies of the invention will generally provide therapeutic benefit without causing substantial toxicity. Toxicity and therapeutic efficacy of an antibody can be determined by standard pharmaceutical procedures in cell culture or experimental animals. Cell culture assays and animal studies can be used to determine the LD_{50} (the dose lethal to 50% of a population) and the ED_{50} (the dose therapeutically effective in 50% of a population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which can be expressed as the ratio LD_{50}/ED_{50}. Antibodies that exhibit large therapeutic indices are preferred. In some aspects, the antibody according to the present invention exhibits a high therapeutic index. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages suitable for use in humans. The dosage lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon a variety of factors, e.g., the dosage form employed, the route of administration utilized, the condition of the subject, and the like. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, in: The Pharmacological Basis of Therapeutics, Ch. 1, p. 1, incorporated herein by reference in its entirety).

The attending physician for patients treated with antibodies of the invention would know how and when to terminate, interrupt, or adjust administration due to toxicity, organ dysfunction, and the like. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated, with the route of administration, and the like. The severity of the condition may, for

example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient.

The antibodies of the invention may be administered in combination with one or more other agents in therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. The term "therapeutic agent" encompasses any agent administered to treat a symptom or disease in an individual in need of such treatment. Such additional therapeutic agent may comprise any active ingredients suitable for the particular disease being treated, preferably those with complementary activities that do not adversely affect each other. In certain aspects, an additional therapeutic agent is an immunomodulatory agent, a cytostatic agent, an inhibitor of cell adhesion, a cytotoxic agent, an activator of cell apoptosis, or an agent that increases the sensitivity of cells to apoptotic inducers. In certain aspects, the additional therapeutic agent is an anti-cancer agent, for example a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, or an antiangiogenic agent.

Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of antibody used, the type of disorder or treatment, and other factors discussed above. The antibodies are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of

manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this aspect of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

As set of clauses defining the invention and its preferred aspects and embodiments is as follows:

1. A set of antibodies comprising:

i) a first antibody that binds to an antigen expressed on the surface of a target cell, and which further comprises a VH domain of an antigen binding site for a radiolabeled compound, but which does not comprise a VL domain of an antigen binding site for the radiolabeled compound, or comprises a VL domain which is not effective to form, together with the VH domain, an antigen binding site for said radiolabeled compound; and

ii) a second antibody that binds to said antigen expressed on the surface of the target cell, and which further comprises a VL domain of an antigen binding site for the radiolabeled compound, but which does not comprise a VH domain of the antigen binding site for the radiolabeled

compound, or comprises a VH domain which is not effective to form, together with the VL domain, an antigen binding site for said radiolabeled compound,

wherein said VH domain of the first antibody and said VL domain of the second antibody are together capable of forming a functional antigen binding site for the radiolabeled compound, and wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by a peptide linker selected from GGSGGGGSGGGGSGGGGSGG (SEQ ID NO: 35) or GGGGSGGGGSGGGGSGGSGG (SEQ ID NO: 36).

2. The set of antibodies according to clause 1, wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by the peptide linker GGSGGGGSGGGGSGGGGSGG (SEQ ID NO: 35)

3. The set of antibodies according to clause 1, wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by the peptide linker GGGGSGGGGSGGGGSGGSGG (SEQ ID NO: 36).

4. The set of antibodies of any one of clauses 1 to 3, wherein the radiolabeled compound comprises Pb-DOTAM.

5. The set of antibodies according to clause 4, wherein the functional binding site for Pb-DOTAM binds with a Kd value of the binding affinity of 100pM, 50pM, 20pM, 10pM, 5pM, 1pM or less, e.g, 0.9pM or less, 0.8pM or less, 0.7pM or less, 0.6pM or less or 0.5pM or less.

6. The set of antibodies according to clause 4 or clause 5, wherein the functional binding site for Pb-DOTAM binds to Pb-DOTAM and to Bi-DOTAM.

7. The set of antibodies of any one of clauses 4 to 7, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises:

a) heavy chain CDR2 of the amino acid sequence FIGSRGDTYYASWAKG (SEQ ID NO: 42), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 42, wherein these substitutions do not include Phe50, Asp56 and/or Tyr58, and optionally also do not include Gly52 and/or Arg54; or wherein the variant is the amino acid sequence AIGSRGDTAYASWAKG (SEQ ID NO: 47).

b) heavy chain CDR3 of the amino acid sequence ERDPYGGGAYPPHL (SEQ ID NO:43), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 43, wherein these substitutions do not include Glu95, Arg96, Asp97, Pro98, and optionally also do not include Ala100C, Tyr100D, and/or Pro100E and/or optionally also do not include Tyr99; and

c) a heavy chain CDR1 of the amino acid sequence TYSMS (SEQ ID NO:41);

8. The set of antibodies of clause 6, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises (a) VH CDR1 comprising the amino acid sequence of TYSMS (SEQ ID NO: 41); (b) VH CDR2 comprising the amino acid sequence of FIGSRGDTYYASWAKG (SEQ ID NO: 42) and (c) VH CDR3 comprising the amino acid sequence of ERDPYGGGAYPPHL (SEQ ID NO: 43).

9. The set of antibodies of any one of clauses 4 to 8, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 44 and SEQ ID NO 48, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 44 or SEQ ID NO: 48.

10. The set of antibodies of any one of clauses 4 to 9, wherein the VH domain of the antigen binding site for the radiolabeled compound consists of an amino acid sequence selected from SEQ ID NO: 44 or SEQ ID NO 48.

11. The set of antibodies of clause 10, wherein residues A or AST are added to the C-terminus of the VH domain of the antigen binding site for the radiolabeled compound.

12. The set of antibodies of any one of clauses 4 to 11, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises

a) light chain CDR1 comprising the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO: 37) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 37, wherein these substitutions do not include Tyr28 and Asp32; or the amino acid sequence QSSHSVASDNRLA (SEQ ID NO: 45)

b) light chain CDR3 comprising the amino acid sequence LGGYDDESDTYG (SEQ ID NO: 39) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 39, wherein these substitutions do not include Gly91, Tyr92, Asp93, Thr95c and Tyr96, and

c) light chain CDR 2 comprising the amino acid sequence QASKLAS (SEQ ID NO: 38) or a variant thereof having at least 1, 2 or 3 substitutions in SEQ ID NO: 5, optionally not including Gln50.

13. The set of antibodies of clause 12, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises (a) LC CDR1 of the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO: 37); (b) LC CDR2 of the amino acid sequence QASKLAS (SEQ ID NO: 38); and (c) LC CDR3 of the amino acid sequence LGGYDDESDTYG (SEQ ID NO: 39).

14. The set of antibodies of any one of clauses 4 to 13, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises an amino acid sequence of SEQ ID NO: 40 or 46, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 40 or 46.

15. The set of antibodies of any one of clauses 4 to 13, wherein the VL domain of the antigen binding site for the radiolabeled compound consists of the amino acid sequence of SEQ ID NO: 40 or 46.

16. The set of antibodies of any one of clauses 1 to 15, wherein the first and second antibody bind to the same epitope of the antigen expressed on the surface of a target cell.

17. The set of antibodies of any one of clauses 1 to 15, wherein the first antibody binds to a different epitope of the antigen from the second antibody.

18. The set of antibodies of any one of clauses 1 to 17, wherein the antigen expressed on the surface of a target cell is a tumor-associated antigen.

19. The set of antibodies of clause 18, wherein the antigen expressed on the surface of a target cell is selected from the group consisting of carcinoembryonic antigen (CEA), CD20, HER2, EGP-1 (epithelial glycoprotein-1, also known as trophoblast-2), colon-specific antigen-p (CSAp), a pancreatic mucin MUC1, GPRC5D and FAP.

20. The set of antibodies of clause 19, wherein the antigen expressed on the surface of a target cell is carcinoembryonic antigen (CEA).

21. The set of antibodies according to clause 20, wherein one or both of the first and second antibody comprises an antigen-binding site which binds to CEA, comprising

a light chain variable region comprising (a) LC CDR1 comprising the amino acid sequence KASAAVGTYVA (SEQ ID NO:1); (b) LC CDR2 comprising the amino acid sequence SASYRKR (SEQ ID NO:2); and (c) LC CDR3 comprising the amino acid sequence HQYYTYPLFT (SEQ ID NO:3); and

a heavy chain variable region comprising (d) HC CDR1 comprising the amino acid sequence EFGMN (SEQ ID NO:6); (e) HC CDR2 comprising the amino acid sequence WINTKTGEATYVEEFKG (SEQ ID NO:7); and (f) HC CDR3 comprising the amino acid sequence WDFAYYVEAMDY (SEQ ID NO:8).

22. The set of antibodies according to clause 21, wherein the first antibody comprises the LC - and HC CDR's disclosed in clause 21, and the second antibody binds to a different CEA epitope.

23. The set of antibodies according to clause 21, wherein both, the first - and second antibody comprise the LC - and HC CDR's disclosed in clause 21.

24. The set of antibodies according to anyone of clauses 20-23, wherein the antigen-binding site for CEA comprises a VH sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 9, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 9.

25. The set of antibodies according to anyone of clauses 20-23, wherein the antigen-binding site for CEA comprises the VH sequence having the amino acid sequence of SEQ ID NO: 9.

26. The set of antibodies according to any one of clauses 20-25, wherein the antigen-binding site for CEA comprises a VL sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 4.

27. The set of antibodies according to anyone of clauses 20-25, wherein the antigen-binding site for CEA comprises the VL sequence having the amino acid sequence of SEQ ID NO: 4.

28. The set of antibodies according to clause 20, wherein one or both of the first and second antibody comprises an antigen-binding site which binds to CEA, comprising

a light chain variable region comprising (a) LC CDR1 comprising the amino acid sequence RAGESVDIFGVGFLH (SEQ ID NO:11); (b) LC CDR2 comprising the amino acid sequence RGSNRAT (SEQ ID NO:12); and (c) LC CDR3 comprising the amino acid sequence QQTSEYPYT (SEQ ID NO:13); and

a heavy chain variable region comprising (d) HC CDR1 comprising the amino acid sequence DTYMH (SEQ ID NO:16); (e) HC CDR2 comprising the amino acid sequence RIDPANGNSKYADSVKG (SEQ ID NO:17); and (f) HC CDR3 comprising the amino acid sequence FGYYVSDYAMAY (SEQ ID NO:18).

29. The set of antibodies according to clause 28, wherein the first antibody comprises the LC - and HC CDR's disclosed in clause 28, and the second antibody binds to a different CEA epitope.

30. The set of antibodies according to clause 28, wherein both, the first - and second antibody comprise the LC - and HC CDR's disclosed in clause 28.

31. The set of antibodies according to anyone of clauses 28-30, wherein the antigen-binding site for CEA comprises a VH sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 19.

32. The set of antibodies according to anyone of clauses 28-30, wherein the antigen-binding site for CEA comprises the VH sequence having the amino acid sequence of SEQ ID NO: 19.

33. The set of antibodies according to any one of clauses 28-32, wherein the antigen-binding site for CEA comprises a VL sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 14, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 14.

34. The set of antibodies according to anyone of clauses 28-32, wherein the antigen-binding site for CEA comprises the VL sequence having the amino acid sequence of SEQ ID NO: 14.

35. The set of antibodies according to clause 1 or 4, wherein

the first antibody comprises or consists of a first heavy chain of SEQ ID NO: 21, a second heavy chain of SEQ ID NO: 23, and a light chain of SEQ ID NO: 5; and

the second antibody comprises or consists of a first heavy chain of SEQ ID NO: 22, a second heavy chain of SEQ ID NO: 24, and a light chain of SEQ ID NO: 5.

36. The set of antibodies according to clause 1 or 4, wherein

the first antibody comprises or consists of a first heavy chain of SEQ ID NO: 25, a second heavy chain of SEQ ID NO: 26, and a light chain of SEQ ID NO: 15; and

the second antibody comprises or consists of a first heavy chain of SEQ ID NO: 27, a second heavy chain of SEQ ID NO: 28, and a light chain of SEQ ID NO: 15.

37. The set of antibodies according to clause 1 or 4, wherein

the first antibody comprises or consists of a first heavy chain of SEQ ID NO: 29, a second heavy chain of SEQ ID NO: 23, and a light chain of SEQ ID NO: 5; and

the second antibody comprises or consists of a first heavy chain of SEQ ID NO: 30, a second heavy chain of SEQ ID NO: 31, and a light chain of SEQ ID NO: 5.

38. The set of antibodies according to clause 1 or 4, wherein

the first antibody comprises or consists of a first heavy chain of SEQ ID NO: 32, a second heavy chain of SEQ ID NO: 26, and a light chain of SEQ ID NO: 15; and

the second antibody comprises or consists of a first heavy chain of SEQ ID NO: 33, a second heavy chain of SEQ ID NO: 34, and a light chain of SEQ ID NO: 15.

39. A pharmaceutical composition comprising the set of antibodies according to any one of clauses 1-38 together with pharmaceutically acceptable excipients.

40. A pharmaceutical composition comprising a first or a second antibody according to any one of clauses 1-38 together with pharmaceutically acceptable excipients.

41. A pharmaceutical product for use in the combined, simultaneous or sequential, administration to a patient with cancer, such product comprising A) as a first component a pharmaceutical composition comprising the first antibody according to clauses 1-38; and B) as a second component a pharmaceutical composition comprising the second antibody according to clauses 1-38.

42. A pharmaceutical product for use in the combined, simultaneous or sequential, administration to a patient with cancer, such product comprising A) as a first component a pharmaceutical composition comprising the first antibody according to clauses 35-38; and B) as a second component a pharmaceutical composition comprising the second antibody according to clauses 35-38.

43. A set of nucleic acids expressing the set of antibodies of any one of clauses 1-38.

44. An expression vector or set of expression vectors comprising the set of nucleic acids according to clause 43.

45. A host cell or set of host cells comprising the expression vector or set of expression vectors of clause 44.

46. A method of pretargeting radioimmunotherapy, comprising

i) administering to a subject the set of antibodies according to any one of clauses 1 to 38, wherein the first and second antibodies are administered simultaneously or sequentially, in either order, wherein the antibodies bind to the target antigen and localize to the surface of a cell expressing the target antigen; and wherein association of the first and second antibody forms a functional binding site for the radiolabeled compound; and

ii) subsequently administering a radiolabeled compound, wherein the radiolabeled compound binds to functional binding site for the radiolabeled compound.

47. The method according to clause 46, wherein the radiolabeled compound is led-DOTAM (Pb-DOTAM).

48. The method of clause 46 or 47, wherein the subject is human.

49. The method of any one of clauses 46 to 48, wherein the target antigen is a cancer- or tumor-associated antigen and the method is a method of radioimmunotherapy of a tumor or cancer.

50. The set of antibodies according to any one of clauses 1 to 38, for use in a method of pretargeting radioimmunotherapy according to any one of clauses 46 to 49.

Examples

I. Efficacy and Biodistribution

Materials and Methods

General

Health monitoring and termination criteria

All experimental protocols were reviewed and approved by the local authorities (Comité Régional d'Ethique de l'Expérimentation Animale du Limousin [CREEAL], Laboratoire Départemental d'Analyses et de Recherches de la Haute-Vienne). Female severe combined immunodeficiency (SCID) mice (Charles River) were maintained under specific and opportunistic pathogen free (SOPF) conditions with daily cycles of light and darkness (12 h/12 h), in line with ethical guidelines. No manipulations were performed during the first 5 days after arrival, to allow the animals to acclimatize to the new environment. Animals were controlled daily for clinical symptoms and detection of adverse events.

Solid xenografts were established by subcutaneous (SC) injection of CEA-expressing tumor cells in cell culture media mixed 1:1 with Corning® Matrigel® basement membrane matrix (growth factor reduced; cat No. 354230). Tumor volumes were estimated through manual calipering 3 times per week, calculated according to the formula: $volume = 0.5 \times length \times width^2$. Additional tumor measurements were made as needed depending on the tumor growth rate.

To minimize re-ingestion of radioactive urine/feces, mice were placed in cages with wire grill floors for 4 hours after ^{212}Pb-DOTAM administration, before being transferred to new cages with standard bedding. All cages were then changed 24 hours post injection (p.i.). A nutritional and hydration solution (SAFE® gel diet Energy) was provided to all mice from the day after the radioactive injection, for 7 days or until all individuals had recovered sufficiently from any acute BW loss.

Mice were euthanized before the scheduled endpoint if they showed signs of unamenable distress or pain due to tumor burden, side effects of the injections, or other causes. Indications of pain, distress, or discomfort include, but are not limited to, acute body weight (BW) loss, scruffy fur,

diarrhea, hunched posture, and lethargy. General criteria for immediate euthanasia were BW loss exceeding 20% of the initial BW or a tumor volume reaching 2000 mm^3. The BW of treated animals was measured 3 times per week, with additional measurements as needed depending on the health status. Other factors taken into account for euthanasia for ethical reasons were tumor status (e.g. ulceration, necrotic areas, blood/liquid leaking out, signs of automutilation) and general appearance of the animal (e.g. fur, posture, movement).

Tissue harvest

After each PRIT cycle, scout mice were sacrificed and necropsied 24 hours after injection of ^{212}Pb-DOTAM.

Blood was collected at termination from the venous sinus using retro-orbital bleeding on anesthetized mice, before termination through cervical dislocation followed by additional tissue harvest for radioactive measurements and/or histological analysis. Unexpected or abnormal conditions were documented. Organs and tissues collected for biodistribution purposes were weighed and measured for radioactivity using a 2470 WIZARD^2 automatic gamma counter (PerkinElmer), and the percent injected activity per gram of tissue (% IA/g) subsequently calculated, including corrections for decay and background.

Test compounds

The compounds utilized in the described studies are presented in Table 1 (SPLIT antibodies) and Table 2 (radiolabeled chelates).

All antibody constructs were stored at –80°C until the day of injection when they were thawed and diluted in standard vehicle buffer (20 mM Histidine, 140 mM NaCl; pH 6.0), mixed together into one single injection solution for intraperitoneal (IP) administration (5 mg/kg of each antibody in a total of 200 µL).

The DOTAM chelate for radiolabeling was provided by Macrocyclics and maintained at –20°C before radiolabeling, performed by Orano Med (Razès, France). ^{212}Pb-DOTAM (RO7205834) was generated by elution with DOTAM from a thorium generator, and subsequently quenched with Cu or Ca after labeling. The ^{212}Pb-DOTAM solution was diluted with 0.9% NaCl to obtain the desired ^{212}Pb activity concentration for IV injection.

Mice in vehicle control groups received multiple injections of vehicle buffer instead of SPLIT antibodies or ^{212}Pb-DOTAM.

Table 1: SPLIT antibodies

Compound (Protein ID)	**Target**	**Protocols**	**Supplier**
P1AI0084-096	CH1A1A	1819	Roche Pharma Research PZ
P1AI0446-037	CH1A1A	1819	Roche Pharma Research PZ
P1AI1622-018	CH1A1A	1819	Roche Pharma Research PZ
P1AI0034-019	CH1A1A	1819	Roche Pharma Research PZ
P1AJ6062-096	T84.66v delta T	1819	Roche Pharma Research PZ
P1AJ6059-081	T84.66v delta T	1819	Roche Pharma Research PZ
P1AJ6063-079	T84.66v delta T	1819, 1917	Roche Pharma Research PZ
P1AJ6064-094	T84.66v delta T	1819, 1917	Roche Pharma Research PZ

Table 2: Radiolabeled chelates

Compound	**Quenching**	**Protocols**	**Supplier**
^{212}Pb-DOTAM (RO7205834)	Ca	1819, 1917	Orano Med

Tumor models

The tumor cell line used for inoculation in mice is described in Table 3. BxPC3 is a human primary pancreatic adenocarcinoma cell line, naturally expressing CEA. Cells were cultured in RPMI 1640 Medium, GlutaMAX™ Supplement, HEPES (Gibco, ref. No. 72400-021) enriched with 10% fetal bovine serum (GE Healthcare Hyclone SH30088.03). Solid xenografts were established in each SCID mouse on study day 0 by subcutaneous injection of cells in RPMI

media mixed 1:1 with Corning® Matrigel® basement membrane matrix (growth factor reduced; cat No. 354230), into the right flank.

Table 3: Tumor cell lines

Cell line	Cells per mouse	Injected volume	Protocols	Supplier
BxPC3	5×10^6	100 µL	1819, 1917	ECACC*

*European Collection of Authenticated Cell Cultures (Salisbury, UK)

Example 1 (Protocol 1819): In vivo distribution of ^{212}Pb and efficacy

The aim of this Example is to assess the in vivo distribution of ^{212}Pb and efficacy following three cycles of the present antibodies for treatment of CEA-expressing SC xenografts in immunodeficient mice.

SCID mice were injected SC with BxPC3 tumor cells and the tumor development followed by manual calipering. Two-step PRIT was performed by co-injection of the SPLIT antibodies, followed 7 days later by radioactive ^{212}Pb-DOTAM. Scout mice were taken for biodistribution assessment to confirm ^{212}Pb-DOTAM targeting and clearance after all three treatment cycles. Treatment efficacy was assessed in terms of tumor growth inhibition (TGI) and survival. The study outline is shown in Figure 1.

The time course and design of protocol 1819 are shown in Table 5 and Table 6.

Table 4: Time course of protocol 1819

Study day	Experimental procedure
0	Preparation of BxPC3 cells and filling of syringes
0	SC injection* of BxPC3 cells
14	IP injection** of SPLIT antibodies (groups B, C, D, E, F, G, H, I, J, K, L, M, N) and vehicle buffer (group A)
21	Elution of ^{212}Pb-DOTAM

21	IV injection* of ^{212}Pb-DOTAM (groups B, C, D, E, F, G, H, I, J, K, L, M, N) and vehicle buffer (group A)
22	Bleeding followed by euthanasia and tissue harvest (24 h p.i.) + gamma counting
28	IP injection** of SPLIT antibodies (groups B, C, D, F, G, I, J, L, M) and vehicle buffer (group A)
35	Elution of ^{212}Pb-DOTAM
35	IV injection* of ^{212}Pb-DOTAM (groups B, C, D, F, G, I, J, L, M) and vehicle buffer (group A)
36	Bleeding followed by euthanasia and tissue harvest (24 h p.i.) + gamma counting
42	IP injection** of SPLIT antibodies (groups B, C, D, G, J, M) and vehicle buffer (group A)
49	Elution of ^{212}Pb-DOTAM
49	IV injection* of ^{212}Pb-DOTAM; (groups B, C, D, G, J, M) and vehicle buffer (group A)
50	Bleeding followed by euthanasia and tissue harvest (24 h p.i.) + gamma counting
158	End of study

*Injection volume 100 µL; **Injection volume 200 µL

Table 5: Study groups* in protocol 1819

Group	**SPLIT (VH) (Protein ID)**	**SPLIT (VL) (Protein ID)**	**SPLIT (VH)** (mg/kg)	**SPLIT (VL)** (mg/kg)	**^{212}Pb** (µCi)	**Cycles** (#)	**n** (mice)
A	—	—	0	0	0	0	10
B	P1AI0084	P1AI0446	5	5	20	3	10
C	P1AI1622	P1AI0034	5	5	20	3	10
D	P1AJ6062	P1AJ6059	5	5	20	3	10
E	P1AI0084	P1AI0446	5	5	20	1	3

F	P1AI0084	P1AI0446	5	5	20	2	3
G	P1AI0084	P1AI0446	5	5	20	3	3
H	P1AI1622	P1AI0034	5	5	20	1	3
I	P1AI1622	P1AI0034	5	5	20	2	3
J	P1AI1622	P1AI0034	5	5	20	3	3
K	P1AJ6062	P1AJ6059	5	5	20	1	3
L	P1AJ6062	P1AJ6059	5	5	20	2	3
M	P1AJ6062	P1AJ6059	5	5	20	3	3
N	P1AJ6063	P1AJ6064	5	5	20	1	5

*Groups A–D = therapy mice; groups E–N = biodistribution scouts

Primary solid xenografts were established in each SCID mouse (age 8 weeks) on study day 0 through injection of BxPC3 cells (passage 21) in RPMI media mixed 1:1 with Corning® Matrigel® basement membrane matrix (growth factor reduced; cat No. 354230). 13 days after tumor cell injection, mice were sorted into four efficacy groups with comparable average tumor size (~180 mm^3; n=10) and ten biodistribution scout groups (n=3).

Mice in groups A–D were followed to assess therapeutic efficacy until the end of the study or until one or several of the termination criteria were reached.

Mice in groups E–N were sacrificed and necropsied 24 hours after the 1st, 2nd, or 3rd ^{212}Pb-DOTAM injection to confirm tumor uptake and retention of ^{212}Pb in normal tissues. The following organs and tissues were harvested and measured for radioactivity: blood, spleen, pancreas, kidneys, liver, muscle, tail and tumor.

Biodistribution

The average ^{212}Pb accumulation and retention in all collected tissues 24 hours after ^{212}Pb-DOTAM injection in cycles 1–3 is displayed in Figure 2. The tumor uptake was specific for all tested SPLIT antibody pairs, with an average of 20.5%, 25.5%, and 22.5% IA/g in the tumor after pretargeting with P1AI0084 + P1AI0446 in cycles 1, 2, and 3, respectively; for P1AI1622 + P1AI0034 the corresponding averages were 7.4%, 14.3%, and 13.5% IA/g in tumor; for P1AJ6062 + P1AJ6059 the corresponding averages were 17.2%, 28.3%, and 22.5% IA/g in tumor; for P1AJ6063 + P1AJ6064 only one cycle was administered, and the average tumor accumulation was 11.7% IA/g.

Tumor development and survival

The average tumor volume after CEA-PRIT and control treatments is shown in Figure 3, with the corresponding individual curves are shown in Figure 4. Tumor growth inhibition was demonstrated for all treatment groups compared with vehicle. The overall survival is shown in Figure 5, based on the termination criteria of tumor volume $\geq$ 2000 mm^3. The study was terminated on day 158 after cell injection, at which point 1/10 mice in each group B and D were alive with developing tumors.

Adverse events and toxicity

The average BW development in all therapy groups is shown in Figure 6. Injection of ^{212}Pb-DOTAM caused transient weight loss in irradiated mice, slightly more pronounced in group D until ca day 100 after cell injection. No mice were euthanized due to acute post-injection BW loss in any group.

From all groups, a total of 4 mice were euthanized due to tumor ulceration, 1 mouse due to loss of activity, 1 mouse due to a spontaneous tumor in the thymus; 1 vehicle mouse was found dead in the cage. All adverse events are described in Table 6.

Table 6: Adverse events in protocol 1819

Group	**Mice sacrificed** (n per group)	**Study day**	**Reason for termination**

A: Vehicle	1(10)	20	Mouse found dead
B: P1AI0084 + P1AI0446	1(10)	70	Tumor ulceration
B: P1AI0084 + P1AI0446	1(10)	158	Loss of activity
C: P1AI1622 + P1AI0034	1(10)	56	Tumor ulceration
C: P1AI1622 + P1AI0034	1(10)	93	Tumor in thymus
D: P1AJ6062 + P1AJ6059	2(10)	107, 158	Tumor ulceration

^{212}Pb irradiation (20 μCi) was performed on study days 21, 35, and 49.

Example 2 (Protocol 1917): In vivo distribution of ^{212}Pb and efficacy for antibody pairs P1AJ6063 and P1AJ6064

The aim of this Example is to assess the in vivo distribution of ^{212}Pb and efficacy following three cycles of SPLIT PRIT for treatment of CEA-expressing SC xenografts in immunodeficient mice. SCID mice were injected SC with BxPC3 tumor cells and the tumor development followed by manual calipering. Two-step PRIT was performed by co-injection of the SPLIT antibodies, followed 7 days later by radioactive ^{212}Pb-DOTAM. Scout mice were taken for biodistribution assessment to confirm ^{212}Pb-DOTAM targeting and clearance after all three treatment cycles. Treatment efficacy was assessed in terms of tumor growth inhibition (TGI) and survival. The study outline is shown in Figure 7.

The time course and design of protocol 1917 are shown in Table 7 and Table 8, respectively.

Table 7: Time course of protocol 1917

Study day	**Experimental procedure**
0	Preparation of BxPC3 cells and filling of syringes
0	SC injection* of BxPC3 cells
14	IP injection** of SPLIT antibodies (groups B C, D, E) and vehicle buffer (group A)

21	Elution of ^{212}Pb-DOTAM
21	IV injection* of ^{212}Pb-DOTAM (groups B, C, D, E) and vehicle buffer (group A)
22	Bleeding followed by euthanasia and tissue harvest (24 h p.i.) + gamma counting
28	IP injection** of SPLIT antibodies (group B, D, E) and vehicle buffer (group A)
35	Elution of 212Pb-DOTAM
35	IV injection* of 212Pb-DOTAM; groups (B, C, D), scouts groups (F, G, I, J, L, M) and vehicle buffer (A)
36	Bleeding followed by euthanasia and tissue harvest (24 h p.i.) + gamma counting
42	IP injection** of SPLIT antibodies (groups B, D) and vehicle buffer (group A)
49	Elution of ^{212}Pb-DOTAM
49	IV injection* of ^{212}Pb-DOTAM (groups B, D) and vehicle buffer (group A)
50	Bleeding followed by euthanasia and tissue harvest (24 h p.i.) + gamma counting
128	End of study

*Injection volume 100 µL; **Injection volume 200 µL;

Table 8: Study groups* in protocol 1917

Group	**SPLIT (VH) (Protein ID)**	**SPLIT (VL) (Protein ID)**	**SPLIT (VH)** (mg/kg)	**SPLIT (VL)** (mg/kg)	**^{212}Pb** (µCi)	**Cycles** (#)	**n** (mice)
A	—	—	0	0	0	0	10
B	P1AJ6063	P1AJ6064	5	5	20	3	10
C	P1AJ6063	P1AJ6064	5	5	20	1	3
D	P1AJ6063	P1AJ6064	5	5	20	2	3
E	P1AJ6063	P1AJ6064	5	5	20	3	3

*Groups A–B = therapy mice; groups C–E = biodistribution scouts

Primary solid xenografts were established in each SCID mouse (age 11 weeks) on study day 0 through injection of BxPC3 cells (passage 19) in RPMI media mixed 1:1 with Corning® Matrigel® basement membrane matrix (growth factor reduced; cat No. 354230). 14 days after tumor cell injection, mice were sorted into two efficacy groups (~180 mm^3; n=10) and three biodistribution scout groups (n=3).

Mice in groups A and B were followed to assess therapeutic efficacy until the end of the study or until one or several of the termination criteria were reached.

Mice in groups C–E were sacrificed and necropsied 24 hours after the 1st, 2nd, or 3rd ^{212}Pb-DOTAM injection to confirm tumor uptake and retention of ^{212}Pb in normal tissues. The following organs and tissues were harvested and measured for radioactivity: blood, spleen, pancreas, kidneys, liver, muscle, tail and tumor.

Biodistribution

The average ^{212}Pb accumulation and retention in all collected tissues 24 hours after ^{212}Pb-DOTAM injection in cycles 1–3 is displayed in Figure 8. The tumor uptake was specific in all cycles, with an average of 21.1%, 16.9%, and 15.4% IA/g in the tumor after pretargeting with P1AJ6063 + P1AJ6064 in cycles 1, 2, and 3, respectively.

Tumor development and survival

The average tumor volume after CEA-PRIT and control treatments is shown in Figure 9, with the corresponding individual curves are shown in Figure 10. Tumor growth inhibition was demonstrated for all treatment groups compared with vehicle. The overall survival is shown in Figure 11, based on the termination criteria of tumor volume ≥ 2000 mm^3.

Adverse events and toxicity

The average BW development in all therapy groups is shown in Figure 12. Injection of ^{212}Pb-DOTAM caused transient weight loss in irradiated mice. No mice were euthanized due to acute post-injection BW loss in any group.

From all groups, a total of 6 mice were euthanized due to tumor ulceration and 1 mouse due to a wound in the area around the vulva combined with BW loss. All adverse events are described in Table 9.

Table 9: Adverse events in protocol 1917

Group	**Mice sacrificed** (n per group)	**Study day**	**Reason for termination**
A: Vehicle	1(10)	63	Tumor ulceration
B: P1AJ6063 + P1AJ6064	5(10)	72, 77, 79, 89, 94	Tumor ulceration
B: P1AJ6063 + P1AJ6064	1(10)	47	Wound around vulva + 17% BW loss

^{212}Pb irradiation (20 µCi) was performed on study days 21, 35, and 49.

Example 3: Generation of CEA-Split-DOTAM VH/VL antibodies

Generation of plasmids for the recombinant expression of antibody heavy or light chains

Desired proteins were expressed by transient transfection of human embryonic kidney cells (HEK 293). For the expression of a desired gene/protein (e.g. full length antibody heavy chain, full length antibody light chain, or a full length antibody heavy chain containing an additional domain

(e.g. an immunoglobulin heavy or light chain variable domain at its C-terminus) a transcription unit comprising the following functional elements was used:

- the immediate early enhancer and promoter from the human cytomegalovirus (P-CMV) including intron A,
- a human heavy chain immunoglobulin 5'-untranslated region (5'UTR),
- a murine immunoglobulin heavy chain signal sequence (SS),
- a gene/protein to be expressed, and
- the bovine growth hormone polyadenylation sequence (BGH pA).

In addition to the expression unit/cassette including the desired gene to be expressed the basic/standard mammalian expression plasmid contained

- an origin of replication from the vector pUC18 which allows replication of this plasmid in E. coli, and
- a beta-lactamase gene which confers ampicillin resistance in E. coli.

Transient expression of the SPLIT antibody molecules

Transient expression of the SPLIT molecules was performed in suspension-adapted HEK Expi293F™ (Life Technologies) cells with transfection reagent Expifectamine™ 293 (Life Technologies).

Cells were passaged, by dilution, at least four times (volume 30 ml) after thawing in a 125 ml shake flask (Incubate/Shake at 37 °C, 7 % CO_2, 85 % humidity, 135 rpm). The cells were expanded to 0.5-2.4x10^6 cells/ml in 250 ml volume. Then cells were split and seeded with a density of 6x10^5 cells/ml in a 250 ml volume in a 1 liter shake flask. Transfection was performed 24 hours later at a cell density around 2.2-2.8x10^6 cells/ml.

Before transfection 250 µg plasmid-DNA were diluted in a final volume of 12.5 ml with pre-heated (water bath; 37 °C) Opti-MEM (Life Technologies). In parallel, the ExpiFectamine transfection reagent was prepared in 12.5 ml Optim-MEM. Both solutions were incubated at room temperature for not longer than 5 min and then combined by gentle mixing, followed by an

incubation at room temperature for 15-20 minutes. The whole volume of mixture was added to 1 L shake flask with 250 ml HEK-cell-culture-volume in a dropwise manner.

Incubate/Shake at 37 °C, 7 % CO_2, 85 % humidity, 135 rpm for 6 or 7 days. After 16-24 h 1.25 ml Enhancer 1 (Life Technologies) and 12.5 ml Enhancer 2 (Life Technologies) was added to each 250 ml culture.

The supernatants were harvested by filtration through diatomaceous earth Sartoclear Dynamics Lab Filter Aid 10g, Product no. SDLKG-10.0-----2 (Sartorius Stedim Biotech), subsequently filtered through a 0.22-µm bottle top filter and stored in a freezer (-20 °C).

Purification of the SPLIT antibody constructs

The antigen binding molecule-containing culture supernatants were filtered and purified by two or three chromatographic steps. The antibodies were captured by affinity chromatography using HiTrap MabSelectSuRe (GE Healthcare) equilibrated with PBS (1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl), pH 7.4. Unbound proteins were removed by washing with equilibration buffer, and the antigen binding molecule was recovered with 100 mM sodium acetate buffer, pH 3.0, and immediately after elution neutralized to pH 6.0 with 1 M Tris-base, pH 9.0. Ion exchange chromatography on POROS^{TM} 50 XS (ThermoFisher) was used as intermediate purification step. The ion exchange chromatography was performed with 40 mM sodium acetat buffer pH 5.5 as loading and wash buffer. Elution was performed in a gradient to 750 mM sodium acetate pH 5.5. Fractions were collected and analyzed by CE-SDS and size exclusion chromatography. Fractions containing the desired product were pooled and polished in a last chromatography step. Size exclusion chromatography on Superdex 200^{TM} (GE Healthcare) was used as last purification step. The size exclusion chromatography was performed in 20 mM histidine buffer, 0.14 M NaCl, pH 6.0. The bispecificantigen binding molecules containing solutions were concentrated with an Amicon Ultra centrifugal filter unit equipped with a cellulose membrane (Millipore, Billerica, MA) and stored at -80 °C.

Example 4: Side Product Profiling of PRIT molecule candidates

Analysis of the Side product profiles for the candidate molecules

Exemplary antibodies of the present invention, i.e. P1AJ6062 and P1AJ6059, were subjected to a 2-step purification to highlight difficult to remove side products and to support CHO pool selection as well as inform single cell cloning. Both molecules were purified via Protein A chromatography followed by a cation-exchange gradient elution applying a salt gradient. Fractions with highest monomer content were pooled and other fractions analyzed with regard to present side products. Both molecules showed a complex side product profile requiring a sophisticated purification process to reach desired product quality, such as e.g. a good bioprocessing yield and adequate protein purity.

Table 10: Side Product Profile after Protein A chromatography (SE-HPLC purity):

Protein ID	**HMW 5+6**	**HMW 4**	**HMW 3**	**HMW 2**	**HMW 1**	**Mono-mer**	**LMW 1**	**LMW 2**	**LMW 3**	**LMW 4**
	un-known	un-known	un-known	un-known	un-known	target protein	Hole-Hole	Half MAb	un-known	un-known
P1AJ6062	1.34	1.54	3.62	9.10	3.57	73.20	-	7.64	-	-
P1AJ6062	1.62	4.40	10.42	5.07	7.02	64.80	5.63	-	-	0.03
	un-known	Tetra-mer	Tri- mer	Di- mer	Hole-Hole	target protein	Half MAb	Half MAb	un-known	un-known
P1AJ6059	-	0.28	1.43	2.37	1.14	86.61	6.63	1.54	-	-

Table 11: Side Product Profile after cation-exchange chromatography (SE-HPLC purity):

Protein ID	**HMW 5+6**	**HMW 4**	**HMW 3**	**HMW 2**	**HMW 1**	**Mono-mer**	**LMW 1**	**LMW 2**	**LMW 3**	**LMW 4**
	un-known	un-known	un-known	un-known	un-known	target protein	Hole-Hole	Half MAb	un-known	un-known
P1AJ6062	0.20	0.90	2.70	8.80	2.90	77.30	4.00	2.90	-	0.10
P1AJ6062	1.40	3.30	9.90	4.00	1.30	77.80	2.30	-	-	-
	un-known	Tetra-mer	Tri- m er	Di- mer	Hole-Hole	target protein	Half MAb	Half MAb	un-known	un-known
P1AJ6059	-	0.10	1.10	1.80	2.30	88.10	5.80	0.90	-	-

Example 5: Developability assessment of the present antibodies

To asses the developability of the antibodies in accordance with the present invention, the following tests were performed:

Apparent hydrophobicity - The apparent hydrophobicity is evaluated by an appropriate hydrophobic interaction chromatography (HIC) HPLC method. Relative retention time (RRT) for each molecule is calculated after normalizing it's absolute retention time to that of standards with known low and high hydrophobicity. The analyzed molecules are ranked based on their RRT.

Surface charge distribution and pharmacokinetic prediction - The interaction of the molecules with immobilized heparin is evaluated by HPLC analysis using TSKgel Heparin-5PW columns (Tosoh) and a gradient with buffers with increasing salt concentration (A = 50 mM Tris pH 7.4, and B = 1 M sodium chloride, 50 mM Tris pH 7.4, 0.8 ml/min, 32 min) in a standard HPLC system (UltiMate 3000, Thermo Fisher Scientific) and considering the manufacturer's

instructions, and as described previously (doi: 10.1080/19420862.2019.1683432). Relative retention time (RRT) for each molecule is calculated after normalizing it's absolute retention time to that of a standard with known high heparin affinity (RRT = 1.0).

Thermal stability and aggregation propensity - To determine the thermal stability and aggregation propensity of the molecules, they are exposed to increasing temperature in a controlled gradient using an UNCLE instrument (UnchainedLabs). Structural unfolding as measured by increase in intrinsic fluorescence indicates the melting temperature (T_m), and aggregation propensity as measured by increase in static light scattering (SLS) indicates the aggregation temperature (T_{agg}). The instrument is operated under standard conditions taking into account the manufacturer's instructions, the samples measured in triplicate at a concentration of 1 mg/ml. Alternatively, thermal stability was assessed using a dynamic light scattering (DLS) based readout on a DynaPro Plate Reader II (Wyatt Technology). Samples at approximately 1 mg/ml in 20 mM His pH6.0 and 140 mM NaCl were subjected to a temperature gradient from 25 °C to 80 °C with 0,05 °C/min increment. The aggregation and unfolding temperatures were determined by an experienced/skilled analyst based on the evaluation of the resulting DLS curve and the observed signal increase.

Small scale stability assessment - To assess the stability of the molecules, they are stored under conditions mimicking both physiological conditions (37°C in 1x phosphate buffer saline, pH 7.4) and shelf life indicating conditions (40°C, 140 mM sodium chloride, 20 mM Histidine buffer, pH 6.0) for two weeks at concentrations of 1 mg/ml or 10 mg/ml. Subsequent analysis focuses on changes observed in the aggregation of the stored samples compared to an untreated control stored at -80°C. Aggregation of the molecules is assessed by analyzing each sample with a size exclusion chromatography (SEC) method appropriate for IgG antibodies, specifically using TSKgel UP-SW3000 or TSKgel G3000PWxl columns (Tosoh) with an isocratic elution (200 mM potassium phosphate buffer, pH 6.2 with 250 mM potassium chloride, 0.3 or 0.5 ml/min) in a standard HPLC system (UltiMate 3000, Thermo Fisher Scientific) and considering the manufacturer's instructions. High molecular weight (HMW) species with retention time shorter than the main species are monitored by UV A280 nm signals. Obtained peaks were integrated using a custom baseline and calculating the area under the curve using Chromeleon (Thermo Fisher Scientific).

Results

For both Heparin and HIC the present antibodies are ranked using relative retention time compared to reference molecules with known properties. High RRT value indicates stronger binding/retention, therefore for both methods lower RRT is a desired property.

Strong binding in **Heparin chromatography** indicates the affinity of the antibodies for a strongly negatively charged matrix. This is used as a surrogate to indicate likely fast clearance of the antibodies in the bloodstream. Therefore an antibody with lower binding to heparin is expected to have better pharmacokinetic (PK) properties (MAbs 2020 Jan-Dec; 12(1):1683432. doi: 10.1080/19420862.2019.1683432). Here the new CEA binder (T84.66v) used in the present antibodies is the likely contributor to the improvement demonstrated with these data.

Hydrophobic interaction chromatography (HIC) is used to determine and rank the hydrophobicity of monoclonal antibodies (mAbs). Stronger retention is a function of higher hydrophobicity, and indicates higher propensity for aggregation, lower stability and potentially poorer PK properties. (MAbs. 2020 Jan-Dec; 12(1): 1743053 doi: 10.1080/19420862.2020.1743053) Based on this, poorer CMC properties (e.g. in downstream processing, formulation, concentrability, overall stability) can be expected for the antibodies with higher relative retention. The data in Table 12 demonstrate an improvement from the reference antibodies (e.g. PRIT1 split constructs, VL 0.82 and VH 0.97) to the present antibodies (e.g. VL 0.55 and VH 0.85 for P1AJ6059 and P1AJ6062).

Thermal stability: High thermal stability is the ability of the protein to maintain its integrity under increased temperature. Thermal stability is indicative of lower tendency for partial unfolding and long term aggregation resistance. Moreover, antibodies with lower thermal stability were shown to have lower expression. (mAbs, 11:2, 239-264, DOI: 10.1080/19420862.2018.1553476). In particular the data for the present antibodies comprising the VH of the DOTAM binder (60.8 °C and 63.8 °C) improve significantly when compared to the reference compounds (50.7 °C). See Table 12.

Table 12: Results of the developability assessment

Description	Protein ID	Heparin [RRT]	Hydrophobic interaction chromatography (HIC) [RRT]	Termal stability	
				T_{agg} [°C]	T_m [°C]
PRIT-0213[+] (Reference compound)	P1AA7386[+]	0.59	0.44	51.0*	54.0*
PRIT1 Split VL (Reference compound)	P1AD8592[++]	0.94	0.82	65.5	73.0
PRIT1 Split VH (Reference compound)	P1AF0171[++]	0.96	0.97	50.7	62.1
	P1AI0446	0.94	0.80	65.0	68.5
	P1AI0084	1.01	1.05	60.8	67.0
	P1AJ6059	0.53	0.55	63.6	64.7
	P1AJ6062	0.61	0.85	63.8	65.1

*measured using DLS based readout as described herein

[+] PRIT-0213 (P1AA7386) is an antibody which binds to CEA ("old" T84.66) and comprises functional VH and VL DOTAM binders *within the same* antibody. PRIT-0213 is for example disclosed in WO2019/201959.

[++] P1AD8592 and P1AD8749 is a pair of antibodies which bind to CEA (CH1A1A), and wherein one antibody comprises a functional VL DOTAM binder (P1AD8592) and the other comprises a functional VH DOTAM binder (P1AD8749). These antibodies are for example disclosed in WO2021/009047. P1AF0171 used herein is identical to P1AD8749 except that for P1AF0171 an extension (AST) was added at the C-terminus of the DOTAM-VH domain. This extension reduces the pre-existing ADA response and has no substantial influence on the data shown in this Table.

Forced stress studies are routinely used to subject antibodies to harsh conditions in order to identify potential downstream liabilities. Storage at elevated temperature for prolonged periods of time under different buffer conditions simulates physiological stress (37 °C, pH 7.4), and manufacturing, transport and storage stress (40 °C, pH 6). High % of aggregation and fragmentation under these conditions leads to high (HMW) and low (LMW) molecular weight

species, respectively, as measured by size exclusion chromatography (SEC), and is indicative of lower long term stability. Specifically, measuring aggregation at increased temperature (40 °C) can be predictive of the stability of the antibodies at 5 °C or 25 °C under long term conditions (mAbs, 11:2, 239-264, DOI: 10.1080/19420862.2018.1553476) (mAbs, 12:1, DOI: 10.1080/19420862.2020.1743053) Therefore, higher percentage of HMW means higher aggregation and thus lower stability. The data in Table 13 demonstrate improved values for HMW and LMW of the present DOTAM-VH comprising antibodies when compared to the corresponding reference compounds (i.e. from up to 42.4% to up to 9.9% for HMW). Table 13 also shows higher percentage of HMW for the present antibodies comprising the DOTAM-VL binder (e.g. up to 19.7%) when compared to the reference compounds (e.g. up to 8.8%). However, here it is important to consider that the present antibodies were stressed at much higher concentration (10 mg/ml), which is by itself a more stringent stress condition. The higher percentage of HMW for the DOTAM-VL comprising antibodies of the present invention can thus be interpreted as a function of the higher concentration during stress.

Table 13:

	Description	**CEA binder**	**Protein ID**	**Forced stress conditions**	**SEC [summed peak %]**		
Antibody concentration					**HMW**	**LMW**	**Main Peak**
1 mg/ml	PRIT-0213*	T84.66	P1AA7386	-80 °C Ref.	1.7	3.6	94.6
	(Reference)			37 °C pH7.4	7.4	3.7	88.9
				40 °C pH6.0	6.8	4.0	89.3
1 mg/ml	PRIT1 Split VL*	CH1A1A	P1AD8592	-80 °C Ref.	2.5	0.9	96.7
	(Reference)			37 °C pH7.4	3.3	0.8	95.8
				40 °C pH6.0	8.8	1.4	89.8
1 mg/ml	PRIT1 Split VH*	CH1A1A	P1AF0171	-80 °C Ref.	3.0	15.7	81.3
	(Reference)			37 °C pH7.4	42.4	18.2	39.4
				40 °C pH6.0	28.5	12.6	58.9

10 mg/ml	with DOTAM-VL	CH1A1A	P1AI0446	-80 °C Ref.	0.0	1.4	98.5
				37 °C pH7.4	0.5	3.5	95.9
				40 °C pH6.0	16.5	3.1	80.3
10 mg/ml	with DOTAM-VH	CH1A1A	P1AI0084	-80 °C Ref.	0.7	0.6	98.7
				37 °C pH7.4	5.7	1.1	93.3
				40 °C pH6.0	7.6	1.6	90.8
10 mg/ml	with DOTAM-VL	T84.66v	P1AJ6059	-80 °C Ref.	0.1	0.7	99.2
				37 °C pH7.4	0.9	2.8	96.3
				40 °C pH6.0	19.7	2.5	77.8
10 mg/ml	with DOTAM-VH	T84.66v	P1AJ6062	-80 °C Ref.	0.7		99.3
				37 °C pH7.4	6.3		93.8
				40 °C pH6.0	9.9	2.0	88.1

* See Table 12

-80 °C Ref.: reference sample stored at -80°C
37 °C pH7.4: physiological stress 1x PBS pH7.4, 37°C
40 °C pH6.0: CMC, or shelf life-indicating stress 140 mM sodium chloride, 20 mM Histidine buffer 40°C

Glossary of Abbreviations

BD	Biodistribution
BsAb	Bispecific antibody
BW	Body weight
CEA	Carcinoembryonic antigen
DOTAM	1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane
huCEA	Human carcinoembryonic antigen
IA	Injected activity
IP	Intraperitoneal
IV	Intravenous
NBF	Neutral buffered formalin
PBS	Phosphate-buffered saline
p.i.	Post injection
PRIT	Pretargeted radioimmunotherapy
RCF	Relative centrifugal force (G-force)
RNCB	Roche non-clinical biorepository
SC	Subcutaneous
SD	Standard deviation
SOPF	Specific and opportunistic pathogen-free
SPLIT	SeParated v-domains LInkage Technology
TGI	Tumor growth inhibition

Sequences

Description	Sequence	SEQ ID NO
Light Chain CDR1 <CEA> CH1A1A	KASAAVGTYVA	1
Light Chain CDR2 <CEA> CH1A1A	SASYRKR	2
Light Chain CDR3 <CEA> CH1A1A	HQYYTYPLFT	3
Light Chain Variable Domain (VL) <CEA> CH1A1A	DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVA WYQQKPGKAPKLLIYSASYRKRGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCHQYYTYPLFTFGQGT KLEIK	4
Light Chain (LC) <CEA> CH1A1A (D1AD3421)	DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVA WYQQKPGKAPKLLIYSASYRKRGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCHQYYTYPLFTFGQGT KLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC	5
Heavy Chain CDR1 <CEA> CH1A1A	EFGMN	6

Heavy Chain CDR2 <CEA> CH1A1A	WINTKTGEATYVEEFKG	7
Heavy Chain CDR3 <CEA> CH1A1A	WDFAYYVEAMDY	8
Heavy Chain Variable Domain (VH) <CEA> CH1A1A	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW DFAYYVEAMDYWGQGTTVTVSS	9
Heavy Chain (HC) <CEA> CH1A1A (D1AE0755)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW DFAYYVEAMDYWGQGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	10
Light Chain CDR1	RAGESVDIFGVGFLH	11

<CEA> T84.66v		
Light Chain CDR2 <CEA> T84.66v	RGSNRAT	12
Light Chain CDR3 <CEA> T84.66v	QQTSEYPYT	13
VL <CEA> T84.66v	EIVLTQSPATLSLSPGERATLSCRAGESVDIFGVGF LHWYQQKPGQAPRLLIYRGSNRATGIPARFSGSGS GTDFTLTISSLEPEDFAVYYCQQTSEYPYTFGQGT KLEIK	14
LC <CEA> T84.66v (D1AM8341)	EIVLTQSPATLSLSPGERATLSCRAGESVDIFGVGF LHWYQQKPGQAPRLLIYRGSNRATGIPARFSGSGS GTDFTLTISSLEPEDFAVYYCQQTSEYPYTFGQGT KLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC	15
Heavy Chain CDR1 <CEA> T84.66v	DTYMH	16
Heavy Chain CDR2 <CEA> T84.66v	RIDPANGNSKYADSVKG	17
Heavy Chain CDR3 <CEA> T84.66v	FGYYVSDYAMAY	18

VH <CEA> T84.66v	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSS	19
HC <CEA> T84.66v (D1AJ5331)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG	20
HCknob <CEA> CH1A1A (D1AC4023)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW DFAYYVEAMDYWGQGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS	21

	DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG	
HChole <CEA> CH1A1A (D1AC4022)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW DFAYYVEAMDYWGQGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG	22
HChole <CEA> CH1A1A with <Dotam> VL (D1AK6882)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW DFAYYVEAMDYWGQGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGGGSGGGGSGGGGSGGGGSGGDI QMTQSPSSLSASVGDRVTITCQSSHSVYSDNDLA	23

	WYQQKPGKAPKLLIYQASKLASGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCLGGYDDESDTYGFGG GTKVEIK	
HCknob <CEA> CH1A1A with <Dotam> VH (D1AK7032)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW DFAYYVEAMDYWGQGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGGGSGGGGSGGGGSGGGGSGG VQLVESGGGLVQPGGSLRLSCAASGFSLSTYSMS WVRQAPGKGLEWVGFIGSRGDTYYASWAKGRFT ISRDNSKNTVYLQMNSLRAEDTAVYYCAKERDP YGGGAYPPHLWGRGTLVTVSSA	24
HCknob <CEA> T84.66v (D1AS9437)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK	25

	TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPG	
HChole <CEA> T84.66v with <Dotam> VL (D1AS9439)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGGGSGGGGSGGGGSGGGGSGGDIQM TQSPSSLSASVGDRVTITCQSSHSVYSDNDLAWY QQKPGKAPKLLIYQASKLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCLGGYDDESDTYGFGGGTK VEIK	26
HChole <CEA> T84.66v (D1AN8651)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR	27

	VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG	
HCknob <CEA> T84.66v with <Dotam> VH (D1AS9435)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGGGSGGGGSGGGGSGGGGSGGVQ LVESGGGLVQPGGSLRLSCAASGFSLSTYSMSWV RQAPGKGLEWVGFIGSRGDTYYASWAKGRFTISR DNSKNTVYLQMNSLRAEDTAVYYCAKERDPYGG GAYPPHLWGRGTLVTVSSA	28
HCknob <CEA> CH1A1A with <Dotam> dematured VH (D1AK6974)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW DFAYYVEAMDYWGQGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV	29

	SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGGGSGGGGSGGGGSGGGGSGG VQLVESGGGLVQPGGSLRLSCAASGFSLSTYSMS WVREAPGKGLEWVGAIGSRGDTAYASWAKGRFT ISRDSNTVYLQMNSLRAEDTAVYYCAKERDPYG GGAYPPHLWGRGTLVTVSSA	
HCknob <CEA> CH1A1A with <Dotam> VH (D1AH1088)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW DFAYYVEAMDYWGQGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGGGGSGGGGSGGGGSGGSGG VQLVESGGGLVQPGGSLRLSCAASGFSLSTYSMS WVRQAPGKGLEWVGFIGSRGDTYYASWAKGRFT ISRDSNTVYLQMNSLRAEDTAVYYCAKERDPYG GGAYPPHLWGRGTLVTVSSAST	30
HChole	QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFG MNWVRQAPGQGLEWMGWINTKTGEATYVEEFK GRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARW	31

<CEA> CH1A1A with <Dotam> dematured VL (D1AK6959)	DFAYYVEAMDYWGQGTTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPI EKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGGGSGGGGSGGGGSGGGGSGGDI QMTQSPSSLSASVGDRVTITCQSSHSVASDNRLA WYQEKPGKAPKLLIYQASKLASGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCLGGYDDESDTYGFGG GTKVEIK	
HCknob <CEA> T84.66v with <Dotam> dematured VH (D1AS9441)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGGGSGGGGSGGGGSGGGGSGGVQ LVESGGGLVQPGGSLRLSCAASGFSLSTYSMSWV REAPGKGLEWVGAIGSRGDTAYASWAKGRFTISR	32

	DSNTVYLQMNSLRAEDTAVYYCAKERDPYGGGA YPPHLWGRGTLVTVSSA	
HCknob <CEA> T84.66v with <Dotam> VH (D1AS9447)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGGGSGGGGSGGGGSGGGGSGGVQ LVESGGGLVQPGGSLRLSCAASGFSLSTYSMSWV RQAPGKGLEWVGFIGSRGDTYYASWAKGRFTISR DSNTVYLQMNSLRAEDTAVYYCAKERDPYGGGA YPPHLWGRGTLVTVSSA	33
HChole <CEA> T84.66v with <Dotam> dematured VL (D1AS9449)	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYM HWVRQAPGKGLEWVGRIDPANGNSKYADSVKG RFTISADTSKNTAYLQMNSLRAEDTAVYYCAPFG YYVSDYAMAYWGQGTTVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAV	34

	KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGGGSGGGGSGGGGSGGGGSGGDIQM TQSPSSLSASVGDRVTITCQSSHSVASDNRLAWYQ EKPGKAPKLLIYQASKLASGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCLGGYDDESDTYGFGGGTK VEIK	
Linker 1	GGSGGGGSGGGGSGGGGSGG	35
Linker 2 (in D1AH1088)	GGGGSGGGGSGGGGSGGSGG	36
<Dotam> VL CDR1	QSSHSVYSDNDLA	37
<Dotam> VL CDR2	QASKLAS	38
<Dotam> VL CDR3	LGGYDDESDTYG	39
<Dotam> VL	DIQMTQSPSSLSASVGDRVTITCQSSHSVYSDNDL AWYQQKPGKAPKLLIYQASKLASGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCLGGYDDESDTYGFG GGTKVEIK	40
<Dotam> VH CDR1	TYSMS	41
<Dotam> VH CDR2	FIGSRGDTYYASWAKG	42
<Dotam> VH CDR3	ERDPYGGGAYPPHL	43
<Dotam> VH	VQLVESGGGLVQPGGSLRLSCAASGFSLSTYSMS WVRQAPGKGLEWVGFIGSRGDTYYASWAKGRFT	44

	ISRDNSKNTVYLQMNSLRAEDTAVYYCAKERDP YGGGAYPPHLWGRGTLVTVSS	
<Dotam> dematured VL CDR1	QSSHSVASDNRLA	45
<Dotam> dematured VL	DIQMTQSPSSLSASVGDRVTITCQSSHSVASDNRL AWYQEKPGKAPKLLIYQASKLASGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCLGGYDDESDTYGFG GGTKVEIK	46
<Dotam> dematured VH CDR2	AIGSRGDTAYASWAKG	47
<Dotam> dematured VH	VQLVESGGGLVQPGGSLRLSCAASGFSLSTYSMS WVREAPGKGLEWVGAIGSRGDTAYASWAKGRFT ISRDSNTVYLQMNSLRAEDTAVYYCAKERDPYG GGAYPPHLWGRGTLVTVSS	48

Claims

1. A set of antibodies comprising:

i) a first antibody that binds to an antigen expressed on the surface of a target cell, and which further comprises a VH domain of an antigen binding site for a radiolabeled compound, but which does not comprise a VL domain of an antigen binding site for the radiolabeled compound, or comprises a VL domain which is not effective to form, together with the VH domain, an antigen binding site for said radiolabeled compound; and

ii) a second antibody that binds to said antigen expressed on the surface of the target cell, and which further comprises a VL domain of an antigen binding site for the radiolabeled compound, but which does not comprise a VH domain of the antigen binding site for the radiolabeled compound, or comprises a VH domain which is not effective to form, together with the VL domain, an antigen binding site for said radiolabeled compound,

wherein said VH domain of the first antibody and said VL domain of the second antibody are together capable of forming a functional antigen binding site for the radiolabeled compound, and wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by a peptide linker selected from GGSGGGGSGGGGSGGGGSGG (SEQ ID NO: 35) or GGGGSGGGGSGGGGSGGSGG (SEQ ID NO: 36).

2. The set of antibodies according to claim 1, wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by the peptide linker GGSGGGGSGGGGSGGGGSGG (SEQ ID NO: 35)

3. The set of antibodies according to claim 1, wherein each of the VH – or VL domains is attached via its N-terminal region to the C-terminus of one of the two heavy chains of the first or second antibody by the peptide linker GGGGSGGGGSGGGGSGGSGG (SEQ ID NO: 36).

4. The set of antibodies of any one of claims 1 to 3, wherein the radiolabeled compound comprises Pb-DOTAM.

5. The set of antibodies according to claim 4, wherein the functional binding site for Pb-DOTAM binds with a Kd value of the binding affinity of 100pM, 50pM, 20pM, 10pM, 5pM, 1pM or less, e.g, 0.9pM or less, 0.8pM or less, 0.7pM or less, 0.6pM or less or 0.5pM or less.

6. The set of antibodies according to claim 4 or claim 5, wherein the functional binding site for Pb-DOTAM binds to Pb-DOTAM and to Bi-DOTAM.

7. The set of antibodies of any one of claims 4 to 7, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises:

a) heavy chain CDR2 of the amino acid sequence FIGSRGDTYYASWAKG (SEQ ID NO: 42), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 42, wherein these substitutions do not include Phe50, Asp56 and/or Tyr58, and optionally also do not include Gly52 and/or Arg54; or wherein the variant is the amino acid sequence AIGSRGDTAYASWAKG (SEQ ID NO: 47).

b) heavy chain CDR3 of the amino acid sequence ERDPYGGGAYPPHL (SEQ ID NO:43), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 43, wherein these substitutions do not include Glu95, Arg96, Asp97, Pro98, and optionally also do not include Ala100C, Tyr100D, and/or Pro100E and/or optionally also do not include Tyr99; and

c) a heavy chain CDR1 of the amino acid sequence TYSMS (SEQ ID NO:41);

8. The set of antibodies of claim 6, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises (a) VH CDR1 comprising the amino acid sequence of TYSMS (SEQ ID NO: 41); (b) VH CDR2 comprising the amino acid sequence of FIGSRGDTYYASWAKG (SEQ ID NO: 42) and (c) VH CDR3 comprising the amino acid sequence of ERDPYGGGAYPPHL (SEQ ID NO: 43).

9. The set of antibodies of any one of claims 4 to 8, wherein the VH domain of the antigen binding site for the radiolabeled compound comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 44 and SEQ ID NO 48, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 44 or SEQ ID NO: 48.

10. The set of antibodies of any one of claims 4 to 9, wherein the VH domain of the antigen binding site for the radiolabeled compound consists of an amino acid sequence selected from SEQ ID NO: 44 or SEQ ID NO 48.

11. The set of antibodies of claim 10, wherein residues A or AST are added to the C-terminus of the VH domain of the antigen binding site for the radiolabeled compound.

12. The set of antibodies of any one of claims 4 to 11, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises

a) light chain CDR1 comprising the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO: 37) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 37, wherein these substitutions do not include Tyr28 and Asp32; or the amino acid sequence QSSHSVASDNRLA (SEQ ID NO: 45)

b) light chain CDR3 comprising the amino acid sequence LGGYDDESDTYG (SEQ ID NO: 39) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 39, wherein these substitutions do not include Gly91, Tyr92, Asp93, Thr95c and Tyr96, and

c) light chain CDR 2 comprising the amino acid sequence QASKLAS (SEQ ID NO: 38) or a variant thereof having at least 1, 2 or 3 substitutions in SEQ ID NO: 5, optionally not including Gln50.

13. The set of antibodies of claim 12, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises (a) LC CDR1 of the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO: 37); (b) LC CDR2 of the amino acid sequence QASKLAS (SEQ ID NO: 38); and (c) LC CDR3 of the amino acid sequence LGGYDDESDTYG (SEQ ID NO: 39).

14. The set of antibodies of any one of claims 4 to 13, wherein the VL domain of the antigen binding site for the radiolabeled compound comprises an amino acid sequence of SEQ ID NO: 40 or 46, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 40 or 46.

15. The set of antibodies of any one of claims 4 to 13, wherein the VL domain of the antigen binding site for the radiolabeled compound consists of the amino acid sequence of SEQ ID NO: 40 or 46.

16. The set of antibodies of any one of claims 1 to 15, wherein the first and second antibody bind to the same epitope of the antigen expressed on the surface of a target cell.

17. The set of antibodies of any one of claims 1 to 15, wherein the first antibody binds to a different epitope of the antigen from the second antibody.

18. The set of antibodies of any one of claims 1 to 17, wherein the antigen expressed on the surface of a target cell is a tumor-associated antigen.

19. The set of antibodies of claim 18, wherein the antigen expressed on the surface of a target cell is selected from the group consisting of carcinoembryonic antigen (CEA), CD20, HER2, EGP-1 (epithelial glycoprotein-1, also known as trophoblast-2), colon-specific antigen-p (CSAp), a pancreatic mucin MUC1, GPRC5D and FAP.

20. The set of antibodies of claim 19, wherein the antigen expressed on the surface of a target cell is carcinoembryonic antigen (CEA).

21. The set of antibodies according to claim 20, wherein one or both of the first and second antibody comprises an antigen-binding site which binds to CEA, comprising

a light chain variable region comprising (a) LC CDR1 comprising the amino acid sequence KASAAVGTYVA (SEQ ID NO:1); (b) LC CDR2 comprising the amino acid sequence SASYRKR (SEQ ID NO:2); and (c) LC CDR3 comprising the amino acid sequence HQYYTYPLFT (SEQ ID NO:3); and

a heavy chain variable region comprising (d) HC CDR1 comprising the amino acid sequence EFGMN (SEQ ID NO:6); (e) HC CDR2 comprising the amino acid sequence WINTKTGEATYVEEFKG (SEQ ID NO:7); and (f) HC CDR3 comprising the amino acid sequence WDFAYYVEAMDY (SEQ ID NO:8).

22. The set of antibodies according to claim 21, wherein the first antibody comprises the LC - and HC CDR's disclosed in claim 21, and the second antibody binds to a different CEA epitope.

23. The set of antibodies according to claim 21, wherein both, the first - and second antibody comprise the LC - and HC CDR's disclosed in claim 21.

24. The set of antibodies according to anyone of claims 20-23, wherein the antigen-binding site for CEA comprises a VH sequence comprising an amino acid sequence selected from the

group consisting of SEQ ID NO: 9, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 9.

25. The set of antibodies according to anyone of claims 20-23, wherein the antigen-binding site for CEA comprises the VH sequence having the amino acid sequence of SEQ ID NO: 9.

26. The set of antibodies according to any one of claims 20-25, wherein the antigen-binding site for CEA comprises a VL sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 4.

27. The set of antibodies according to anyone of claims 20-25, wherein the antigen-binding site for CEA comprises the VL sequence having the amino acid sequence of SEQ ID NO: 4.

28. The set of antibodies according to claim 20, wherein one or both of the first and second antibody comprises an antigen-binding site which binds to CEA, comprising

a light chain variable region comprising (a) LC CDR1 comprising the amino acid sequence RAGESVDIFGVGFLH (SEQ ID NO:11); (b) LC CDR2 comprising the amino acid sequence RGSNRAT (SEQ ID NO:12); and (c) LC CDR3 comprising the amino acid sequence QQTSEYPYT (SEQ ID NO:13); and

a heavy chain variable region comprising (d) HC CDR1 comprising the amino acid sequence DTYMH (SEQ ID NO:16); (e) HC CDR2 comprising the amino acid sequence RIDPANGNSKYADSVKG (SEQ ID NO:17); and (f) HC CDR3 comprising the amino acid sequence FGYYVSDYAMAY (SEQ ID NO:18).

29. The set of antibodies according to claim 28, wherein the first antibody comprises the LC - and HC CDR's disclosed in claim 28, and the second antibody binds to a different CEA epitope.

30. The set of antibodies according to claim 28, wherein both, the first - and second antibody comprise the LC - and HC CDR's disclosed in claim 28.

31. The set of antibodies according to anyone of claims 28-30, wherein the antigen-binding site for CEA comprises a VH sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 19.

32. The set of antibodies according to anyone of claims 28-30, wherein the antigen-binding site for CEA comprises the VH sequence having the amino acid sequence of SEQ ID NO: 19.

33. The set of antibodies according to any one of claims 28-32, wherein the antigen-binding site for CEA comprises a VL sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 14, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 14.

34. The set of antibodies according to anyone of claims 28-32, wherein the antigen-binding site for CEA comprises the VL sequence having the amino acid sequence of SEQ ID NO: 14.

35. The set of antibodies according to claim 1 or 4, wherein

the first antibody comprises or consists of a first heavy chain of SEQ ID NO: 21, a second heavy chain of SEQ ID NO: 23, and a light chain of SEQ ID NO: 5; and

the second antibody comprises or consists of a first heavy chain of SEQ ID NO: 22, a second heavy chain of SEQ ID NO: 24, and a light chain of SEQ ID NO: 5.

36. The set of antibodies according to claim 1 or 4, wherein

the first antibody comprises or consists of a first heavy chain of SEQ ID NO: 25, a second heavy chain of SEQ ID NO: 26, and a light chain of SEQ ID NO: 15; and

the second antibody comprises or consists of a first heavy chain of SEQ ID NO: 27, a second heavy chain of SEQ ID NO: 28, and a light chain of SEQ ID NO: 15.

37. The set of antibodies according to claim 1 or 4, wherein

the first antibody comprises or consists of a first heavy chain of SEQ ID NO: 29, a second heavy chain of SEQ ID NO: 23, and a light chain of SEQ ID NO: 5; and

the second antibody comprises or consists of a first heavy chain of SEQ ID NO: 30, a second heavy chain of SEQ ID NO: 31, and a light chain of SEQ ID NO: 5.

38. The set of antibodies according to claim 1 or 4, wherein

the first antibody comprises or consists of a first heavy chain of SEQ ID NO: 32, a second heavy chain of SEQ ID NO: 26, and a light chain of SEQ ID NO: 15; and

the second antibody comprises or consists of a first heavy chain of SEQ ID NO: 33, a second heavy chain of SEQ ID NO: 34, and a light chain of SEQ ID NO: 15.

39. A pharmaceutical composition comprising the set of antibodies according to any one of claims 1-38 together with pharmaceutically acceptable excipients.

40. A pharmaceutical composition comprising a first or a second antibody according to any one of claims 1-38 together with pharmaceutically acceptable excipients.

41. A pharmaceutical product for use in the combined, simultaneous or sequential, administration to a patient with cancer, such product comprising A) as a first component a pharmaceutical composition comprising the first antibody according to claims 1-38; and B) as a second component a pharmaceutical composition comprising the second antibody according to claims 1-38.

42. A pharmaceutical product for use in the combined, simultaneous or sequential, administration to a patient with cancer, such product comprising A) as a first component a pharmaceutical composition comprising the first antibody according to claims 35-38; and B) as a second component a pharmaceutical composition comprising the second antibody according to claims 35-38.

43. A set of nucleic acids expressing the set of antibodies of any one of claims 1-38.

44. An expression vector or set of expression vectors comprising the set of nucleic acids according to claim 43.

45. A host cell or set of host cells comprising the expression vector or set of expression vectors of claim 44.

46. A method of pretargeting radioimmunotherapy, comprising

i) administering to a subject the set of antibodies according to any one of claims 1 to 38, wherein the first and second antibodies are administered simultaneously or sequentially, in either order, wherein the antibodies bind to the target antigen and localize to the surface of a cell expressing the target antigen; and wherein association of the first and second antibody forms a functional binding site for the radiolabeled compound; and

ii) subsequently administering a radiolabeled compound, wherein the radiolabeled compound binds to functional binding site for the radiolabeled compound.

47. The method according to claim 46, wherein the radiolabeled compound is led-DOTAM (Pb-DOTAM).

48. The method of claim 46 or 47, wherein the subject is human.

49. The method of any one of claims 46 to 48, wherein the target antigen is a cancer- or tumor-associated antigen and the method is a method of radioimmunotherapy of a tumor or cancer.

50. The set of antibodies according to any one of claims 1 to 38, for use in a method of pretargeting radioimmunotherapy according to any one of claims 46 to 49.

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