ANTI-CD19 ANTIBODIES AND CAR-T STRUCTURES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of the filing date of U.S. Provisional Application Serial No. 63/171,520, filed on April 6, 2021, the disclosure of which is incorporated by reference herein in its entirety. This application also claims priority benefit of the filing date of U.S. Provisional Application Serial No. 63/311,913, filed on February 18, 2022, the disclosure of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 8, 2022, is named TNO-0040-WO_SL.txt and is 74,739 bytes in size.

FIELD OF THE INVENTIONp

[0002] The present invention concerns antibodies (e.g., UniAbs™) and CAR-T structures that bind to CD19. The invention further concerns methods of making such antibodies and CAR-T structures, compositions, including pharmaceutical compositions, comprising such antibodies and CAR-T structures, and their use to treat disorders that are characterized by the expression of CD19.

BACKGROUND OF THE INVENTION

Cluster of differentiation 19 (CD19)

[0003] CD19, also known as B-Lymphocyte Surface Antigen B4 (UniProt P15391), is a cell surface receptor that is expressed on all human B-cells, but not plasma cells. CD19 is a transmembrane protein that recruits cytoplasmic signaling proteins to the membrane, and it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways. CD19 has a relatively large, 240 amino acid, cytoplasmic tail. The extracellular Ig-like domains are divided by a potential disulfide linked non-Ig-like domain and N-linked carbohydrate addition sites. The cytoplasmic tail contains at least nine tyrosine residues near the C-terminus, some of which have been shown to be phosphorylated. Along with CD20 and CD22, the restricted expression of CD19 to the B-cell lineage makes it an attractive target for the therapeutic treatment of B-cell malignancies. Many monoclonal antibodies and antibody-drug conjugates specific to CD19 have been described (Naddafi et al. 2015, PMC4644525). In addition, anti-CD19 chimeric antigen receptor T-cells have been approved to treat leukemia (Sadelain et al. 2017, PMID: 29245005).

Heavy Chain Antibodies

[0004] In a conventional IgG antibody, the association of the heavy chain and light chain is due in part to a hydrophobic interaction between the light chain constant region and the CH1 constant domain of the heavy chain. There are additional residues in the heavy chain framework 2 (FR2) and framework 4 (FR4) regions that also contribute to this hydrophobic interaction between the heavy and light chains.

[0005] It is known, however, that sera of camelids (sub-order Tylopoda which includes camels, dromedaries and llamas) contain a major type of antibodies composed solely of paired H-chains (heavy-

used in reference to a heavy chain-only variable region domain means that two heavy chain-only variable region domains are present (each having a single binding site), and are connected by a linker sequence. Non-limiting examples of linker sequences are discussed further herein, and include, without limitation, GS linker sequences of various lengths. When a heavy chain-only variable region is in a bivalent configuration, each of the two heavy chain-only variable region domains can bind to the same antigen, or to different antigens (e.g., to different epitopes on the same protein; to two different proteins, etc.). However, unless specifically noted otherwise, a heavy chain-only variable region denoted as being in a "bivalent configuration" is understood to contain two identical heavy chain-only variable region domains, connected by a linker sequence, wherein each of the two identical heavy chain-only variable region domains binds to the same target antigen.

[0072] Aspects of the invention include antibodies having multi-specific configurations, which include, without limitation, bispecific, trispecific, etc. A large variety of methods and protein configurations are known and used in bispecific monoclonal antibodies (BsMAB), tri-specific antibodies, etc.

[0073] Various methods for the production of multivalent artificial antibodies have been developed by recombinantly fusing variable domains of two or more antibodies. In some embodiments, a first and a second antigen-binding domain on a polypeptide are connected by a polypeptide linker. One non-limiting example of such a polypeptide linker is a GS linker, having an amino acid sequence of four glycine residues, followed by one serine residue, and wherein the sequence is repeated n times, where n is an integer ranging from 1 to about 10, such as 2, 3, 4, 5, 6, 7, 8, or 9 (SEQ ID NO: 42). Non-limiting examples of such linkers include GGGGS (SEQ ID NO: 43) (n=1) and GGGGSGGGGS (SEQ ID NO: 44 (n=2). Other suitable linkers can also be used, and are described, for example, in Chen et al., Adv Drug Deliv Rev. 2013 October 15; 65(10): 1357-69, the disclosure of which is incorporated herein by reference in its entirety.

[0074] The term "three-chain antibody-like molecule" or "TCA" is used herein to refer to antibody-like molecules comprising, consisting essentially of, or consisting of three polypeptide subunits, two of which comprise, consist essentially of, or consist of one heavy and one light chain of a monoclonal antibody, or functional antigen-binding fragments of such antibody chains, comprising an antigen-binding region and at least one CH domain. This heavy chain/light chain pair has binding specificity for a first antigen. The third polypeptide subunit comprises, consists essentially of, or consists of a heavy-chain only antibody comprising an Fc portion comprising CH2 and/or CH3 and/or CH4 domains, in the absence of a CH1 domain, and one or more antigen binding domains (e.g., two antigen binding domains) that binds an epitope of a second antigen or a different epitope of the first antigen, where such binding domain is derived from or has sequence identity with the variable region of an antibody heavy or light chain. Parts of such variable region may be encoded by V_H and/or V_L gene segments, D and J_H gene segments, or J_L gene segments. The variable region may be encoded by rearranged V_HDJ_H, V_LDJ_H, V_HJ_L, or V_LJ_L gene segments.