# TUMOR NECROSIS FACTOR (TNF) SUPERFAMILY RECEPTOR BINDING MOLECULES AND USES THEREOF

## BACKGROUND

**[0001]** Since the advent of humanized antibodies, the therapeutic use of antibodies such as Rituxan® (rituximab), Herceptin® (trastuzumab) and Avastin® (bevacizumab), has revolutionized the fields of medicine, including oncology, the treatment of inflammatory disorders, such as rheumatoid arthritis, and many other indications. In the United States, more than 30 human or humanized antibodies have been approved for clinical use, and more than 600 new antibodies or antibody-like molecules are in various stages of development. Some antibodies have antagonistic function on soluble target molecules such as vascular endothelial growth factor (VEGF) or tumor necrosis factor (TNF), whose actions are part of the pathologic process of a disease. Alternatively, antibodies can bind, block and/or induce destruction of pathologic cells in certain diseases, such as cancer. The main functions of these therapeutic antibodies are binding through the Fab region, and recruitment of effector function via the Fc domain (which also mediates the long circulating half-life of antibodies). One of the major advantages of antibodies compared to small molecule drugs can be their exquisite specificity. Antibodies can very accurately target selected protein antigens, such as oncogenes, to the exclusion of very similar homologs, allowing for benign safety profiles. Hence, antibodies are well characterized for specific single targeting function.

**[0002]** As the field has progressed, antibody function has been enhanced through creative means of protein engineering, such as to provide higher affinity, longer half-life, and/or better tissue distribution, as well as combination of small and large molecule technologies for increased focus of cell destruction via toxic payload delivery (e.g. antibody-drug conjugates). Another approach to improving antibody function takes advantage of the multivalent binding capabilities of the immunoglobulin A (IgA) or immunoglobulin M (IgM) structure which allows one IgA or IgM molecule to bind multiple antigens. Heavy and light chain variable domains of interest can be expressed as an IgA or IgM isotype antibody, thereby creating a multimeric binding molecule with the same specificity as a monomeric antibody, e.g., an IgG antibody.

**[0003]** The multivalent nature of IgA or IgM molecules presents a useful tool for application to specific biological systems in which multiple components necessarily must be bound simultaneously to transmit biological signals. For instance, many receptor proteins on the surface of eukaryotic cells require the simultaneous activation of multiple monomers or subunits to achieve activation and transmission of a biological signal across a cell membrane, to the cytoplasm of the cell.

**[0004]** One such system of cell surface protein receptors requiring multimerization prior to, or commensurate with, activation is found in the Tumor Necrosis Factor (TNF) superfamily of receptor proteins. Within this superfamily of receptor proteins are members which, upon activation, transmit a signal to the nucleus of the cell causing apoptosis. Other family members of this superfamily cause activation of NF-κB, apoptosis pathways, extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38MAPK), and c-Jun N-terminal kinase (JNK). Examples of TNF superfamily receptor members which regulate apoptosis of the cell when activated are the following: TNFR1 (DR1), TNFR2, TNFR1/2, CD40 (p50), Fas (CD95, Apo1, DR2), CD30, 4-1BB (CD137, ILA), TRAILR1 (DR4, Apo2), DR5 (TRAILR2), TRAILR3 (DcR1), TRAILR4 (DcR2), OPG (OCIF), TWEAKR (FN14), LIGHTR (HVEM), DcR3, DR3, EDAR, and XEDAR. (*See*, Aggarwal et al., *Blood*, 119:651-665, 2012).

**[0005]** More particularly, it is postulated that activation of the TNF superfamily receptor protein members mentioned above requires that at least three non-interacting receptor monomers be cross-linked, e.g., by a ligand, to form a stabilized receptor trimer, resulting in signal transduction across the cell membrane. Clustering of these TNF superfamily receptor protein trimers into "rafts" of trimers has been observed and has been postulated to lead to more effective activation of this TNF superfamily receptor protein-dependent signaling cascade. (*See*, Valley et al., *J. Biol. Chem.*, 287(25):21265-21278, 2012). Additional modes of activation have been discussed. (*See*, for instance, Lewis et al., *Biophys. J.*, 106(6):L21-L24, 2014).

**[0006]** Signaling through certain of the TNF superfamily receptor proteins noted above can lead to cell apoptosis. In the treatment of cancer, one therapeutic strategy is to activate an apoptotic signaling cascade in cancer cells, thereby halting progression. One manner in which this can be accomplished is by the binding of TNF superfamily receptor proteins expressed (or over-expressed) in cancer cells with a multivalent or multimeric agonist binding molecule, which can promote receptor trimerization and activation, leading to

apoptosis. One TNF superfamily receptor protein that is activated upon cross-linking resulting in apoptosis is DR5 (TRAILR2).

**[0007]** Interest- in DR5 is heightened due to the finding that it is expressed at a higher level in various cancers than in normal tissue, such as bladder cancer (Y et al., *Urology*, 79(4):968.e7-15, 2012), gastric cancer (Lim et al., *Carcinogen.*, 32(5):723-732, 2011), ovarian cancer (Jiang et al., *Mol. Med. Rep.*, 6(2):316-320, 2012), pancreatic ductal adenocarcinoma (Rajeshkumar et al., *Mol. Cancer Ther.*, 9(9):2583-92, 2010), oral squamous cell carcinoma (Chen et al. *Oncotarget* 4:206-217, 2013) and non-small cell lung cancer (Reck et al., *Lung Canc.*, 82(3):441-448, 2013). It is of additional importance to the medical community that the observed higher level of expression of this family of receptor proteins, especially family member DR5, occurs in some of the most difficult to detect and treat cancers, such as pancreatic and gastric cancer.

**[0008]** While certain monoclonal antibodies, such as Tigatuzumab (CS-1008, Daiichi Sankyo Co. Ltd., disclosed in U.S. Patent No. 7,244,429, VH and VL presented herein as SEQ ID NO: 7 and SEQ ID NO: 8, respectively), have been found to be effective *in vitro* and *in vivo* even without additional cross-linkers added, these antibodies have not resulted in significant clinical efficacy. (*See*, Reck et al., 2013). Examples of such anti-DR5 agonistic monoclonal IgG antibodies are Conatumumab (Amgen, described in US Patent No. 7,521,048, VH and VL presented herein as SEQ ID NO: 5 and SEQ ID NO: 6, respectively), Drozitumab (Genentech, as described in U.S. Patent No. 8,029,783, VH and VL presented herein as SEQ ID NO: 3 and SEQ ID NO: 4, respectively), and Lexatumumab (Human Genome Sciences, as disclosed in U.S. Patent Application Publication No. 2006/0269555, VH and VL presented herein as SEQ ID NO: 1 and SEQ ID NO: 2, respectively).

**[0009]** Better binding molecules are needed to achieve the benefits of the basic research performed which provided a critical understanding of this subset of the TNF superfamily receptor proteins. Additional binding molecules are disclosed herein which, based on the understanding of the underlying biochemical mechanism of the TNF superfamily of receptor proteins, are capable of addressing this need.

## SUMMARY

**[0010]** This disclosure provides a multimeric, e.g., dimeric, pentameric, or hexameric binding molecule including two, five, or six bivalent binding units or variants or fragments thereof,

where each binding unit includes two IgA or IgM heavy chain constant regions or fragments thereof, each associated with an antigen-binding domain, where at least three of the antigen-binding domains of the binding molecule specifically and agonistically bind to a tumor necrosis factor (TNF) superfamily receptor protein that can induce apoptosis of a cell expressing the TNF superfamily receptor protein, and where the binding molecule can cross-link at least three identical TNF superfamily receptor proteins expressed on the surface of a cell, thereby inducing apoptosis of the cell.

**[0011]** In certain aspects a dimeric, pentameric, or hexameric binding molecule as provided herein can induce TNF superfamily receptor-mediated apoptosis in a TNF receptor superfamily-expressing cell at a higher potency than an equivalent amount of a bivalent IgG antibody or fragment thereof, which also specifically binds to and agonizes the same TNF superfamily receptor protein. In certain aspects, the three or more antigen-binding domains that specifically bind to and agonize the TNF superfamily receptor protein do not cross-react with other TNF superfamily receptor proteins. In certain aspects, the three or more antigen-binding domains that specifically bind to and agonize the TNF superfamily receptor protein can cross-react with other TNF superfamily receptor proteins.

**[0012]** In certain aspects, a dimeric, pentameric, or hexameric binding molecule as provided herein can include at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains that specifically and agonistically bind to a TNF superfamily receptor protein expressed on the surface of the cell, thereby inducing apoptosis of the cell. In certain aspects, the at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains bind to the same extracellular epitope of a single type of TNF superfamily receptor molecule expressed on the surface of the cell. In certain aspects, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains each specifically bind one of a group of two or more different extracellular epitopes of a single type of TNF superfamily receptor molecule expressed on the surface of the cell.

**[0013]** The binding units of a dimeric, pentameric, or hexameric binding molecule provided herein can be human, humanized, or chimeric immunoglobulin binding units.

**[0014]** In certain aspects, a dimeric, pentameric, or hexameric binding molecule as provided herein can bind to, without limitation, TNFR1 (DR1), TNFR2, TNFR1/2, CD40 (p50), Fas (CD95, Apo1, DR2), CD30, 4-1BB (CD137, ILA), TRAILR1 (DR4, Apo2), TRAILR2

(DR5), TRAILR3 (DcR1), TRAILR4 (DcR2), OPG (OCIF), TWEAKR (FN14), LIGHTR (HVEM), DcR3, DR3, EDAR, and XEDAR. In certain aspects, the binding molecule includes at least three antigen-binding domains that can specifically and agonistically bind to DR5. In certain aspects, the antigen binding domains do not bind to DR4, DcR1, or DcR2. In certain aspects the binding molecule includes at least three antigen-binding domains that can also specifically bind to DR4. In certain aspects, DR5 is expressed on a cancer cell.

**[0015]** In certain aspects a dimeric, pentameric, or hexameric binding molecule is provided where at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains include a heavy chain variable region (VH) and a light chain variable region (VL), where the VH and VL include six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, where the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 include the CDRs of an antibody including the VH and VL amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 7 and SEQ ID NO: 8; SEQ ID NO: 9 and SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 13 and SEQ ID NO: 14; SEQ ID NO: 15 and SEQ ID NO: 16; SEQ ID NO: 17 and SEQ ID NO: 18; SEQ ID NO: 19 and SEQ ID NO: 20; SEQ ID NO: 21 and SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 24; SEQ ID NO: 25 and SEQ ID NO: 26; SEQ ID NO: 27 and SEQ ID NO: 28; SEQ ID NO: 29 and SEQ ID NO: 30; SEQ ID NO: 31 and SEQ ID NO: 32; SEQ ID NO: 33 and SEQ ID NO: 34; SEQ ID NO: 35 and SEQ ID NO: 36; SEQ ID NO: 37 and SEQ ID NO: 38; SEQ ID NO: 39 and SEQ ID NO: 40; SEQ ID NO: 41 and SEQ ID NO: 42; SEQ ID NO: 43 and SEQ ID NO: 44; SEQ ID NO: 45 and SEQ ID NO: 46; SEQ ID NO: 47 and SEQ ID NO: 48; SEQ ID NO: 49 and SEQ ID NO: 50; SEQ ID NO: 51 and SEQ ID NO: 52; SEQ ID NO: 53 and SEQ ID NO: 54; SEQ ID NO: 55 and SEQ ID NO: 56; SEQ ID NO: 82 and SEQ ID NO: 83; SEQ ID NO: 84 and SEQ ID NO: 85; SEQ ID NO: 86 and SEQ ID NO: 87; or SEQ ID NO: 88 and SEQ ID NO: 89; respectively, or the ScFv sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73 or the six CDRs with one or two amino acid substitutions in one or more of the CDRs.

**[0016]** In certain aspects a dimeric, pentameric, or hexameric binding molecule is provided where at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains include an antibody VH and a VL, where the VH and VL include amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 7 and SEQ ID NO: 8; SEQ ID NO: 9 and SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 13 and SEQ ID NO: 14; SEQ ID NO: 15 and SEQ ID NO: 16; SEQ ID NO: 17 and SEQ ID NO: 18; SEQ ID NO: 19 and SEQ ID NO: 20; SEQ ID NO: 21 and SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 24; SEQ ID NO: 25 and SEQ ID NO: 26; SEQ ID NO: 27 and SEQ ID NO: 28; SEQ ID NO: 29 and SEQ ID NO: 30; SEQ ID NO: 31 and SEQ ID NO: 32; SEQ ID NO: 33 and SEQ ID NO: 34; SEQ ID NO: 35 and SEQ ID NO: 36; SEQ ID NO: 37 and SEQ ID NO: 38; SEQ ID NO: 39 and SEQ ID NO: 40; SEQ ID NO: 41 and SEQ ID NO: 42; SEQ ID NO: 43 and SEQ ID NO: 44; SEQ ID NO: 45 and SEQ ID NO: 46; SEQ ID NO: 47 and SEQ ID NO: 48; SEQ ID NO: 49 and SEQ ID NO: 50; SEQ ID NO: 51 and SEQ ID NO: 52; SEQ ID NO: 53 and SEQ ID NO: 54; SEQ ID NO: 55 and SEQ ID NO: 56; SEQ ID NO: 82 and SEQ ID NO: 83; SEQ ID NO: 84 and SEQ ID NO: 85; SEQ ID NO: 86 and SEQ ID NO: 87; or SEQ ID NO: 88 and SEQ ID NO: 89; respectively, or where the VH and VL are contained in an ScFv with an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

**[0017]** In certain aspects, the binding molecule as provided herein as a dimeric IgA molecule further including a J chain

**[0018]** In certain aspects, the binding molecule as provided herein is a pentameric IgM molecule further including a J chain.

**[0019]** In certain aspects, the binding molecule as provided herein is a hexameric IgM molecule.

**[0020]** The disclosure further provides a composition including a dimeric, pentameric, or hexameric binding molecule as provided herein.

[0021] The disclosure further provides a polynucleotide that includes a nucleic acid sequence encoding a polypeptide subunit, e.g., a heavy or light chain of a binding molecule provided herein. In certain aspects, the polypeptide subunit includes a human IgA or IgM constant region or fragment thereof fused to the C-terminal end of a VH including: (a) HCDR1, HCDR2, and HCDR3 regions including the CDRs contained in the VH amino acid sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88, or in the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73; or the CDRs contained in the VH amino acid sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88, or in the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73 with one or two single amino acid substitutions in one or more of the HCDRs; or (b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID

NO: 88, or the VH portion of an ScFv with the amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

**[0022]** In certain aspects, the polypeptide subunit includes a light chain constant region or fragment thereof fused to the C-terminal end of the polypeptide subunit to an antibody VL portion of the antigen-binding domain of the dimeric, pentameric, or hexameric binding molecule. In certain aspects the polypeptide subunit includes a human kappa or lambda light chain constant region or fragment thereof fused to the C-terminal end of a VL including: (a) LCDR1, LCDR2, and LCDR3 regions including the CDRs contained in the VL amino acid sequence SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, or SEQ ID NO: 89, or in the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73; or the CDRs contained in the VL amino acid sequence SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, or SEQ ID NO: 89, or in the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73, with one or two single amino acid substitutions in one or more of the LCDRs; or (b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID

NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, or SEQ ID NO: 89, or the VL portion of an ScFv with the amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

**[0023]** The disclosure further provides a composition including one, two, or more polynucleotides as provided herein. In certain aspects a composition is provided where the polynucleotides are on separate vectors. Such vectors are provided. In certain aspects, a composition is provided where the polynucleotides are on a single vector. Such a vector is provided. In certain aspects, the composition further includes a polynucleotide including a nucleic acid sequence encoding a J chain, or fragment thereof, or variant thereof.

**[0024]** The disclosure also provides a host cell including a polynucleotide as provided herein, a composition as provided herein, or the vector or vectors provided herein, where the host cell can express a dimeric, pentameric, or hexameric binding molecule as provided herein. The disclosure further provides a method of producing the binding molecule as provided herein, where the method includes culturing the host cell and recovering the binding molecule.

**[0025]** In another aspect, the disclosure provides a method of inducing TNF superfamily receptor-mediated apoptosis in a TNF superfamily receptor-expressing cell, where the method includes contacting the TNF superfamily receptor-expressing cell with a dimeric, pentameric, or hexameric binding molecule as provided herein.

**[0026]** In another aspect, the disclosure provides a method of inducing TNF superfamily receptor lipid raft formation in a TNF superfamily receptor-expressing cell, including contacting the TNF superfamily receptor-expressing cell with a dimeric, pentameric, or hexameric binding molecule as provided herein.

**[0027]** In another aspect, the disclosure provides a method of treating cancer, where the method includes administering to a subject in need thereof an effective amount of a dimeric, pentameric, or hexameric binding molecule as provided herein, where the cancer cells

express a TNF superfamily receptor with apoptotic activity. In certain aspects the multimeric binding molecule can induce greater apoptosis of cancer cells than non-cancer cells, e.g., normal hepatocytes, e.g., normal human hepatocytes. In certain aspects, the TNF superfamily receptor is DR5. In certain aspects, the subject is human.

## BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

**[0028]** **Figure 1A and Figure 1B: DR5 Expression Profiling on Cell Lines.** Figure 1A) Surface expression of human DR5 on Colo205 cells, measured by flow cytometry; Figure 1B) Mean fluorescence intensity (MFI) of DR5 expression on a panel of cell lines.

**[0029]** **Figure 2: Anti-DR5 mAb is Specific for DR5.** Anti-human DR5 mAb binds specifically to DR5, and not DR4 or decoy receptors DcR1 and DcR2 as measured by ELISA.

**[0030]** **Figure 3: Anti-DR5 mAb Cell Binding.** Anti-human DR5 mAb or Isotype control were incubated with Colo205 cells for 15 minutes, washed, and stained with an allophycocyanin- conjugated secondary antibody. Binding was measured by flow cytometry.

**[0031]** **Figure 4: Anti-DR5 IgG Requires Crosslinker for Cytotoxicity.** Colo205 cells were incubated with anti-DR5 mAb in the absence or presence of crosslinker. Cell viability was measured after 24 hours. Isotype control displayed no cytotoxicity with or without crosslinker (data not shown).

**[0032]** **Figure 5: Anti-DR5 IgG Requires Crosslinker for Apoptosis.** Colo205 cells were incubated with 5 μg/mL anti-DR5 mAb in the absence or presence of crosslinker. After 4 hour treatment, Annexin V and 7-AAD were used to measure apoptotic and dead cells, respectively.

**[0033]** **Figure 6: Anti-DR5 IgG Requires Crosslinker for Caspase Activation.** Colo205 cells were incubated with 5 μg/mL anti-DR5 mAb in the absence or presence of crosslinker. Caspase activation was measured after 1, 2, 4, and 24 hours of treatment.

**[0034]** **Figure 7A-D: Multimeric Anti-DR5 mAb is More Cytotoxic than Monomeric IgG.**

**[0035]** **Figure 7A**: Non-reducing SDS-PAGE shows one anti-DR5 mAb that is predominantly multimeric (lane 1); Lane 1 corresponds to R&D Systems clone 71903, Lane 2 corresponds with BioLegend clone DJR2-4, Lane 3 corresponds to Acris Antibodies clone B-K29, and Lane 4 corresponds to Acris Antibodies clone B-D37.

**[0036]** **Figure 7B**: Cell viability assay showing that only the multimeric anti-DR5 mAb causes Colo205 cytotoxicity in the absence of crosslinker - R&D Systems clone 71903 (filled squares), BioLegend clone DJR2-4 (open circles, dashed line), Acris Antibodies clone B-K29 (filled diamonds), Acris Antibodies clone B-D37 (open triangles, dashed line).

**[0037]** **Figure 7C**: FACS assay results showing that in the absence of crosslinker, the multimeric anti-DR5 mAb induces apoptosis in Colo205 cells, but similar results are not observed for the monomeric anti-DR5 mAb or isotype control.

**[0038]** **Figure 7D**: Caspase activation luminescence assay showing that in the absence of crosslinker the multimeric, but not monomeric, anti-DR5 mAb induces caspase activation in Colo205 cells.

**[0039]** **Figure 8A-B:** DR5 MAb IgM is Specific for DR5. DR5 MAb IgG (Panel A) and IgM (Panel B) #2 binds specifically to human DR5, and not DR4 or decoy receptors DcR1 and DcR2 as measured by ELISA. DR5, filled circles; DR4, filled triangles; DcR1, open squares; DcR2, open triangles.

**[0040]** **Figure 9**: DR5 MAb IgM Target Cell Binding. DR5 MAb IgM #1 (filled circles) or DR5 MAb IgG #1 (open squares) were incubated with Colo205 cells for 15 minutes, washed, and stained with an Anti-Human IgM or Anti-Human IgG Fc Alexa 647-conjugated secondary antibody. Binding (expressed as % of cells bound) was measured by flow cytometry.

**[0041]** **Figure 10A-E**: DR5 MAb IgM Superagonists are More Cytotoxic than Monomeric IgG. Multimeric DR5 MAbs IgM #1 (Panel A), IgM #2 (Panel B), IgM #3 (Panel C), and IgM #4 (Panel D) are more cytotoxic than IgG equivalents on Colo205 cells. Panel E shows that DR5 MAb IgM #1 is more cytotoxic than crosslinked IgG. DR5 MAb IgM, filled circles; DR5 MAb IgG, open squares; DR5 MAb IgG + crosslinker, open triangles.

**[0042]** **Figure 11A-D:** DR5 MAb IgM Superagonists are More Cytotoxic on Colo205 Tumor Cells than Primary Human Hepatocytes. Multimeric DR5 MAb IgM #1 (Panel A), DR5 MAb IgM #2 (Panel B), DR5 MAb IgM #3 (Panel C), and DR5 MAb IgM #4 (Panel D) were incubated with Colo205 tumor cells or primary human hepatocytes and cell viability was measured after 24 hours. DR5 MAb IgM treated Colo205 cells, filled circles; DR5 MAb IgM treated hepatocytes, open circles.

# DETAILED DESCRIPTION

## Definitions

**[0043]** It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule," is understood to represent one or more binding molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

**[0044]** Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

**[0045]** Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

**[0046]** Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

**[0047]** As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides,

oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of "polypeptide,” and the term “polypeptide” can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, and derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

**[0048]** A polypeptide as disclosed herein can be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides can have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded. As used herein, the term glycoprotein refers to a protein coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid, *e.g.*, a serine or an asparagine.

**[0049]** By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated as disclosed herein, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

**[0050]** As used herein, the term “a non-naturally occurring polypeptide” or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the polypeptide that are, or might be, determined or interpreted by a judge or an administrative or judicial body, to be “naturally-occurring.”

**[0051]** Other polypeptides disclosed herein are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" as disclosed herein include any polypeptides which retain at least

some of the properties of the corresponding native antibody or polypeptide, for example, specifically binding to an antigen. Fragments of polypeptides include, for example, proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of, e.g., a polypeptide include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. In certain aspects, variants can be non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives are polypeptides that have been altered so as to exhibit additional features not found on the original polypeptide. Examples include fusion proteins. Variant polypeptides can also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a polypeptide can also refer to a subject polypeptide having one or more amino acids chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides that contain one or more derivatives of the twenty standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

**[0052]** A "conservative amino acid substitution" is one in which one amino acid is replaced with another amino acid having a similar side chain. Families of amino acids having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. In certain embodiments, conservative substitutions in the sequences of the polypeptides and antibodies of the present disclosure do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen to which the binding molecule binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen-binding are well-known in the art (see, *e.g.*, Brummell *et al.*, *Biochem.* 32: 1180-1 187 (1993); Kobayashi *et*

*al.*, *Protein Eng.* 12(10):879-884 (1999); and Burks *et al.*, *Proc. Natl. Acad. Sci. USA* 94:.412-417 (1997)).

**[0053]** The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA), cDNA, or plasmid DNA (pDNA). A polynucleotide can comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The terms "nucleic acid" or "nucleic acid sequence" refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide.

**[0054]** By an "isolated" nucleic acid or polynucleotide is intended any form of the nucleic acid or polynucleotide that is separated from its native environment. For example, gel-purified polynucleotide, or a recombinant polynucleotide encoding a polypeptide contained in a vector would be considered to be "isolated." Also, a polynucleotide segment, e.g., a PCR product, which has been engineered to have restriction sites for cloning is considered to be "isolated." Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in a non-native solution such as a buffer or saline. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides, where the transcript is not one that would be found in nature. Isolated polynucleotides or nucleic acids further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid can be or can include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

**[0055]** As used herein, the term "a non-naturally occurring polynucleotide" or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the nucleic acid or polynucleotide that are, or might be, determined or interpreted by a judge, or an administrative or judicial body, to be "naturally-occurring."

**[0056]** 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 can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 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 can

contain a single coding region, or can comprise two or more coding regions, *e.g.*, a single vector can separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid can include heterologous coding regions, either fused or unfused to another coding region. Heterologous coding regions include without limitation, those encoding specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

**[0057]** In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. 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 can be a cell-specific promoter that directs substantial transcription of the DNA 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.

**[0058]** 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 (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-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 lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

**[0059]** 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 picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

**[0060]** In other embodiments, a polynucleotide can be RNA, for example, in the form of messenger RNA (mRNA), transfer RNA, or ribosomal RNA.

**[0061]** Polynucleotide and nucleic acid coding regions can be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide as disclosed herein. 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 can have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, 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, can be used. For example, the wild-type leader sequence can be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse ß-glucuronidase.

**[0062]** As used herein, the terms “TNF superfamily of receptor proteins,” “TNF superfamily,” “TNF receptor family,” “TNF receptors” or any combination of such phrases, refer to the family of Tumor Necrosis Factor transmembrane receptor proteins expressed on the surface of various cells and tissues. Family members of this superfamily include those that, upon activation by ligand binding trigger apoptosis, proliferation and/or morphogenesis in the cell in which the receptor protein is expressed. TNF superfamily receptor protein members that trigger apoptosis upon activation include, but are not limited to the following receptors: TNFR1 (DR1), TNFR2, TNFR1/2, CD40 (p50), Fas (CD95, Apo1, DR2), CD30, 4-1BB (CD137, ILA), TRAILR1 (DR4, Apo2), TRAILR2 (DR5), TRAILR3 (DcR1), TRAILR4 (DcR2), OPG (OCIF), TWEAKR (FN14), LIGHTR (HVEM), DcR3, DR3, EDAR, and

XEDAR. TNF superfamily receptor protein members which, upon activation, trigger proliferation include, but are not limited to the following receptors: TNFR1/2, GITR (AITR), TACI, BCMA, TWEAKR (FN14), RANK (TRANCER), CD27, CD40 (p50), OX40 (CD134), LT-βR, TNFR1 (DR1) and TNFR2. TNF superfamily receptor protein members which, upon activation, are believed to trigger morphogenesis include, but are not limited to the following receptors: Fas (CD95, Apo1, DR2), TRAILR1 (DR4, Apo2), DR5 (TRAILR2), TRAILR3 (DcR1), TRAILR4 (DcR2), OPG (OCIF), CD40 (p50), EDAR, XEDAR, and TNFR1/2.

**[0063]** Disclosed herein are certain binding molecules, or antigen-binding fragments, variants, or derivatives thereof that bind to certain TNF superfamily receptor proteins, thereby eliciting cellular apoptosis. Unless specifically referring to full-sized antibodies, the term "binding molecule" encompasses full-sized antibodies as well as antigen-binding subunits, fragments, variants, analogs, or derivatives of such antibodies, e.g., engineered antibody molecules or fragments that bind antigen in a manner similar to antibody molecules, but which use a different scaffold.

**[0064]** As used herein, the term “binding molecule” refers in its broadest sense to a molecule that specifically binds to a receptor, e.g., an epitope or an antigenic determinant. As described further herein, a binding molecule can comprise one of more “antigen binding domains” described herein. A non-limiting example of a binding molecule is an antibody or fragment thereof that retains antigen-specific binding.

**[0065]** As used herein, the terms “binding domain” or “antigen binding domain” refer to a region of a binding molecule that is necessary and sufficient to specifically bind to an epitope. For example, an “Fv,” e.g., a variable heavy chain and variable light chain of an antibody, either as two separate polypeptide subunits or as a single chain, is considered to be a “binding domain.” Other binding domains include, without limitation, the variable heavy chain (VHH) of an antibody derived from a camelid species, or six immunoglobulin complementarity determining regions (CDRs) expressed in a fibronectin scaffold. A “binding molecule” as described herein can include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more “antigen binding domains.”

**[0066]** The terms "antibody" and "immunoglobulin" can be used interchangeably herein. An antibody (or a fragment, variant, or derivative thereof as disclosed herein) includes at least the variable domain of a heavy chain (for camelid species) or at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are

relatively well understood. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988). Unless otherwise stated, the term "antibody" encompasses anything ranging from a small antigen-binding fragment of an antibody to a full sized antibody, e.g., an IgG antibody that includes two complete heavy chains and two complete light chains, an IgA antibody that includes four complete heavy chains and four complete light chains and optionally includes a J chain and/or a secretory component, or an IgM antibody that includes ten or twelve complete heavy chains and ten or twelve complete light chains and optionally includes a J chain.

**[0067]** As will be discussed in more detail below, the term "immunoglobulin" comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, ($\gamma$, $\mu$, $\alpha$, $\delta$, $\varepsilon$) with some subclasses among them (*e.g.*, $\gamma$1-$\gamma$4 or $\alpha$1-$\alpha$2)). It is the nature of this chain that determines the "isotype" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (subtypes) *e.g.*, $IgG_1$, $IgG_2$, $IgG_3$, $IgG_4$, $IgA_1$, $IgA_2$, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these immunoglobulins are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of this disclosure.

**[0068]** Light chains are classified as either kappa or lambda ($\kappa$, $\lambda$). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are expressed, e.g., by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. The basic structure of certain antibodies, e.g., IgG antibodies, includes two heavy chain subunits and two light chain subunits covalently connected via disulfide bonds to form a "Y" structure, also referred to herein as an "H2L2" structure, or a "binding unit."

**[0069]** The term "binding unit" is used herein to refer to the portion of a binding molecule, e.g., an antibody or antigen-binding fragment thereof, which corresponds to a standard "H2L2" immunoglobulin structure, i.e., two heavy chains or fragments thereof and two light chains or fragments thereof. In certain aspects, e.g., where the binding molecule is a bivalent

IgG antibody or antigen-binding fragment thereof, the terms "binding molecule" and "binding unit" are equivalent. In other aspects, e.g., where the binding molecule is an IgA dimer, an IgM pentamer, or an IgM hexamer, the binding molecule comprises two or more "binding units." Two in the case of an IgA dimer, or five or six in the case of an IgM pentamer or hexamer, respectively. A binding unit need not include full-length antibody heavy and light chains, but will typically be bivalent, i.e., will include two "binding domains," as defined below. Certain binding molecules provided in this disclosure are dimeric, and include two bivalent binding units that include IgA constant regions or fragments thereof. Certain binding molecules provided in this disclosure are pentameric or hexameric, and include five or six bivalent binding units that include IgM constant regions or fragments thereof. A binding molecule comprising two or more, e.g., two, five, or six binding units, is referred to herein as "multimeric."

**[0070]** The terms "valency," "bivalent," "multivalent" and grammatical equivalents, refer to the number of binding domains in given binding molecule or binding unit. As such, the terms "bivalent", "tetravalent", and "hexavalent" in reference to a given binding molecule, e.g., an IgM antibody or fragment thereof, denote the presence of two binding domains, four binding domains, and six binding domains, respectively. In a typical IgM-derived binding molecule where each binding unit is bivalent, the binding molecule itself can have 10 or 12 valencies. A bivalent or multivalent binding molecule can be monospecific, i.e., all of the binding domains are the same, or can be bispecific or multispecific, e.g., where two or more binding domains are different, e.g., bind to different epitopes on the same antigen, or bind to entirely different antigens.

**[0071]** The term "epitope" includes any molecular determinant capable of specific binding to an antibody. In certain aspects, an epitope can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain aspects, can have a three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of a target that is bound by an antibody.

**[0072]** The term "target" is used in the broadest sense to include substances that can be bound by a binding molecule. A target can be, e.g., a polypeptide, a nucleic acid, a carbohydrate, a lipid, or other molecule. Moreover, a "target" can, for example, be a cell, an organ, or an organism that comprises an epitope bound that can be bound by a binding molecule.

**[0073]** Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will

be appreciated that the variable domains of both the variable light (VL) and variable heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (e.g., CH1, CH2 or CH3) confer biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 (or CH4 in the case of IgM) and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

**[0074]** A "full length IgM antibody heavy chain" is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable domain (VH), an antibody constant heavy chain constant domain 1 (CM1 or Cμ1), an antibody heavy chain constant domain 2 (CM2 or Cμ2), an antibody heavy chain constant domain 3 (CM3 or Cμ3), and an antibody heavy chain constant domain 4 (CM4 or Cμ4) that can include a tailpiece.

**[0075]** A "full length IgA antibody heavy chain" is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable domain (VH), an antibody constant heavy chain constant domain 1 (CA1 or Cα1), an antibody heavy chain constant domain 2 (CA2 or Cα2), and an antibody heavy chain constant domain 3 (CA3 or Cα3) that can include a tailpiece.

**[0076]** As indicated above, variable region(s) allows a binding molecule to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs), of a binding molecule, *e.g.*, an antibody, combine to form the antigen binding domain. More specifically, an antigen binding domain can be defined by three CDRs on each of the VH and VL chains. Certain antibodies form larger structures. For example, IgA can form a molecule that includes two H2L2 binding units and a J chain covalently connected via disulfide bonds, which can be further associated with a secretory component, and IgM can form a pentameric or hexameric molecule that includes five or six H2L2 binding units and optionally a J chain covalently connected via disulfide bonds.

**[0077]** The six "complementarity determining regions" or "CDRs" present in an antibody antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in

the binding domain, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids that make up the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been defined in various different ways (*see*, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, *196*:901-917 (1987), which are incorporated herein by reference in their entireties).

**[0078]** In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described, for example, by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference. The Kabat and Chothia definitions include overlapping or subsets of amino acids when compared against each other. Nevertheless, application of either definition (or other definitions known to those of ordinary skill in the art) to refer to a CDR of an antibody or variant thereof is intended to be within the scope of the term as defined and used herein, unless otherwise indicated. The appropriate amino acids which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact amino acid numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which amino acids comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1 CDR Definitions*

| | Kabat | Chothia |
|---|---|---|
| VH CDR1 | 31-35 | 26-32 |
| VH CDR2 | 50-65 | 52-58 |
| VH CDR3 | 95-102 | 95-102 |
| VL CDR1 | 24-34 | 26-32 |
| VL CDR2 | 50-56 | 50-52 |
| VL CDR3 | 89-97 | 91-96 |

*Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat *et al.* (see below).

**[0079]** Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless use of the Kabat numbering system is explicitly noted, however, consecutive numbering is used for all amino acid sequences in this disclosure.

**[0080]** Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof include, but are not limited to, polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

**[0081]** By "specifically binds," it is generally meant that a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof binds to an epitope via its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, a binding molecule is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain binding molecule binds to a certain epitope. For example, binding molecule "A" can be deemed to have a higher specificity for a given

epitope than binding molecule "B," or binding molecule "A" can be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

**[0082]** A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof disclosed herein can be said to bind a target antigen with an off rate (k(off)) of less than or equal to 5 X $10^{-2}$ $sec^{-1}$, $10^{-2}$ $sec^{-1}$, 5 X $10^{-3}$ $sec^{-1}$, $10^{-3}$ $sec^{-1}$, 5 X $10^{-4}$ $sec^{-1}$, $10^{-4}$ $sec^{-1}$, 5 X $10^{-5}$ $sec^{-1}$, or $10^{-5}$ $sec^{-1}$ 5 X $10^{-6}$ $sec^{-1}$, $10^{-6}$ $sec^{-1}$, 5 X $10^{-7}$ $sec^{-1}$ or $10^{-7}$ $sec^{-1}$.

**[0083]** A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target antigen with an on rate (k(on)) of greater than or equal to $10^{3}$ $M^{-1}$ $sec^{-1}$, 5 X $10^{3}$ $M^{-1}$ $sec^{-1}$, $10^{4}$ $M^{-1}$ $sec^{-1}$, 5 X $10^{4}$ $M^{-1}$ $sec^{-1}$, $10^{5}$ $M^{-1}$ $sec^{-1}$, 5 X $10^{5}$ $M^{-1}$ $sec^{-1}$, $10^{6}$ $M^{-1}$ $sec^{-1}$, or 5 X $10^{6}$ $M^{-1}$ $sec^{-1}$ or $10^{7}$ $M^{-1}$ $sec^{-1}$.

**[0084]** A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof is said to competitively inhibit binding of a reference antibody or antigen binding fragment to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody or antigen binding fragment to the epitope. Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays. A binding molecule can be said to competitively inhibit binding of the reference antibody or antigen binding fragment to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

**[0085]** As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with one or more binding domains, e.g., of an immunoglobulin molecule. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of binding domains and an antigen. *See*, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual binding domains in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity. An interaction between a between a bivalent monoclonal antibody with a receptor present at a high density on a cell surface would also be of high avidity.

**[0086]** Binding molecules or antigen-binding fragments, variants or derivatives thereof as disclosed herein can also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of a binding molecule, *e.g.*, an

antibody or fragment, variant, or derivative thereof, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, a binding molecule is cross reactive if it binds to an epitope other than the one that induced its formation. The cross reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, can actually fit better than the original.

**[0087]** A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can also be described or specified in terms of their binding affinity to an antigen. For example, a binding molecule can bind to an antigen with a dissociation constant or $K_D$ no greater than 5 x $10^{-2}$ M, $10^{-2}$ M, 5 x $10^{-3}$ M, $10^{-3}$ M, 5 x $10^{-4}$ M, $10^{-4}$ M, 5 x $10^{-5}$ M, $10^{-5}$ M, 5 x $10^{-6}$ M, $10^{-6}$ M, 5 x $10^{-7}$ M, $10^{-7}$ M, 5 x $10^{-8}$ M, $10^{-8}$ M, 5 x $10^{-9}$ M, $10^{-9}$ M, 5 x $10^{-10}$ M, $10^{-10}$ M, 5 x $10^{-11}$ M, $10^{-11}$ M, 5 x $10^{-12}$ M, $10^{-12}$ M, 5 x $10^{-13}$ M, $10^{-13}$ M, 5 x $10^{-14}$ M, $10^{-14}$ M, 5 x $10^{-15}$ M, or $10^{-15}$ M.

**[0088]** Antibody fragments including single-chain antibodies or other binding domains can exist alone or in combination with one or more of the following: hinge region, CH1, CH2, CH3, or CH4 domains, J chain, or secretory component. Also included are antigen-binding fragments that can include any combination of variable region(s) with one or more of a hinge region, CH1, CH2, CH3, or CH4 domains, a J chain, or a secretory component. Binding molecules, *e.g.*, antibodies, or antigen-binding fragments thereof can be from any animal origin including birds and mammals. The antibodies can be human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region can be condricthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and can in some instances express endogenous immunoglobulins and some not, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.*

**[0089]** As used herein, the term “heavy chain subunit” includes amino acid sequences derived from an immunoglobulin heavy chain, a binding molecule, *e.g.*, an antibody comprising a heavy chain subunit can include at least one of: a VH domain, a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH4 domain, or a variant or fragment thereof. For example, a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can include without limitation, in addition to a VH

domain:, a CH1 domain; a CH1 domain, a hinge, and a CH2 domain; a CH1 domain and a CH3 domain; a CH1 domain, a hinge, and a CH3 domain; or a CH1 domain, a hinge domain, a CH2 domain, and a CH3 domain. In certain aspects a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can include, in addition to a VH domain, a CH3 domain and a CH4 domain; or a CH3 domain, a CH4 domain, and a J chain. Further, a binding molecule for use in the disclosure can lack certain constant region portions, *e.g.*, all or part of a CH2 domain. It will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain subunit) can be modified such that they vary in amino acid sequence from the original immunoglobulin molecule.

**[0090]** As used herein, the term "light chain subunit" includes amino acid sequences derived from an immunoglobulin light chain. The light chain subunit includes at least a VL, and can further include a CL (e.g., Cκ or Cλ) domain.

**[0091]** Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof can be described or specified in terms of the epitope(s) or portion(s) of an antigen that they recognize or specifically bind. The portion of a target antigen that specifically interacts with the antigen binding domain of an antibody is an "epitope," or an "antigenic determinant." A target antigen can comprise a single epitope or at least two epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen.

**[0092]** As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term "VH domain" includes the amino terminal variable domain of an immunoglobulin heavy chain and the term "CH1 domain" includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of a typical IgG heavy chain molecule.

**[0093]** As used herein the term "CH2 domain" includes the portion of a heavy chain molecule that extends, *e.g.*, from about amino acid 244 to amino acid 360 of an IgG antibody using conventional numbering schemes (amino acids 244 to 360, Kabat numbering system; and amino acids 231-340, EU numbering system; see Kabat EA *et al.*, *op. cit.* The CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 amino acids. Certain immunoglobulin classes, e.g., IgM, further include a CH4 region.

**[0094]** As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain in IgG, IgA, and IgD heavy chains. This hinge region comprises approximately 25 amino acids and is flexible, thus allowing the two N-terminal antigen binding regions to move independently.

**[0095]** As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group.

**[0096]** As used herein, the term "chimeric antibody" refers to an antibody in which the immunoreactive region or site is obtained or derived from a first species and the constant region (which can be intact, partial or modified) is obtained from a second species. In some embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

**[0097]** The terms "multispecific antibody" or "bispecific antibody" refer to an antibody that has binding domains for two or more different epitopes within a single antibody molecule. Other binding molecules in addition to the canonical antibody structure can be constructed with two binding specificities. Epitope binding by bispecific or multispecific antibodies can be simultaneous or sequential. Triomas and hybrid hybridomas are two examples of cell lines that can secrete bispecific antibodies. Bispecific antibodies can also be constructed by recombinant means. (Ströhlein and Heiss, *Future Oncol.* *6*:1387-94 (2010); Mabry and Snavely, *IDrugs.* *13*:543-9 (2010)). A bispecific antibody can also be a diabody.

**[0098]** As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy and light chain or both is altered by at least partial replacement of one or more amino acids in either the CDR or framework regions. In certain aspects entire CDRs from an antibody of known specificity can be grafted into the framework regions of a heterologous antibody. Although alternate CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, CDRs can also be derived from an antibody of different class, *e.g.*, from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity are grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." In certain aspects not all of the CDRs are replaced with the complete CDRs from the donor variable region and yet the antigen binding capacity of the donor can still be transferred to the recipient variable domains. Given the explanations set forth in, *e.g.*, U. S.

Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of those skilled in the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional engineered or humanized antibody.

**[0099]** As used herein the term “engineered” includes manipulation of nucleic acid or polypeptide molecules by synthetic means (*e.g.* by recombinant techniques, *in vitro* peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques).

**[0100]** As used herein, the terms "linked," "fused" or "fusion" or other grammatical equivalents can be used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the translational reading frame of the original ORFs. Thus, a recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments can be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region can be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide.

**[0101]** As used herein, the term “cross-linked” refers to joining together of two or more molecules by a third molecule. For example, a bivalent antibody with two binding domains that specifically bind to the same antigen can “cross-link” two copies of that antigen, e.g., as they are expressed on a cell. Many TNF superfamily receptor proteins require cross-linking of three or more receptors on the surface of a cell for activation. Cross-linking of TNF superfamily receptor proteins means, for instance, contacting a binding molecule, as disclosed herein, with TNF superfamily receptors expressed on the surface of a cell such that at least three such family members are simultaneously bound together by one or more binding molecules, thereby activating the receptors.

**[0102]** In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which amino acids that neighbor each other in the sequence are contiguous in the primary structure of the

polypeptide. A portion of a polypeptide that is “amino-terminal” or “N-terminal” to another portion of a polypeptide is that portion that comes earlier in the sequential polypeptide chain. Similarly a portion of a polypeptide that is “carboxy-terminal” or “C-terminal” to another portion of a polypeptide is that portion that comes later in the sequential polypeptide chain. For example in a typical antibody, the variable domain is “N-terminal” to the constant region, and the constant region is “C-terminal” to the variable domain.

**[0103]** The term “expression” as used herein refers to a process by which a gene produces a biochemical, for example, a polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into RNA, e.g., messenger RNA (mRNA), and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide that is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

**[0104]** Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt or slow the progression of an existing diagnosed pathologic condition or disorder. Terms such as “prevent,” “prevention,” “avoid,” “deterrence” and the like refer to prophylactic or preventative measures that prevent the development of an undiagnosed targeted pathologic condition or disorder. Thus, “those in need of treatment” can include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

**[0105]** By "subject" or "individual" or "animal" or "patient" or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

**[0106]** As used herein, phrases such as “a subject that would benefit from therapy” and “an animal in need of treatment” includes subjects, such as mammalian subjects, that would

benefit from administration of a binding molecule such as an antibody, comprising one or more antigen binding domains. Such binding molecules, *e.g.*, antibodies, can be used, *e.g.*, for a diagnostic procedures and/or for treatment or prevention of a disease.

IgM Binding Molecules

**[0107]** IgM is the first immunoglobulin produced by B cells in response to stimulation by antigen, and is present at around 1.5 mg/ml in serum with a half-life of 5 days. IgM is a pentameric or hexameric molecule. An IgM binding unit includes two light and two heavy chains. While IgG contains three heavy chain constant domains (CH1, CH2 and CH3), the heavy (μ) chain of IgM additionally contains a fourth constant domain (CH4), that includes a C-terminal "tailpiece." The human IgM constant region typically comprises the amino acid sequence SEQ ID NO: 74. The human Cμ1 region ranges from about amino acid 5 to about amino acid 102 of SEQ ID NO: 74; the human Cμ2 region ranges from about amino acid 114 to about amino acid 205 of SEQ ID NO: 74, the human Cμ3 region ranges from about amino acid 224 to about amino acid 319 of SEQ ID NO: 74, the Cμ 4 region ranges from about amino acid 329 to about amino acid 430 of SEQ ID NO: 74, and the tailpiece ranges from about amino acid 431 to about amino acid 453 of SEQ ID NO: 74. SEQ ID NO: 74 is presented below:

GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITLSW
KYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGT
DEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGF
FGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQV
QAEAKESGPTTYKVTSTLTIKESDWLGQSMFTCRVDHRGLT
FQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTD
LTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASI
CEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDV
YLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPL
SPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTC
VAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

**[0108]** Five IgM binding units can form a complex with an additional small polypeptide chain (the J chain) to form an IgM antibody. The human J chain comprises the amino acid sequence SEQ ID NO: 76. Without the J chain, IgM binding units typically assemble into a hexamer. While not wishing to be bound by theory, the assembly of IgM binding units into a pentameric or hexameric binding molecule is thought to involve the Cμ3 and Cμ4 domains. Accordingly, a pentameric or hexameric binding molecule provided in this disclosure

typically includes IgM constant regions that include at least the Cμ3 and Cμ4 domains. SEQ ID NO: 76 is presented below:

MKNHLLFWGVLAVFIKAVHVKAQEDERIVLVDNKCKCARI
TSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVY
HLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTY
DRNKCYTAVVPLVYGGETKMVETALTPDACYPD

**[0109]** An IgM heavy chain constant region can additionally include a Cμ2 domain or a fragment thereof, a Cμ1 domain or a fragment thereof, and/or other IgM heavy chain domains. In certain aspects, a binding molecule as provided herein can include a complete IgM heavy (μ) chain constant domain, e.g., SEQ ID NO: 74, or a variant, derivative, or analog thereof.

Pentameric or Hexameric TNF Superfamily Binding Molecules

**[0110]** This disclosure provides a pentameric or hexameric binding molecule, i.e., a binding molecule with five or six "binding units" as defined herein, that can specifically bind to one or more TNF superfamily receptor proteins, e.g., DR5. A binding molecule as provided herein can possess improved binding characteristics or biological activity as compared to a binding molecule composed of a single binding unit, e.g., a bivalent IgG antibody. For example, a pentameric or hexameric binding molecule can more efficiently cross-link three or more TNF superfamily receptor molecules on the surface of a cell, e.g., a tumor cell, thereby facilitating apoptosis of the cell.

**[0111]** A binding molecule as provided herein can likewise possess distinctive characteristics compared to multivalent binding molecule composed of synthetic or chimeric structures. For example, use of human IgM constant regions can afford reduced immunogenicity and thus increased safety relative to a binding molecule containing chimeric constant regions or synthetic structures. Moreover, an IgM-based binding molecule can consistently form hexameric or pentameric oligomers resulting in a more homogeneous expression product. Superior complement fixation can also be an advantageous effector function of IgM-based binding molecules.

**[0112]** In certain aspects, the disclosure provides a pentameric or hexameric binding molecule comprising five or six bivalent binding units, respectively, where each binding unit includes two IgM heavy chain constant regions or fragments thereof. In certain aspects, the two IgM heavy chain constant regions are human heavy chain constant regions.

**[0113]** Where the binding molecule provided herein is pentameric, the binding molecule can further comprise a J chain, or fragment thereof, or variant thereof.

**[0114]** An IgM heavy chain constant region can include one or more of a Cμ1 domain, a Cμ2 domain, a Cμ3 domain, and/or a Cμ4 domain, provided that the constant region can serve a desired function in the binding molecule, e.g., associate with second IgM constant region to form a binding domain, or associate with other binding units to form a hexamer or a pentamer. In certain aspects the two IgM heavy chain constant regions or fragments thereof within an individual binding unit each comprise a Cμ3 domain or fragment thereof, a Cμ4 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a Cμ3 domain a Cμ domain, and a TP or fragment thereof. In certain aspects the two IgM heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cμ2 domain or fragment thereof, a Cμ1 domain or fragment thereof, or a Cμ1 domain or fragment thereof and a Cμ2 domain or fragment thereof.

**[0115]** In certain aspects each of the two IgM heavy chain constant regions in a given binding unit is associated with an antigen-binding domain, for example an Fv portion of an antibody, e.g., a VH and a VL of a human or murine antibody, where the VL can be associated with a light chain constant region. In a binding molecule as provided herein at least one antigen-binding domain of the binding molecule is a TNF superfamily receptor protein binding domain, i.e., a binding domain that can specifically bind to a member of the TNF superfamily of receptor proteins, e.g., human DR5.

IgA Binding Molecules

**[0116]** IgA plays a critical role in mucosal immunity, and comprises about 15% of total immunoglobulin produced. IgA is a monomeric or dimeric molecule. An IgA binding unit includes two light and two heavy chains. IgA contains three heavy chain constant domains (Cα1, Cα2 and Cα3), and includes a C-terminal "tailpiece." Human IgA has two subtypes, IgA1 and IgA2. The human IgA1 constant region typically comprises the amino acid sequence SEQ ID NO: 78. The human Cα1 region ranges from about amino acid 6 to about amino acid 98 of SEQ ID NO: 78; the human Cα2 region ranges from about amino acid 125 to about amino acid 220 of SEQ ID NO: 78, the human Cα3 region ranges from about amino acid 228 to about amino acid 330 of SEQ ID NO: 78, and the tailpiece ranges from about amino acid 331 to about amino acid 352 of SEQ ID NO: 78. The human IgA2 constant region typically comprises the amino acid sequence SEQ ID NO: 79. The human Cα1 region

ranges from about amino acid 6 to about amino acid 98 of SEQ ID NO: 79; the human Cα2 region ranges from about amino acid 112 to about amino acid 207 of SEQ ID NO: 79, the human Cα3 region ranges from about amino acid 215 to about amino acid 317 of SEQ ID NO: 79, and the tailpiece ranges from about amino acid 318 to about amino acid 340 of SEQ ID NO: 79. SEQ ID NOS: 78 and 79 are presented below:

SEQ ID NO: 78

ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWS
ESGQGVTARNFPPSQDASGDLYTTSSQLTLPATQCLAGKSV
TCHVKHYTNPSQDVTVPCPVPSTPPTPSPSTPPTPSPSCCHPR
LSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPERDLCGCYSVSSVLPGCAEPWNHGKTFTCTAA
YPESKTPLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTC
LARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTT
TFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
LAGKPTHVNVSVVMAEVDGTCY

SEQ ID NO: 79

ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFFPQEPLSVTW
SESGQNVTARNFPPSQDASGDLYTTSSQLTLPATQCPDGKS
VTCHVKHYTNPSQDVTVPCPVPPPPPCCHPRLSLHRPALED
LLLGSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGPPER
DLCGCYSVSSVLPGCAQPWNHGETFTCTAAHPELKTPLTAN
ITKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVL
VRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAA
EDWKKGDTFSCMVGHEALPLAFTQKTIDRMAGKPTHVNVS
VVMAEVDGTCY

**[0117]** Two IgA binding units can form a complex with two additional polypeptide chains, the J chain (SEQ ID NO: 76) and the secretory component (precursor, SEQ ID NO: 80, mature, SEQ ID NO: 81) to form a secretory IgA (sIgA) antibody. While not wishing to be bound by theory, the assembly of IgA binding units into a dimeric sIgA binding molecule is thought to involve the Cα3 and tailpiece domains. Accordingly, a dimeric sIgA binding molecule provided in this disclosure typically includes IgA constant regions that include at least the Cα3 and tailpiece domains. SEQ ID NO: 80 and SEQ ID NO: 81 are presented below:

SEQ ID NO: 80:

MLLFVLTCLLAVFPAISTKSPIFGPEEVNSVEGNSVSITCYYP
PTSVNRHTRKYWCRQGARGGCITLISSEGYVSSKYAGRANL
TNFPENGTFVVNIAQLSQDDSGRYKCGLGINSRGLSFDVSLE
VSQGPGLLNDTKVYTVDLGRTVTINCPFKTENAQKRKSLYK
QIGLYPVLVIDSSGYVNPNYTGRIRLDIQGTGQLLFSVVINQL

RLSDAGQYLCQAGDDSNSNKKNADLQVLKPEPELVYEDLR
GSVTFHCALGPEVANVAKFLCRQSSGENCDVVVNTLGKRA
PAFEGRILLNPQDKDGSFSVVITGLRKEDAGRYLCGAHSDG
QLQEGSPIQAWQLFVNEESTIPRSPTVVKGVAGGSVAVLCP
YNRKESKSIKYWCLWEGAQNGRCPLLVDSEGWVKAQYEG
RLSLLEEPGNGTFTVILNQLTSRDAGFYWCLTNGDTLWRTT
VEIKIIEGEPNLKVPGNVTAVLGETLKVPCHFPCKFSSYEKY
WCKWNNTGCQALPSQDEGPSKAFVNCDENSRLVSLTLNLV
TRADEGWYWCGVKQGHFYGETAAVYVAVEERKAAGSRD
VSLAKADAAPDEKVLDSGFREIENKAIQDPRLFAEEKAVAD
TRDQADGSRASVDSGSSEEQGGSSRALVSTLVPLGLVLAVG
AVAVGVARARHRKNVDRVSIRSYRTDISMSDFENSREFGA
NDNMGASSITQETSLGGKEEFVATTESTTETKEPKKAKRSS
KEEAEMAYKDFLLQSSTVAAEAQDGPQEA

SEQ ID NO: 81:

KSPIFGPEEVNSVEGNSVSITCYYPPTSVNRHTRKYWCRQG
ARGGCITLISSEGYVSSKYAGRANLTNFPENGTFVVNIAQLS
QDDSGRYKCGLGINSRGLSFDVSLEVSQGPGLLNDTKVYTV
DLGRTVTINCPFKTENAQKRKSLYKQIGLYPVLVIDSSGYVN
PNYTGRIRLDIQGTGQLLFSVVINQLRLSDAGQYLCQAGDD
SNSNKKNADLQVLKPEPELVYEDLRGSVTFHCALGPEVAN
VAKFLCRQSSGENCDVVVNTLGKRAPAFEGRILLNPQDKD
GSFSVVITGLRKEDAGRYLCGAHSDGQLQEGSPIQAWQLFV
NEESTIPRSPTVVKGVAGGSVAVLCPYNRKESKSIKYWCLW
EGAQNGRCPLLVDSEGWVKAQYEGRLSLLEEPGNGTFTVIL
NQLTSRDAGFYWCLTNGDTLWRTTVEIKIIEGEPNLKVPGN
VTAVLGETLKVPCHFPCKFSSYEKYWCKWNNTGCQALPSQ
DEGPSKAFVNCDENSRLVSLTLNLVTRADEGWYWCGVKQ
GHFYGETAAVYVAVEERKAAGSRDVSLAKADAAPDEKVL
DSGFREIENKAIQDPR

**[0118]** An IgA heavy chain constant region can additionally include a Cα2 domain or a fragment thereof, a Cα1 domain or a fragment thereof, and/or other IgA heavy chain domains. In certain aspects, a binding molecule as provided herein can include a complete IgA heavy (α) chain constant domain (e.g., SEQ ID NO: 78 or SEQ ID NO: 79), or a variant, derivative, or analog thereof.

Dimeric TNF Superfamily Receptor Binding Molecules

**[0119]** This disclosure provides a dimeric binding molecule, e.g., a binding molecule with two IgA "binding units" as defined herein, that can specifically bind to one or more TNF superfamily receptor proteins, e.g., DR5. A binding molecule as provided herein can possess improved binding characteristics or biological activity as compared to a binding molecule

composed of a single binding unit, e.g., a bivalent IgG antibody. For example, an IgA binding molecule can more efficiently cross-link three or more TNF superfamily receptors on the surface of a cell, e.g., a tumor cell, thereby facilitating apoptosis of the cell. Moreover, an IgA binding molecule can reach mucosal sites providing greater tissue distribution for the binding molecules provided herein. Use of an IgA-based binding molecule can allow, for example, greater tissue distribution for a binding molecule provided herein. Mucosal distribution could be beneficial for certain cancers, e.g., lung cancer, ovarian cancer, colorectal cancer, or squamous cell carcinoma. Likewise, a dimeric binding molecule as provided herein can possess binding characteristics or biological activity that can be distinguished from a binding molecule comprising five or six binding units, e.g., a hexameric or pentameric IgM antibody. For example, a dimeric binding molecule would be smaller, and could, for example, achieve better tissue penetration in solid tumors.

**[0120]** In certain aspects, the disclosure provides a dimeric binding molecule comprising two bivalent binding units, where each binding unit includes two IgA heavy chain constant regions or fragments thereof. In certain aspects, the two IgA heavy chain constant regions are human heavy chain constant regions.

**[0121]** A dimeric IgA binding molecule as provided herein can further comprise a J chain, or fragment thereof, or variant thereof. A dimeric IgA binding molecule as provided herein can further comprise a secretory component, or fragment thereof, or variant thereof.

**[0122]** An IgA heavy chain constant region can include one or more of a Cα1 domain, a Cα2 domain, and/or a Cα3 domain, provided that the constant region can serve a desired function in the binding molecule, e.g., associate with a light chain constant region to facilitate formation of an antigen binding domain, or associate with another IgA binding unit to form a dimeric binding molecule. In certain aspects the two IgA heavy chain constant regions or fragments thereof within an individual binding unit each comprise a Cα3 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a Cα3 domain, a TP, or fragment thereof. In certain aspects the two IgA heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cα2 domain or fragment thereof, a Cα1 domain or fragment thereof, or a Cα1 domain or fragment thereof and a Cα2 domain or fragment thereof.

**[0123]** In certain aspects each of the two IgA heavy chain constant regions in a given binding unit is associated with an antigen binding domain, for example an Fv portion of an antibody, e.g., a VH and a VL of a human or murine antibody, where the VL can be associated with a

light chain constant region. In a binding molecule as provided herein at least one antigen-binding domain of the binding molecule is a TNF superfamily receptor protein binding domain, i.e., a binding domain that can specifically bind to a member of the TNF superfamily of receptor proteins, e.g., human DR5.

TNF Superfamily Receptor Binding Domains

**[0124]** A TNF superfamily receptor protein binding molecule as provided herein can be dimeric, pentameric, or hexameric, comprising two, five, or six bivalent binding units, respectively. The binding units can be full length or variants or fragments thereof that retain binding function.

**[0125]** Each binding unit comprises two IgA or IgM heavy chain constant regions or fragments thereof, each associated with an antigen-binding domain. As noted above, an antigen binding domain is a region of a binding molecule that is necessary and sufficient to specifically bind to an epitope. A "binding molecule" as described herein can include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more "antigen binding domains."

**[0126]** A dimeric, pentameric, or hexameric binding molecule as provided herein can include at least three antigen-binding domains which specifically and agonistically bind to a tumor necrosis factor (TNF) superfamily receptor protein. As noted above, some of these TNF superfamily receptor proteins, upon activation, can induce apoptosis of the cell expressing the TNF superfamily receptor protein which was bound. Apoptosis will occur, as presently understood, when multiple receptor proteins are bound together, causing cross-linking of the receptor molecules such that a signal is transmitted across the cell membrane into the cytosol of the cell expressing the TNF superfamily receptor protein.

**[0127]** A dimeric, pentameric, or hexameric binding molecule as provided herein can cross-link at least three identical TNF superfamily receptor proteins expressed on the surface of a cell. Due to the dimeric, pentameric, or hexameric nature of a TNF superfamily receptor protein binding molecule as provided herein can cross-link as many as three, four, five, six, seven, eight, nine, ten, eleven, or twelve TNF superfamily receptor proteins, the receptor proteins are necessarily spatially brought into proximity of each other, thereby contributing to their cross-linking and activation. When all five or all six of the bivalent binding units a TNF superfamily receptor protein binding molecule as provided herein binds to up to ten or

twelve TNF superfamily receptor proteins on a single cell, respectively, cross-linking and activation of the receptors can occur.

**[0128]** Because each of the binding units is bivalent, each binding molecule can bind to as many as 10 (for pentameric binding molecules) or 12 (for hexameric binding molecules) TNF superfamily receptor proteins.

**[0129]** Upon activation of the receptors by the binding of a dimeric, pentameric, or hexameric binding molecule as provided herein, the cell can either undergo apoptosis, activation or morphogenesis, as described above, depending on which receptor of the superfamily is bound.

**[0130]** In certain aspects, a dimeric, pentameric, or hexameric binding molecule as presently disclosed can induce TNF superfamily receptor-mediated apoptosis in a TNF receptor superfamily-expressing cell at a higher potency than an equivalent amount of a bivalent IgG antibody or fragment thereof, which also specifically binds to and agonizes the same TNF superfamily receptor protein. Not wishing to be bound by theory, because a provided binding molecule is dimeric, pentameric, or hexameric, and because each binding unit is bivalent, such a binding molecule can induce receptor-mediated functions previously characterized for this superfamily of receptor proteins at a higher potency than any single binding unit alone, such as an equivalent IgG binding unit. IgG binding units are bivalent, containing two binding sites, but as previous clinical studies have shown, binding of two receptors of this superfamily with a single IgG molecule can be ineffective without addition of other components, such as cross-linkers, etc.

**[0131]** By "potency" or "improved binding characteristics" is meant the least amount of a given binding molecule necessary to achieve a given biological result, e.g., activation of 20%, 50%, or 90% of a TNF superfamily receptor protein in a given assay, e.g., a ELISA or Western blot based caspase assays, annexin-v staining as seen by FACS analysis, or other assay as provided in the examples below. For instance, when the TNF superfamily receptor protein is one which, when activated, causes apoptosis of the cell in which it is activated, potency can be expressed as a curve in which % survival of cells is on the Y axis, and binding molecule concentration (in, e.g., μg/ml or μM) is on the X axis.

**[0132]** Because a binding molecule as provided herein is dimeric, pentameric, or hexameric, it can contain as many as 4, 10, or 12, respectively, antigen-binding domains. Each of the antigen-binding domains can specifically bind to and agonize the TNF superfamily receptor. Further, each antigen-binding domain can be specific for one particular epitope of the TNF

superfamily receptor protein. In certain aspects, the binding molecule does not cross-react with other TNF superfamily receptor proteins. However, in other aspects, two or more of the antigen-binding domains can be specific for different epitopes and/or different TNF superfamily receptor proteins.

**[0133]** Thus, a single dimeric, pentameric, or hexameric binding molecule can: a) simultaneously bind a single epitope on many identical receptor proteins, b) bind many different epitopes on the same identical receptor protein, or c) can bind different epitopes on different TNF superfamily receptor proteins. In embodiment a), a TNF superfamily receptor protein binding molecule as provided herein can bind multiple copies of an identical TNF superfamily receptor at the same location for each identical copy, thereby forming a raft of such receptor proteins in a single location and likely increasing the likelihood that the receptor proteins will be activated. In other embodiments, such as embodiment c), a dimeric, pentameric, or hexameric binding molecule as provided herein can be used to contact multiple different TNF superfamily receptor proteins, thereby activating more than one pathway through the various targeted receptors, to achieve the desired biological response in the cells. Of course, in these embodiments, a TNF superfamily receptor protein binding molecule as provided herein can contact and agonize such receptors all on one single cell, or across multiple cells.

**[0134]** Thus, a dimeric, pentameric, or hexameric binding molecule as provided herein can comprise three, four, five, six, seven, eight, nine, ten, or in the case of the hexameric binding molecules, as many as eleven, or twelve antigen-binding domains that specifically and agonistically bind to one or more TNF superfamily receptor proteins expressed on the surface of one or more cells, thereby inducing the intended or desired biological response in the cell(s).

**[0135]** The binding units of a dimeric, pentameric, or hexameric binding molecule as provided herein can be human, humanized, or chimeric immunoglobulin binding units. Methods of humanizing immunoglobulin sequences are well known in the art. Thus, the nucleotide sequences encoding a dimeric, pentameric, or hexameric binding molecule polypeptide can be directly from human sequences, or can be humanized or chimeric, i.e., encoded by sequences from multiple different species.

**[0136]** A dimeric, pentameric, or hexameric binding molecule as provided herein can specifically bind any one of the known TNF superfamily receptor proteins. These receptor proteins can be grouped into specific functions of triggering either morphogenesis, apoptosis

or proliferation. Thus, a TNF superfamily receptor protein binding molecule as provided herein can, for instance, specifically bind to any one or more of the following receptors: TNFR1 (DR1), TNFR2, TNFR1/2, CD40 (p50), Fas (CD95, Apo1, DR2), CD30, 4-1BB (CD137, ILA), DR4 (TRAILR1, Apo2), DR5 (TRAILR2), DcR1 (TRAILR3), DcR2 (TRAILR4), OPG (OCIF), TWEAKR (FN14), LIGHTR (HVEM), DcR3, DR3, EDAR, and XEDAR.

**[0137]** In one embodiment, a TNF superfamily receptor protein binding molecule as provided herein specifically and agonistically binds to DR5 but does not specifically bind to other receptors, e.g., DR4 (TRAILR1, Apo2), decoy receptor DcR1 (TRAILR3) or decoy receptor DcR2 (TRAILR4). In certain aspects the TNF superfamily receptor protein binding molecule as provided herein can specifically and agonistically bind to DR5 and can also specifically bind to DR4.

**[0138]** The cells which express TNF superfamily receptor proteins can be any animal cell. For instance, in one embodiment, the cell is a human cell. For example, the cell can be any one or more of primate, rodent, canine, equine, etc., cells. Further, the cell expressing the TNF superfamily receptor protein can be a cancer cell. That is, the cell can be a cell in a tumor which is malignant or benign.

**[0139]** A dimeric, pentameric, or hexameric binding molecule as provided herein can be genetically engineered such that its antigen-binding domains are encoded by sequences known to specifically bind a TNF superfamily receptor protein. Many groups have published sequences of variable regions of monoclonal antibodies, most of the IgG isotype that are characterized and are known to specifically bind to a TNF superfamily receptor, e.g., DR5. Non-limiting immunoglobulin variable domain sequences that are known to specifically bind to DR5 are provided in Tables 2 and 3. Other monoclonal antibody sequences specific for other members of the TNF superfamily of receptor proteins have been published. One of skill in the art is capable of engineering these published sequences into immunoglobulin structures, such as an IgG, IgA, IgM structure, or biologically active or functional fragments thereof (such as scFv fragments and the like, as discussed above). Methods for genetically engineering cloned variable regions into immunoglobulin domains, and expressing and purifying such constructs are published and within the capability of one skilled in the art.

**[0140]** Thus, in certain aspects, a TNF superfamily receptor protein binding domain as provided herein comprises six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, or the six immunoglobulin

complementarity determining regions with one, two, three, four, or five single amino acid substitutions in one or more CDR, of an anti-DR5 mAb comprising the VH and VL amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 7 and SEQ ID NO: 8; SEQ ID NO: 9 and SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 13 and SEQ ID NO: 14; SEQ ID NO: 15 and SEQ ID NO: 16; SEQ ID NO: 17 and SEQ ID NO: 18; SEQ ID NO: 19 and SEQ ID NO: 20; SEQ ID NO: 21 and SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 24; SEQ ID NO: 25 and SEQ ID NO: 26; SEQ ID NO: 27 and SEQ ID NO: 28; SEQ ID NO: 29 and SEQ ID NO: 30; SEQ ID NO: 31 and SEQ ID NO: 32; SEQ ID NO: 33 and SEQ ID NO: 34; SEQ ID NO: 35 and SEQ ID NO: 36; SEQ ID NO: 37 and SEQ ID NO: 38; SEQ ID NO: 39 and SEQ ID NO: 40; SEQ ID NO: 41 and SEQ ID NO: 42; SEQ ID NO: 43 and SEQ ID NO: 44; SEQ ID NO: 45 and SEQ ID NO: 46; SEQ ID NO: 47 and SEQ ID NO: 48; SEQ ID NO: 49 and SEQ ID NO: 50; SEQ ID NO: 51 and SEQ ID NO: 52; SEQ ID NO: 53 and SEQ ID NO: 54; SEQ ID NO: 55 and SEQ ID NO: 56; SEQ ID NO: 82 and SEQ ID NO: 83; SEQ ID NO: 84 and SEQ ID NO: 85; SEQ ID NO: 86 and SEQ ID NO: 87; or SEQ ID NO: 88 and SEQ ID NO: 89; respectively, or the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

Table 2 Anti-DR5 Antibody VH and VL Sequences

| SEQ ID | VH or Heavy Chain | SEQ ID | VL or Light Chain | Reference |
|---|---|---|---|---|
| 1 | EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYGMS WVRQAPGKGLEWVSGINWNGGSTGYADSVKGRV TISRDNAKNSLYLQMNSLRAEDTAVYYCAKILGAG RGWYFDLWGKGTTVTVSS | 2 | SSELTQDPAVSVALGQTVRITCQGDSLRSYYAS WYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSG NTASLTITGAQAEDEADYYCNSRDSSGNHVVFG GGTKLTVL | U.S. Patent App Pub. No. 20060269555A1 |
| 3 | EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMS WVRQAPGKGLEWVSGINWQGGSTGYADSVKGRV TISRDNAKNSLYLQMNSLRAEDTAVYYCAKILGAG RGWYFDYWGKGTTVTVSS | 4 | SELTQDPAVSVALGQTVRITCSGDSLRSYYASW YQQKPGQAPVLVIYGANNRPSGIPDRFSGSSSGN TASLTITGAQAEDEADYYCNSADSSGNHVVFGG GTKLTVL | U.S. Patent No. 8,029,783 |
| 5 | QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGDYTW SWIRQLPGKGLECIGHIHINSGTTYYNPSLKSRVTISV DTSKKQFSLRLSSVTAADTAVYYCARDRGGDYYY GMDVWGQGTTVTVSS | 6 | EIVLTQSPGTLSLSPGERATLSCRASQGISRSYLA WYQQKPGQAPSLLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQFGSSPWTFGQGT KVEIK | U.S. Patent No. 7,521,048 |
| 7 | EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYVMS WVRQAPGKGLEWVATISSGGSYTYYPDSVKGRFTI SRDNAKNTLYLQMNSLRAEDTAVYYCARRGDSMI TTDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK | 8 | DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVA WYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCQQYSSYRTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC | U.S. Patent No. 7,790,165 |
| 9 | QIQLVQSGPELKKPGETVKISCKASGYTFT DFSMNWVKQAPGKGLKWMGWINTETGEPTY ADDFKGRFALSMETSASTAYLQINNLKNED TATYFCVRIDYWGQGTTLTVSS | 10 | DVVMTQTPLSLPVSLGDQASISCRSSQSLV HSNGNTYLHWYLQKPGQSPKLLIYKVSNRF SGVPDRFSGSGSGTDFTLKISRVEAEDLGV YFCFQSTHVPHTFGGGTKLEIKR | U.S. Patent No. 7,893,216 |

| SEQ ID | VH or Heavy Chain | SEQ ID | VL or Light Chain | Reference |
|---|---|---|---|---|
| 11 | MDWTWRILFLVAAATSAHSQVQLVQSGAEMKKPG ASVKVSCKTSGYTFTNYKINWVRQAPGQGLEWMG WMNPDTDSTGYPQKFQGRVTMTRNTSISTAYMELS SLRSEDTAVYYCARSYGSGSYYRDYYYGMDVWG QGTTVTVSS | 12 | MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLS PGERATLSCRASQSVSSYLAWYQQKPGQAPRLL IYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDF AVYYCQQRSNWPLTFGGGTKVEIKR | U.S. Patent No. 7,115,717 |
| 13 | MKHLWFFLLLVAAPRWVLSEVQLQQSGPEL VKPGASVKIS CKASGYSFIG YFMNWMKQSH GKSLEWIGRF NPYNGDTFYN QKFKGKATLT VDKSSTTAHM ELLSLTSEDS AVYFCGRSAY YFDSGGYFDY WGQGTTLTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K | 14 | MVLQTQVFIS LLLWISGAYG DVVMTQTPLS LPVSLGDQAS ISCRSSQSLV HSNGNTYLHW YLQKPGQSPK LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGI YFCSQSTHVP WTFGGGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC | EP Patent Publication No. EP2636736A1 |
| 15 | QVQLVQSGSELKKPGASVKVSCKASGYTFTDFSMN WVRQAPGQGLEWMGWINTETGEPTYADDFKGRFV FSLDTSVSTAYLQISSLKAEDTAVYYCARIDYWGQ GTTVTVSS | 16 | DIVMTQSPLSLPVTPGEPASISCRSSQSLVHSNGN TYLHWYLQKPGQSPQLLIYKVSNRFSGVPDRFS GSGSGTDFTLKISRVEAEDVGVYYCFQSTHVPHT FGQGTKLEIKR | PCT Publication No. WO 2014/063368 A1 |

| SEQ ID | VH or Heavy Chain | SEQ ID | VL or Light Chain | Reference |
|---|---|---|---|---|
| 17 | MGXLGLSWVFLVVILEGVQCEVHLVESGGGLVRPG GSLKLSCAASGFAFSSYDMSWVRQTPEKRLEWVA YISDGGGITYYPDTMKGRFTISRDNAKNTLSLQMSS LKSEDTAMYYCARHTMVVGPFAYWGQGTLVTVS AASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTMDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK | 18 | MRLPAQLLGLLMLWVSGSSGDIQMTQSSSSFSV SLGDRVTITCKASEDIYNRLAWYQQKPGNAPRL LISGATSLETGVPSRFSGSGSGKDYTLSITSLQTE DVATYYCQQYWSTPLTFGAGTKLELKRAVAAP SVDIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C | U.S. Patent No. 7,897,730 |
| 19 | MELGLSWVFLVVILEGVQCEVQLQQSGPELVKPGA SVRMSCKASGYTFTSYFIHWVKQRPGQGLEWIGWI YPGNVNTKYSEKFKGKATLTADKSSSTAYMQFSSL TSEDSAVYFCARGEAGYFDYWGQGTTLTVSSASTK GPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTMDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK | 20 | MRLPAQLLGLLMLWVSGSSGDIVMTQSHKFMS TSVGDRVSITCKASQDVSTAVAWYQQKPGQSPR LLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQ AEDQALYYCQQHYRTPWTFGGGTKLEIKRAVA APSVDIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC | U.S. Patent No. 7,897,730 |
| 21 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDIN WVRQATGQGLEWMGWMNPNSDNTGYAQKFQGR VTMTRNTSISTAYMELSSLRSEDTAVYYCARWNHY GSGSHFDYWGQGTLVTVSS | 22 | DIQMTQSPSSLSASVGDRVTITCRASQSISIYLNW YQQKPGKAPKLLIYAASSLQSGVPLRFSGSGSGT DFTLTISSLQPEDIATYYCQQSYKTPLTFGGGTK VEIK | U.S. Patent No. 7,521,048 |

| SEQ ID | VH or Heavy Chain | SEQ ID | VL or Light Chain | Reference |
|---|---|---|---|---|
| 23 | QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGHYW SWIRQHPGKGLEWIGYIYYSGSTYYNPSLKSRVTIS VDTSKNQFSLKLSSVTAADTAVYYCARDDSSGWGF DYWGQGILVTVSS | 24 | DIQMTQSPSSLSASVGDRVTITCRASQGLRNDLG WFQQKPGKVTKRLIYAASSLQRGVPSRFSGSGS GTEFTLTISSLQPEDFATYYCLQHYSFPWTFGQG TKVEIK | U.S. Patent No. 7,521,048 |
| 25 | QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGHYW SWIRQHPGKGLEWIGYIYYSGSAYYNPSLKSRVTIS VDTSKNQFSLKLSSVTAADTAVYYCARDDSSGWGF DYWGQGILVTVSS | 26 | DIQMTQSPSSLSASVGDRVTITCRASQGLRNDLG WFQQKPGKAPKRLIYAASSLQRGVPSRFSGSGS GTEFTLTISSLQPEDFTTYFCLQHNSFPWTFGQGT KVEIK | U.S. Patent No. 7,521,048 |
| 27 | QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGHYW SWIRQHPGKGLEWIGYIYYSGSAYYNPSLKSRVTIS VDTSKNQFSLKLSSVTAADTAVYYCARDDSSGWGF DYWGQGILVTVSS | 28 | DIQMTQSPSSLSASVGDRVTITCRASQGLRNDLG WFQQKPGKAPKRLIYAASSLQRGVPSRFSGSGS GTEFTLTISSLQPEDFTTYFCLQHNSFPWTFGQGT KVEIK | U.S. Patent No. 7,521,048 |
| 29 | QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMN WIRQAPGKGLEWVSHISSSGSILDYADSVKGRFTISR DNAKNSLYLQMNSLRVEDTAVYYCARDGAAAGT DAFDLWGQGTMVTVSS | 30 | DIQMTQSPSSLSASVGDRVTITCRSSQSISNYINW YQQRPGKAPNLLIHDVSSFQSAVPSRFSRSGSGT VFTLTISSLQPEDFATYFCQQTYITPFTFGPGTKV DIK | U.S. Patent No. 7,521,048 |
| 31 | QVQLVESGGGVVQPGRSLRLSCAASGFTFSYYGIH WVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCARGRYSS SSWWYFDLWGRGTLVTVSS | 32 | DIQMTQSPSSLSASVGDRVTITCRASQGISNYLA WYQQKPGKVPKLLIYAASTLQSGVPSRFSGSGS GTDFTLTISSLQPEDVATYYCQKYNSAPLTFGGG TKVEIK | U.S. Patent No. 7,521,048 |
| 33 | QVQAEQSGPGLVKPSETLSLTCTVSGGSISNYYWS WIRQPPGKGLEWIGYIYYSGSTKYNPSLKSRVTISV DTSKNQFSLKLTSVTTADTAVYYCARDSPRGFSGY EAFDSWGQGTLVTVSS | 34 | DIVMTQSPDSLAVSLGERATINCKSSQSVLYRSN NKIYLAWYQQKPGQPPKLLIYWASTRESGVPDR FSGSGSGTDFTLTISSLLAEDVAVYYCQQYYSTP FTFGPGTKVDIK | U.S. Patent No. 7,521,048 |
| 35 | QVQLQESGPGLVKPSQTLSLTCTVSGGSISSDNYYW SWIRQHPGKGLEWIGYIYYSGSTYYNPSLKSRVTIS VDTSKNQFSLKLSSVTAADTAVYYCARGVNWNFL FDIWGQGTMVTVSS | 36 | DIVMTQSPLSLPVTPGEPASISCRSSQSLLRRNGY NYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFS GSGSGTDFTLKISRVEAEDVGVYYCMQALQTPL TFGGGTEVEIK | U.S. Patent No. 7,521,048 |
| 37 | QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS WIRQAPGKGLEWVSYISRSGSTIYYADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCARSLGGMDV WGQGTTVTVSS | 38 | DIVMTQFPDSLAVSLGERATINCKSSQSVLHSSN NKNYLTWYQLKPGQPPKLLIYWASTRESGVPDR FSGSGSGTDFTLTISSLQAEDVAVYYCHQYYSTP SSFGQGTKLEIK | U.S. Patent No. 7,521,048 |

| SEQ ID | VH or Heavy Chain | SEQ ID | VL or Light Chain | Reference |
|---|---|---|---|---|
| 39 | QVQLVESGGGVVQPGRSLRLSCAASGFTFNNYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRTVYSNSSPFYYYYYGMDVWGQGTTVTVSS | 40 | DIQMTQSPSSLSASVGDRVTITCRTSQSISTYLNWYQQKPGKAPKLLISATSSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIK | U.S. Patent No. 7,521,048 |
| 41 | QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRTVYSSSSPFYYYYYGMDVWGQGTTVTVSS | 42 | DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLISATSSFQSGVPSRFSGSGSGTDFTLTISSLQPEDFAAYYCQQSYSTPLTFGGGTKVEIK | U.S. Patent No. 7,521,048 |
| 43 | QVQLQQWGARLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVDTSKNQFSLKLRSVTAADTAVYYCARGGSSGYWYFDLWGRGTLVTVSS | 44 | DIVMTQSPDSLAVSLGERATINCKSSQSVLHSSNNKNYLVWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPLTFGGGTKVEIK | U.S. Patent No. 7,521,048 |
| 45 | EVQVVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGGSSWYGDWFDPWGQGTLVTVSS | 46 | DIQMTQSPSSVSASVGDRVTITCRASQGISSWLVWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPFTFGGGTKVEIK | U.S. Patent No. 7,521,048 |
| 47 | QLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGRNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREVGYCTNGVCSYYYYGMDVWGQGTTVTVSS | 48 | DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSKFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPLTFGGGTKVEIK | U.S. Patent No. 7,521,048 |
| 49 | QVQLQESGPGLVKPSQTLSLTCSVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSGSTYCNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDNGSGSYDWFDPWGQGILVTVSS | 50 | DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKFLIFVASSFQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPRTFGQGTKVEIK | U.S. Patent No. 7,521,048 |
| 51 | QVQMQESGPGLVKPSQTLSLTCTVSGGSISSGDYYWSWIRQHPGKNLEWIGYIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDNGSGSYDWFDPWGQGTLVTVSS | 52 | DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKFLIFVASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPRTFGQGTKVEIK | U.S. Patent No. 7,521,048 |
| 53 | KVQLQQSGAELVKPGASVKLSCKASGYTFTDYTIHWVKQRSGQGLEWIGWFYPGGGYIKYNEKFKDRATLTADKSSNTVYMELSRLTSEGSAVYFCARHEEGIYFDYWGQGTTLTVSS | 54 | DIAMTQSHKFMSTLVGDRVSITCKASQDVNTAIAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKRA | U.S. Patent No. 7,229,617 |

| SEQ ID | VH or Heavy Chain | SEQ ID | VL or Light Chain | Reference |
|---|---|---|---|---|
| 55 | KVQLQQSGAELVKPGASVKLSCKASGYTFTDYTIH WVKQRSGQGLEWIGWFYPGGGYIKYNEKFKDRAT LTADKSSNTVYMELSRLTSEDSAVYFCARHEEGIYF DYWGQGTTLTVSS | 56 | DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAI AWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSG SGTDYTLTISSVQAEDLALYYCQQHYTTPFTFGS GTKL | U.S. Patent No. 7,229,618 |
| 82 | MDLMCKKMKHLWFFLLLVAAPRWVLSQLQLQES GPGLVKPSETLSLTCTVSGGSIISKSSYWGWIRQPPG KGLEWIGSIYYSGSTFYNPSLKSRVTISVDTSKNQFS LKLSSVTAADTAVYYCARLTVAEFDYWGQGTLVT VSSAS | 83 | MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLS PGERATLSCRASQSVSSFLAWYQQKPGQAPRLLI YDASNRATGIPARFSGSGSGTDFTLTISSLEPEDF AVYYCQQRSNWPLTFGPGTKVDIKRT | U.S. Patent No. 7,115,717 |
| 84 | MDLMCKKMKHLWFFLLLVAAPRWVLSQLQLQES GPGLVKPSETLSLTCTVSGGSISSRSNYWGWIRQPP GKGLEWIGNVYYRGSTYYNSSLKSRVTISVDTSKN QFSLKLSSVTVADTAVYYCARLSVAEFDYWGQGIL VTVSSAS | 85 | MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLS PGERATLSCRASQSVSSFLAWYQQKPGQAPRLLI YDASNRATGSPARFSGSGSGTDFTLTISSLEPEDF AVYYCQQRSDWPLTFGPGTKVDIKRT | U.S. Patent No. 7,115,717 |
| 86 | MDLMCKKMKHLWFFLLLVAAPRWVLSQLQLQES GPGLVKPSETLSLTCTVSGGSISSSSYYWGWVRQPP GKGLEWIGSIHYSGSTFYNPSLKSRVTISVDTSKNQF SLKLSSVTAADTTVYYCARQGSTVVRGVYYYGMD VWGQGTTVTVSSAS | 87 | METPAQLLFLLLLWLPDTTGEIVLTQSPGTLSLSP GERATLSCRASQSVSSSYLAWYQQKPGQAPRLL IYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQYGSSPLYTFGQGTKLEIKRT | U.S. Patent No. 7,115,717 |
| 88 | MEFGLSWLFLVAILKGVQCEVQLLESGGGLVQPGR SLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAI SGSGGSRYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKESSGWFGAFDYWGQGTLVTVSS | 89 | MSPSQLIGFLLLWVPASRGEIVLTQSPDFQSVTPK EKVTITCRASQSIGSSLHWYQQKPDQSPKLLIKY ASQSFSGVPSRFSGSGSGTDFTLTINSLEAEDAAA YYCHQSSSLPITFGQGTRLEIKR | U.S. Patent No. 7,115,717 |

Table 3: Anti DR5 ScFv Sequences

| SEQ ID | SEQUENCE | Reference |
|---|---|---|

| SEQ ID | SEQUENCE | Reference |
|---|---|---|
| 57 | EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYAD<br>SVKGRVTISRDNAKNSLYLQMNSLRAEDTAVYYCAKILGAGRGWYFDLWGKGTTVTVSSGGG<br>GSGGGGSGGGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNN<br>RPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVLG | U.S. Patent Application Publication No. 2006/0269555 |
| 58 | EVQLVETGGGLVQPGGSLRLSCAAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS<br>VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYHCARGGYSSSRSAAYDIWGQGTLVTVSSGGGG<br>SGGGGSGGGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNR<br>PSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVLG | U.S. Patent Application Publication No. 2006/0269556 |
| 59 | QVQLVQSGAEVKKPGASVKISCEGSGYTFNSYTLHWLRQAPGQRLEWMGRINAGNGNTKYSQ<br>NFQGRLSITRDTSATTAYMELSSLRSEDTGVYYCARVFTYSFGMDVWGRGTLVTVSSGGGGSG<br>GGGSGGGGSAQSVLTQPPSASGTPGQRVTISCSGGSNIGRNSVSWYQQLPGTAPKLLIYSNNQR<br>PSGVPDRFSGSKSGTSASLAISGLRSEDEALYYCAAWDDSLSGGVFGGGTKLTVLG | U.S. Patent Application Publication No. 2006/0269557 |
| 60 | QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS<br>VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVHRPGRSGYFDYWGRGTLVTVSSGGGGS<br>GGGGSGGGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRP<br>SGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVLG | U.S. Patent Application Publication No. 2006/0269558 |
| 61 | QVQLQQSGAEVKKPGASVRVSCQASGYSLSEYYIHWVRQAPGQGLEWMGWLNPNSGVTDYA<br>QKFQGRVSMTRDTSISTAYMELSSLTFNDTAVYFCARGNGDYWGKGTLVTVSPGGGSGGGGS<br>GGGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYTNWFQQKPGQAPLLVVYAKNKRPSGIPDR<br>FSGSSSGNTASLTITGAQAEDEADYYCHSRDSSGWVFGGGTKLTVLG | U.S. Patent Application Publication No. 2006/0269559 |
| 62 | QVQLVQSGGGVVQPGRSLRLSCAASGFTFSPDAMHWVRQAPGKGLEWMGVISFDGSQIFYADS<br>VKGRFTISRDNSQNTLYLQMNSLRSDDTAVYYCARAPARFFPLHFDIWGRGTMVTVSSGGGGS<br>GGGGSGGGGSALSSELTQDPAVSVALGQTVRITCQGDSLRTHYASWYHQRPGRAPVLVNYPKD<br>SRPSGIPDRFSGSSSGNTASLTIIGAQAADEGDYYCQSRDSSGVLFGGGTKVTVLG | U.S. Patent Application Publication No. 2006/0269560 |
| 63 | EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYVD<br>SVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDFSGYGDYLDYWGKGTLVTVSSGGGG<br>SGGGGSGGGGSAQSALTQPPSASGSPGQSVTISCTGTSSDIGNYNYVSWYQQHPGKAPKLMIYE<br>VNERPSGVPDRFSGSKSGNTASLTVSGLRPEDEADYYCSSYAGNNAVIFGGGTQLTVLG | U.S. Patent Application Publication No. 2006/0269561 |

| SEQ ID | SEQUENCE | Reference |
|---|---|---|
| 64 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTTHAMHWVRQAPGQSLEWMGWINTGNGNTKYS QSFQGRVSITRDTSANTAYMELSSLKSEDTAMYYCARASRDSSGYYYVPPGDFFDIWGQGTLVT VSSGGGGSGGGGSGGGGSAQSALTQPASVSGSPGQSITISCTGSRSDIGGYNFVSWYQQHPGKAP KLLIYDVYNRPSGISDHFSGSKSDNTASLTISGLQSEDDADYYCSSYAGYHTWIFGGGTKVTVLG | U.S. Patent Application Publication No. 2006/0269562 |
| 65 | EVQLVQSGAEVKKPGASVKLSCKASGYTLVNYFMHWVRQAPGQGPEWMGMINPSGGTTKNR QKFQDRVTMTRDTSTRTVYMELSGLTSEDTAVYYCATDFKGTDILFRDWGRGTLVTVSSGGGG SGGGGSGGGGSAQSVLTQPPSASGTPGQRVSISCSGSSNIGSNTVIWYQQLPGTAPKLLMYSND RRPSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCATWDDSLNGHYVFGTGTKLTVLG | U.S. Patent Application Publication No. 2006/0269563 |
| 66 | QMQLVQSGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSAISGSGGSTYYADS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGSTFDIWGRGTMVTVSSGGGGSGGGGS GGGGSAQPVLTQPPSASGTPGQRVTISCSGSNSNIGSRPVNWYQQLPGTAPKLLIQGNNQRPSGV PDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLTGYVFGPGTKLTVLG | U.S. Patent Application Publication No. 2006/0269564 |
| 67 | QMQLVQSGGAVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSIKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARERLRGLDPWGQGTMVTVSSGGGGSGG GGSGGGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGI PDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVLG | U.S. Patent Application Publication No. 2006/0269565 |
| 68 | EVQLVETGGGLVQPGGSLRLSCAASGFTFSPYYMSWVRQAPGKGLEWVSAISGSGGSIYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTALYYCARGASGPDYWGRGTMVTVSSGGGSGGGGS GGGGSAQSVLTQPPSVSAAPGQKVTISCSGSTSNIGNNYVSWYQQVPGTAPKLLIYDNNKRPSGI PDRFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSALVFGGGTKVTVLG | U.S. Patent Application Publication No. 2006/0269566 |
| 69 | QVQLQQSGAEVKTPGSSVKVSCKASGGTFRNNAISWVRQAPGQGLEWMGGFIPKFGTTNHAQK FQGRVTMTADDSTNTVYMELSSLRSEDTAVYYCARGGAYCGGRCYLYGMDVWGQGTLVTV SSGGGGSGGGGSGGGGSAQAVVIQEPSLTVSPGGTVTLTCGSSTGAVTSGHYPYWFQQKPGQAP RTLIYDTSNKRSWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCLVSYSGSLVVFGGGTKLTVL G | U.S. Patent Application Publication No. 2006/0269567 |

| SEQ ID | SEQUENCE | Reference |
|---|---|---|
| 70 | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVKGAWLDYWGRGTMVTVSSGGGGSGGGGS GGGGSALNFMLTQPHSVSESPGKTVTISCTGSSGSVARNYVQWYQQRPGSAPTIVIYEDNRRPSG VPGRFSGSIDRSSNSASLTISGLQTEDEADYYCQSYNYNTWVFGGGTKLTVLG | U.S. Patent Application Publication No. 2006/0269568 |
| 71 | EVQLVQSGAEVKKPGASVKVSCRASGYTFTSYGITWVRQAPGQGLEWMGWISAYNGKTNYVQ ELQGRVTMTTDTSTSTVYMELTSLRSDDTAVYYCARRGNNYRFGYFDFWGQGTLVTVSSGGG GSGGGGSGGGGSALETTLTQSPGTLSLSPGERATLSCRASQSISSSNLAWYQQKPGRAPRLLIYG ASSRAIGIPDRFSGSGSGTDFTLTISRLEAEDFAVYYCQQYGSSPITFGQGTRLEIKR | U.S. Patent Application Publication No. 2006/0269569 |
| 72 | QVQLQQSGPG LVKPSQTLSL TCAISGDSVS STTVAWDWIR QSPSRGLEWL GRTYYRSKWY NEYAVSVKSR ITINVDTSKN QISLQLNSVT PEDTAVYYCA REPDAGRGAF DIWGQGTTVT SPLRWGRFGW RGLGRGWLRS PVTQSPGTLS LSPGERATLS CRASQSVSSS HLAWYQQKPG QAPRLLIYGA SSRATGIPDR FSGSGSGTDF TLTISSLEPE DFAVYYCQQR SNWPPRAVFG QGTRLEIK | U.S. Patent No. 8,097,704 |
| 73 | QVQLQQSGPG RVQPSQTLSL TCAISGDSVS NNNAAWYWIR QSPSRGLEWL GRTYYRSKWY NDYAVSVKSR ITISPDTSKN QFSLQLNSVT PEDTAVYYCA RRGDGNSYFD YWGQGTLVTV SSGGLRWGRF GWRGLGRGWL EIVLTQSPGT LSLSPGERAT LSCRASQSVS SGYVSWYRQK PGQAPRLLIY GASTRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCH QYGSSPNTYG QGTKVGIK | U.S. Patent No 8,097,705 |

**[0141]** In certain aspects the DR5 binding domain comprises a VH and a VL, wherein the VH and VL comprise amino acid sequences at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 7 and SEQ ID NO: 8; SEQ ID NO: 9 and SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 13 and SEQ ID NO: 14; SEQ ID NO: 15 and SEQ ID NO: 16; SEQ ID NO: 17 and SEQ ID NO: 18; SEQ ID NO: 19 and SEQ ID NO: 20; SEQ ID NO: 21 and SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 24; SEQ ID NO: 25 and SEQ ID NO: 26; SEQ ID NO: 27 and SEQ ID NO: 28; SEQ ID NO: 29 and SEQ ID NO: 30; SEQ ID NO: 31 and SEQ ID NO: 32; SEQ ID NO: 33 and SEQ ID NO: 34; SEQ ID NO: 35 and SEQ ID NO: 36; SEQ ID NO: 37 and SEQ ID NO: 38; SEQ ID NO: 39 and SEQ ID NO: 40; SEQ ID NO: 41 and SEQ ID NO: 42; SEQ ID NO: 43 and SEQ ID NO: 44; SEQ ID NO: 45 and SEQ ID NO: 46; SEQ ID NO: 47 and SEQ ID NO: 48; SEQ ID NO: 49 and SEQ ID NO: 50; SEQ ID NO: 51 and SEQ ID NO: 52; SEQ ID NO: 53 and SEQ ID NO: 54; SEQ ID NO: 55 and SEQ ID NO: 56; SEQ ID NO: 82 and SEQ ID NO: 83; SEQ ID NO: 84 and SEQ ID NO: 85; SEQ ID NO: 86 and SEQ ID NO: 87; or SEQ ID NO: 88 and SEQ ID NO: 89; respectively, or where the VH and VL are situated in an ScFv comprising an amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

**[0142]** While a variety of different dimeric, pentameric, and hexameric binding molecules can be contemplated by a person of ordinary skill in the art based on this disclosure, and as such are included in this disclosure, in certain aspects, a binding molecule as described above is provided in which each binding unit comprises two IgA or IgM heavy chains each comprising a VH situated amino terminal to the IgA or IgM constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

**[0143]** Moreover in certain aspects, at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule, comprises or comprise two of the DR5 binding domains as described above. In certain aspects the two DR5 binding domains in the at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule, can be different from each other, or they can be identical.

**[0144]** In certain aspects, the two IgA or IgM heavy chains within the at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule, are identical. In certain aspects, two identical IgA or IgM heavy chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule comprise the heavy chain variable domain amino acid sequences as disclosed in Tables 2 and 3.

**[0145]** In certain aspects, the two light chains within the at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule, are identical. In certain aspects, two identical light chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule are kappa light chains, e.g., human kappa light chains, or lambda light chains, e.g., human lambda light chains. In certain aspects, two identical light chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule each comprise the light chain variable domain amino acid sequences as disclosed in Tables 2 and 3.

**[0146]** In certain aspects at least one, at least two, at least three, at least four, at least five, or at least six binding units of a dimeric, pentameric, or hexameric binding molecule provided by this disclosure comprises or each comprise two identical IgA or IgM heavy chain constant regions each comprising identical heavy chain variable domain amino acid sequences as disclosed in Tables 2 and 3, and two identical light chains each comprising identical heavy chain variable domain amino acid sequences as disclosed in Tables 2 and 3. According to this aspect, the DR5 binding domains in the at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule, can be identical. Further according to this aspect, a dimeric, pentameric, or hexameric binding

molecule as provided herein can comprise at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve copies of an DR5 binding domain as described above. In certain aspects at least two, at least three, at least four, at least five, or at least six of the binding units can be identical and, in certain aspects the binding units can comprise identical binding domains, e.g., at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve DR5 binding domains can be identical.

**[0147]** In certain aspects, a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule as provided herein can possess advantageous structural or functional properties compared to other binding molecules. For example, the dimeric, pentameric, or hexameric TNF superfamily receptor protein binding relative to a corresponding bivalent binding molecule having the same antigen binding domains. Biological assays include, but are not limited to ELISA and Western blot caspase assays, and FACS analyses using stains indicative of apoptotic cell death such as annexin-v. In certain aspects a dimeric, pentameric, or hexameric binding molecule as provided herein can trigger apoptosis of a TNF superfamily receptor protein-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same TNF superfamily receptor protein epitope as the TNF superfamily receptor protein binding domain. In certain aspects a dimeric, pentameric, or hexameric binding molecule as provided herein can trigger apoptosis of a DR5-expressing cell at higher potency than an equivalent amount of monospecific, bivalent anti-DR5 monoclonal antibody or fragment thereof, where the antibody is, or comprises the same VH and VL regions as, the antibodies provided in Tables 2 and 3, e.g., Conatumumab (Amgen), Drozitumab (Genentech), or Lexatumumab (HGS/GlaxoSmithKline).

Polynucleotides, Vectors, and Host Cells

**[0148]** The disclosure further provides a polynucleotide, e.g., an isolated, recombinant, and/or non-naturally-occurring polynucleotide, comprising a nucleic acid sequence that encodes a polypeptide subunit of a dimeric, pentameric, or hexameric binding molecule as provided herein. By “polypeptide subunit” is meant a portion of a binding molecule, binding unit, or binding domain that can be independently translated. Examples include, without limitation, an

antibody VH, an antibody VL, a single chain Fv, an antibody heavy chain, an antibody light chain, an antibody heavy chain constant region, an antibody light chain constant region, and/or any fragment thereof.

**[0149]** The disclosure further provides a composition comprising two or more polynucleotides, where the two or more polynucleotides collectively can encode a dimeric, pentameric, or hexameric binding molecule as described above. In certain aspects the composition can include a polynucleotide encoding an IgA or IgM heavy chain or fragment thereof, e.g, a human IgA or IgM heavy chain as described above where the IgA or IgM heavy chain comprises at least the VH of a TNF superfamily receptor protein binding domain, and a polynucleotide encoding a light chain or fragment thereof, e.g., a human kappa or lambda light chain that comprises at least the VL of a TNF superfamily receptor protein binding domain. A polynucleotide composition as provided can further include a polynucleotide encoding a J chain, e.g., a human J chain, or a fragment thereof or a variant thereof. In certain aspects the polynucleotides making up a composition as provided herein can be situated on two or three separate vectors, e.g., expression vectors. Such vectors are provided by the disclosure. In certain aspects two or more of the polynucleotides making up a composition as provided herein can be situated on a single vector, e.g., an expression vector. Such a vector is provided by the disclosure.

**[0150]** The disclosure further provides a host cell, e.g., a prokaryotic or eukaryotic host cell, comprising a polynucleotide or two or more polynucleotides encoding a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule as provided herein, or any subunit thereof, a polynucleotide composition as provided herein, or a vector or two, three, or more vectors that collectively encode a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule as provided herein, or any subunit thereof. In certain aspects a host cell provided by the disclosure can express a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule as provided by this disclosure, or a subunit thereof.

**[0151]** In a related aspect, the disclosure provides a method of producing a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule as provided by this disclosure, where the method comprises culturing a host cell as described above, and recovering the binding molecule.

Methods of Use

**[0152]** This disclosure provides improved methods for triggering apoptosis of cells that express TNF superfamily receptor proteins, e.g., malignant or immortalized cells, using a dimeric, pentameric, or hexameric IgA- or IgM-based TNF superfamily receptor protein binding molecule as provided herein. The methods described below can utilize binding molecules comprising TNF superfamily receptor protein binding domains derived from any existing anti-TNF superfamily receptor protein antibodies, including without limitation the antibodies provided in Tables 2 and 3, or variants, derivatives, or analogs thereof, where the dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule can provide improved apoptosis-mediated cell death TNF superfamily receptor protein-expressing cells as compared to an equivalent bivalent antibody, fragment, variant, derivative, or analog. Based on this disclosure, construction of a dimeric, pentameric, or hexameric IgA- or IgM-based TNF superfamily receptor protein binding molecule comprising any TNF superfamily receptor protein binding domain of interest is well within the capabilities of a person of ordinary skill in the art. The improved activity can, for example, allow a reduced dose to be used, or can result in more effective killing of cells that are resistant to killing by the original antibody. By "resistant" is meant any degree of reduced activity of an anti-TNF superfamily receptor protein antibody on the TNF superfamily receptor protein-expressing cell.

**[0153]** In certain aspects, this disclosure provides a method for triggering apoptosis, morphogenesis or proliferation in cells which express TNF superfamily receptor proteins, where the method includes contacting a TNF superfamily receptor protein-expressing cell with a dimeric, pentameric, or hexameric binding molecule as described herein, where the binding molecule triggers apoptosis, morphogenesis or proliferation of a TNF superfamily receptor protein-expressing cell at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same TNF superfamily receptor protein epitope as the TNF superfamily receptor protein binding domain.

**[0154]** In yet another aspect a TNF superfamily receptor protein binding molecule as provided herein can facilitate cancer treatment, *e.g.*, by slowing tumor growth, stalling tumor growth, or reducing the size of existing tumors, when administrated as an effective dose to a subject in need of cancer treatment. The disclosure provides a method of treating cancer comprising

administering to a subject in need of treatment an effective dose of a TNF superfamily receptor protein binding molecule as provided herein, e.g., a DR5 binding molecule as provided herein.

**[0155]** In certain aspects the TNF superfamily receptor protein-expressing cell is an immortalized cell line, i.e. a cancer cell. The terms "cancer", "tumor", "cancerous", and "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancers include but are not limited to, carcinoma including adenocarcinomas, lymphomas, blastomas, melanomas, sarcomas, and leukemias. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, Hodgkin's and non-Hodgkin's lymphoma, pancreatic cancer, glioblastoma, glioma, cervical cancer, ovarian cancer, liver cancer such as hepatic carcinoma and hepatoma, bladder cancer, breast cancer (including hormonally mediated breast cancer, see, *e.g.*, Innes *et al.* (2006) Br. J. Cancer 94:1057-1065), colon cancer, colorectal cancer, endometrial carcinoma, myeloma (such as multiple myeloma), salivary gland carcinoma, kidney cancer such as renal cell carcinoma and Wilms' tumors, basal cell carcinoma, melanoma, prostate cancer, vulval cancer, thyroid cancer, testicular cancer, esophageal cancer, various types of head and neck cancer including, but not limited to, squamous cell cancers, and cancers of mucinous origins, such as, mucinous ovarian cancer, cholangiocarcinoma (liver) and renal papillary carcinoma. Mucosal distribution, for example as provided by an IgA-based binding molecule as provided herein, could be beneficial for certain cancers, e.g., lung cancer, ovarian cancer, colorectal cancer, or squamous cell carcinoma.

**[0156]** This disclosure further provides a method of preventing or treating a cancer in a subject in need thereof, comprising administering to the subject an effective amount of a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule as provided herein or an antigen-binding fragment thereof, a composition or formulation comprising the binding molecule, or a polynucleotide, a vector, or a host cell as described herein.

**[0157]** Effective doses of compositions for treatment of cancer vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. In certain aspects the treatment methods provided herein can provide increased safety, in that the composition exhibits greater cytotoxicity (e.g.,

induces apoptosis to a greater extent) on cancer cells than on non-cancer cells, e.g., normal human hepatocytes. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated. Treatment dosages can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

**[0158]** The compositions of the disclosure can be administered by any suitable method, *e.g.*, parenterally, intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

**[0159]** The subject to be treated can be any animal, *e.g.*, mammal, in need of treatment, in certain aspects, subject is a human subject.

**[0160]** In its simplest form, a preparation to be administered to a subject is a dimeric, pentameric, or hexameric binding molecule as provided herein, or an antigen-binding fragment thereof, administered in conventional dosage form, which can be combined with a pharmaceutical excipient, carrier or diluent as described elsewhere herein.

**[0161]** A TNF superfamily receptor protein binding molecule as provided herein or an antigen-binding fragment thereof can be administered by any suitable method as described elsewhere herein, *e.g.*, via IV infusion. In certain aspects, a TNF superfamily receptor protein binding molecule as provided herein or an antigen-binding fragment thereof can be introduced into a tumor, or in the vicinity of a tumor cell.

**[0162]** All types of tumors are potentially amenable to treatment by this approach including, without limitation, carcinoma of the breast, lung, pancreas, ovary, kidney, colon and bladder, as well as melanomas, sarcomas and lymphomas. Mucosal distribution could be beneficial for certain cancers, e.g., lung cancer, ovarian cancer, colorectal cancer, or squamous cell carcinoma.

**[0163]** A dimeric, pentameric, or hexameric binding molecule for use in the methods provided herein, is a binding molecule with two, five, or six "binding units" as defined herein, that can specifically bind to a TNF superfamily receptor protein, e.g., human DR5. In certain aspects, a dimeric, pentameric, or hexameric binding molecule for use in the methods provided herein comprises two, five, or six bivalent binding units, respectively, where each binding unit includes

two IgA or IgM heavy chain constant regions or fragments thereof. In certain aspects, the two IgA or IgM heavy chain constant regions are human heavy chain constant regions.

**[0164]** Where the binding molecule for use in the methods provided herein is a dimeric IgA-based binding molecule, the binding molecule can further comprise a J chain, or fragment thereof, or variant thereof, and can further comprise a secretory component, or fragment thereof, or variant thereof.

**[0165]** Where the binding molecule for use in the methods provided herein is pentameric IgM-based binding molecule, the binding molecule can further comprise a J chain, or fragment thereof, or variant thereof.

**[0166]** An IgA heavy chain constant region of a binding molecule for use in the methods provided herein can include one or more of a Cα1 domain, a Cα2 domain, and/or a Cα3 domain, provided that the constant region can serve a desired function in the binding molecule, e.g., associate with a light chain constant region to facilitate formation of a binding domain, or associate with another binding unit to form a dimer. In certain aspects the two IgA heavy chain constant regions or fragments thereof within an individual binding unit each comprise a Cα3 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a Cα3 domain and a TP or fragment thereof. In certain aspects the two IgA heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cα2 domain or fragment thereof, a Cα1 domain or fragment thereof, or a Cα1 domain or fragment thereof and a Cα2 domain or fragment thereof.

**[0167]** An IgM heavy chain constant region of a binding molecule for use in the methods provided herein can include one or more of a Cμ1 domain, a Cμ2 domain, a Cμ3 domain, and/or a Cμ4 domain, provided that the constant region can serve a desired function in the binding molecule, e.g., associate with a light chain constant region to facilitate formation of a binding domain, or associate with other binding units to form a hexamer or a pentamer. In certain aspects the two IgM heavy chain constant regions or fragments thereof within an individual binding unit each comprise a Cμ3 domain or fragment thereof, a Cμ4 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a Cμ3 domain a Cμ4 domain, and a TP or fragment thereof. In certain aspects the two IgM heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cμ2

domain or fragment thereof, a Cμ1 domain or fragment thereof, or a Cμ1 domain or fragment thereof and a Cμ2 domain or fragment thereof.

**[0168]** While a variety of different dimeric, pentameric, and hexameric binding molecules for use in the methods provided herein can be contemplated by a person of ordinary skill in the art based on this disclosure, and as such are included in this disclosure, in certain aspects, a binding molecule for use in the methods provided herein is provided in which each binding unit comprises two IgA or IgM heavy chains each comprising a VH situated amino terminal to the IgA or IgM constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

**[0169]** Moreover in certain aspects, at least one binding unit of the binding molecule for use in the methods provided herein, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein, comprises or comprise two of the TNF superfamily receptor protein binding domains as described above. In certain aspects the two TNF superfamily receptor protein binding domains in at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein for use in the methods provided herein can be different from each other, or they can be identical.

**[0170]** In certain aspects, the two IgA or IgM heavy chains within at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein are identical.

**[0171]** In certain aspects, the two light chains within at least one binding unit of the binding molecule, or at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein are identical. In certain aspects, two identical light chains within at least one binding unit, or within at least two, at least three, at least four, at least five, or at least six binding units of the binding molecule for use in the methods provided herein are kappa light chains, e.g., human kappa light chains, or lambda light chains, e.g., human lambda light chains.

**[0172]** Dimeric, pentameric, or hexameric TNF receptor binding molecule for use in the methods provided herein can possess advantageous structural or functional properties compared to other binding molecules. For example, a dimeric, pentameric, or hexameric TNF receptor binding

molecule for use in the methods provided herein can possess improved activity in a biological assay, either in vitro or in vivo, than a corresponding binding molecule, e.g., Lexatumumab or a variant, analog, or derivative thereof. Biological assays include, but are not limited to ELISA or Western blot caspase assays, and FACS analyses using stains indicative of apoptotic cell death such as annexin-v.

Pharmaceutical Compositions and Administration Methods

**[0173]** Methods of preparing and administering a dimeric, pentameric, or hexameric TNF receptor binding molecule as provided herein to a subject in need thereof are well known to or are readily determined by those skilled in the art in view of this disclosure. The route of administration of a TNF receptor binding molecule can be, for example, oral, parenteral, by inhalation or topical. The term parenteral as used herein includes, *e.g.*, intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal, or vaginal administration. While these forms of administration are contemplated as suitable forms, another example of a form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. A suitable pharmaceutical composition can comprise a buffer (*e.g.* acetate, phosphate or citrate buffer), a surfactant (*e.g.* polysorbate), optionally a stabilizer agent (*e.g.* human albumin), etc.

**[0174]** As discussed herein, a dimeric, pentameric, or hexameric TNF receptor binding molecule as provided herein can be administered in a pharmaceutically effective amount for the *in vivo* treatment of cancers expressing TNF superfamily receptor proteins. In this regard, it will be appreciated that the disclosed binding molecules can be formulated so as to facilitate administration and promote stability of the active agent. Pharmaceutical compositions accordingly can comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives and the like. A pharmaceutically effective amount of a dimeric, pentameric, or hexameric TNF receptor binding molecule as provided herein means an amount sufficient to achieve effective binding to a target and to achieve a therapeutic benefit. Suitable formulations are described in Remington's Pharmaceutical Sciences (Mack Publishing Co.) 16th ed. (1980).

**[0175]** Certain pharmaceutical compositions provided herein can be orally administered in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also can be administered by nasal aerosol or inhalation. Such compositions can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other conventional solubilizing or dispersing agents.

**[0176]** The amount of a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule that can be combined with carrier materials to produce a single dosage form will vary depending, e.g., upon the subject treated and the particular mode of administration. The composition can be administered as a single dose, multiple doses or over an established period of time in an infusion. Dosage regimens also can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

**[0177]** In keeping with the scope of the present disclosure, a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule as provided herein can be administered to a subject in need of therapy in an amount sufficient to produce a therapeutic effect. A dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule as provided herein can be administered to the subject in a conventional dosage form prepared by combining the antibody or antigen-binding fragment, variant, or derivative thereof of the disclosure with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. The form and character of the pharmaceutically acceptable carrier or diluent can be dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

**[0178]** By "therapeutically effective dose or amount" or "effective amount" is intended an amount of a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule, that when administered brings about a positive therapeutic response with respect to treatment of a patient with cancer expressing TNF superfamily receptor protein.

**[0179]** Therapeutically effective doses of the compositions disclosed herein for treatment of cancer can vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. In certain

aspects, the subject or patient is a human, but non-human mammals including transgenic mammals can also be treated. Treatment dosages can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

**[0180]** The amount of a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule to be administered is readily determined by one of ordinary skill in the art without undue experimentation given this disclosure. Factors influencing the mode of administration and the respective amount of a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule include, but are not limited to, the severity of the disease, the history of the disease, and the age, height, weight, health, and physical condition of the individual undergoing therapy. Similarly, the amount of a dimeric, pentameric, or hexameric TNF receptor binding molecule to be administered will be dependent upon the mode of administration and whether the subject will undergo a single dose or multiple doses of this agent.

**[0181]** This disclosure also provides for the use of a dimeric, pentameric, or hexameric TNF superfamily receptor protein binding molecule in the manufacture of a medicament for treating, preventing, or managing cancer where the cancer expresses TNF superfamily receptor proteins.

**[0182]** This disclosure employs, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook *et al.*, ed. (1989) Molecular Cloning A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) Molecular Cloning: A Laboratory Manual, (Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) DNA Cloning, Volumes I and II; Gait, ed. (1984) Oligonucleotide Synthesis; Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) Nucleic Acid Hybridization; Hames and Higgins, eds. (1984) Transcription And Translation; Freshney (1987) Culture Of Animal Cells (Alan R. Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) A Practical Guide To Molecular Cloning; the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian Cells, (Cold Spring Harbor Laboratory); Wu *et al.*, eds., Methods In Enzymology, Vols. 154 and 155; Mayer and Walker, eds. (1987) Immunochemical Methods In Cell And

Molecular Biology (Academic Press, London); Weir and Blackwell, eds., (1986) Handbook Of Experimental Immunology, Volumes I-IV; Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel *et al.* (1989) Current Protocols in Molecular Biology (John Wiley and Sons, Baltimore, Md.).

**[0183]** General principles of antibody engineering are set forth in Borrebaeck, ed. (1995) Antibody Engineering (2nd ed.; Oxford Univ. Press). General principles of protein engineering are set forth in Rickwood *et al.*, eds. (1995) Protein Engineering, A Practical Approach (IRL Press at Oxford Univ. Press, Oxford, Eng.). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff (1984) Molecular Immunology (2nd ed.; Sinauer Associates, Sunderland, Mass.); and Steward (1984) Antibodies, Their Structure and Function (Chapman and Hall, New York, N.Y.). Additionally, standard methods in immunology known in the art and not specifically described can be followed as in Current Protocols in Immunology, John Wiley & Sons, New York; Stites *et al.*, eds. (1994) Basic and Clinical Immunology (8th ed; Appleton & Lange, Norwalk, Conn.) and Mishell and Shiigi (eds) (1980) Selected Methods in Cellular Immunology (W.H. Freeman and Co., NY).

**[0184]** Standard reference works setting forth general principles of immunology include Current Protocols in Immunology, John Wiley & Sons, New York; Klein (1982) J., Immunology: The Science of Self-Nonself Discrimination (John Wiley & Sons, NY); Kennett *et al.*, eds. (1980) Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses (Plenum Press, NY); Campbell (1984) "Monoclonal Antibody Technology" in Laboratory Techniques in Biochemistry and Molecular Biology, ed. Burden *et al.*, (Elsevier, Amsterdam); Goldsby *et al.*, eds. (2000) Kuby Immunology (4th ed.; H. Freemand & Co.); Roitt *et al.* (2001) Immunology (6th ed.; London: Mosby); Abbas *et al.* (2005) Cellular and Molecular Immunology (5th ed.; Elsevier Health Sciences Division); Kontermann and Dubel (2001) Antibody Engineering (Springer Verlag); Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press); Lewin (2003) Genes VIII (Prentice Hall, 2003); Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Press); Dieffenbach and Dveksler (2003) PCR Primer (Cold Spring Harbor Press).

**[0185]** All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

**[0186]** The following examples are offered by way of illustration and not by way of limitation.

## Examples

### Example 1: DR5 Expression Profiling

**[0187]** DR5 cell surface expression was quantitated by fluorescence activated cell sorting (FACS) analysis. Briefly, FACS stain buffer (BD Pharmigen Catalog #554656) was used for staining and wash steps. Tumor cells ($1x10^5$-$5x10^{5)}$ were stained with 0.25 μg of anti-human DR5-PE (eBioscience Catalog #12-9908-42) or isotype-PE control (eBioscience Catalog #12-4714-42) for 15 minutes at 4°C, protected from light. Cells were washed twice, resuspended in FACS stain buffer, and results were acquired by flow cytometry. **Figure 1A** shows the peak shift with the anti-human DR5 antibody (lower panel) as opposed to the isotype control (upper panel). **Figure 1B** shows that DR5 expression varies by cell line, with 293F cells expressing the most DR5 among this set of cell lines.

### Example 2: Anti-DR5 mAb Specificity

Specificity ELISA

**[0188]** The purpose of this assay is to demonstrate anti-DR5 mAb binding to DR5, but not to DR4 or decoy receptors DcR1 and DcR2. Soluble DR5, DR4, DcR1, or DcR2 protein (R&D Systems Catalog #631-T2-100/CF, 347-DR-100/CF, 630-TR-100/CF, 633-TR-100 respectively) were coated on an ELISA plate at 2 μg/mL in 100 mM $NaHCO_3$ pH 9.5 overnight at 4°C. A solution of 2% BSA in PBS was used for the blocking and antibody incubation steps. The plate was blocked for 1 hour at room temperature, then incubated with mouse anti-human DR5 mAb (Acris Antibodies Catalog #AM31206AF-N) for 1 hour at room temperature. anti-DR5 mAb was 3-fold serially diluted to concentrations ranging from 1 to 200 ng/mL. After washing 3 times with PBS plus 0.05% Tween-20, the plate was incubated with rat anti-mouse kappa-HRP (Southern Biotech Catalog #1180-05) for 1 hour at room temperature, protected from light. After washing 3 times with PBS plus 0.05% Tween-20, the plate was incubated with TMB substrate (BD Biosciences Catalog #555214) for 20 minutes at room temperature. The reaction

was stopped with 1M $H_2SO_4$ and absorbance at 450nm was determined using a microtiter plate reader. **Figure 2** shows that the mouse anti-human DR5 mAb bound to DR5, but not to DR4, DcR1 or DcR2.

**[0189]** For Human Anti-DR5 mAb ELISAs, 1:2000 Mouse Anti-Human Lambda-HRP (Southern Biotech Catalog #9180-05) or 1:6000 Mouse Anti-Human Kappa-HRP (Southern Biotech Catalog #9230-05) was used for detection.

Cell Binding

**[0190]** This example was used to confirm anti-DR5 mAb binding to cells. FACS Stain Buffer (BD Pharmigen Catalog #554656) was used for staining and washing steps. Colo205 cells ($2x10^5$ cells from ATCC Catalog #CCL-222) were stained with 10 µg/mL of mouse anti-human DR5 mAb (Acris Antibodies Catalog #AM31206AF-N) or an isotype control (Invivogen Catalog #hcd20-mab9) for 15 minutes at 4°C, protected from light. Cells were washed twice, then stained with goat anti-mouse IgG-APC (Jackson ImmunoResearch Catalog #115-136-071) at a final dilution of 1:200 for 15 minutes at 4°C, protected from light. Cells were washed twice, then resuspended in FACS stain buffer, and results were acquired by flow cytometry. Results are provided in **Figure 3**, the lower panel showing the peak shift observed for the anti-DR5 mAb.

**[0191]** For Human Anti-DR5 mAb binding, Goat Anti-Human IgG Fc-Alexa 647 (Southern Biotech Catalog #2014-31) or Rabbit Anti-Human IgM-Alexa 647 (Abcam Catalog #ab 150191) was used for detection.

## Example 3: Anti-DR5 mAb Functional Activity and Cytotoxicity Assay

**[0192]** This example demonstrates that cross-linking is required to achieve DR5 mAb cytotoxicity. Colo205 cells (ATCC Catalog #CCL-222) were seeded, $1x10^4$ cells per well, in a 96-well plate and allowed to attach overnight. The next day, cells were treated with serially diluted mouse anti-human DR5 mAb (Acris Antibodies Catalog #AM31206AF-N, R&D Systems Catalog #MAB631, BioLegend Catalog #307402, or Acris Antibodies Catalog # AM31205AF-N) and incubated for 24 hours at 37°C. Colorimetric readout: three hours prior to harvest, CCK-8 cell viability reagent (Dojindo CK04-13) was added at 10 percent of the total

reaction volume and the plate was incubated at 37°C for the remaining 3 hours. Absorbance at 450 nm was evaluated on a plate reader. Results are shown in **Figure 4** (open circles), showing little or no cytotoxicity in the absence of a cross-linker.

**[0193]** The assay was then performed as above, except that after pre-incubation of cells with serially diluted mouse anti-human DR5 mAb for 20 minutes at room temperature, goat anti-mouse IgG1 Fc (Jackson ImmunoResearch Catalog #115-005-205) cross-linking agent was added at 3 fold the concentration of the highest dose of anti-DR5 mAb. Results are provided in **Figure 4** (closed circles), showing 100% cytotoxicity with the cross-linker.

**[0194]** For human anti-DR5 mAb induced cytotoxicity, Anti-Human IgG Fc (Biolegend Catalog #409302) was used as the cross-linking agent. Alternatively, goat anti-human IgG plus IgM (H+L) Fab2 (Jackson ImmunoResearch Catalog #109-006-127) can be used as cross-linking agent.

**[0195]** Luminescent readout: at time of harvest, Cell Titer Glo viability reagent (Promega G7572) was added at a volume equal to that of the medium in the well. Cells were lysed for 10 min and luminescence was read on a plate reader.

## Example 4: Apoptosis Assays

**[0196]** Anti-DR5-induced apoptosis in the presence or absence of cross linker was measured using the following methods. Colo205 cells ($1x10^5$ cells from ATCC, Catalog #CCL-222) were treated with 5 μg/mL mouse anti-human DR5 mAb (Acris Antibodies Catalog #AM31206AF-N or R&D Systems Catalog #MAB631) or an isotype control (Invivogen Catalog #hcd20-mab9) for up to 4 hours at 37°C. Cells were washed twice with cold PBS, then stained with Annexin V-PE and 7-AAD (1 μL each per sample) in the supplied binding buffer (BD Pharmigen Catalog #559763) for 15 minutes at 4°C, protected from light. Annexin V and 7-AAD were used to measure apoptotic (x-axis) and dead cells (y-axis), respectively, using flow cytometry. Results with untreated cells are shown in the leftmost panel of **Figure 5** (untreated), and results using anti-human DR5 mAb in the absence of cross linker are shown in the second panel from the left in **Figure 5** (Anti-DR5 IgG only). Little or no change in the pattern was observed.

**[0197]** The assay was then performed as above, except that after pre-incubation of cells with serially diluted mouse anti-human DR5 mAb for 20 minutes at room temperature, goat anti-

mouse IgG1 Fc (Jackson ImmunoResearch Catalog #115-005-205) cross-linking agent was added at 3 fold increased concentration over of the highest dose of anti-DR5 mAb. The third panel from the left in **Figure 5** shows results from cross-linker only (no apoptosis). The right-most panel of **Figure 5** shows significant annexin V-stained cells, indicating that apoptosis occurred in the presence of the cross-linker (Anti-DR5 IgG + crosslinker).

**[0198]** For human anti-DR5 mAb induced apoptosis, Anti-Human IgG Fc (Biolegend Catalog #409302) was used as the cross-linking agent. Alternatively, goat anti-human IgG plus IgM (H+L) Fab2 (Jackson ImmunoResearch Catalog #109-006-127) can be used as cross-linking agent.

## Example 5: Caspase Activation Assay

**[0199]** This example shows anti-DR5 induced apoptosis demonstrated by caspase activation. Colo205 cells (ATCC Catalog #CCL-222) were seeded with $1x10^4$ cells per well in a 96-well plate and allowed to attach overnight. The next day, cells were treated with 5 µg/mL mouse anti-human DR5 mAb (Acris Antibodies Catalog #AM31206AF-N or R&D Systems Catalog #MAB631) for up to 24 hours at 37°C. At time of harvest, Caspase Glo 3/7 reagent (Promega Catalog #G8090) was added at a volume equal to the total media in each well. Reaction was incubated with shaking, for 30 minutes at room temperature and luminescence was evaluated on a plate reader. In the absence of cross-linking little or no caspase activity was observed (**Figure 6**, crosshatch bars).

**[0200]** The assay was then performed as above, except that after pre-incubation of cells with serially diluted mouse anti-human DR5 mAb for 20 minutes at room temperature, goat anti-mouse IgG1 Fc (Jackson ImmunoResearch Catalog #115-005-205) cross-linking agent was added at 3 fold over the concentration of anti-DR5 mAb. The results are shown as solid bars in **Figure 6**. In the presence of cross linking, significant caspase activity was observed.

**[0201]** For human anti-DR5 mAb induced caspase activation, Anti-Human IgG Fc (Biolegend Catalog #409302) was used as the cross-linking agent. Alternatively, goat anti-human IgG plus IgM (H+L) Fab2 (Jackson ImmunoResearch Catalog #109-006-127) can be used as cross-linking agent.

## Example 6: Multimeric Anti-DR5 Antibodies Have Superior Activity

**[0202]** This example shows the superior in vitro activity of multimeric anti-DR5 antibodies. **Figure 7A**, is a photograph of a non-reducing SDS-PAGE showing one anti-DR5 mAb that is predominantly multimeric (lane 1, R&D Systems clone 71903), lane 2 corresponds to BioLegend clone DJR2-4, lane 3 corresponds to Acris Antibodies clone B-K29, and lane 4 corresponds to Acris Antibodies clone B-D37.

### Anti-DR5 mAb Functional Activity and Cytotoxicity Assay

**[0203]** Using similar methods as in Example 3, it is shown that only the multimeric Anti-DR5 mAb causes Colo205 cytotoxicity in the absence of cross-linker. (*See*, **Figure 7B**; R&D Systems clone 71903, filled squares; BioLegend clone DJR2-4, open circles, dashed line; Acris Antibodies clone B-K29, filled diamonds; Acris Antibodies clone B-D37, open triangles, dashed line).

### Apoptosis Assays

**[0204]** Using similar methods as in Example 4, it is shown that in the absence of cross-linker, the multimeric anti-DR5 mAb induces apoptosis in Colo205 cells over time, but not the monomeric anti-DR5 mAb or isotype control. (*See*, **Figure 7C**; 1 hr, 2 hr and 4 hr time points shown).

### Caspase Activation Assay

**[0205]** Using methods similar to those provided in Example 5, it is shown that in the absence of crosslinker the multimeric, but not monomeric, anti-DR5 mAb induces caspase activation in Colo205 cells. (*See*, **Figure 7D**).

## Example 7: Construction of an IgM Anti-DR5 Antibody

### Generation of DNA constructs

**[0206]** The VH and VL sequences according to SEQ ID NO: 1 and SEQ ID NO: 2 (Anti-DR5 mAb #1), the VH and VL sequences according to SEQ ID NO; 5 and SEQ ID NO: 6 (Anti-DR5 mAb #2), the VH and VL sequences according to SEQ ID NO; 84 and SEQ ID NO: 85 (Anti-

DR5 mAb #3), and the VH and VL sequences according to SEQ ID NO; 88 and SEQ ID NO: 89 (Anti-DR5 mAb #4) were inserted into Aragen Biosciences and Lake Pharma proprietary IgG and IgM vectors by standard cloning methods.

Transfection

**[0207]** Mammalian cells are co-transfected with equal molar ratios of different expression vectors by standard procedures.

Purification of Human Anti-DR5 IgG

**[0208]** Human anti-DR5 IgG is purified using the MabSelectSuRe affinity matrix (GE Life Sciences Catalog #17-5438-01) according to manufacturer's recommendation.

Purification of Human Anti-DR5 IgM.

**[0209]** Human anti-DR5 IgM with or without J chain is purified using the Capture Select IgM affinity matrix (BAC, Thermo Fisher Catalog #2890.05) according to manufacturer's recommendation.

## Example 8: IgM Anti-DR5 mAb #2 is Specific for DR5

**[0210]** An IgG version and pentameric IgM-J chain version of anti-DR5 mAb #2 were tested for binding specificity by ELISA as described in Example 2. As shown in **Figure 8A** (IgG) and **Figure 8B** (IgM), the antibodies bound to human DR5 but not human DR4 or either of the decoy receptors DcR1 or DcR2.

**[0211]** IgG and IgM versions of anti-DR5 Mab #1 were tested for binding to the target cell Colo205 by the method described in Example 2. The results are shown in **Figure 9**.

## Example 9: IgM Anti-DR5 mAbs are More Cytotoxic than the Corresponding IgG Versions

**[0212]** IgG versions and pentameric IgM-J chain versions of anti-DR5 Mabs #1, #2, #3, and #4 were tested for cytotoxicity on Colo205 cells using the bioluminescence assay described in Example 3. As shown in **Figure 10A-D**, anti-DR5 IgMs are more cytotoxic than the IgG

counterparts. In **Figure 10E**, the IgM version of anti-DR5 Mab #1 was compared to the corresponding IgG version with and without a crosslinker. Even with the crosslinker, the IgM version was more cytotoxic.

Hepatotoxicity Assay

**[0213]** Moreover, DR5 mAb IGM superagonists are more cytotoxic on Colo205 tumor cells than on primary human hepatocytes. The Colo205 cytotoxicity assays were carried out using the bioluminescence assay described in Example 3. About $3.5x10^4$ primary human hepatocytes (Bioreclamation Catalog #X008001-P) were seeded in a collagen coated 96-well plate and allowed to attach overnight. The next day, the Colo205 cells and hepatocytes were treated with serially diluted IgM Anti-DR5 mAbs #1-#4, and incubated for 24 hours at 37°C. At time of harvest, Cell Titer Glo viability reagent (Promega G7572) was added at a volume equal to that of the medium in the well. The cells were lysed for 10 min and luminescence was read on a plate reader. As shown in Figure 11A-D, the cytotoxicity of the four IgM anti-DR5 mAbs was consistently greater on Colo205 tumor cells (closed circles) than on primary human hepatocytes (open circles).

WHAT IS CLAIMED IS:

1. A multimeric binding molecule comprising two, five, or six bivalent binding units or variants or fragments thereof,

wherein each binding unit comprises two IgA or IgM heavy chain constant regions or fragments thereof, each associated with an antigen-binding domain,

wherein at least three of the antigen-binding domains of the binding molecule specifically and agonistically bind to a tumor necrosis factor (TNF) superfamily receptor protein that can induce apoptosis of a cell expressing the TNF superfamily receptor protein, and

wherein the binding molecule can cross-link at least three identical TNF superfamily receptor proteins expressed on the surface of a cell, thereby inducing apoptosis of the cell.

2. The multimeric binding molecule of claim 1, which induces TNF superfamily receptor-mediated apoptosis in a TNF receptor superfamily-expressing cell at a higher potency than an equivalent amount of a bivalent IgG antibody or fragment thereof, which also specifically binds to and agonizes the same TNF superfamily receptor protein.

3. The multimeric binding molecule of claim 1 or claim 2, wherein the at least three antigen-binding domains that specifically bind to and agonize the TNF superfamily receptor protein do not cross-react with other TNF superfamily receptor proteins.

4. The multimeric binding molecule of claim 1 or claim 2, wherein the at least three antigen-binding domains that specifically bind to and agonize the TNF superfamily receptor protein can cross-react with other TNF superfamily receptor proteins.

5. The multimeric binding molecule of claim 3, which comprises at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains that specifically and agonistically bind to a TNF superfamily receptor protein expressed on the surface of the cell, thereby inducing apoptosis of the cell.

6. The multimeric binding molecule of claim 5, wherein the at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or

twelve antigen-binding domains bind to the same extracellular epitope of a single type of TNF superfamily receptor molecule expressed on the surface of the cell.

7. The multimeric binding molecule of claim 5, wherein at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains each specifically bind one of a group of two or more different extracellular epitopes of a single type of TNF superfamily receptor molecule expressed on the surface of the cell.

8. The multimeric binding molecule of any one of claims 1 to 7, wherein the two, five, or six binding units are human, humanized, or chimeric immunoglobulin binding units.

9. The multimeric binding molecule of any one of claims 1 to 8, wherein the TNF superfamily receptor protein is TNFR1 (DR1), TNFR2, TNFR1/2, CD40 (p50), Fas (CD95, Apo1, DR2), CD30, 4-1BB (CD137, ILA), TRAILR1 (DR4, Apo2), TRAILR2 (DR5), TRAILR3 (DcR1), TRAILR4 (DcR2), OPG (OCIF), TWEAKR (FN14), LIGHTR (HVEM), DcR3, DR3, EDAR, XEDAR, or a combination thereof.

10. The multimeric binding molecule of any one of claims 1 to 9, wherein the at least three antigen-binding domains specifically and agonistically bind to DR5.

11. The multimeric binding molecule of claim 10, wherein the at least three antigen-binding domains do not specifically bind to DR4, DcR1, or DcR2.

12. The multimeric binding molecule of claim 10, wherein the at least three antigen-binding domains can also specifically bind to DR4.

13. The multimeric binding molecule of any one of claims 1 to 11, wherein the cell is a cancer cell.

14. The multimeric binding molecule of any one of claims 1 to 13, wherein at the least three antigen-binding domains of the binding molecule are DR5 binding domains, and wherein at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains comprise a heavy

chain variable region (VH) and a light chain variable region (VL), wherein the VH and VL comprise six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprise the CDRs of an antibody comprising the VH and VL amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 7 and SEQ ID NO: 8; SEQ ID NO: 9 and SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 13 and SEQ ID NO: 14; SEQ ID NO: 15 and SEQ ID NO: 16; SEQ ID NO: 17 and SEQ ID NO: 18; SEQ ID NO: 19 and SEQ ID NO: 20; SEQ ID NO: 21 and SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 24; SEQ ID NO: 25 and SEQ ID NO: 26; SEQ ID NO: 27 and SEQ ID NO: 28; SEQ ID NO: 29 and SEQ ID NO: 30; SEQ ID NO: 31 and SEQ ID NO: 32; SEQ ID NO: 33 and SEQ ID NO: 34; SEQ ID NO: 35 and SEQ ID NO: 36; SEQ ID NO: 37 and SEQ ID NO: 38; SEQ ID NO: 39 and SEQ ID NO: 40; SEQ ID NO: 41 and SEQ ID NO: 42; SEQ ID NO: 43 and SEQ ID NO: 44; SEQ ID NO: 45 and SEQ ID NO: 46; SEQ ID NO: 47 and SEQ ID NO: 48; SEQ ID NO: 49 and SEQ ID NO: 50; SEQ ID NO: 51 and SEQ ID NO: 52; SEQ ID NO: 53 and SEQ ID NO: 54; SEQ ID NO: 55 and SEQ ID NO: 56; SEQ ID NO: 82 and SEQ ID NO: 83; SEQ ID NO: 84 and SEQ ID NO: 85; SEQ ID NO: 86 and SEQ ID NO: 87; or SEQ ID NO: 88 and SEQ ID NO: 89; respectively, or the ScFv sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73 or the six CDRs with one or two amino acid substitutions in one or more of the CDRs.

15. The multimeric binding molecule of any one of claims 1 to 14, wherein at the least three antigen-binding domains of the binding molecule are anti-DR5 binding domains, and wherein at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen-binding domains comprise an antibody VH and a VL, wherein the VH and VL comprise amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 7 and SEQ ID NO: 8; SEQ ID NO: 9 and SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 13 and SEQ ID NO: 14; SEQ ID NO: 15 and SEQ ID NO: 16; SEQ ID NO: 17 and SEQ ID NO: 18; SEQ

ID NO: 19 and SEQ ID NO: 20; SEQ ID NO: 21 and SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 24; SEQ ID NO: 25 and SEQ ID NO: 26; SEQ ID NO: 27 and SEQ ID NO: 28; SEQ ID NO: 29 and SEQ ID NO: 30; SEQ ID NO: 31 and SEQ ID NO: 32; SEQ ID NO: 33 and SEQ ID NO: 34; SEQ ID NO: 35 and SEQ ID NO: 36; SEQ ID NO: 37 and SEQ ID NO: 38; SEQ ID NO: 39 and SEQ ID NO: 40; SEQ ID NO: 41 and SEQ ID NO: 42; SEQ ID NO: 43 and SEQ ID NO: 44; SEQ ID NO: 45 and SEQ ID NO: 46; SEQ ID NO: 47 and SEQ ID NO: 48; SEQ ID NO: 49 and SEQ ID NO: 50; SEQ ID NO: 51 and SEQ ID NO: 52; SEQ ID NO: 53 and SEQ ID NO: 54; SEQ ID NO: 55 and SEQ ID NO: 56; SEQ ID NO: 82 and SEQ ID NO: 83; SEQ ID NO: 84 and SEQ ID NO: 85; SEQ ID NO: 86 and SEQ ID NO: 87; or SEQ ID NO: 88 and SEQ ID NO: 89; respectively, or wherein the VH and VL are contained in an ScFv with an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

16. The multimeric binding molecule of claim 15, wherein at least four, at least ten, or twelve antigen-binding domains comprise antibody VH and VL regions comprising the amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO; 5 and SEQ ID NO: 6, SEQ ID NO; 84 and SEQ ID NO: 85, or SEQ ID NO; 88 and SEQ ID NO: 89, respectively.

17. The multimeric binding molecule of any one of claims 1 to 16, which is a dimeric binding molecule comprising two bivalent IgA binding units or fragments thereof and a J chain or fragment or variant thereof, wherein each binding unit comprises two IgA heavy chain constant regions or fragments thereof each associated with an antigen-binding domain.

18. The multimeric binding molecule of claim 17, further comprising a secretory component, or fragment or variant thereof.

19. The multimeric binding molecule of claim 17 or claim 18, wherein the IgA heavy chain constant regions or fragments thereof each comprise a Cα2 domain or a Cα3-tp domain.

20. The multimeric binding molecule of claim 19, wherein one or more IgA heavy chain constant regions or fragments thereof further comprise a Cα1 domain.

21. The multimeric binding molecule of any one of claims 17 to 20, wherein the IgA heavy chain constant region is a human IgA constant region.

22. The multimeric binding molecule of any one of claims 17 to 21, wherein each binding unit comprises two IgA heavy chains each comprising a VH situated amino terminal to the IgA constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

23. The multimeric binding molecule of any one of claims 1 to 16, which is a pentameric or a hexameric binding molecule comprising five or six bivalent IgM binding units, respectively, wherein each binding unit comprises two IgM heavy chain constant regions or fragments thereof each associated with an antigen-binding domain.

24. The multimeric binding molecule of claim 23, wherein the IgM heavy chain constant regions or fragments thereof each comprise a Cμ3 domain and a Cμ4-tp domain.

25. The multimeric binding molecule of claim 23 or claim 24, wherein one or more IgM heavy chain constant regions or fragments thereof further comprise a Cμ2 domain, a Cμ1 domain, or any combination thereof.

26. The multimeric binding molecule of any one of claims 23 to 25, wherein the binding molecule is pentameric, and further comprises a J chain, or fragment thereof, or variant thereof.

27. The multimeric binding molecule of any one of claims 23 to 26, wherein the IgM heavy chain constant region is a human IgM constant region.

28. The multimeric binding molecule of any one of claims 23 to 27, wherein each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the IgM constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

29. The multimeric binding molecule of any one of claims 1 to 28, wherein each binding unit comprises two heavy chains and two light chains, wherein the heavy chains and light chains comprise VH and VL amino acid sequences at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 7 and SEQ ID NO: 8; SEQ ID NO: 9 and SEQ ID NO: 10; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 13 and SEQ ID NO: 14; SEQ ID NO: 15 and SEQ ID NO: 16; SEQ ID NO: 17 and SEQ ID NO: 18; SEQ ID NO: 19 and SEQ ID NO: 20; SEQ ID NO: 21 and SEQ ID NO: 22; SEQ ID NO: 23 and SEQ ID NO: 24; SEQ ID NO: 25 and SEQ ID NO: 26; SEQ ID NO: 27 and SEQ ID NO: 28; SEQ ID NO: 29 and SEQ ID NO: 30; SEQ ID NO: 31 and SEQ ID NO: 32; SEQ ID NO: 33 and SEQ ID NO: 34; SEQ ID NO: 35 and SEQ ID NO: 36; SEQ ID NO: 37 and SEQ ID NO: 38; SEQ ID NO: 39 and SEQ ID NO: 40; SEQ ID NO: 41 and SEQ ID NO: 42; SEQ ID NO: 43 and SEQ ID NO: 44; SEQ ID NO: 45 and SEQ ID NO: 46; SEQ ID NO: 47 and SEQ ID NO: 48; SEQ ID NO: 49 and SEQ ID NO: 50; SEQ ID NO: 51 and SEQ ID NO: 52; SEQ ID NO: 53 and SEQ ID NO: 54; SEQ ID NO: 55 and SEQ ID NO: 56; SEQ ID NO: 82 and SEQ ID NO: 83; SEQ ID NO: 84 and SEQ ID NO: 85; SEQ ID NO: 86 and SEQ ID NO: 87; or SEQ ID NO: 88 and SEQ ID NO: 89; respectively, or wherein the heavy chains and light chains comprise VH and VL amino acid sequences contained in an ScFv with an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

30. The multimeric binding molecule of claim 29, wherein the heavy chains and light chains comprise the VH and VL amino acid sequences SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO; 5 and SEQ ID NO: 6, SEQ ID NO; 84 and SEQ ID NO: 85, or SEQ ID NO; 88 and SEQ ID NO: 89, respectively.

31. The multimeric binding molecule of any one of claims 1 to 16 or 23 to 30, wherein the binding molecule is a pentameric IgM molecule, further comprising a J chain.

32. A composition comprising the multimeric binding molecule of any one of claims 1 to 31.

33. A polynucleotide comprising a nucleic acid sequence that encodes a polypeptide subunit of the binding molecule of any one of claims 1 to 31.

34. The polynucleotide of claim 33, wherein the polypeptide subunit comprises an IgM heavy chain constant region and at least an antibody VH portion of the antigen-binding domain of the multimeric binding molecule.

35. The polynucleotide of claim 34, wherein the polypeptide subunit comprises a human IgM constant region or fragment thereof fused to the C-terminal end of a VH comprising:

(a) HCDR1, HCDR2, and HCDR3 regions comprising the CDRs contained in the VH amino acid sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88, or in the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73; or the CDRs contained in the VH amino acid sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88, or in the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ

ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73 with one or two single amino acid substitutions in one or more of the HCDRs; or

(b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 88, or the VH portion of an ScFv with the amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

36. The polynucleotide of any one of claims 33 to 35, wherein the polypeptide subunit comprises a light chain constant region and an antibody VL portion of the antigen-binding domain of the multimeric binding molecule.

37. The polynucleotide of claim 36, wherein the polypeptide subunit comprises a human kappa or lambda light chain constant region or fragment thereof fused to the C-terminal end of a VL comprising:

(a) LCDR1, LCDR2, and LCDR3 regions comprising the CDRs contained in the VL amino acid sequence SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, or SEQ ID NO: 89, or in the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67,

SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73; or the CDRs contained in the VL amino acid sequence SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, or SEQ ID NO: 89, or in the ScFv amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73, with one or two single amino acid substitutions in one or more of the LCDRs; or

(b) an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, or SEQ ID NO: 89, or the VL portion of an ScFv with the amino acid sequence SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 73.

38. A composition comprising the polynucleotide of any one of claims 33 to 35, and the polynucleotide of any one of claims 33, 36, or 37.

39. The composition of claim 38, wherein the polynucleotides are on separate vectors.

40. The composition of claim 38, wherein the polynucleotides are on a single vector.

41. The composition of any one of claims 38 to 40, further comprising a polynucleotide comprising a nucleic acid sequence encoding a J chain, or fragment thereof, or variant thereof.

42. The vector of claim 40.

43. The vectors of claim 39.

44. A host cell comprising the polynucleotide of any one of claims 33 to 37, the composition of any one of claims 38 to 41, or the vector or vectors of any one of claims 42 or 43, wherein the host cell can express the binding molecule of any one of claims 1 to 31, or a subunit thereof.

45. A method of producing the binding molecule of any one of claims 1 to 31, comprising culturing the host cell of claim 44, and recovering the binding molecule.

46. A method of inducing TNF superfamily receptor-mediated apoptosis in a TNF superfamily receptor-expressing cell, which comprises contacting the TNF superfamily receptor - expressing cell with the multimeric binding molecule of any one of claims 1 to 31.

47. A method of inducing TNF superfamily receptor lipid raft formation in a TNF superfamily receptor-expressing cell, which comprises contacting the TNF superfamily receptor-expressing cell with the multimeric binding molecule of any one of claims 1 to 31.

48. A method of treating cancer comprising administering to a subject in need of treatment an effective amount of the multimeric binding molecule of any one of claims 1 to 31, wherein the cancer cells in the subject express a TNF superfamily receptor with apoptotic activity, and wherein the multimeric binding molecule can induce apoptosis of the cancer cells.

49. The method of claim 48, wherein the multimeric binding molecule can induce greater apoptosis of cancer cells than non-cancer cells.

50. The method of claim 49, wherein the non-cancer cells are normal hepatocytes.

51. The method of any one of claims 48 to 50, wherein the TNF superfamily receptor is DR5.

52. The method of any one of claims 48 to 51, wherein the subject is human.