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(54) Title: ANTI-TENASCIN-C A2 ANTIBODIES AND METHODS OF USE

(57) Abstract: The invention provides antibodies against the A2 domain of tenascin-C and methods of using the same.

ANTI-TENASCIN-C A2 ANTIBODIES AND METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to antibodies specific for the A2 domain of tenascin-C (TNC A2). In addition, the invention relates to polynuleotides encoding such antibodies, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for
5   producing the antibodies and methods of using them in the treatment of disease.

BACKGROUND

Tenascin C and anti-Tenascin C Antibodies

Tenascins are a highly conserved family of large multimeric extracellular matrix (ECM)
10   glycoproteins, which is found in vertebrates. Four tenascin paralogues have been identified in mammals, termed tenascin-C, tenascin-R, tenascin-X and tenascin-W. Tenascin family proteins have a common primary structure, comprising N-terminal heptad repeats, epidermal growth factor (EGF)-like repeats, fibronectin type III domain repeats and a C-terminal fibrinogen-like globular domain. Via an N-terminal oligomerization domain, individual subunits assemble into
15   trimers or, as is the case for tenascin-C, even hexamers.

Mammalian tenascin-C monomers typically have 14.5 EGF-like repeats and 8 fibronectin type III domain repeats that are shared by all tenascin-C isoforms. However, up to 9 additional fibronectin type III domain repeats (domains A1 to D) can be independently included or excluded by alternative splicing, giving rise to a large number of tenascin-C isoforms (see e.g.
20   Hsia and Schwarzbauer, J Biol Chem 280, 26641-26644 (2005)).

Tenascin-C is transiently expressed in the developing embryo, but virtually absent from adult tissues. It reappears, however, in tissues undergoing remodeling processes, including certain pathological conditions such as wound healing, inflammation and cancer (reviewed in Chiquet-Ehrismann & Chiquet, J Pathol 200, 488-499 (2003)).

25   Importantly, tenascin-C is highly expressed in the majority of malignant solid tumors, including tumors of the brain, breast, colon, lung, skin and other organs (reviewed in Orend and Chiquet-Ehrismann, Cancer Letters 244, 143-163 (2006)), where it may be expressed by transformed

epithelial cells as well as stromal cells in the tumor microenvironment (Yoshida et al., J Pathol 182, 421-428 (1997), Hanamura et al., Int J Cancer 73, 10-15 (1997)). In particular, the "large isoform" of tenascin-C, containing the alternatively spliced domains A1 to D, is expressed in invasive carcinomas while being nearly undetectable in healthy adult tissues (Borsi et al., Int J

5   Cancer 52, 688-692 (1992), Carnemolla et al., Eur J Biochem 205, 561-567 (1992)).

Its expression pattern makes tenascin-C, in particular its alternatively spliced domains, a promising antigen for tumor targeting applications, and accordingly a number of antibodies against several domains of the protein have been developed (see e.g. Brack et al., Clin Cancer Res 12, 3200-3208 (2006) or EP 1 817 345, describing antibodies against the A1 domain of

10   tenascin-C; Silacci et al., Prot Eng Des Sel 19, 471-478 (2006), or EP 1 173 766, describing antibodies against the C domain of tenascin-C; Wang et al., Hybridoma 29, 13-16 (2010), describing an antibody against the D domain of tenascin C; or Balza et al., FEBS 332, 39-43 (1993), describing several antibodies against different domains of human tenascin).

Recently, also an antibody recognizing a specific epitope in the A2 domain of human tenascin-C

15   has been described (WO 2009/089998).

Antibody Glycosylation

The oligosaccharide component can significantly affect properties relevant to the efficacy of a therapeutic glycoprotein, including physical stability, resistance to protease attack, interactions

20   with the immune system, pharmacokinetics, and specific biological activity. Such properties may depend not only on the presence or absence, but also on the specific structures, of oligosaccharides. Some generalizations between oligosaccharide structure and glycoprotein function can be made. For example, certain oligosaccharide structures mediate rapid clearance of the glycoprotein from the bloodstream through interactions with specific carbohydrate binding

25   proteins, while others can be bound by antibodies and trigger undesired immune reactions (Jenkins et al., Nature Biotechnol 14, 975-81 (1996)).

IgG1 type antibodies, the most commonly used antibodies in cancer immunotherapy, are glycoproteins that have a conserved N-linked glycosylation site at Asn 297 in each CH2 domain. The two complex biantennary oligosaccharides attached to Asn 297 are buried between the CH2

30   domains, forming extensive contacts with the polypeptide backbone, and their presence is essential for the antibody to mediate effector functions such as antibody dependent cell-mediated cytotoxity (ADCC) (Lifely et al., Glycobiology 5, 813-822 (1995); Jefferis et al., Immunol Rev 163, 59-76 (1998); Wright and Morrison, Trends Biotechnol 15, 26-32 (1997)). Protein

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engineering studies have shown that FcγRs interact with the lower hinge region of the IgG CH2 domain. Lund *et al.*, *J. Immunol. 157*:4963-69 (1996). However, FcγR binding also requires the presence of the oligosaccharides in the CH2 region. Lund *et al.*, *J. Immunol. 157*:4963-69 (1996); Wright and Morrison, *Trends Biotech. 15*:26-31 (1997), suggesting that either

5   oligosaccharide and polypeptide both directly contribute to the interaction site or that the oligosaccharide is required to maintain an active CH2 polypeptide conformation. Modification of the oligosaccharide structure can therefore be explored as a means to increase the affinity of the interaction between IgG1 and FcγR, and to increase ADCC activity of IgG1s.

A way to obtain large increases in the potency of monoclonal antibodies, is to enhance their

10   natural, cell-mediated effector functions by engineering their oligosaccharide component as described in Umaña et al., Nat Biotechnol 17, 176-180 (1999) and U.S. Patent No. 6,602,684 (WO 99/54342), the contents of which are hereby incorporated by reference in their entirety. Umaña et al. showed that overexpression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, in Chinese hamster

15   ovary (CHO) cells significantly increases the *in vitro* ADCC activity of antibodies produced in those cells. Overexpression of GnTIII in production cell lines leads to antibodies enriched in bisected oligosaccharides, which are generally also non-fucosylated and of the hybrid type. If in addition to GnTIII, mannosidase II (ManII) is overexpressed in production cell lines, antibodies enriched in bisected, non-fucosylated oligosaccharides of the complex type are obtained (Ferrara

20   et al., Biotechn Bioeng 93, 851-861 (2006)). Both types of antibodies show strongly enhanced ADCC, as compared to antibodies with unmodified glycans, but only antibodies in which the majority of the N-glycans are of the complex type are able to induce significant complement-dependent cytotoxicity (Ferrara et al., Biotechn Bioeng 93, 851-861 (2006)). Alterations in the composition of the Asn 297 carbohydrate or its elimination also affect binding of the antibody

25   Fc-domain to Fcγ-receptor (FcγR) and complement C1q protein, which is important for ADCC and CDC, respectively (Umaña et al., Nat Biotechnol 17, 176-180 (1999); Davies et al., Biotechnol Bioeng 74, 288-294 (2001); Mimura et al., J Biol Chem 276, 45539-45547 (2001); Radaev et al., J Biol Chem 276, 16478-16483 (2001); Shields et al., J Biol Chem 276, 6591-6604 (2001); Shields et al., J Biol Chem 277, 26733-26740 (2002); Simmons et al., J Immunol

30   Methods 263, 133-147 (2002)).

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BRIEF SUMMARY OF THE INVENTION

The present invention provides antibodies that specifically bind to the A2 domain of tenascin-C, having a high affinity and/or enhanced effector function.

In one aspect, the invention is directed to an antibody that specifically binds toTNC A2,

5   comprising at least one (i.e. one, two, three, four, five or six) of the complementarity determining regions (CDRs) set forth in SEQ ID NOs 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47. In one embodiment, the antibody comprises three heavy chain CDRs (i.e. HCDR1, HCDR2, and HCDR3) and/or three light chain CDRs (i.e. LCDR1, LCDR2, and LCDR3) selected from SEQ ID NOs 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33,

10   35, 37, 39, 41, 43, 45 and 47. In a more particular embodiment, the antibody comprises an antibody heavy chain variable region and/or an antibody light chain variable region, particularly both a heavy and light chain variable region, selected from the heavy and light chain variable region sequences set forth in SEQ ID NOs 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89 and 91. In one embodiment, the antibody comprises an Fc region, particularly an

15   IgG Fc region. In a further embodiment, the antibody is a full-length antibody, particularly an IgG class antibody. In another embodiment, the antibody comprises a human antibody constant region. In one embodiment, the antibody is human. In one embodiment, the antibody is glycoengineered to have modified oligosaccharides in the Fc region. In one embodiment the antibody has an increased proportion of non-fucosylated and/or bisected oligosaccharides in the

20   Fc region, as compared to a non-glycoengineered antibody. In a further embodiment, the antibody has increased effector function and/or increased Fc receptor binding affinity. In a particular embodiment, the increased effector function is increased antibody-dependent cell-mediated cytotoxicity (ADCC). In another embodiment the antibody binds to human TNC A2 with a K_D value of lower than about 1 μM, preferably lower than about 100 nM, most preferably

25   lower than about 1 nM. In one embodiment, the antibody is affinity matured. In one embodiment, the antibody binds to TNC A2 in human tissues.

In other aspects, the invention is also directed to polypeptides, polynucleotides, host cells, and expression vectors related to the antibodies. In a further aspect, the invention relates to methods of making the antibodies. In a further aspect, the invention is directed to methods of using the

30   antibodies, particularly for the treatment of diseases characterized by expression of TNC A2, such as cancer.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 shows Surface Plasmon Resonance (SPR)-based kinetic analyses of affinity-matured anti-TNC A2 Fab fragments binding to human (hu) TNC A2. Processed kinetic data sets are presented for clone 2B10_C3B6 (A), clone 2B10_6A12 (B), clone 2B10_C3A6 (C), clone

5   2B10_O7D8 (D), clone 2B10_O1F7 (E) and clone 2B10_6H10 (F). Smooth lines represent a global fit of the data to a 1:1 interaction model.

FIGURE 2 (A) shows SPR-based kinetic analyses of 2B10 anti-TNC A2 Fab fragments binding to human, murine and cynomolgus TNC A2. Panel (B) shows SPR-based kinetic analyses of 2B10 anti-TNC A2 human IgG binding to human, murine and cynomolgus TNC A2, as

10   described in Example 7.

FIGURE 3 (A) shows immunohistochemical images of normal (upper panels) and tumor (lower panels) human uterus tissue at 100X (left panels) and 400X (middle panels) magnification as stained with 2B10 variable region in a Fab fragment fused to a FLAG fragment (SHD2B10-FLAG). Right panels: control, 100X magnification. (B) shows the expression levels of TNC A2

15   in various human tissue samples in terms of % of immunofluorescence surface area as stained with 2B10 variable region in a Fab fragment fused to a FLAG fragment (SHD2B10-FLAG). Various human tissue samples from healthy individuals and cancer patients were stained with the SHD2B10-FLAG Fab fragment as described in Example 8.

FIGURE 4 (A) to (N), shows immunohistochemical images of human tissue at 100X (left panels)

20   and 400X (middle panels) magnification stained with SHD2B10-mouse IgG as described in Example 8. Staining with isotype control antibody is shown at 100X magnification (right panels). Upper panels: normal tissue, lower panels: tumor tissue. (A) brain, (B) breast, (C) colon, (D) kidney, (E) liver, (F) lung, (G) ovary, (H) pancreas, (I) prostate, (J) skeletal muscle, (K) skin, (L) small intestine, (M) stomach, (N) uterus.

25   FIGURE 5 shows binding of 2B10-derived human IgG to TNC A2 expressed on U87MG glioblastoma tumor cells as determined by flow cytometry (see Example 9). Mean fluorescence intensity of cells treated with different concentrations of 2B10 IgG compared to untreated cells and cells stained only with the secondary antibody (negative controls) is shown.

FIGURE 6 shows the purification and analysis of the wild-type 2B10 human IgG. A) Protein A

30   affinity chromatography purification step. B) Size exclusion chromatography purification step. C) Analytical SDS PAGE. Experimental prodecures are described in Example 1.

FIGURE 7 shows the purification and analysis of the glycoengineered 2B10 human IgG. A) Protein A affinity chromatography purification step. B) Size exclusion chromatography

purification step. C) Analytical SDS PAGE. D) Analytical size exclusion chromatography. Experimental prodecures are described in Example 1.

FIGURE 8 shows binding of anti-TNC A2 antibody 2B10 as wildtype (wt) and glycoengineered (ge) version to TNC A2 on U87MG cells.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I.   DEFINITIONS

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D), which is the ratio of dissociation and association rate constants (k_{off} and k_{on}, respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An "affinity matured" antibody refers to an antibody with one or more alterations (e.g. amino acid mutations) in one or more hypervariable regions (HVRs) (e.g. CDRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen. Typically, the affinity matured antibody binds to the same epitope as the parent antibody.

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The terms "anti-TNC A2 antibody" and "an antibody that binds to the A2 domain of Tenascin-C" refer to an antibody that is capable of binding TNC A2 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting TNC A2. In one embodiment, the extent of binding of an anti-TNC A2 antibody to an unrelated, non-TNC A2

5 protein is less than about 10% of the binding of the antibody to TNC A2 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to TNC A2 has a dissociation constant (K_D) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-TNC A2 antibody binds to an epitope of TNC A2 that is conserved

10 among TNC A2 from different species.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity. Also included are antibody fragments having an Fc

15 region, and fusion proteins that comprise a region equivalent to the Fc region of an immunoglobulin.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')$_2$, single-chain

20 antibody molecules (e.g. scFv), diabodies, and multispecific antibodies formed from antibody fragments.

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a

25 competition assay by 50% or more. An exemplary competition assay is provided herein.

The term "antigen binding domain" refers to the part of an antigen binding molecule that comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antigen binding molecule may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be provided by, for

30 example, one or more antibody variable domains (also called antibody variable regions). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species. For chimeric antibodies, for example, the non-antigen binding components may be derived from a wide variety of species, including

5   primates such as chimpanzees and humans. Humanized antibodies are a particularly preferred form of chimeric antibodies.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4,

10   IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ϵ, γ, and μ, respectively.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and

15   radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments

20   and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

"Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; cytokine secretion;

25   immune-complex-mediated antigen uptake by antigen presenting cells; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

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

30   The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However,

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the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National

5   Institutes of Health, Bethesda, MD, 1991.

A "region equivalent to the Fc region of an immunoglobulin" is intended to include naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants having alterations which produce substitutions, additions, or deletions but which do not decrease substantially the ability of the immunoglobulin to mediate effector functions (such as antibody-

10   dependent cellular cytotoxicity). For example, one or more amino acids can be deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of biological function. Such variants can be selected according to general rules known in the art so as to have minimal effect on activity (see, e.g., Bowie, J. U. et al., Science 247:1306-10 (1990)). "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR)

15   (or CDR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native

20   antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages.

25   Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. In one embodiment, the host cell is engineered to allow the production of an antibody with modified oligosaccharides. In certain embodiments, the host cells have been further manipulated to express increased levels of

30   one or more polypeptides having $\beta(1,4)$-N-acetylglucosaminyltransferase III (GnTIII) activity. Host cells include cultured cells, *e.g.*, mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6

cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that

5   utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework

10   sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the VH, the subgroup is

15   subgroup III as in Kabat et al., *supra*.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human

20   antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR", as used herein, refers to each of the regions of an

25   antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition.

30   With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. Hypervariable regions (HVRs) are also referred to as complementarity determining regions (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has

been described 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), where the definitions include overlapping or subsets of amino acid residues when

compared against each other. Nevertheless, application of either definition to refer to a CDR of

5   an antibody or variants thereof is intended to be within the scope of the term as defined and used

herein. The appropriate amino acid residues 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 residue

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 residues comprise a particular CDR

10   given the variable region amino acid sequence of the antibody.

TABLE 1. CDR Definitions[1]

CDR	Kabat	Chothia	AbM[2]
V_H CDR1	31-35	26-32	26-35
V_H CDR2	50-65	52-58	50-58
V_H CDR3	95-102	95-102	95-102
V_L CDR1	24-34	26-32	24-34
V_L CDR2	50-56	50-52	50-56
V_L CDR3	89-97	91-96	89-97

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

[2] "AbM" with a lowercase "b" as used in Table 1 refers to the CDRs as
15   defined by Oxford Molecular's "AbM" antibody modeling software.

Kabat *et al.* also defined a numbering system for variable region 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 region sequence, without reliance on any experimental data beyond

20   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 otherwise specified, references to the numbering of

specific amino acid residue positions in an antibody variable region are according to the Kabat

numbering system.

25   CDRs also comprise "specificity determining residues," or "SDRs," which are residues that

contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-

CDRs. In general, only one-fifth to one-third of the residues in a given CDR participate in

antigen binding. The specificity-determining residues in a particular CDR can be identified by,

for example, computation of interatomic contacts from three-dimensional modeling and

determination of the sequence variability at a given residue position in accordance with the methods described in Padlan *et al.*, *FASEB J. 9(1)*:133-139 (1995). Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3

5   (see Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra.*

An "antibody conjugate" is an antibody conjugated to a cytotoxic agent.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to,

10   domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity

15   as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC) methods. For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated" polynucleotide refers to a polynucleotide molecule that has been separated from a

20   component of its natural environment. An isolated polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated polynucleotide encoding an anti-TNC A2 antibody" refers to one or more

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

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the

30   population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different

determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular

5   method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

10   A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

       "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about

15   150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a

20   light chain variable domain, followed by a constant light (CL) domain, also called a light chain constant region. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

       "No substantial cross-reactivity" means that a molecule (e.g., an antibody) does not recognize or specifically bind an antigen different from the actual target antigen of the molecule (e.g. an

25   antigen closely related to the target antigen), particularly when compared to that target antigen. For example, an antibody may bind less than about 10% to less than about 5% to an antigen different from the actual target antigen, or may bind said antigen different from the actual target antigen at an amount selected from the group consisting of less than about 10%, 9%, 8% 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1%, preferably less than about 2%, 1%, or 0.5%,

30   and most preferably less than about 0.2% or 0.1% antigen different from the actual target antigen.

       The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage,

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administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

The term "parent" antibody refers to an antibody that is used as the starting point or basis for the preparation of a variant.

5   "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for

10   purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes

15   herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly

20   available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino

25   acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

30   100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid

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sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

5   Similarly, by a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide

10   sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal

15   positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether any particular polynucleotide or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence or polypeptide sequence of the present invention can be determined conventionally using known computer programs, such as the ones listed above.

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

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation,

25   other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

The term "A2 domain of tenascin-C (TNC A2)" as used herein, refers to any native TNC A2 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed

30   TNC A2 as well as any form of TNC A2 that results from processing in the cell. The term also encompasses naturally occurring variants of TNC A2, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human TNC A2 (with a C-terminal avi-tag and 6x His-tag) is shown in SEQ ID NO: 97. In the human tenascin-C molecule, the A2 domain is the

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second (counted from the N-terminus) of the up to nine alternatively spliced fibronectin-type III domains, which may be inserted between the fifth and the sixth of the constant fibronectin-type III domains (for a schematic representation of the domain structure of tenascin-C, see e.g. Orend and Chiquet-Ehrismann, Cancer Letters 244, 143-163 (2006). Similarly, in the mouse tenascin-C

5   molecule, the A2 is the second of up to six alternatively spliced fibronectin-type III domains (described e.g. in Joestner and Faissner, J Biol Chem 274, 17144-17151 (1999)).

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of disease of the individual being treated, and can be performed either for prophylaxis or during the course of clinical

10   pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or

15   to slow the progression of a disease.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and

20   three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.*

25   150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to

30   which they are operatively linked. Such vectors are referred to herein as "expression vectors."

As used herein, the term "polypeptide having GnTIII activity" refers to polypeptides that are able to catalyze the addition of a N-acetylglucosamine (GlcNAc) residue in β-1-4 linkage to the β-linked mannoside of the trimannosyl core of N-linked oligosaccharides. This includes fusion

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polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an activity of β(1,4)-N-acetylglucosaminyltransferase III, also known as β-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyl-transferase (EC 2.4.1.144), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), as

5   measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of GnTIII, but rather substantially similar to the dose-dependence in a given activity as compared to the GnTIII (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less

10   activity relative to the GnTIII).

       As used herein, the term "Golgi localization domain" refers to the amino acid sequence of a Golgi resident polypeptide which is responsible for anchoring the polypeptide to a location within the Golgi complex. Generally, localization domains comprise amino terminal "tails" of an enzyme.

15   As used herein, the terms "engineer, engineered, engineering," particularly with the prefix "glyco-," as well as the term "glycosylation engineering" are considered to include any manipulation of the glycosylation pattern of a naturally occurring or recombinant polypeptide or fragment thereof. Glycosylation engineering includes metabolic engineering of the glycosylation machinery of a cell, including genetic manipulations of the oligosaccharide synthesis pathways

20   to achieve altered glycosylation of glycoproteins expressed in cells. Furthermore, glycosylation engineering includes the effects of mutations and cell environment on glycosylation. In one embodiment, the glycosylation engineering is an alteration in glycosyltransferase activity. In a particular embodiment, the engineering results in altered glucosaminyltransferase activity and/or fucosyltransferase activity.

25   As used herein, the term "Fc-mediated cellular cytotoxicity" includes antibody-dependent cell-mediated cytotoxicity (ADCC) and cellular cytotoxicity mediated by a soluble Fc-fusion protein containing a human Fc-region. It is an immune mechanism leading to the lysis of "targeted cells" by "human immune effector cells."

       As used herein, the term "human immune effector cells" refers to a population of leukocytes that

30   display Fc receptors on their surfaces, through which they bind to the Fc-region of antibodies or of Fc-fusion proteins and perform effector functions. Such a population may include, but is not limited to, peripheral blood mononuclear cells (PBMC) and/or natural killer (NK) cells.

As used herein, the term "targeted cells" refers to cells to which antigen binding molecules comprising an Fc region (*e.g.*, antibodies or fragments thereof comprising an Fc region) or Fc-fusion proteins specifically bind. The antigen binding molecules or Fc fusion-proteins bind to target cells via the protein part that is N-terminal to the Fc region.

As used herein, the term "increased Fc-mediated cellular cytotoxicity" is defined as either an increase in the number of "targeted cells" that are lysed in a given time, at a given concentration of antibody or of Fc-fusion protein in the medium surrounding the target cells, by the mechanism of Fc-mediated cellular cytotoxicity defined above, and/or a reduction in the concentration of antibody or of Fc-fusion protein, in the medium surrounding the target cells, required to achieve the lysis of a given number of "targeted cells," in a given time, by the mechanism of Fc-mediated cellular cytotoxicity. The increase in Fc-mediated cellular cytotoxicity is relative to the cellular cytotoxicity mediated by the same antigen binding molecule or Fc-fusion protein produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, (which are known to those skilled in the art) but that has not been produced by host cells engineered to have an altered pattern of glycosylation (e.g., to express the glycosyltransferase, GnTIII, or other glycosyltransferases) by the methods described herein.

By "antibody having increased antibody dependent cell-mediated cytotoxicity (ADCC)" is meant an antibody, as that term is defined herein, having increased ADCC as determined by any suitable method known to those of ordinary skill in the art. One accepted *in vitro* ADCC assay is as follows:

1)   the assay uses target cells that are known to express the target antigen recognized by the antigen-binding region of the antibody;

2)   the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;

3)   the assay is carried out according to following protocol:

i)   the PBMCs are isolated using standard density centrifugation procedures and are suspended at 5×10^6 cells/ml in RPMI cell culture medium;

ii)   the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of ^{51}Cr, washed twice with cell culture medium, and resuspended in cell culture medium at a density of 10^5 cells/ml;

iii)   100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;

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iv)   the antibody is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting antibody solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various antibody concentrations covering the whole concentration range above;

v)   for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of a 2% (V/V) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the antibody solution (point iv above);

vi)   for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of RPMI cell culture medium instead of the antibody solution (point iv above);

vii)   the 96-well microtiter plate is then centrifuged at 50 x g for 1 minute and incubated for 1 hour at 4°C;

viii)   50 microliters of the PBMC suspension (point i above) are added to each well to yield an effector:target cell ratio of 25:1 and the plates are placed in an incubator under 5% CO_2 atmosphere at 37°C for 4 hours;

ix)   the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;

x)   the percentage of specific lysis is calculated for each antibody concentration according to the formula (ER-MR)/(MR-SR) x 100, where ER is the average radioactivity quantified (see point ix above) for that antibody concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point v above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);

4)   "increased ADCC" is defined as either an increase in the maximum percentage of specific lysis observed within the antibody concentration range tested above, and/or a reduction in the concentration of antibody required to achieve one half of the maximum percentage of specific lysis observed within the antibody concentration range tested above. The increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same antibody, produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been produced by host cells engineered to overexpress GnTIII.

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II.   COMPOSITIONS AND METHODS

Tenascin-C, in particular the A2 domain and other alternatively spliced domains of tenascin-C, is specifically expressed in certain pathological conditions but essentially absent from healthy adult tissues, thus antibodies targeting this antigen have great therapeutic potential. The present invention provides antibodies that bind to the A2 domain of tenascin-C, in particular antibodies with high affinity and strong effector functions. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of diseases characterized by expression of TNC A2, such as cancer.

A.   Exemplary Anti-TNC A2 Antibodies

The present invention provides for antibodies that specifically bind to the A2 domain of tenascin C (TNC A2). Particularly, the present invention provides for antibodies that specifically bind TNC A2, wherein said antibodies are glycoengineered to have increased effector function.

In one embodiment, an anti-TNC A2 antibody of the invention comprises at least one (e.g. one, two, three, four, five, or six) heavy or light chain complementarity determining region (CDR) selected from the group of 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, and SEQ ID NO: 47, or a variant or truncated form thereof containing at least the specificity-determining residues (SDRs) for said CDR. In one embodiment, an anti-TNC A2 antibody of the invention comprises at least one (e.g. one, two, three, four, five, or six) heavy or light chain complementarity determining region (CDR) selected from the group of 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, and SEQ ID NO: 47, wherein the antibody does not comprise the combination of a heavy chain CDR1 (HCDR1) selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, a heavy chain CDR2 (HCDR2) selected from the group of SEQ ID NO: 9, SEQ ID NO: 15, and SEQ ID NO: 21, the heavy chain CDR3 (HCDR3) of SEQ ID NO: 27, the light chain CDR1 (LCDR1) of SEQ ID NO: 29, the light chain CDR2 (LCDR2) of SEQ ID NO: 35, and the light chain CDR3 (LCDR3) of SEQ ID NO:47.

In one embodiment, said at least one CDR is a heavy chain CDR, particularly a heavy chain CDR3 of SEQ ID NO: 27. In another embodiment, the antibody comprises at least one heavy chain CDR and at least one light chain CDR, particularly a heavy chain CDR3 of SEQ ID NO:27 and a light chain CDR3 of SEQ ID NO: 47.

5   In one embodiment, an antibody of the invention comprises at least one, at least two, or all three heavy chain CDR (HCDR) sequences selected from (a) HCDR1 comprising an amino acid sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7; (b) HCDR2 comprising an amino acid sequence selected from the group of 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,

10   SEQ ID NO: 23, and SEQ ID NO: 25; and (c) HCDR3 comprising the amino acid sequence of SEQ ID NO: 27. In a further embodiment, the antibody comprises a heavy chain variable region comprising (a) a heavy chain CDR1 selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7; (b) a heavy chain CDR2 selected from the group of 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,

15   SEQ ID NO: 23, and SEQ ID NO: 25; and (c) the heavy chain CDR3 of SEQ ID NO: 27, or variants or truncated forms thereof containing at least the SDRs for said CDRs.

In one embodiment, an antibody of the invention comprises at least one, at least two, or all three light chain CDR (LCDR) sequences selected from (a) LCDR1 comprising an amino acid sequence selected from the group of SEQ ID NO: 29, SEQ ID NO: 31, and SEQ ID NO: 33; (b)

20   LCDR2 comprising an amino acid sequence selected from the group of SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 45; and (c) LCDR3 comprising the amino acid sequence of SEQ ID NO: 47. In a further embodiment, the antibody comprises a light chain variable region comprising (a) a light chain CDR1 selected from the group of SEQ ID NO: 29, SEQ ID NO: 31, and SEQ ID NO: 33; (b) a light chain CDR2

25   selected from the group of SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 45; and (c) the light chain CDR3 of SEQ ID NO:47, or variants or truncated forms thereof containing at least the SDRs for said CDRs.

In one embodiment, an antibody of the invention comprises a heavy chain variable region comprising a heavy chain CDR1 selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, and

30   SEQ ID NO: 7; a heavy chain CDR2 selected from the group of 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, and SEQ ID NO: 25; and the heavy chain CDR3 of SEQ ID NO: 27, and a light chain variable region comprising a light chain CDR1 selected from the group of SEQ ID NO: 29, SEQ

ID NO: 31, and SEQ ID NO: 33; a light chain CDR2 selected from the group of SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 45; and the light chain CDR3 of SEQ ID NO:47, or variants or truncated forms thereof containing at least the SDRs for said CDRs.

5   In another embodiment, an antibody of the invention comprises a heavy chain variable region comprising a heavy chain CDR1 selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7; a heavy chain CDR2 selected from the group of 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, and SEQ ID NO: 25; and the heavy chain CDR3 of SEQ ID NO: 27, and a light chain

10   variable region comprising a light chain CDR1 selected from the group of SEQ ID NO: 29, SEQ ID NO: 31, and SEQ ID NO: 33; a light chain CDR2 selected from the group of SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 45; and the light chain CDR3 of SEQ ID NO:47, wherein said antibody does not comprise the combination of a heavy chain CDR1 (HCDR1) selected from the group of SEQ ID NO: 3, SEQ

15   ID NO: 5, SEQ ID NO: 7, a heavy chain CDR2 (HCDR2) selected from the group of SEQ ID NO: 9, SEQ ID NO: 15, and SEQ ID NO: 21, the heavy chain CDR3 (HCDR3) of SEQ ID NO: 27, the light chain CDR1 (LCDR1) of SEQ ID NO: 29, the light chain CDR2 (LCDR2) of SEQ ID NO: 35, and the light chain CDR3 (LCDR3) of SEQ ID NO:47.

In a specific embodiment, an antibody of the invention comprises a heavy chain variable region

20   comprising a heavy chain CDR1 selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7; a heavy chain CDR2 selected from the group of SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, and SEQ ID NO: 25; and the heavy chain CDR3 of SEQ ID NO: 27, and a light chain variable region comprising the light chain CDR1 of SEQ ID NO: 29, the light chain CDR2 of SEQ ID NO: 35, and the light chain CDR3 of SEQ ID

25   NO:47. In another specific embodiment, an antibody of the invention comprises a heavy chain variable region comprising a heavy chain CDR1 selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7; a heavy chain CDR2 selected from the group of SEQ ID NO: 9, SEQ ID NO: 15, and SEQ ID NO: 21; and the heavy chain CDR3 of SEQ ID NO: 27, and a light chain variable region comprising a light chain CDR1 selected from the group of SEQ ID NO: 29,

30   SEQ ID NO: 31, and SEQ ID NO: 33; a light chain CDR2 selected from the group of SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 45; and the light chain CDR3 of SEQ ID NO:47. In yet another specific embodiment, an antibody of the invention comprises a heavy chain variable region comprising a heavy chain CDR1 selected from the

group of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7; a heavy chain CDR2 selected from the group of SEQ ID NO: 9, SEQ ID NO: 15, and SEQ ID NO: 21; and the heavy chain CDR3 of SEQ ID NO: 27, and a light chain variable region comprising the light chain of SEQ ID NO: 29, the light chain CDR2 of SEQ ID NO: 35; and the light chain CDR3 of SEQ ID NO:47.

5   In one embodiment, an antibody of the invention comprises a heavy chain variable region (VH) comprising an amino acid sequence having at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a sequence selected from the group of SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63. In one embodiment, the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group of: SEQ ID NO: 59,

10   SEQ ID NO: 67, and SEQ ID NO: 63.

In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-TNC A2 antibody comprising that sequence retains the ability to bind to TNC A2. In certain embodiments, a total of 1 to 10 amino

15   acids have been substituted, inserted and/or deleted in SEQ ID NO 59, 63 or 67. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs or CDRs (i.e., in the FRs). Optionally, an anti-TNC A2 antibody according to the invention comprises the VH sequence in SEQ ID NO 59, 63 or 67, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three heavy chain CDRs

20   selected from the sequences set forth in SEQ ID NOs 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27 for the HCDR1, HCDR2 and HCDR3.

In another embodiment, an antibody of the invention comprises a light chain variable region comprising an amino acid sequence having at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a sequence selected from the group of SEQ ID NO: 55, SEQ

25   ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89. In yet another embodiment, the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group of: SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89.

30   In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-TNC A2 antibody comprising that sequence retains the ability to bind to TNC A2. In certain embodiments, a total of 1 to 10 amino

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acids have been substituted, inserted and/or deleted in SEQ ID NO 55, 57, 69, 73, 77, 81, 85 or 89. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs or CDRs (i.e., in the FRs). Optionally, an anti-TNC A2 antibody of the invention comprises the VL sequence in SEQ ID NO 55, 57, 69, 73, 77, 81, 85 or 89, including post-

5   translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three light chain CDRs selected from sequences set forth in SEQ ID NOs 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47 for the LCDR1, LCDR2 and LCDR3.

In another aspect, an anti-TNC A2 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided

10   above. In one embodiment, the antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of: SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63, and a light chain variable region comprising an amino acid sequence that is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical

15   to a sequence selected from the group of: SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO 59, 63 or 67 and SEQ ID NO 55, 57, 69, 73, 77, 81, 85 or 89, respectively, including post-translational modifications of those sequences.

20   In one embodiment, the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group of: SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63, and a light chain variable region comprising an amino acid sequence selected from the group of: SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89, wherein said antibody does not comprise a

25   combination of a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 59 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 55 or SEQ ID NO: 57.

In a specific embodiment, an antibody of the invention comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 59 and a light chain variable region

30   comprising an amino acid sequence selected from the group of SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89. In another specific embodiment, an antibody of the invention comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:63 or SEQ ID NO:67, and a light chain variable region

comprising the amino acid sequence of SEQ ID NO: 57. In yet another specific embodiment an antibody of the invention comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:59, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 55 or SEQ ID NO:57. In a particular embodiment, the antibody

5   according to any of the above embodiments additionally comprises an Fc region or a region equivalent to the Fc region of an immunoglobulin.

In one embodiment an antibody of the invention comprises an Fc region, particularly a IgG Fc region, most particularly a IgG1 Fc region.

In a particular embodiment, the antibody of the invention is a full length antibody, particularly an

10   IgG class antibody, most particularly an IgG1 isotype antibody. In another embodiment, the antibody of the invention is an antibody fragment, selected from the group of: an scFv fragment, an Fv fragment, a Fab fragment, and a F(ab')2 fragment. In a further embodiment, the antibody of the invention is an antibody fragment having an Fc region, or a fusion protein that comprises a region equivalent to the Fc region of an immunoglobulin. In one embodiment, the antibody of

15   the invention is a monoclonal antibody.

In one embodiment, an antibody of the invention is chimeric, more specifically humanized. In a particular embodiment, an antibody of the invention is human. In another embodiment, an antibody of the invention comprises a human constant region. In one embodiment the antibody of the invention comprises a human Fc region, preferably a human IgG Fc region, most

20   particularly a human IgG1 Fc region.

In one embodiment, an antibody of the invention comprises a heavy chain constant region, wherein said heavy chain constant region is a human IgG constant region, particularly a human IgG1 constant region, comprising an Fc region. In a specific embodiment, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO:

25   93. In another specific embodiment an antibody of the invention comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO: 95. In yet another specific embodiment, an antibody of the invention comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 93, and a light chain constant region comprising the amino acid sequence of SEQ ID NO: 95.

30   In a particular embodiment, the invention provides an antibody that specifically binds to TNC A2, wherein said antibody comprises a) a heavy chain variable region comprising an amino acid sequence that is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID

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NO: 63, or a light chain variable region comprising an amino acid sequence that is at least about

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence

selected from the group of SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73,

SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89, or a combination

5   thereof, and b) an Fc region or a region equivalent to the Fc region of an immunoglobulin.

In one embodiment, an antibody of the invention comprises an Fc region, wherein said Fc region

is a glycoengineered Fc region. In a further embodiment, an antibody of the invention is

glycoengineered to have modified oligosaccharides in the Fc region. In a specific embodiment,

the antibody has an increased proportion of bisected oligosaccharides in the Fc region, compared

10   to a non-glycoengineered antibody. In a more specific embodiment, at least about 10%, about

15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about

55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about

95%, or about 100%, preferably at least about 50%, more preferably at least about 70%, of the

N-linked oligosaccharides in the Fc region of the antibody are bisected. The bisected

15   oligosaccharides may be of the hybrid or complex type.

In another specific embodiment, an antibody of the invention has an increased proportion of non-

fucosylated oligosaccharides in the Fc region, compared to a non-glycoengineered antibody. In a

more specific embodiment, at least about 20%, about 25%, about 30%, about 35%, about 40%,

about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%,

20   about 85%, about 90%, about 95%, or about 100%, preferably at least about 50%, more

preferably at least about 70%, of the N-linked oligosaccharides in the Fc region of the antibody

are non-fucosylated. The non-fucosylated oligosaccharides may be of the hybrid or complex

type.

In a particular embodiment, an antibody of the invention has an increased proportion of bisected,

25   non-fucosylated oligosaccharides in the Fc region, compared to a non-glycoengineered antibody.

Specifically, the antibody comprises an Fc region in which at least about 10%, about 15%, about

20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about

60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or

about 100%, preferably at least about 15%, more preferably at least about 25%, at least about

30   35% or at least about 50%, of the N-linked oligosaccharides are bisected, non-fucosylated. The

bisected, non-fucosylated oligosaccharides may be of the hybrid or complex type.

In one embodiment, an antibody of the invention has increased effector function and/or increased

Fc receptor binding affinity. Increased effector function and/or increased Fc receptor binding can

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result e.g. from glycoengineering and/or affinity maturation of antibodies. In one embodiment, the increased effector function and/or increased Fc receptor binding is a result of glycoengineering of the Fc region of the antibody. In another embodiment, the increased effector function and/or increased Fc receptor binding is a result of a combination of increased affinity

5   and glycoengineering. The increased effector function can include, but is not limited to, one or more of the following: increased Fc-mediated cellular cytotoxicity (including increased antibody-dependent cell-mediated cytotoxicity (ADCC)), increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased binding to NK cells, increased binding to

10   macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, increased direct signaling inducing apoptosis, increased crosslinking of target-bound antibodies, increased dendritic cell maturation, or increased T cell priming. In a particular embodiment, the increased effector function is increased ADCC. The increased Fc receptor binding preferably is increased binding to an activating Fc receptor, most preferably FcγRIIIa.

15   In one embodiment, an antibody of the invention does not cause a clinically significant level of toxicity when administered to an individual in a therapeutically effective amount.

In one embodiment, an antibody of the invention is affinity matured. In a further embodiment, an antibody of the invention binds to the A2 domain of tenascin C (TNC A2) with a dissociation constant (K_D) value lower than about 1 μM to about 0.001 nM, particularly a K_D value lower

20   than about 100 nM, lower than about 20 nM, lower than about 10 nM, or lower than about 1 nM. In one embodiment, an antibody of the invention binds to the A2 domain of human, mouse, and cynomolgus TNC A2. In one embodiment, an antibody of the invention binds to the A2 domain of human and cynomolgus TNC A2. In a more specific embodiment, an antibody of the invention binds to the A2 domain of human and cynomolgus TNC A2 with a K_D value lower

25   than about lower than about 20 nM, lower than about 10 nM, or lower than about 1 nM. K_D values are determined by Surface Plasmon Resonance, using the antibodies as Fab or IgG, particularly as IgG.

In one embodiment, an anti-TNC A2 antibody of the invention binds TNC A2 in human tissues. In a particular embodiment, the invention provides an antibody that specifically binds to the A2

30   domain of tenascin C (TNC A2), wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group of SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63, a light chain variable region comprising an amino acid sequence selected from the group of SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID

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NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89, and a human IgG Fc region, and

wherein optionally said antibody is glycoengineered to have increased effector function and/or

Fc receptor binding affinity. In another particular embodiment, the invention provides an

antibody that specifically binds to the A2 domain of tenascin C (TNC A2), wherein said

antibody comprises a heavy chain variable region comprising an amino acid sequence selected

from the group of SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63, a light chain variable

region comprising an amino acid sequence selected from the group of SEQ ID NO: 55, SEQ ID

NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85,

and SEQ ID NO: 89, and a human IgG Fc region, and wherein said antibody has an increased

proportion of non-fucosylated oligosaccharides and/or an increased proportion of bisected

oligosaccharides in said Fc region.

In one aspect, the invention provides for an antibody that specifically bind to the A2 domain of

tenascin C (TNC A2), wherein said antibody is derived from a parent antibody comprising the

heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 9, the heavy chain

CDR3 of SEQ ID NO: 27, the light chain CDR1 of SEQ ID NO: 29, the light chain CDR2 of

SEQ ID NO: 35, and the light chain CDR3 of SEQ ID NO: 47, and wherein said antibody

comprises at least one amino acid substitution in at least one heavy or light chain CDR of to the

parent antibody. For example, the antibody may comprise at least one, *e.g.* from about one to

about ten (i.e., about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10), and particularly from about two to about five,

substitutions in one or more hypervariable regions or CDRs (i.e., 1, 2, 3, 4, 5, or 6 hypervariable

regions or CDRs) of the parent antibody. In certain embodiments, any one or more amino acids

of the parent antibody as provided above are substituted at the following CDR positions:

- Heavy chain CDR2 (SEQ ID NO: 9): positions 1, 6 and 8

- Light chain CDR1 (SEQ ID NO: 29): positions 5 and 9

- Light chain CDR1 (SEQ ID NO: 35): positions 1, 2 and 3

In certain embodiments, the substitutions are conservative substitutions, as provided herein. In

certain embodiments, any one or more of the following substitutions may be made in any

combination:

- Heavy chain CDR2 (SEQ ID NO: 9): G1A or V, F6L, T8I

- Light chain CDR1 (SEQ ID NO: 29): G5S, D9V

- Light chain CDR1 (SEQ ID NO: 35): A1D, A2S or V, S3Y or T

Additionally, the antibody may also comprise one or more additions, deletions and/or

substitutions in one or more framework regions of either the heavy or the light chain, compared

to the parent antibody. In one embodiment, said at least one amino acid substitution in at least one CDR contributes to increased binding affinity of the antibody compared to its parent antibody. In another embodiment said antibody has at least about 2-fold to about 10-fold greater affinity for TNC A2 than the parent antibody (when comparing the antibody of the invention and

5   the parent antibody in the same format, e.g. the Fab format). Further, the antibody derived from a parent antibody may incorporate any of the features, singly or in combination, described in the preceding paragraphs in relation to the antibodies of the invention.

The present invention also provides for polynucleotides encoding antibodies that specifically bind to the A2 domain of tenascin-C. In one aspect, the invention is directed to an isolated

10   polynucleotide encoding a polypeptide that forms part of an anti-TNC A2 antibody according to the invention as described hereinbefore. In one embodiment, the isolated polynucleotide encodes an antibody heavy chain and/or an antibody light chain that forms part of an anti-TNC A2 antibody according to the invention as described hereinbefore.

In one embodiment, the invention is directed to an isolated polynucleotide comprising a

15   sequence encoding one or more (e.g. one, two, three, four, five, or six) of the heavy or light chain complementarity determining regions (CDRs) set forth in SEQ ID NOs 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or a variant or truncated form thereof containing at least the specificity-determining residues (SDRs) for said CDR. In another embodiment, the polynucleotide comprises a sequence that encodes three heavy chain CDRs

20   (e.g., HCDR1, HCDR2, and HCDR3) or three light chain CDRs (e.g. LCDR1, LCDR2, and LCDR3) selected from SEQ ID NOs 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or variants or truncated forms thereof containing at least the SDRs for each of said three complementarity determining regions. In yet another embodiment, the polynucleotide comprises a sequence encoding three heavy chain CDRs (e.g., HCDR1, HCDR2,

25   and HCDR3) and three light chain CDRs (e.g. LCDR1, LCDR2, and LCDR3) selected from SEQ ID NOs 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47. In a particular embodiment the polynucleotide encoding one or more CDRs comprises a sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to one or more of the CDR nucleotide sequences of SEQ ID NOs 4, 6 ,8, 10, 12, 14, 16, 18, 20, 22, 24, 26,

30   28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 49, 50, 51, 52, 53 and 54.

In a further embodiment, the polynucleotide comprises a sequence encoding a heavy chain variable region selected from the group of SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63, and/or a sequence encoding a light chain variable region selected from the group of SEQ ID

NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89. In a particular embodiment, the polynucleotide encoding a heavy chain and/or light chain variable region comprises a sequence selected from the group of variable region nucleotide sequences consisting of SEQ ID NOs 56, 58, 60, 62, 64, 66, 68, 70,

5   72, 74, 76, 78, 80, 82, 84, 86, 88, 90 and 92, or a combination thereof.

In a specific embodiment, the polynucleotide comprises a sequence encoding a heavy chain variable region selected from the group of SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63, and a sequence encoding a heavy chain constant region, particularly a human heavy chain constant region. In a particular embodiment, said heavy chain constant region is a human IgG

10   heavy chain constant region, specifically a human IgG1 heavy chain constant region, comprising an Fc region. In a specific embodiment, said heavy chain constant region comprises the sequence of SEQ ID NO: 93. In another specific embodiment, the polynucleotide comprises a sequence encoding a light chain variable region selected from the group of SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and

15   SEQ ID NO: 89, and a sequence encoding a light chain constant region, particularly a human light chain constant region. In a specific embodiment, said light chain constant region comprises the sequence of SEQ ID NO: 95.

In one embodiment, the invention is directed to a composition that comprises a first isolated polynucleotide encoding a polypeptide comprising an amino acid sequence that is at least about

20   90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63, and a second isolated polynucleotide encoding a polypeptide comprising an amino acid sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO:

25   77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89.

In one embodiment, the invention is directed to a composition that comprises a first isolated polynucleotide comprising a sequence that is at least about 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 60, SEQ ID NO: 68, and SEQ ID NO: 64, and a second isolated polynucleotide comprising a sequence that is

30   at least about 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, and SEQ ID NO: 90.

In a further aspect, the invention is also directed to isolated polypeptides, encoded by any of the polynucleotides according the invention as described hereinbefore.

In a further aspect, an anti-TNC A2 antibody according to any of the above embodiments may

5   incorporate any of the features, singly or in combination, as described in Sections 1-6 below:

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant (K_D) of $\leq 1 \mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from

10   10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). Preferably, the antibodies provided herein bind to the A2 domain of tenascin-C (TNC A2), in particular human TNC A2, with a K_D value lower than 1 nM, as determined by Surface Plasmon Resonance (SPR).

According to one embodiment, K_D is measured using surface plasmon resonance. Such an assay can be performed, for example, using a BIACORE®-T100 machine (GE Healthcare) at 25°C

15   with biotinylated antigen immobilized on streptavidin chips at ~80 response units (RU). Briefly, for immobilization antigen is diluted with 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, pH 7.4, to 0.1 μg/ml before injection at a flow rate of 10 μl/minute to achieve approximately 80 response units (RU) of coupled protein. Protein and DNA sequences of suitable antigen constructs are shown in SEQ ID NOs 99-104. For kinetics measurements, two-

20   fold serial dilutions of Fab (1.56 nM to 100 nM) or IgG (0.39 nM to 25 nM) are injected in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, pH 7.4 at 25°C at a flow rate of approximately 50 μl/min. Association and dissociation times are 180 sec and a regeneration with 10 mM glycine pH 1.5 for 60 sec is performed between the cycles. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model

25   (BIACORE® T100 Evaluation Software version 1.1.1) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). .

2. Antibody Fragments

In certain embodiments, an antibody provided herein is an antibody fragment. Antibody

30   fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')$_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003), or Carter, *Nat. Rev. Immunol.* 6:343-357 (2006).

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Single-chain Fv or scFv fragments comprise a VH domain and a VL domain as a single polypeptide chain. Typically, the VH and VL domains are joined by a linker sequence. For a review of scFv fragments, see, e.g., Plückthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')$_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

A minibody is a bivalent, homodimeric scFv derivative that contains a constant region, typically the CH3 region of an immunoglobulin, preferably IgG, more preferably IgG1, as the dimerisation region. Generally, the constant region is connected to the scFv via a hinge region and/or a linker region. Examples of minibody proteins can be found in Hu *et al.*, *Cancer Res.* 56: 3055-61 (1996).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see*, e.g., U.S. Patent No. 6,248,516 B1).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof. In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and

affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region.

5   In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity. Humanization may be achieved by various methods including, but not limited to (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies, (b)

10   grafting only the non-human (e.g., donor antibody) CDRs onto human (e.g., recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g., those that are important for retaining good antigen binding affinity or antibody functions), (c) grafting only the non-human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (d)

15   transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and

20   7,087,409; Jones et al., *Nature* 321:522-525 (1986); Morrison et al., *Proc. Natl. Acad. Sci.* 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.* 44:65-92 (1988); Verhoeyen et al., *Science* 239:1534-1536 (1988); Padlan, *Molec. Immun.* 31(3):169-217 (1994); Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing

25   "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer* 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies

30   of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions

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derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

In certain embodiments, an antibody provided herein is a human antibody. Human antibodies

5   can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable

10   regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat.*

15   *Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HuMab® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further

20   modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York,

25   1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas).

30   Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

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Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5.   *Library-Derived Antibodies*

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

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Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. *Multispecific Antibodies*

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific
5   antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for TNC A2 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of TNC A2. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express TNC A2. Bispecific antibodies can be prepared as full
10   length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305:537 (1983), WO 93/08829, and Traunecker et al., *EMBO J.* 10:3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-
15   specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science* 229:81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.* 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody
20   fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g. Gruber et al., *J. Immunol.* 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147:60 (1991). Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

25   The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to TNC A2 as well as another, different antigen (see, US 2008/0069820, for example).

7. *Antibody Variants*

In certain embodiments, amino acid sequence variants of the antibodies provided herein are
30   contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the

antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g.,

5   antigen-binding.

a)   Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs.

Amino acid substitutions can result in replacing one amino acid with another amino acid having

10   similar structural and/or chemical properties, e.g., conservative amino acid replacements. *"Conservative"* amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, phenylalanine, tryptophan, and methionine; polar neutral amino acids include serine,

15   threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Conservative substitutions are shown in Table 2 under the heading of "preferred substitutions." More substantial changes are provided in Table 2 under the heading of "exemplary substitutions," and as further described below in reference to amino acid

20   side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 2.

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile

Original Residue	Exemplary Substitutions	Preferred Substitutions
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

5 (3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for
10 another class. For example, amino acid substitutions can also result in replacing one amino acid with another amino acid having different structural and/or chemical properties, for example, replacing an amino acid from one group (*e.g.*, polar) with another amino acid from a different group (*e.g.*, basic). The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using
15 recombinant DNA techniques and assaying the resulting recombinant variants for activity.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent
20 antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding
25 affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH

5   or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed

10   mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often

15   targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations

20   may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells

25   (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or

30   additionally, it may be beneficial to analyze a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

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Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants

5   of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

10   In one aspect, the present invention provides glycoforms of anti-TNC A2 antibodies having increased effector function, including antibody-dependent cellular cytotoxicity. Glycosylation engineering of antibodies has been previously described. See, *e.g.*, U.S. Patent No. 6,602,684, incorporated herein by reference in its entirety. Methods of producing anti-TNC A2 antibodies from host cells that have altered activity of genes involved in glyocsylation are also described

15   herein in detail (see, e.g, section entitled "Recombinant Methods and Compositions" below). An IgG molecule carries two N-linked oligosaccharides in its Fc region, one on each heavy chain. As any glycoprotein, an antibody is produced as a population of glycoforms which share the same polypeptide backbone but have different oligosaccharides attached to the glycosylation sites. The oligosaccharides normally found in the Fc region of serum IgG are of complex bi-

20   antennary type (Wormald *et al.*, *Biochemistry 36*:130-38 (1997), with a low level of terminal sialic acid and bisecting N-acetylglucosamine (GlcNAc), and a variable degree of terminal galactosylation and core fucosylation (fucose attached to a GlcNAc residue in the "stem" of the biantennary oligosaccharide structure). Some studies suggest that the minimal carbohydrate structure required for FcγR binding lies within the oligosaccharide core. Lund *et al.*, *J. Immunol.*

25   *157*:4963-69 (1996).

The mouse- or hamster-derived cell lines used in industry and academia for production of antibodies normally attach the required oligosaccharide determinants to Fc sites. IgGs expressed in these cell lines lack, however, the bisecting GlcNAc found in low amounts in serum IgGs. Lifely *et al.*, *Glycobiology 318*:813-22 (1995). In the N-linked glycosylation pathway, a

30   bisecting GlcNAc is added by GnTIII. Schachter, *Biochem. Cell Biol. 64*:163-81 (1986). Umaña et al. used a single, antibody-producing CHO cell line that was previously engineered to express, in an externally-regulated fashion, different levels of a cloned GnTIII enzyme gene (Umaña, P., *et al., Nature Biotechnol. 17*:176-180 (1999)). This approach established for the

first time a rigorous correlation between expression of a glycosyltransferase (e.g., GnTIII) and the ADCC activity of the modified antibody. Thus, the invention contemplates anti-TNC A2 antibodies, comprising an Fc region or region equivalent to an Fc region having altered glycosylation resulting from changing the expression level of a glycosyltransferase gene in the

5   antibody-producing host cell. In a specific embodiment, the change in gene expression level is an increase in GnTIII activity. Increased GnTIII activity results in an increase in the percentage of bisected oligosaccharides, as well as a decrease in the percentage of fucosylated oligosaccharides, in the Fc region of the antibody. This antibody, or fragment thereof, has increased Fc receptor binding affinity and increased effector function.

10   Antibodies are provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umaña et al.); and US 2005/0123546 (Umaña et al.).

15   In one embodiment, the anti-TNC A2 antibodies of the invention have an increased proportion of bisected oligosaccharides in the Fc region as a result of the modification of their oligosaccharides by the methods of the present invention. In one embodiment, the percentage of bisected N-linked oligosaccharides in the Fc region of the anti-TNC A2 antibodies of the invention is at least about 10% to about 100%, specifically at least about 50%, more specifically,

20   at least about 60%, at least about 70%, at least about 80%, or at least about 90-95% of the total oligosaccharides. The bisected oligosaccharides may be of the hybrid or complex type.

In another embodiment, the anti-TNC A2 antibodies of the invention have an increased proportion of nonfucosylated oligosaccharides in the Fc region as a result of the modification of their oligosaccharides by the methods of the present invention. In one embodiment, the

25   percentage of nonfucosylated oligosaccharides is at least about 20% to about 100%, specifically at least about 50%, at least about 60% to about 70%, and more specifically, at least about 75%. The nonfucosylated oligosaccharides may be of the hybrid or complex type.

The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g.

30   complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described for example in WO 2008/077546. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e.,

between positions 294 and 300, due to minor sequence variations in antibodies. The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures identified in an N-Glycosidase F treated sample (e. g. complex, hybrid and high mannose structures) by MALDI-TOF MS. Such fucosylation variants may have improved ADCC

5   function.

The glycoengineering methodology that can be used with the anti-TNC A2 antibodies of the present invention has been described in greater detail in U.S. Pat. No. 6,602,684, U.S. Pat. Appl. Publ. No. 2004/0241817 A1, U.S. Pat. Appl. Publ. No. 2003/0175884 A1, Provisional U.S. Patent Application No. 60/441,307 and WO 2004/065540, the entire contents of each of which is

10   incorporated herein by reference in its entirety. The anti-TNC A2 antibodies of the present invention can alternatively be glycoengineered to have reduced fucose residues in the Fc region according to the techniques disclosed in U.S. Pat. Appl. Pub. No. 2003/0157108 (Genentech), or in EP 1 176 195 A1 , WO 03/084570, WO 03/085119 and U.S. Pat. Appl. Pub. Nos. 2003/0115614, 2004/093621, 2004/110282, 2004/110704, 2004/132140, Niwa et al., J Immunol

15   Methods 306, 151/160 (2006), US Pat. No. 6,946,292 (Kyowa). Glycoengineered anti-TNC A2 antibodies of the invention may also be produced in expression systems that produce modified glycoproteins, such as those taught in U.S. Pat. Appl. Pub. No. 60/344,169 and WO 03/056914 (GlycoFi, Inc.) or in WO 2004/057002 and WO 2004/024927 (Greenovation).

Further examples of publications related to "defucosylated" or "fucose-deficient" antibody

20   variants include: WO 2000/61739; WO 2001/29246; US 2002/0164328; US 2004/0109865; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No

25   US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

In a particular embodiment, the anti-TNC A2 antibodies of the invention have an increased

30   proportion of bisected, nonfucosylated oligosaccharides in the Fc region. The bisected, nonfucosylated oligosaccharides may be either hybrid or complex. Specifically, the methods of the present invention may be used to produce anti-TNC A2 antibodies in which at least about 10% to about 100%, specifically at least about 15%, more specifically at least about 20% to

about 25%, and more specifically at least about 30% to about 35% of the oligosaccharides in the Fc region of the antigen binding molecule are bisected, nonfucosylated. The anti-TNC A2 antibodies of the present invention may also comprise an Fc region in which at least about 10% to about 100%, specifically at least about 15%, more specifically at least about 20% to about 25%, and more specifically at least about 30% to about 35% of the oligosaccharides in the Fc region of the antibody are bisected hybrid nonfucosylated.

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

Increases in ADCC or other effector functions of the anti-TNC A2 antibodies of the present invention can also achieved by increasing affinity of the antigen binding molecule for TNC A2, for example by affinity maturation or other methods of improving affinity (*see* Tang *et al., J. Immunol.* 2007, 179:2815-2823), or by amino acid modifications in the Fc region as described below. Combinations of these approaches are also encompassed by the present invention.

c) Fc region variants

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII

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and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and

5   Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include

10   peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and

15   WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

20   Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

25   Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of

30   the Fc region (EU numbering of residues).

In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g.,

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as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

For further examples concerning Fc region variants see also U.S. Pat. Appl. Nos. 60/439,498; 60/456,041; 60/514,549; or WO 2004/063351 (variant Fc regions with increased binding affinity due to amino acid modification); or U.S. Pat. Appl. No. 10/672,280 or WO 2004/099249 (Fc variants with altered binding to FcγR due to amino acid modification), Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351.

d) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an antibody conjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol,

carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers,

5   polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for

10   derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the

15   nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B.   Recombinant Methods and Compositions

20   Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated polynucleotide encoding an anti-TNC A2 antibody described herein is provided. Such polynucleotide may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more

25   vectors (e.g., cloning vectors or expression vectors) comprising such polynucleotide are provided. In a further embodiment, a host cell comprising such polynucleotide or such vector is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a polynucleotide that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody (e.g. a polycistronic

30   vector), or (2) a first vector comprising a polynucleotide that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a polynucleotide that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is a eukaryotic cell, particularly a mammalian cell, e.g. a Chinese Hamster Ovary (CHO), a baby

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hamster kidney (BHK) cell or lymphoid cell (e.g., YO, NSO, Sp20 cell). In one embodiment, a method of making an anti-TNC A2 antibody is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

5

For recombinant production of an anti-TNC A2 antibody, one or more polynucleotide(s) encoding an antibody, e.g., as described above, are isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of an anti-TNC A2 antibody along with appropriate transcriptional/translational control signals.

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These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. *See*, for example, the techniques described in Maniatis *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, N.Y. (1989) and Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing

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Associates and Wiley Interscience, N.Y (1989).

In one embodiment, one or several polynucleotides encoding an anti-TNC A2 antibody may be expressed under the control of a constitutive promoter or, alternatively, a regulated expression system. Suitable regulated expression systems include, but are not limited to, a tetracycline-regulated expression system, an ecdysone-inducible expression system, a lac-switch expression

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system, a glucocorticoid-inducible expression system, a temperature-inducible promoter system, and a metallothionein metal-inducible expression system. If several different polynucleotides encoding an antibody of the present invention are comprised within the host cell system, some of them may be expressed under the control of a constitutive promoter, while others are expressed under the control of a regulated promoter.

25

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C.

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Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

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In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.*

5   22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006). Such expression systems are also taught in U.S. Pat. Appl. No. 60/344,169 and WO 03/056914 (methods for producing human-like glycoprotein in a non-human eukaryotic host cell).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and

10   insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts. *See,* e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

15   Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-

20   251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese

25   hamster ovary (CHO) cells, including DHFR⁻ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

30   Stable expression is generally preferred to transient expression because it typically achieves more reproducible results and also is more amenable to large-scale production; however, it is within the skill of one in the art to determine whether transient expression is better for a particular situation.

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The present invention is further directed to a method for modifying the glycosylation profile of the anti-TNC A2 antibodies of the present invention that are produced by a host cell, comprising expressing in said host cell one or more polynucleotide(s) encoding an anti-TNC A2 antibody and one or more polynucleotide(s) encoding a polypeptide with a glycosyltransferase activity, or

5   a vector comprising such polynucleotides. Generally, any type of cultured cell line, including the cell lines discussed above, can be used to generate cell lines for the production of anti-TNC A2 antibodies with altered glycosylation pattern. Preferred cell lines include CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, and other mammalian cells. Polypeptides with glycosyltransferase

10   activity include $\beta(1,4)$-N-acetylglucosaminyltransferase III (GnTIII), α-mannosidase II (ManII), $\beta(1,4)$-galactosyltransferase (GalT), $\beta(1,2)$-N-acetylglucosaminyltransferase I (GnTI), and $\beta(1,2)$-N-acetylglucosaminyltransferase II (GnTII). In one embodiment, a combination of polynucleotides encoding for polynucleotides with glycosyltransferase activity are expressed in the host cell (e.g., GnTIII and Man II). Likewise, the method also encompasses expression of

15   one or more polynucleotide(s) encoding the anti-TNC A2 antibody in a host cell in which a glycosyltransferase gene has been disrupted or otherwise deactivated (e.g., a host cell in which the activity of the gene encoding $\alpha1,6$ core fucosyltransferase has been knocked out). In a particular embodiment, the anti-TNC A2 antibodies of the present invention can be produced in a host cell that further expresses a polynucleotide encoding a polypeptide having GnTIII activity to

20   modify the glycosylation pattern of said antibodies. In a specific embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the Golgi localization domain of a Golgi resident polypeptide. In another particular embodiment, the expression of the anti-TNC A2 antibody of the present invention in a host cell that expresses a polynucleotide encoding a polypeptide having GnTIII activity results in anti-TNC A2 antibodies with increased Fc receptor

25   binding affinity and/or increased effector function. Accordingly, in one embodiment, the present invention is directed to a host cell comprising (a) one or more isolated polynucleotide(s) comprising a sequence encoding a polypeptide having GnTIII activity; and (b) one or more isolated polynucleotide(s) encoding an anti-TNC A2 antibody of the present invention. In a particular embodiment, the polypeptide having GnTIII activity is a fusion polypeptide

30   comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide. Particularly, said Golgi localization domain is the Golgi localization domain of mannosidase II. Methods for generating such fusion polypeptides and using them to produce antibodies with increased effector functions are disclosed in WO2004/065540, U.S.

Provisional Pat. Appl. No. 60/495,142 and U.S. Pat. Appl. Publ. No. 2004/0241817, the entire

contents of which are expressly incorporated herein by reference. In another embodiment, the

host cell additionally comprises an isolated polynucleotide comprising a sequence encoding a

polypeptide having mannosidase II (ManII) activity. The polynucleotide(s) encoding

5   polypeptide(s), like the polynucleotide(s) encoding the anti-TNC A2 antibody, may be expressed

under the control of a constitutive promoter or, alternately, a regulated expression system. Such

systems are well known in the art, and include the systems discussed above.

The host cells which contain the coding sequence of the anti-TNC A2 antibody and/or the coding

sequence of polypeptides having glycosyltransferase activity, and which express the biologically

10   active gene products may be identified e.g. by DNA-DNA or DNA-RNA hybridization; the

presence or absence of "marker" gene functions; assessing the level of transcription as measured

by the expression of the respective mRNA transcripts in the host cell; or detection of the gene

product as measured by immunoassay or by its biological activity - methods which are well

known in the art. GnTIII or Man II activity can be detected e.g. by employing a lectin which

15   binds to biosynthetis products of GnTIII or ManII, respectively. An example for such a lectin is

the E_4-PHA lectin which binds preferentially to oligosaccharides containing bisecting GlcNAc.

Biosynthesis products (i.e. specific oligosaccharide structures) of polypeptides having GnTIII or

ManII activity can also be detected by mass spectrometric analysis of oligosaccharides released

from glycoproteins produced by cells expressing said polypeptides. Alternatively, a functional

20   assay which measures the increased Fc receptor binding or increased effector function mediated

by antibodies produced by the cells engineered with the polynucleotide encoding a polypeptide

having GnTIII activity may be used.

The present invention is also directed to a method for producing an anti-TNC A2 antibody

having modified oligosaccharides, comprising (a) culturing a host cell engineered to express at

25   least one polynucleotide encoding a polypeptide having glycosyltransferase activity under

conditions which permit the production of an anti-TNC A2 antibody according to the present

invention, wherein said polypeptide having glycosyltransferase activity is expressed in an

amount sufficient to modify the oligosaccharides in the Fc region of said anti-TNC A2 antibody

produced by said host cell; and (b) isolating said anti-TNC A2 antibody. In one embodiment,

30   the polypeptide having glycosyltransferase activity is GnTIII. In another embodiment, there are

two polypeptides having glycosyltransferase activity. In a particular embodiment, the two

peptides having glycosyltransferase activity are GnTIII and ManII. In another embodiment, the

polypeptide having glycosyltransferase activity is a fusion polypeptide comprising the catalytic

domain of GnTIII. In a more specific embodiment, the fusion polypeptide further comprises the Golgi localization domain of a Golgi resident polypeptide. Particularly, the Golgi localization domain is the localization domain of mannosidase II or GnTI, most particularly the localization domain of mannosidase II. Alternatively, the Golgi localization domain is selected from the

5   group consisting of: the localization domain of mannosidase I, the localization domain of GnTII, and the localization domain of α1,6 core fucosyltransferase.

In a particular embodiment, the modified anti-TNC A2 antibody produced by the host cell or method described above has an IgG constant region or a fragment thereof comprising the Fc region. In another particular embodiment the anti-TNC A2 antibody is a humanized or human

10   antibody or a fragment thereof comprising an Fc region.

The anti-TNC A2 antibody with altered glycosylation produced by the host cell or method described above typically exhibit increased Fc receptor binding affinity and/or increased effector function as a result of the modification of the host cell (e.g., by expression of a glycosyltransferase gene). Preferably, the increased Fc receptor binding affinity is increased

15   binding to an activating Fcγ receptor, most preferably the FcγRIIIa receptor. The increased effector function is preferably an increase in one or more of the following: increased antibody-dependent cellular cytotoxicity, increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune-complex-mediated antigen uptake by antigen-presenting cells, increased Fc-mediated cellular cytotoxicity, increased binding to NK cells,

20   increased binding to macrophages, increased binding to polymorphonuclear cells (PMNCs), increased binding to monocytes, increased crosslinking of target-bound antibodies, increased direct signaling inducing apoptosis, increased dendritic cell maturation, and increased T cell priming.

C.   Assays

25   Anti-TNC A2 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

30   In another aspect, competition assays may be used to identify an antibody that competes with another specific anti-TNC A2 antibody for binding to TNC A2. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is

bound by said other specific anti-TNC A2 antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

In an exemplary competition assay, immobilized TNC A2 is incubated in a solution comprising a

5   first labeled antibody that binds to TNC A2 (e.g. the 2B10 antibody described in the Examples) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to TNC A2. The second antibody may be present in a hybridoma supernatant. As a control, immobilized TNC A2 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions

10   permissive for binding of the first antibody to TNC A2, excess unbound antibody is removed, and the amount of label associated with immobilized TNC A2 is measured. If the amount of label associated with immobilized TNC A2 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to TNC A2. See Harlow and Lane (1988) *Antibodies: A Laboratory*

15   *Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

2. *Activity assays*

In one aspect, assays are provided for identifying anti-TNC A2 antibodies thereof having biological activity. Biological activity may include, e.g., lysis of targeted cells, antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), or

20   induction of apoptosis. Antibodies having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, an antibody of the invention is tested for such biological activity. Exemplary assays for testing ADCC are described hereinbefore (see under "Definitions": "antibody having increased ADCC") and in Example 11. Assays for detecting cell lysis (e.g. by

25   measurement of LDH release) or apoptosis (e.g. using the TUNEL assay) are well known in the art. Assays for measuring ADCC or CDC are also described in WO 2004/065540 (see Example 1 therein), the entire content of which is incorporated herein by reference.

D.   Antibody conjugates

The invention also provides conjugates comprising an anti-TNC A2 antibody herein conjugated

30   to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

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In one embodiment, in an antibody-drug conjugate (ADC) an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588,

5   and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006);

10   Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

15   In another embodiment, an antibody conjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and

20   PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an antibody conjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186},

25   Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

30   Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as

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disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be

5   prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or

10   disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The antibody conjugates herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-

15   GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E.   Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-TNC A2 antibodies provided herein is useful for

20   detecting the presence of TNC A2 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as cells or tissues from brain, breast, colon, kidney, liver, lung, ovary, pancreas, prostate, skeletal muscle, skin, small intestine, stomach or uterus, including also cells or tissues tumors of these organs.

25   In one embodiment, an anti-TNC A2 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of TNC A2 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample, optionally with a control sample, with an anti-TNC A2 antibody as described herein under conditions permissive for binding of the anti-TNC A2 antibody to TNC A2, and detecting

30   whether a complex is formed between the anti-TNC A2 antibody and TNC A2. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-TNC A2 antibody is used to select subjects eligible for therapy with an anti-TNC A2 antibody, e.g. where TNC A2 is a biomarker for selection of patients.

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Exemplary disorders that may be diagnosed using an antibody of the invention include disorders associated with the expression of TNC A2, such as cancer and inflammatory conditions.

In one aspect, a method of diagnosing disease in a subject is provided, said method comprising administering to said subject an effective amount of a diagnostic agent, wherein said diagnostic

5   agent comprises an anti-TNC A2 antibody as described herein and a label, typically an imaging agent, that allows detection of a complex of said diagnostic agent and TNC A2.

In certain embodiments, labeled anti-TNC A2 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes

10   or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish

15   peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the

20   like.

F.   Pharmaceutical Formulations

Pharmaceutical formulations of an anti-TNC A2 antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol,

25   A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, acetate and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride;

30   benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as

glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as

5   polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186

10   and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

15   The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, if the disease to be treated is cancer, it may be desirable to further provide one or more anti-cancer agents, e.g. a chemotherapeutic agent, an inhibitor of tumor cell proliferation, or an activator of tumor cell apoptosis. Such active

20   ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug

25   delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the

30   antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

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The molecules described herein may be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application, but will typically

5   be injectable or infusible solutions.

G.   Therapeutic Methods and Compositions

Any of the anti-TNC A2 antibodies or pharmaceutical formulations comprising the anti- TNC A2 antibodies provided herein may be used in therapeutic methods.

The anti-TNC A2 antibodies provided herein can be used for treating diseases characterized by

10   TNC A2 expression, particularly by abnormal expression (e.g. overexpression, or expression in a different pattern in the cell) of TNC A2 compared to normal tissue of the same cell type. TNC A2 is abnormally expressed (e.g. overexpressed) in many human tumors compared to non-tumor tissue of the same cell type. Thus, the anti-TNC A2 antibodies provided herein are particularly useful in the prevention of tumor formation, eradication of tumors and inhibition of tumor

15   growth or metastasis. The anti-TNC A2 antibodies provided herein can be used to treat any tumor expressing TNC A2. Particular malignancies that can be treated by the anti-TNC A2 antibodies provided herein include, for example, lung cancer, colon cancer, gastric cancer, breast cancer, head and neck cancer, skin cancer, liver cancer, kidney cancer, prostate cancer, pancreatic cancer, brain cancer, cancer of the skeletal muscle.

20   The anti-TNC A2 antibodies disclosed herein can be used to inhibit tumor growth or kill tumor cells. For example, the anti-TNC A2 antibodies can bind to TNC A2 that is on the membrane or cell surface of cancerous cells (tumor cells or cells of the tumor stroma) and elicit, e.g., ADCC or other effector mediated killing of the cancerous cells.

The anti-TNC A2 antibodies can alternatively be used in order to block the function of TNC A2,

25   particularly by physically interfering with its binding of another compound. For example, the antigen binding molecules can be used to block TNC A2 mediated cell adhesion, spreading or migration.

In one aspect, an anti-TNC A2 antibody for use as a medicament is provided. In further aspects, an anti-TNC A2 antibody for use in treating a disease characterized by expression of TNC A2 is

30   provided. In certain embodiments, an anti-TNC A2 antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-TNC A2 antibody for use in a method of treating an individual having a disease characterized by expression of TNC A2, comprising administering to the individual an effective amount of the anti- TNC A2 antibody. In

one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-TNC A2 antibody for use in inducing lysis of a cell. In certain embodiments, the invention provides an anti-TNC A2 antibody for use in a method of

5   inducing lysis of a cell in an individual comprising administering to the individual an effective amount of the anti TNC A2 antibody to induce lysis of a cell. An "individual" according to any of the above embodiments is preferably a human. A "disease characterized by expression of TNC A2" according to any of the above embodiments is preferably cancer, most preferably a cancer selected from the group of lung cancer, colon cancer, gastric cancer, breast cancer, head

10   and neck cancer, skin cancer, liver cancer, kidney cancer, prostate cancer, pancreatic cancer, brain cancer, cancer of the skeletal muscle. A "cell" according to any of the above embodiments is preferably a cell present in a tumor, such as a tumor cell or a cell of the tumor stroma, most preferably a tumor cell. "TNC A2 expression" according to any of the above embodiments preferably is abnormal expression, such as overexpression or expression in a different pattern in

15   the cell, compared to normal tissue of the same cell type.

In a further aspect, the invention provides for the use of an anti-TNC A2 antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of a disease characterized by expression of TNC A2. In a further embodiment, the medicament is for use in a method of treating a disease characterized by expression of TNC A2

20   comprising administering to an individual having a disease characterized by expression of TNC A2 an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for inducing lysis of a cell. In a further embodiment, the medicament is for use in a method of

25   inducing lysis of a cell in an individual comprising administering to the individual an amount effective of the medicament to inducing lysis of a cell. An "individual" according to any of the above embodiments is preferably a human. A "disease characterized by expression of TNC A2" according to any of the above embodiments is preferably cancer, most preferably a cancer selected from the group of lung cancer, colon cancer, gastric cancer, breast cancer, skin cancer,

30   liver cancer, kidney cancer, prostate cancer, pancreatic cancer, brain cancer, cancer of the skeletal muscle. A "cell" according to any of the above embodiments is preferably a cell present in a tumor, such as a tumor cell or a cell of the tumor stroma, most preferably a tumor cell. "TNC A2 expression" according to any of the above embodiments preferably is abnormal expression,

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such as overexpression or expression in a different pattern in the cell, compared to normal tissue of the same cell type.

In a further aspect, the invention provides a method for treating a disease characterized by expression of TNC A2. In one embodiment, the method comprises administering to an individual having such disease characterized by expression of TNC A2 an effective amount of an anti-TNC A2 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. In a further embodiment, the invention provides a method for inducing lysis of a cell in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-TNC A2 antibody to induce lysis of a cell. An "individual" according to any of the above embodiments may be a human. A "disease characterized by expression of TNC A2" according to any of the above embodiments is preferably cancer, most preferably a cancer selected from the group of lung cancer, colon cancer, gastric cancer, breast cancer, skin cancer, liver cancer, kidney cancer, prostate cancer, pancreatic cancer, brain cancer, cancer of the skeletal muscle. A "cell" according to any of the above embodiments is preferably a cell present in a tumor, such as a tumor cell or a cell of the tumor stroma, most preferably a tumor cell. "TNC A2 expression" according to any of the above embodiments preferably is abnormal expression, such as overexpression or expression in a different pattern in the cell, compared to normal tissue of the same cell type.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-TNC A2 antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti- TNC A2 antibodies provided herein and one or more pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-TNC A2 antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is an anti-cancer agent. Suitable anti-cancer agents are e.g. a chemotherapeutic agent, an inhibitor of tumor cell proliferation, or an activator of tumor cell apoptosis.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to,

simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies of the invention can also be used in combination with radiation therapy. An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local

5   treatment, intralesional administration. Parenteral administration includes intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Intravenous administration is typically preferred. However, the intraperitoneal route is expected to be particularly useful, for example, in the treatment of colorectal tumors. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in

10   part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular

15   disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other

20   agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

25   For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the

30   attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous

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infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the

5   range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (*e.g.* such that the patient receives from about two to about twenty, or *e.g.* about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be

10   administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using an antibody conjugate of the invention in place of or in addition to an anti-TNC A2 antibody.

15   **H.   Articles of Manufacture**

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags,

20   etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of

25   the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of

30   manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection

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(BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an antibody conjugate of the invention in place of or in addition to an anti-TNC A2 antibody.

III.   EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1

Recombinant DNA Techniques

Standard methods were used to manipulate DNA as described in Sambrook, J. et al., Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer's instructions. DNA sequences were determined by double strand sequencing. In some cases desired gene segments were prepared by Geneart AG (Regensburg, Germany) from synthetic oligonucleotides and PCR products by automated gene synthesis. The gene segments which are flanked by singular restriction endonuclease cleavage sites were cloned into pGA18 (ampR) plasmids. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The DNA sequence of the subcloned gene fragments was confirmed by DNA sequencing. Gene Segments were designed with suitable restriction sites to allow sub-cloning into the respective expression vectors.

General information regarding the nucleotide sequences of human immunoglobulin light and heavy chains is given in: Kabat, E.A. et al., (1991) Sequences of Proteins of Immunological Interest, Fifth Ed., NIH Publication No 91-3242. For expression, all constructs contained a 5'-end DNA sequence coding for a leader peptide which targets proteins for secretion in eukaryotic cells. Exemplary leader peptides and polynucleotide sequences encoding them are given in SEQ ID NOs 107 to 115..

Preparation of (glycoengineered) antibodies

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The full antibody heavy and light chain DNA sequences have been obtained by subcloning the variable regions in frame with either the constant heavy chain or the constant light chain pre-inserted into the respective recipient mammalian expression vector. The antibody expression was driven by an MPSV promoter and the vector carries a synthetic polyA signal sequence at the 3'

5   end of the CDS. In addition each vector contains an EBV OriP sequence.

Antibodies are produced by co-transfecting HEK293-EBNA cells with the mammalian antibody expression vectors using a calcium phosphate-transfection. Exponentially growing HEK293-EBNA cells are transfected by the calcium phosphate method. Alternatively, HEK293 cells growing in suspension are transfected by polyethylenimine. For the production of unmodified

10 non-glycoengineered antibody, the cells are transfected only with antibody heavy and light chain expression vectors in a 1:1 ratio.

For the production of the glycoengineered antibody, the cells are co-transfected with two additional plasmids, one for a fusion GnTIII polypeptide expression (a GnT-III expression vector), and one for mannosidase II expression (a Golgi mannosidase II expression vector) at a

15 ratio of 4:4:1:1, respectively. Cells are grown as adherent monolayer cultures in T flasks using DMEM culture medium supplemented with 10% FCS, and are transfected when they are between 50 and 80% confluent. For the transfection of a T150 flask, 15 million cells are seeded 24 hours before transfection in 25 ml DMEM culture medium supplemented with FCS (at 10% V/V final), and cells are placed at 37°C in an incubator with a 5% CO_2 atmosphere overnight.

20 For each T150 flask to be transfected, a solution of DNA, $CaCl_2$ and water is prepared by mixing 94 µg total plasmid vector DNA divided equally between the light and heavy chain expression vectors, water to a final volume of 469 µl and 469 µl of a 1M $CaCl_2$ solution. To this solution, 938 µl of a 50 mM HEPES, 280 mM NaCl, 1.5 mM Na_2HPO_4 solution at pH 7.05 are added, mixed immediately for 10 sec and left to stand at room temperature for 20 sec. The suspension is

25 diluted with 10 ml of DMEM supplemented with 2% FCS, and added to the T150 in place of the existing medium. Then additional 13 ml of transfection medium are added. The cells are incubated at 37°C, 5% CO_2 for about 17 to 20 hours, then medium is replaced with 25 ml DMEM, 10% FCS. The conditioned culture medium is harvested approx. 7 days post-media exchange by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 um filter)

30 and sodium azide in a final concentration of 0.01 % w/v is added, and kept at 4°C.

The secreted wildtype or glycoengineered afucosylated antibodies are purified from cell culture supernatants by affinity chromatography using Protein A (HiTrap ProtA, GE Healthcare) affinity chromatography. Briefly, the column was equilibrated with 20 mM sodium phosphate, 20 mM

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sodium citrate pH 7.5, the cell supernatant was loaded, followed by a first wash with 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5, and a second wash with 13.3 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride pH 5.45. The antibodies were eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine pH 3. In a subsequent

5   size exclusion chromatographic step on a HiLoad Superdex 200 column (GE Healthcare) the buffer was exchanged to 25 mM potassium phosphate, 125 mM sodium chloride, 100 mM glycine solution of pH 6.7 or alternatively 140 mM sodium chloride, 20 mM histidine, pH 6.0 and the pure monomeric IgG1 antibodies were collected. If required an additional cation exchange chromatography step is included between the two standard purification steps.

10   The protein concentration of purified protein samples is determined by measuring the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of antibodies are analyzed by SDS-PAGE in the presence and absence of a reducing agent (5 mM 1,4-dithiotreitol) and staining with Coomassie (SimpleBlue™ SafeStain from Invitrogen). The NuPAGE® Pre-Cast gel system

15   (Invitrogen, USA) is used according to the manufacturer's instruction (4-20% Tris-Glycine gels or 3-12% Bis-Tris). The aggregate content of antibody samples is analyzed using a Superdex 200 10/300GL analytical size-exclusion column (GE Healthcare, Sweden) in 2 mM MOPS, 150 mM NaCl, 0.02% NaN$_3$, pH 7.3 running buffer at 25°C. The integrity of the amino acid backbone of reduced antibody light and heavy chains is verified by NanoElectrospray Q-TOF mass

20   spectrometry after removal of N-glycans by enzymatic treatment with Peptide-N Glycosidase F (Roche Molecular Biochemicals).

The results of the purification and analysis of the wild-type and glycoengineered 2B10 human IgG antibodies are shown in Figures 6 and 7.

The oligosaccharides attached to the Fc region of the antibodies are analysed by MALDI TOF-

25   MS as described below. Oligosaccharides are enzymatically released from the antibodies by PNGaseF digestion. The resulting digest solution containing the released oligosaccharides is either prepared directly for MALDI TOF-MS analysis or is further digested with EndoH glycosidase prior to sample preparation for MALDI TOF-MS analysis.

30   **Analysis of glycostructure of (glycoengineered) antibodies**

For determination of the relative ratios of fucose- and non-fucose (a-fucose) containing oligosaccharide structures, released glycans of purified antibody material are analyzed by MALDI-Tof-mass spectrometry. The antibody sample (about 50 µg) is incubated overnight at

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37°C with 5 mU N-Glycosidase F (QAbio; PNGaseF: E-PNG01) in 2 mM Tris, pH 7.0, in order

to release the oligosaccharide from the protein backbone. For deamination of glycans acetic acid

to a final concentration of 150 mM is added and incubated for 1h at 37°C. For analysis by

MALDI TOF mass spectrometry, 2 μL of the sample are mixed on the MALDI target with 2 μL

5   DHB matrix solution (2, 5-dihydroxybenzoic acid [Bruker Daltonics #201346] dissolved in 50

% ethanol/5 mM NaCl at 4 mg/ml) and analysed with MALDI TOF Mass Spectrometer Autoflex

II instrument [Bruker Daltonics]. Routinely, 50-300 shots are recorded and summed up to a

single experiment. The spectra obtained are evaluated by the flex analysis software (Bruker

Daltonics) and masses are determined for the each of the peaks detected. Subsequently, the peaks

10   are assigned to fucose or a-fucose (non-fucose) containing carbohydrate structures by

comparing the masses calculated and the masses theoretically expected for the respective

structures (e.g. complex, hybrid and oligo-or high-mannose, respectively, with and without

fucose).

For determination of the ratio of hybrid structures, the antibody samples are digested with N-

15   Glycosidase F and Endo-Glycosidase H [QAbio; EndoH: E-EH02] concomitantly. N-

Glycosidase F releases all N-linked glycan structures (complex, hybrid and oligo- and high

mannose structures) from the protein backbone and the Endo-Glycosidase H cleaves all the

hybrid type glycans additionally between the two N-acetylglucosamine (GlcNAc) residues at the

reducing end of the glycan. This digest is subsequently treated and analysed by MALDI TOF

20   mass spectrometry in the same way as described above for the N-Glycosidase F digested sample.

By comparing the pattern from the N-Glycosidase F digest and the combined N-glycosidase F /

Endo H digest, the degree of reduction of the signals of a specific carbohydrate structure is used

to estimate the relative content of hybrid structures. The relative amount of each carbohydrate

structure is calculated from the ratio of the peak height of an individual structure and the sum of

25   the peak heights of all oligosaccharides detected. The amount of fucose is the percentage of

fucose-containing structures related to all carbohydrate structures identified in the N-Glycosidase

F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures, resp.). The

amount of non-fucosylation is the percentage of fucose-lacking structures related to all

carbohydrates identified in the N-Glycosidase F treated sample (e.g. complex, hybrid and oligo-

30   and high-mannose structures, resp.).

The degree of non-fucosylation as determined by MALDI-TOF MS for the 2B10 human IgG

antibodies was 8.1% for the wild-type version and 83% for the glycoengineered version.

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Example 2

Construction of Generic Fab-Libraries

Generic antibody libraries in the Fab-format were constructed on the basis of human germline genes using the following V-domain pairings: Vk3_20 kappa light chain with VH3_23 heavy

5   chain for the DP47-3 library and Vk1_17 kappa light chain with VH1_69 heavy chain for the DP88-3 library. See SEQ ID NOs 1 and 2.

Both libraries were randomized in CDR3 of the light chain (L3) and CDR3 of the heavy chain (H3) and were assembled from 3 fragments per library by splicing by overlapping extension (SOE) PCR. Fragment 1 comprises the 5' end of the antibody gene including randomized L3,

10   fragment 2 is a central constant fragment spanning from L3 to H3, whereas fragment 3 comprises randomized H3 and the 3' portion of the antibody gene.

The following primer combinations were used to generate library fragments for DP47-3 library: fragment 1 (LMB3 – LibL1b_new), fragment 2 (MS63 – MS64), fragment 3 (Lib2H - fdseqlong). See Table 3. The following primer combinations were used to generate library

15   fragments for the DP88-3 library: fragment 1 (LMB3 – RJH_LIB3), fragment 2 (RJH31 – RJH32) and fragment 3 (LIB88_2 - fdseqlong). See Table 4.

TABLE 3.

Primers Used In the DP47-3 Library		SEQ ID NO
LMB3	CAGGAAACAGCTATGACCATGATTAC	116
LibL1b_new	CACTTTGGTCCCCTGGCCGAACGTMNNGGGMNNMNNMNNA CCCTGCTGACAGTAATACACTGC	117
MS63	TTTCGCACAGTAATATACGGCCGTGTCC	118
MS64	ACGTTCGGCCAGGGGACCAAAGTGG	119
Lib2H	GGCCGTATATTACTGTGCGAAANNKNNKNNKNNKNNKTTTG ACTACTGGGGCCAAGGAAC	120
fdseqlong	GACGTTAGTAAATGAATTTTCTGTATGAGG	121

20

TABLE 4.

Primers Used in DP88-3 Library		SEQ ID NO
LMB3	CAGGAAACAGCTATGACCATGATTAC	116
RJH_LIB3	GACTTTGGTGCCCTGGCCAAACGT MNN GGG MNN MNN ACC MNN CTGCAAGCAGTAATAGGTGGCAAATC	122
RJH31	ACGTTTGGCCAGGGCACCAAAGTCGAG	123
RJH32	TCTCGCACAGTAATACACGGCGGTGTCC	124
LIB88_2	GGACACCGCCGTGTATTACTGTGCGAGA –[(33% GAC Asp; 26% GGT Gly; 10% GAA Glu; 9% CGT Arg; 7% Lys; 6% GTT Val; 5% TCT Ser; 4% CTG Leu)1 - (23% GGT Gly; 17% TAC Tyr; 16% TCT Ser; 11% GCT Ala; 9% CGT Arg; 7% AAC Asn; 6% ACT Thr; 6% GTT Val; 5% CCG Pro)8]-	125

	TTTGACTACTGGGGCCAAGGGACCACCGTGACCGTCTCC	
fdseqlong	GACGTTAGTAAATGAATTTTCTGTATGAGG	121

The PCR protocol for the production of library fragments included: 5 minutes of initial denaturation at 94°C; 25 cycles of 1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C; and terminal elongation for 10 minutes at 72 °C. For assembly PCR, equimolar ratios of the 3

5   fragments were used as template. The assembly PCR protocol included: 3 minutes of initial denaturation at 94°C; and 5 cycles of 30 seconds at 94°C, 1 minute at 58°C, and 2 minutes at 72°C. At this stage, primers complementary to sequence outside fragments 1-3 were added and an additional 20 cycles were performed prior to a terminal elongation for 10 min at 72 °C. After assembly of sufficient amounts of full length randomized Fab constructs, the Fab

10   constructs were digested with NcoI / NotI for the DP47-3 library and with NcoI / NheI for the DP88-3 library alongside with similarly treated acceptor phagemid vector. For the DP47-3 library, 22.8 μg of Fab library was ligated with 16.2 μg of phagemid vector. For the DP88-3 library, 30.6 μg of Fab library was ligated with 30.6 μg of phagemid vector. Purified ligations were used for 68 transformations for the DP47-3 library and 64

15   transformations for the DP88-3 library, respectively, to obtain final library sizes of 4.2×10^{10} for DP47-3 and 3.3×10^9 for DP88-3. Phagemid particles displaying the Fab libraries were rescued and purified by PEG/NaCl purification to be used for selections.

Example 3

Selection of Anti-TNC A2 Clone 2B10 (primary selections)

20   Selections were carried out against E. coli-expressed human TNC-A2 which was subcloned 5' of an avi-tag and 6×his-tag. See SEQ ID NO: 97.

The antigen was biotinylated in vivo upon expression. Selections have been carried out in solution according to the following protocol: (i) binding of ~ 10^{12} phagemid particles of library DP88-3 and 100nM biotinylated human TNC A2 for 0.5 hours in a total volume of 1ml; (ii)

25   capture of biotinylated human TNC-A2 and attached phage by the addition of 5.4×10^7 streptavidin-coated magnetic beads for 10 minutes; (iii) washing of beads using 5x 1ml PBS/Tween20 and 5x 1ml PBS; (iv) elution of phage particles by the addition of 1 mL 100mM TEA (triethylamine) for 10 minutes and neutralization by the addition of 500 μL 1M Tris/HCl pH 7.4; and (v) re-infection of log-phase E. coli TG1 cells, infection with helperphage VCSM13

30   and subsequent PEG/NaCl precipitation of phagemid particles to be used in subsequent selection rounds.

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Selections were carried out over 3 rounds using constant antigen concentrations at 100 nM. In round 2, capture of antigen:phage complexes was performed on neutravidin plates instead of streptavidin beads. Specific binders were identified by ELISA as follows using: 100 μl of 100 nM biotinylated human TNC-A2 was coated in each well of neutravidin plates.

5   Fab-containing bacterial supernatants were added and binding Fabs were detected via their Flag-tags by using an anti-Flag/HRP secondary antibody. Once identified, clone 2B10 was bacterially expressed in a 0.5 litre culture volume, affinity purified and further characterized by SPR-analysis using BIACORE T100. See SEQ ID NOs: 56 and 60.

Example 4

10   **Construction of Anti-TNC A2 Affinity Maturation Libraries based on clone 2B10**

An affinity maturation library was constructed on the basis of a pre-selected antibody from the primary TNC A2 selections. More precisely, it was based on parental clone 2B10 and consisted of two sub-libraries: 1) V_L sub-library, randomized in CDR1 and CDR2 of the light chain (L1/L2) and 2) V_H sub-library, randomized in CDR1 and CDR2 of the heavy chain (H1/H2).

15   These sub-libraries were pooled upon transformation. Each of these sub-libraries was constructed by four subsequent steps of amplification and assembly. For L1/L2 libraries: 1) amplification of fragment 1 (LMB3 – AM_Vk1A30_L1_ba) and fragment 2 (RJH50 (Vk1A30_L1/L2_fo) – RJH51 (Vk1A30_BsiWI_ba)), 2) assembly of fragments 1 and 2 using outer primers LMB3 and RJH51 (Vk1A30_BsiWI_ba) to create the template for 3) amplification

20   of fragment 3 (LMB3 – AM_Vk1A30_L2_ba) and fragment 4 (RJH52 (Vk1A30_L2/L3) – RJH51 (Vk1A30_BsiWI_ba)) and 4) final assembly of fragments 3 and 4 using the same outer primers as above. For H1/H2 libraries: 1) amplification of fragment 1 (RJH53 – AM_DP88_H1_ba_opt) and fragment 2 (RJH54(DP88_H1/H2_fo) – MS52), 2) assembly of fragments 1 and 2 using outer primers RJH53 and MS52 to create the template for 3)

25   amplification of fragment 3 (RJH53 – AM_DP88_H2_ba) and fragment 4 (RJH55 (DP88_H2H3_fo) – MS52) and 4) final assembly of fragments 3 and 4 using the same outer primers as above. Final assembly products have been digested *Nco*I / *Bsi*WI for V_L sub-libraries and *Mun*I and *Nhe*I for V_H sub-libraries and were cloned in similarly digested acceptor vectors. Library size resulted in 1.16×10^{10} independent clones.

30
TABLE 5.

Primers used in L1/L2 Affinity Maturation Libraries for Anti-TNC A2	SEQ ID NO

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binder 2B10		
LMB3	CAGGAAACAGCTATGACCATGATTAC	116
AM_Vk1A30_L1_ba	CCTGGCTTCTGCTGGTACCAGCCTAA<u>A</u>**TC**<u>A</u>**TT**A CG**AAT**G<u>C</u>CCTGACTTGCCCGGCAGGTGATG	126
RJH50(Vk1A30_L1/L2_fo)	GCTGGTACCAGCAGAAGCCAGGGAAAG	127
RJH51(Vk1A30_BsiWI_ba)	GGTGCAGCCACCGTACGCTTGATCTC	128
AM_Vk1A30_L2_ba	CTTGATGGGACGCCACTCTGCAA<u>A</u>**CT**GGA**C**GC<u>A</u> GC<u>A</u>**TA**GATCAGGCGCTTAGGGGCTTTCC	129
RJH52(Vk1A30_L2/L3)	TTGCAGAGTGGCGTCCCATCAAGGTTC	130

Underline: 60% original base and 40% randomization as V
Bold: 60% original base and 40% randomization as N

TABLE 6.

Primers used in H1/H2 Affinity Maturation Libraries for Anti-TNC A2 binder 2B10		SEQ ID NO
RJH53	CATCAGGGCCTGAGCTCGCCCGTCAC	131
AM_DP88_H1_ba_opt	GTCCAGGGGCCTGTCGCACCCAGCTTAT<u>A</u>**GC**G**TA**GC TGCTGAATGTGCCTCCGGAGGCCTTG	132
RJH54(DP88_H1/H2_fo)	ATAAGCTGGGTGCGACAGGCCCCTGGAC	133
MS52	GAAGACCGATGGGCCTTTGGTGCTAG	134
AM_DP88_H2_ba	GACCCTGCCCTGGAACTTCTGTGCGTAGTTTGC<u>G</u>GTA CC**AAA**G<u>A</u>**TA**GG<u>G</u>**AT**GATC<u>C</u>CTCCCATCCACTCGAGC CCTTGTCCAG	135
RJH55 (DP88_H2H3_fo)	TACGCACAGAAGTTCCAGGGCAGGGTCAC	136

Underligned: 60% original base and 40% randomization as V
Bold: 60% original base and 40% randomization as N

Example 5

Selection of Affinity-Matured Anti-TNC A2 Clones

Selections were carried out against *E. coli* expressed human TNC A2 which was cloned upstream an avi-tag and 6×his-tag (see SEQ ID NO: 97). The antigen was biotinylated *in vivo* upon expression. Selections have been carried out in solution as described for the primary TNC A2 selections using decreasing concentrations of human TNC A2 ranging from 100 to 2 nM. After identification of affinity-matured clones by ELISA, secondary screenings were carried out using a ProteOn XPR36 biosensor (Biorad) and kinetic rate constants and affinities were determined analyzing affinity-purified Fab preparations on the same instrument. K_D was measured by surface plasmon resonance using a ProteOn XPR36 machine (Biorad) at 25°C with antigens immobilized on NLC sensor chips (Biorad). Briefly, recombinant antigen was diluted to

10 µg/ml in PBS / 0.005% Tween-20, pH 7.4 and injected at 30 µg/min for 100 to 300 s to achieve approximately 200-800 response units (RU) of coupled antigen. For one-shot kinetic measurements, two-fold dilution series of purified Fabs (concentration range between ~0.01 nM and 25 nM) were injected at a flow rate of 100 µl/min. Association time was 210 s, dissociation

5   time 600 s. The sensorchip was regenerated by injection of 50 mM NaOH for 18s at a flow rate of 100 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (ProteOn manager software version 2.1) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on}.

10   The following affinity-matured clones were identified: 2B10_O1F7 (see SEQ ID NOs: 85 and 87), 2B10_6H10 (see SEQ ID NOs: 89 and 91), 2B10_C3A6 (see SEQ ID NOs: 69 and 71), 2B10_D1A2 (see SEQ ID NOs: 73 and 75), and 2B10_O7D8 (see SEQ ID NOs: 81 and 83) (all of these are derived from the V_L sub-library), as well as 2B10_C3B6 (see SEQ ID NOs: 61 and 63) and 2B10_6A12 (see SEQ ID NOs: 65 and 67) (these two clones are derived from the V_H

15   sub-library). Moreover, for clone 2B10_D1A2, a V32D mutant was generated (see SEQ ID NOs: 77 and 79) (numbering according to Kabat).

Figure 1 shows the Surface Plasmon Resonance sensorgrams of the selected affinity matured Fabs binding to immobilized TNC A2, and Table 7 gives the respective affinities derived. The selected Fabs span a high affinity range in the pM range.

20

TABLE 7.

*Summary of kinetic equilibrium constants (K_D) of affinity-matured
anti-TNC A2 antibodies as Fab fragments (monovalent binding).*

antibody	affinity (KD) to hu TNC A2 [pM]
2B10_C3B6	191
2B10_6A12	290
2B10_C3A6	497
2B10_O7D8	147
2B10_O1F7	56
2B10_6H10	810

25

Example 6

IgG Conversion of 2B10 Fabs Binding TNC A2

The parental 2B10 Fab and the affinity matured 2B10 Fab derivatives have been converted into a human IgG1 format, a mouse IgG2a format and a human IgG1 format with an Avi Tag fused to the C-terminus of the heavy chain. The affinity matured 2B10 Fabs were converted into mouse IgG to allow immunohistochemical analysis (see Example 8)

5   The full antibody heavy and light chain DNA sequences were obtained either by subcloning the variable regions in frame with the respective constant heavy and the constant light chain regions pre-inserted into different recipient mammalian expression vectors or were recombined by fusing a short sequence stretch homologous to the recipient vectors insertion site. The recombination was performed according to the "In-Fusion Cloning System" from Invitrogen.

10   In all vectors the antibody expression is driven by an MPSV promoter and all vectors carry a synthetic polyA signal sequence at the 3' end of the CDS. In addition each vector contains an EBV OriP sequence.

Example 7

Determination of Affinity of 2B10 Fab and IgG to TNC A2

15   The affinity of the TNC A2 2B10 Fab fragment as well as of the human IgG1 converted TNC A2 2B10 antibody was subsequently determined and confirmed for human, murine and cynomolgus TNC A2 domain by surface plasmon resonance using a BIACORE® T100 machine (GE Healthcare) at 25°C. For this purpose the biotinylated antigens (expressed in *E. coli*, antigen in context but not glycosylated) were immobilized on a streptavidin chip and the constructs were

20   used as analytes. For immobilization the antigens were diluted with HBS-EP+ (GE Healthcare, 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, pH 7.4) to 0.1 µg/ml before injection at a flow rate of 10 µl/minute to achieve approximately 80 response units (RU) of coupled protein. The TNC A2 domain from man, mouse and cynomolgus monkey was expressed in the context of the domains A1 and A3. The antigen constructs comprising the fifth

25   fibronectin type III domain of human TNC (Fn5), the A1 and A2 domain of either human, murine or cynomolgus TNC, and the A3 domain of human TNC (TNC Fn5-A1-human/murine/cynoA2-A3) are shown in SEQ ID NOs 99-104.

For kinetics measurements, two-fold serial dilutions of Fab fragments (range between 1.56 nM to 100 nM) or IgG (range between 0.39 nM to 25 nM) were injected in HBS-EP+ at 25°C at a flow

30   rate of 50 µl/min. Association and dissociation times were 180 s and a regeneration with 10 mM glycine pH 1.5 for 60 s was performed between cycles. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIACORE®

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T100 Evaluation Software version 1.1.1) by simultaneously fitting the association and

dissociation sensorgrams. The equilibrium dissociation constant (K_D) was calculated as the ratio

k_{off}/k_{on}. See, e.g., Chen et al., J Mol Biol 293, 865-881 (1999)

TNC domains 1 to 8 produced in HEK served as negative control (see SEQ ID 105 and 106).

5   The K_D values of binding are given in Table 8. Figure 2 A-B shows the corresponding SPR-

based kinetic analyses.

TABLE 8.

Summary of kinetic equilibrium constants (K_D) of

10   *2B10 anti-TNC A2 antibodies as Fab fragments and as IgG*

Construct	Human TNC	Murine TNC	Cyno TNC
IgG 2B10 Immob E. coli Ag	Avidity: 0.29 nM	Avidity: 1.9 nM	Avidity: 0.21 nM
Fab fragment 2B10 Immob E. coli Ag	Affinity: 1.8 nM	Affinity: 180 nM	Affinity: 17 nM

Example 8

Binding of anti-TNC A2 antibodies on human tumor tissue versus normal tissue

15   **sections**

In order to check the specificity of the selected 2B10 Fab for the A2 domain of human tenascin

(TNC-A2), and to evaluate its capability to bind selectively to tumor tissues versus normal

tissues, immunohistochemical analyses were performed. Briefly, the 2B10 variable region in a

Fab fragment was fused to a FLAG fragment (SHD2B10-FLAG). Healthy and cancerous human

20   uterine tissue samples were prepared for immunohistochemical staining. Subsequently, the

samples were incubated with the SHD2B10-FLAG Fab fragment. The samples were then washed

and incubated with a fluorescent antibody specific for the FLAG epitope. Cancerous tissue

samples exhibited higher expression levels of TNC A2 as compared to the healthy tissue samples

(Figure 3A). Various human tissue samples from healthy individuals and cancer patients were

25   incubated with the SHD2B10-FLAG Fab fragment as described. Figure 3B gives the quantified

expression levels of TNC A2 in various human tissue samples in terms of % of

immunofluorescence surface area. In a another set of experiments the 2B10 variable region was

used in a mouse IgG format. Healthy and cancerous human tissue samples from different organs

were prepared for immunohistochemical staining. Subsequently, the samples were incubated

with the SHD2B10-mouse IgG. The samples were then washed and incubated with a peroxidase developing system. Figure 4 A-N shows that cancerous tissue samples exhibited higher expression levels of TNC A2 as compared to the healthy tissue samples.

Example 9

Binding of anti-TNC A2 antibodies to Tenascin-C on U87 MG glioblastoma cells

The 2B10 Fab was converted to a human IgG1 antibody and tested for binding to TNC A2 expressed on U87MG glioblastoma tumor cells by FACS. Briefly, 200.000 cells per well were incubated with the indicated concentration of the anti-TNC A2 antibody 2B10 in a round bottom 96-well plate, incubated for 30 min at 4°C and washed once with PBS/0.1 % BSA. Bound antibody was detected with FITC-conjugated AffiniPure F(ab')2 Fragment goat anti-human IgG Fcγ Specific (Jackson Immuno Research Lab #109-096-098, working solution: 1:20 diluted in PBS/0.1% BSA, freshly prepared) after incubation for 30 min at 4°C, using a FACS CantoII (Software FACS Diva). Figure 5 shows the mean fluorescence intensity of binding in a dose-dependent manner compared to untreated cells and cells stained only with the secondary antibody.

Using the same experimental procedure, the glycoengineered 2B10 human IgG antibody was tested for binding to TNC A2 on U87MG cells, compared to the wild-type 2B10 human IgG antibody. Figure 8 shows that both the wild-type and the glycoengineered 2B10 antibody bind to TNC A2 on U87MG cells.

Example 10

Binding of affinity matured anti-TNC A2 antibodies to Tenascin-C on tumor cells

Binding of human IgG1 antibodies derived from affinity matured 2B10 Fabs to human TNC A2 on U87MG glioblastoma cells or SK-Mel5 melanoma cells is measured by FACS. Briefly, 200.000 cells per well are incubated with the indicated concentration of the 2B10 derived anti-TNC A2 antibodies in a round bottom 96-well-plate, incubated for 30 min at 4°C and washed once with PBS/0.1 % BSA. Bound antibody is detected with FITC-conjugated AffiniPure F(ab')2 Fragment goat anti-human IgG Fcγ Specific (Jackson Immuno Research Lab # 109-096-098, working solution: 1:20 diluted in PBS/0.1% BSA, freshly prepared) after incubation for 30 min at 4°C using a FACS CantoII (Software FACS Diva).

Example 11

Antibody-dependent cell-mediated cytotoxicity mediated by glycoengineered anti-TNC A2 IgG1 antibodies

Human IgG1 antibodies against TNC A2 derived from 2B10 are glycoengineered by co-
5   transfection with plasmids encoding for GnTIII and ManII as described in Example 1.
Subsequently, glycoengineered 2B10 parental and affinity matured human IgG1 antibodies
derived from 2B10 are compared in an ADCC assay for their potential to mediate superior
antibody mediated cellular cytotoxicity compared to their non-glycoengineered wildtype version.
Briefly, U87MG glioblastoma cells or SK-Mel5 melanoma cells as target cells are collected,
10   washed and resuspended in culture medium and stained with freshly prepared Calcein AM
(Molecular Probes) at 37°C for 30 min, washed three times, counted and diluted to 300.000
cells/ml. This suspension is transferred to a round bottom 96-well plate (30.000 cells/well), the
respective antibody dilution is added and incubated for 10 min to facilitate the binding of the
tested antibody to the cells prior to contact with effector cells. Effector to target ratio is 30 to 1
15   for PBMCs; alternatively NK92 cells can be applied. Co-incubation is performed for 4 hours. As
read-out the release of lactate dehydrogenase (LDH) into supernatant after disintegration of the
attacked cells is used. LDH from co-culture supernatant is collected and analyzed with a LDH
detection Kit (Roche Applied Science). Substrate conversion by the LDH enzyme is measured
with an ELISA absorbance reader (SoftMaxPro software, reference wavelengths: 490 nm versus
20   650 nm).

*   *   *

Although the foregoing invention has been described in some detail by way of illustration and
example for purposes of clarity of understanding, the descriptions and examples should not be
25   construed as limiting the scope of the invention. The disclosures of all patent and scientific
literature cited herein are expressly incorporated in their entirety by reference.

Claims

1. An antibody that specifically binds to the A2 domain of tenascin A2 (TNC A2), wherein said antibody is glycoengineered to have increased effector function.

2. An antibody that specifically binds to the A2 domain of tenascin C (TNC A2), wherein said antibody comprises at least one heavy or light chain complementarity determining region (CDR) selected from the group of 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, and SEQ ID NO: 47, or a combination thereof.

3. The antibody of claim 1 or 2, wherein said antibody comprises a heavy chain variable region comprising
(a) a heavy chain CDR1 selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7;
(b) a heavy chain CDR2 selected from the group of 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, and SEQ ID NO: 25; and
(c) the heavy chain CDR3 of SEQ ID NO: 27.

4. The antibody of any one of claims 1 to 3, wherein said antibody comprises a light chain variable region comprising
(a) a light chain CDR1 selected from the group of SEQ ID NO: 29, SEQ ID NO: 31, and SEQ ID NO: 33;
(b) a light chain CDR2 selected from the group of SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 45; and
(c) the light chain CDR3 of SEQ ID NO:47.

5. The antibody of any one of claims 1 to 4, wherein said antibody does not comprise the combination of a heavy chain CDR1 selected from the group of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, a heavy chain CDR2 selected from the group of SEQ ID NO: 9,

SEQ ID NO: 15, and SEQ ID NO: 21, the heavy chain CDR3 of SEQ ID NO: 27, the light chain CDR1 of SEQ ID NO: 29, the light chain CDR2 of SEQ ID NO: 35, and the light chain CDR3 of SEQ ID NO:47.

6. The antibody of any one of claims 1 to 5, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group of: SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63.

7. The antibody of any one of claims 1 to 6, wherein said antibody comprises a light chain variable region comprising an amino acid sequence selected from the group of: SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89.

8. The antibody of any one of claims 1 to 7, wherein said antibody does not comprise a combination of a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 59 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 55 or SEQ ID NO: 57.

9. The antibody of any one of claims 1 to 8, wherein said antibody comprises an Fc region or a region equivalent to the Fc region of an immunoglobulin.

10. The antibody of claim 9, wherein said Fc region is an IgG Fc region.

11. The antibody of any one of claims 1 to 10, wherein said antibody is a full-length IgG class antibody.

12. The antibody of any one of claims 1 to 11, wherein said antibody comprises a human constant region.

13. The antibody of claim 1 to 12, wherein said antibody is a human antibody.

14. The antibody of any one of claims 1 to 13, wherein said antibody comprises a glycoengineered Fc region.

15. The antibody of claim 14, wherein said antibody has an increased proportion of non-fucosylated oligosaccharides in said Fc region, as compared to a non-glycoengineered antibody.

16. The antibody of claims 14 or 15, wherein at least about 20% to about 100% of the N-linked oligosaccharides in said Fc region are non-fucosylated.

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17. The antibody of any one claims 14 to 16, wherein said antibody has an increased proportion of bisected oligosaccharides in said Fc region, as compared to a non-glycoengineered antibody.

18. The antibody of any one of claims 14 to 17, wherein at least about 20% to about 100% of the N-linked oligosaccharides in said Fc region are bisected.

19. The antibody of any one of claims 14 to 18, wherein at least about 20% to about 50% of the N-linked oligosaccharides in said Fc region are bisected, non-fucosylated.

20. The antibody of any one of claims 1 to 19, wherein said antibody has increased effector function and/or increased Fc receptor binding affinity.

21. The antibody of claim 20, wherein said increased effector function is increased ADCC.

22. The antibody of any one of claims 1 to 21, wherein said antibody is affinity matured.

23. The antibody of any one of claims 1 to 22, wherein said antibody binds to human TNC A2 with a K_D value lower than about 1 μM.

24. The antibody of any one of claims 1 to 23, wherein said antibody binds TNC A2 in human tissue.

25. An isolated polynucleotide encoding a polypeptide that forms part of the antibody according to any one of claims 1 to 24.

26. An isolated polypeptide encoded by the polynucleotide of claim 25.

27. A composition comprising a first isolated polynucleotide encoding a polypeptide comprising a sequence selected from the group of SEQ ID NO: 59, SEQ ID NO: 67, and SEQ ID NO: 63, and a second isolated polynucleotide encoding a polypeptide comprising a sequence selected from the group of SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, and SEQ ID NO: 89.

28. A vector comprising the polynucleotide of claim 25.

29. A host cell comprising the polynucleotide of claim 25, the composition of claim 27, or the vector of claim 28.

30. The host cell of claim 29, wherein said host cell has been manipulated to express increased levels of one or more polypeptides having GnTIII activity.

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31. The host cell of claim 30, wherein said polypeptide having GnTIII activity is a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain of ManII.

32. The host cell of claim 30 or 31, wherein said host cell has been further manipulated to express increased levels of one or more polypeptides having ManII activity.

33. A method of producing an antibody that specifically binds to the A2 domain of tenascin A2, said method comprising

a) culturing the host cell of claim 29 in a medium under conditions allowing the expression of the antibody, and

b) recovering the antibody.

34. A method of producing an antibody that specifically binds to the A2 domain of tenascin A2, said method comprising

a) culturing the host cell of any one of claims 32 to 32 in a medium under conditions allowing the expression of the antibody and the modification of the oligosaccharides present on the Fc region of said antibody by said polypeptide having GnTIII activity, and

b) recovering the antibody

35. An antibody that specifically binds to the A2 domain of tenascin A2, wherein said antibody is produced by the method of claim 33 or 34.

36. An antibody conjugate comprising the antibody of any one of claims 1 to 24 and a cytotoxic agent.

37. A pharmaceutical formulation comprising the antibody of any one of claims 1 to 24 and a pharmaceutically acceptable carrier.

38. The pharmaceutical formulation of claim 37, further comprising an additional therapeutic agent.

39. The antibody of any one of claims 1 to 24 for use as a medicament.

40. The antibody of any one of claims 1 to 24 for treatment of a disease characterized by expression of TNC A2.

41. The antibody of claim 40, wherein said disease is cancer.

42. The antibody of any one of claims 1 to 24 for use in inducing cell lysis of a tumor cell or a stromal cell of a tumor.

43. Use of the antibody of any one of claims 1 to 24 in the manufacture of a medicament.

44. Use of the antibody of any one of claims 1 to 24 for the manufacture of a medicament for treatment of a disease characterized by expression of TNC A2.

45. The use of claim 44, wherein said disease is cancer.

46. Use of the antibody of any one of claims 1 to 24 for the manufacture of a medicament for inducing lysis of a tumor cell or a stromal cell of a tumor.

47. A method of treating an individual having a disease characterized by TNC A2 expression, comprising administering to the individual an effective amount of the antibody of any one of claims 1 to 24, or the pharmaceutical formulation of claim 37 or 38.

48. The method of claim 47 further comprising administering an additional therapeutic agent to the individual.

49. The method of claim 47 or 48, wherein said disease is cancer.

50. A method of inducing cell lysis of a tumor cell or a stromal cell of a tumor, said method comprising contacting said tumor cell or stromal cell with the antibody of any one of claims 1 to 24.

51. The method of claim 50, wherein said cell lysis is induced by antibody dependent cytotoxicity of the antibody.

52. A method of diagnosing disease in an individual, said method comprising administering to the individual an effective amount of a diagnostic agent, wherein said diagnostic agent comprises the antibody of any one of claims 1 to 24 and a label that allows detection of a complex of said diagnostic agent and TNC A2

53. The invention as described hereinbefore.

Figure 1
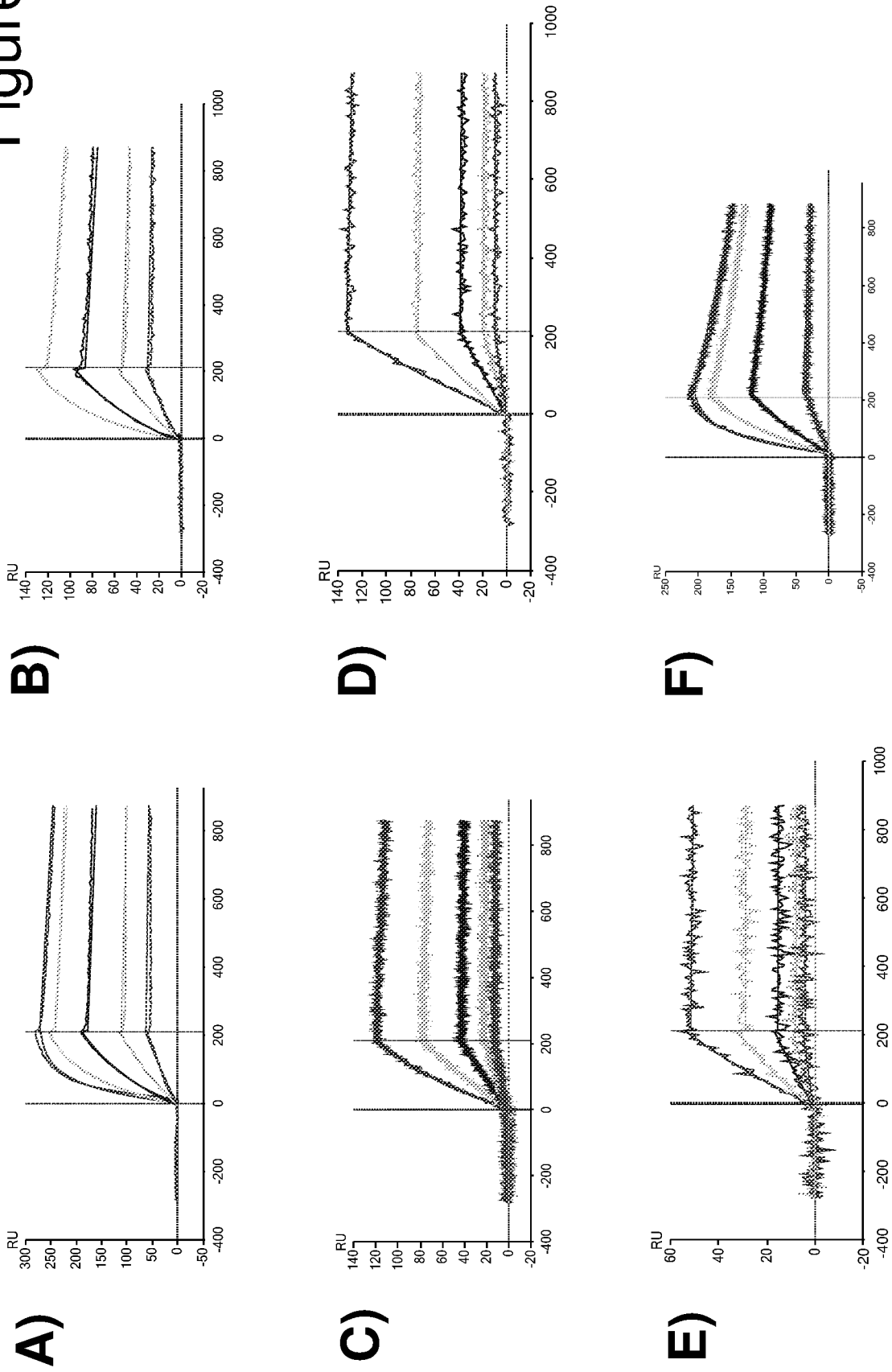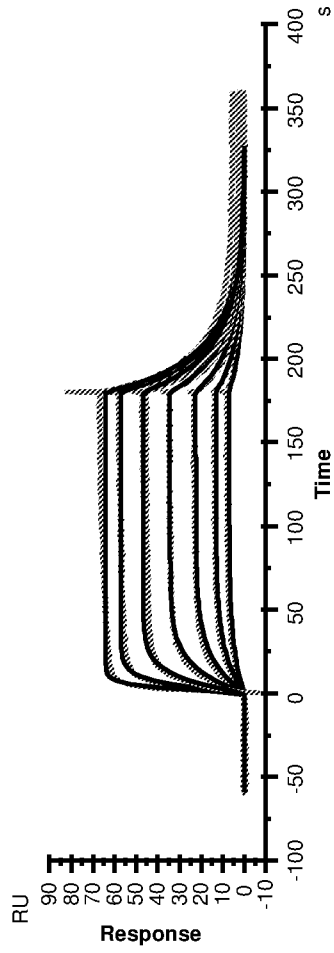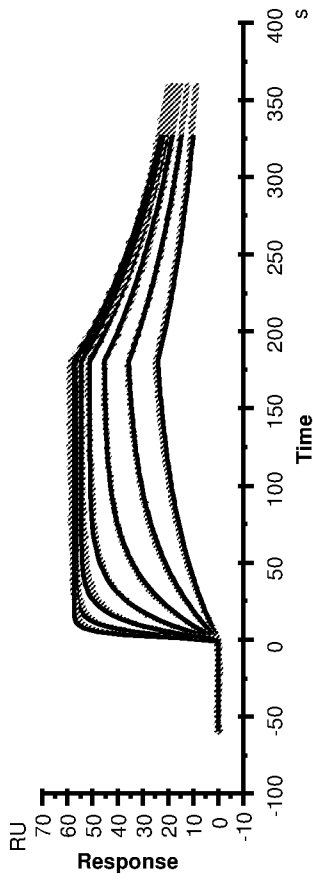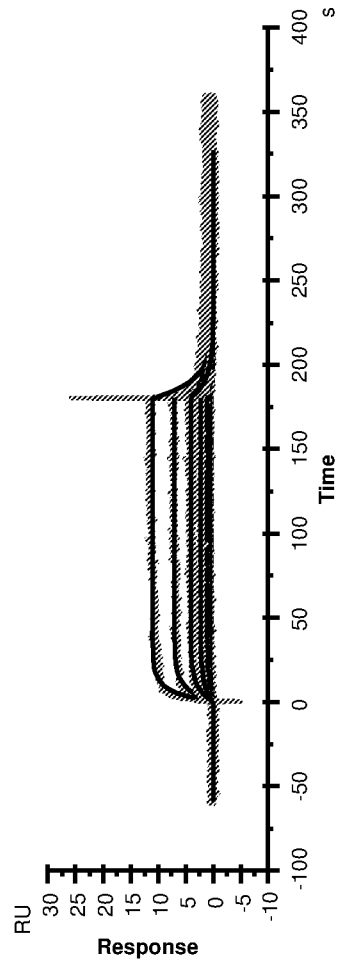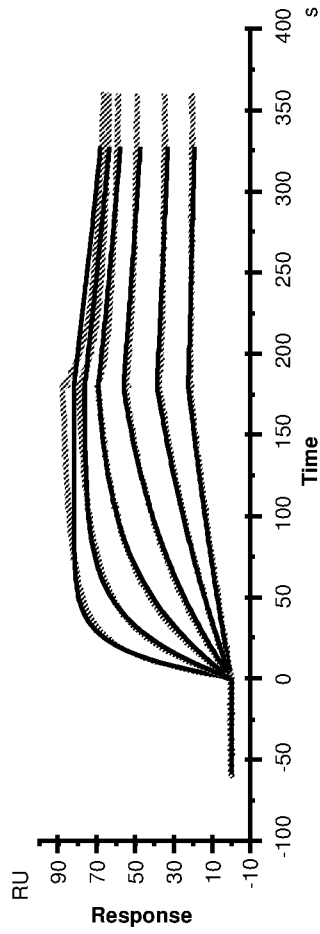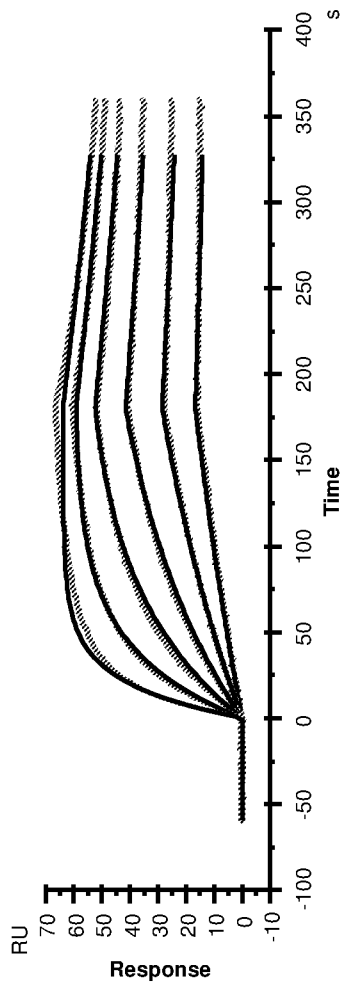
Figure 2A

Figure 2B

Figure 3A

	Brain	Breast	Colon	Kidney	Liver	Lung	Ovary	Pancreas	Prostate	Skeletal muscle	Skin	Small intestine	Stomach	Uterus
Tumor	2.22	11.09	17.74	9.24	5.19	9.39	0.87	20.27	9.31	41.72	19.61	8.72	12.81	33.77
Normal	0.45	1.38	2.85	7.59	0.45	1.06	1.08	0.65	2.96	0.83	0.98	0.52	2.77	11.09
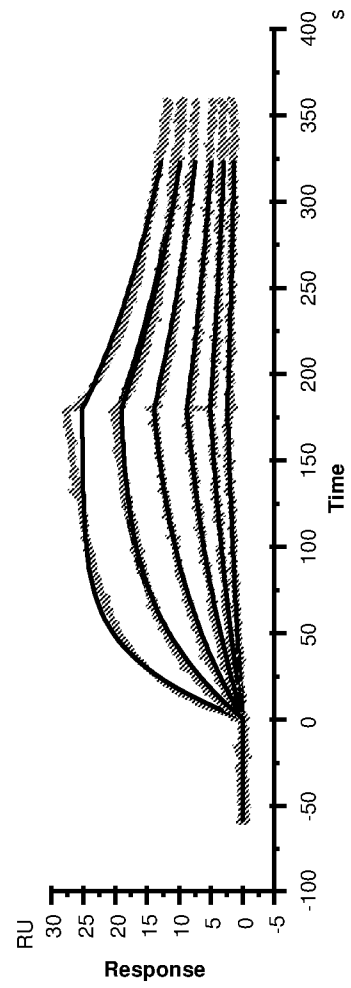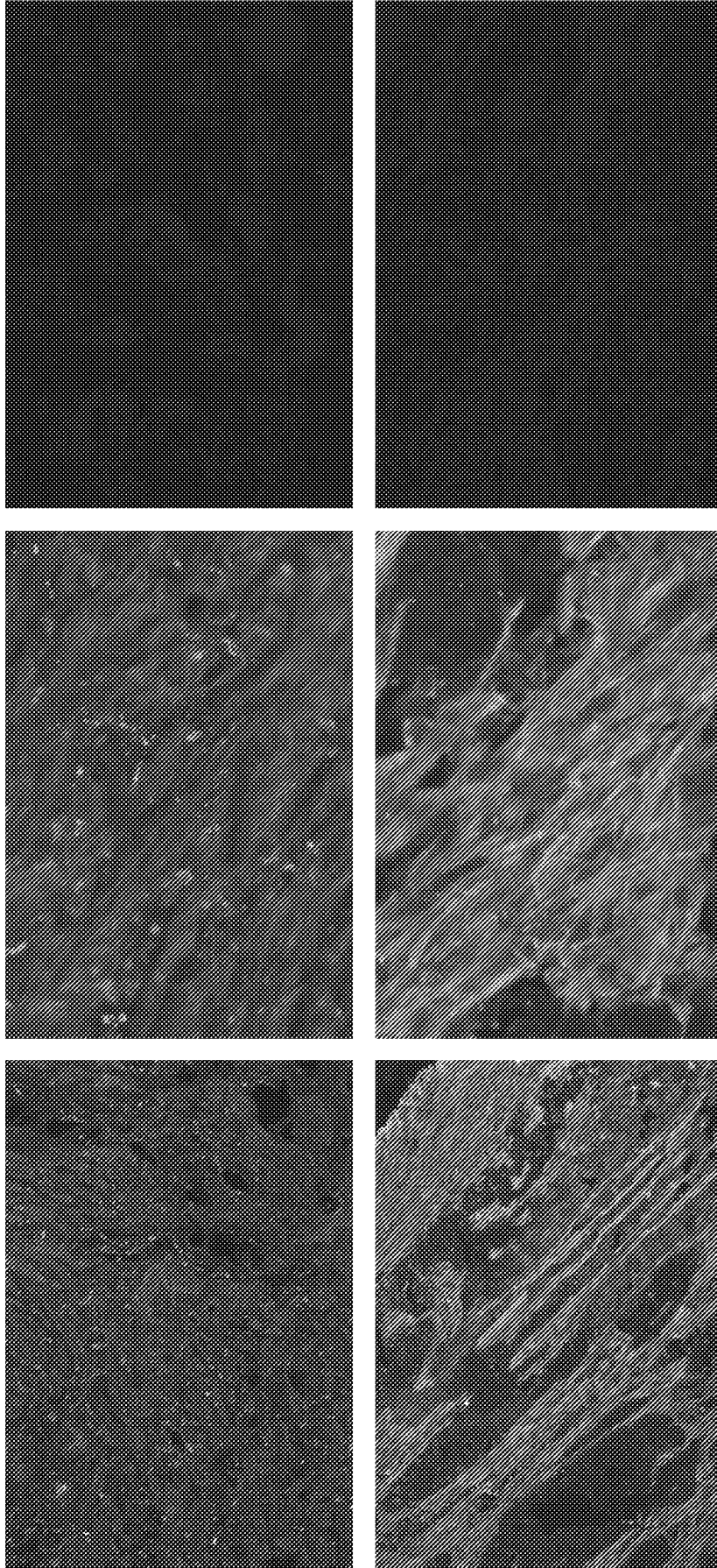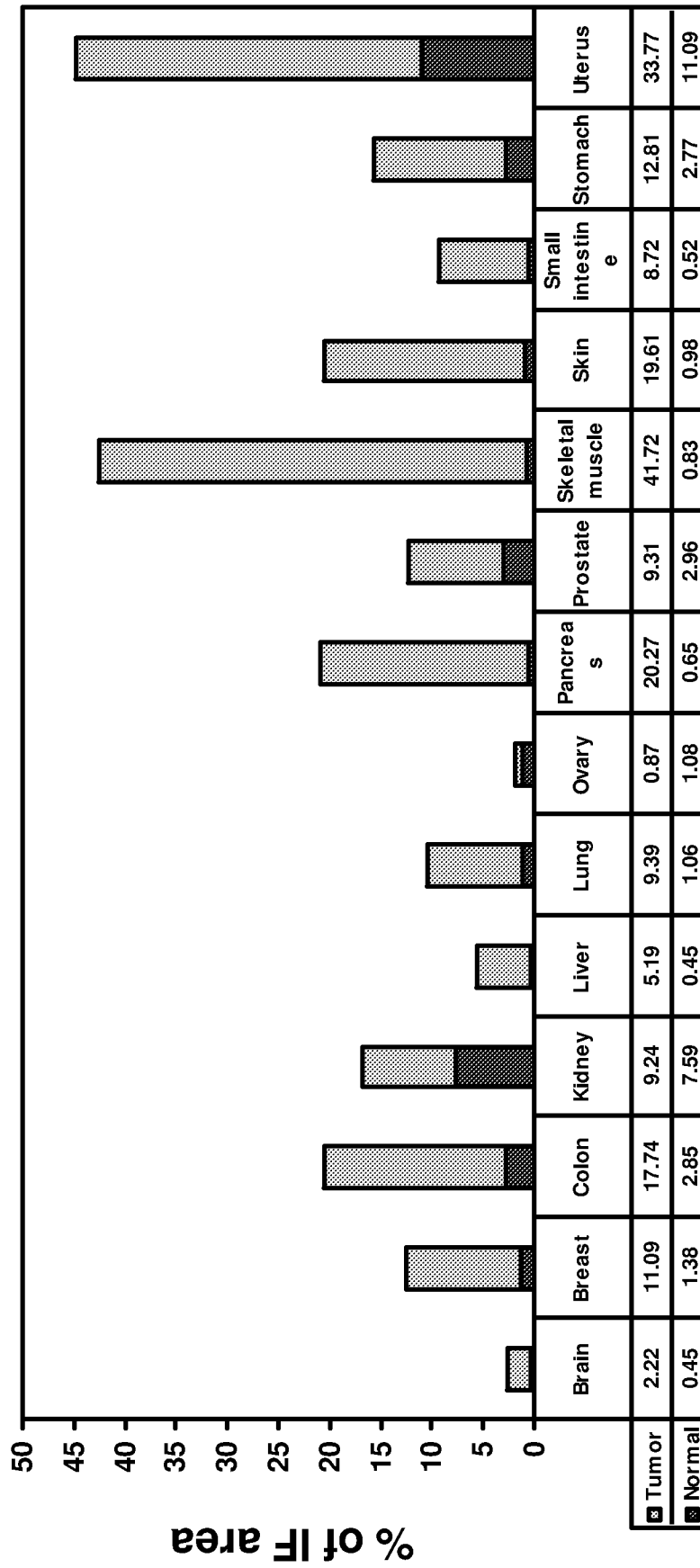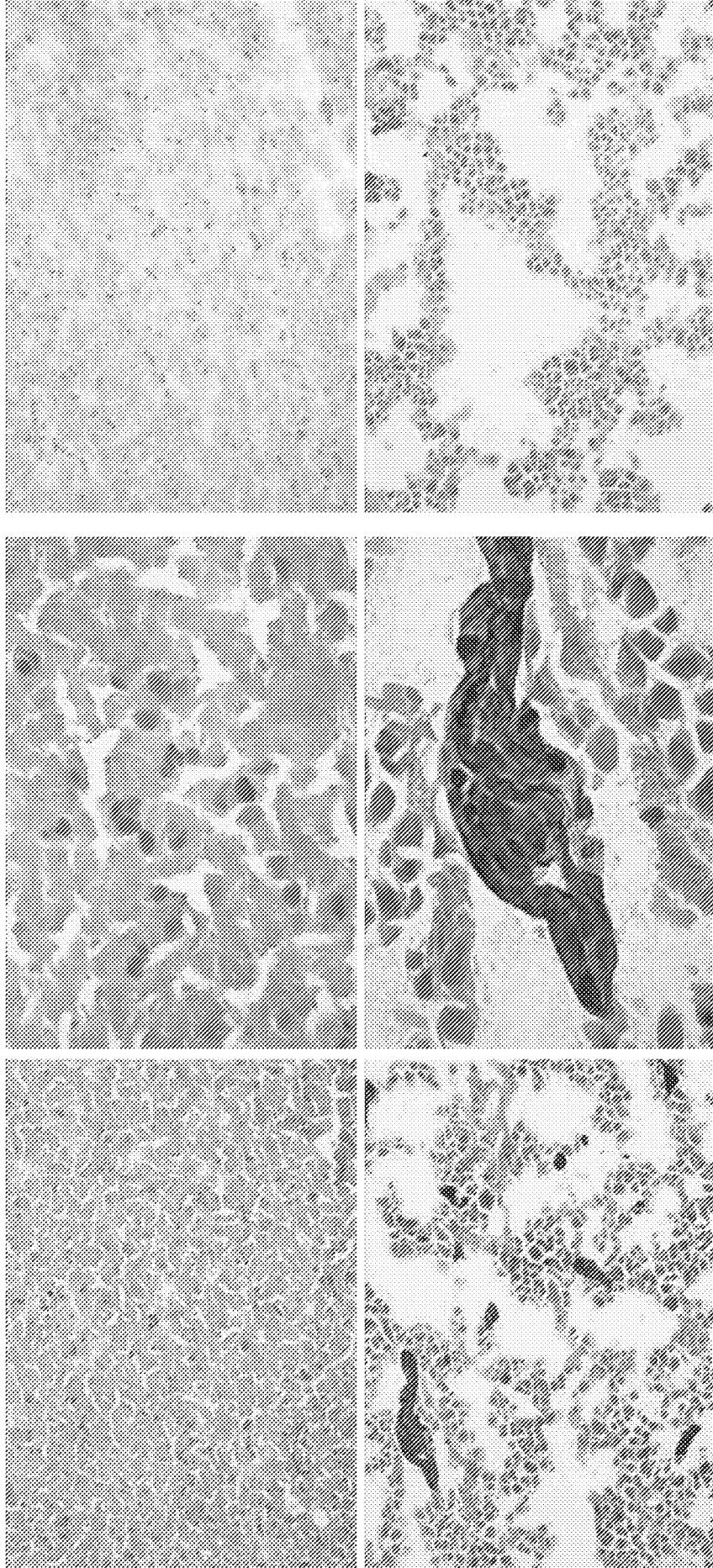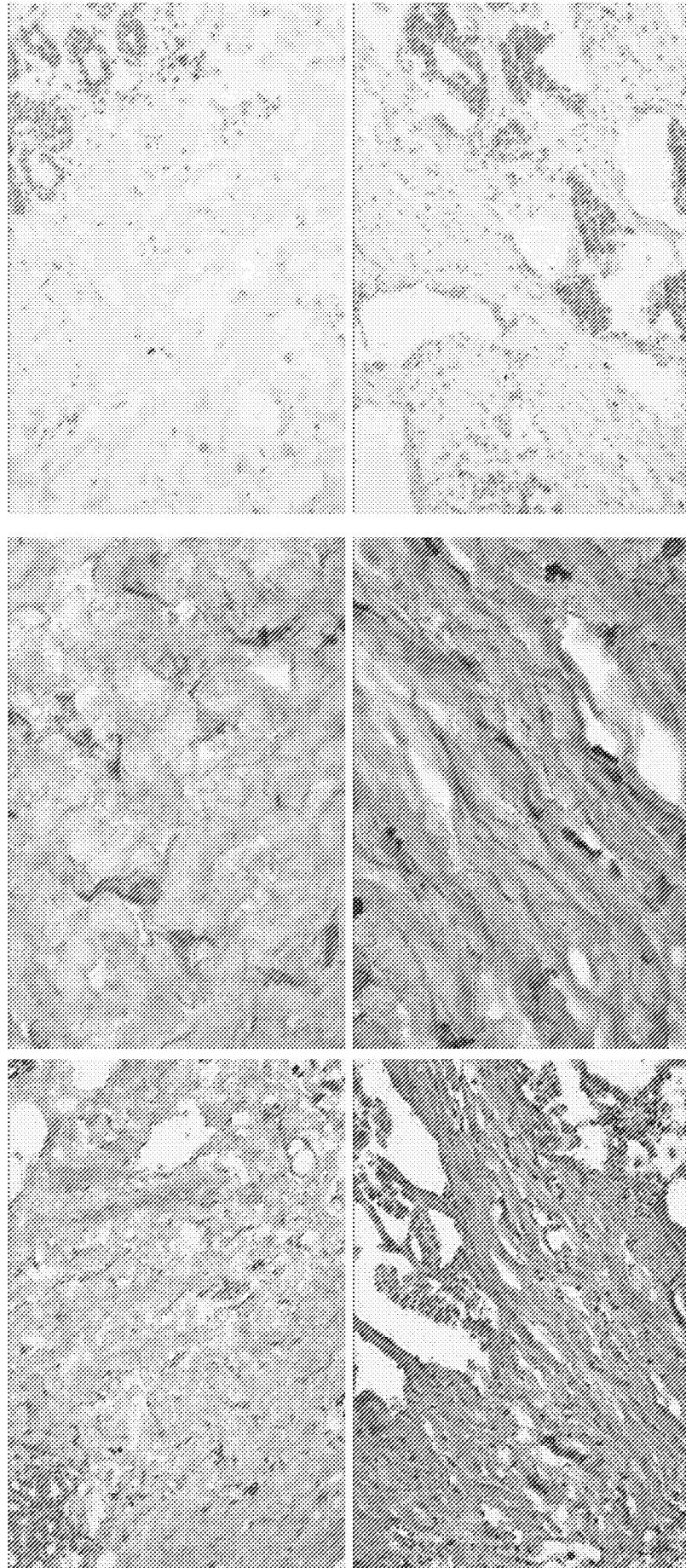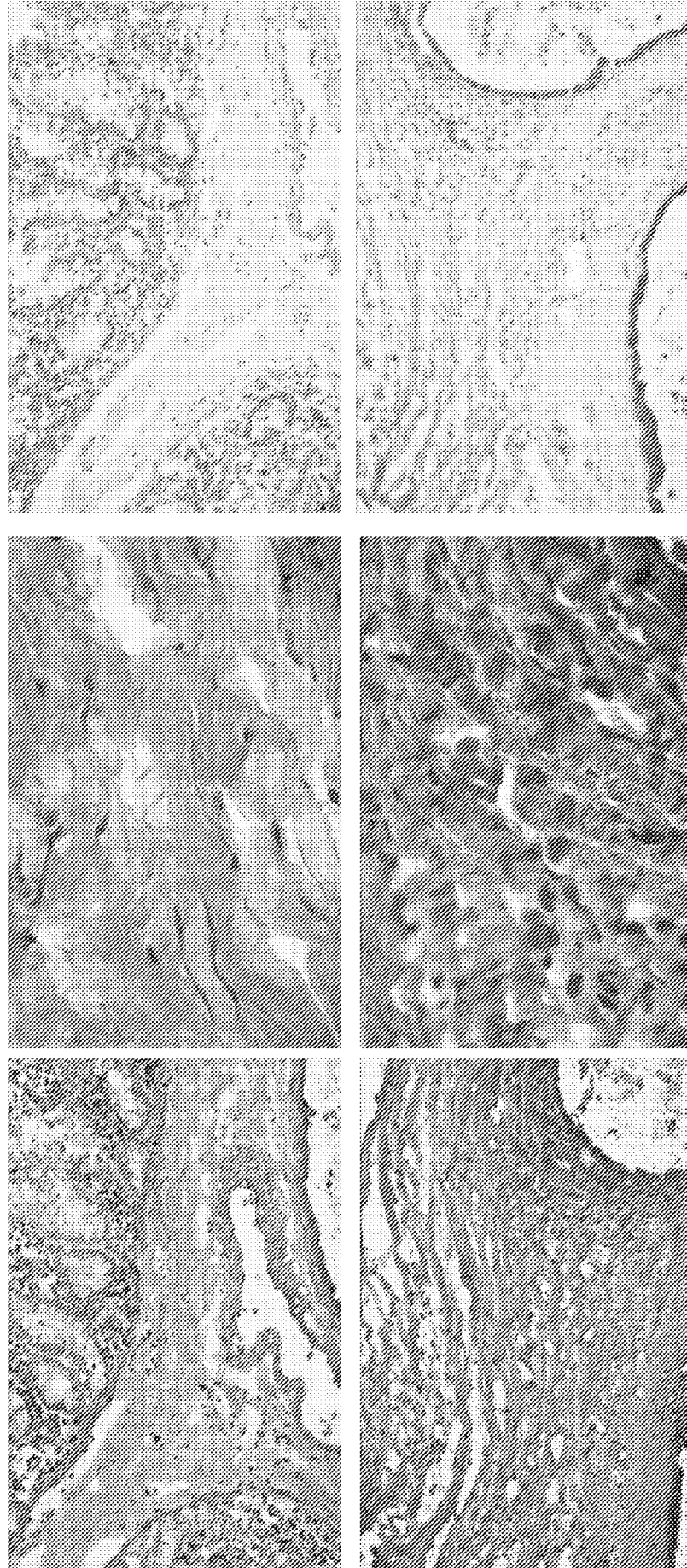
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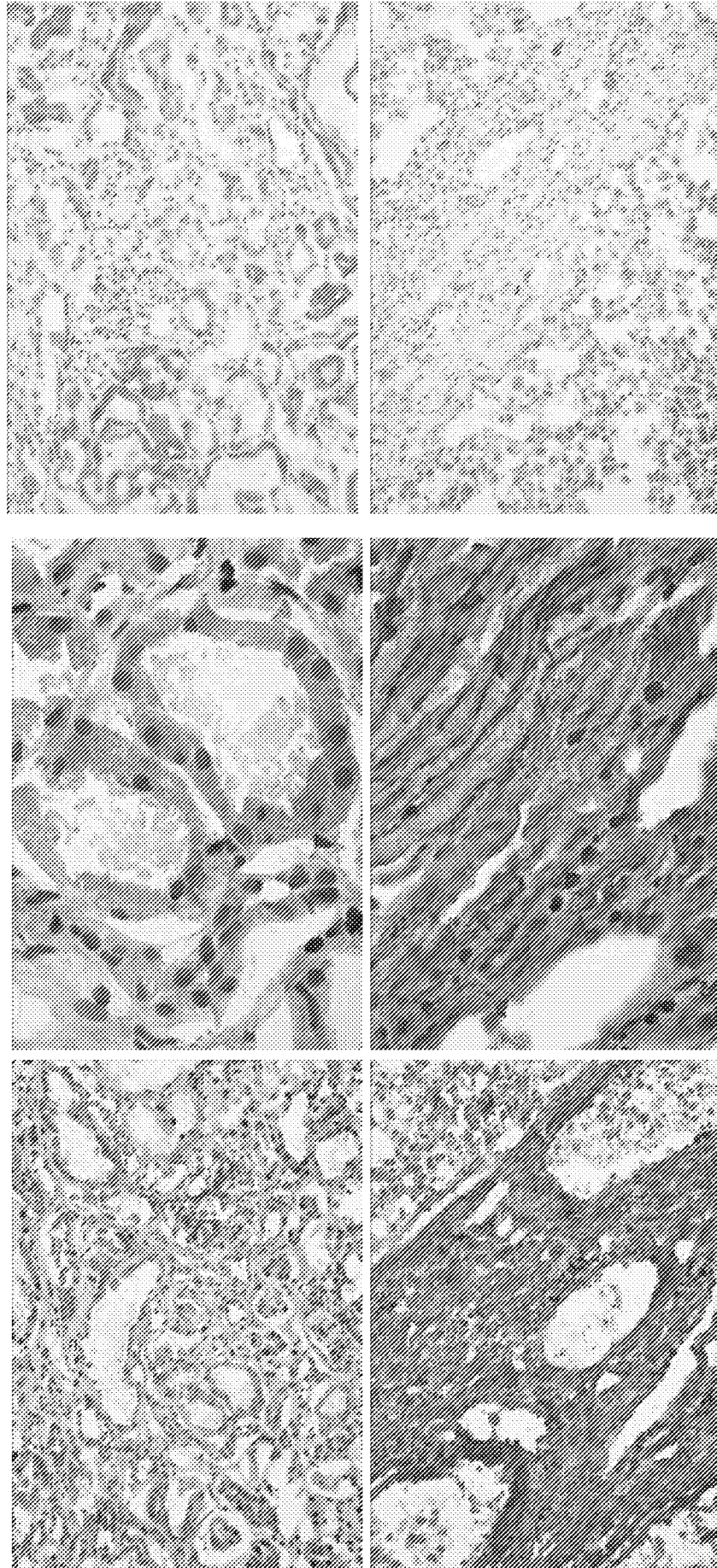
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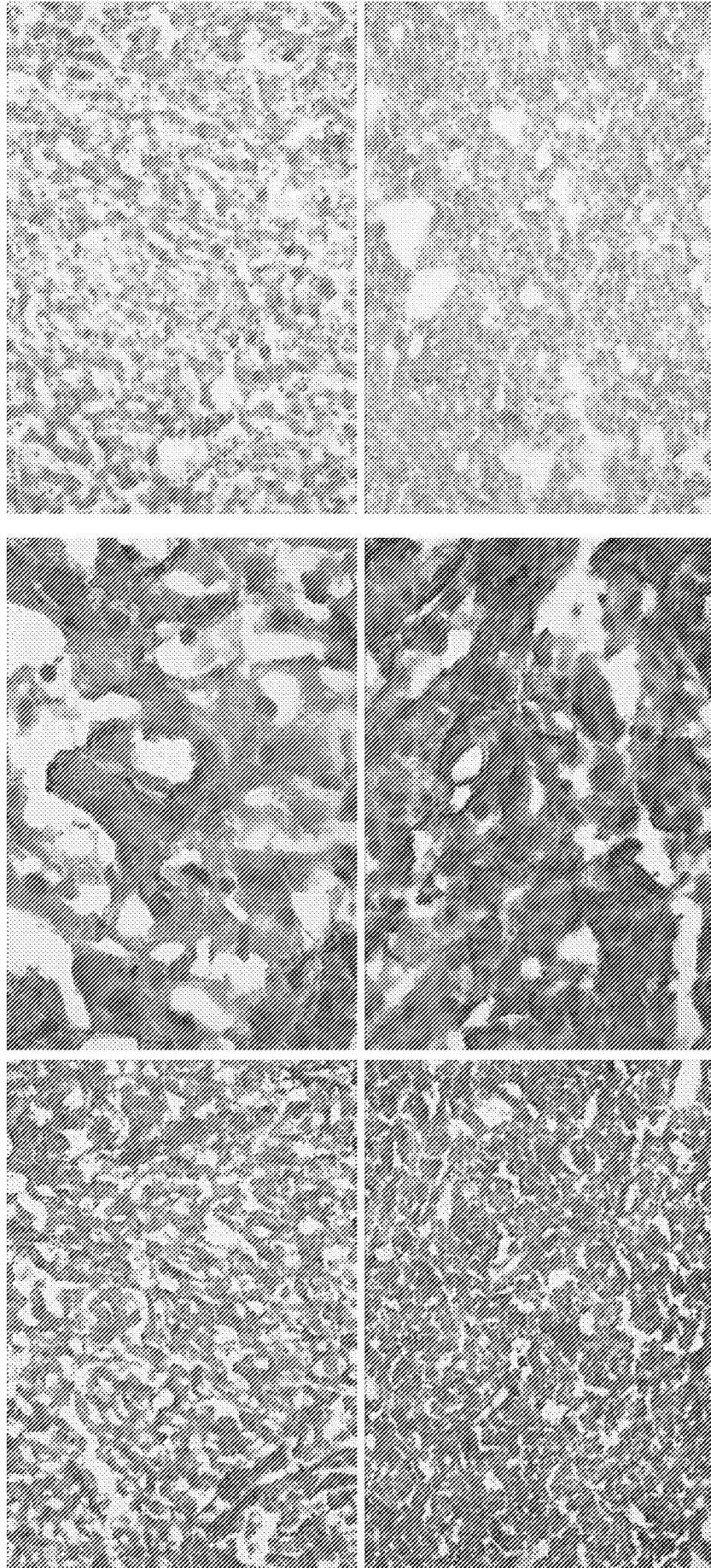
Figure 4B

Figure 4C
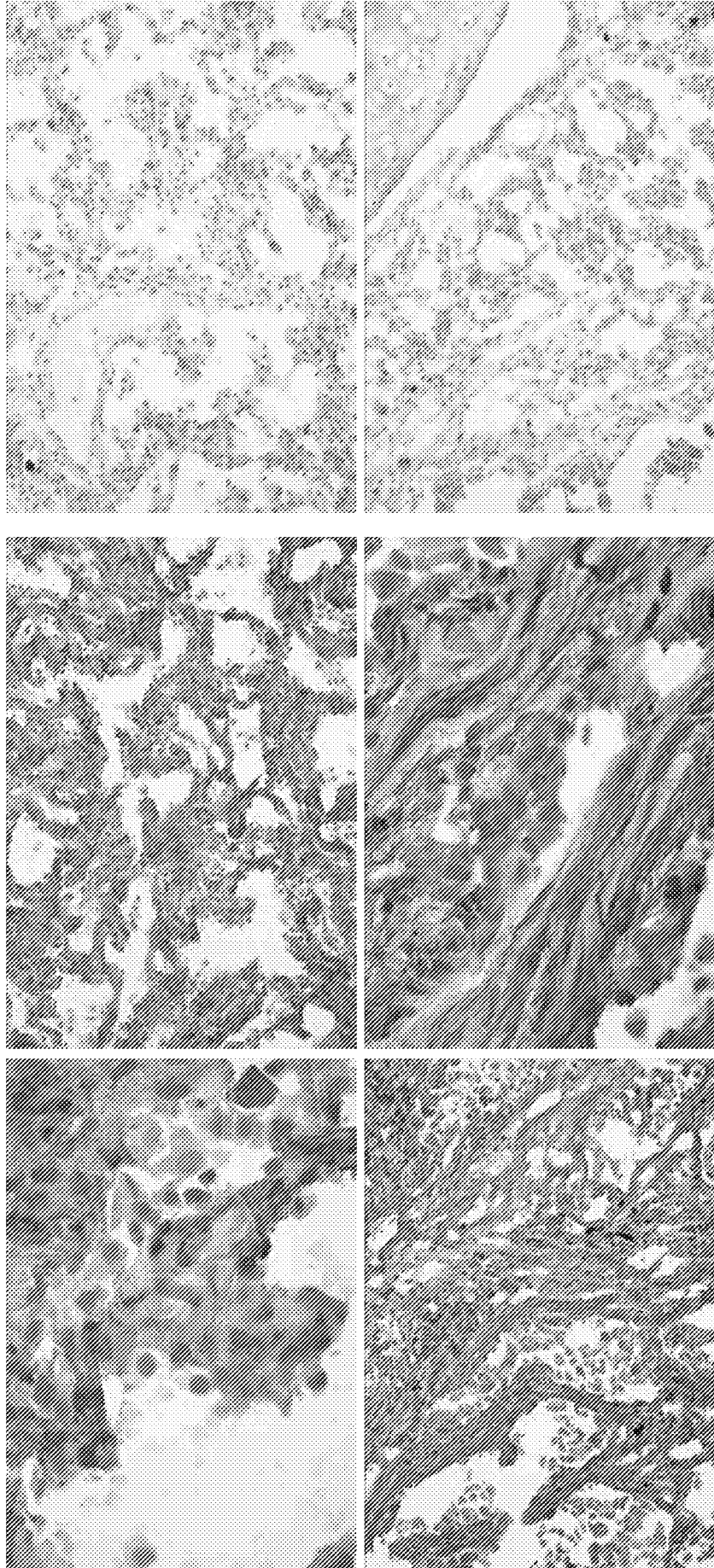
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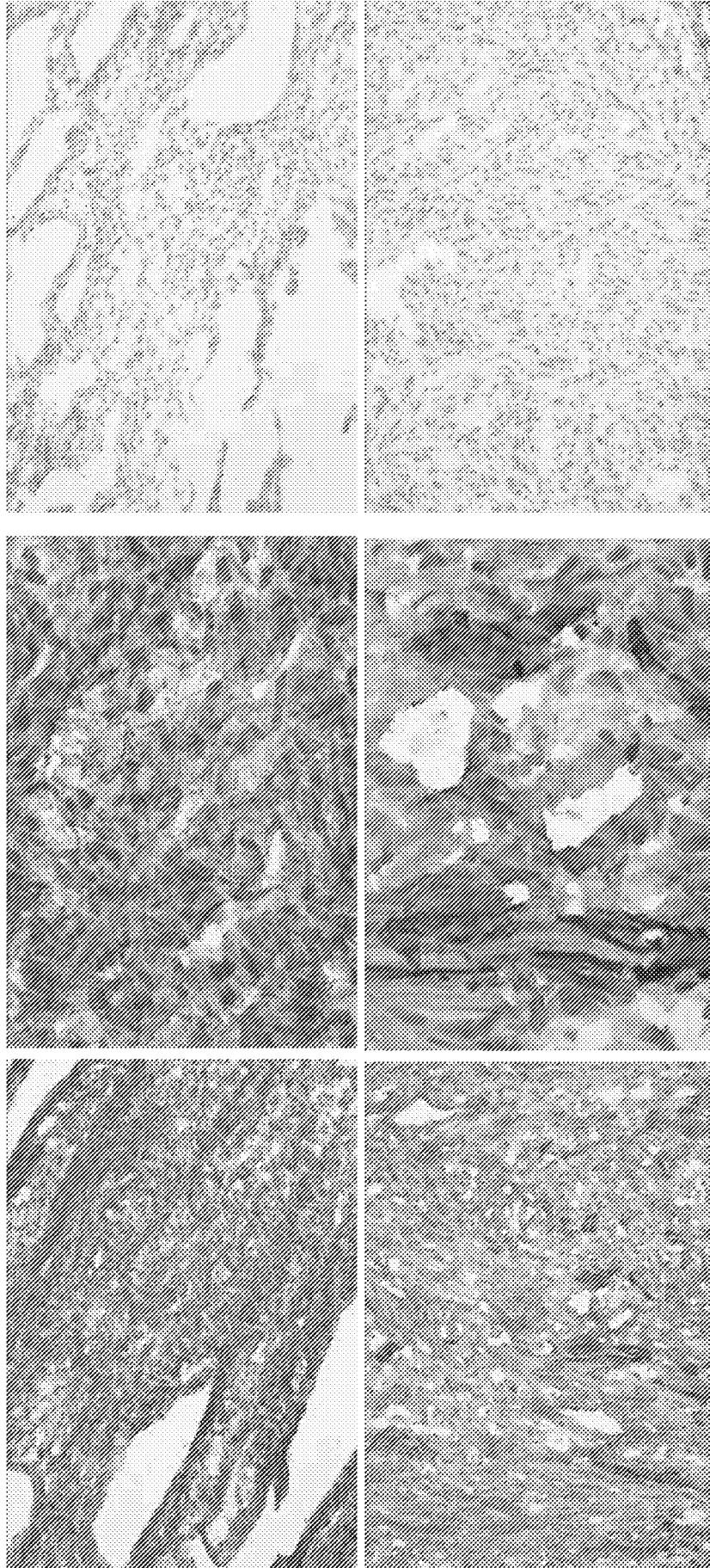
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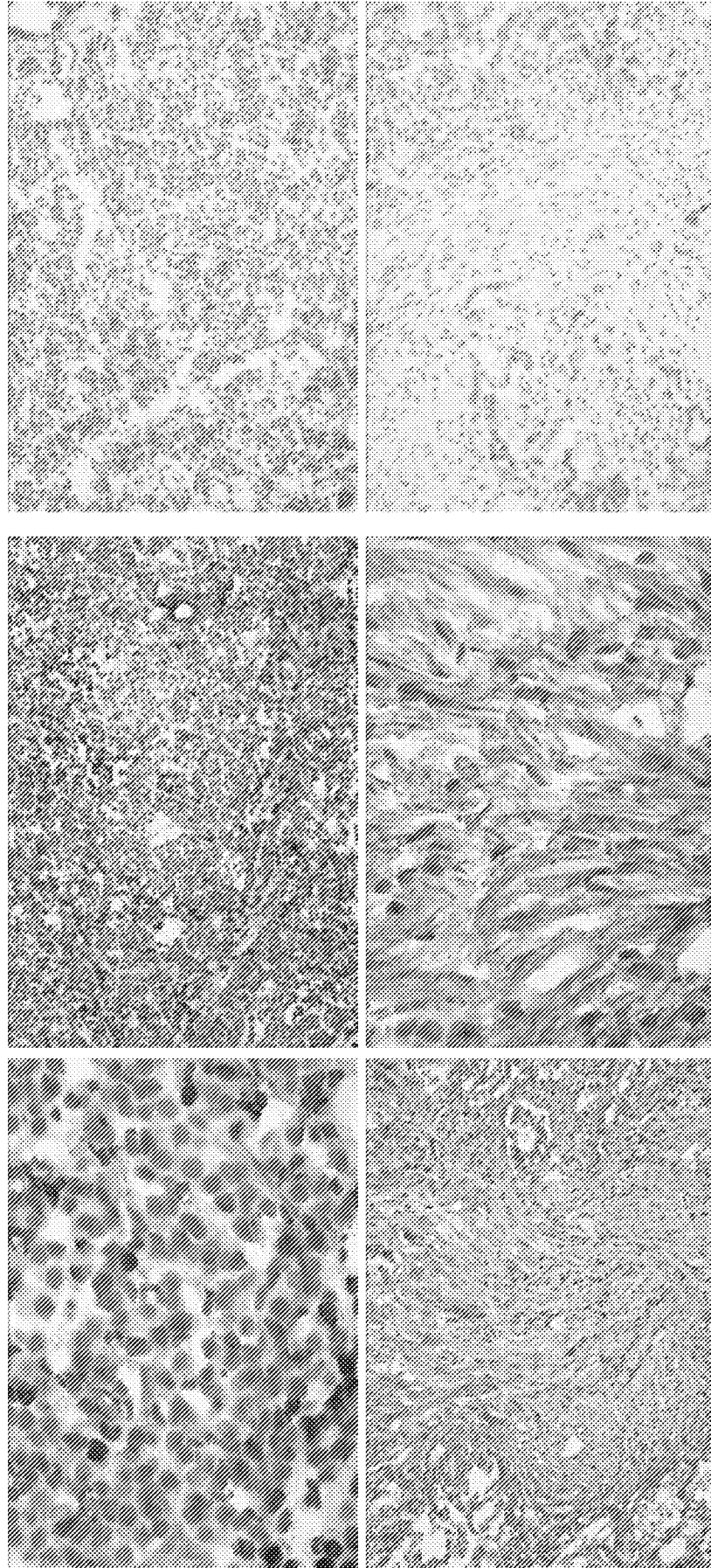
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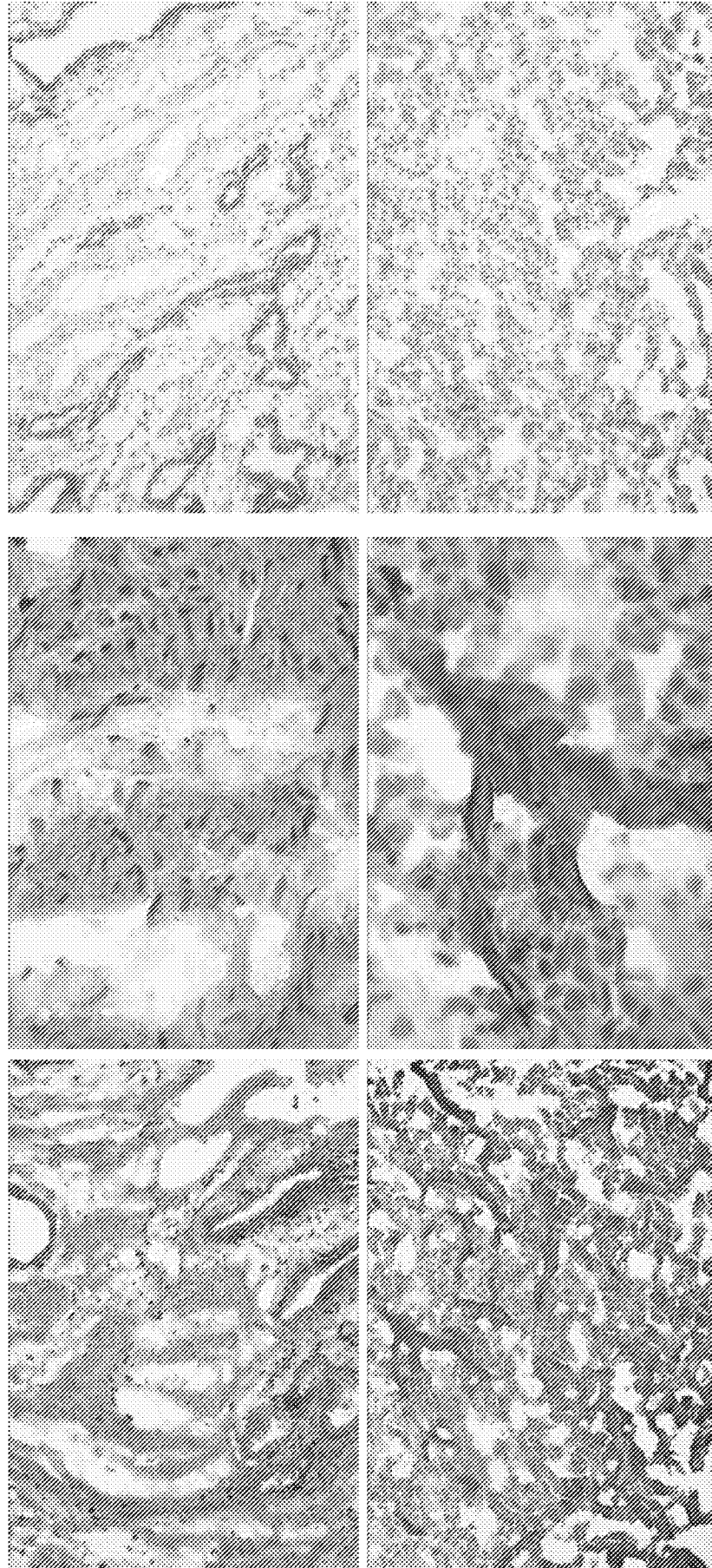
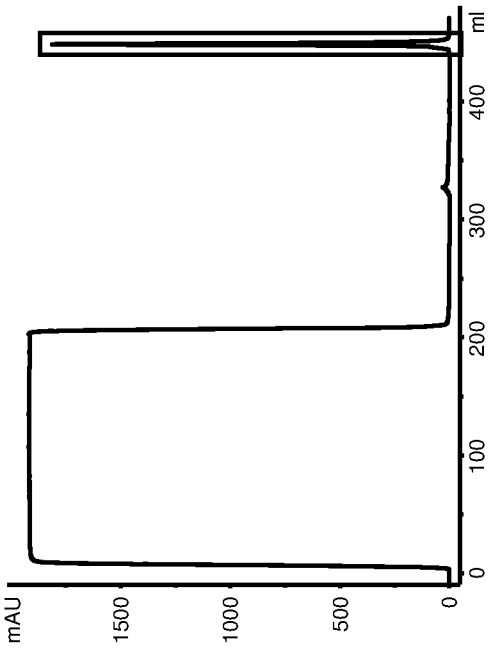Figure 4G

Figure 4H

Figure 4I
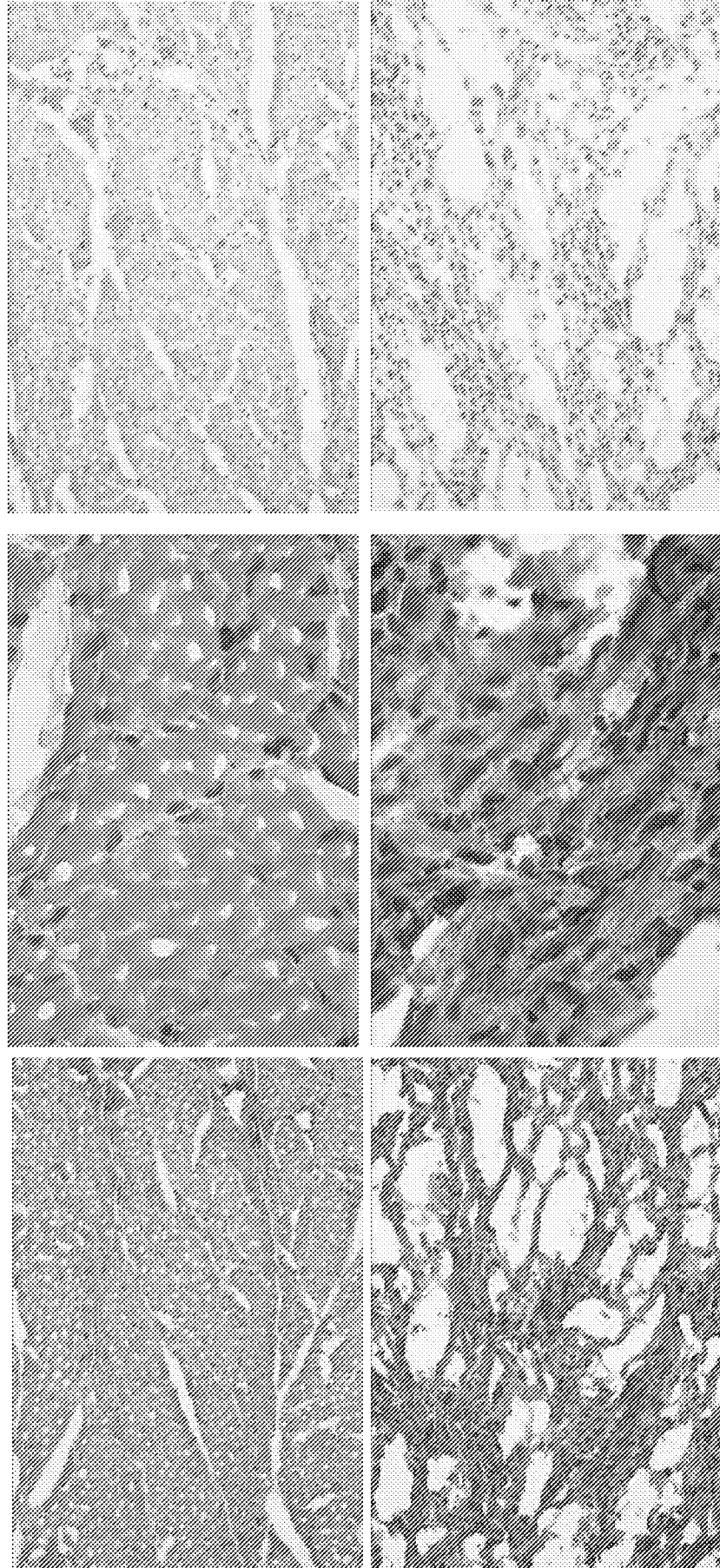
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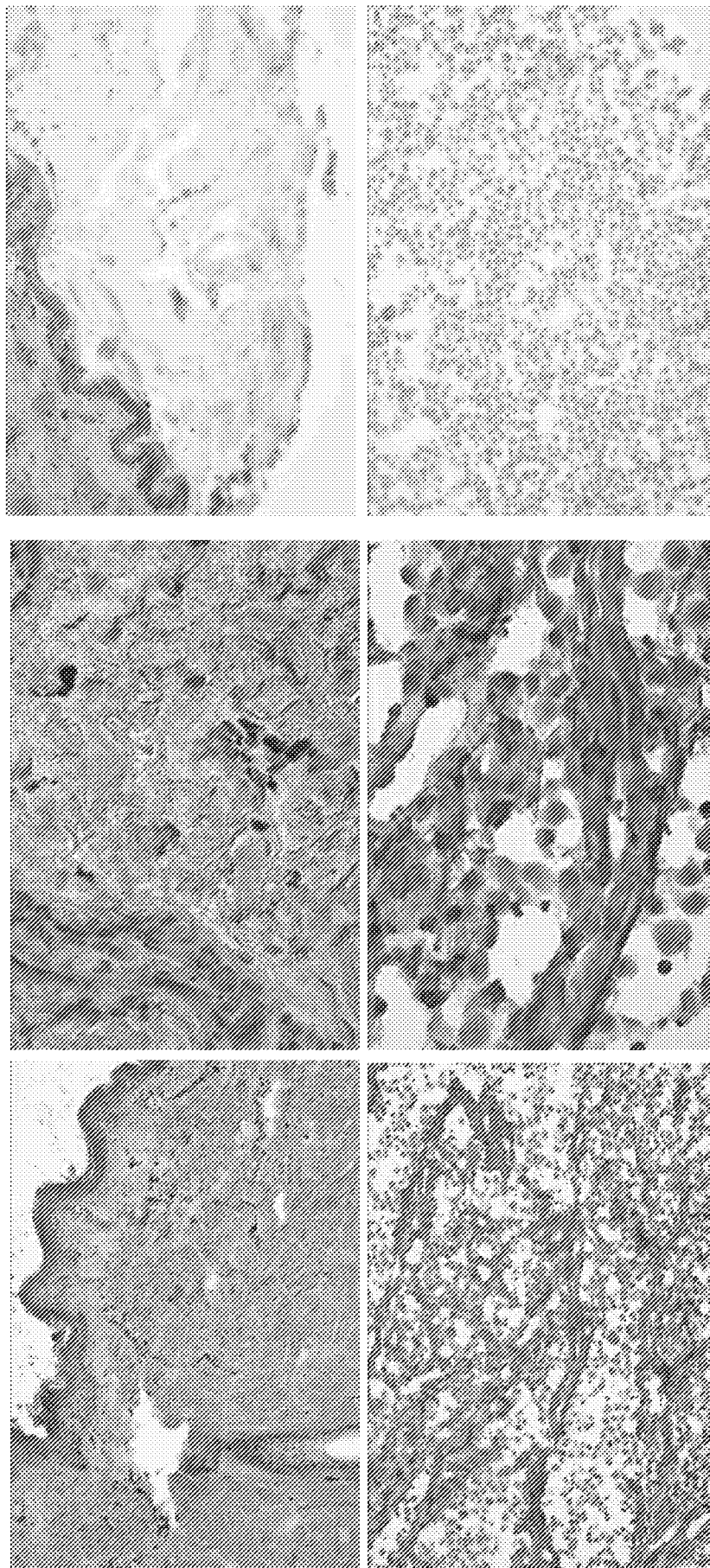
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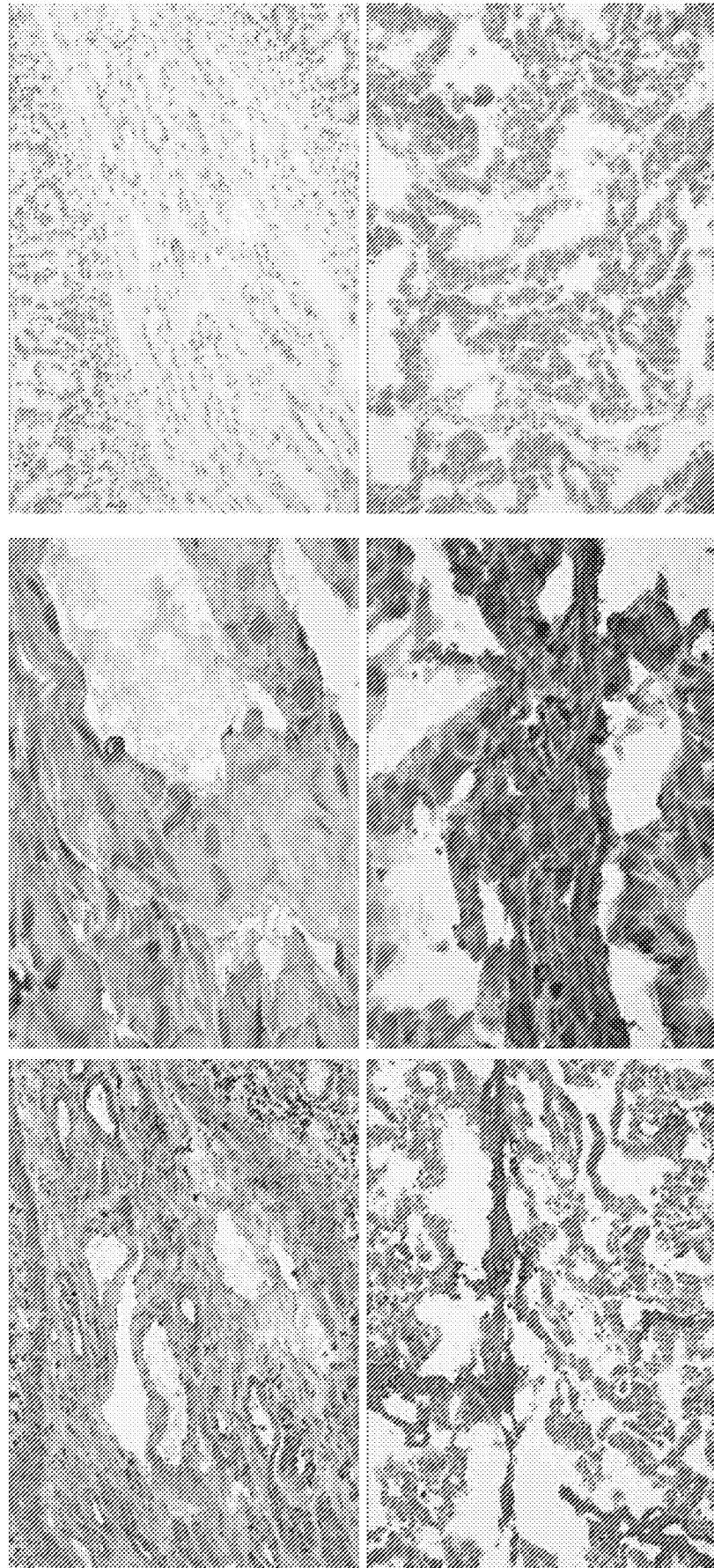
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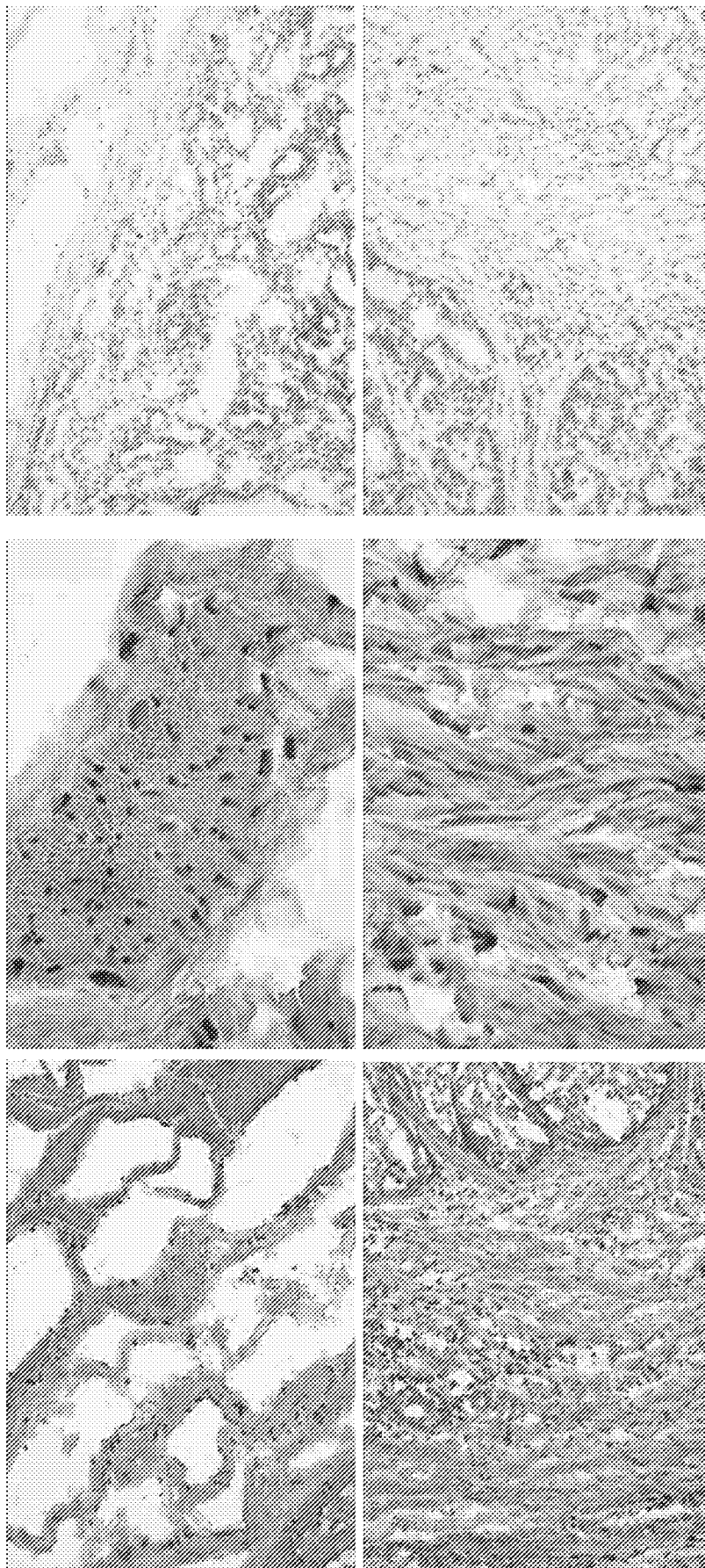
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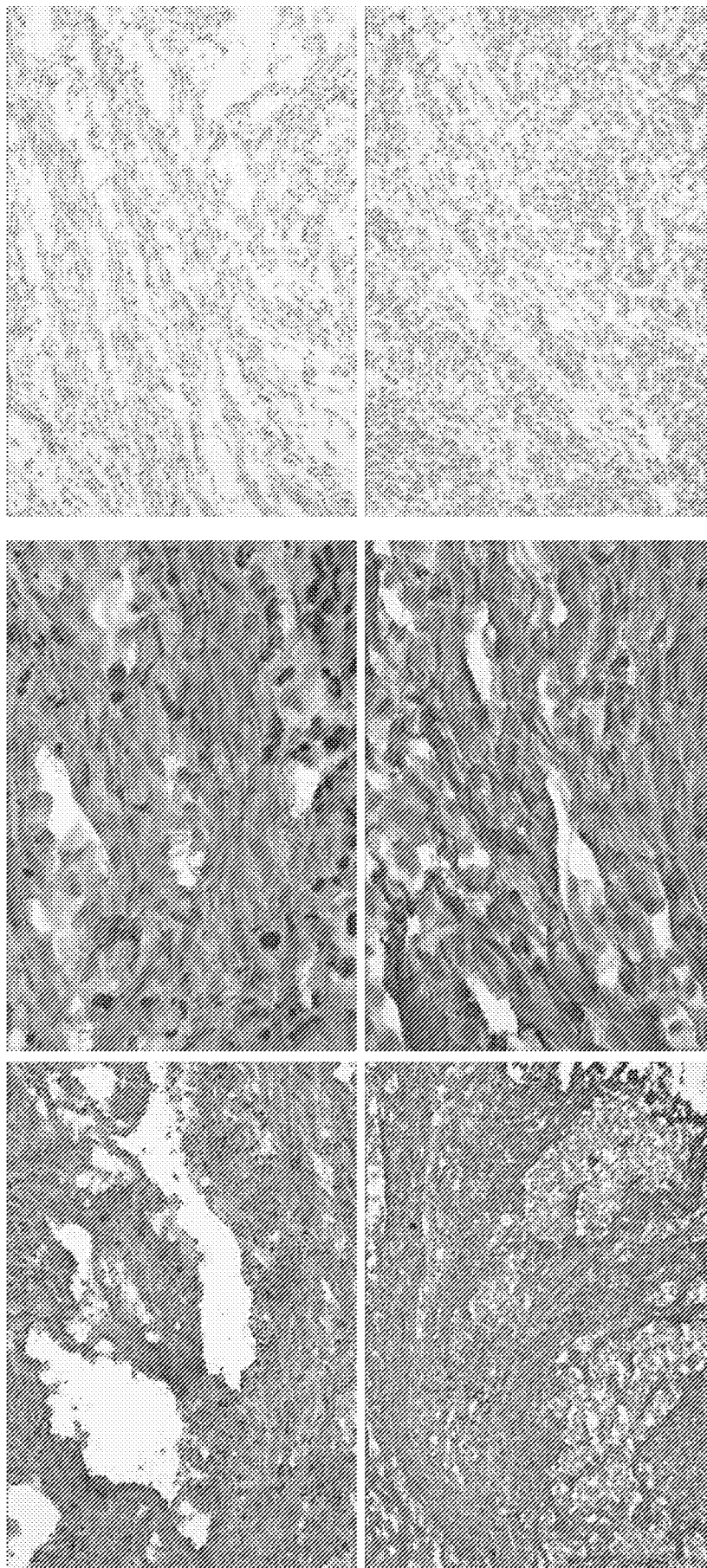
Figure 4N
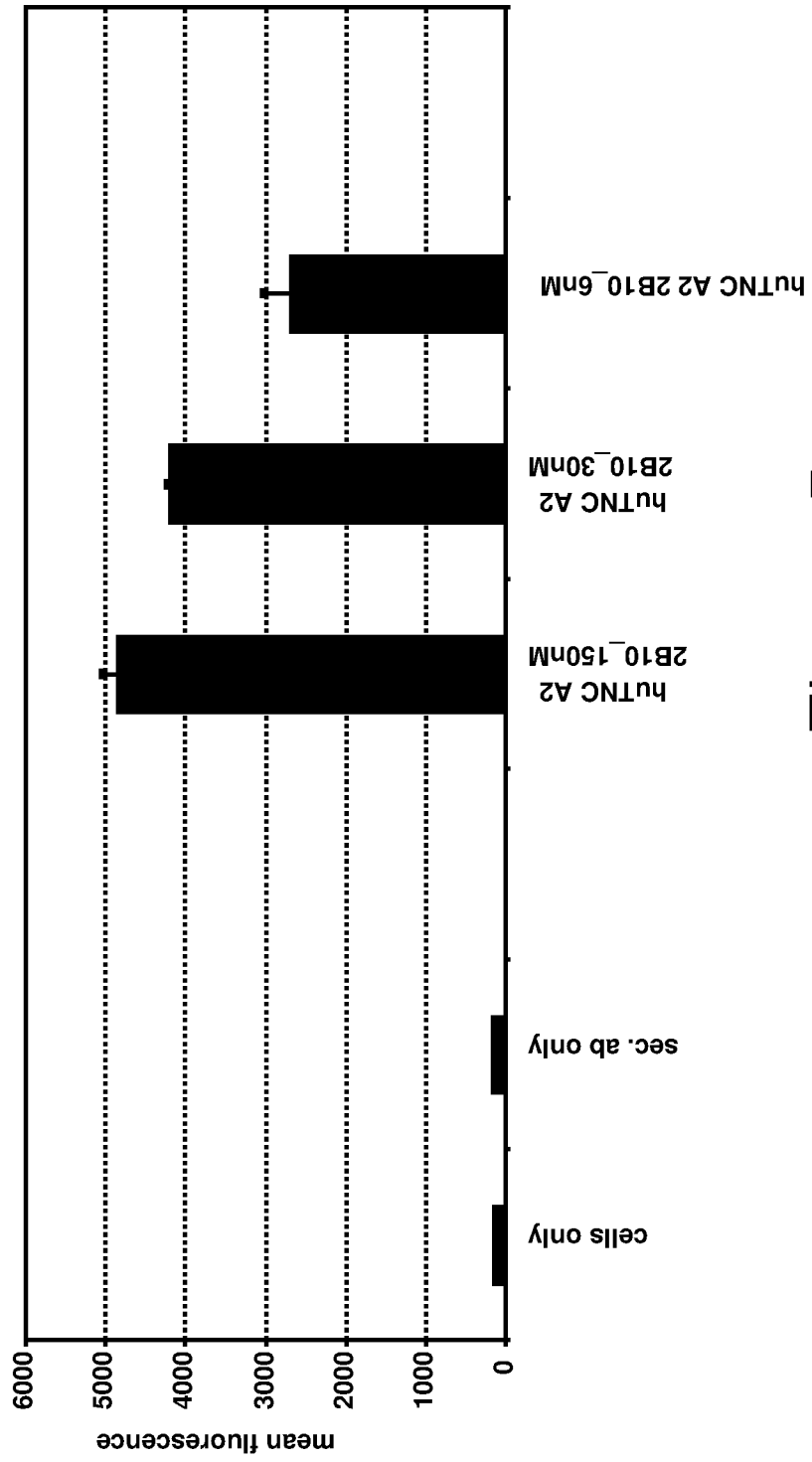
Figure 5
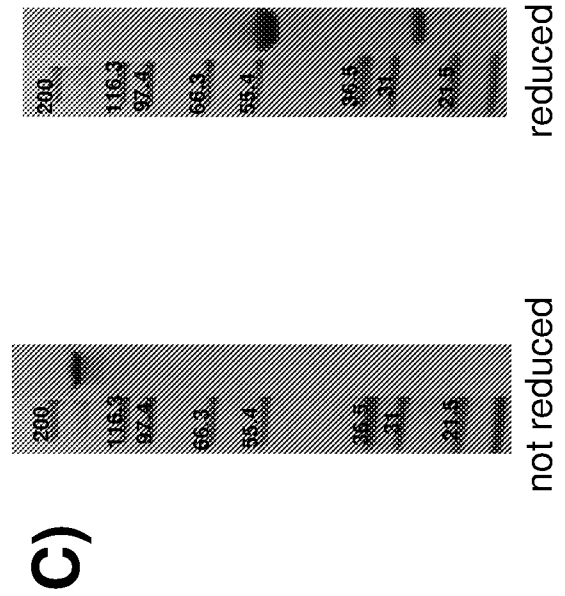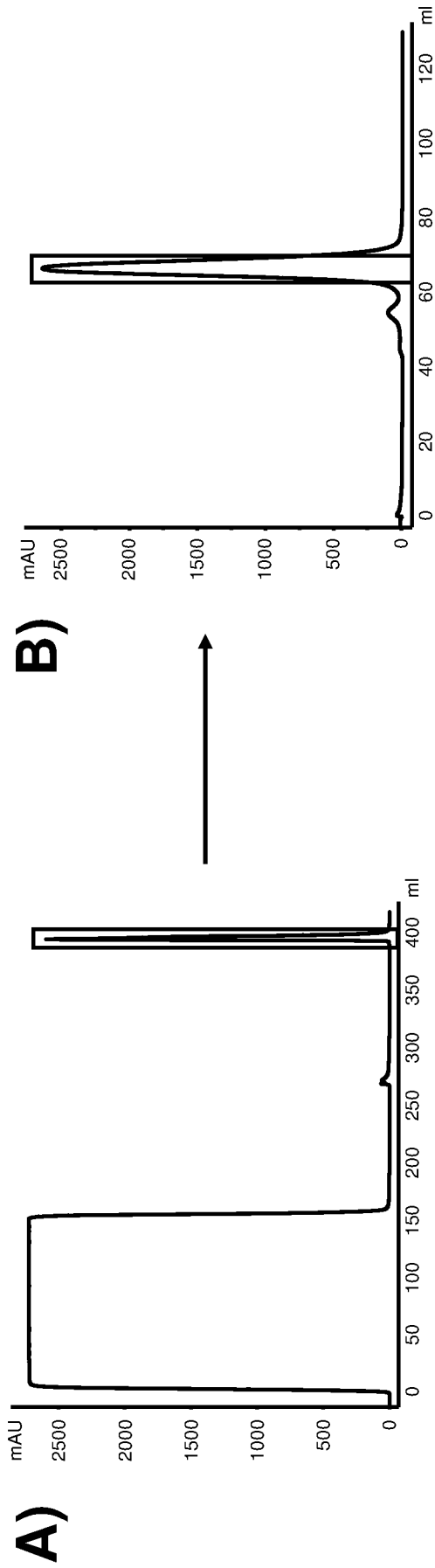
Figure 6
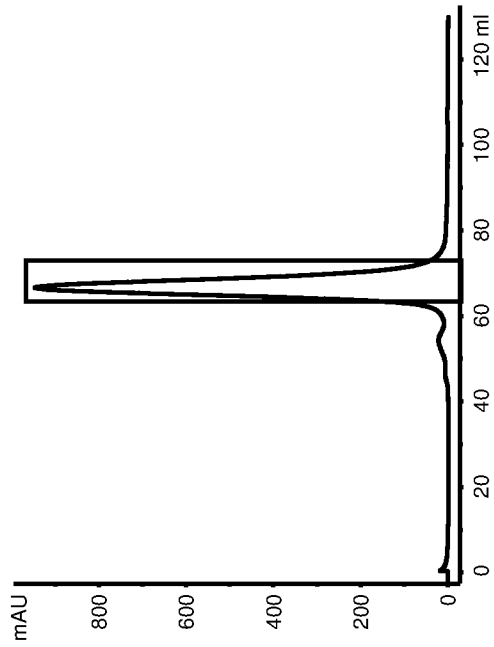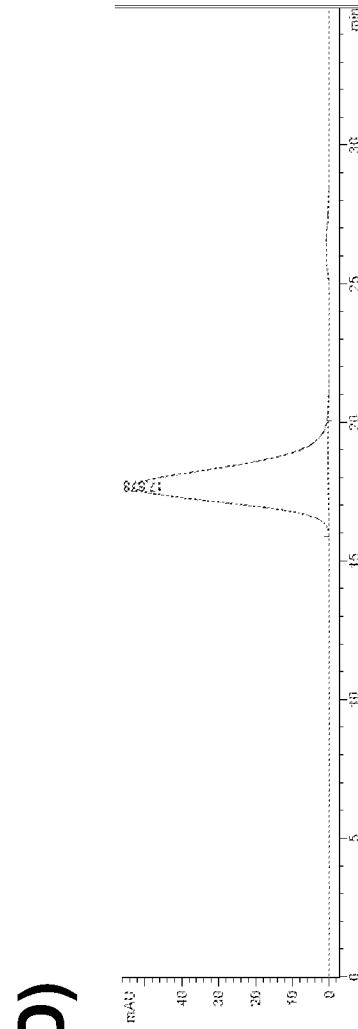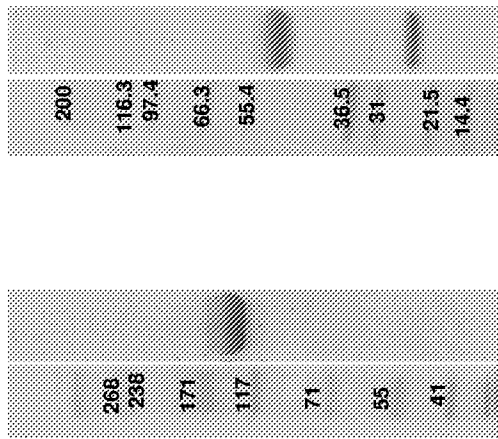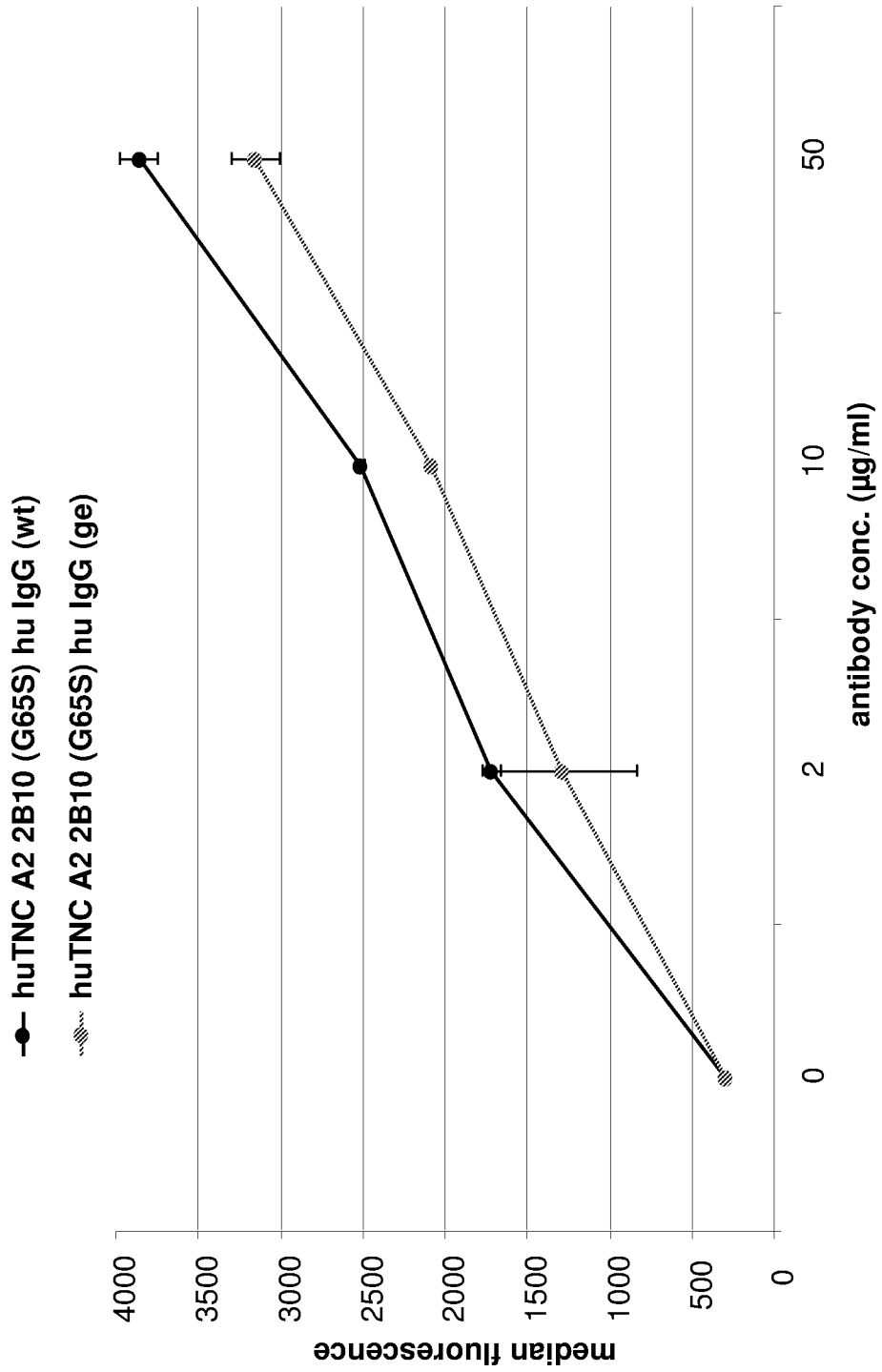
Figure 7

Figure 8

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/30   A61K39/395   G01N33/53
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/089998 A1 (PHILOCHEM AG [CH]; VILLA ALLESSANDRA [CH]; TRACHSEL EVELINE [CH]; KASP) 23 July 2009 (2009-07-23) examples 1-4 -----	1-53
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[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2011	27/10/2011

| Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016 | Authorized officer Le Flao, Katell |

Form PCT/ISA/210 (second sheet) (April 2005)

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	PUENTE NAVAZO M D ET AL: "The alternatively spliced domain TnFnIII A1A2 of the extracellular matrix protein tenascin-C suppresses activation-induced T lymphocyte proliferation and cytokine production.", JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 DEC 2001 LNKD- PUBMED:11714809, vol. 167, no. 11, 1 December 2001 (2001-12-01), pages 6431-6440, XP002661475, ISSN: 0022-1767 the whole document -----	1-53
A	SCHLIEMANN C ET AL: "Three clinical-stage tumor targeting antibodies reveal differential expression of oncofetal fibronectin and tenascin-C isoforms in human lymphoma", LEUKEMIA RESEARCH, NEW YORK,NY, US, vol. 33, no. 12, 1 December 2009 (2009-12-01), pages 1718-1722, XP026611698, ISSN: 0145-2126, DOI: 10.1016/J.LEUKRES.2009.06.025 [retrieved on 2009-07-22] the whole document -----	1-53
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