

INHIBITORS AND USES THEREOF

TECHNICAL FIELD

The present invention relates to inhibitors of IGFBP5 and/or of at least one of its
5 receptors, wherein said inhibitor has anti-fibrotic effect, methods for their production,
pharmaceutical compositions containing said inhibitors, and uses thereof. In particular,
the invention relates to antibodies or antigen binding fragments thereof that bind
specifically to human IGFBP5 and/or to at least one of its receptors.

10 BACKGROUND ART

IGFBP5

The insulin-like growth factor binding proteins constitute a family of six binding proteins
which modulate the bioavailability of insulin-like growth factors (IGFs). Among them,
Insulin-like growth factor-binding protein 5 (IGFBP5) is the most conserved member of
15 the IGFBP family (Schneider, et al. Journal of Endocrinology 172, 423–440 (2002)); it
binds IGFs with high affinity forming a ternary complex with IGF-1 or -2 and the acid
labile subunit (ALS) (Baxter et al. Am J Physiol Endocrinol Metab. 278: E967–E976
(2000)).

In addition to its ability to regulate availability of IGFs, IGFBP5 has also been shown to
20 have IGF-independent functions (Schneider, et al. Journal of Endocrinology 172, 423–
440 (2002)). Indeed, it is able to promote fibrosis directly by inducing the expression of
extracellular matrix (ECM) genes and indirectly by increasing levels of other growth
factors with pro-fibrotic activity, such as connective tissue growth factor (CTGF),
resulting in further increase of ECM production. Besides regulating ECM and growth
25 factor genes, IGFBP5 also increases the expression of Lysyl oxidase (LOX), an enzyme
responsible for cross-linking the matrix, rendering the ECM more resistant to proteolytic
degradation, thus promoting ECM accumulation and fibrosis, and altering tissue
structure and function. Further, IGFBP5 promotes its own expression, generating a
30 positive feedback loop (Nguyen et al. Frontiers in Endocrinology. 9: 601 (2018)). IGFBP5
as an important mediator in fibrosis that is upstream of known pro-fibrotic factors, as
transforming growth factor beta (TGF- β) signaling. All these data strongly suggest that
IGFBP5 exerts its pro-fibrotic activity by directly inducing expression of the majority of
the ECM and pro-fibrotic genes. Finally, while majority of the drugs are targeting only
35 one single pro-fibrotic factor downstream the cascade, by targeting IGFBP5 we will be

able to block the entire pathway with consequent downregulation of several pro-fibrotic factors and ECM genes.

To the best of our knowledge there are no commercially available monoclonal antibodies against IGFBP5 capable of inhibiting IGFBP5 pro-fibrotic activities, and other detrimental effects on target tissues/cells.

IGFBP5 in Idiopathic Pulmonary fibrosis

Idiopathic Pulmonary fibrosis (IPF) is a nonneoplastic chronic lung syndrome that is characterized by aberrant accumulation of fibroblasts/myofibroblasts and progressive abnormal remodeling of lung parenchyma, with subsequent scarring and disruption of its structure and function. Despite considerable recent progress, current treatment regimens are largely unpromising with a median survival rate of less than three years from the date of diagnosis (Sureshababu et al. *Pulmonary Medicine*. 517687 (2011)).

A central event in IPF is the abnormal proliferation and migration of fibroblasts into the alveolar space after lung injury. Although under normal circumstances fibroblasts are important for wound healing and connective tissue production, their function in the fibrotic lung is out of control, leading to formation of fibroblastic foci which consist of highly proliferative fibroblasts, certain immune cells, and excessive ECM protein deposition. The consequences of these processes are disproportionate levels of scar tissue, alterations of the alveolar framework, changes in the lung epithelium structure, stiffening of the functional lung tissue, loss of the gas exchange function of the lung, and dramatically decreased oxygen saturation of the blood (Coward, et al. *Ther. Adv. Respir. Dis.* 4, 367–388 (2010)).

It has been shown that IGFBP5 is increased in the extracellular milieu when primary fibroblasts are cultured from fibrotic lung tissues of patients with IPF, and that IGFBP5 is deposited in ECM produced by these cells.

Moreover, adenoviral mediated expression of IGFBP5, or addition of recombinant IGFBP5, leads to increased ECM production by primary normal adult lung fibroblasts (Pilewski et al. *Am J Pathol* 166(2):399-407 (2005)).

Interestingly, it has been also observed that transgenic mice for murine dermal fibrosis model utilizes overexpression of IGFBP5 to promote a pro-fibrotic environment leading to fibroblast activation, myofibroblast conversion, and increased deposition of ECM (Yasuoka et al., *Am. J. of Pathology* 169(5):1633-42. (2006)). Using this model, it has been shown that IGFBP5 exerts its pro-fibrotic effects in an IGF-independent manner (Yasuoka et al. *Plos One* 9(2) 2014).

IGFBP5 in scleroderma

Systemic sclerosis (SSc) is a rare systemic autoimmune connective tissue disease characterized by vasculopathy, immune dysregulation and progressive fibrosis, affecting primarily the skin, gastrointestinal tract, lungs, heart and kidneys (Henes et al. Haematologica. (2020)). It is associated with high disease related mortality and the main causes of death are related to cardiac, pulmonary and renal involvement. There are still no effective treatments to prevent or halt the progression of fibrosis in SSc and other fibrosing diseases (Tyndall et al. Ann Rheum Dis. 69(10):1809-1815 (2010)).

The excessive fibrosis of the skin and internal organs is due to fibroblast proliferation and excessive production of ECM (Henes et al. Haematologica. (2020)). Fibrotic changes lead to destruction of normal histologic structures in the skin and other organs. IGFBP5 appears to be involved in the early stages of fibrosis and it could be an initiating event in ECM production, suggesting its involvement in the development of fibrosis in SSc. In fact, it has been shown that the expression of IGFBP5 at mRNA and protein levels is increased *in vitro* in primary fibroblasts cultured from disease-affected skin of patients with SSc, compared with the skin from their healthy twins. It has been also demonstrated that IGFBP5 induces collagen and fibronectin (FN1) production from fibroblasts and induces fibroblast/myofibroblast transdifferentiation *in vitro* and *in vivo* (Yasuoka et al., American Journal Of Pathology 169(5):1633-42. (2006)). Moreover, *in vivo* overexpression of IGFBP5, using replication-deficient adenovirus, induced skin fibrosis in mice which included increased thickness of the dermis and increased collagen bundle thickness (Henes et al. Haematologica. (2020)). Increased expression of alpha smooth muscle actin (α -SMA) and vimentin in dermal fibroblasts was also evident with overexpression of IGFBP5.

IGFBP5 is overexpressed in SSc and IPF (Nguyen X-X, Muhammad L, Nietert PJ and Feghali-Bostwick C (2018) Front. Endocrinol. 9:601.doi: 10.3389/fendo.2018.00601 suggesting that strategies to inhibit IGFBP5 function might be effective for the amelioration of fibrosis.

Thus, there is the need for potent and specific IGFBP5 inhibitors, in particular neutralizing antibodies.

SUMMARY OF THE INVENTION

In the present invention it was surprisingly found that disruption, neutralization or blockade of the IGFBP5 axis, including IGFBP5 and any of its receptors has a beneficial effect on fibrosis.

Disclosed herein are inhibitors of IGFBP5 and/or of at least one of its receptors, wherein
5 said inhibitors have anti-fibrotic activity.

The anti-fibrotic activity may be measured by any means known in the art, including as described herein. The isolated antibody or antigen binding fragment thereof is considered to have anti-fibrotic effect when fibrosis is inhibited by at least 10 % when compared to a control condition without antibody or antigen binding fragment thereof.

10 Preferably the inhibitor is considered to have anti-fibrotic effect when fibrosis is inhibited by at least 15, 20, 25, 30, 35, 40, 50, 60, 70, 80 or 90 % when compared to a control condition without antibody or antigen binding fragment thereof.

Preferably the inhibitor of IGFBP5 and/or of at least one of its receptors is for use in the treatment and/or prevention of fibrosis or of a fibrotic condition.

15 Still preferably said inhibitor is selected from the group consisting of:

- a) a polypeptide;
- b) a polynucleotide or a polynucleotide coding for said polypeptide;
- c) a vector comprising or expressing said polynucleotide;
- d) a host cell genetically engineered expressing said polypeptide or said
20 polynucleotide;
- e) a small molecule;
- f) a peptide, a protein, an antibody, an antisense oligonucleotide, a siRNA, antisense expression vector or recombinant virus.

More preferably said inhibitor is an isolated antibody or antigen binding fragment thereof
25 that binds to human IGFBP5 or to at least one of its receptors, wherein said isolated antibody or antigen binding fragment thereof has anti-fibrotic effect.

Preferably the inhibitor inhibits, reduces, or neutralizes the formation of α SMA induced by a pro-fibrotic mediator and /or that inhibits, reduces, or neutralizes the formation of FN1 induced by a pro-fibrotic mediator .

30 Preferably the inhibitor is an isolated antibody or antigen binding fragment thereof that binds to human IGFBP5 and has anti-fibrotic activity.

Preferably the isolated antibody or antigen binding fragment thereof comprises:

- a. a heavy chain variable domain (VH) comprising:
 - i. a CDR1 sequence of the amino acid sequence selected from the group
35 consisting of: SEQ ID NO: 1, 2, 3, 4 and 5;

ii. a CDR2 sequence of the amino acid sequence selected from the group consisting of: SEQ ID NO: 6, 7, 8, 9, 10 and 11; and

iii. a CDR3 sequence of the amino acid sequence selected from the group consisting of: SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26; and/or

b. a light chain variable domain (VL) comprising:

i. a CDR1 sequence of the amino acid sequence selected from the group consisting of: SEQ ID NO: 27, 28, 29, 30, 31, 32, 33, 34, 35 and 36;

ii. a CDR2 sequence of the amino acid sequence selected from the group consisting of: SEQ ID NO: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47; and

iii. a CDR3 sequence of the amino acid sequence selected from the group consisting of: SEQ ID NO: 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60.

Still preferably, the isolated antibody or antigen binding fragment thereof comprises:

a. a heavy chain variable domain (VH) comprising:

i. a CDR1 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 4.1;

ii. a CDR2 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 4.1; and

iii. a CDR3 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 4.1; and/or

b. a light chain variable domain (VL) comprising:

i. a CDR1 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 4.1;

ii. a CDR2 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 4.1; and

iii. a CDR3 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 4.1.

Still preferably, the isolated antibody or antigen binding fragment thereof comprises:

a. a heavy chain variable domain (VH) comprising:

i. a CDR1 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 5.1;

5 ii. a CDR2 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 5.1; and

10 iii. a CDR3 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 5.1; and/or

b. a light chain variable domain (VL) comprising:

i. a CDR1 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 5.1;

15 ii. a CDR2 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 5.1; and

20 iii. a CDR3 sequence of the amino acid sequence selected from the group consisting of a sequence as defined using abysis tool analysis (www.abysis.org) preferably as shown in Table 5.1.

Still preferably, the isolated antibody or antigen binding fragment thereof comprises:

a. a heavy chain variable domain sequence of the amino acid sequence selected from the group consisting of: SEQ ID NO:61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74 and 75 or

25 b. a light chain variable domain sequence of the amino acid sequence selected from the group consisting of: SEQ ID NO: 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, and 90 or

c. the heavy chain variable domain of (a) and the light chain variable domain of (b).

30 Still preferably, the isolated antibody or antigen binding fragment thereof is selected from the group consisting of: A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G01, preferably the isolated antibody or antigen binding fragment thereof is the antibody B06 or B09.

Still preferably, the isolated antibody or antigen binding fragment thereof VH CDR1, CDR2 and CDR3 selected from Table 2 and VL CDR1, CDR2 and CDR3 selected from Table 3.

Yet preferably, the isolated antibody or antigen binding fragment thereof having an
5 affinity constant lower than or equal to 10^{-8} M for human IGFBP5.

It is also disclosed an isolated antibody or antigen binding fragment thereof that:

- (a) binds specifically to an epitope on IGFBP5, said epitope being the same or similar epitope as the epitope recognized by the monoclonal antibody A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G01 as defined in Tables 2-8; or
- 10 (b) cross-competes for binding with the monoclonal antibody A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G01 as defined in Tables 2-8; or
- (c) shows the same or similar binding affinity or specificity, or both, as any of A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G0 as defined in Tables 2-8; or
- 15 (d) has one or more biological properties of an antibody molecule described herein, e.g., an antibody molecule chosen from, e.g., any of A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G01 as defined in Tables 2-8 and; or
- (e) has one or more pharmacokinetic properties of an antibody molecule described herein, e.g., an antibody molecule chosen from, e.g., any of A09, B06, C02, D10, F05,
20 B09, B11, C11, C12, D12, E02, E03, E05, F04, G01 as defined in Tables 2-8.

In a preferred embodiment the isolated antibody or antigen binding fragment as described above:

- (a) binds specifically to an epitope on IGFBP5, said epitope being the same or similar epitope as the epitope recognized by the monoclonal antibody A09, B06, C02, D10, F05,
25 B09, B11, C11, C12, D12, E02, E03, E05, F04, G01 as defined in Tables 2-8; or
- (b) cross-competes for binding with the monoclonal antibody A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G01 as defined in Tables 2-8; or
- (c) shows the same or similar binding affinity or specificity, or both, as any of A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G0 as defined in Tables
30 2-8; or
- (d) has one or more biological properties of an antibody molecule described herein, e.g., an antibody molecule chosen from, e.g., any of A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G01 as defined in Tables 2-8 and; or

(e) has one or more pharmacokinetic properties of an antibody molecule described herein, e.g., an antibody molecule chosen from, e.g., any of A09, B06, C02, D10, F05, B09, B11, C11, C12, D12, E02, E03, E05, F04, G01 as defined in Tables 2-8.

Preferably the isolated antibody or antigen binding fragment thereof as defined above is
5 a human or a humanized antibody, preferably it is an IgG2 or IgG4 antibody, preferably an IgG2 kappa antibody, an IgG2 lambda antibody, an IgG4 kappa antibody or an IgG4 lambda antibody.

Preferably the inhibitor as described above reduces or inhibits the binding of IGFBP5 to at least one of its receptors, preferably the at least one receptor is the $\alpha 2\beta 1$ integrin or
10 $\alpha v\beta 6$ integrin.

Still preferably the inhibitor as defined above i) reduces or inhibits extracellular matrix production and/or ii) reduces or inhibits extracellular matrix deposition and/or iii) reduces or inhibits collagen and/or fibronectin production in primary lung fibroblasts and/or iv) reduces or inhibits collagen and/or fibronectin deposition in primary lung fibroblasts.

15 It is herein disclosed an isolated polynucleotide comprising at least one sequence that encodes the antibody or antigen binding fragment thereof as defined above, preferably said polynucleotide is a cDNA.

It is herein disclosed a vector comprising the polynucleotide as defined above, preferably said vector being selected from the group consisting of a plasmid, a viral vector, a non-episomal mammalian vector, an expression vector and a recombinant expression
20 vector.

It is herein disclosed an isolated cell comprising the polynucleotide or the vector as defined above, preferably said isolated cell being a hybridoma or a Chinese Hamster Ovary (CHO) cell or a Human Embryonic Kidney cells (HEK293).

25 Preferably the inhibitor, the antibody or antigen binding fragment thereof or the polynucleotide or the vector or the cell as defined above is for use as a medicament, preferably for use in the treatment and/or prevention of fibrosis or of a fibrotic condition, preferably scleroderma or pulmonary fibrosis, preferably morphea, fibrosis as a result of Graft-Versus-Host Disease (GVHD), keloid and hypertrophic scar, subepithelial fibrosis, endomyocardial fibrosis, uterine fibrosis, myelofibrosis, retroperitoneal fibrosis,
30 nephrogenic systemic fibrosis, scarring after surgery, asthma, aberrant wound healing, and multifocal fibrosclerosis, cryptogenic fibrosing alveolitis (CFA), and idiopathic pulmonary fibrosis (IPF).

It is herein disclosed a pharmaceutical composition comprising the inhibitor, isolated
35 antibody or antigen binding fragment thereof or the polynucleotide or the vector or the

cell as described above and pharmaceutically acceptable carrier, preferably for use in the treatment of fibrosis or of a fibrotic condition, preferably said composition further comprises a therapeutic agent.

The therapeutic agent may be nintedanib and pirfenidone.

- 5 Antibodies and antigen binding fragment thereof are disclosed herein that have anti-fibrotic activity.

In some embodiments, methods are disclosed for inhibiting fibrosis in vivo or in vitro. In additional embodiments, methods are disclosed for the treatment of fibrosis in a subject.

- 10 In some specific non-limiting examples, the subject has scleroderma or pulmonary fibrosis.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

15 BRIEF DESCRIPTION OF THE DRAWINGS

- Figure 1.** Effect of anti-IGFBP5 mAbs on Fibroblast-to-Myofibroblast Transition (FMT) assay upon TGF- β 1 exposure. Human primary lung fibroblasts from three IPF donors (IPF05, IPF06 and IPF07) were triggered with 1.25 ng/mL of TGF- β 1 to induce FMT. The anti-IGFBP5 mAbs, B06 (A) and B09 (B) were tested in an FMT assay to check their capacity to inhibit α SMA expression. Vehicle 2: cells treated with 3.6 % PBS and 1.25 ng/mL of TGF- β 1.

- Figure 2.** Effect of anti-IGFBP5 mAbs on Epithelial-to-Mesenchymal Transition (EMT) assay upon TGF- β 1 exposure. Human primary bronchial epithelial cells (HBECs) from three IPF donors (IPF05, IPF06 and IPF07) were triggered with 5 ng/mL of TGF- β 1 to induce EMT. The anti-IGFBP5 mAbs, B06 (A) and B09 (B) were tested in an EMT assay to check its capacity to inhibits the FN1 expression. Anti-IGFBP5 mAb B06 showed a concentration-dependent inhibition of TGF- β 1-mediated FN1 expression in tissue of IPF patient donors. Vehicle 2: cells treated with 3.6 % PBS and 5 ng/mL of TGF- β 1.

30 DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise noted, technical terms are used according to conventional usage in the art.

The antibodies of the invention specifically bind human IGFBP5.

- 35 As discussed herein, these antibodies are collectively referred to as “anti-IGFBP5 antibodies”. All of such antibodies are encompassed by the discussion herein. The

respective antibodies can be used alone or in combination in the methods of the invention.

By "antibodies that specifically bind" IGFBP5 is intended that the antibodies will not substantially cross react with another, non-homologous, human polypeptide. By "not substantially cross react" is intended that the antibody or fragment has a binding affinity for a non-homologous protein which is less than 10%, more preferably less than 5%, and even more preferably less than 1%, of the binding affinity for IGFBP5.

In various embodiments, an antibody that "specifically binds" IGFBP5, as used herein, includes antibodies that bind human IGFBP5 with a K_D of less than about 10^{-6} M, less than about 5×10^{-7} M, less than about 3×10^{-7} M, less than about 2×10^{-7} M, less than about 10^{-7} M, less than about 9×10^{-8} M, less than about 8×10^{-8} M, less than about 7×10^{-8} M, less than about 6×10^{-8} M, less than about 5×10^{-8} M, less than about 4×10^{-8} M, less than about 3×10^{-8} M, less than about 2×10^{-8} M, less than about 10^{-8} M, less than about 5×10^{-9} M, less than about 4×10^{-9} M, less than about 3×10^{-9} M, less than about 2×10^{-9} M, less than about 10^{-9} M or about 5×10^{-10} M, as measured in a surface plasmon resonance assay, for example using the BIAcore™ system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ) or kinetic exclusion assays or using Biomolecular Interaction Analysis performed by an Octet platform as described herein.

The term "antibody" herein is used in the broadest sense understood in the art, including all polypeptides described as antibodies in Sumit G, Wei W, Tsutomu and Satoshi O, Antibodies 2013; 2: 452-500, incorporated herein by reference.

For example, the term "antibody", as used herein encompasses monoclonal antibodies, polyclonal antibodies, monospecific and multi-specific antibodies (e.g., bispecific antibodies), and antibody fragments so long as the fragment exhibits the desired antigen-binding activity (antigen-binding fragments).

The terms "antigen-binding fragment" of an antibody or equivalently "antigen-binding portion" of an antibody and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that comprises a portion of an antibody and that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, e.g., from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, e.g., commercial sources, DNA libraries (including, e.g., phage-antibody libraries), or can be synthesized. The DNA

may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

- 5 As with full antibody molecules, antigen-binding fragments may be monospecific or multi-specific (e.g., bispecific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different antigen binding moieties, wherein each antigen binding moiety is capable of specifically binding to a separate antigen or to a different epitope on the same antigen.
- 10 In particular embodiments, an antigen-binding fragment of an antibody comprises at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody include: (i) VH- CH1; (ii) VH-CH2; (iii) VH-CH3; (iv) VH-CH1-CH2; (v) VH-CH1-CH2-CH3; (vi) VH- CH2-CH3; (vii) VH-CL; (viii) VL-CH1; (ix) VL-CH2; (x) VL-CH3; (xi) VL-CH1-CH2; (xii) VL-CH1-CH2-CH3; (xiii) VL-CH2-CH3; and (xiv) VL-CL. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may in various embodiments consist of at least 2 (e.g., 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody may in various embodiments comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric VH or VL domain (e.g., by disulfide bond(s)).

The term "antigen-binding fragment" of an antibody further includes single domain antibodies.

- A single-domain antibody is an antibody fragment consisting of a single monomeric variable antibody domain. In some embodiments, the single-domain antibody is derived from the variable domain of the antibody heavy chain from camelids (also termed nanobodies, or VHH fragments). In some embodiments, the single-domain antibody is an autonomous human heavy chain variable domain (aVH) or VNAR fragments derived from sharks.
- 30

- Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')₂ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv)
- 35

- molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (e.g., monovalent nanobodies, and bivalent nanobodies), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.
- 10 An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a VH domain associated with a VL domain, the VH and VL domains may be situated relative to one another in
- 15 any suitable arrangement. For example, the variable region may be dimeric and contain VH-VH, VH-VL or VL-VL dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric VH or VL domain.
- The term "antibody," as used herein, also includes ADC (antibody drug conjugate) and payload fusion antibodies.
- 20 As used herein, the term "antigen binding molecule" refers in its broadest sense to a molecule that specifically binds an antigenic determinant. Examples of antigen binding molecules are antibodies, including antigen-binding antibody fragments, and scaffold antigen binding proteins.
- The term "antigen binding moiety" refers to the portion of an antigen binding molecule
- 25 that specifically binds to an antigenic determinant. Antigen binding moieties include antibodies and antigen-binding fragments thereof, such as scFv, that are capable of specific binding to an antigen on a target cell. In a particular aspect, the antigen binding moiety is able to direct the entity to which it is attached, such as a cell, to a target site.
- In addition, antigen binding moieties capable of specific binding to a target cell antigen
- 30 include scaffold antigen binding proteins as defined herein below, e.g. binding domains which are based on designed repeat proteins or designed repeat domains such as designed ankyrin repeat proteins (DARPs) (see e.g. WO 2002/020565) or Lipocalins (Anticalin).
- Designed Ankyrin Repeat Proteins (DARPs), which are derived from Ankyrin, which is
- 35 a family of proteins that mediate attachment of integral membrane proteins to the

cytoskeleton. A single ankyrin repeat is a 33-residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different target antigens by randomizing residues in the first alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. 332, 489-503 (2003), PNAS 100(4), 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007) and US20040132028.

In certain embodiments, antibodies and antigen binding molecules provided herein are altered to increase or decrease the extent to which the antigen binding moiety is glycosylated. Glycosylation variants of the molecules may be conveniently obtained by altering the amino acid sequence such that one or more glycosylation sites is created or removed. Where the antigen binding molecule comprises an Fc region, the carbohydrate attached thereto may be altered. In one aspect, variants of antigen binding molecules are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. Such fucosylation variants may have improved ADCC function, see e.g. US Patent Publication Nos. US 2003/0157108 (Presta, L.) or US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Further variants of antigen binding molecules of the invention include those with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region is bisected by GlcNAc. Such variants may have reduced fucosylation and/or improved ADCC function, see for example WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). 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 and are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.).

In certain embodiments, it may be desirable to create cysteine engineered variants of the antibody or antigen binding molecule of the invention, e.g., "thioMAbs," in which one or more residues of the molecule are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the molecule. 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 immunoconjugate. 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 antigen binding molecules may be generated as described, e.g., in U.S. Patent No. 7,521,541.

In certain aspects, the antibody or antigen binding molecules provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody or antigen binding molecule 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, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, 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 is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for 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 aspect, conjugates of an antibody and non-proteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the non-proteinaceous moiety is a carbon nanotube (Kam, N.W. et al., Proc. Natl. Acad. Sci. USA 102 (2005) 11600-11605). 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 non-proteinaceous moiety to a temperature at which cells proximal to the antibody-non-proteinaceous moiety are killed. In another aspect, immunoconjugates of the antigen binding molecules provided herein may be obtained. An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate

cytotoxicity. In certain embodiments, the constant region is an IgG1, IgG2, IgG3, IgG4 constant region.

The instant invention encompasses in various embodiments antibodies having one or more mutations in the hinge, CH2 or CH3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form. In some embodiments, for example, the antibodies described herein comprise a human IgG4 constant region. In particular embodiments, the IgG4 constant region has a single amino acid substitution in the hinge region of the human IgG4 hinge which reduced Fab arm exchange (Angal et al. (1993) *Molecular Immunology* 30:105) to levels typically observed using a human IgG1 hinge.

In certain embodiments, the antibody comprises one or more mutations in the constant region that increase serum half-life, including those described in US Patent Nos. 7,083,784, 8,323,962 and Dall'Aqua et al., *J. Biol. Chem.* 281(33):23514-23524 (2006); Hinton et al., *J. Immunology* 176:346-356 (2006); Yeung et al., *J. Immunology* 182:7663-7671 (2009); and Petkova et al., *Intn'l Immunology*, 18: 1759-1769 (2006), incorporated herein by reference in their entireties.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies featured in the invention may in various embodiments nonetheless include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and, in some embodiments, CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences are derived from the germline of another mammalian species, such as a mouse, which have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences.

Such recombinant human antibodies have variable and constant regions derived from

human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody." In various embodiments, the isolated antibody also includes an antibody in situ within a recombinant cell. In other embodiments, isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. In various embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

The anti-IGFBP5 antibodies described herein and useful for the methods featured herein may in various embodiments include one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases.

The present invention includes in various embodiments antibodies and methods involving the use of antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the

corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations").

5 Numerous antibodies and antigen-binding fragments may be constructed which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the VH and/or VL domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are
10 mutated back to the original germline sequence, e.g., only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (i.e., a germline sequence that is different
15 from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies may contain any combination of two or more germline mutations within the framework and/or CDR regions, e.g., wherein certain individual residues are mutated to the corresponding residue of a certain germline sequence while certain other residues that differ from the original germline sequence are maintained or
20 are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. The
25 use of antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

The present invention also includes anti-IGFBP5 antibodies and methods involving the use of anti-IGFBP5 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative
30 substitutions. For example, the present invention includes the use of anti-IL-6R antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, e.g., 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. The term "bioequivalent" as used herein, refers to a molecule having similar
35 bioavailability (rate and extent of availability) after administration at the same molar dose

and under similar conditions (e.g., same route of administration), such that the effect, with respect to both efficacy and safety, can be expected to be essentially same as the comparator molecule. Two pharmaceutical compositions comprising an anti- IGFBP5 antibody are bioequivalent if they are pharmaceutically equivalent, meaning they contain the same amount of active ingredient (e.g., IGFBP5 antibody), in the same dosage form, for the same route of administration and meeting the same or comparable standards. Bioequivalence can be determined, for example, by an in vivo study comparing a pharmacokinetic parameter for the two compositions. Parameters commonly used in bioequivalence studies include peak plasma concentration (C_{max}) and area under the plasma drug concentration time curve (AUC).

The invention in certain embodiments relates to antibodies and methods comprising administering to the subject an antibody which comprises the heavy chain variable region comprising a sequence chosen from the group of: SEQ ID No. 61 to SEQ ID No. 75 and the light chain variable region comprising a sequence chosen from the group of: SEQ ID No. 76 to SEQ ID No. 90. The disclosure provides pharmaceutical compositions comprising such antibody, and methods of using these compositions.

The antibody is administered to the subject in various embodiments in a formulation comprising suitable carriers, excipients, and other agents to provide improved transfer, delivery, tolerance, and the like, and suitable for an intravenous or subcutaneous injection.

The injectable preparations may be prepared by methods publicly known. For example, injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 20 or 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injectable preparation thus prepared can be filled in an appropriate ampoule.

The antibody according to the invention can be administered to the subject using any acceptable device or mechanism. For example, the administration can be accomplished using a syringe and needle or with a reusable pen and/or autoinjector delivery device.

The methods of the present invention include the use of numerous reusable pen and/or autoinjector delivery devices to administer an antibody (or pharmaceutical formulation comprising the antibody). Examples of such devices include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-Aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen and/or autoinjector delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to, the SOLOSTAR™ pen (Sanofi-Aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), the HUMIRA™ Pen (Abbott Labs, Abbott Park, IL), the DAI® Auto Injector (SHL Group) and any auto-injector featuring the PUSHCLICK™ technology (SHL Group), to name only a few.

In one embodiment, the antibody is administered with a prefilled syringe. In another embodiment, the antibody is administered with a prefilled syringe containing a safety system. For example, the safety system prevents an accidental needlestick injury. In various embodiments, the antibody is administered with a prefilled syringe containing an ÈRIST™ safety system (West Pharmaceutical Services Inc.). See also U.S. patent numbers 5,215,534 and 9,248,242, incorporated herein by reference in their entireties.

In another embodiment, the antibody is administered with an auto-injector. In various embodiments, the antibody is administered with an auto-injector featuring the PUSHCLICK™ technology (SHL Group). In various embodiments, the auto-injector is a device comprising a syringe that allows for administration of a dose of the composition and/or antibody to a subject. See also U.S. patent numbers 9,427,531 and 9,566,395, incorporated herein by reference in their entireties.

According to the invention, "subject" means a human subject or human patient.

"Fibrosis" is the formation or development of excess fibrous connective tissue in an organ or tissue as a reparative or reactive process, as opposed to a formation of fibrous tissue as a normal constituent of an organ or tissue. It is the major histopathologic feature of a variety of clinical conditions. Many organs are susceptible to pathological fibrosis,

including but not limited to skin, lungs, heart, and the gastrointestinal tract. The invention relates to an inhibitor that specifically targets the pathogenesis of fibrosis, having “anti-fibrotic activity or effects”, by targeting IGFBP5, which is a pro-fibrotic mediator.

Fibrosis is defined by accumulation of fibrous connective tissue (components of the extracellular matrix (ECM) such as collagen and fibronectin) in and around inflamed or damaged tissue, which can lead to permanent scarring, organ malfunction and, ultimately, death. Fibrosis is a pathological feature of most chronic inflammatory diseases. Fibrosis affects nearly every tissue in the body. (Schruf et al. Respiratory Research (2019) 20:87).

Following activation by pro-fibrotic stimuli, fibroblasts differentiate into an aggressive phenotype, which secrete excessive amounts of collagen-rich extracellular matrix and thereby constitute the primary pathologic fibroblast phenotype (Geng et al. Respiratory Research (2015) 16:124).

Exemplary fibrotic conditions are scleroderma, idiopathic pulmonary fibrosis, morphea, fibrosis as a result of Graft-Versus-Host Disease (GVHD), keloid and hypertrophic scar, and subepithelial fibrosis, endomyocardial fibrosis, uterine fibrosis, myelofibrosis, retroperitoneal fibrosis, nephrogenic systemic fibrosis, scarring after surgery, asthma, aberrant wound healing, glomerulonephritis, and multifocal fibrosclerosis.

“Idiopathic Pulmonary Fibrosis” is a condition also known as cryptogenic fibrosing alveolitis (CFA) that is a chronic, progressive form of lung disease characterized by fibrosis of the supporting framework (interstitium) of the lungs. By definition, the term is used only when the cause of the pulmonary fibrosis is unknown (“idiopathic”). When lung tissue from patients with IPF is examined under a microscope by a pathologist, it shows a characteristic set of histologic/pathologic features known as usual interstitial pneumonia (UIP). UIP is characterized by progressive scarring of both lung that involves the supporting framework (interstitium) of the lung.

Inhibiting or treating a disease: Inhibiting a disease, such as fibrosis, refers to inhibiting or slowing the full development of a disease. In several examples, inhibiting a disease refers to lessening symptoms of a fibrosis, such as the formation of scar tissue or an increase in range of motion or a decrease in pain. “Treatment” refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition related to the disease, such as the fibrosis.

“Scleroderma” is a chronic autoimmune disease characterized by fibrosis (or hardening), vascular alterations, and autoantibodies. There are two major forms, one is a limited systemic scleroderma form that includes limited cutaneous scleroderma-mainly affects

the hands, arms and face, although pulmonary hypertension is frequent. While, diffuse cutaneous scleroderma (or systemic sclerosis) is rapidly progressing and affects a large area of the skin and one or more internal organs, frequently the kidneys, esophagus, heart and lungs. Systemic scleroderma in both of its forms can be fatal. Other forms of scleroderma include systemic scleroderma, which lacks skin changes with systemic manifestations and two localized form that affect the skin, but not internal organs: morphea and linear scleroderma. The disclosed antibodies can be used to treat any form of scleroderma.

10 Therapeutic Methods and Pharmaceutical Compositions

The antibodies disclosed herein can be used to treat fibrosis occurring in several disease conditions. The antibodies disclosed herein may decrease fibrosis that has already occurred, resolving existing fibrosis and/or may decrease the rate or amount of additional fibrosis. In several examples, the antibodies are of use to decrease fibrosis in pathogenic processes, such as in a subject

Thus, in several embodiments, the methods include administering to a subject a therapeutically effective amount of one or more of the antibodies disclosed herein, in order to decrease fibrosis. Any of the antibodies disclosed herein can be used to decrease fibrosis. In some embodiments, the antibodies can be administered as a unit dose.

Suitable subjects include those with a fibrosis of the skin or lungs, but fibrosis of any tissue can be treated using the methods disclosed herein. In one example, the subject has scleroderma. In other examples, the subject has idiopathic pulmonary fibrosis, morphea, fibrosis as a result of Graft-Versus-Host Disease (GVHD), a keloid or hypertrophic scar, subepithelial fibrosis, endomyocardial fibrosis, uterine fibrosis, myelofibrosis, retroperitoneal fibrosis, nephrogenic systemic fibrosis, scarring after surgery, asthma, aberrant wound healing, glomerulonephritis, and multifocal fibrosclerosis, .

In further examples, the methods are used to treat the systemic form of scleroderma, such as limited cutaneous scleroderma or diffuse cutaneous scleroderma (or systemic sclerosis). The methods can be used to treat the localized form of scleroderma, including morphea and linear scleroderma.

The methods can include selecting a subject in need of treatment, such as a subject with a fibrotic disease, such as scleroderma, idiopathic pulmonary fibrosis, morphea, a keloid scar, a hypertrophic scar, or subepithelial fibrosis. In exemplary applications,

compositions are administered to a subject having a fibrotic disease, such as scleroderma, idiopathic pulmonary fibrosis, morphea, a keloid scar, a hypertrophic scar, or subepithelial fibrosis, or any of the disorders listed above, in an amount sufficient to reduce the fibrosis. Amounts effective for this use will depend upon the severity of the disease, the general state of the patient's health, and the robustness of the patient's immune system. In one example, a therapeutically effective amount of the compound is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer.

A method is provided herein for decreasing skin thickness. The method includes administering a therapeutically effective amount of an antibody, thereby decreasing skin thickness. In another embodiment, a method is provided for decreasing lung fibrosis. The method includes administering a therapeutically effective amount of an antibody, thereby decreasing skin thickness. Any of the antibody disclosed herein can be used in these methods.

Methods are provided herein for decreasing α SMA or FN1 expression, such as transforming growth factor (TGF)- β induced α SMA or FN1 expression. The method includes contacting a cell with an effective amount of an antibody, thereby decreasing α SMA or FN1 expression. The methods can be practiced *in vivo* or *in vitro*. In some embodiments, the methods include comparing the amount of α SMA or FN1 expression produced by a cell contacted with an antibody to a control. The control can be a standard value, or the amount of α SMA or FN1 produced by a cell not contacted with the antibody, such as a cell contacted with a carrier.

An antibody herein disclosed can be administered by any means known to one of skill in the art either locally or systemically, such as by intradermal, intrathecal, intramuscular, subcutaneous, intraperitoneal or intravenous injection, but even oral, nasal, transdermal or anal administration is contemplated. In one embodiment, administration is by subcutaneous, intradermal, or intramuscular injection. In another embodiment, administration is by intraperitoneal or intrathecal administration. To extend the time during which the antibody is available to stimulate a response, the antibody can be provided as an implant, an oily injection, or as a particulate system. The particulate system can be a microparticle, a microcapsule, a microsphere, a nanocapsule, or similar particle. (see, e.g., Banga, *supra*).

For treatment of the skin, a therapeutically effective amount of at least antibody can be locally administered to the affected area of the skin, such as in the form of an ointment (RGA Jones and A Marino, "Targeted localized use of therapeutic antibodies: a review

of non-systemic, topical and oral applications, *Crit. Rev. Biotechnol.* 36(3):506-520 (2016).

In one embodiment, the ointment is an entirely homogenous semi-solid external agent with a firmness appropriate for easy application to the skin. Such an ointment can include
5 fats, fatty oils, lanoline, Vaseline, paraffin, wax, hard ointments, resins, plastics, glycols, higher alcohols, glycerol, water or emulsifier and a suspending agent. Using these ingredients as a base, a decoy compound can be evenly mixed. Depending on the base, the mixture can be in the form of an oleaginous ointment, an emulsified ointment, or a water-soluble ointment. Oleaginous ointments use bases such as plant and animal oils
10 and fats, wax, Vaseline and liquid paraffin. Emulsified ointments are comprised of an oleaginous substance and water, emulsified with an emulsifier. They can take either an oil-in-water form (O/W) or a water-in-oil-form (W/O). The oil-in-water form (O/W) can be a hydrophilic ointment. The water-in-oil form (W/O) initially lacks an aqueous phase and can include hydrophilic Vaseline and purified lanoline, or it can contain a water-
15 absorption ointment (including an aqueous phase) and hydrated lanoline. A water-soluble ointment can contain a completely water-soluble Macrogol base as its main ingredient.

Pharmaceutically acceptable carriers include a petroleum jelly, such as VASELINE®, wherein the petroleum jelly contains 5% stearyl alcohol, or petroleum jelly alone, or
20 petroleum jelly containing liquid paraffin. Such carriers enable pharmaceutical compositions to be prescribed in forms appropriate for consumption, such as tablets, pills, sugar-coated agents, capsules, liquid preparations, gels, ointments, syrups, slurries, and suspensions. When locally administered into cells in an affected area or a tissue of interest, the at least one C-terminal endostatin polypeptide, or polynucleotide
25 encoding the peptide can be administered in a composition that contains a synthetic or natural hydrophilic polymer as the carrier. Examples of such polymers include hydroxypropyl cellulose and polyethylene glycol. One or more antibody can be mixed with a hydrophilic polymer in an appropriate solvent. The solvent is then removed by methods such as air-drying, and the remainder is then shaped into a desired form (for
30 example, a sheet) and applied to the target site. Formulations containing such hydrophilic polymers keep well as they have a low water-content. At the time of use, they absorb water, becoming gels that also store well. In the case of sheets, the firmness can be adjusted by mixing a polyhydric alcohol with a hydrophilic polymer similar to those above, such as cellulose, starch and its derivatives, or synthetic polymeric compounds.

Hydrophilic sheets thus formed can be used. A therapeutically effective amount of one or more antibodies can also be incorporated into bandages and dressings.

For administration by inhalation, the antibody can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of
5 a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such
10 as lactose or starch.

In some embodiments, the antibody, can be administered by inhalation. For example, it can be administered in an aerosolized form, such as using a nebulizer or a metered dose inhaler. Technologies of use include micropump nebulizers (such as the AEROGENTM GO system), jet nebulizers designed to produce large fine particle fractions
15 (such as the PARI LC STAR), jet nebulizers developing less shear during atomization (such as the HUDSON MICROMIST), and ultrasonic nebulizers (such as the DeVilbiss ULTRA-NEB).

The antibody can be dissolved in a carrier, such as saline, and atomized using the devices above. The associated aerosols can be collected using a NEXT GENERATION
20 IMPACTOR (NGI) (MSP Corp., Shoreview, Minn.), which uses a series of aerodynamic stages to separate and collect the aerosol into separate fractions based on droplet size. Since droplet size is the primary determinant of deposition location in the lungs, this device allows us to specifically isolate the portion of the liquid aerosol that will deposit in the small airways and alveoli.

Aerosol particle size is often expressed in terms of mass median aerodynamic diameter (MMAD), a parameter that is based on particle size, shape, and density. For a spherical particle, MMAD is equal to $MMD (p < 1/2)^{1/3}$, in which MMD is mass median diameter and ρ is the bulk density. For a non-spherical particle, MMAD is equal to $MMD (p/x)^{1/3}$, in which X is the shape factor. Thus, particles with larger than unit density will have actual
25 diameters smaller than their MMAD.

The site of particle deposition within the respiratory tract is demarcated based on particle size. In one example, particles of about 1 to about 500 microns are utilized, such as particles of about 25 to about 250 microns, or about 10 to about 25 microns are utilized. In other embodiments, particles of about 1 to 50 microns are utilized. For use in a
30 metered dose inhaler, for administration to lungs particles of less than about 10 microns,

such as particles of about 2 to about 8 microns, such as about 1 to about 5 microns, such as particles of 2 to 3 microns, can be utilized.

A therapeutically effect amount of an antibody can be administered in the pharmaceutically acceptable carrier. Pharmacologically acceptable carriers (e.g.,
5 physiologically or pharmaceutically acceptable carriers) are well known in the art, and include, but are not limited to buffered solutions as a physiological pH (e.g. from a pH of about 7.0 to about 8.0, or at a pH of about 7.4). One specific, non-limiting example of a physiologically compatible buffered solution is phosphate buffered saline. Other pharmacologically acceptable carriers include penetrants, which are particularly suitable
10 for pharmaceutical formulations that are intended to be topically applied (for example in the application of surgical wounds to promote healing).

The pharmacological compositions disclosed herein facilitate the use of at least one antibody, either in vivo or ex vivo, to decrease fibrosis. Such a composition can be suitable for delivery of the active ingredient to any suitable subject, and can be
15 manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmacological compositions can be formulated in a conventional manner using one or more pharmacologically (e.g., physiologically or pharmaceutically) acceptable carriers, as well as optional auxiliaries that facilitate processing of the active compounds
20 into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Thus, for injection, the active ingredient can be formulated in aqueous solutions. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the active ingredient can be combined with carriers suitable for
25 incorporation into tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like. The active ingredient can be formulated for parenteral administration by injection, such as by bolus injection or continuous infusion. Such compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can
30 contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Other pharmacological excipients are known in the art.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the compositions of the invention described above, increasing convenience to the subject and the
35 physician. Many types of release delivery systems are available and known to those of

ordinary skill in the art. They include polymer-based systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems, such as lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the at least one C-terminal endostatin polypeptide, or polynucleotide encoding the peptide is contained in a form within a matrix such as those described in U.S. Pat. Nos. 4,452,775; 4,667,014; 4,748,034; 5,239,660; and 6,218,371 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. Nos. 3,832,253 and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Use of a long-term sustained release implant may be particularly suitable for treatment of chronic conditions, such as scleroderma. Long-term release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well known to those of ordinary skill in the art and include some of the release systems described above. These systems have been described for use with oligodeoxynucleotides (see U.S. Pat. No. 6,218,371). For use in vivo, nucleic acids and peptides are preferably relatively resistant to degradation (such as via endo- and exo-nucleases). Thus, modifications, such as the inclusion of a C-terminal amide, can be used.

The therapeutically effective amount of the antibody will be dependent on the antibody that is utilized, the subject being treated, the severity and type of the affliction, and the manner of administration. For example, a therapeutically effective amount of an antibody can vary from about 0.01 µg per kilogram (kg) body weight to about 1 g per kg body weight, such as about 1 µg to about 5 mg per kg body weight, or about 5 µg to about 1 mg per kg body weight. The exact dose is readily determined by one of skill in the art based on the potency of the specific compound the age, weight, sex and physiological condition of the subject.

Single or multiple administrations of the compositions are administered depending on the dosage and frequency as required and tolerated by the subject. In one embodiment, the dosage is administered once as a bolus, but in another embodiment can be applied periodically until a therapeutic result is achieved. Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the subject. Systemic or local administration can be utilized.

In a further method, an additional agent is administered. In one example, this administration is sequential. In other examples, the additional agent is administered simultaneously with the antibody.

For the treatment of scleroderma, examples of additional agents that can be used with the antibody include nifedipine, amlodipine, diltiazem, felodipine, or nicardipine. An investigational drug Gleevec, is also used for the treatment of scleroderma. Gleevec or other tyrosine kinase inhibitors can be used with the antibodies disclosed herein. Patients with lung involvement of scleroderma benefit from oxygen therapy; the C-terminal endostatin polypeptides disclosed herein can be administered with this therapy. For the treatment of fibrosis of the skin and scleroderma, additional agents of use are d-penicillamine, colchicine, Relaxin, steroids, and cyclosporine. In some embodiments, the additional agent is SAR100842 (see Allanore et al., *Arthr. Rheum.* 70(10):1634-1643 (2018)). C-terminal endostatin polypeptides also can be used in combination with immunosuppressive agents. Additionally, the antibodies can be used with methotrexate, cyclophosphamide, azathioprine, mycophenolate, glitazones, endothelin receptor antagonists, or Fulvestrant (ICI-182,780).

The disclosure is illustrated by the following non-limiting Examples.

EXAMPLES

Example 1: Monoclonal antibodies development

Monoclonal anti-IGFBP5 antibodies were discovered from naïve human phage-display libraries. First, unspecific or cross-reactive antibody-phage was cleared from the library. For that purpose, the library was incubated in presence of: a) Streptavidin-Beads and BSA, or b) Streptavidin-Beads and BSA, IGFBP1 (R&D, cat n° 871-B1), IGFBP2 (R&D, cat n° 674-B2), IGFBP3 (R&D, cat n° 675-B3), IGFBP4 (R&D, cat n° 804-GB) and IGFBP6 (R&D, cat n° 876-B6).

Antibody-phage that bound to the negative antigens were removed from further selection. After that, the cleared library was selected for target antigen-specific antibodies. First, biotinylated IGFBP5 (R&D, human: cat n° 875-B5, mouse: cat n° 578-

B5) was added in presence of the negative antigens to the library preparations a) and b). Antibody-phage that bound to the biotinylated target antigen were captured and recovered from the solution using magnetic streptavidin beads. The beads were washed multiple times with a BSA solution (containing 0.05% Tween20) and BSA in order to remove unspecific or weakly bound antibody-phage particles. Antigen-specific antibody phage were eluted from the beads by Trypsin treatment and rescued by E. coli infection. After short propagation, the bacteria were coinfecting with M13K07 helper phage and antibody-phage amplification was induced. The amplified phage were used for two more selection cycles as described above. An overview of the antibody selection strategies is given in Table 1:

Table 1: Overview of the antibody selection strategy			
Negative selection in selection cycle 1-3	Positive selection in selection cycle 1	Positive selection in selection cycle 2	Positive selection in selection cycle 3
a) Streptavidin-Beads, BSA	Human IGFBP5	Human IGFBP5	Human IGFBP5 or Mouse IGFBP5
b) Streptavidin-Beads, BSA, IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP6	Human IGFBP5	Human IGFBP5	Human IGFBP5 or Mouse IGFBP5

At the end of the discovery process, each selection output was screened for antigen-specific antibodies and the binding characteristics of monoclonal antibody clones was analysed. For this purpose, 3072 single colonies were used for scFv antibody production. These were then tested for specific antigen binding by ELISA on Streptavidin + biotinylated human IGFBP5, Streptavidin + biotinylated murine IGFBP5, Streptavidin and BSA and 842 clones showed cross-reactive binding between human and murine IGFBP5 with a S/N ratio of > 10. 192 of those ELISA positive clones were selected for a secondary screening. For that purpose, a second batch of scFv were produced and tested on a broader panel of antigens: Streptavidin + biotinylated human IGFBP5, Streptavidin + biotinylated murine IGFBP5, Streptavidin, BSA, Human IGFBP1, Human IGFBP2, Human IGFBP3, Human IGFBP4, Human IGFBP5 and Human IGFBP6. 17 lead antibody clones were identified that showed binding to the Streptavidin-captured and directly immobilized human and murine IGFBP5. Binding to human IGFBP1, human IGFBP2, human IGFBP3, human IGFBP4 and human IGFBP6 was not detected.

DNA sequence analysis of the 17 lead antibody clones revealed presence of 15 candidates with a unique antibody sequence (≥ 1 amino acid difference in the CDRs). The scFv DNA sequence was amplified by PCR and cloned into YUMAB's mammalian scFv-Fc expression vector, resulting in a genetic fusion of the scFv with a human IgG4 Fc. Bacterial clones with a correctly cloned scFv gene were used for isolation of transfection grade DNA. The DNA was used for the transient transfection of HEK cells which produced and secreted the antibody into the culture medium. The antibodies were purified by affinity chromatography (Protein A) and re-buffered in PBS. The protein concentration was determined by UV/VIS spectrometry and purity was checked by Coomassie staining. 13 of the 15 antibodies could be successfully produced in the scFv-Fc format.

The sequences of the 15 novel anti-IGFBP5 antibodies are reported in Tables 2-8.

Table 2: VH CDR Sequences of exemplified antibodies (Kabat definitions)			
Antibody	VH CDR1	VH CDR2	VH CDR3
A09	SYGIS (SEQ ID No. 1)	WISAYNGNTNYAQKLQG (SEQ ID No. 6)	EWELLGGLDY (SEQ ID No. 12)
B06	SYAMS (SEQ ID No. 2)	AISGSGGSTYYADSVKG (SEQ ID No. 7)	GAGTLDY (SEQ ID No. 13)
C02	SYGIS (SEQ ID No. 1)	WISAYNGNTNYAQKLQG (SEQ ID No. 6)	GRGVIDY (SEQ ID No. 14)
D10	SYGIS (SEQ ID No. 1)	WISAYNGNTNYAQKLQG (SEQ ID No. 6)	DRYLGWFDP (SEQ ID No. 15)
F05	SYGIS (SEQ ID No. 1)	WISAYNGNTNYAQKLQG (SEQ ID No. 6)	GRGSFDP (SEQ ID No. 16)
B09	SYGIS (SEQ ID No. 1)	WISAYNGNTNYAQKLQG (SEQ ID No. 6)	DLAVGTHGDY (SEQ ID No. 17)
B11	SYAIS (SEQ ID No. 3)	GIPIFGTANYAQKFQG (SEQ ID No. 8)	NVRLPGWFDP (SEQ ID No. 18)
C11	SYGMH (SEQ ID No. 4)	VISYDGSNKYYADSVKG (SEQ ID No. 9)	GDYSDTSGTDFQH (SEQ ID No. 19)
C12	SYAIS (SEQ ID No. 3)	GIPIFGTANYAQKFQG (SEQ ID No. 8)	LGRELSFDY (SEQ ID No. 20)
D12	SYAIS (SEQ ID No. 3)	GIPIFGTANYAQKFQG (SEQ ID No. 8)	FGRPQLGYAFDI (SEQ ID No. 21)
E02	SYAIS (SEQ ID No. 3)	GIPIFGTANYAQKFQG (SEQ ID No. 8)	MSVAGPSISFDY (SEQ ID No. 22)
E03	SYAIS (SEQ ID No. 3)	GITPIFGTANYAQKFQG (SEQ ID No. 10)	AGILTGYFDY (SEQ ID No. 23)
E05	GYGMN (SEQ ID No. 5)	YVGSSSSTIYYADSVKG (SEQ ID No. 11)	GLGNN (SEQ ID No. 24)
F04	SYGMH (SEQ ID No. 4)	VISYDGSNKYYADSVKG (SEQ ID No. 9)	LSGPNGVVDY (SEQ ID No. 25)
G01	SYAIS (SEQ ID No. 3)	GIPIFGTANYAQKFQG (SEQ ID No. 8)	ITEGAFDY (SEQ ID No. 26)

Table 3: VL CDR sequences of exemplified antibodies (Kabat definitions)			
Antibody	VL CDR1	VL CDR2	VL CDR3
A09	TGTSSDVGGYNYVS (SEQ ID No. 27)	EVSNRPS (SEQ ID No. 37)	SSYTSSSTVV (SEQ ID No. 48)
B06	KSSQSVLYSSNNKNYLS (SEQ ID No. 28)	WASTRES (SEQ ID No. 38)	QYYSTPLT (SEQ ID No. 49)
C02	KSSQSVLYSSNNKNYLA (SEQ ID No. 29)	WASTRES (SEQ ID No. 38)	QYYSTPLT (SEQ ID No. 49)
D10	TGTSSDVGGYNYVS (SEQ ID No. 27)	EVSNRPS (SEQ ID No. 39)	SSYTSSSTPYV (SEQ ID No. 50)
F05	KSSQSVLYSSNNKNYLA (SEQ ID No. 29)	WASTRES (SEQ ID No. 38)	QYYSTPLT (SEQ ID No. 49)
B09	TGTSSDVGGYNYVS (SEQ ID No. 27)	EVSNRPS (SEQ ID No. 39)	SSYTSSSTLEV (SEQ ID No. 51)
B11	RASQSISSWLA (SEQ ID No. 30)	KASSLES (SEQ ID No. 40)	QYNSYPLT (SEQ ID No. 52)
C11	RASQSISSYLN (SEQ ID No. 31)	AASSLQS (SEQ ID No. 41)	QSYSTPG (SEQ ID No. 53)
C12	RASQSISSYLN (SEQ ID No. 31)	AASSLQS (SEQ ID No. 41)	QSYSESWT (SEQ ID No. 54)
D12	RASQSLADYLA (SEQ ID No. 32)	GASTRAT (SEQ ID No. 42)	QNDNWPYT (SEQ ID No. 55)
E02	RASQSISSYLN (SEQ ID No. 31)	AASSLQR (SEQ ID No. 43)	QETYSNLWT (SEQ ID No. 56)
E03	TRSSGSIASNYVQ (SEQ ID No. 33)	EDNQRPS (SEQ ID No. 44)	QSYDSSNRPWV (SEQ ID No. 57)
E05	SGRSNIGSNYVH (SEQ ID No. 34)	RNDQRPS (SEQ ID No. 45)	AAWDDGLSVL (SEQ ID No. 58)
F04	QGDSVRKYTN (SEQ ID No. 35)	GKNYRPS (SEQ ID No. 46)	HSRDTSDIHLNV (SEQ ID No. 59)
G01	RASRTVGSWLA (SEQ ID No. 36)	DASNLQS (SEQ ID No. 47)	QYNSSPYT (SEQ ID No. 60)

Table 4: VH amino acid sequences of exemplified antibodies	
Antibody	AA of VH
A09	QVQLVESGAIEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQGLEWMGWISAYNG NTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAREWELLGGLDYWGQG TLTVTVSS (SEQ ID No. 61)
B06	EVQLLESGLLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGG STYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARGAGTLDYWGQGT VSS (SEQ ID No. 62)
C02	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQGLEWMGWISAYN GNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARGRVIDYWGQGT LTVTVSS (SEQ ID No. 63)
D10	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQGLEWMGWISAYN GNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDRLGWFDPWGQ GTLTVTVSS (SEQ ID No. 64)
F05	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQGLEWMGWISAYN GNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARGRGSFDPWGQGT LTVTVSS (SEQ ID No. 65)
B09	QVQLVQSGAEVKKPGASVKVSCASGYTFTSYGISWVRQAPGQGLEWMGWISAYN GNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDLAVGTHGDYWGQ GTLTVTVSS (SEQ ID No. 66)
B11	QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNVRLPGWFDPWGQGT LTVTVSS (SEQ ID No. 67)

Table 4: VH amino acid sequences of exemplified antibodies	
Antibody	AA of VH
C11	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDG SNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDYS DTS G T D F F Q H WGQGTQVTVSS (SEQ ID No. 68)
C12	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCATLGRELSFDYWGQGT L V TVSS (SEQ ID No. 69)
D12	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARFGRPQLGYAFDIWGQG TMVTVSS (SEQ ID No. 70)
E02	QMQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCATMSVAGPSISFDYWGQG TLVTVSS (SEQ ID No. 71)
E03	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGITPIFG TANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARAGILTGYFDYWGQG TLVTVSS (SEQ ID No. 72)
E05	EVQLVESGGGLVQPGGSLKLSAASGFTFSGYGMNHWVRQAPGKGLEWVS YV G S S S STIYYADSVKGRFTISRDNANNSLYLQMNSLRAEDTAVYYCARGLGGNWGQGTMTV SS (SEQ ID No. 73)
F04	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDG SNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKLSGPN G V D Y W G Q G TLVTVSS (SEQ ID No. 74)
G01	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCASITEGA F D Y W G Q G T L V T V SS (SEQ ID No. 75)

Table 5: VL amino acid sequences of exemplified antibodies	
Antibody	AA of VL
A09	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLM IYEVS NR PSGVSNRFGSGSKSGNTASLTISGLQAEDEADY Y C S S Y T S S S T V F G G G T K L T V L (SEQ ID No. 76)
B06	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLSWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPLTFGGG TKVEIK (SEQ ID No. 77)
C02	EIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWA STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPLTFGGG TKVDIK (SEQ ID No. 78)
D10	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLM IYEVS NR PSGVSNRFGSGSKSGNTASLTISGLQAEDEADY Y C S S Y T S S S T P Y V F G T G T K L T V L (SEQ ID No. 79)
F05	DIQLTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWA STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPLTFGGG TKVEIK (SEQ ID No. 80)
B09	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLM IYEVS NR PSGVSNRFGSGSKSGNTASLTISGLQAEDEADY Y C S S Y T S S S T L E V F G G G T K L T V L (SEQ ID No. 81)
B11	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYKASSLESG VPSRFSGSGSGTEFTLTISSLQPD F A T Y Y C Q Q Y N S Y P L T F G G G T K V E I K (SEQ ID No. 82)
C11	DIVMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSG VPSRFSGSGSGTDFTLTISSLQPD F A T Y Y C Q Q S Y S T P G F G G G T K V E I K (SEQ ID No. 83)
C12	DIVMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSG VPSRFSGSGSGTDFTLTISSLQPD F A T Y Y C Q Q S Y S E S W T F G Q G T T V D I K (SEQ ID No. 84)

Table 5: VL amino acid sequences of exemplified antibodies	
Antibody	AA of VL
D12	ETTLTQSPGTLVSPGDRATLSCRASQSLSDYLAQYQQKPGQAPRLLIYGASTRA TGIPARFSGSGSGTEFTLTVSSSLQSEDFAVYYCQQNDNWPYTFGQGTKLEIK (SEQ ID No. 85)
E02	DIQMTQSPSSLSASVGDRAIPCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQRG VPSRFSGSGSGTDFTLTISLQPEDFATYYCQETYSNLWTFGQGTKVEIK (SEQ ID No. 86)
E03	NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRP SGVPDRFSGSIDSSNSASLTISGLKTEDEADYYCQSYDSSNRPWVFGGGTKLTVL (SEQ ID No. 87)
E05	QPGLTQPPSASGAPGQRVTISCSGSRNIGSNYVHWYQQLPGTAPKLIYRNDQRP SGVPARFSASKSGTSASLAISGLRSEDEADYYCAAWDDGLSVLFGGGTKLTVL (SEQ ID No. 88)
F04	SSELTQDPSVSVALGQTVTITCQGDSVRKYYTNWYQQKPGQAPILVLYGKNYRPSG IPDRFSGSTSGDTAYLTITGAQAEDAADYYCHSRDTSIHLNVFGTGKTVL (SEQ ID No. 89)
G01	AIQLTQSPSTLSASVGDRTVITCRASRTVGSWLAQYQQKPGKAPKLLIYDASNLQSG VPSRFSGSGSGTEFTLTISLQPDLLSTYYCQYNSPYTFGQGTKVEIK (SEQ ID No. 90)

CDR definitions are also provided using the annotation tool from <http://www.abysis.org/> based on full VH and VL amino acid sequences as defined in Tables 4 and 5.

For example, the VH or VL amino acid sequence of any antibody disclosed herein is plugged into the annotation tool and Kabat defined CDR sequences, or IMGT, or Chothia, or AbM or Contact defined CDR sequences are provided. Using the “All, side by side” feature, defined CDR sequences are provided.

Table 4.1 shows such sequences related to B06 VH and VL .

10

Table 4.1 B-06 VH and VL CDRs according to different definitions as sorted by inserting aa VH (SEQ ID No. 62) and VL (SEQ ID No. 77) sequence in the www.abysis.org annotation tool.

V_H

15

Region	Definition	Sequence Fragment	Residues	Length
CDR-H1	Chothia	GFTFSSY---	26 - 32	7
	AbM	GFTFSSYAMS	26 - 35	10
	Kabat	----SYAMS	31 - 35	5
	Contact	----SSYAMS	30 - 35	6
	IMGT	GFTFSSYA--	26 - 33	8
CDR-H2	Chothia	----SGSGGS-----	52 - 57	6
	AbM	---AISGSGGSTY-----	50 - 59	10
	Kabat	---AISGSGGSTYYADSVKG	50 - 66	17
	Contact	WVSAISGSGGSTY-----	47 - 59	13
	IMGT	---ISGSGGST-----	51 - 58	8
CDR-H3	Chothia	--GAGTLDY	99 - 105	7
	AbM	--GAGTLDY	99 - 105	7

Kabat	--GAGTLDY	99 - 105	7
Contact	ARGAGTLD-	97 - 104	8
IMGT	ARGAGTLDY	97 - 105	9

V_L

Region	Definition	Sequence Fragment	Residues	Length
CDR-L1	Chothia	KSSQSVLYSSNNKNYLS--	24 - 40	17
	AbM	KSSQSVLYSSNNKNYLS--	24 - 40	17
	Kabat	KSSQSVLYSSNNKNYLS--	24 - 40	17
	Contact	-----LYSSNNKNYLSWY	30 - 42	13
	IMGT	---QSVLYSSNNKNY----	27 - 38	12
CDR-L2	Chothia	----WASTRES	56 - 62	7
	AbM	----WASTRES	56 - 62	7
	Kabat	----WASTRES	56 - 62	7
	Contact	LLIYWASTRE-	52 - 61	10
	IMGT	----WA-----	56 - 57	2
CDR-L3	Chothia	QQYYSTPLT	95 - 103	9
	AbM	QQYYSTPLT	95 - 103	9
	Kabat	QQYYSTPLT	95 - 103	9
	Contact	QQYYSTPL-	95 - 102	8
	IMGT	QQYYSTPLT	95 - 103	9

5

Table 5.1 shows such sequences related to B09 VH and VL .

Table 5.1 B-09 VH and VL CDRs according to different definitions as sorted by inserting aa VH (SEQ ID No. 66) and VL (SEQ ID No. 81) sequence in the www.abysis.org annotation tool.

10

V_H

Region	Definition	Sequence Fragment	Residues	Length
CDR-H1	Chothia	GYTFTSY---	26 - 32	7
	AbM	GYTFTSYGIS	26 - 35	10
	Kabat	-----SYGIS	31 - 35	5
	Contact	----TSYGIS	30 - 35	6
	IMGT	GYTFTSYG--	26 - 33	8
CDR-H2	Chothia	-----SAYNGN-----	52 - 57	6
	AbM	---WISAYNGNTN-----	50 - 59	10
	Kabat	---WISAYNGNTNYAQKLQG	50 - 66	17
	Contact	WMGWISAYNGNTN-----	47 - 59	13
	IMGT	----ISAYNGNT-----	51 - 58	8
CDR-H3	Chothia	--DLAVGTHGDY	99 - 108	10
	AbM	--DLAVGTHGDY	99 - 108	10
	Kabat	--DLAVGTHGDY	99 - 108	10
	Contact	ARDLAVGTHGD-	97 - 107	11
	IMGT	ARDLAVGTHGDY	97 - 108	12

V_L

Region	Definition	Sequence Fragment	Residues	Length
CDR-L1	Chothia	TGTSSDVGGYNYVS--	23 - 36	14
	AbM	TGTSSDVGGYNYVS--	23 - 36	14
	Kabat	TGTSSDVGGYNYVS--	23 - 36	14
	Contact	-----VGGYNYVSWY	29 - 38	10
	IMGT	---SSDVGGYNY----	26 - 34	9
CDR-L2	Chothia	----EVSNRPS	52 - 58	7
	AbM	----EVSNRPS	52 - 58	7
	Kabat	----EVSNRPS	52 - 58	7
	Contact	LMIYEVSNRP-	48 - 57	10
	IMGT	----EV-----	52 - 53	2
CDR-L3	Chothia	SSYTSSSTLEV	91 - 102	12
	AbM	SSYTSSSTLEV	91 - 102	12
	Kabat	SSYTSSSTLEV	91 - 102	12
	Contact	SSYTSSSTLEV-	91 - 101	11
	IMGT	SSYTSSSTLEV	91 - 102	12

Table 6: VH nucleotide sequences of exemplified antibodies	
Antibody	DNA of VH
A09	CAGGTGCAGCTGGTGGAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG AAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGG TGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACA ATGGTAACACAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGA CACATCCACGAGCACAGCCTACATGGAGCTGAGAAGCCTGAGATCTGACGACAC GGCCGTGTATTACTGTGCGAGAGAGTGGGAGCTACTTGGGGGCTTGACTACTG GGGCCAGGGAACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 91)
B06	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGCTCCCT GAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGG GTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGT GGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGA GACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA CGGCCGTATATTACTGTGCGAGAGGGGCTGGTACGCTTGACTACTGGGGCCAGG GAACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 92)
C02	CAGGTTTCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG AAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGG TGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACA ATGGTAACACAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGA CACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC GGCCGTGTATTACTGTGCGAGAGGTGCGGGAGTTATAGACTACTGGGGCCAGGG AACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 93)
D10	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGT GAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGG GTGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTAC AATGGTAACACAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAG ACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC CGGCCGTGTATTACTGTGCGAGAGATCGATATTTGGGGTGGTTTCGACCCCTGGG GCCAGGGAACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 94)
F05	CAGGTTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG AAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTACGGTATCAGCTGGG TGCGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACA ATGGTAACACAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGA CACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC GGCCGTGTATTACTGTGCGAGAGGTAGAGGTAGTTTCGACCCCTGGGGCCAGGG AACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 95)

Table 6: VH nucleotide sequences of exemplified antibodies	
Antibody	DNA of VH
B09	CAGGTGTAGCTGGTGCAATCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTG AAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGG TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACA ATGGTAACACAACTATGCACAGAAGCTCCAGGGCAGAGTTACCATGACCACAGA CACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC GGCCGTGTATTACTGTGCGAGAGATCTGGCAGTTGGGACCCACGGTGACTACTG GGGCCAGGGAACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 96)
B11	CAGGTCCAGCTGGTGCACTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTG AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCT TTGGTACAGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGA CAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACAC GGCCGTGTATTACTGTGCGAGAAACGTCCGTCTCCCAGGCTGGTTCGACCCCTG GGGCCAGGGAACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 97)
C11	GAGGTGCAGCTGGTGCACTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCT GAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTAGCTATGGCATGCACTGG GTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGAT GGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAG ACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACAC AGCTGTATACTACTGTGCGAGAGGGGACTATTCTGATACCACTGGTACCGATTCT TCCAGCACTGGGGCCAGGGCACCCAGGTCACCGTGTCTCTCA (SEQ ID No. 98)
C12	CAGGTGCAGCTGGTGCACTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTG AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCT TTGGTACAGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGA CGAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACAC GGCCGTGTATTACTGTGCGACCTCGGGAGGGAGCTCAGCTTTGACTACTGGGG CCAGGGAACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 99)
D12	CAGGTCCAGCTGGTGCACTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTG AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCT TTGGTACAGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGA CGAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACAC GGCCGTGTATTACTGTGCGAGATTCGGAAGGCCGAGCTGGGCTATGCTTTTGAT ATCGGGGCCAAGGGACAATGGTCACCGTGTCTCTCA (SEQ ID No. 100)
E02	CAAATGCAGCTGGTGCAATCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTG AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCT TTGGTACAGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGA CGAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACAC GGCCGTGTATTACTGTGCAACAATGTCACTGGCTGGTCTTCAATATCTTTTGACT ACTGGGGCCAGGGAACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 101)
E03	CAGGTGCAGCTGGTGCACTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTG AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCACCCCTATCT TTGGTACAGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGA CGAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACAC GGCCGTGTATTACTGTGCGAGACGGGAATTTTGACTGGTTATTACTTTGACTACT GGGGCCAGGGAACCCTGGTCACCGTGTCTCTCA (SEQ ID No. 102)
E05	GAGGTGCAGCTGGTGCACTCTGGGGGAGGCTTGGTACAGCCTGGGGGTCCCT GAAACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTGGCTATGGCATGAACTGG GTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACGTTGGTAGTAGT AGTAGTACCATATACTACGCAGATTCTGTGAAGGGCCGATTACCATCTCCAGAG ACAATGCCAACAACCTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACAC GGCTGTGTATTACTGTGCGAGAGGCCTCGGTGGTAATTGGGGCCAAGGGACAAT GGTCACCGTGTCTCTCA (SEQ ID No. 103)
F04	GAGGTGCAGCTGGTGCACTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCT GAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTAGCTATGGCATGCACTGG GTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGAT

Table 6: VH nucleotide sequences of exemplified antibodies	
Antibody	DNA of VH
	GGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAG ACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACAC GGCTGTGTATTACTGTGCGAAACTTTCCGGGCCCAACGGTGTGGACTACTGGGG CCAGGGAACCCTGGTCACCGTGTCTCA (SEQ ID No. 104)
G01	CAGGTTTCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCTCGGTG AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG TGCGACAGGCCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCT TTGGTACAGCAAACCTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGA CGAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACAC GGCCGTGTATTACTGTGCGAGCATTACGGAGGGGGCCTTTGACTACTGGGGCCA GGGAACCCTGGTCACCGTGTCTCA (SEQ ID No. 105)

Table 7: VL nucleotide sequences	
Antibody	DNA of VL
A09	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCA CCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTG GTACCAACAGCACCCAGGCAAAGCCCCCAAACCTCATGATTTATGAGGTCAGTAAT CGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCT CCCTGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAGCTC ATATACAAGCAGCAGCACTGTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTA (SEQ ID No. 106)
B06	GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGG GCCACCATCAACTGCAAGTCCAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAA CTACTTATCTTGGTACCAGCAGAAACCAGGTCAGCCTCCTAAGCTGCTCATTTACT GGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATTCAAGTGGCAGCGGGTCTG GGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTA TTACTGTCAGCAATATTATAGTACTCCGCTCACTTTCCGGCGGAGGGACCAAGGTG GAAATCAAA (SEQ ID No. 107)
C02	GAAATAGTGATGACGCACTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGG GCCACCATCAACTGCAAGTCCAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAA CTACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTACT GGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATTCAAGTGGCAGCGGGTCTG GGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTA TTACTGTCAGCAATATTATAGTACTCCGCTCACTTTCCGGCGGAGGGACCAAGTG GATATCAAA (SEQ ID No. 108)
D10	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCA CCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTG GTACCAACAGCACCCAGGCAAAGCCCCCAAACCTCATGATTTATGAGGTCAGTAAT CGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCT CCCTGACCATCTCTGGGCTCCAGGCTGAGGATGAGGCTGATTATTACTGCAGCTC ATATACAAGCAGCAGCACCCCTTATGTCTTCGGAACCTGGGACCAAGCTGACCGTC CTA (SEQ ID No. 109)
F05	GACATCCAGTTGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGG GCCACCATCAACTGCAAGTCCAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAA CTACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTACT GGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATTCAAGTGGCAGCGGGTCTG GGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCCGAAGATGTGGCAGTTTA TTACTGTCAGCAATATTATAGTACTCCTCTCACTTTCCGGCGGAGGGACCAAGGTG GAGATCAAA (SEQ ID No. 110)
B09	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCA CCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTG GTACCAACAGCACCCAGGCAAAGCCCCCAAACCTCATGATTTATGAGGTCAGTAAT CGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCT CCCTGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAGCTC ATACACAAGCAGCAGCACTCTCAGGTGGTATTCCGGCGGAGGGACCAAGCTGAC CGTCCTA (SEQ ID No. 111)
B11	GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAG TCACCATCACTTGCCGGGCCAGTCAGAGTATTAGTAGCTGGTTGGCCTGGTATCA

Table 7: VL nucleotide sequences	
Antibody	DNA of VL
	GCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATAAGGCGTCTAGTTTAGAA AGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGATCTGGGACAGAATTCAGCTCTCA CCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTATTACTGCCAACAGTATAAT AGTTACCCGCTCACTTTTCGGCGGAGGGACCAAGGTGGAGATCAAA (SEQ ID No. 112)
C11	GACATCGTGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG TCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAG CAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAA GTGGGGTCCCATCAAGGTTTCAGTGGCAGTGATCTGGGACAGATTTCACTCTCAC CATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACA GTACCCAGGGTTCGGCGGAGGGACCAAGGTGGAGATCAAA (SEQ ID No. 113)
C12	GATATTGTGATGACTCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGT CACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGC AGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAG TGGGGTCCCATCAAGGTTTCAGTGGCAGTGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAG TGAGTCGTGGACGTTTCGGCCAGGGACACGGTGGACATCAAA (SEQ ID No. 114)
D12	GAAACGACACTCAGCAGTCTCCAGGCACCCTGTCTGTGTCTCCAGGGGATAGA GCCACCCTCTCCTGCAGGGCCAGTCAGAGCTTAGCGCCGACTACTTAGCCTGGT ACCAGCAGAAACCTGGCCAGGCTCCAGGCTCCTCATCTATGGTGCATCCACAAG GGCCACTGGTATCCAGCCAGGTTTCAGTGGCAGTGATCTGGGACAGATTTCACTCTCA TCTCACCGTCAGCAGCCTGCAGTCTGAAGATTTTGCAAGTTTACTACTGTCAAGAG ATGATAACTGGCCGTACACTTTTGCCAGGGGACCAAGCTGGAGATCAAA (SEQ ID No. 115)
E02	GACATCCAGATGACCCAGTCTCCATCTTCCCTGTCCGCATCTGTGGGAGACAGAG TCGCCATCCCTTGCCGGGCAAGTCAGAGCATTAGCAGCTACTTAAATTGGTATCA GCAGAAACCAGGGAAAGCCCCTAAACTGCTGATCTACGCTGCATCCAGTTTGCAA AGAGGGGTCCCATCAAGGTTTCAGTGGCAGTGATCTGGGACAGATTTCACTCTCA CCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTATTGTCAAGAGACTTAC AGTAACCTGTGGACGTTTCGGCCAAGGGACCAAGGTGGAATCAAA (SEQ ID No. 116)
E03	AATTTTATGCTGACTCAGCCCCACTCTGTGTCTGGAGTCTCCGGGGAAGACGGTAA CCATCTCCTGCACCCGCAGCAGTGGCAGCATTGCCAGCAACTATGTGCAGTGGTA CCAGCAGCGCCCGGGCAGTTCCCCCACCCTGTGATCTATGAGGAATGATCAGCG ACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACAGCTCCTCCAACCTCT GCCTCCCTCACCATCTCTGGACTGAAGACTGAGGACGAGGCTGACTACTACTGTC AGTCTTATGATAGCAGCAATCGGCCCTTGGGTGTTTCGGCGGAGGGACCAAGCTGA CCGTCCTA (SEQ ID No. 117)
E05	CAGCCTGGGCTGACTCAGCCACCCTCAGCGTCTGGGGCCCCCGGGCAGAGGGT CACCATCTCTTGTCTGGAAGCAGGTCCAATATCGGTTCTAATTATGTACACTGGT ACCAACAACCTCCAGGAACGGCCCCCAAACATCATTTATAGGAATGATCAGCG GCCCTCAGGGGTCCCTGCCGATTCTCTGCCTCCAAGTCTGGCACCTCAGCCTC CCTGGCCATCAGTGGGCTCCGGTCCGAGGATGAAGCTGATTACTACTGTGCAGC GTGGGATGACGGAAGTGTCTTTCGGCGGAGGGACCAAGTTGACCGTCTCT A (SEQ ID No. 118)
F04	TCTTCTGAGCTGACTCAGGACCCCTCTGTGTCTGTGGCCTTGGGACAAACAGTCA CTATCACGTGCCAAGGAGACAGTGTGAGAAAGTATTATACGAACTGGTATCAACA GAAGCCAGGACAGGCCCTATCCTTGTCTCTATGGTAAAAACTACCGGCCCTCA GGGATCCAGACCGATTCTCTGGCTCCACGTCCGGAGACACAGCGTACTTGACC ATCACTGGGGCTCAGGCGGAGGATGCGGCAGACTATTATTGTCACTCCCGGGAC ACCAAGTATATCCATCTAAATGTCTTCGGCACTGGGACCAAGGTACCGTCTCTA (SEQ ID No. 119)
G01	GCCATCCAGTTGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAG TCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAG CAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGATGCCTCCAATTTGCAA AGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGATCTGGGACAGAATTCAGCTCTCA CCATCAGCAGCCTGCAGCCTGATGATTTGTCAACTTATTACTGCCAACAGTATAAT

Table 7: VL nucleotide sequences	
Antibody	DNA of VL
	AGTTCCCCGTACACTTTTGGCCAGGGGACCAAGGTGGAGATCAAA (SEQ ID No. 120)

Table 8: Constant region amino acid sequences	
Constant region	AA
Human IgG4 heavy chain P01861.1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRV E SKYGPPCPSPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ E DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNNHYTQKSLSLSLGK (SEQ ID No. 121)
Human IgG2 heavy chain P01859	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVPSNFGTQTYTCNVDHKPSNTKVDKTV E RKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH E DPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGK EYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDISVEWESNGQPENNYKTPPMLSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNNHYTQKSLSLSPGK (SEQ ID No. 122)
Human light chain, lambda 1 P0CG04	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVK AGVETTKPSKQSNKYAASSYLSLTPEQWWSHRYSYSCQVTHEGSTVEKT VAPTECS (SEQ ID No. 123)
Human light chain, lambda 2 P0DOY2	GQPKAAPSVTFLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK AGVETTTTPSKQSNKYAASSYLSLTPEQWWSHRYSYSCQVTHEGSTVEKT VAPTECS (SEQ ID No. 124)
Human light chain, kappa P01834	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC (SEQ ID No. 125)

Method of expressing recombinant protein in CHO cells

- The corresponding B06 and B09 cDNAs were cloned into evitria's vector system using conventional (non-PCR based) cloning techniques. The evitria vector plasmids were gene synthesized. Plasmid DNA was prepared under low-endotoxin conditions based on anion exchange chromatography. Correctness of the sequences was verified with Sanger sequencing (with up to two sequencing reactions per plasmid depending on the size of the cDNA.)
- Suspension-adapted CHO K1 cells (evitria) was used for production. The seed was grown in eviGrow medium, a chemically defined, animal-component free, serum-free medium. Cells were transfected with eviFect, evitria's custom-made, proprietary transfection reagent, and cells were grown after transfection in eviMake, an animal-component free, serum-free medium, at 37°C and 5% CO₂ for 7 days. Supernatant was harvested by centrifugation and subsequent filtration (0.2 µm filter).
- The antibody was purified using MabSelect™ SuRe™ with Dulbecco's PBS (Lonza BE17-512Q) as wash buffer and 0.1 M Glycine pH 3.5 as elution buffer. Subsequent

size exclusion chromatography was performed on a HiLoad Superdex 200 pg column using the final buffer as running buffer.

Monomericity was determined by analytical size exclusion chromatography with an Agilent AdvanceBio SEC column (300A 2.7 μ m 7.8 x 300 mm) and DPBS as running
5 buffer at 0.8 ml/min.

Example 2: Affinity measurement

Octet BLI-based analysis

To assess the binding affinity in more detail, affinity measurements were performed
10 using Biomolecular Interaction Analysis performed by an Octet platform (BLItz System), which is a Biolayer Interferometry (BLI) platform. To establish the assay, the target monoclonal antibody (mAb, 2 identical antigen binding moieties, 2 μ g/ml in 1% BSA containing 0.05% Tween 20) was immobilized via Fc on the via AHC biosensor and the interaction with the antigen human IGFBP5 (R&D, cat n° 875-B5) and mouse IGFBP5
15 (R&D, cat n° 578-B5) at different concentration were measured.

The affinity measurement of B06 and B09 full length mAb for the target human and murine IGFBP5 are reported in the Table 9.

Table 9: Affinity of B06 and B09 for the target human and murine IGFBP5		
Molecule id	B06	B09
hIGFBP-5 KD (M)	1×10^{-10}	1×10^{-10}
mIGFBP-5 KD (M)	1×10^{-10}	1×10^{-10}

20 Newly generated anti-IGFBP5 mAbs show good human antigen binding affinity with KD below 4×10^{-10} M. The antibodies also show murine cross reactivity. This data confirmed that mice can be considered a relevant animal species for testing of the monoclonal antibodies during preclinical development.

25 Example 3: Primary cell-based assay relevant to fibrosis (IPF)

Fibrosis is a process of continuous tissue repair characterized by the formation and deposition of fibrillary collagen-rich extracellular matrix (ECM) leading to a progressive remodeling in most tissues and organs. Formation of fibrotic scar tissue is essential to restore and maintain tissue and organ integrity during wound healing after injury. However,
30 er, when the scarring mechanisms exacerbate after repetitive injury, chronic fibrogenesis results in a shift from supportive fibrotic tissue to scar tissue. This coincides

with an increased number and activity of extracellular matrix producing cells, which finally leads to destruction of normal tissue/organ architecture and function.

In the lung, such as in Idiopathic Pulmonary Fibrosis disease (IPF), tissue scarring/fibrosis occurs. Tissue damage is followed by the activation of the immune system that induces release of several cytokines and growth factors including transforming growth factor beta-1 (TGF β -1) that signals the tissue to repair. Mechanistically, epithelial to mesenchymal transition (EMT) is one of the major drivers of fibrosis, and approximately 30% of ECM-producing (myo-)fibroblasts derive from epithelial cells through EMT. During EMT, tightly organized epithelial cells lose expression of the tight junction marker E-cadherin and transform to mesenchymal cells with expression of the mesenchymal cell markers N-cadherin, vimentin and fibronectin. Furthermore, the cells gain migratory potential, enabling them to migrate throughout the tissue. Another important mechanism during fibrosis is fibroblast to myofibroblast transformation (FMT), when EMT-derived, resident and invading fibroblastoid cells are activated (e.g., by TGF β -1) and start to differentiate towards myofibroblasts. Myofibroblasts are a heterogeneous population derived from different progenitors, and are characterized by formation of alpha smooth muscle actin (α SMA) containing stress fibers and the expression and secretion of fibrillar collagen, fibronectin and additional ECM components. Increased production and accumulation of ECM result in a permanently damaged fibrotic tissue with an aberrant architecture unable to function properly.

FMT and EMT in vitro assays are established models to identify and characterize anti-fibrotic compounds (see Weigle et al. J Biol Methods (2019) Vol. 6(2)).

25 Fibroblast-to-Myofibroblast Transition (FMT) assay

Human bronchial lung fibroblasts derived from three IPF patients were seeded in 384-well plates (Cat. 6057302, Perkin Elmer) at a density of 750 cells/well in 2%FBS-DMEM (DMEM supplemented with 2% fetal bovine serum (Cat. 10100-147, Gibco) and 1% penicillin and streptomycin (Cat. 15140, Gibco)). Two days after seeding, cells were refreshed with 2%FBS-DMEM. Five days after seeding, cells were refreshed with 0.2%FBS-DMEM (DMEM supplemented with 0.2% fetal bovine serum (Cat. 10100-147, Gibco) and 1% penicillin and streptomycin (Cat. 15140, Gibco)) and the anti-IGFBP5 mAbs (B06 and B09) were added in an eight-step concentration response curve using 0.5LogM dilution steps with a top concentration of 1.1 μ M for B06 and 1.5 μ M for B09. ALK5 inhibitor (SB525334; Cat. S8822-25, Sigma at 1 μ M in 0.1% DMSO (positive

control), 0.1% DMSO (vehicle control 1; Cat. D8418, Sigma) and 3.6% PBS (vehicle control 2) were used to assess assay performance. One hour after the anti-IGFBP5 mAbs (B06 and B09), DMSO or PBS addition, cells were triggered with 1.25 ng/mL TGF- β 1 (Cat. 240-B-010, R&D Systems) to induce Fibroblast-to-Myofibroblast Transition (FMT). Three (3) days after TGF- β 1 addition, the cells were fixed with 4% formaldehyde (Cat. 8187081000, Merck) and stained for α -smooth muscle actin (α SMA) as a marker of myofibroblast and nuclei (DAPI). For determination of α SMA expression, fixed lung fibroblasts were incubated in blocking buffer (PBS containing 5 mg/mL BSA (Cat. A2153, Sigma) and 0.2% (vol/vol) Triton X-100 (Cat. T8787, Sigma)) for one hour at room temperature. Cells were then incubated with a monoclonal Alexa Fluor 488-conjugated anti- α SMA antibody (1 μ g/mL; Cat. AB184675, Abcam) for one hour at room temperature, washed with PBS and incubated with 4',6-diamidino-2-phenylindole (DAPI, 0.5 μ g/mL; Cat. D3571, Life Technologies) and imaged on the In Cell Analyzer 2200 (GE Healthcare). Expression was quantified using the IN Cell developer software (GE Healthcare). α SMA expression is presented as the staining intensity multiplied by the stained area (DxA levels). Co-staining of cell nuclei with DAPI was performed to quantify cell number, as a measure of potential cytotoxicity.

After analysis, the anti-IGFBP5 mAbs showed a concentration-dependent inhibition of TGF- β 1-mediated α SMA expression in IPF patient samples. Moreover, the anti-IGFBP5 mAbs showed no modulation of the number of nuclei, indicative of absence of potential cytotoxic side-effects (Figure 1).

Epithelial-to-Mesenchymal Transition (EMT) assay

Human bronchial epithelial cells (HBECs) derived from three IPF patients (IPF05, IPF06, and IPF07) were seeded in 384-well plates (Cat. 6057302, Perkin Elmer) at a density of 900 cells/well in KSFM Complete (keratinocyte serum-free medium supplemented with 0.2 ng/mL epidermal growth factor, 25 μ g/mL bovine pituitary extract (all from Cat. 17005-075, Gibco), 1 μ mol/L isoproterenol (Cat. I6504, Sigma) and 1% penicillin and streptomycin (Cat. 15140, Gibco)). Two days after seeding, cells were refreshed with KSFM Complete. Six days after seeding, cells were refreshed with KSFM Complete and the anti-IGFBP5 mAbs (B06 and B09) were added in an eight-step concentration response curve using 0.5LogM dilution steps with a top concentration of 1.1 μ M for B06 and 1.5 μ M for B09. ALK5 inhibitor (SB525334; Cat. S8822-25, Sigma) at 1 μ M in 0.1% DMSO (positive control), 0.1% DMSO (vehicle control 1; Cat. D8418, Sigma) and 3.6% Limited) was PBS (vehicle control 2) were used to assess assay performance. One hour

after the anti-IGFBP5 mAbs (B06 and B09), DMSO or PBS addition, cells were triggered with 5 ng/mL TGF- β 1 (Cat. 240-B-010, R&D Systems) to induce Epithelial-to-Mesenchymal Transition (EMT). Three days after trigger addition, cells were fixed with 4% formaldehyde (Cat. 8187081000, Merck) and stained for fibronectin (FN1), a mesenchymal cell marker and nuclei (DAPI). For determination of FN1 expression, fixed HBECS were incubated in blocking buffer (PBS containing 5 mg/mL BSA (Cat. A2153, Sigma) and 0.2% (vol/vol) Triton X-100 (Cat. T8787, Sigma)) for one hour at room temperature. Cells were then incubated with a monoclonal anti-FN1 antibody (0.125 μ g/mL; Cat. 610001, Biohit healthcare) for one hour at room temperature, washed with 0.05% Tween-20 (Cat. P1379, Sigma) in PBS and incubated with an Alexa Fluor 546-conjugated donkey anti-mouse secondary antibody (4 μ g/mL; Cat. A10036, Life Technologies) for one hour at room temperature. Plates were then washed with 0.05% Tween in PBS, incubated with 4',6-diamidino-2-phenylindole (DAPI, 0.5 μ g/mL; Cat. D3571, Life Technologies) and imaged on the In Cell Analyzer 2200 (GE Healthcare). Expression was quantified using the IN-Cell developer software (GE Healthcare). FN1 expression is presented as the staining intensity multiplied by the stained area (DxA levels). Co-staining of cell nuclei with DAPI was performed to quantify cell number, as a measure of potential cytotoxicity.

After analysis, the anti-IGFBP5 mAb B06 showed a concentration-dependent inhibition of TGF- β 1-mediated FN1 expression in IPF patient samples of donors IPF05 and IPF06. Moreover, all the anti-IGFBP5 mAbs showed no modulation of the number of nuclei, indicative of absence of potential cytotoxic side-effects (figure 2).

INCORPORATION BY REFERENCE

All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

EQUIVALENTS

While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s). Many variations will become apparent to those skilled in the art upon review of this specification.