



(51) International Patent Classification:

C07K 16/18 (2006.01) A61K 39/00 (2006.01)
A61P 29/00 (2006.01)

(21) International Application Number:

PCT/US2020/042064

(22) International Filing Date:

15 July 2020 (15.07.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/874,340 15 July 2019 (15.07.2019) US

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(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN,
KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO,
NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW,
SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: METALLOTHIONEIN ANTIBODIES AND THEIR USE

(57) Abstract: Disclosed herein are isolated anti-metlothionein antibodies, or fragments thereof, and their use in treating intestinal inflammation, diabetes, pre-diabetes, impaired glucose tolerance, hepatitis, and/or inflammatory liver disease.



Metallothionein antibodies and their use

5

Cross-reference

This application claims priority to U.S. Provisional Patent Application Serial No. 62/874340 filed July 15, 2019, incorporated by referenced herein in its entirety.

Federal Funding Statement

This invention was made with government support under Grant No. ES07408, awarded by the National Institutes of Health. The government has certain rights in the invention.

Reference to Sequence Listing

This application contains a Sequence Listing submitted as an electronic text file named "19-1213-PCT_Sequence-Listing_ST25.txt", having a size in bytes of 21 kb, and created on July 13, 2020. The information contained in this electronic file is hereby incorporated by reference in its entirety pursuant to 37 CFR §1.52(e)(5).

20

Background

Diabetes is most often treated with insulin replacement. There is research being done to replace the islets that are damaged in diabetes, but unless the inflammatory processes that damage the islet producing insulin is arrested, islet transplants or stem cell repopulation of the islets in the pancreas will be short-lived treatments. Similarly, current reagents and methods for treating hepatitis and/or inflammatory liver disease are inadequate.

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Summary

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In one aspect, the disclosure provides isolated anti-metallothionein antibody, or fragment thereof, comprising 1, 2, or all 3, complementarity determining regions (CDRs) selected from the group consisting of:

Light chain CDR1 (L-CDR1) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence QSIVHSNGNTYLE (SEQ ID

35

NO:1);

Light chain CDR2 (L-CDR2) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence KVS (SEQ ID NO:2); and

Light chain CDR3 (L-CDR3) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence FQGSHVPF(A/T) (SEQ ID NO:3),
 5 and/or comprising 1, 2, or all 3, complementarity determining regions (CDRs) selected from the group consisting of:

Heavy chain CDR1 (H-CDR1) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence GFSLSRYG (SEQ ID NO:4);

Heavy chain CDR2 (H-CDR2) comprising an amino acid sequence at least 80%, 85%,
 10 90%, 95%, or 100% identical to the amino acid sequence IWSGGST (SEQ ID NO:5); and

Heavy chain CDR3 (H-CDR3) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence WLLPGY (SEQ ID NO:6).

In various embodiments, the antibody comprises a monoclonal antibody, or fragment thereof, and/or a humanized antibody, or fragment thereof.

15 In other embodiments, the isolated anti-metallothionein antibody, or fragment thereof, comprises a light chain comprising the amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:7-9, wherein the light chain comprises the amino acid of each of SEQ ID NOS:1-3, and wherein the light
 20 chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following residues: D1, V3, T5, S7, T14, Q17, P18, F41, Q42, R44, R50, V88, V90, T102, Q105, and K112; and/or comprises the amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:11-15, wherein the
 25 heavy chain comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or all 43 of the following residues: E1, V5, G9, K13, G15, E16 or G16, T17, R19, L20, S21, A23, S35, I37, P40 or A40, G42, I48 or V48, S49, N58, A60, P61 or
 30 D61, S62, L63 or V63, K64, G65, V67 or F67, T68, S70, V71, T73, A74, N76 or K76, S77, F78, S79 or Y79, L80, Q81, L82, S83 or N83, V85, T86 or R86, A87, A88 or E88, V92, and Q109.

In one embodiment, the isolated anti-metallothionein antibody or fragment thereof comprises a Fab' fragment.

In other aspects, the disclosure provides nucleic acids encoding the isolated antibodies or fragment thereof, vectors comprising the nucleic acids operatively linked to a suitable control sequence, host cells comprising the nucleic acids and/or the vectors, and pharmaceutical composition, comprising

(a) the isolated antibodies or fragments thereof, the nucleic acids, the vectors, or the host cells disclosed herein; and

(b) a pharmaceutically acceptable carrier.

In another aspect, the disclosure provides methods for treating or limiting development of a disorder selected from the group consisting of intestinal inflammation, diabetes, pre-diabetes, impaired glucose tolerance, hepatitis, and/or inflammatory liver disease, comprising administering a subject in need thereof an amount effective to treat the disorder of the isolated antibody or fragment thereof or a pharmaceutically acceptable salt thereof, the nucleic acid, the vector, the host cell, or the pharmaceutical composition of any embodiment or combination of embodiments disclosed herein.

Description of the Figures

Figure 1A-B. (A) Clustal Omega software comparison of humanized kappa light chains with original mouse UC1MT anti-MT L sequences. (B) Phylogram of the relative differences between the variable regions of UC1MT L-Hum1 (VK1), UC1MT L-Hum2 (VK2), and UC1MT L-Hum3 (VK3), in comparison to the sequence of the original hybridoma light chain sequence (UC1MT).

Figure 2A-B. (A) Clustal Omega software comparison of humanized heavy chain V regions with original mouse UC1MT anti-MT H sequence. (B) Phylogram of the relative differences between the humanized variable regions of UC1MT H-Hum1 (VH1), UC1MT H-Hum2 (VH2), UC1MT H-Hum3 (VH3), UC1MT H-Hum4 (VH4), and UC1MT L-Hum5 (VH5), in comparison to the sequence of the original hybridoma heavy chain V sequence (UC1MT)

Figure 3. ELISA measurements of humanized antibodies tested against immobilized metallothionein. Immulon 2HB plate was coated with 10ug/mL MT overnight at 4C, wells washed with PBST and blocked with 2% BSA for 1.5h at RT. Plate was washed,

coated with dilutions of each recombinant Fab fragment, and incubated for 1.5h at RT. Plate was washed and incubated with 1:1000 mouse anti-human Ig light chain kappa-biotin for 1.h at RT, then washed and incubated with streptavidin-HRP (wells containing Fab) for 20m at RT. After a final wash, substrate was added and incubated for 15m at RT before addition of
5 stop solution (2N H₂SO₄) and OD₄₅₀ measured in Spectramax plate reader. The chart represents OD₄₀₅ values. Each clone's reactivity was characterized as Low (L), Medium (M), or High (H).

Figure 4A-B. (A) Graph of Fab' fragment binding to metallothionein from in silico modeling. (B) Individual CDRs are indicated for both the H and L chain of a representative
10 humanized Fab fragment.

Detailed Description

As used herein and unless otherwise indicated, the terms "a" and "an" are taken to
15 mean "one", "at least one" or "one or more". Unless otherwise required by context, singular terms used herein shall include pluralities and plural terms shall include the singular. All embodiments of any aspect of the disclosure can be used in combination, unless the context clearly dictates otherwise.

As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A),
20 asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

25 In one aspect, the disclosure provides anti-metallothionein (MT) antibodies, or fragments thereof. The anti-metallothionein antibodies or fragments thereof are useful, for example, to treat disorders selected from the group consisting of intestinal inflammation, diabetes, pre-diabetes, impaired glucose tolerance, hepatitis, and/or inflammatory liver disease. The use of MT inhibitors for treating such disorders is described in
30 WO2019/143767, incorporated by reference herein in its entirety.

As disclosed herein, "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an epitope in metallothionein. As such, the term antibody encompasses not only whole antibody molecules, but also antibody

fragments as well as variants (including derivatives) of antibodies and antibody fragments. Such antibody or antibody fragments thereof may include, but are not limited to monoclonal antibodies, humanized antibodies, chimeric antibodies, Fab', F(ab')₂, Fab, Fv, rIgG, recombinant single chain Fv fragments (scFv), bivalent or bispecific molecules, diabodies, triabodies, and tetrabodies. In one specific embodiment, the antibodies comprise monoclonal antibodies, or fragments thereof. In a further specific embodiment, the antibodies comprise humanized antibodies, or fragments thereof. In one embodiment, the antibodies comprise Fab' fragments.

In one embodiment, the antibodies are humanized. As used herein, the term "humanized antibody" refers to a chimeric antibody which contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework ("FR") residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity, expression, and reduction in the immunogenicity of a xenogenic antibody, such as a murine antibody, for introduction into a human, while maintaining the full antigen binding affinity and specificity of the antibody.

As used herein, "isolated" means that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type.

In one embodiment, humanized antibodies or fragments thereof are provided, comprising 1, 2, or all 3, complementarity determining regions (CDRs) selected from the group consisting of:

Light chain CDR1 (L-CDR1) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence QSIVHSNGNTYLE (SEQ ID NO:1);

Light chain CDR2 (L-CDR2) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence KVS (SEQ ID NO:2); and

Light chain CDR3 (L-CDR3) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence FQGSHVPPF(A/T) (SEQ ID NO:3).

In another embodiment, humanized anti-metallothionein antibodies or fragment thereof, are provided comprising 1, 2, or all 3 complementarity determining regions (CDRs) selected from the group consisting of:

Heavy chain CDR1 (H-CDR1) comprising an amino acid sequence at least 80%, 85%,
5 90%, 95%, or 100% identical to the amino acid sequence GFSLSRYG (SEQ ID NO:4);

Heavy chain CDR2 (H-CDR2) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence IWSGGST (SEQ ID NO:5); and

Heavy chain CDR3 (H-CDR3) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence WLLPGY (SEQ ID NO:6).

10 In a further embodiment, the humanized anti-metallothionein antibody of claim 1, or fragment thereof, comprising 4, 5, or all 6 complementarity determining regions (CDRs) selected from the group consisting of:

Heavy chain CDR1 (H-CDR1) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence GFSLSRYG (SEQ ID NO:4);

15 Heavy chain CDR2 (H-CDR2) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence IWSGGST (SEQ ID NO:5); and

Heavy chain CDR3 (H-CDR3) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence WLLPGY (SEQ ID NO:6);

Light chain CDR1 (L-CDR1) comprising an amino acid sequence at least 80%, 85%,
20 90%, 95%, or 100% identical to the amino acid sequence QSIVHSNGNTYLE (SEQ ID
NO:1);

Light chain CDR2 (L-CDR2) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence KVS (SEQ ID NO:2); and

Light chain CDR3 (L-CDR3) comprising an amino acid sequence at least 80%, 85%,
25 90%, 95%, or 100% identical to the amino acid sequence FQGSHVPPF(A/T) (SEQ ID NO:3).

In another aspect, isolated anti-metallothionein antibodies, or fragments thereof, are provided that comprise a light chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence selected from the group consisting of SEQ ID NOS: 7-9, as
30 shown in Table 1 (see also Figure 1), wherein the light chain comprises the complementarity determining regions (CDRs) of each SEQ ID NOS:1-3, and wherein the light chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following

residues (noted in bold and enlarged font in Table 1): D1, V3, T5, S7, T14, Q17, P18, F41, Q42, R44, R50, V88, V90, T102, Q105, and K112.

Table 1

DV V M T Q S P L S L P V T L G Q P A S I S C R S S Q S I V H S N G N T Y L E W F Q Q R P G Q S P R L L I Y K V S N RFSGVDPDRFSGSGSGTDFTLKISRVEAED V G V Y Y C F Q G S H V P F T F G Q G T K L E I K UC1MT-L-Hum1 SEQ ID NO:7
DV V M T Q S P L S L P V T L G Q P A S I S C R S S Q S I V H S N G N T Y L E W Y L Q R P G Q S P R L L I Y K V S N RFSGVDPDRFSGSGSGTDFTLKISRVEAED V G V Y Y C F Q G S H V P F A F G Q G T K L E I K UC1MT-L-Hum2 SEQ ID NO:8
DV V M T Q S P L S L P V T L G Q P A S I S C R S S Q S I V H S N G N T Y L E W Y L Q R P G Q S P R L L I Y K V S N RFSGVDPDRFSGSGSGTDFTLKISRVEAED L G I Y Y C F Q G S H V P F A F G Q G T K L E I K UC1MT-L-Hum3 SEQ ID NO:9

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The bolded letters represent changes relative to the parental mouse hybridoma; the CDRs are underlined.

In one specific embodiment, the isolated anti-metallothionein antibody, or fragment thereof, comprises a light chain comprising an amino acid sequence at least 60%, 65%, 70%,
10 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence of SEQ ID NO:8, wherein the light chain comprises the CDRs of each of SEQ ID NOS:1-3, and wherein the light chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following residues (noted in bold and enlarged font in Table 1): D1, V3, T5, S7, T14, Q17, P18, R44, R50, V88, V90, Q105, and K112.

15 In another embodiment, the light chain of the isolated anti-metallothionein antibody, or fragment thereof further comprises a kappa constant region from a human light chain, including but not limited to an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of:

20 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:10)

In another aspect are provided isolated anti-metallothionein antibodies, or fragments thereof, comprising a heavy chain comprising an amino acid sequence at least 60%, 65%,
25 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 11-15, wherein the heavy chain comprises the complementarity determining regions (CDRs) of

each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or all 43 of the following residues (noted in bold and enlarged font in Table 2; see also Figure 2): E1, V5, G9, K13, G15, E16 or G16, T17, R19, L20, S21, A23, S35, I37, P40 or A40, G42, I48 or V48, S49, N58, A60, P61 or D61, S62, L63 or V63, K64, G65, V67 or F67, T68, S70, V71, T73, A74, N76 or K76, S77, F78, S79 or Y79, L80, Q81, L82, S83 or N83, V85, T86 or R86, A87, A88 or E88, V92, and Q109.

In the sequences that follow, the bolded letters represent changes relative to the parental UC1MT mouse hybridoma sequence; the CDRs are underlined.

10 **Table 2**

QVQLQESGPGGLV KPS ET LSLTCTVSGFSLSRYGVS W I RQ PP G KGLEW I GVIWSGGST N YN PS LKSR V T I S VDT SK N Q F SL K LS S V T AADTAVYYCAR W LL P GYFDVWG Q GTTVT VSS UC1MT-H-Hum1 (SEQ ID NO:11)
QVQLQESGPGGLV KPS ET LSITCTVSGFSLSRYG V HW I RQ PP G KGLEW I GVIWSGGSTDY N PS LKSR V T I S VDT SK N Q F SL K LS S V T AADTAVYYCAR W LL P GYFDVWG Q GTTVT SS UC1MT-H-Hum2 (SEQ ID NO:12)
QVQLQESGPGGLV KPS ET LSITCTVSGFSLSRYG V HWVRQ PP G KGLEW I GVIWSGGSTDY N PS F K SR V T I S KDNS K N Q V S F K LS S L T AADTAVYYCAR W LL P GYFDVWG Q GTTVT VS UC1MT-H-Hum3 (SEQ ID NO:13)
QVQLQESGPGGLV KPS ET LSITCTVSGFSLSRYG V HW V RQ SP G KGLEW L GVIWSGGSTDY NAA F K SRL S I S KDNS K N Q V F F K S SL T AADTARYYCAR W LL P GYFDVWG Q GTTVT VSS UC1MT-H-Hum4 (SEQ ID NO:14)
E VQL V ES G GGLVQ P G SL R LS C AVSGFSLSRYG V HWVRQ A P G KGLEW V S VIWSGGSTD Y A D S V K G R F T I SKDN A K S V Y L Q M N S L R AEDTAVYYCAR W LL P GYFDVWG Q GTTVT VSS UC1MT-H-Hum5 (SEQ ID NO:15)

In one specific embodiment, the isolated anti-metallothionein antibody, or fragment thereof, comprises a heavy chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid of SEQ ID NOS:14, wherein the heavy chain comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following residues (noted in bold and enlarged font in Table 2; see also Figure 2): K13, E16, V37, G42, K64, S70, N76, V78, S83, T86, A87, A88, and Q109.

In another specific embodiment, the isolated anti-metallothionein antibody, or fragment thereof, comprises a heavy chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

100% identical the amino acid sequence of SEQ ID NOS:15, wherein the heavy chain comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or all 34 of the following
 5 residues (noted in bold and enlarged font in Table 2; see also Figure 2): E1, V5, G9, G15, G16, R19, L20, S21, A23, A40, G42, V48, S49, A60, D61, S62, V63, K64, G65, F67, T68, A74, K76, S77, V78, Y79, L80, Q81, N83, R86, A87, E88, V92, and Q109.

In another embodiment, the heavy chain further comprises a human IgG1 constant sequence that includes a mutation that prevents glycosylation, including but not limited to the
 10 amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:27.

astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsгалtsgv htfpavlqss
 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkkvep kscdkthtcp pcpapellgg
 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreegyQ
 15 styrvvsvlt vlhqdwlngk eyckvsnka lpapiektis kakgqprepq vytlppsrd
 ltknqvsltc lvkgfyfysdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
 qqgnvfscsv mhealhnhyt qkslsislspgk (SEQ ID NO:16)

In a further embodiment, the isolated anti-metallothionein antibody, or fragment
 20 thereof, comprises:

(a) a light chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence selected from the group consisting of SEQ ID NOS:7-9, wherein the light chain comprises the complementarity determining regions (CDRs) of each SEQ ID
 25 NOS:1-3, and wherein the light chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following residues: D1, V3, T5, S7, T14, Q17, P18, F41, Q42, R44, R50, V88, V90, T102, Q105, and K112; and

(b) comprises a heavy chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or
 30 100% identical the amino acid sequence selected from the group consisting of SEQ ID NOS:16-26, or selected from the group consisting of SEQ ID NOS:11-15, wherein the heavy chain comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37,

38, 39, 40, 41, 42, or all 43 of the following residues (noted in bold and enlarged font in Table 2; see also Figure 2): E1, V5, G9, K13, G15, E16 or G16, T17, R19, L20, S21, A23, S35, I37, P40 or A40, G42, I48 or V48, S49, N58, A60, P61 or D61, S62, L63 or V63, K64, G65, V67 or F67, T68, S70, V71, T73, A74, N76 or K76, S77, F78, S79 or Y79, L80, Q81,
 5 L82, S83 or N83, V85, T86 or R86, A87, A88 or E88, V92, and Q109.

In another specific embodiment, the isolated anti-metallothionein antibody, or fragment thereof, comprises

- (a) a light chain comprising an amino acid sequence at least 60%, 65%, 70%,
 10 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence of SEQ ID NO:8, wherein the light chain comprises the CDRs of each of SEQ ID NOS:1-3, and wherein the light chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following residues (noted in bold and enlarged font in Table 1): D1, V3, T5, S7, T14, Q17, P18, R44, R50, V88, V90, Q105, and K112; and
- 15 (b) comprises a heavy chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence selected from the group consisting of SEQ ID NOS:14-15, wherein the heavy chain comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises
 - 20 (i) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following residues: K13, E16, V37, G42, K64, S70, N76, V78, S83, T86, A87, A88, and Q109; or
 - (ii) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or all 34 of the following residues (noted in bold and enlarged font in Table 2; see also Figure 2): E1, V5, G9, G15, G16, R19, L20, S21, A23,
 25 A40, G42, V48, S49, A60, D61, S62, V63, K64, G65, F67, T68, A74, K76, S77, V78, Y79, L80, Q81, N83, R86, A87, E88, V92, and Q109.

The anti-MT antibodies of the disclosure may be linked to any other moiety as deemed suitable for an intended purpose. In one embodiment, the isolated antibody or fragment thereof further comprises a detectable label. Such embodiments may be useful for
 30 monitoring a course of treatment, for example. Any suitable detectable label may be bound, either covalently, genetically, or by any other means, to the antibody, including but not limited to radioactive isotopes, fluorescent molecules, magnetic particles (including

nanoparticles), metal particles (including nanoparticles), phosphorescent molecules, and enzymes.

In another embodiment, the isolated antibody or fragment thereof further comprises an additional therapeutic agent. Any therapeutic agent suitable for an intended purpose may be linked either covalently, genetically, or by any other means, to the antibody, including but not limited to insulin, metformin, pramlintide, a sulfonylurea (including but not limited to glyburide, glipizide, and glimepiride), a meglitinide (including but not limited to repaglinide and nateglinide), a thiazolidinedione (including but not limited to rosiglitazone and pioglitazone), a DPP-4 inhibitor (including but not limited to sitagliptin, saxagliptin, and linagliptin), a GLP-1 receptor agonist (including but not limited to exenatide, liraglutide, and semaglutide), a SGLT2 inhibitor (including but not limited to canagliflozin, dapagliflozin, and empagliflozin), entecavir, tenofovir, lamivudine, adefovir, telbivudine, simeprevir, sofosbuvir, cytokines, chemokines, interferon or ribavirin. Such conjugates may be useful, for example, in the methods disclosed herein.

In another embodiment, the isolated antibody or fragment thereof further comprises a cell targeting moiety. Any cell targeting moiety may be used as deemed suitable for an intended cell target, including but not limited to a pancreatic or hepatic cell targeting moiety (such as a pancreatic cell targeting moiety comprising one or more peptides or other moieties that preferentially bind pancreatic β cells, including but not limited to glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), peptide YY (PYY), neuropeptide Y (NPY), pancreatic peptide (PPY), and exendin-4; or a hepatic cell targeting moiety, and wherein the hepatic cell targeting moiety includes but is not limited to circumsporozoite protein (CSP), CSP region I, CSP I plus, lactosaminated human serum albumin, glycosylated lipoprotein, and arabinogalactan). Such conjugates may be useful, for example, in the methods disclosed herein.

In another aspect isolated nucleic acids are disclosed encoding the antibody of any embodiment or combination of embodiments disclosed herein. The isolated nucleic acid sequence may comprise RNA or DNA. Such isolated nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals.

In a further aspect expression vectors comprising the isolated nucleic acid of the disclosure are provided. "Expression vectors" include vectors that operatively link a nucleic acid coding region or gene to any promoter capable of effecting expression of the gene product. The promoter sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline, ecdysone, steroid-responsive). The expression vector is replicable in a suitable host organism either as an episome or by integration into host chromosomal DNA. In various embodiments, the expression vector comprises a plasmid or viral vector.

In a further aspect, host cells comprising the antibody, nucleic acid, and/or vector of the disclosure are provided. The host cells can be either prokaryotic or eukaryotic. The cells can be transiently or stably transfected. In one embodiment, the cells are hybridoma cells that express and secrete antibodies of the present disclosure. Thus, the recombinant host cells can be used, for example in methods for producing antibody, comprising:

- (a) culturing the recombinant host cell under conditions suitable for expression of the nucleic-acid encoded antibody; and
- (b) isolating the antibody from the cultured cells.

Suitable conditions for expression of the nucleic-acid encoded antibody can be determined by those of skill in the art based on the teachings herein, the specific host cells and vectors used, and the general knowledge of those of skill in the art.

In another aspect pharmaceutical compositions are provided, comprising:

- (a) the antibody, isolated nucleic acid, expression vector, or host cell of any embodiment or combination of embodiments disclosed herein; and
- (b) a pharmaceutically acceptable carrier.

For example, the antibodies of the disclosure may be present in a pharmaceutical formulation. In this embodiment, the antibodies are combined with a pharmaceutically acceptable carrier. Suitable acids which are capable of forming such salts include inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming such salts include

inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanol-amines (e.g., ethanolamine, diethanolamine and the like).

5 The pharmaceutical composition may comprise in addition to the composition of the invention (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The pharmaceutical composition may also include a
10 lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the pharmaceutical composition includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the pharmaceutical composition
15 includes a bulking agent, like glycine. In yet other embodiments, the pharmaceutical composition includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate- 60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The pharmaceutical
20 composition may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the pharmaceutical composition additionally includes a stabilizer, e.g., a molecule which, when
25 combined with a protein of interest substantially prevents or reduces chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

 The pharmaceutical compositions of the invention may be made up in any suitable
30 formulation, preferably in formulations suitable for administration by injection. Such pharmaceutical compositions can be used, for example, in the therapeutic methods disclosed herein.

The pharmaceutical compositions may contain any other components as deemed appropriate for a given use, such as additional therapeutics. In one embodiment, the pharmaceutical compositions further comprise an additional therapeutic selected from the group consisting of insulin, metformin, pramlintide, a sulfonylurea (including but not limited to glyburide, glipizide, and glimepiride), a meglitinide (including but not limited to repaglinide and nateglinide), a thiazolidinedione (including but not limited to rosiglitazone and pioglitazone), a DPP-4 inhibitor (including but not limited to sitagliptin, saxagliptin, and linagliptin), a GLP-1 receptor agonist (including but not limited to exenatide, liraglutide, and semaglutide), a SGLT2 inhibitor (including but not limited to canagliflozin, dapagliflozin, and empagliflozin), entecavir, tenofovir, lamivudine, adefovir, telbivudine, simeprevir, sofosbuvir, cytokines, chemokines, interferon or ribavirin.

In another aspect, the disclosure provides methods for treating or limiting development of a disorder selected from the group consisting of intestinal inflammation, diabetes, pre-diabetes, impaired glucose tolerance, hepatitis, and/or inflammatory liver disease, comprising administering a subject in need thereof an amount effective to treat the disorder of the isolated antibody or fragment thereof, the nucleic acid, the vector, the host cell, and/or the pharmaceutical composition of any embodiment or combination of embodiments disclosed herein.

As used herein, a "therapeutically effective amount" refers to an amount of the composition that is effective for treating and/or limiting the relevant disorder.

As used herein, "treat" or "treating" means accomplishing one or more of the following in an individual that has one or more of the recited disorders: (a) reducing the severity of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in patients that have previously had the disorder(s); and/or (e) limiting or preventing recurrence of symptoms in patients that were previously symptomatic for the disorder(s). Any amount of such "treating" is of great benefit to a subject one of the recited disorders.

As used herein, "limit" or "limiting development of" means accomplishing one or more of the following in an individual that is at risk one or more of the recited disorders: (a) slowing progression to the disorder and/or (b) limiting or preventing development of symptoms characteristic of progression to the disorder. Any amount of such "limiting development" is of great benefit to a subject at risk of one of the recited disorders.

Such treating or limiting development of may comprise use of the extracellular MT inhibitor as the sole therapeutic, or may comprise its use to complement or augment other therapeutic interventions, as deemed appropriate by attending medical personnel.

In one embodiment, the subject has diabetes or is at risk of developing diabetes, and
5 the method serves to treat or limit development of diabetes.

In one such embodiment, the subject is at risk of type 1 diabetes, and the method serves to limit development of type I diabetes in the subject. As described in WO2019/143767, human MT1 inhibitors prevented development of type 1 diabetes in the NOD mouse model. Thus, the methods of this embodiment can be used to limit development
10 of type 1 diabetes (T1D) in subjects at risk of T1D. Limiting development of T1D may include, but is not limited to, slowing progression to T1D and/or slowing development of symptoms characteristic of T1D. In this embodiment, the subject at risk for T1D has one or more risk factor for T1D from which attending medical personnel deems the treatment to be appropriate. Such risk T1D risk factors include, but are not limited to: a parent or sibling
15 with type 1 diabetes, a pancreatic tumor, pancreatitis, pancreatic islet cell autoantibodies, insulin autoantibodies, glutamic acid decarboxylase autoantibodies (GADA), insulinoma-associated (IA-2) autoantibodies, zinc transporter autoantibodies (ZnT8), and/or variants of the IDDM1 gene selected from the group consisting of DRB1 0401, DRB1 0402, DRB1 0405, DQA 0301, DQB1 0302 and DQB1 0201. Alternatively, or in combination, the subject
20 may exhibit one or more symptom of T1D (but not yet be diagnosed with T1D); such symptoms may include, but are not limited to polyuria (increased urination), polydipsia (increased thirst), dry mouth, polyphagia (increased hunger), fatigue, and weight loss. As will be understood by those of skill in the art, any limit on the development of T1D or its symptoms provides a great benefit a subject at risk

25 The Insulin-dependent (type I) diabetes mellitus 1 (IDDM1) gene is located in the MHC Class II region on chromosome 6. Certain variants of this gene increase the risk for decreased histocompatibility characteristic of type 1 diabetes. Such variants include DRB1 0401, DRB1 0402, DRB1 0405, DQA 0301, DQB1 0302 and DQB1 0201. Similarly, the appearance of diabetes-related autoantibodies such as pancreatic islet cell autoantibodies,
30 insulin autoantibodies, glutamic acid decarboxylase autoantibodies (GADA), insulinoma-associated (IA-2) autoantibodies, zinc transporter autoantibodies (ZnT8) often predate the hyperglycemia diabetes type 1 before any hyperglycemia arises. The risk of T1D increases with the number of antibody types, and the time interval from emergence of autoantibodies to

clinically diagnosable T1D can be a few months in infants and young children, but in some people it may take years. Such autoantibodies can be detected by, for example, immunofluorescence or binding assays.

In another embodiment, the subject has type 1 diabetes, and the method serves to treat
5 type I diabetes in the subject. In this embodiment, the subject has already been diagnosed with T1D, and the methods can be used to treat T1D. T1D involves autoimmune destruction of beta cells in the pancreas, little to no insulin production, and hyperglycemia. Treating T1D thus involves administration of insulin to the subject. Subjects with T1D may have
10 symptoms or complications including but not limited to hypoglycemia, polyuria, polyphagia, polydipsia, weight loss, blurred vision, fatigue, decreased wound healing capability, urinary tract infections, sexual dysfunction, dry mouth, diabetic ketoacidosis, cardiovascular disease, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, stroke, kidney failure, and foot ulcers. In some cases, a subject having T1D may require a pancreatic or pancreatic islet transplant. Thus, in various embodiments, the treating may comprise one or more of:
15 reducing frequency of need for insulin injection; slowing development or progression of type 1 diabetes complications in the subject including but not limited to destruction of pancreatic beta cells, hyperglycemia, hypoglycemia, polyuria, polyphagia, polydipsia, weight loss, blurred vision, fatigue, decreased wound healing capability, urinary tract infections, sexual dysfunction, dry mouth, diabetic ketoacidosis, cardiovascular disease, diabetic nephropathy,
20 diabetic neuropathy, diabetic retinopathy, stroke, kidney failure, and foot ulcers; and delaying need for a pancreatic or pancreatic islet cell transplant. In one embodiment, the treating may comprise a reduction of 10%, 15%, 20% or greater in blood glucose levels (mg/dL), such as, for example, within 20-120 minutes after administration of the inhibitor.

In another embodiment, the subject is at risk of type 2 diabetes, and the method serves
25 to limit development of type 2 diabetes (T2D) in the subject. T2D is a metabolic disorder characterized by hyperglycemia, insulin resistance, and relative lack of insulin. Symptoms and/or complications include, but are not limited to, hypoglycemia, insulin resistance, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, proteinuria, impaired glomerular clearance, diabetic circulatory disorders, kidney failure, cardiovascular disease,
30 polyuria, polydipsia, weight loss, and stroke. In one embodiment, the limiting development of type 2 diabetes may comprise a reduction of 10%, 15%, 20% or greater in blood glucose levels (mg/dL), such as, for example, within 20-120 minutes after administration of the inhibitor.

Risk factors for T2D include, but are not limited to, obesity, smoking, a sedentary lifestyle, a parent or sibling with type 2 diabetes, pre-diabetes, a parent or sibling with pre-diabetes, poor eating habits (ex: too much fat, not enough fiber, too many simple carbohydrates, etc.), age 50 or older, high blood pressure, high cholesterol, testosterone deficiency, metallothionein 1 A (MT1A) rs8052394 locus (G alteration) single nucleotide polymorphism, and a history of gestational diabetes. Thus, in various embodiments the subject has one or more of these risk factors, and the method serves to slow progression to T2D and/or (b) limit or prevent development of symptoms characteristic of T2D.

In another embodiment, the subject has T2D, and the method serves to treat T2D in the subject. In this embodiment, the treating may comprise limiting one or more of type 2 diabetic complications, including but not limited to hyperglycemia, hypoglycemia, insulin resistance, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, proteinuria, impaired glomerular clearance, diabetic circulatory disorders, kidney failure, cardiovascular disease, polyuria, polydipsia, weight loss, stroke, and reducing frequency of need for insulin or other therapy. Any amount of limiting of these symptoms/complications is of great benefit to a subject with T2D. In one embodiment, the treating may comprise a reduction of 10%, 15%, 20% or greater in blood glucose levels (mg/dL), such as, for example, within 20-120 minutes after administration of the inhibitor.

In another embodiment, the subject has pre-diabetes, and the method serves to treat pre-diabetes in the subject. In this embodiment, the subject is one who has pre-diabetes. As used herein, "pre-diabetes" is the state in which some but not all of the diagnostic criteria for diabetes are met. Thus, the prediabetic subject may: (a) have impaired fasting glucose tolerance, which is a condition whereby the response of beta cells to an oral glucose challenge (OGT) is deficient or (b) may have consistently elevated fasting glucose (IFG), which is a condition in which the fasting blood glucose is elevated above what is considered normal levels but is not high enough to be classified as diabetes mellitus. The pre-diabetic state may be associated with insulin resistance and increased risk of cardiovascular pathology. Individuals with a pre-diabetic state are at a relatively high risk of developing T2D. The methods of the disclosure can be used to treat a subject with pre-diabetes by, for example, limiting or slowing progression of one or more complications of pre-diabetes, including but not limited to T2D, hyperglycemia, insulin resistance, and/or cardiovascular disease. . In one embodiment, the treating may comprise a reduction of 10%, 15%, 20% or

greater in blood glucose levels (mg/dL), such as, for example, within 20-120 minutes after administration of the inhibitor.

In another embodiment, the subject has impaired glucose intolerance, and the method serves to treat impaired glucose tolerance in the subject. As used herein, impaired glucose tolerance is defined as two-hour glucose levels of 140 to 199 mg per dL (7.8 to 11.0 mmol/l) on the 75-g oral glucose tolerance test. A patient is said to be under the condition of IGT when he/she has an intermediately raised glucose level after 2 hours, but less than the level that would qualify for type 2 diabetes mellitus. The fasting glucose may be either normal or mildly elevated. Impaired glucose tolerance is a pre-diabetic state of hyperglycemia that is associated with insulin resistance and increased risk of cardiovascular pathology. IGT may precede type 2 diabetes mellitus by many years. In this embodiment, the treating may comprise limiting or slowing progression of one or more complications of impaired glucose tolerance, including but not limited to type 2 diabetes, hyperglycemia, insulin resistance, and/or cardiovascular disease. In one embodiment, the treating may comprise a reduction of 10%, 15%, 20% or greater in blood glucose levels (mg/dL), such as, for example, within 20-120 minutes after administration of the inhibitor.

In another embodiment, the subject has hepatitis, and the method serves to treat hepatitis in the subject. As described in WO2019/143767, MT inhibitors are effective in limiting tissue inflammation, and decreased pro-inflammatory cytokines MCP-1 and TNF- α while increasing the anti-inflammatory IL-10 signal in liver tissue. Hepatitis is an inflammation of the liver tissue. Symptoms include, but are not limited to yellow discoloration of the skin and whites of the eyes, poor appetite, vomiting, tiredness, abdominal pain, diarrhea, acute liver failure, scarring of the liver, liver failure, or liver cancer. The most common causes of hepatitis are viral infections (types A, B, C, D, and E), heavy alcohol use, certain medications, toxins, other infections, autoimmune diseases, and non-alcoholic steatohepatitis (NASH). Thus, in various embodiments the treating may comprise limiting or slowing progression of one or more complications of hepatitis, including but not limited to yellow discoloration of the skin and/or whites of the eyes, poor appetite, vomiting, fatigue, abdominal pain, diarrhea, acute liver failure, scarring of the liver, liver failure, and liver cancer.

In a further embodiment, the subject has an inflammatory liver disease, and the method serves to treat the inflammatory liver disease in the subject. As used herein, "Inflammatory liver disease" is a condition associated with intracytoplasmic accumulation of

large vacuoles of triglyceride fat in liver cells via steatosis (i.e., abnormal retention of lipids within a cell). The liver plays a large role in systemic metabolism and energy imbalance is particularly associated with defects in liver lipid metabolism. Specifically, obesity and insulin resistance are often associated by increased lipid deposition in the liver characteristic of nonalcoholic fatty liver disease (NAFLD). Although lipid metabolism is highly dynamic, chronic lipid overload causes tissue damage in the liver resulting in recruitment of liver-resident and non-resident immune cells which can cause fibrosis characteristic of nonalcoholic steatohepatitis (NASH). Liver fibrosis can lead to cirrhosis, cancer and significantly increases the risk of cardiovascular disease. This raises the potential for blocking recruitment of immune cells to the liver to ameliorate the risks of non-alcoholic fatty liver disease (NAFLD). As shown in the examples that follow, MT inhibitor treatment was responsible for an increase in the wet tissue weight of epididimal white adipose tissue, and decreased total triglyceride levels, as well as decreased pro-inflammatory cytokines MCP-1 and TNF- α while increasing the anti-inflammatory IL-10 signal. The inflammatory liver disease may be steatosis (non-alcoholic fatty liver (NAFL)). In another embodiment, fatty liver disease may non-alcoholic fatty liver disease (NAFLD), including but not limited to non-alcoholic steatohepatitis (NASH), the most extreme form of NAFLD. NAFLD is one of the types of inflammatory liver disease which occurs when fat is deposited (steatosis) in the liver due to causes other than excessive alcohol use. Symptoms of NASH and NAFLD may include, but are not limited to, fatigue, malaise, dull right-upper-quadrant abdominal discomfort, mild jaundice, and abnormal liver function tests during routine blood tests; complications of NASH and NAFLD may include, but are not limited to hepatic fibrosis, hepatic cancer, and/or cirrhosis of the liver. Thus, in one embodiment, the inflammatory liver disease is selected from the group consisting of nonalcoholic steatohepatitis (NASH) and/or non-alcoholic fatty liver disease (NAFLD). In a further embodiment, the treating comprises limiting or slowing progression of one or more complications of inflammatory liver disease including but not limited to fatigue, malaise, hepatic fibrosis, hepatic cancer, and/or cirrhosis of the liver.

In another embodiment, the subject has intestinal inflammation. Such intestinal inflammation may include, but is not limited to Crohn's disease, ulcerative colitis, collagenous-, lymphocytic-, ischemic-, diversion- and indeterminate colitis, and Behcet's disease.

In one embodiment, the MT inhibitor, including but not limited to an anti-MT antibody, may be linked to a pancreatic cell targeting moiety, to specifically target pancreatic cells producing MT, such as pancreatic beta cells. This embodiment will be particularly useful for treating or limiting development of T1D, T2D, pre-diabetes, and/or impaired glucose tolerance. In one embodiment, the pancreatic β cell specific targeting moiety comprises one or more peptides or other moieties that preferentially bind pancreatic β cells, including but not limited to glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), peptide YY (PYY), neuropeptide Y (NPY), pancreatic peptide (PPY), and exendin-4. Exemplary amino acid sequences of such targeting moieties are provided in Table 3.

Table 3

Glucagon-like peptide 1; GLP1(aa92-128) HDEFERHAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG (SEQ ID NO:17)
Glucagon-like peptide 2; GLP2(aa146-178) HADGSFSDEMNTILDNLAARDFINWLIQTKITD (SEQ ID NO:18)
pancreatic peptide (PPY) MAAARLCLSLLLLSTCVALLLQPLLGAQGAPLEPVYPGDNATPEQMAQYAADLRRYINMLTRPRYGKRHKEDTLA FSEWGSPPHAAVPRELSPLDL (SEQ ID NO:19)
neuropeptide Y (NPY) MLGNKRLGLSGLTLALSLLVCLGALAEAYPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRYGKRSSPETLIS DLLMRESTENVPRTRLEDPAW (SEQ ID NO:20)
peptide YY (PYY) MVFVRRPWPALTTVLLALLVCLGALVDAYPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRYGKRDPDTLLS KTFFPDGEDRPVRSRSEGPDLW (SEQ ID NO: 21)
exendin-4 MKIILWLCVFGFLATLFPISWQMPVESGLSSEDSASSESFASKIKRHGEGTFTSDLSKQMEEEAVRLFIEWLKN GGPSSGAPPPSG (SEQ ID NO: 22)

Attaching the pancreatic cell targeting moiety to the MT inhibitor, including but not limited to the MT antibody or fragment thereof, may be accomplished by any chemical reaction that will bind the two molecules so long as the pancreatic cell targeting moiety and the MT antibody or fragment thereof, retain their respective activities. In one embodiment where the extracellular MT inhibitor comprises an antibody or fragment thereof, the composition comprises a recombinant fusion protein. In other embodiments, a linkage between the pancreatic cell targeting moiety and the MT antibody or fragment thereof can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. Covalent binding can be achieved either

by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies, to other molecules. For example, representative, non-limiting examples of coupling agents can be organic compounds such as thioesters, carbodiimides, succinimide esters, disocyanates, glutaraldehydes, diazobenzenes and hexamethylene diamines.

In all embodiments, the subject may be any subject that can benefit from the methods of treatment disclosed herein, including mammals, humans, cattle, dogs, cats, horses, chickens, and so on. In one embodiment, the subject is human.

The compositions for administration are typically formulated as a pharmaceutical composition to include a pharmaceutically acceptable carrier. Suitable acids which are capable of forming pharmaceutically acceptable salts include inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming such salts include inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanol-amines (e.g., ethanolamine, diethanolamine and the like).

The pharmaceutical composition may comprise in addition to the composition and carrier (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The pharmaceutical composition may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the pharmaceutical composition includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the pharmaceutical composition includes a bulking agent, like glycine. In yet other embodiments, the pharmaceutical composition includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate- 60,

polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The pharmaceutical composition may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the pharmaceutical composition additionally includes a stabilizer, e.g., a molecule which, when combined with a protein-based composition substantially prevents or reduces chemical and/or physical instability of the protein in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

The compositions can be administered via any suitable route, including orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intra-arterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). A suitable dosage range may, for instance, be 0.1 ug/kg-100 mg/kg body weight; alternatively, it may be 0.5 ug/kg to 50 mg/kg; 1 ug/kg to 25 mg/kg, or 5 ug/kg to 10 mg/kg body weight. The compositions can be delivered in a single bolus, or may be administered more than once (e.g., 2, 3, 4, 5, or more times) as determined by attending medical personnel. The compositions may be the sole therapeutic administered, or may be administered together with one or more other therapeutic (either separately or as a combination) as deemed appropriate by attending medical personnel. In one non-limiting embodiment, the subject has or is at risk of T1D and the inhibitor may be used together with one or more of insulin, metformin, or pramlintide. In another embodiment, the subject has or is at risk of T2D, pre-diabetes, and/or impaired glucose tolerance, and the inhibitor may be used together with one or more of metformin, sulfonylureas (including but not limited to glyburide, glipizide, and glimepiride), meglitinides (including but not limited to repaglinide and nateglinide), thiazolidinediones (including but not limited to rosiglitazone and pioglitazone), DPP-4 inhibitors (including but not limited to sitagliptin, saxagliptin, and linagliptin), GLP-1 receptor agonists (including but not limited to exenatide, liraglutide, and

semaglutide), SGLT2 inhibitors (including but not limited to canagliflozin, dapagliflozin, and empagliflozin), or insulin. In one non-limiting embodiment, the subject has or is at risk of hepatitis, and the inhibitor may be used in combination with one or more of entecavir, tenofovir, lamivudine, adefovir, telbivudine, simeprevir, sofosbuvir, interferon or ribavirin.

- 5 In another embodiment, compositions are provided comprising
- (a) an anti-metallothionein antibody disclosed herein; and
 - (b) one or more of insulin, metformin, pramlintide, a sulfonylurea (including but not limited to glyburide, glipizide, and glimepiride), a meglitinide (including but not limited to repaglinide and nateglinide), a thiazolidinedione (including but not limited to rosiglitazone and pioglitazone), a DPP-4 inhibitor (including but not limited to sitagliptin, saxagliptin, and linagliptin), a GLP-1 receptor agonist (including but not limited to exenatide, liraglutide, and semaglutide), a SGLT2 inhibitor (including but not limited to canagliflozin, dapagliflozin, and empagliflozin), entecavir, tenofovir, lamivudine, adefovir, telbivudine, simeprevir, sofosbuvir, interferon or ribavirin. The compositions may be used, for example, in the
- 10 methods of the disclosure. All embodiments and combinations of embodiments of antibodies disclosed above are suitable for inclusion in the compositions of this aspect. In one embodiment, the anti-MT antibody or an antigen-binding fragment thereof comprises a monoclonal antibody or an antigen binding fragment thereof. In another embodiment the anti-MT antibody comprises a humanized anti-MT antibody, or an antigen binding fragment
- 15 thereof.

In another aspect, the disclosure provides compositions, comprising:

- (a) an anti-metallothionein antibody disclosed herein; and
 - (b) a pancreatic or hepatic cell targeting moiety linked to the inhibitor of extracellular human MT.
- 20
- 25 The compositions may be used, for example, in the methods of the disclosure. All embodiments and combinations of embodiments of antibodies disclosed above are suitable for inclusion in the compositions of this aspect. In one embodiment, the anti-MT antibody or an antigen-binding fragment thereof comprises a monoclonal antibody or an antigen binding fragment thereof. In another embodiment the anti-MT antibody comprises a humanized anti-
- 30 MT antibody, or an antigen binding fragment thereof.

In one embodiment, the cell targeting moiety is a hepatic cell targeting moiety. In exemplary such embodiments, wherein the hepatic cell targeting moiety includes but is not limited to circumsporozoite protein (CSP), CSP region I, CSP region I-plus, lactosaminated

human serum albumin, glycosylated lipoprotein, and/or arabinogalactan. In one embodiment, the hepatic cell binding moiety is peptidic and selected from CSP, CSP region I, CSP region I plus, or hepatic cell binding fragments thereof. CSP targets *Plasmodium* sporozoite to the liver is attributed to the circumsporozoite protein (CSP), which is present on the surface of *Plasmodium* sporozoite (Rathore D, et al. *The Journal of Biological Chemistry*. 2005;280(21):20524–20529) CSP is approximately 400 amino acids long organized into three domains: the N-terminal domain containing a conserved KLKQP motif named “region I”, a highly repetitive central domain, and a C-terminal domain containing another conserved sequence named “region II” (Singh et al. *Cell*. 2007;131(3):492–504). In addition to the conserved region I KLKQP sequence, the N-terminal region also contains upstream from region I, two consensus heparin sulfate binding sequences. Peptides containing both the conserved region I amino acids and two consensus heparin binding sequences upstream from region I have been named “region I-plus” (Prudêncio et al., *Nature Reviews Microbiology*. 2006;4(11):849–856).

In another embodiment, the cell targeting moiety is a pancreatic cell targeting moiety, including but not limited to glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), peptide YY (PYY), neuropeptide Y (NPY), pancreatic peptide (PPY), and exendin-4; exemplary amino acid sequences for such peptides are described above.

Attaching the cell targeting moiety to the anti-MT antibody or fragment thereof, may be accomplished by any chemical reaction that will bind the two molecules so long as the cell targeting moiety and the anti-MT antibody or fragment thereof, retain their respective activities. In one embodiment, the composition comprises a recombinant fusion protein. In other embodiments, a linkage between the cell targeting moiety and anti-MT antibody or fragment thereof can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies, to other molecules. For example, representative, non-limiting examples of coupling agents can be organic compounds such as thioesters, carbodiimides, succinimide esters, disocyanates, glutaraldehydes, diazobenzenes and hexamethylene diamines.

In all embodiments, the subject may be any subject that can benefit from the methods of treatment disclosed herein, including mammals, humans, cattle, dogs, cats, horses, chickens, and so on. In one embodiment, the subject is human.

5

Examples

Humanizing the UC1MT monoclonal anti-metallothionein antibody

The mouse UC1MT monoclonal anti-metallothionein (MT) antibody was humanized using sequences PromabKV6,10 (light chain) and MHC2822HC/Promab (heavy chain). Three human light chain sequences and 5 human heavy chain sequences were designed. The sequences were roughly designed in a nested manner, from more to fewer changes. The heavy chain and light chain designs were tested in all possible pairwise combinations along with the parental V regions, resulting in 24 combinations. The constant regions used were consensus human heavy chains and are shared by all of the heavy and light chains used in these permutations.

Parental sequences of the original UC1MT mouse hybridoma (of the V region of the heavy and light chains)

20

A. Primary UC1MT hybridoma source sequences for the heavy chain (ProMab)

GSEVQLQESGPGGLVQPSQSL SITCTVSGFSLSR YGVHWRQSPVKGLEWLGVIWSGGSTDYNAAFISR
LSINKDNSKSQVFFKMHS LQTYDTARYYCARW LLPGYFDVWGAGTTVTVSS (SEQ ID NO:23)

25

(Lake MHC2822HC replicated sequence to original ProMab sequence):

QVQLTQSGPGLVQPSQSL SITCTVSGFSLSR YGVHWRQSPVKGLEWLGVIWSGGSTDYNAAFISRLS
INKDNSKSQVFFKMHS LQTYDTARYYCARW LLPGYFDVWGAGTTVTVSS (SEQ ID NO:24)

Light chain source sequence from original UC1MT hybridoma for the CDRs (underlined)

AVLMSQTPLSLPVS LGDQASISCRSSQSI VHSNGNTYLEWYLQKPGQSPKLLIYKVS NRFGVPDRFS
GSGSGTDFTLKISRVEAEDLGIYYCFQGSHPV FAFGSGTKLEIE (SEQ ID NO:25)

35

Sequence design considerations

The starting sequences were first compared to the mouse germline V regions to identify potential mutations in the framework regions that could cause poor folding. The heavy chain was most similar to the murine IGHV2-2 germline sequence and J1 J chain. Four mutations in the framework regions were noted. The light chain was most similar to the murine Ig kappa light chains IGKV6-13 and IGKV6-15. Six mutations were noted. Also, there appeared to have been a cross-over event between IGKV6-13 and IGKV6-15 near the 5' end of the coding sequence to generate the final light chain sequence. The J5 light chain murine J region appears to have been used.

Sequences were mutated for codon usage in human immunoglobulins, sequences were also selected with small, medium and larger numbers of modifications, and sequences were mutated to orient the antigen binding regions (ABR)/complementary determining regions (CDR) toward the antigen binding pocket of the antibody

Each of these 3 humanized light chains and 5 humanized heavy chains generated was co-expressed in H,L pairwise fashion in HEK 294 cells, and immunoglobulin was affinity-purified from these cells. These antibodies were tested against MT in a standard ELISA, and results are shown in Figure 3. Combinations of humanized heavy and light chain bind to metallothionein, with some differences in the binding potential between the different permutations of H and L chains. When the parental sequences of mouse H and L V regions are combined with human immunoglobulin H and L constant regions, the antibodies perform similarly, so variations in the constant regions do not significantly contribute to antigen recognition.

We used software to identify the antigen binding regions complementarity determining regions (CDRs), which are described herein as SEQ ID NOS:1-6. (See Kunik V, et al., (2012). PLoS Comput Biol 8(2): e1002388.

doi:10.1371/journal.pcbi.1002388; Kunik V, et al., Y (2012). Nucleic Acids Res. 2012 Jul;40(Web Server issue):W521-4.)

We evaluated the humanized antibodies showing the best reactivity with MT for their binding to other metalloproteins (alcohol dehydrogenase (ADH), carbonic anhydrase (CA), superoxide dismutase (SOD), Cytochrome C Oxide (CC), metallothionein (MT) and human insulin). All of the tested antibodies retain high specificity for MT (data not shown). Finally, Fab' fragments were made of the best binding versions of heavy and light chain

combinations, and binding to metallothionein tested. Data is presented in Figure 4, showing that they bind to metallothionein with improved binding relative to Fab' fragments of parental UC1MT.

We claim

1. An isolated anti-metallothionein antibody, or fragment thereof, comprising 1, 2, or all
3, complementarity determining regions (CDRs) selected from the group consisting of:
 - 5 Light chain CDR1 (L-CDR1) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence QSIVHSNGNTYLE (SEQ ID
NO:1);
Light chain CDR2 (L-CDR2) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence KVS (SEQ ID NO:2); and
 - 10 Light chain CDR3 (L-CDR3) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence FQGSHVPF(A/T) (SEQ ID NO:3).
2. An isolated anti-metallothionein antibody, or fragment thereof, comprising 1, 2, or all
3 complementarity determining regions (CDRs) selected from the group consisting of:
 - 15 Heavy chain CDR1 (H-CDR1) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence GFSLSRYG (SEQ ID NO:4);
Heavy chain CDR2 (H-CDR2) comprising an amino acid sequence at least 80%, 85%,
90%, 95%, or 100% identical to the amino acid sequence IWSGGST (SEQ ID NO:5); and
Heavy chain CDR3 (H-CDR3) comprising an amino acid sequence at least 80%, 85%,
20 90%, 95%, or 100% identical to the amino acid sequence WLLPGY (SEQ ID NO:6).
3. The isolated anti-metallothionein antibody of claim 1 or 2, or fragment thereof,
comprising 4, 5, or all 6 complementarity determining regions (CDRs) selected from the
group consisting of SEQ ID NOS:1-6.
25
4. The isolated antibody or fragment thereof of any one of claims 1-3, wherein the
antibody comprises a monoclonal antibody, or fragment thereof.
5. The isolated antibody or fragment thereof of any one of claims 1-4, wherein the
30 antibody comprises a humanized antibody, or fragment thereof.
6. The isolated anti-metallothionein antibody, or fragment thereof, of any one of claims
1-5, comprising a light chain comprising the amino acid sequence at least 60%, 65%, 70%,

75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:7-9, wherein the light chain comprises the amino acid of each of SEQ ID NOS:1-3, and wherein the light chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following residues: D1, V3, T5, S7, T14, Q17, P18, F41, Q42, R44, R50, V88, V90, T102, Q105, and K112.

7. The isolated anti-metallothionein antibody, or fragment thereof of claim 6, comprising a light chain comprising the amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:8, wherein the light chain comprises the CDRs of SEQ ID NOS:1-3, and wherein the light chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following residues D1, V3, T5, S7, T14, Q17, P18, R44, R50, V88, V90, Q105, and K112.

8. The isolated anti-metallothionein antibody, or fragment thereof of claim 6 or 7, further comprising a kappa constant region from a human light chain, including but not limited to the amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:10.

9. The isolated anti-metallothionein antibody, or fragment thereof, of any one of claims 1-8, comprising a heavy chain comprising the amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:11-15, wherein the heavy chain comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or all 43 of the following residues: E1, V5, G9, K13, G15, E16 or G16, T17, R19, L20, S21, A23, S35, I37, P40 or A40, G42, I48 or V48, S49, N58, A60, P61 or D61, S62, L63 or V63, K64, G65, V67 or F67, T68, S70, V71, T73, A74, N76 or K76, S77, F78, S79 or Y79, L80, Q81, L82, S83 or N83, V85, T86 or R86, A87, A88 or E88, V92, and Q109.

10. The isolated anti-metallothionein antibody or fragment thereof of claim 9, comprising a heavy chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid
 5 sequence selected from the group consisting of SEQ ID NOS:14, wherein the heavy chain comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13, 14 of the following residues: K13, E16, V37, G42, K64, S70, N76, V78, S83, T86, A87, A88, and Q109.
- 10
11. The isolated anti-metallothionein antibody or fragment thereof of claim 9 or 10, comprising a heavy chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence selected from the group consisting of SEQ ID NOS:15, wherein the
 15 heavy chain comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or all 43 of the following residues: E1, V5, G9, K13, G15, E16 or G16, T17, R19, L20, S21, A23, S35, I37, P40 or A40, G42, I48 or V48, S49, N58, A60, P61 or
 20 D61, S62, L63 or V63, K64, G65, V67 or F67, T68, S70, V71, T73, A74, N76 or K76, S77, F78, S79 or Y79, L80, Q81, L82, S83 or N83, V85, T86 or R86, A87, A88 or E88, V92, and Q109.
12. The isolated anti-metallothionein antibody or fragment thereof of any one of claims 9-
 25 11, wherein the heavy chain further comprises a human IgG1 constant sequence that includes a mutation that prevents glycosylation, including but not limited to the amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:16.
13. The isolated anti-metallothionein antibody or fragment thereof of any one of claims 1-
 30 12, comprising:
- (a) a light chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical

the amino acid sequence selected from the group consisting of SEQ ID NOS:7-9, wherein the light chain comprises the amino acid of each of SEQ ID NOS:1-3, and wherein the light chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following residues: D1, V3, T5, S7, T14, Q17, P18, F41, Q42, R44, R50, V88, V90, T102,

5 Q105, and K112; and

(b) a heavy chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence selected from the group consisting of SEQ ID NOS:16-26, or selected from the group consisting of SEQ ID NOS:11-15, wherein the heavy chain

10 comprises the complementarity determining regions (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or all 43 of the following residues: E1, V5, G9, K13, G15, E16 or G16, T17, R19, L20, S21, A23, S35, I37, P40 or A40, G42, I48 or V48, S49, N58, A60, P61 or D61, S62, 15 L63 or V63, K64, G65, V67 or F67, T68, S70, V71, T73, A74, N76 or K76, S77, F78, S79 or Y79, L80, Q81, L82, S83 or N83, V85, T86 or R86, A87, A88 or E88, V92, and Q109.

14. The isolated anti-metallothionein antibody or fragment thereof of any one of claims 1-13, comprising:

20 (a) a light chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence of SEQ ID NO:8, wherein the light chain comprises the CDRs of SEQ ID NOS:1-3, and wherein the light chain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following residues D1, V3, T5, S7, T14, Q17, P18, R44, R50, V88, 25 V90, Q105, and K112; and

(b) comprises a heavy chain comprising an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical the amino acid sequence selected from the group consisting of SEQ ID NOS:14-15, wherein the heavy chain comprises the complementarity determining regions

30 (CDRs) of each of SEQ ID NOS:4-6, and wherein the heavy chain comprises

(i) at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following residues: K13, E16, V37, G42, K64, S70, N76, V78, S83, T86, A87, A88, and Q109; or

(ii) at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or all 43 of the following residues: E1, V5, G9, K13, G15, E16 or G16, T17, R19, L20, S21, A23, S35, I37, P40 or A40, G42, I48 or V48, S49, N58, A60, P61 or D61, S62, L63 or V63, K64, G65, V67 or F67, T68, S70, V71, T73, A74, N76 or K76, S77, F78, S79 or Y79, L80, Q81, L82, S83 or N83, V85, T86 or R86, A87, A88 or E88, V92, and Q109.

15. The isolated anti-metallothionein antibody or fragment thereof of claim 13 or 14, wherein the antibody comprises a monoclonal antibody, or fragment thereof, and/or a humanized antibody or fragment thereof.

16. The isolated anti-metallothionein antibody or fragment thereof of any one of claims 1-14, wherein the antibody comprises a Fab' fragment.

17. The isolated antibody or fragment thereof of any one of claims 1-16, further comprising a detectable label.

18. The isolated antibody or fragment thereof of any one of claims 1-17, further comprising (a) a therapeutic agent conjugated to the antibody or fragment thereof, including but not limited to insulin, metformin, pramlintide, a sulfonylurea (including but not limited to glyburide, glipizide, and glimepiride), a meglitinide (including but not limited to repaglinide and nateglinide), a thiazolidinedione (including but not limited to rosiglitazone and pioglitazone), a DPP-4 inhibitor (including but not limited to sitagliptin, saxagliptin, and linagliptin), a GLP-1 receptor agonist (including but not limited to exenatide, liraglutide, and semaglutide), a SGLT2 inhibitor (including but not limited to canagliflozin, dapagliflozin, and empagliflozin), entecavir, tenofovir, lamivudine, adefovir, telbivudine, simeprevir, sofosbuvir, cytokines, chemokines, interferon or ribavirin, and/or

(b) a cell targeting moiety conjugated to the antibody or fragment thereof, including but not limited to a pancreatic or hepatic cell targeting moiety (including but not limited to a pancreatic cell targeting moiety comprising one or more peptides or other moieties that preferentially bind pancreatic β cells, including but not limited to glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), peptide YY (PYY), neuropeptide Y (NPY), pancreatic peptide (PPY), and exendin-4; or a hepatic cell targeting moiety, and wherein the

hepatic cell targeting moiety includes but is not limited to circumsporozoite protein (CSP), CSP region I, CSP I plus, lactosaminated human serum albumin, glycosylated lipoprotein, and arabinogalactan).

- 5 19. A nucleic acid encoding the isolated antibody or fragment thereof of any one of claims 1-18.
20. A vector comprising the nucleic acid of claim 19 operatively linked to a suitable control sequence.
- 10 21. A host cell comprising the nucleic acid of claim 19 and/or the vector of claim 20.
22. A pharmaceutical composition, comprising
- (a) the isolated antibody or fragment thereof of any one of claims 1-18 or a
- 15 pharmaceutically acceptable salt thereof, the nucleic acid of claim 19, the vector of claim 20, or the host cell of claim 21; and
- (b) a pharmaceutically acceptable carrier.
23. A method for treating or limiting development of a disorder selected from the group
- 20 consisting of intestinal inflammation, diabetes, pre-diabetes, impaired glucose tolerance, hepatitis, and/or inflammatory liver disease, comprising administering a subject in need thereof an amount effective to treat the disorder of the isolated antibody or fragment thereof of any one of claims 1-18 or a pharmaceutically acceptable salt thereof, the nucleic acid of claim 19, the vector of claim 20, the host cell of claim 21, or the pharmaceutical composition
- 25 of claim 22.
24. The method of claim 23, wherein the subject has diabetes or is at risk of developing diabetes, and the method serves to treat or limit development of diabetes.
- 30 25. The method of claim 24, wherein the subject is at risk of type 1 diabetes, and the method serves to limit development of type I diabetes in the subject.

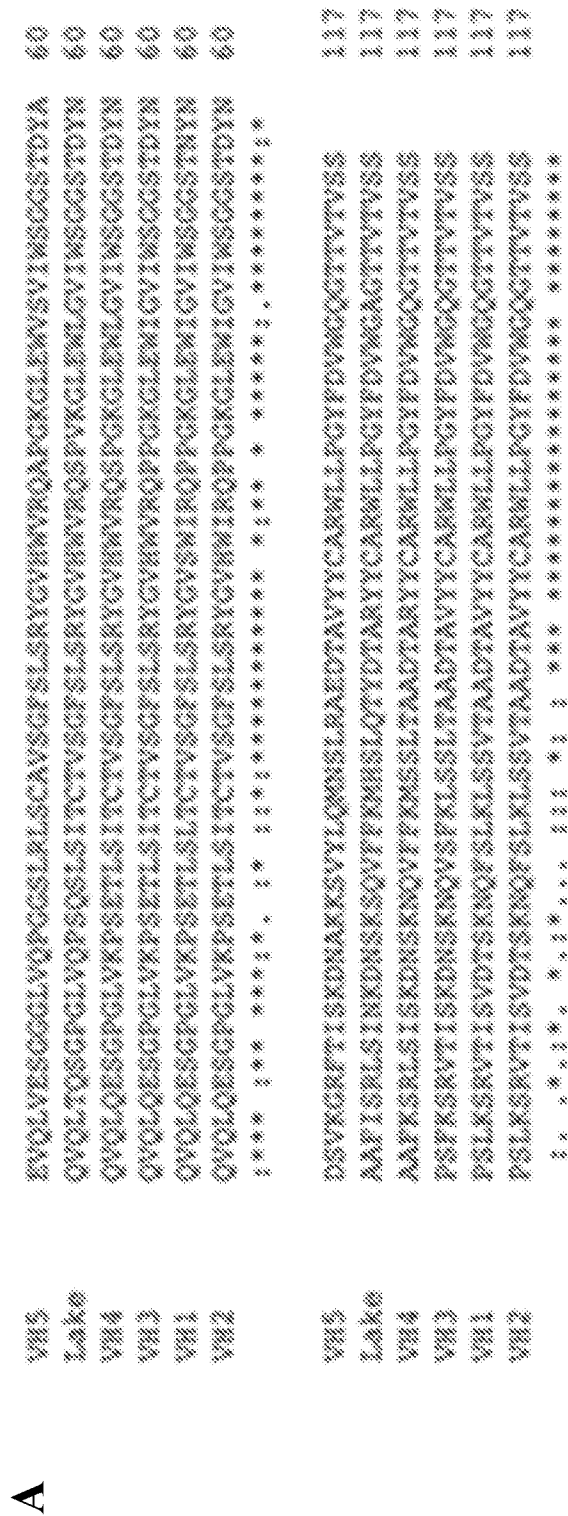
26. The method of claim 25, wherein the subject has one or more of the risk factors for type 1 diabetes, including but not limited to a parent or sibling with type 1 diabetes, a pancreatic tumor, pancreatitis, pancreatic islet cell autoantibodies, insulin autoantibodies, glutamic acid decarboxylase autoantibodies (GADA), insulinoma-associated (IA-2)
5 autoantibodies, zinc transporter autoantibodies (ZnT8), variants of the IDDM1 gene selected from the group consisting of DRB1 0401, DRB1 0402, DRB1 0405, DQA 0301, DQB1 0302 and DQB1 0201; polyuria, polydipsia, dry mouth, polyphagia, fatigue, or weight loss.
27. The method of claim 23, wherein the subject has type 1 diabetes, and the method
10 serves to treat type I diabetes in the subject.
28. The method of claim 27, wherein the treating comprises one or more of reducing frequency of need for insulin injection; slowing development or progression of type 1 diabetes complications in the subject including but not limited to destruction of pancreatic
15 beta cells, hyperglycemia, hypoglycemia, polyuria, polyphagia, polydipsia, weight loss, blurred vision, fatigue, decreased wound healing capability, urinary tract infections, sexual dysfunction, dry mouth, diabetic ketoacidosis, cardiovascular disease, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, stroke, kidney failure, and foot ulcers; and delaying need for a pancreatic or pancreatic islet cell transplant.
20
29. The method of claim 23, wherein the subject is at risk of type 2 diabetes, and the method serves to limit development of type 2 diabetes in the subject.
30. The method of claim 29, wherein the subject has one or more risk factors for type 2
25 diabetes, including but not limited to obesity, smoking, a sedentary lifestyle, a parent or sibling with type 2 diabetes, pre-diabetes, a parent or sibling with pre-diabetes, poor eating habits (ex: too much fat, not enough fiber, too many simple carbohydrates, etc.), age 50 or older, high blood pressure, high cholesterol, testosterone deficiency, metallothionein 1 A (MT1A) rs8052394 locus (G alteration) single nucleotide polymorphism, and a history of
30 gestational diabetes.
31. The method of claim 23, wherein the subject has type 2 diabetes, and the method serves to treat type 2 diabetes in the subject.

32. The method of claim 31, wherein the treating comprises limiting one or more of type 2 diabetic complications, including but not limited to hyperglycemia, hypoglycemia, insulin resistance, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, proteinuria,
5 impaired glomerular clearance, diabetic circulatory disorders, kidney failure, cardiovascular disease, polyuria, polydipsia, weight loss, stroke, and reducing frequency of need for insulin or other therapy.
33. The method of claim 32, wherein the subject has pre-diabetes, and the method serves
10 to treat pre-diabetes in the subject.
34. The method of claim 33, where the treating comprises limiting or slowing progression of one or more complications of pre-diabetes, including but not limited to type 2 diabetes, hyperglycemia, insulin resistance, and/or cardiovascular disease.
15
35. The method of claim 23, wherein the subject has impaired glucose intolerance, and the method serves to treat impaired glucose tolerance in the subject.
36. The method of claim 35, wherein the treating comprises limiting or slowing
20 progression of one or more complications of impaired glucose tolerance, including but not limited to type 2 diabetes, hyperglycemia, insulin resistance, and/or cardiovascular disease.
37. The method of claim 23, wherein the subject has hepatitis, and the method serves to treat hepatitis in the subject.
25
38. The method of claim 37, wherein the treating comprises limiting or slowing progression of one or more complications of hepatitis, including but not limited to yellow discoloration of the skin and/or whites of the eyes, poor appetite, vomiting, fatigue, abdominal pain, diarrhea, acute liver failure, scarring of the liver, liver failure, and liver
30 cancer.
39. The method of claim 23, wherein the subject has an inflammatory liver disease, and the method serves to treat the inflammatory liver disease in the subject.

40. The method of claim 39, wherein the inflammatory liver disease is selected from the group consisting of nonalcoholic steatohepatitis (NASH) and/or non-alcoholic fatty liver disease (NAFLD).
- 5 41. The method of claim 39 or 40, wherein the treating comprises limiting or slowing progression of one or more complications of inflammatory liver disease including but not limited to fatigue, malaise, hepatic fibrosis, hepatic cancer, and/or cirrhosis of the liver.
42. The method of claim 23, wherein the subject has intestinal inflammation.
- 10 43. The method of claim 42, wherein the intestinal inflammation is selected from the group consisting of Crohn's disease, ulcerative colitis, collagenous-, lymphocytic-, ischemic-, diversion- and indeterminate colitis, and Behcet's disease.
- 15 44. The method according to any one of claims 23-43, wherein the subject is a mammal.
45. The method according to any one of claims 23-44, wherein the subject is a human.

Figure 2

CUSTAL O(1.2.4) multiple sequence alignment



B

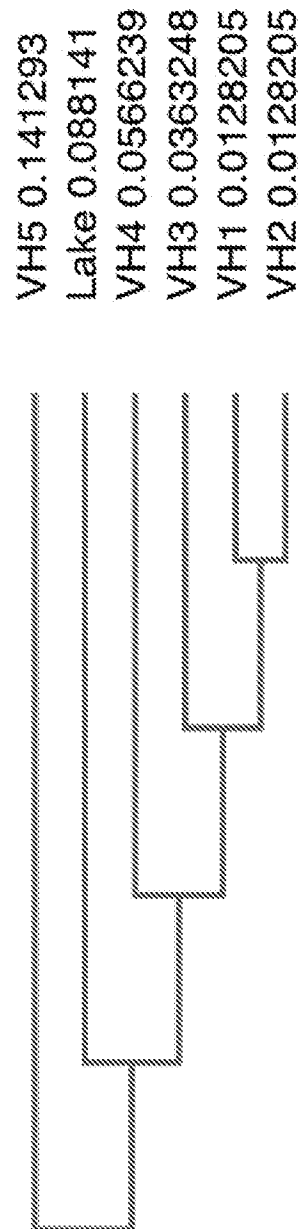
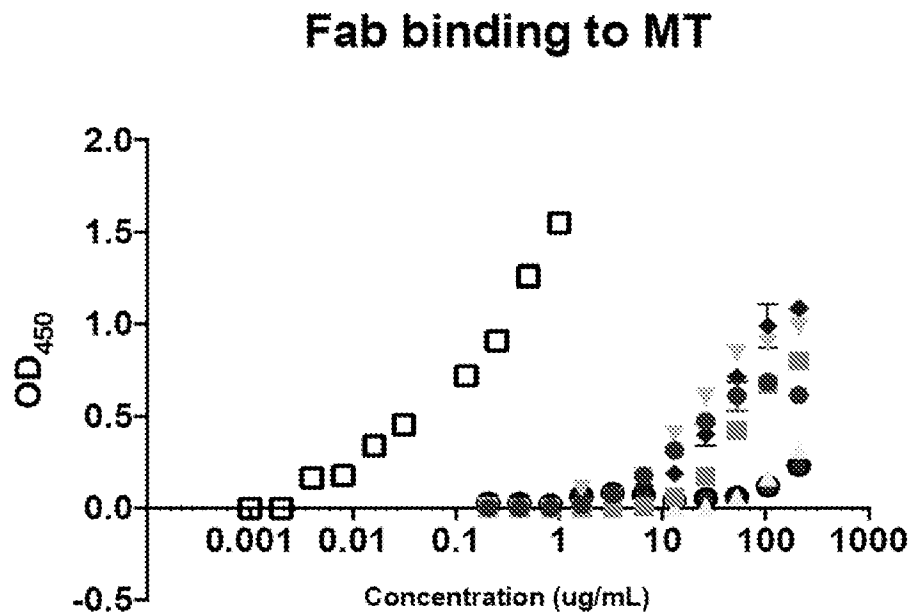
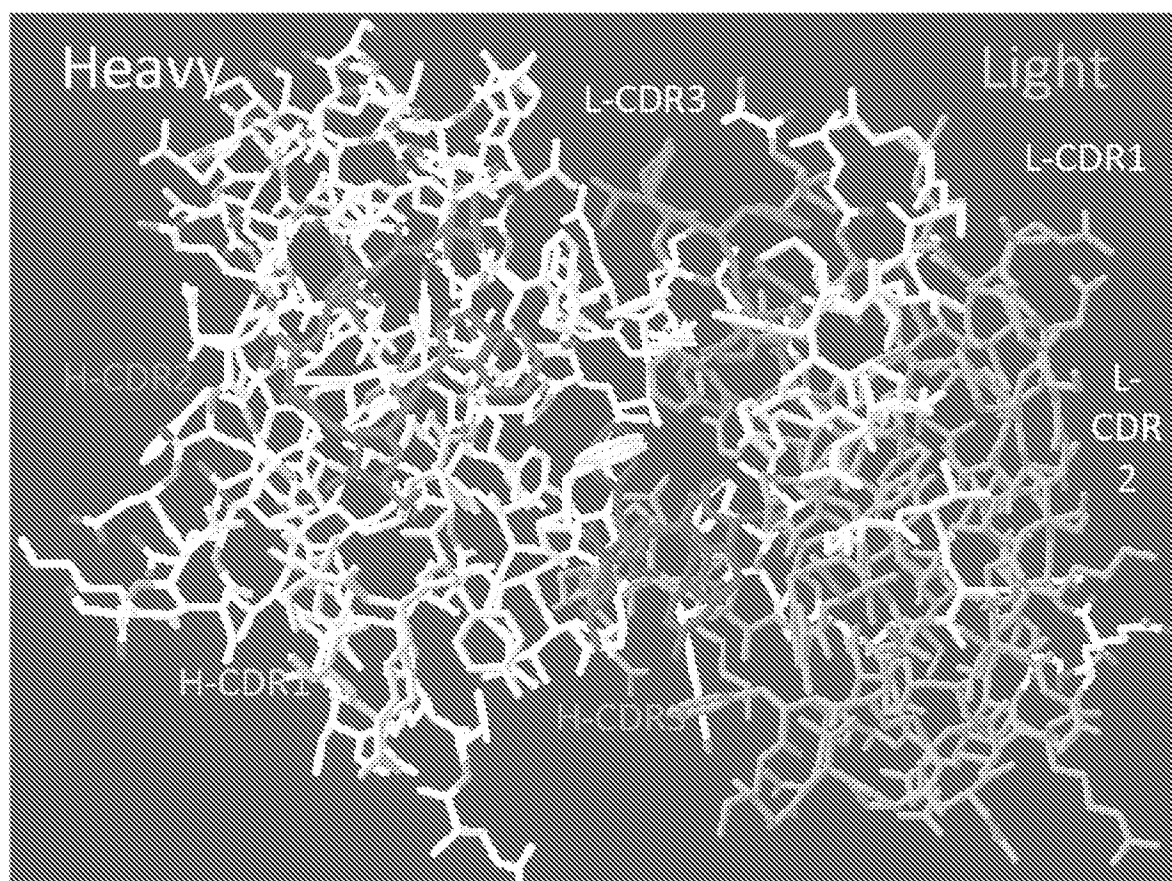


Figure 3

	L-Hum1	L-Hum2	L-Hum3	L-P
H-Hum1	0.09&.07/L,L	0.288&.106/L,L	0.01&.007/L,L	0.107/L
H-Hum2	0.143&.088/L,L	0.22&.212/M,M	0.12&.016/M,L	0.254/M
H-Hum3	0.542&.31/L,M	0.547&.504/M,M	0.478&.443/M,M	0.613/H
H-Hum4	0.225&.124/L,M	0.59&.551/H,H	0.548&.491/M,M	0.617/H
H-Hum5	0.618&.475/M,H	0.612 &.578/H,H	0.577&.374/M,H	0.681/H
H-P	0.466&.281/M,M	0.613/H	0.668/H	0.665/H

Figure 4A

- Fab1 (UC1MT-L-Hum2_UC1MT-H-Hum4)
- ▨ Fab2 (UC1MT-L-Hum2_UC1MT-H-Hum5)
- ◊ Fab3 (UC1MT-L-P_UC1MT-H-P)
- ▼ Fab4 (UC1MT-L-Hum3_UC1MT-H-Hum5)
- ◆ Fab5 (UC1MT-L-Hum3_UC1MT-H-Hum4)
- Fab6 (UC1MT-L-Hum2_UC1MT-H-Hum3)
- Full IgG (UC1MT-L-Hum2_UC1MT-H-Hum5)

Figure 4B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/042064

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 A61P29/00 A61K39/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

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

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

EPO-Internal, BIOSIS, Sequence Search, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/007678 A1 (UNIV GENT [BE]; UNIV CONNECTICUT [US] ET AL.) 17 January 2013 (2013-01-17) claims 2,4; example 1 -----	1-45
A	HAYNES VANESSA ET AL: "Metallothionein 2a gene expression is increased in subcutaneous adipose tissue of type 2 diabetic patients", MOLECULAR GENETICS AND METABOLISM, vol. 108, no. 1, 25 July 2012 (2012-07-25) , pages 90-94, XP028962327, ISSN: 1096-7192, DOI: 10.1016/J.YMGME.2012.10.012 Abstract and last paragraph of page 93 ----- -/-	1-45



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 October 2020

Date of mailing of the international search report

19/10/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Renggli-Zulliger, N

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/042064

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LINA YANG ET AL: "Polymorphisms in metallothionein-1 and -2 genes associated with the risk of type 2 diabetes mellitus and its complications", AMERICAN JOURNAL OF PHYSIOLOGY: ENDOCRINOLOGY AND METABOLISM., vol. 294, no. 5, 1 May 2008 (2008-05-01), pages E987-E992, XP055735414, US ISSN: 0193-1849, DOI: 10.1152/ajpendo.90234.2008 abstract	1-45
X,P	----- WO 2019/143767 A1 (UNIV CONNECTICUT [US]; JOSLIN DIABETES CENTER INC [US]) 25 July 2019 (2019-07-25) cited in the application figures 7,8,9; example all -----	1-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/042064

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 6-15(partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 6-15(partially)

Present claim 6-15 relate to an extremely large number of possible compounds.

Claims 6-15 refer to antibodies wherein the antibody is defined in terms of their CDRs, and where each CDR or VH, VL that can differ from those of certain recited sequences by addition, deletion or substitution of 1, 2 or 3 or more amino acids as well as by homology or less than 100% identity.

In view of the importance of the role played by each single amino acid in the binding of an antibody to its target, particularly at the level of the CDRs, claims to antibodies (or epitope binding domains) which are structurally characterized by less than the full sequence of the CDRs are considered to lack essential features necessary to properly bind the requisite targets (Article 6 PCT). Support and disclosure in the sense of Articles 6 and 5 PCT is to be found however for only a very small proportion of the compounds claimed, SEQ. IDs, 7-9, 10, 11-15, humanised variants of UC1 MT of the example and Figures 2,3.

The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of claim 6-15 PCT (Guidelines 9.19 and 9.23).

The search of claim 6-15 was restricted to those claimed compounds which appear to be supported i.e. SEQ. IDs 7-9, 10, 11-15 and the combination thereof as disclosed on page 26 of the example.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/042064

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013007678 A1	17-01-2013	CA 2840282 A1	17-01-2013
		EP 2729171 A1	14-05-2014
		US 2014141009 A1	22-05-2014
		US 2015158940 A1	11-06-2015
		WO 2013007678 A1	17-01-2013

WO 2019143767 A1	25-07-2019	AU 2019209491 A1	23-07-2020
		CA 3088661 A1	25-07-2019
		SG 11202006421R A	28-08-2020
		WO 2019143767 A1	25-07-2019
