(54) Titre : ANTICORPS DE CONNEXINE 43 ET LEUR UTILISATION
(54) Title: CONNEXIN 43 ANTIBODIES AND USE THEREOF

FIG. 1
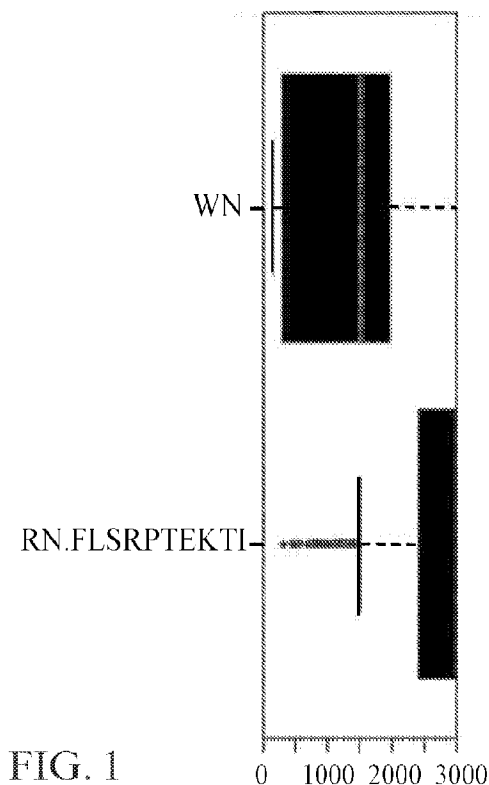
(57) Abrégé/Abstract:
The present disclosure generally relates to compositions and methods for treating a disease or condition associated with opening of Cx43 hemichannels in astrocytes or osteocytes, preferably for treating an inflammatory disease or condition or a neurodegenerative disease such as spinal cord injury.

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(54) **Title:** CONNEXIN 43 ANTIBODIES AND USE THEREOF

(57) **Abstract:** The present disclosure generally relates to compositions and methods for treating a disease or condition associated with opening of Cx43 hemichannels in astrocytes or osteocytes, preferably for treating an inflammatory disease or condition or a neurodegenerative disease such as spinal cord injury.

FIG. 1
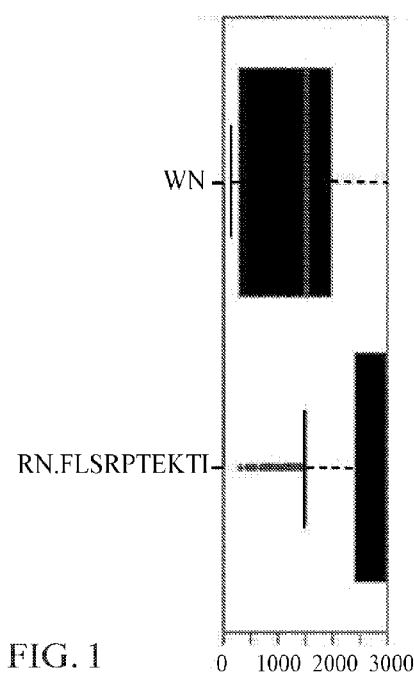
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CONNEXIN 43 ANTIBODIES AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to and the benefit of U.S. Provisional Patent
Application No. 62/800,869 filed February 4, 2019, incorporated herein by reference in its
entirety.

FIELD

The present disclosure generally relates to anti-connexin (Cx) 43 antibodies and their
use in the treatment of, e.g., a disease or condition associated with opening of Cx43
hemichannels in cells such as astrocytes and osteocytes.

BACKGROUND

It is estimated that in the United States alone about 400,000 individuals are living
with spinal cord injury (SCI) with more than 14,000 new cases occurring each year.
Although acute inflammatory response is a defense mechanism aimed at preserving tissue
integrity and demarcating the traumatic lesion, an exaggerated response may limit the
potential for successful recovery. Tissue swelling, especially within the tight confines of the
vertebral canal, can reduce tissue perfusion and cause secondary ischemia. The delayed loss
of tissue affects functional recovery in most patients, and no effective treatment options
currently exist.

It was shown 50 years ago that injection of ATP in the absence of injury was
sufficient to induce acute inflammatory responses. A key observation linking purine
signaling to inflammatory mediators was that activation of a purinergic receptor P2RX7
triggers maturation and secretion of IL-1β from microglial cells. Although it is recognized
that adenine nucleotides (i.e., ATP and its metabolites) are inflammatory mediators, the role
of purinergic signaling in spinal cord injury has received relatively little. Spinal cord injury
leads to excessive and sustained ATP release in peritraumatic regions and inhibition of
P2RX7 reduces inflammatory responses and improves functional recovery.

Previous studies showed that astrocytes release ATP, at least in part, by the opening
of connexin43 (Cx43) hemichannels. Connexins are a family of proteins with dual channel
functions. The traditional role is to form gap junctions, which are composed of two docked
hemichannels linking the cytosol of two neighboring cells. Gap junctions allow cell-to-cell

passage of ions and small molecules, including Ca^{2+} cAMP, IP_3, ATP, glutamate, and glucose. It has been acknowledged that unopposed hemichannels constitute a pathway for regulated gliotransmitter release. Because of their relatively large inner-pore diameter (about 10 Å), open hemichannels facilitate efflux of small cytosolic compounds, and many of these, including ATP and glutamate, will act as transmitters once released. Hemichannel openings are normally tightly controlled, because prolonged opening of many hemi-channels is incompatible with cellular survival.

Cx43 has also been implicated in other inflammatory disorders, including sterile as well as infectious inflammation, such as inflammatory lung diseases, osteoarthritis, and spinal cord injury.

As such, a need exists for effective methods and compositions to treat spinal cord injury, such as by inhibiting the opening Cx43 hemichannels.

SUMMARY

Provided herein are compositions and methods for treating a disease or condition associated with opening (e.g., excessive or prolonged opening) of Cx43 hemichannels, such as inflammatory diseases or conditions and neurodegenerative diseases, e.g., spinal cord injury, Alzheimer's disease, osteoarthritis, etc.

In one aspect, provided herein is an anti-Cx43 antibody, or antigen binding fragment thereof, comprising:

a first, second and third heavy chain complementarity determining region (CDR) sequence having the amino acid sequence of SEQ ID NOs: 1, 2, and 3, respectively; and

a first, second and third light chain CDR sequence having the amino acid sequence of SEQ ID NOs: 4, 5, and 6, respectively.

In certain embodiments, the antibody or fragment thereof can have a heavy chain variable domain having the amino acid sequence of SEQ ID NO: 7, and a light chain variable domain having the amino acid sequence of SEQ ID NO: 8.

A further aspect relates to an anti-Cx43 antibody, or antigen binding fragment thereof, comprising a heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, and a light chain having the amino acid sequence of SEQ ID NO: 18.

In another aspect, provided herein is an antibody that binds an epitope located within the amino acid sequence of FLSRPTEKTI (SEQ ID NO: 19). In some embodiments, the

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epitope can include one or more amino acids selected from the group consisting of R4, P5, E7, K8 and I10 of SEQ ID NO: 19. In one embodiment, the epitope consists of R4, P5, E7, K8 and I10 of SEQ ID NO: 19. In some embodiments, the epitope can include all ten amino acids of SEQ ID NO: 19. In certain embodiments, the epitope consists of all ten amino

5   acids of SEQ ID NO: 19.

Another aspect relates to an isolated anti-Cx43 antibody, or antigen binding fragment thereof, wherein the antibody or fragment thereof cross-competes for binding to Cx43 with any antibody or fragment thereof disclosed herein. In various embodiments, the antibody or fragment thereof can inhibit or reduce or block opening of Cx43 hemichannels

10   in cells.

A further aspect relates to an isolated anti-Cx43 antibody, or antigen binding fragment thereof, wherein the antibody or fragment thereof cross-competes for binding to Cx43 with the antibody or fragment thereof disclosed herein having one or more of SEQ ID NOs: 1-18, wherein preferably the antibody or fragment thereof binds to an epitope located

15   within the amino acid sequence of FLSRPTEKTI (SEQ ID NO: 19), wherein more preferably the epitope comprises one or more amino acids selected from the group consisting of R4, P5, E7, K8 and I10 of SEQ ID NO: 19, wherein even more preferably the epitope comprises all ten amino acids of SEQ ID NO: 19.

In various embodiments, the antibody or fragment thereof disclosed herein can

20   inhibit or reduce or prevent opening of Cx43 hemichannels in cells. In some embodiments, said opening is excessive or chronic opening.

Also disclosed herein is a pharmaceutical composition for inhibiting opening of Cx43 hemichannels in cells, preferably for treating an inflammatory disease or condition or a neurodegenerative disease such as spinal cord injury, comprising one or more of the

25   antibodies or fragments thereof disclosed herein and a pharmaceutically acceptable carrier. In some embodiments, said opening is excessive or chronic opening.

A further aspect relates to use of the antibody or fragment thereof disclosed herein for the manufacture of a medicament for inhibiting opening of Cx43 hemichannels in cells, preferably for treating an inflammatory disease or condition or a neurodegenerative disease

30   such as spinal cord injury. In some embodiments, said opening is excessive or chronic opening.

Another aspect relates to a method of inhibiting opening of Cx43 hemichannels in cells, preferably for treating an inflammatory disease or condition or a neurodegenerative

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disease such as spinal cord injury, comprising contacting the cells with an effective amount of the antibody or fragment thereof disclosed herein. In some embodiments, said opening is excessive or chronic opening.

A further aspect relates to a method for treating a disease or condition associated with opening of Cx43 hemichannels in astrocytes or osteocytes, preferably for treating an inflammatory disease or condition or a neurodegenerative disease such as spinal cord injury, comprising administering a therapeutically effective amount of the antibody or fragment thereof disclosed herein to a patient in need thereof. In some embodiments, said opening is excessive or chronic opening.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Box plot graphs of raw data of antibody screening. The bottom and top of the boxes are the 25th and 75th percentile of the data. The band near the middle of the box is the 50th percentile (the median). The whiskers are at 1.5 the inter-quantile range, an indication of statistical outliers within the dataset (Mcgill et al., (1978) The American Statistician, **32**: 12-16).

Figure 2. Letterplot graph of a full substitution analysis of peptide FLSRPTEKTI probed with antibody under high stringency conditions. The base sequence is listed below the graph, mean signal for the base sequence is at the red line. Substitutions at a given position are plotted at the signal intensity recorded for that replacement.

Figure 3. Antibody probed on set 2 peptides shows increased binding to peptides containing the C-terminal residues of the target sequence. Lines are drawn from the starting position to the end position in the peptide, at the height on which the signal was recorded.

DETAILED DESCRIPTION

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the compositions and methods of the present disclosure.

Disclosed herein are compositions and methods related to anti-Cx43 antibodies, or antigen-binding fragment thereof. In some embodiments, the compositions disclosed herein display unexpectedly superior activity, drugability (e.g., reduced toxicity), stability and/or developability (e.g., reduced cost of production) over those disclosed in PCT Publication

Nos. WO 2015/027120 and WO 2017/147561, both of which are incorporated herein by reference in their entirety. In certain embodiments, the advantages are unexpected.

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

As used herein, the following terms and phrases are intended to have the following meanings:

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "about" means acceptable variations within 20%, more preferably within 10% and most preferably within 5% of the stated value.

An "anti-Cx43 antibody" is an antibody that immunospecifically binds to Cx43 (e.g., its extracellular domain). The antibody may be an isolated antibody. Such binding to Cx43 exhibits a K_D with a value of, e.g., no greater than 1 μM, no greater than 100 nM or no greater than 50 nM. K_D can be measured by any methods known to one skilled in the art, such as a surface plasmon resonance assay or a cell binding assay. An anti-Cx43 antibody may be a monoclonal antibody, or antigen-binding fragments thereof.

An "antibody," as used herein is a protein comprising binding domains that bind to a target epitope. The term antibody includes monoclonal antibodies comprising immunoglobulin heavy and light chain molecules, single heavy chain variable domain antibodies, and variants and derivatives thereof, including chimeric variants of monoclonal and single heavy chain variable domain antibodies. Binding domains are substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes, wherein the protein immunospecifically binds to an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. For most vertebrate organisms, including humans and murine species, the typical

immunoglobulin structural unit comprises a tetramer that is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). "V_L" and V_H'' refer to the variable domains of these light and heavy chains respectively. "C_L" and C_H'' refer to the constant domains of the light and heavy chains. Loops of β-strands, three each on the V_L and V_H are responsible for binding to the antigen, and are referred to as the "complementarity determining regions" or "CDRs". The "Fab" (fragment, antigen-binding) region includes one constant and one variable domain from each heavy and light chain of the antibody, i.e., V_L, C_L, V_H and C_H1.

Antibodies include intact immunoglobulins as well as antigen-binding fragments thereof. The term "antigen-binding fragment" refers to a polypeptide fragment of an antibody which binds antigen or competes with intact antibody (i.e., with the intact antibody from which they were derived) for antigen binding (i.e., specific binding). Antigen binding fragments can be produced by recombinant or biochemical methods that are well known in the art. Exemplary antigen-binding fragments include Fv, Fab, Fab', (Fab')$_2$, CDR, paratope and single chain Fv antibodies (scFv) in which a V_H and a V_L chain are joined together (directly or through a peptide linker) to form a continuous polypeptide.

Antibodies also include variants, chimeric antibodies and humanized antibodies. The term "antibody variant" as used herein refers to an antibody with single or multiple mutations in the heavy chains and/or light chains. In some embodiments, the mutations exist in the variable region. In some embodiments, the mutations exist in the constant region. "Chimeric antibodies" refers to those antibodies wherein one portion of each of the amino acid sequences of heavy and light chains is homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular class, while the remaining segment of the chains is homologous to corresponding sequences in another. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals, while the constant portions are homologous to the sequences in antibodies derived from another. One clear advantage to such chimeric forms is that, for example, the variable regions can conveniently be derived from presently known sources using readily available hybridomas or B cells from non-human host organisms in combination with constant regions derived from, for example, human cell preparations. While the variable region has the advantage of ease of preparation, and the specificity is not affected by its source, the constant region being human, is less likely to elicit an immune response from a human subject when the

antibodies are injected than would the constant region from a non-human source. However, the definition is not limited to this particular example. "Humanized" antibodies refer to a molecule having an antigen-binding site that is substantially derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and/or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete variable domains fused onto constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the variable domains. Antigen binding sites may be wild type or modified by one or more amino acid substitutions, e.g., modified to resemble human immunoglobulin more closely. Some forms of humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs.

As described herein, the amino acid residues of an antibody can be numbered according to the general numbering of Kabat (Kabat, et al. (1991) Sequences of Proteins of Immunological Interest, 5th edition. Public Health Service, NIH, Bethesda, MD).

The term "binding" as used herein in the context of binding between an antibody and an epitope of Cx43 as a target, refers to the process of a non-covalent interaction between molecules. Preferably, said binding is specific. The specificity of an antibody can be determined based on affinity. A specific antibody can have a binding affinity or dissociation constant K_D for its epitope of less than 10^{-7} M, preferably less than 10^{-8} M.

The term "affinity" refers to the strength of a binding reaction between a binding domain of an antibody and an epitope. It is the sum of the attractive and repulsive forces operating between the binding domain and the epitope. The term affinity, as used herein, refers to the dissociation constant, K_D.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term "epitope" includes any determinant, preferably a polypeptide determinant, capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as

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amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics. In one embodiment, an epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it

5   preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. Methods for epitope mapping are well known in the art, such as X-ray co-crystallography, array-based oligo-peptide scanning, site-directed mutagenesis, high throughput mutagenesis mapping and hydrogen–deuterium exchange. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by

10   tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

The site on the antibody that binds the epitope is referred to as "paratope," which

15   typically include amino acid residues that are in close proximity to the epitope once bound. See Sela-Culang et al., Front Immunol. 2013; 4: 302.

"Immunohistochemistry" or "IHC" refers to the process of detecting an antigen in cells of a tissue section allowing the binding and subsequent detection of antibodies immunospecifically recognizing the antigen of interest in a biological tissue. For a review

20   of the IHC technique, see, e.g., Ramos-Vara et al., Veterinary Pathology January 2014 vol. 51 no. 1, 42-87, incorporated herein by reference in its entirety. To evaluate IHC results, different qualitative and semi-quantitative scoring systems have been developed. See, e.g., Fedchenko et al., Diagnostic Pathology, 2014; 9: 221, incorporated herein by reference in its entirety. One example is the H-score, determined by adding the results of multiplication of

25   the percentage of cells with staining intensity ordinal value (scored from 0 for "no signal" to 3 for "strong signal") with 300 possible values.

"Immunospecific" or "immunospecifically" (sometimes used interchangeably with "specifically") refer to antibodies that bind via domains substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes to one or more epitopes of a

30   protein of interest, but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic molecules. Typically, an antibody binds immunospecifically to a cognate antigen with a K_D with a value of no greater than 50 nM, as measured by, e.g., real-time, label free bio-layer interferometry assay, e.g., an Octet® HTX

biosensor, or by surface plasmon resonance, e.g., BIACORE™, or by solution-affinity ELISA. The use of such assays is well known in the art.

The term "surface plasmon resonance", refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

Bio-layer interferometry is a label-free technology for measuring biomolecular interactions. It is an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer. Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time (Abdiche, Y. N., et al. Analytical Biochemistry, (2008), 377(2), 209-217). In certain embodiments, a "real-time bio-layer interferometer based biosensor (Octet HTX assay)" was used to assess the binding characteristics of certain anti-Cx43 antibodies disclosed herein.

The terms "cross-compete", "cross-competition", "cross-block", "cross-blocked" and "cross-blocking" are used interchangeably herein to mean the ability of an antibody or fragment thereof to interfere with the binding directly or indirectly through allosteric modulation of the anti-Cx43 antibodies of the present disclosure to the target Cx43. The extent to which an antibody or fragment thereof is able to interfere with the binding of another to the target, and therefore whether it can be said to cross-block or cross-compete according to the present disclosure, can be determined using competition binding assays. One particularly suitable quantitative cross-competition assay uses a FACS- or an AlphaScreen-based approach to measure competition between the labelled (e.g. His tagged, biotinylated or radioactive labelled) an antibody or fragment thereof and the other an antibody or fragment thereof in terms of their binding to the target. In general, a cross-competing antibody or fragment thereof is for example one which can bind to the target in the cross-competition assay such that, during the assay and in the presence of a second antibody or fragment thereof, the recorded displacement of the immunoglobulin single variable domain or polypeptide according to the disclosure is up to 100% (e.g., in FACS based competition assay) of the maximum theoretical displacement (e.g., displacement by cold (e.g., unlabeled) antibody or fragment thereof that needs to be cross-blocked) by the to be tested potentially cross-blocking antibody or fragment thereof that is present in a given amount. Preferably, cross-competing antibodies or fragments thereof have a recorded

displacement that is between 10% and 100%, more preferred between 50% to 100%.

Cross-competition between antibodies may also be measured by a real-time, label-free bio-layer interferometry assay. Cross-competition between two antibodies may be expressed as the binding of the second antibody that is less than the background signal due to self-self binding (wherein first and second antibodies is the same antibody). Cross-competition between 2 antibodies may be expressed, for example, as % binding of the second antibody that is less than the baseline self-self background binding (wherein first and second antibodies is the same antibody).

The terms "inhibit," "block" and "reduce" as used interchangeably herein, refer to any statistically significant decrease in a biological activity (e.g., hemichannel opening). For example, "inhibition" or "blockage" can refer to a decrease of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in biological activity.

The term "subject" or "patient" includes a human or other mammalian animal that receives either prophylactic or therapeutic treatment.

The terms "treat," "treating," and "treatment," as used herein, refer to therapeutic or preventative measures such as those described herein. The methods of "treatment" employ administration to a patient a Cx43 ligand provided herein, for example, a patient having an inflammatory disease or condition or a neurodegenerative disease, in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of the inflammatory disease or condition or neurodegenerative disease, or in order to prolong the survival of a patient beyond that expected in the absence of such treatment. The methods of "treatment" also employ administration to a patient a Cx43 ligand provided herein (e.g., an antibody) to provide therapy in a patient beyond that expected in the absence of such treatment.

The term "effective amount," as used herein, refers to that amount of an agent, such as a Cx43 ligand, for example an anti-Cx43 antibody, which is sufficient to effect treatment, prognosis or diagnosis of a disease, when administered to a patient. A therapeutically effective amount will vary depending upon the patient and disease condition being treated, the weight and age of the patient, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The dosages for administration can range from, for example, about 1 ng to about 10,000 mg, about 5 ng to about 9,500 mg, about 10 ng to about 9,000 mg, about 20 ng to about 8,500 mg, about 30 ng to about 7,500 mg, about 40 ng to about 7,000 mg, about 50 ng to about 6,500 mg, about 100 ng to about 6,000 mg, about 200 ng to about 5,500 mg, about

300 ng to about 5,000 mg, about 400 ng to about 4,500 mg, about 500 ng to about 4,000 mg, about 1 μg to about 3,500 mg, about 5 μg to about 3,000 mg, about 10 μg to about 2,600 mg, about 20 μg to about 2,575 mg, about 30 μg to about 2,550 mg, about 40 μg to about 2,500 mg, about 50 μg to about 2,475 mg, about 100 μg to about 2,450 mg, about 200 μg to about 2,425 mg, about 300 μg to about 2,000, about 400 μg to about 1,175 mg, about 500 μg to about 1,150 mg, about 0.5 mg to about 1,125 mg, about 1 mg to about 1,100 mg, about 1.25 mg to about 1,075 mg, about 1.5 mg to about 1,050 mg, about 2.0 mg to about 1,025 mg, about 2.5 mg to about 1,000 mg, about 3.0 mg to about 975 mg, about 3.5 mg to about 950 mg, about 4.0 mg to about 925 mg, about 4.5 mg to about 900 mg, about 5 mg to about 875 mg, about 10 mg to about 850 mg, about 20 mg to about 825 mg, about 30 mg to about 800 mg, about 40 mg to about 775 mg, about 50 mg to about 750 mg, about 100 mg to about 725 mg, about 200 mg to about 700 mg, about 300 mg to about 675 mg, about 400 mg to about 650 mg, about 500 mg, or about 525 mg to about 625 mg, of an antibody or antigen binding portion thereof, as provided herein. Dosing may be, e.g., every week, every 2 weeks, every three weeks, every 4 weeks, every 5 weeks or every 6 weeks. Dosage regimens may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any toxic or detrimental effects (side effects) of the agent are minimized and/or outweighed by the beneficial effects. Administration may be intravenous at exactly or about 6 mg/kg or 12 mg/kg weekly, or 12 mg/kg or 24 mg/kg biweekly. Additional dosing regimens are described below.

Other terms used in the fields of recombinant nucleic acid technology, microbiology, immunology, antibody engineering, and molecular and cell biology as used herein will be generally understood by one of ordinary skill in the applicable arts. For example, conventional techniques may be used for preparing recombinant DNA, performing oligonucleotide synthesis, and practicing tissue culture and transformation (*e.g.*, electroporation, transfection or lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Sambrook et al., 2001, *Molecular Cloning: A Laboratory Manual,* 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference for any purpose. Unless specific definitions

are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for chemical syntheses, chemical analyses,

5   pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are present in a given embodiment, yet open to the inclusion of unspecified elements.

As used herein the term "consisting essentially of" refers to those elements required

10   for a given embodiment. The term permits the presence of additional elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the disclosure.

The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in

15   that description of the embodiment.

As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon

20   reading this disclosure and so forth.

Various aspects and embodiments are described in further detail in the following subsections.

Cx43

25   Various cells are able to communicate with each other and with the extracellular environment through hemichannels and gap junctions formed by the protein connexin. Connexin proteins are ubiquitously expressed throughout the body. Six connexin proteins make up one hemichannel, and 2 hemichannels make up 1 gap junction channel. Gap junctions are a cluster of channels that are located in the plasma membrane between

30   adjoining cells and they mediate intercellular communication. Hemichannels are a separate entity from gap junction channels. Hemichannels permit the exchange of molecules between the intracellular compartments and the extracellular environment.

Many cells express hemichannels known as connexin (Cx) 43 hemichannels.

Connexin-43 is also known as gap junction alpha-1 protein (GJA1), which is a 43.0 kDa protein composed of 382 amino acids (NCBI Reference Sequence: NP_000156.1). GJA1 contains a long C-terminal tail, an N-terminal domain, and multiple transmembrane domains. The protein passes through the phospholipid bilayer four times, leaving its C- and N-
5   terminals exposed to the cytoplasm. The C-terminal tail is composed of 50 amino acids and includes post-translational modification sites, as well as binding sites for transcription factors, cytoskeleton elements, and other proteins. As a result, the C-terminal tail is central to functions such as regulating pH gating and channel assembly. Notably, the DNA region of the *GJA1* gene (NCBI Gene ID: 2697) encoding this tail is highly conserved, indicating
10   that it is either resistant to mutations or becomes lethal when mutated. Meanwhile, the N-terminal domain is involved in channel gating and oligomerization and, thus, may control the switch between the channel's open and closed states. The transmembrane domains form the gap junction channel while the extracellular loops facilitate proper channel docking. Moreover, two extracellular loops form disulfide bonds that interact with two hexamers to
15   form a complete gap junction channel.

     Cx43 plays an important role in the regulation of various immune processes. A recent report shows the pathogenic role of Cx43 hemichannels in sterile injuries as well as infectious inflammatory diseases (Li et al., Scientific Reports (2018) 8:166, doi:10.1038/s41598-017-18452-1). Sterile inflammation is a common event, triggered by
20   physical, chemical or metabolic noxiae. The different noxiae cause cell stress and hence stress responses. Many types of stress response exist (e.g. unfolding protein response, integrated stress response, oxidative stress), often entangled among each other. Stress responses trigger inflammation. When noxiae persist, inflammation does not resolve, resulting in a vicious circle that has a key role in the pathophysiology of many human
25   disorders, including cancer, metabolic and genetic diseases.

     The acute conditions that result from sterile inflammation include ischemia reperfusion injury (IRI), trauma (e.g., spinal cord injury, traumatic brain injury, peripheral nerve injury), crystal-induced inflammation, and toxin exposure. Acute myocardial infarctions, cerebral infarctions, acute kidney injury and solid organ transplantation are all
30   conditions in which IRI occurs. Crystal deposition within joints leads to gouty arthritis and elicits the classic clinical signs of inflammation including redness, pain, heat, swelling and loss of function. Toxins, such as acetaminophen or cobra venom, induce hepatic and muscle injury, respectively. Trauma, including crush injury, triggers an abrupt inflammatory

response, and endogenous and microbial triggers (from bacterial exposure) may contribute to inflammation in this context.

Chronic conditions that trigger or result from sterile inflammation include particle-induced lung diseases such as asbestosis and silicosis, chronic pulmonary diseases such as cystic fibrosis and idiopathic pulmonary fibrosis, cardiovascular diseases such as atherosclerosis, some causes of chronic heart failure, certain cases of tumors, arthritis (e.g., osteoarthritis and rheumatoid arthritis (RA)), and autoimmune conditions.

Infectious inflammation can be caused by various pathogens such as bacteria and fungi in a number of tissues.

In various embodiments, regardless of the cause, inflammatory diseases can be treated by the anti-Cx43 antibodies disclosed herein. Inflammatory diseases as used herein refer to a vast array of disorders and conditions that are characterized by inflammation. Examples include arthritis, allergy, asthma, autoimmune diseases, coeliac disease, glomerulonephritis, hepatitis, inflammatory bowel disease (including Crohn's disease and Ulcerative Colitis), reperfusion injury and transplant rejection. Autoimmune diseases are diseases in which the immune system attacks its own proteins, cells, and tissues, or in which immune effector T cells are autoreactive to endogenous self peptides and cause destruction of tissue. Thus an immune response is mounted against a subject's own antigens, referred to as self antigens. A comprehensive listing and review of autoimmune diseases can be found in *The Autoimmune Diseases* (Rose and Mackay, 2014, Academic Press). Autoimmune diseases include but are not limited to rheumatoid arthritis, Crohn's disease, Type 1 diabetes, alopecia, multiple sclerosis, lupus, systemic lupus erythematosus (SLE), autoimmune encephalomyelitis, myasthenia gravis (MG), Hashimoto's thyroiditis, Goodpasture's syndrome, pemphigus (e.g., pemphigus vulgaris), Grave's disease, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis, pernicious anemia, idiopathic Addison's disease, autoimmune-associated infertility, glomerulonephritis (e.g., crescentic glomerulonephritis, proliferative glomerulonephritis), bullous pemphigoid, Sjogren's syndrome, insulin resistance, and autoimmune diabetes mellitus.

Excessive or chronic Cx43 hemichannel opening during chronic neurodegenerative diseases has also been shown to promote disease progression by perturbing metabolic gradients and the exaggerated release of toxic molecules to induce cell death. See, e.g., Orellana et al., (2011), Neurodegenerative Diseases - Processes, Prevention, Protection and

Monitoring, Chapter 10, Role of Connexin Hemichannels in Neurodegeneration, doi: 10.5772/28054; Bosch et al., Front Cell Neurosci. 2014; 8: 242; and Vicario et al., Front. Physiol., 2017, Vol. 8, Article 1060, doi: 10.3389/fphys.2017.01060; all of which are incorporated by reference in their entirety. As such, inhibiting or reducing Cx43

5   hemichannel opening using the compositions disclosed herein can be used to treat various neurodegenerative diseases such as Alzheimer's disease (AD), lysosomal storage disorders, bacterial meningitis, amyotrophic lateral sclerosis, hypoxia, ischemia, glaucoma, schizophrenia, major depression, bipolar disorder, epilepsy, traumatic brain injury, post-traumatic stress disorder, Parkinson's disease, Down syndrome, spinocerebellar ataxia,

10   Huntington's disease, radiation therapy induced neurodegeneration, chronic stress induced neurodegeneration, and neurodegeneration associated with normal aging or abuse of neuro-active drugs (such as alcohol, opiates, methamphetamine, phencyclidine, and cocaine).

Anti-Cx43 Antibody

15   Inhibiting or reducing Cx43 hemichannel opening can reduce or inhibit opening of Cx43 hemichannels in cells, thereby treating, e.g., an inflammatory disease or condition or a neurodegenerative disease. As such, anti-Cx43 antibodies can be used as an effective agent in anti-inflammation and/or neuroprotective therapeutics.

        In certain embodiments, the anti-Cx43 antibody can be a monoclonal antibody or an

20   antigen-binding fragment thereof. In certain embodiments, the anti-Cx43 antibody can be a modified, e.g., chimeric or humanized antibody derived from a mouse anti-Cx43 antibody. In some embodiments, the anti-Cx43 antibody is an antibody or antigen binding fragment thereof which binds to an epitope present on the human Cx43 protein, e.g., the extracellular loops, or a portion thereof.

25   Exemplary anti-Cx43 antibodies can have one or more of the following CDR sequences:

Heavy chain:

CDR1 (SEQ ID NO.: 1): GYTFTSYY
CDR2 (SEQ ID NO.: 2): INPSNAGT
30   CDR3 (SEQ ID NO.: 3): TREGNPYYTMNY

Light chain:

CDR1 (SEQ ID NO.: 4): QSLLNSGNQKTY
CDR2 (SEQ ID NO.: 5): GAS
35   CDR3 (SEQ ID NO.: 6): QNDHSYPFT

In some embodiments, it has been surprisingly discovered that antibodies having the above CDR sequences show superior binding affinity and/or antibody stability, compared to those disclosed in PCT Publication Nos. WO 2015/027120 and WO 2017/147561. Without wishing to be bound by theory, it is believed that the "NG" to "NA" mutation in heavy chain CDR2 can reduce deamidation. Antibody deamidation especially in the CDR region may cause binding affinity change, antibody degradation, and charge variants changes, which can affect antibody function and increase the cost of antibody production. As such, the CDRs disclosed herein provide improved binding affinity and antibody stability, resulting in an advantageous technical effect over those disclosed in PCT Publication Nos. WO 2015/027120 and WO 2017/147561.

Monoclonal antibodies can be humanized and optimized using, e.g., CDR grafting, germline modeling and 3-D structure analysis, to increase the drugability and/or developability of the antibodies. In some embodiments, after humanization, the anti-Cx43 antibody can have one or both of the following variable domains:

Heavy Chain variable domain (SEQ ID NO.: 7):

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGINPSN
AGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYTMNYWG
QGTLVTVSS

Light Chain variable domain (SEQ ID NO.: 8):

DIVMTQSPDSLAVSLGERATISCKSSQSLLNSGNQKTYLAWYQQKPGQPPKLLIYGA
STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDHSYPFTFGQGTKLEIK

In select embodiments, the anti-Cx43 antibody can have a variable domain fused to the constant regain of, e.g., human IgG1, IgG2 or IgG4 that can optionally contain one or more mutations. In some embodiments, the mutations can be designed to reduce or minimize the cytotoxic effector function of the antibody, while maintaining binding affinity and antibody stability. For example, the anti-Cx43 antibody can have one or more of the following heavy chain sequences (wherein the bold portion corresponds to the variable domain and the non-bolded portion corresponds to the constant region):

> **Heavy chain of Ab#C (SEQ ID NO.: 9)**
**EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNgGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT**

16

MNYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
5   ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGK

> Heavy chain of Ab#E (SEQ ID NO.: 10)
10   EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNgGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT
MNYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR
VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
15   NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
LSLGK

20   > Heavy chain of Ab#G (SEQ ID NO.: 11)
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNaGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT
MNYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR
25   VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
LSLGK
30
> Heavy chain of Ab#I (SEQ ID NO.: 12)
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNgGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT
MNYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
35   NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
40   KSLSLSPGK

> Heavy chain of Ab#K (SEQ ID NO.: 13)
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNaGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT
45   MNYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES

NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

> **Heavy chain of Ab#M (SEQ ID NO.: 14)**

5   **EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNgGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT
MNYWGQGTLVTVSS**ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR
VESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
10   NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
LSLGK

15   > **Heavy chain of Ab#O (SEQ ID NO.: 15)**

**EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNaGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT
MNYWGQGTLVTVSS**ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR
20   VESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
LSLGK

25

   > **Heavy chain of Ab#Q (SEQ ID NO.: 16)**

**EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNgGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT
MNYWGQGTLVTVSS**ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW
30   NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR
VESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL
PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKS
35   LSLSLGK

   >**Heavy chain of Ab#S (SEQ ID NO.: 17)**

**EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMYWVRQAPGQGLEWIGGIN
PSNaGTNFNEKFKNRATLTVDKSTSTAYMELSSLRSEDTAVYYCTREGNPYYT
MNYWGQGTLVTVSS**ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW
40   NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR
VESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL
PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNG
45   QPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKS
LSLSLGK

In some embodiments, the anti-Cx43 antibody can have the following light chain sequence (wherein the bold portion corresponds to the variable domain and the non-bolded portion corresponds to the constant region):

>Light chain of **Ab#E, Ab#G, Ab#I, Ab#K, Ab#M, Ab#O, Ab#Q, Ab#S** (SEQ ID NO.: 18)
DIVMTQSPDSLAVSLGERATISCKSSQSLLNSGNQKTYLAWYQQKPGQPPKLLI
YGASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDHSYPFTFGQGT
KLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

In yet another embodiment, the anti-Cx43 antibody can comprise a mixture, or cocktail, of two or more anti-Cx43 antibodies, each of which binds to the same or different epitope on Cx43.

In some embodiments, a bispecific antibody can be made in which at least one of the specificities is an anti-Cx43 antibody or antigen-binding fragment thereof disclosed herein. The other specificity can be directed to another target implicated in the inflammatory disease or neurodegenerative disease being treated.

In one aspect, use of Cx43 ligand for the manufacture of a medicament in the treatment of an inflammatory disease or a neurodegenerative disease is provided. In another aspect, a method of suppressing inflammation or neurodegeneration in a patient is provided, the method comprising administering to the patient an effective amount of a Cx43 ligand.

Preparation of Anti-Cx43 Antibodies

Anti-Cx43 antibodies can be made using various methods generally known in the art. For example, phage display technology can be used to screen a human antibody library, to produce a fully human monoclonal antibody for therapy. High affinity binders can be considered candidates for neutralization studies. Alternatively, a conventional monoclonal approach can be used, in which mice or rabbits can be immunized with the human protein, candidate binders identified and tested, and a humanized antibody ultimately produced by engrafting the combining sites of heavy and light chains into a human antibody encoding sequence.

Antibodies typically comprise two identical pairs of polypeptide chains, each pair having one full-length "light" chain (typically having a molecular weight of about 25 kDa) and one full-length "heavy" chain (typically having a molecular weight of about 50-70 kDa). The amino-terminal portion of each chain typically includes a variable region of about 100 to 110 or more amino acids that typically is responsible for antigen recognition. The

carboxy-terminal portion of each chain typically defines a constant region responsible for effector function. The variable regions of each of the heavy chains and light chains typically exhibit the same general structure comprising four relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair typically are aligned by the framework regions, which alignment may enable binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is typically in accordance with the definitions of *Kabat Sequences of Proteins of Immunological Interest* (1987 and 1991, National Institutes of Health, Bethesda, Md.), Chothia & Lesk, 1987, *J. Mol. Biol.* 196:901-917, or Chothia et al., 1989, *Nature* 342:878-883).

Antibodies became useful and of interest as pharmaceutical agents with the development of monoclonal antibodies. Monoclonal antibodies are produced using any method that produces antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler et al. (1975, *Nature* 256:495-497) and the human B-cell hybridoma method (Kozbor, 1984, *J. Immunol.* 133:3001; and Brodeur et al., 1987, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, pp. 51-63).

Monoclonal antibodies may be modified for use as therapeutics. One example is a "chimeric" antibody in which a portion of the heavy chain and/or light chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Other examples are fragments of such antibodies, so long as they exhibit the desired biological activity. See, U.S. Pat. No. 4,816,567; and Morrison et al. (1985), *Proc. Natl. Acad. Sci. USA* 81:6851-6855. A related development is the "CDR-grafted" antibody, in which the antibody comprises one or more complementarity determining regions (CDRs) from a particular species or belonging to a particular antibody class or subclass, while the remainder of the antibody chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass.

Another development is the "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art (see U.S. Pat. Nos. 5,585,089, and 5,693,762; see also Cécile Vincke et al. J. Biol. Chem. 2009;284:3273-3284 for humanization of llama antibodies). Generally, a humanized antibody is produced by a non-human animal, and then certain amino acid residues, typically from non-antigen recognizing portions of the antibody, are modified to be homologous to said residues in a human antibody of corresponding isotype. Humanization can be performed, for example, using methods described in the art (Jones et al., 1986, *Nature* 321:522-525; Riechmann et al., 1988, *Nature* 332:323-327; Verhoeyen et al., 1988, *Science* 239:1534-1536), by substituting at least a portion of a rodent variable region for the corresponding regions of a human antibody.

More recent is the development of human antibodies without exposure of antigen to human beings ("fully human antibodies"). Using transgenic animals (e.g., mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous mouse immunoglobulin production, such antibodies are produced by immunization with an antigen (typically having at least 6 contiguous amino acids), optionally conjugated to a carrier. See, for example, Jakobovits et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:2551-2555; Jakobovits et al., 1993, *Nature* 362:255-258; and Bruggermann et al., 1993, *Year in Immunol.* 7:33. In one example of these methods, transgenic animals are produced by incapacitating the endogenous mouse immunoglobulin loci encoding the mouse heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals, which have less than the full complement of modifications, are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies that are immunospecific for these antigens having human (rather than murine) amino acid sequences, including variable regions. See PCT Publication Nos. WO96/33735 and WO94/02602, incorporated by reference. Additional methods are described in U.S. Pat. No. 5,545,807, PCT Publication Nos. WO91/10741, WO90/04036, and in EP 546073B1 and EP 546073A1, incorporated by reference. Human antibodies may also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

In some embodiments, phage display technology may be used to screen for therapeutic antibodies. In phage display, antibody repertoires can be displayed on the surface of filamentous bacteriophage, and the constructed library may be screened for

phages that bind to the immunogen. Antibody phage is based on genetic engineering of bacteriophages and repeated rounds of antigen-guided selection and phage propagation. This technique allows *in vitro* selection of Cx43 monoclonal antibodies. The phage display process begins with antibody-library preparation followed by ligation of the variable heavy

5   (VH) and variable light (VL) PCR products into a phage display vector, culminating in analysis of clones of monoclonal antibodies. The VH and VL PCR products, representing the antibody repertoire, are ligated into a phage display vector (*e.g.*, the phagemid pComb3X) that is engineered to express the VH and VL as an scFv fused to the pIII minor capsid protein of a filamentous bacteriophage of *Escherichia coli* that was originally derived

10   from the M13 bacteriophage. However, the phage display vector pComb3X does not have all the other genes necessary to encode a full bacteriophage in *E. coli*. For those genes, a helper phage is added to the *E. coli* that are transformed with the phage display vector library. The result is a library of phages, each expressing on its surface a Cx43 monoclonal antibody and harboring the vector with the respective nucleotide sequence within. The

15   phage display can also be used to produce the Cx43 monoclonal antibody itself (not attached to phage capsid proteins) in certain strains of *E. Coli*. Additional cDNA is engineered, in the phage display vector, after the VL and VH sequences to allow characterization and purification of the mAb produced. Specifically, the recombinant antibody may have a hemagglutinin (HA) epitope tag and a polyhistidine to allow easy purification from solution.

20   Diverse antibody phage libraries are produced from ~10^8 independent *E. coli* transformants infected with helper phage. Using bio-panning, a library can screened for phage binding to the immunogen sequence listed above, or a fragment thereof, through the expressed surface of the monoclonal antibody. Cyclic panning allows for pulling out potentially very rare antigen-binding clones and consists of multiple rounds of phage

25   binding to antigen (immobilized on ELISA plates or in solution on cell surfaces), washing, elution, and reamplification of the phage binders in *E. coli*. During each round, specific binders are selected out from the pool by washing away non-binders and selectively eluting binding phage clones. After three or four rounds, highly specific binding of phage clones through their surface Cx43 monoclonal antibody is characteristic for directed selection on

30   the immobilized immunogen.
            Another method is to add a C-terminal His tag, suitable for purification by affinity chromatography, to the immunogen sequence listed above. Purified protein can be inoculated into mice together with a suitable adjuvant. Monoclonal antibodies produced in

hybridomas can be tested for binding to the immunogen, and positive binders can be screened as described in the assays herein.

Fully human antibodies can also be produced from phage-display libraries (as disclosed in Hoogenboom et al., 1991, *J. Mol. Biol.* 227:381; and Marks et al., 1991, *J. Mol. Biol.* 222:581). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT Publication No. WO99/10494, incorporated by reference, which describes the isolation of high affinity and functional agonistic antibodies for MPL- and msk-receptors using such an approach.

Nucleotide sequences encoding the above antibodies can be determined. Thereafter, chimeric, CDR-grafted, humanized, and fully human antibodies also may be produced by recombinant methods. Nucleic acids encoding the antibodies can be introduced into host cells and expressed using materials and procedures generally known in the art.

The disclosure provides antibodies against Cx43. Preferably, the antibodies bind Cx43. In preferred embodiments, the disclosure provides nucleotide sequences encoding, and amino acid sequences comprising, heavy and light chain immunoglobulin molecules, particularly sequences corresponding to the variable regions thereof. In preferred embodiments, sequences corresponding to CDRs, specifically from CDR1 through CDR3, are provided. In additional embodiments, the disclosure provides hybridoma cell lines expressing such immunoglobulin molecules and monoclonal antibodies produced therefrom, preferably purified human monoclonal antibodies against human Cx43.

The CDRs of the light and heavy chain variable regions of anti-Cx43 antibodies of the disclosure can be grafted to framework regions (FRs) from the same, or another, species. In certain embodiments, the CDRs of the light and heavy chain variable regions of anti-Cx43 antibody may be grafted to consensus human FRs. To create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. The FRs of the anti-Cx43 antibody heavy chain or light chain can be replaced with the FRs from a different heavy chain or light chain. Rare amino acids in the FRs of the heavy and light chains of anti- Cx43 antibody typically are not replaced, while the rest of the FR amino acids can be replaced. Rare amino acids are specific amino acids that are in positions in which they are not usually found in FRs. The grafted variable regions from anti-Cx43 antibodies of the disclosure can be used with a constant region that is different from the constant region of anti-Cx43 antibody. Alternatively, the

grafted variable regions are part of a single chain Fv antibody. CDR grafting is described, e.g., in U.S. Pat. Nos. 6,180,370, 5,693,762, 5,693,761, 5,585,089, and 5,530,101, which are hereby incorporated by reference for any purpose.

In some embodiments, antibodies of the disclosure can be produced by hybridoma lines. In these embodiments, the antibodies of the disclosure bind to Cx43 with a dissociation constant (K_D) of between approximately 4 pM and 1 µM. In certain embodiments of the disclosure, the antibodies bind to Cx43 with a K_D of less than about 100 nM, less than about 50 nM or less than about 10 nM.

In embodiments, the antibodies of the present disclosure are of the IgG1, IgG2, IgG3, or IgG4 isotype, such as the IgG1 isotype. In certain embodiments, the antibodies comprise a human kappa or lambda light chain and a human IgG1, IgG2, or IgG4 heavy chain. In embodiments, the variable regions of the antibodies are ligated to a constant region of the IgG1, IgG2, or IgG4 isotype. In particular embodiments, the variable regions of the antibodies are ligated to a constant region other than the constant region for the IgG1, IgG2, or IgG4 isotype. In certain embodiments, the antibodies of the disclosure have been cloned for expression in mammalian cells.

In alternative embodiments, antibodies of the disclosure can be expressed in cell lines other than hybridoma cell lines. In these embodiments, sequences encoding particular antibodies can be used for transformation of a suitable host cell, e.g., mammalian cell. According to these embodiments, transformation can be achieved using any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art. Such procedures are exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (all of which are hereby incorporated herein by reference for any purpose). Generally, the transformation procedure used may depend upon the host to be transformed. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

According to certain embodiments of the methods of the disclosure, a nucleic acid molecule encoding the amino acid sequence of a heavy chain constant region, a heavy chain variable region, a light chain constant region, or a light chain variable region of a Cx43

antibody of the disclosure is inserted into an appropriate expression vector using standard ligation techniques. In a preferred embodiment, the Cx43 antibody heavy or light chain constant region is appended to the C-terminus of the appropriate variable region and is ligated into an expression vector. The vector is typically selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). For a review of expression vectors, see, Goeddel (ed.), 1990, *Meth. Enzymol. Vol.* 185, Academic Press. N.Y.

Typically, expression vectors used in any of the host cells can contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. These sequences are well known in the art.

Expression vectors of the disclosure may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the flanking sequences described herein are not already present in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

After the vector has been constructed and a nucleic acid molecule encoding light chain or heavy chain or light chain and heavy chain comprising an anti-Cx43 antibody has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression. The transformation of an expression vector for an anti- Cx43 antibody into a selected host cell may be accomplished by well-known methods including transfection, infection, calcium phosphate co-precipitation, electroporation, microinjection, lipofection, DEAE-dextran mediated transfection, or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook et al., supra.

The host cell, when cultured under appropriate conditions, synthesizes an anti-Cx43 antibody that can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). The selection of an appropriate host cell will depend upon various factors, such as desired

5   expression levels, polypeptide modifications that are desirable or necessary for activity (such as glycosylation or phosphorylation) and ease of folding into a biologically active molecule.

Mammalian cell lines available as hosts for expression are well known in the art and include, but are not limited to, many immortalized cell lines available from the American

10   Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, human embryonic kidney cells (HEK), monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. In certain embodiments, one may select cell lines by determining which cell lines have high expression levels and produce antibodies with

15   constitutive Cx43 binding properties. In another embodiment, one may select a cell line from the B cell lineage that does not make its own antibody but has a capacity to make and secrete a heterologous antibody (e.g., mouse myeloma cell lines NS0 and SP2/0).

Epitope Mapping and Related Technologies

20   The present disclosure provides anti-Cx43 antibodies, which interact with one or more amino acids found within one or more domains, e.g., extracellular loops, of the Cx43 molecule. The epitope to which the antibodies bind may include one or more contiguous sequences of 2 or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or more) amino acids located within one or more extracellular loops. Alternatively or additionally, the epitope may include 1 or more

25   non-contiguous amino acids (or amino acid sequences) located within one or more extracellular loops (e.g. a conformational epitope).

Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking

30   assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.). Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol. Biol. 248: 443-63), peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope

excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Prot. Sci. 9: 487-496).

Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. See, e.g., Ehring (1999) Analytical Biochemistry 267: 252-259; Engen and Smith (2001) Anal. Chem. 73: 256A-265A.

Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the invention into groups of antibodies binding different epitopes.

The present disclosure provides anti-Cx43 antibodies that bind to the same epitope, or a portion of the epitope. Likewise, the present disclosure also includes anti-Cx43 antibodies that compete for binding to Cx43 or a fragment thereof with any of the specific exemplary antibodies described herein. For example, the present disclosure includes anti-

Cx43 antibodies that cross-compete for binding to Cx43 with one or more antibodies obtained from those antibodies described herein.

One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-Cx43 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-Cx43 antibody of the invention, the reference antibody can be allowed to bind to Cx43 or peptide under saturating conditions. Next, the ability of a test antibody to bind to the Cx43 molecule is assessed. If the test antibody is able to bind to Cx43 following saturation binding with the reference anti-Cx43 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-Cx43 antibody. On the other hand, if the test antibody is not able to bind to the Cx43 following saturation binding with the reference anti-Cx43 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-Cx43 antibody of the present disclosure.

To determine if an antibody competes for binding with a reference anti-Cx43 antibody, the above-described binding methodology can be performed in two orientations: In a first orientation, the reference antibody can be allowed to bind to Cx43 under saturating conditions followed by assessment of binding of the test antibody to the Cx43 molecule. In a second orientation, the test antibody can be allowed to bind to a Cx43 molecule under saturating conditions followed by assessment of binding of the reference antibody to the Cx43 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the Cx43 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to Cx43. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes

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if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

Additional routine experimentation (e.g., peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

In various embodiments, provided herein is an antibody that binds an epitope located within the amino acid sequence of FLSRPTEKTI (SEQ ID NO: 19). In some embodiments, the epitope can include one or more amino acids selected from the group consisting of R4, P5, E7, K8 and I10 of SEQ ID NO: 19. In one embodiment, the epitope consists of R4, P5, E7, K8 and I10 of SEQ ID NO: 19. In some embodiments, the epitope can include all ten amino acids of SEQ ID NO: 19. In certain embodiments, the epitope consists of all ten amino acids of SEQ ID NO: 19.

Pharmaceutical Compositions and Use Thereof

In another aspect, pharmaceutical compositions are provided that can be used in the methods disclosed herein, i.e., pharmaceutical compositions for inhibiting opening of Cx43 hemichannels in astrocytes or osteocytes, preferably for treating an inflammatory disease or condition or a neurodegenerative disease.

In some embodiments, the pharmaceutical composition comprises a Cx43 ligand and a pharmaceutically acceptable carrier. The Cx43 ligand can be formulated with the pharmaceutically acceptable carrier into a pharmaceutical composition. Additionally, the pharmaceutical composition can include, for example, instructions for use of the composition for the treatment of patients to inhibit opening of Cx43 hemichannels in astrocytes or osteocytes, preferably for treating an inflammatory disease or condition or a neurodegenerative disease.

In one embodiment, the Cx43 ligand can be an anti-Cx43 antibody or antigen-binding fragment thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, and other excipients that are physiologically compatible. Preferably,

the carrier is suitable for parenteral, oral, or topical administration. Depending on the route of administration, the active compound, e.g., small molecule or biologic agent, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

5   Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion, as well as conventional excipients for the preparation of tablets, pills, capsules and the like. The use of such media and agents for the formulation of pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is

10   incompatible with the active compound, use thereof in the pharmaceutical compositions provided herein is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutically acceptable carrier can include a pharmaceutically acceptable antioxidant. Examples of pharmaceutically-acceptable antioxidants include: (1) water

15   soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric

20   acid, and the like.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions provided herein include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, and injectable organic esters, such as ethyl oleate. When required, proper fluidity can be

25   maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. In many cases, it may be useful to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the

30   composition an agent that delays absorption, for example, monostearate salts and gelatin.

These compositions may also contain functional excipients such as preservatives, wetting agents, emulsifying agents and dispersing agents.

Therapeutic compositions typically must be sterile, non-phylogenic, and stable under

the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization, e.g., by microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation include vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The active agent(s) may be mixed under sterile conditions with additional pharmaceutically acceptable carrier(s), and with any preservatives, buffers, or propellants which may be required.

Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Pharmaceutical compositions comprising a Cx43 ligand can be administered alone or in combination therapy. For example, the combination therapy can include a composition provided herein comprising a Cx43 ligand and at least one or more additional therapeutic agents, e.g., anti-inflammatory agents known in the art.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

Exemplary dosage ranges for administration of an antibody include: 10-1000 mg (antibody)/kg (body weight of the patient), 10-800 mg/kg, 10-600 mg/kg, 10-400 mg/kg, 10-200 mg/kg, 30-1000 mg/kg, 30-800 mg/kg, 30-600 mg/kg, 30-400 mg/kg, 30-200 mg/kg, 50-1000 mg/kg, 50-800 mg/kg, 50-600 mg/kg, 50-400 mg/kg, 50-200 mg/kg, 100-1000 mg/kg, 100-900 mg/kg, 100-800 mg/kg, 100-700 mg/kg, 100-600 mg/kg, 100-500 mg/kg,

100-400 mg/kg, 100-300 mg/kg and 100-200 mg/kg. Exemplary dosage schedules include once every three days, once every five days, once every seven days (i.e., once a week), once every 10 days, once every 14 days (i.e., once every two weeks), once every 21 days (i.e., once every three weeks), once every 28 days (i.e., once every four weeks) and once a month.

5   It may be advantageous to formulate parenteral compositions in unit dosage form for ease of administration and uniformity of dosage. Unit dosage form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit contains a predetermined quantity of active agent calculated to produce the desired therapeutic effect in association with any required pharmaceutical carrier. The specification

10   for unit dosage forms are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

       Actual dosage levels of the active ingredients in the pharmaceutical compositions

15   disclosed herein may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. "Parenteral" as used herein in the context of administration means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous,

20   intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

       The phrases "parenteral administration" and "administered parenterally" as used herein refer to modes of administration other than enteral (i.e., via the digestive tract) and

25   topical administration, usually by injection or infusion, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Intravenous injection and infusion are often (but not exclusively) used for antibody

30   administration.

       When agents provided herein are administered as pharmaceuticals, to humans or animals, they can be given alone or as a pharmaceutical composition containing, for example, 0.001 to 90% (e.g., 0.005 to 70%, e.g., 0.01 to 30%) of active ingredient in

combination with a pharmaceutically acceptable carrier.

In one aspect, the improved effectiveness of a combination according to the disclosure can be demonstrated by achieving therapeutic synergy.

The term "therapeutic synergy" is used when the combination of two products at given doses is more efficacious than the best of each of the two products alone at the same doses. In one example, therapeutic synergy can be evaluated by comparing a combination to the best single agent using estimates obtained from a two-way analysis of variance with repeated measurements (e.g., time factor) on desired parameters.

The term "additive" refers to when the combination of two or more products at given doses is equally efficacious than the sum of the efficacies obtained with of each of the two or more products, whilst the term "superadditive" refers to when the combination is more efficacious than the sum of the efficacies obtained with of each of the two or more products.

Also provided herein is a method for inhibiting opening of Cx43 hemichannels in cells, preferably for treating an inflammatory disease or condition or a neurodegenerative disease, comprising administering any one or more of the anti-Cx43 antibodies disclosed herein in a subject in need thereof.

In various embodiments, the methods disclosed herein can include administering to the subject an effective amount of anti-Cx43 antibody or antigen-binding fragment thereof. In general, the effective amount can be administered therapeutically and/or prophylactically.

Treatment can be suitably administered to subjects, particularly humans, suffering from, having, susceptible to, or at risk of developing such disease. Determination of those subjects "at risk" can be made by any objective or subjective determination by a diagnostic test or opinion of a subject or health care provider (e.g., genetic test, enzyme or protein marker, family history, and the like). Identifying a subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (e.g. opinion) or objective (e.g. measurable by a test or diagnostic method).

Administration of the Formulation

The formulations of the present disclosure, including but not limited to reconstituted and liquid formulations, are administered to a mammal in need of treatment with the anti-Cx43 antibodies, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular,

intraventricular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

In embodiments, the formulations are administered to the mammal by intravenous or subcutaneous (i.e., beneath the skin) administration. For such purposes, the formulation may

5   be injected using a syringe. However, other devices for administration of the formulation are available such as injection devices (e.g., the INJECT-EASE™ and GENJECT™ devices); injector pens (such as the GENPEN™); auto-injector devices, needleless devices (e.g., MEDIJECTOR™ and BIOJECTOR™); and subcutaneous patch delivery systems.

In a specific embodiment, the present disclosure is directed to kits for a single dose-

10   administration unit. Such kits comprise a container of an aqueous formulation of therapeutic protein or antibody, including both single or multi-chambered pre-filled syringes. Exemplary pre-filled syringes are available from Vetter GmbH, Ravensburg, Germany.

The appropriate dosage ("therapeutically effective amount") of the protein will depend, for example, on the condition to be treated, the severity and course of the condition,

15   whether the protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to anti-Cx43 antibody, the format of the formulation used, and the discretion of the attending physician. The anti-Cx43 antibody is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The anti-Cx43 antibody

20   may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

For anti-Cx43 antibodies, an initial candidate dosage can range from about 0.1-100 or 1-20 mg/kg for administration to the patient, which can take the form of one or more separate administrations. However, other dosage regimens may be useful. The progress of

25   such therapy is easily monitored by conventional techniques.

According to certain embodiments of the present disclosure, multiple doses of an anti-Cx43 antibody (or a pharmaceutical composition comprising a combination of an anti-Cx43 antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject over a defined time course. The methods according to this

30   aspect of the disclosure comprise sequentially administering to a subject multiple doses of an anti-Cx43 antibody of the disclosure. As used herein, "sequentially administering" means that each dose of anti-Cx43 antibody is administered to the subject at a different point in time, e.g., on different days separated by a predetermined interval (e.g., hours, days, weeks

or months). The present disclosure includes methods which comprise sequentially administering to the patient a single initial dose of an anti-Cx43 antibody, followed by one or more secondary doses of the anti-Cx43 antibody, and optionally followed by one or more tertiary doses of the anti-Cx43 antibody. The anti-Cx43 antibody may be administered at a dose of between 0.1 mg/kg to about 100 mg/kg.

The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-Cx43 antibody of the disclosure. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of anti-Cx43 antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-Cx43 antibody contained in the initial, secondary and/or tertiary doses varies from one another (e.g., adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (e.g., 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (e.g., "maintenance doses").

In certain exemplary embodiments of the present disclosure, each secondary and/or tertiary dose is administered 1 to 26 (e.g., 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 1 1, 1 1 ½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21 , 21 ½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of anti-Cx43 antibody which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

The methods according to this aspect of the disclosure may comprise administering to a patient any number of secondary and/or tertiary doses of an anti-Cx43 antibody. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks or 1 to 2 months after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 12 weeks after the immediately preceding dose. In certain embodiments of the disclosure, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

The present disclosure includes administration regimens in which 1-10 or 2-6 loading doses are administered to a patient at a first frequency (e.g., once a week, once every two weeks, once every three weeks, once a month, once every two months, etc.), followed by administration of two or more maintenance doses to the patient on a less frequent basis. For example, according to this aspect of the disclosure, if the loading doses are administered at a frequency of, e.g., once a month (e.g., two, three, four, or more loading doses administered once a month), then the maintenance doses may be administered to the patient once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every ten weeks, once every twelve weeks, etc.).

EXAMPLES

The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting the disclosure.

Example 1: Antibody binding affinity

Optimized antibody sequences were tested for binding affinity to Cx43 according to the following protocol.

Equilibrium dissociation constants (K_D values) for Cx43 binding to selected purified anti-Cx43 antibodies were determined using a real-time bio-layer interferometer based biosensor (Octet HTX) assay. Octet Streptavidin (SA) biosensors coated with biotinylated-antigen (0.01 ug/mL) were used to capture anti-Cx43 antibodies. Loaded sensors were

dipped into a two-fold dilution series of purified control antibody (starting at 100 nM). All the binding studies were performed in assay buffer (PBS with 0.1% BSA, 0.02% Tween-20 (pH 7.2)) at 25° C with plates shaking at a speed of 1000 rpm. Kinetic association (K_a) and dissociation (K_d) rate constants were determined by processing and fitting the data to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium constants (K_D) were calculated from the kinetic rate constants as:

$$K_D (M) = Kd/Ka$$

Binding affinity results (Table 1) show that in general binding affinity was at least maintained and in many cases, surprisingly enhanced.

Table 1. Binding affinity of various antibodies

Sample ID	K_D (M)	Ka (1/Ms)	Kd (1/s)
Control	8.84E-09	1.87E+05	1.65E-03
Ab#E	2.15E-09	2.10E+05	4.51E-04
Ab#C	2.76E-09	2.54E+05	7.03E-04
Ab#G	2.31E-09	1.91E+05	4.42E-04
Ab#I	3.06E-09	2.00E+05	6.11E-04
Ab#M	2.98E-09	2.00E+05	5.95E-04
Ab#K	2.83E-09	2.22E+05	6.29E-04
Ab#O	3.36E-09	2.01E+05	6.77E-04
Ab#Q	2.53E-09	2.55E+05	6.47E-04
Ab#S	3.42E-09	1.96E+05	6.71E-04

Example 2. Fc receptor binding analysis

The Fc effector functions are mediated by binding of Fc to receptors. The receptors include FCRI, FCRIIa, FCRIIb, FCRIIIa, FCRIIIb, C1q, and FcRn. It is generally desirable to reduce binding affinities to most of the Fc receptors expect FcRn to minimize potential in-vivo toxicity while maintaining antibody half-life. The following surface plasmon resonance (SPR) and enzyme-linked immunosorbent assay (ELISA) protocols were used to test different Fc receptor binding for various antibodies.

A. FCRI binding

Experiment: Biacore 8K

Chip: CM5

(1) Immobilization

The activator was prepared by mixing 400 mM EDC and 100 mM NHS immediately prior to injection. The CM5 sensor chip was activated for 420 s with the mixture. 30 µg/mL of

5   THE™ His tag antibody in 10 mM NaAc (pH 4.5) was then injected to channels 1-8 for 400 s at a flow rate of 30 µL/min. The chip was deactivated by 1 M ethanolamine-HCl (GE).

(2) Capturing Ligand and Running analyte

2 µg/mL CD64 in running buffer (1×HBS-EP+) was injected to Fc2 of channel 1- 4 at a flow rate of 10 µL/min for 30 s. 6 concentrations (40, 20, 10, 5, 2.5 and 1.25 nM) of analyte

10   Ab#C, Ab#G and running buffer were injected orderly to Fc1 - Fc2 of channel 1 - 4 at a flow rate of 30 µL/min for an association phase of 180, followed by 400 dissociation. Repeat 6 cycles of capturing ligand and running analyte according to analyte concentrations in ascending order. 10 mM glycine pH 1.5 as regeneration buffer was injected following every dissociation phase.

15   2 µg/mL CD64 in running buffer (1×HBS-EP+) was injected to Fc2 of channel 1- 6 at a flow rate of 10 µL/min for 30 s. 8 concentrations (10240, 5120, 2560, 1280, 640, 320, 160 and 80 nM) of analyte Ab#K, Ab#O, Ab#S and running buffer were injected orderly to Fc1 - Fc2 of channel 1 - 6 at a flow rate of 30 µL/min for an association phase of 60, followed by 90 dissociation. Repeat 8 cycles of capturing ligand and running analyte according to

20   analyte concentrations in ascending order. 10 mM glycine pH 1.5 as regeneration buffer was injected following every dissociation phase.

(3) Regeneration

The chip was regenerated with 10 mM glycine pH 1.5.

(4) Data analysis

25   Surface channels Fc1 without capturing ligand was used as control surface for reference subtraction. Final data of each interaction was deducted from reference channel and buffer channel data. The experimental data of Ab#C, Ab#G binding to CD64 was fitted by 1:1 binding mode. The 10240 nM curves of analyte Ab#K, Ab#O, Ab#S were removed to allow a better fit. The relative experimental data was fitted by steady state affinity and shown in

30   Table 2 below.

Table 2. FCRI binding

Analyte	Ka (1/Ms)	Kd (1/s)	K_D (M)	Comment

Ab#C	4.39E+05	7.27E-04	1.66E-09	1:1 binding
Ab#G	4.80E+05	2.23E-03	4.65E-09	
Ab#K			1.76E-06	steady state affinity
Ab#O	NA		2.35E-06	
Ab#S			2.61E-06	

All antibodies showed low or no FCRI binding which is advantageous.

B.   Binding to FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb

5   Experiment: Biacore 8K

Chip: CM5

(1) Immobilization

The activator was prepared by mixing 400 mM EDC and 100 mM NHS immediately prior

to injection. The CM5 sensor chip was activated for 420 s with the mixture. 30 μg/mL of

10   THE™ His tag antibody in 10 mM NaAc (pH 4.5) was then injected to channels 1-8 for 400

s at a flow rate of 30 μL/min. The chip was deactivated by 1 M ethanolamine-HCl (GE).

(2) Capturing Ligand and Running analyte

1 μg/mL FcγRIIa , FcγRIIb, FcγRIIIa, or FcγRIIIb in running buffer (1×HBS-EP+) was

injected to Fc2 of channel 1 - 8 at a flow rate of 10 μL/min for 15 s. Analytes were injected

15   to channel 1 - 8 respectively. A series of analyte concentrations (seeing Table 3 below) were

monitored at a flow rate of 30 μL/min for an association phase of 60 s, followed by 90 s

dissociation. 10 mM glycine pH 1.5 as regeneration buffer was injected following every

dissociation phase.

Table 3. Analyte concentrations

Analyte	Tested Concentration (nM)	20
Ab#C	0, 160, 320, 640, 1280, 2560, 5120, 10240	
Others	0, 320, 640, 1280, 2560, 5120, 10240, 20480, 40960	

25   (3) Regeneration

The chip was regenerated with 10 mM glycine pH 1.5.

(4) Data analysis

Surface channels Fc1 without capturing ligand was used as control surface for reference subtraction. Final data of each interaction was deducted from reference channel and buffer channel data. The experimental data of antibodies binding to FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb was fitted by steady state affinity mode and shown in Table 4 below.

Table 4. FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb binding

Sample ID	binding to FcγRIIa	binding to FcγRIIb	binding to FcγRIIIa	binding to FcγRIIIb
Ab#C	3.03E-06	9.72E-06	1.78E-06	4.31E-06
Ab#G	1.79E-05	1.32E-05	no/very weak binding	no/very weak binding
Ab#K	no/very weak binding	no/very weak binding	2.64E-05	no/very weak binding
Ab#O	3.30E-05	1.78E-05	no/very weak binding	no/very weak binding
Ab#S	no/very weak binding	no/very weak binding	no/very weak binding	no/very weak binding

All antibodies showed low or no FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb binding which is advantageous.

C. Binding to FcRn

Experiment: Biacore 8K

Chip: CM5

(1) Buffer exchange

Buffer of human FcRn was exchanged to running buffer (50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 150 mM NaCl, 0.05% Tween20, pH 6.0) using desalting column according to the instruction manual. The concentration was determined by Nanodrop.

(2) Immobilization

The activator was prepared by mixing 400 mM EDC and 100 mM NHS (GE) immediately prior to injection. The CM5 sensor chip was activated for 420 s with the mixture at a flow rate of 10 µL/min. 5 µg/mL of antibodies in 10 mM NaAc (pH 5.5) were then injected to Fc2 of channel 1-8 respectively at a flow rate of 10 µL/min for 60 s. The relative Fc1 was blocked. The chip was deactivated by 1 M ethanolamine-HCl (GE) at a flow rate of 10

μL/min for 420 s.

(2) Running analyte

Analyte FcRn was injected to channel 1 - 8 respectively. 8 concentrations of FcRn (0, 93.75, 187.5, 375, 750, 1500, 3000 and 6000 nM) were monitored at a flow rate of 30 μL/min for an association phase of 60 s, followed by 90 s dissociation. After each cycle of interaction analysis, the sensor chip surface was regenerated with 1×PBS (pH 7.4) at a flow rate of 10 μL/min for 30 s.

(3) Regeneration

The chip was regenerated with 1×PBS (pH 7.4).

(4) Data analysis

Surface channels Fc1 without immobilized antibodies were used as control surface for reference subtraction. Final data of each interaction was deducted from reference channel and buffer channel data. The experimental data was fitted by steady state affinity mode and shown in Table 5 below.

Table 5. FcRn binding

Ligand	K_D (M)
Ab#C	2.40E-06
Ab#G	2.39E-06
Ab#K	2.56E-06
Ab#O	2.57E-06
Ab#S	2.43E-06

All antibodies showed similar FcRn binding which is desirable.

D. Binding to C1q by ELISA

Plates (Nunc) were coated with antibodies at 3μg/mL overnight at 4 °C. After blocking and washing, C1q was half-log titrated in blocking buffer (600, 189.75, 60.01, 18.98, 6.00, 1.90, 0.60, 0.19, 0.06 and 0.02 μg/mL) and incubated at room temperature for 2 h. The plates were then washed and subsequently incubated with secondary antibody Sheep anti-human C1q Ab-HRP for 1 h. After washing, TMB substrate was added and the interaction was stopped by 2M HCl. The absorbance at 450 nm was read using a microplate reader (Molecular Device) and shown in Table 6 below.

Table 6. C1q binding

Antibody	K_D
Ab#C	61 nM
Ab#G	no binding
Ab#K	weak binding
Ab#O	no binding
Ab#S	no binding

All antibodies showed low or no C1q binding which is advantageous.

Example 3. Epitope mapping

To reconstruct epitopes of the target molecule a library of peptide based epitope mimics was synthesized using solid-phase Fmoc synthesis. An amino functionalized polypropylene support was obtained by grafting with a proprietary hydrophilic polymer formulation, followed by reaction with t- butyloxycarbonyl-hexamethylenediamine (BocHMDA) using dicyclohexylcarbodiimide (DCC) with *N*-hydroxybenzotriazole (HOBt) and subsequent cleavage of the Boc-groups using trifluoroacetic acid (TFA). Standard Fmoc-peptide synthesis was used to synthesize peptides on the amino-functionalized solid support by custom modified JANUS liquid handling stations (Perkin Elmer).

Synthesis of structural mimics was done using Chemically Linked Peptides on Scaffolds (CLIPS) technology. CLIPS technology allows to structure peptides into single loops, double loops, triple loops, sheet-like folds, helix-like folds and combinations thereof. CLIPS templates are coupled to cysteine residues. The side-chains of multiple cysteines in the peptides are coupled to one or two CLIPS templates. For example, a 0.5 mM solution of the P2 CLIPS (2,6-bis(bromomethyl)pyridine) is dissolved in ammonium bicarbonate (20 mM, pH 7.8)/acetonitrile (1:3(v/v)). This solution is added onto the peptide arrays. The CLIPS template will bind to side-chains of two cysteines as present in the solid-phase bound peptides of the peptide-arrays (455 wells plate with 3 μl wells). The peptide arrays are gently shaken in the solution for 30 to 60 minutes while completely covered in solution. Finally, the peptide arrays are washed extensively with excess of H_2O and sonicated in disrupt-buffer containing 1% SDS/0.1 % 2,2'-

(Ethylenedioxy)diethanethiol in PBS (pH 7.2) at 70°C for 30 minutes, followed by sonication in H₂O for another 45 minutes. The T3 CLIPS carrying peptides were made in a similar way but now with three cysteines.

Different sets of peptides were synthesized according to the following designs. Note that actual order of peptides on mini-cards in some was randomized.

	Set 1	Set 2
Label	RN.FLSRPTEKTI	WN
Description	Single residue substitution variants derived from the lead sequence FLSRPTEKTI. In this series every residue within the peptide is replaced by all other proteogenic residues with an offset of one residue.	Peptides of length 5, 6, 7, 8 and 9 derived from the lead peptide sequence FLSRPTEKTI with an offset of one residue.
Sequences (first 10)	FWSRPTEKTI (SEQ ID NO. 20) FLSRPTEKTC (SEQ ID NO. 21) FLGRPTEKTI (SEQ ID NO. 22) FLSRPTEKDI (SEQ ID NO. 23) FLSRPTEKYI (SEQ ID NO. 24) FLSRWTEKTI (SEQ ID NO. 25) FLSRPSEKTI (SEQ ID NO. 26) FLNRPTEKTI (SEQ ID NO. 27) FLSRPFEKTI (SEQ ID NO. 28) FLSRPTEKTG (SEQ ID NO. 29)	FLSRP (SEQ ID NO. 30) LSRPT (SEQ ID NO. 31) SRPTE (SEQ ID NO. 32) RPTEK (SEQ ID NO. 33) PTEKT (SEQ ID NO. 34) TEKTI (SEQ ID NO. 35) FLSRPT (SEQ ID NO. 36) LSRPTE (SEQ ID NO. 37) SRPTEK (SEQ ID NO. 38) RPTEKT (SEQ ID NO. 39)

The binding of antibody to each of the synthesized peptides was tested in ELISA. The peptide arrays were incubated with primary antibody solution (overnight at 4°C). After washing, the peptide arrays were incubated with a 1/1000 dilution of an appropriate antibody peroxidase conjugate (SBA; goat anti-human HRP conjugate, Southern Biotech) for one hour at 25°C. After washing, the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 20 µl/ml of 3 percent H₂O₂ were added. After one hour, the color development was measured. The color development was quantified with a charge coupled device (CCD) - camera and an image processing system.

The values obtained from the CCD camera range from 0 to 3000 mAU, similar to a standard 96-well plate ELISA-reader. The results are quantified and stored into the lab database. Occasionally a well contains an air-bubble resulting in a false-positive value, the cards are manually inspected and any values caused by an air-bubble are scored as 0.

To verify the quality of the synthesized peptides, a separate set of positive and negative control peptides was synthesized in parallel. These were screened with commercial antibodies 3C9 and 57.9 (*ref.* Posthumus *et al.* (1990) *J. Virol.* **64**:3304–3309).

A graphical overview of the complete dataset is given in **Figure 1.** Here a box plot depicts each dataset and indicates the average ELISA signal, the distribution and the outliers within each dataset. Depending on experiment conditions (amount of antibody, blocking strength etc) different distributions of ELISA data are obtained.

Antibody was tested under high stringency conditions and yielded binding with arrayed peptides (**Figure 2 and Figure 3**). Each of two peptide sets was analyzed separately.

Analysis of data recoded with substitution variants of the lead sequence FLSRPTEKTI suggested that positions R4, P5, E7, K8 and I10 are crucial for binding of the antibody, as most replacements on these positions reduced signal intensities (Figure 2) except for homologous replacements, e. g., R4K.

Analysis of data recorded with truncation variants of the lead sequence FLSRPTEKTI indicated that the C-terminus of the sequence is essential for binding to occur (**Figure 3**), as well as presence of at least five residues, which do not tolerate replacements.

In summary, the antibody was tested on a peptide array comprised of two types of peptide variants derived from the lead sequence FLSRPTEKTI – single residue mutants and truncation variants. The antibody yielded detectable binding under high stringency conditions. Binding of the antibody is sensitive to replacements of residues R4, P5, E7, K8 and I10 with preference towards the C-terminal part of the lead sequence.

Example 4. Antibody stability

Antibody stability is an important factor affecting development, efficacy, production cost, etc. After sequence optimization, key stability parameters were evaluated. The species distribution profiles of various antibodies under acidic and heat conditions were tested. All antibodies show improved stability.

A. SE-UPLC (size-exclusion ultra performance liquid chromatograph)

Formulation: PBS, pH 6.5 or 7.2

Concentration (mg/mL): 5.28, 5.13, 5.00, 5.17, 5.01, 5.26, 4.92, 5.04, 4.99, 5.12 (all at about 5 mg/mL)

Condition: room temperature, acid treatment then storage at 4 °C for 1 week or at 40 °C for 1 week

2 μL of sample was injected into ACQUITY UPLC Protein BEH SEC 200, 1.7 μm, 4.6 x 150 mm column with a flow of 0.3 mL/min for 10 minutes. A mobile phase of 50 mM Sodium Phosphate, 500 mM NaCl, pH 6.2 was used. All antibodies show desirable stability under various pH, heat and storage conditions.

B. rCE-SDS (reduced capillary electrophoresis-sodium dodecyl sulfate)

Formulation: PBS, pH 6.5, 7.2, 6.2

Concentration (mg/mL): 0.5

Condition: room temperature, acidic treatment then storage at 4 °C for 1 week or at 40 °C for 1 week

The sample was prepared in reducing labeling buffer before being submitted to the LabChip GXII system (PerkinElmer). All antibodies show desirable stability under various pH, heat and storage conditions.

Example 5. Assays for hemichannel opening

A. In vitro assays

The antibodies disclosed herein can be tested in vitro for their effect on hemichannel opening or blockage using, e.g., a dye-uptake assay. The dye can be a fluorescent tracer dye (e.g., ethidium bromide or Lucifer yellow or Alexa dyes).

In one example, a fluid flow loop apparatus (FFLA) (Parrallel Plate Flow Chamber), or modification thereof, can be used. FFLA mimics dynamic fluid microenvironment in the bone to produce fluid flow shear stress (FFSS). Cells are cultured in a parallel plate flow chamber, exposing the cells to steady laminar fluid flow.

Osteocytes sense mechanical strain produced by FFSS in the osteocyte lacuna/canalicular network. It has been proposed that bone fluid flow is driven by extravascular pressure as well as applied cyclic mechanical loading of osteocytes and that the peak physiologic loads are 8 to 30 dyn/cm². In certain aspects FFSS levels were in range of physiological values reported from previous studies measuring fluid flow within bone. Fluid shear stress magnitude can be changed by adjusting column height of the flow loop.

Assays used to assess the functionality of the hemichannels can use a fluorescent tracer molecule that is small enough to pass through the pore of the hemichannel. If the hemichannel is closed the molecules cannot pass. If the hemichannel is open the dye can pass through and cause the cell to fluoresce, allowing quantification of the fluorescence.

5   When ethidium bromide attaches to DNA it becomes fluorescent. Lucifer yellow fluoresces once it is located inside of a cell.

Dye transfer methods can comprise exposing cells to extracellular fluorescent permeability tracers. Extracellular permeability tracers are molecules that remain outside of cell unless some condition increases the permeability of the cell membrane. In certain

10   aspects the tracers have a mass of less than 1, 2, or 3 kDa. In other aspect the tracer will have a net charge. Such permeability tracers include, but are not limited to the anionic dyes Lucifer yellow (LY; net charge = −1) and cationic probes ethidium bromide (Etd; net charge = +1), propidium iodide (PI; net charge = +2). The fluorescence of EtBr is enhanced upon binding to DNA, increasing the contrast and allowing more easy identification. In certain

15   aspects extracellular dye is removed at different time periods or after the application of stimuli to open hemichannels and the fluorescence intensity retained by each cell is quantified. In certain aspects fluorescence intensity is quantified in snap shot images.

The materials used in in vitro assays to test hemichannel opening/blockage include:

Hemichannel expressing cells or cell lines. Cells or cell lines expressing the various

20   connexin hemichannels can be obtained, isolated, or engineered using methods and/or expression vectors known in the art.

Osteocytes: Primary osteocytes isolated from animals (including mouse, rats, rabbits, chicken) etc. or osteocytic cell lines including, but not limited to MLO-Y4 cells and others.

Osteoblasts: MLO-A5 osteoblasts are used as a control because they express

25   Connexin 43, but they do not appear to open when stimulated by alendronate.

Tracer Molecules include, but are not limited to lucifer yellow, ethidium bromide, Evans Blue, Alexa350, Alexa488 and Alexa594.

Cx43(E2): The Cx43(E2) antibody is specific for Cx43 hemichannels. Cx43E2 binds the 2nd extracellular loop of Cx43 hemichannels and prevents hemichannel opening.

30   Methods for determining if an antibody opens or closes/blocks hemichannels include one or more of the following steps:

(a) Isolating, obtaining, or producing a connexin expressing cell or cell line. For example, primary osteocytes can be isolated from calveria. Other cell types can be isolated

using other methods known in the art. In certain aspects calvarial osteocytes are isolated from animals (e.g., 16-day embryonic chicken calvaria or new-born mice). Animals are decapitated and calvarial bone is dissected and quickly dipped in 70% alcohol. The calvarial bone is then put in αMEM and washed multiple times with PBS. Cleaned bones are placed in fresh αMEM. The bones are minced and cut into 1.5 mm area size. The bone pieces can be treated with collagenase to remove soft tissues and osteoid followed by decalcification using EDTA. Finally, osteocytes are released from the bone chips by treating with collagenase and vigorous agitation.

(b) Isolating primary osteocytes from long bone. Long bone osteocytes can be isolated from 2-3 week old mice or rats. For example, mice are given an overdose of anesthesia, and cervically dislocated, decapitated, and dipped into 70% Ethanol. The femur and tibia with the end of the joints still intact are isolated. The leg is quickly dipped in 70% alcohol and then placed into αMEM. Legs in αMEM are washed with PBS. The major portion of muscle is removed, and detached from the tendons/ligaments. Cleaned bones are placed in fresh αMEM. Once all bones are cleaned, both ends of each bone are cut off using a scalpel just prior to flushing out the marrow using PBS. Bones are cut into 1.5 to 2 mm lengths and treated with collagenase. In one example, the bone pieces are treated with collagenase sequentially 9 times to remove all other tissues and osteoid followed by decalcification using EDTA.

(c) Culturing the cells or cell lines. For example, primary and/or osteocytic cell lines are cultured on collagen-coated plates and are bathed in recording medium (HCO_3-free α-MEM medium buffered with HEPES) containing a permeability tracer.

(d) Administering a test antibody. The cultured cells are placed contacted with a test antibody for desirable amount of time.

(e) Determining permeability tracer uptake. Permeability tracer uptake is determined by detecting the amount of tracer inside the cells. In certain aspects time-lapse recording is used. Fluorescence can be recorded at regions of interest in different cells with an eclipse filter on a microscope based on the wavelength of the fluorescence of the tracer or other probe(s) being used. In certain aspects images are captured by fast cooled digital camera every 2 minutes and image processing is performed with ImageJ software. The collected data can be illustrated as fold difference of initial fluorescence and fluorescence at the time of interest versus the basal fluorescence.

For snapshot images, cells can be exposed to permeability tracer for 5-10 minutes, rinsed multiple times with PBS, and fixed with formaldehyde. In certain aspects, at least three microphotographs of fluorescence fields are taken with a microscope. Image analysis is done with ImageJ software. The average of pixel density of random cells is measured.

Confirmation of the opening or blockage of connexin hemichannels can be obtain by, for example, treating osteocytes with fluid flow shear stress and/or AD, both known to open Cx43 hemichannels, along with the test antibody. If the test antibody blocks Cx43 hemichannel, this channel blockage will be reversed by fluid flow shear stress and/or AD. To control for the blockage of Cx43 hemichannels, osteocytes are treated with Cx43(E2) antibody, a polyclonal antibody specifically inhibiting opening of Cx43 hemichannels.

In a particular example, MLO-Y4 osteocytic cells were treated with 1 µg/ml Cx43(E2) antibody or a test antibody for 30 min in the absence or presence of 20 µM AD. Ethidium bromide dye uptake was conducted and quantified as compared to non-treated basal level of uptake. The assay was carried out in presence of calcium. Low calcium conditions can be used as control (opens hemichannels). The opening of osteocytic hemichannels induced by AD is blocked by Cx43(E2) antibody or a test antibody.

Inhibiting the opening of Cx43 hemichannels in chondrocytes (e.g., by chemical reagents, etc.), can suppress the inflammation and the development of osteoarthritis. The hemichannel opening in chondrocytes can be detected using the methods described herein. The release of pro-inflammatory factors (PGE2 and ATP) by Cx43 hemichannels is measured using ELISA assays. Agents that block the opening of hemichannels can be used a therapeutic for inflammatory disorders such as OA.

In vitro cell models for evaluating Cx43 channel activity. Primary chondrocytes are isolated from joints of mouse leg bones. An agent's effect on Cx43 hemichannel opening and gap junction coupling in chondrocytes can be detected and the time and dosage-dependent effects evaluated. For example, hemichannel opening is assessed by dye uptake assay, using Lucifer yellow or Alexa dyes. Downstream effects are measured by detecting release of PGE2 and ATP using ELISA assays.

Specific protocols are shown below.

Immunoblots. MLO-Y4 Cells were seeded 3×10^5 at 60 mm dishes for 48 h. Mouse heart tissues were collected in lysis buffer (5 mM Tris, 5 mM EDTA, 5 mM EGTA plus protease inhibitors, 20 µl/ml phenylmethylsulfonyl fluoride (PMSF), 20 µl/ml N-ethylmaleimide, 10 µl/ml NaVO4 and 10 µl/ml leupeptin), homogenized and centrifuged at

100,000 x g at 4°C for 30 min and resuspended in lysis buffer. Crude membrane proteins were separated by 10% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes and blotted with anti-Cx43 CT (1:300 dilution) recognizing the C-terminus of Cx43 or anti-Cx43 E2 (1:500 dilution) recognizing the second extracellular loop

5   of Cx43 or the monoclonal antibodies against the second extracellular loop of Cx43 (1:100 dilution). Secondary antibodies, infrared IRDye® 800 anti-rabbit IgG (1:15000) (LI-COR, Lincoln, NE, USA), fluorescence was detected with Odyssey infrared detection system (LI-COR, Lincoln, NE, USA).

Immunofluorescence. MLO-Y4 cells were cultured on collagen-coated glass

10   coverslip. The cells were rinsed 2 times with PBS and incubated with cold 70% ethanol for 20 min at -20 °C. The use of PFA destroys the epitope, which is lysine rich, therefore not recommended. Then the cells were rinsed twice with PBS in order to remove the ethanol. After that the cells were blocked with blocking solution (2% goat serum, 2% fish skin gelatin, and 1% bovine serum albumin in PBS) overnight. Then, the cells were labeled with

15   monoclonal antibodies at different concentrations in PBS, followed by FITC-conjugated goat anti-mouse antibody and WGA-alexa594 (Invitrogen) (1:400 and 1:1500 in blocking solution respectively). The cells were observed by Olympus BH-2 fluorescence microscopy and the images were processed offline with NIH Image J software.

Dye uptake for hemichannel activities. Dye uptake measurements were evaluated

20   using snap shot photographs. MLO-Y4 cells were plated on the collagen coated 35 mm dish and incubated with recording medium, HCO_3^- free saline medium buffered with 10 mM HEPES salt composition in mM, 154 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5 Glucose. Medium with low concentration of divalent cation (low$[X^2]$) was added 0.5 mM EGTA but not $CaCl_2$ and $MgCl_2$. The recording or low$[X^2]$ containing 50 μM EtBr for snap shot

25   recording. Cells were exposed to 100 μM of EtBr during 5 min, then rinsed 3 times with PBS and fixed with 2% formamide. At least 3 microphotographs of fluorescence fields were taken with a 10X dry objective in an inverted microscope (Carl Zeiss) with a rhodamine filter. The image analysis was made offline with the software image J. The average of pixel density of 30 random cells was measured.

30   *Dye coupling assay for gap junctions.* MLO-Y4 cells were plated on collagen coated 35 mm dish and incubated with recording medium (HCO_3^- free αMEM medium buffered with 10 mM HEPES). Cells were microinjected using a micromanipulator

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InjectMan NI 2 and Femtojet both from Eppendorf (Eppendorf) at 37°C with alexafluor 350 (Invitrogen, Eugene, Oregon, USA) (10 mM in PBS). Dye transfer was measured after 2 minutes of alexafluor 350 injection. The index of dye coupling was scored counting the number of cells that were dye transferred. Dye coupling was observed under an inverted
5   microscope equipped with Xenon arc lamp illumination and a Nikon eclipse (Nikon, Japan) (excitation wavelengths 330-380 nm; emission wavelengths above 420 nm).

Cell parachute dye-transfer assay for gap junctions. MLO-Y4 cells were grown to confluence in 12 well plates. The donor cells were incubated with 5 µM calcein red-orange-AM (790 Da) and 5 µM Oregon green 488 BAPTA-2-AM (1752 Da) for 40 minutes at
10   37°C. Gap junction intercellular communication can be followed by simultaneously labeling cells with calcein red-orange as a gap junction permeable tracer dye and the gap junction channel impermeable dye Oregon green 488 BAPTA-2. Donor cells preloaded were remove form the plate by trypsinization. Preloaded cells were layered ('parachuted') over the top of the unlabeled recipient cells cultured in at a 1:4 donor to receiver ratio. Cells were allowed
15   to attach for various periods 1 hours, and then carefully washed 3 times and fixed in fresh 2% PFA 10 min RT and rinse 3 times again. The cells were examined with a fluorescence microscope. For calcein red-orange transfer, the threshold was adjusted to clearly distinguish the dye-transfer boundaries. The dye transfer positive criterion was detecting the calcein red-orange/Oregon green 488 BAPTA-2 with contact cells calcein red-orange
20   positive and Oregon green 488 BAPTA-2 negative. The dye transfer was almost undetectable (< 1%). The images were taken in places where we found Oregon green 488 BAPTA-2 green positive cells.

Fluid flow shear stress to open hemichannels. Fluid flow was created by parallel-plate flow chambers separated by a gasket of defined thickness with gravity-driven fluid
25   flow using a peristaltic pump. The thickness of the gasket determined the channel height, which was adjusted along with flow rate to generate stress levels of 16 dyn/cm^2. The circulating medium was α-MEM buffered with 10 mM HEPES.

B. In vivo assays

30   In certain aspects Cx43 modulation in osteocytes is determined by injecting candidate reagents into a long bone and using fluorescence tracer dyes (e.g., calcein or Evans blue) to detect the opening or blockage of hemichannels in osteocytes in situ.

One example of an in vivo assay to analyze hemichannels in osteocytes uses 3-4 month old mice or rats. The animals are weighed. A test antibody is introduced into the animal through intraperitoneal (IP) injection. After 2-4 hours, fluorescence tracer dyes (i.e. Evans blue, Alexa 594) are injected into lateral tail vein of the animal or by IP injection. Note: up to 1% of animal's body weight in volume can be injected. In certain aspects the animal is warmed prior to tail vein injection to dilate the tail vein. After 2-4 hours, the animal is scarified and tibial and femur bones free of muscle tissues are dissected and washed multiple times with PBS. The bone is fixed in paraformaldehyde and decalcified in 14% EDTA solution at 4°C for two weeks or room temperature under constant agitation for 3-5 days. The bone is washed in PBS and soaked in 30% sucrose in PBS overnight and embedded in OCT compound. Position of the bone is typically adjusted in the mold as needed. Five μm thick frozen sections are cut using a cryostat, the sections rinsed in PBS, and mounted using 50% glycerol in PBS. The bone sections can be examined under fluorescence microscope and the degree of osteocytes in the bone taking up tracer dyes are quantified using Image J.

The opening of Cx43 hemichannels in osteocytes can be confirmed by mechanical loading on tibias opening Cx43 hemichannels in osteocytes. This can serve as a negative control for hemichannel opening in osteocytes in vivo. For positive control, mice with the deficiency of Cx43 in osteocytes are used. This mouse is generated by crossing with 10-kb DMP-1 Cre and Cx43 flox mice.

Cx43 conditional knock out (cKO) mice. Because homozygous Cx43 global knockouts are lethal, to examine the role of Cx43 expressed in osteocytes, osteocyte-specific Cx43 knockout mice were generated. Crossing mice homozygous for the floxed Cx43 gene with Cx43 global heterozygous mice to facilitate the complete deletion of Cx43 in osteocytes. Cx43fl/- mice (50% of progeny) were then crossed with mice expressing Cre recombinase driven by the human DMP-1 promoter. This created mice that were Cx43 fl/-, DMP1 Cre+ or Cx43 fl/-, DMP1 Cre- (small percentage are Cx43fl/fl or Cx43-/-). Cx43 deficient osteocytes were confirmed by immunohistochemistry.

Studies can include 8 groups of mice: WT treated with Cx43(E2), WT without Cx43(E2), cKO treated with Cx43(E2), cKO without AD Cx43(E2) WT treated with test antibody (TA), WT without TA, cKO treated with TA, and cKO without TA. Cx43(E2) or TA was administered to the mice at 150 μg/kg body weight. With Cx43(E2) or TA treatment it is expected inflammation will increase in KO compared to WT mice. And

without Cx43(E2) or TA treatment bone metastasis should be similar between WT and knockout mice.

Spinal cord injury animal models that can be used to test the Cx43 antibodies disclosed herein are reviewed by, e.g., Sharif-Alhoseini et al., Spinal Cord. 2017 Aug;55(8):714-721, incorporated herein by reference in its entirety. Inflammation (e.g., rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis) animal models for use to test the Cx43 antibodies disclosed herein are reviewed by, e.g., Webb, Biochem Pharmacol. 2014 Jan 1;87(1):121-30, incorporated herein by reference in its entirety.

Various aspects of the present disclosure may be used alone, in combination, or in a variety of arrangements not specifically discussed in the embodiments described in the foregoing and is therefore not limited in its application to the details and arrangement of components set forth in the foregoing description or illustrated in the drawings. For example, aspects described in one embodiment may be combined in any manner with aspects described in other embodiments.

While specific embodiments of the subject disclosure have been discussed, the above specification is illustrative and not restrictive. Many variations of the disclosure will become apparent to those skilled in the art upon review of this specification. The full scope of the disclosure should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

INCORPORATION BY REFERENCE

All publications, patents and patent applications referenced in this specification are incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically indicated to be so incorporated by reference.

CLAIMS

1.   An anti-Cx43 antibody, or antigen binding fragment thereof, comprising:

a first, second and third heavy chain complementarity determining region (CDR) sequence having the amino acid sequence of SEQ ID NOs: 1, 2, and 3, respectively; and

a first, second and third light chain CDR sequence having the amino acid sequence of SEQ ID NOs: 4, 5, and 6, respectively.

2.   The antibody or fragment thereof of claim 1, comprising a heavy chain variable domain having the amino acid sequence of SEQ ID NO: 7, and a light chain variable domain having the amino acid sequence of SEQ ID NO: 8.

3.   An anti-Cx43 antibody, or antigen binding fragment thereof, comprising a heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-17, and a light chain having the amino acid sequence of SEQ ID NO: 18.

4.   An anti-Cx43 antibody, or antigen binding fragment thereof, wherein, when bound to Cx43, binds to an epitope located within the amino acid sequence of FLSRPTEKTI (SEQ ID NO: 19).

5.   The antibody or fragment thereof of claim 4, wherein the epitope comprises one or more amino acids selected from the group consisting of R4, P5, E7, K8 and I10 of SEQ ID NO: 19.

6.   The antibody or fragment thereof of claim 4, wherein the epitope consists of R4, P5, E7, K8 and I10 of SEQ ID NO: 19.

7.   The antibody or fragment thereof of claim 4, wherein the epitope comprises all ten amino acids of SEQ ID NO: 19.

8.   The antibody or fragment thereof of claim 4, wherein the epitope consists of all ten amino acids of SEQ ID NO: 19.

9.   An isolated anti-Cx43 antibody, or antigen binding fragment thereof, wherein the antibody or fragment thereof cross-competes for binding to Cx43 with the antibody or fragment thereof of any one of claims 1-8.

10.   An isolated anti-Cx43 antibody, or antigen binding fragment thereof, wherein the antibody or fragment thereof cross-competes for binding to Cx43 with the antibody or fragment thereof of any one of claims 1-3, wherein preferably the antibody or fragment thereof binds to an epitope located within the amino acid sequence of FLSRPTEKTI (SEQ ID NO: 19), wherein more preferably the epitope comprises one or more amino acids selected from the group consisting of R4, P5, E7, K8 and I10 of SEQ ID NO: 19, wherein

even more preferably the epitope comprises all ten amino acids of SEQ ID NO: 19.

11.   The antibody or fragment thereof of any one of claims 1-10, which inhibits opening of Cx43 hemichannels in cells.

12.   A pharmaceutical composition for inhibiting opening of Cx43 hemichannels in cells, preferably for treating an inflammatory disease or condition or a neurodegenerative disease, comprising the antibody or fragment thereof of any one of claims 1-11 and a pharmaceutically acceptable carrier.

13.   The pharmaceutical composition of claim 12, wherein the neurodegenerative disease is spinal cord injury.

14.   Use of the antibody or fragment thereof of any one of claims 1-11 for the manufacture of a medicament for inhibiting opening of Cx43 hemichannels in cells, preferably for treating an inflammatory disease or condition or a neurodegenerative disease such as spinal cord injury.

15.   A method of inhibiting opening of Cx43 hemichannels in cells, preferably for treating an inflammatory disease or condition or a neurodegenerative disease such as spinal cord injury, comprising contacting the cells with an effective amount of the antibody or fragment thereof of any one of claims 1-11.

16.   A method for treating a disease or condition associated with opening of Cx43 hemichannels in astrocytes or osteocytes, preferably for treating an inflammatory disease or condition or a neurodegenerative disease such as spinal cord injury, comprising administering a therapeutically effective amount of the antibody or fragment thereof of any one of claims 1-11 to a patient in need thereof, wherein preferably said opening is excessive or chronic opening.
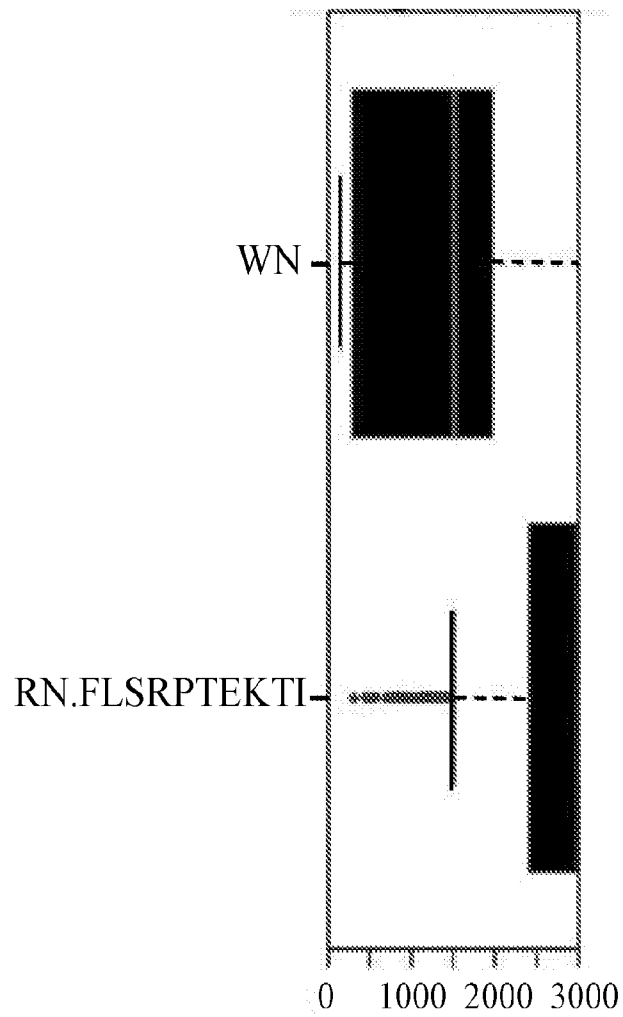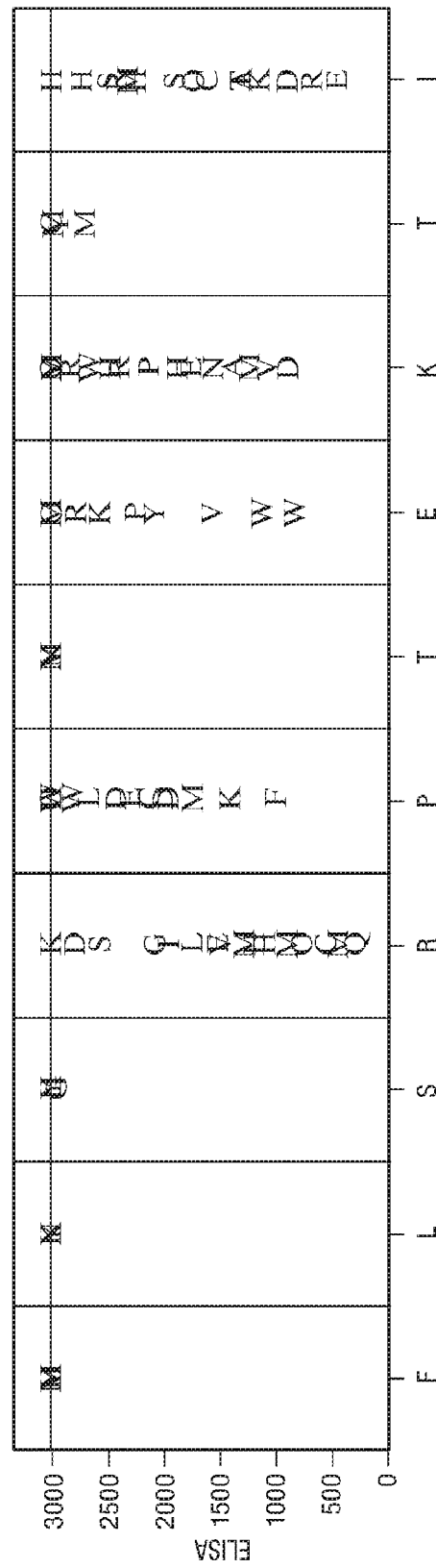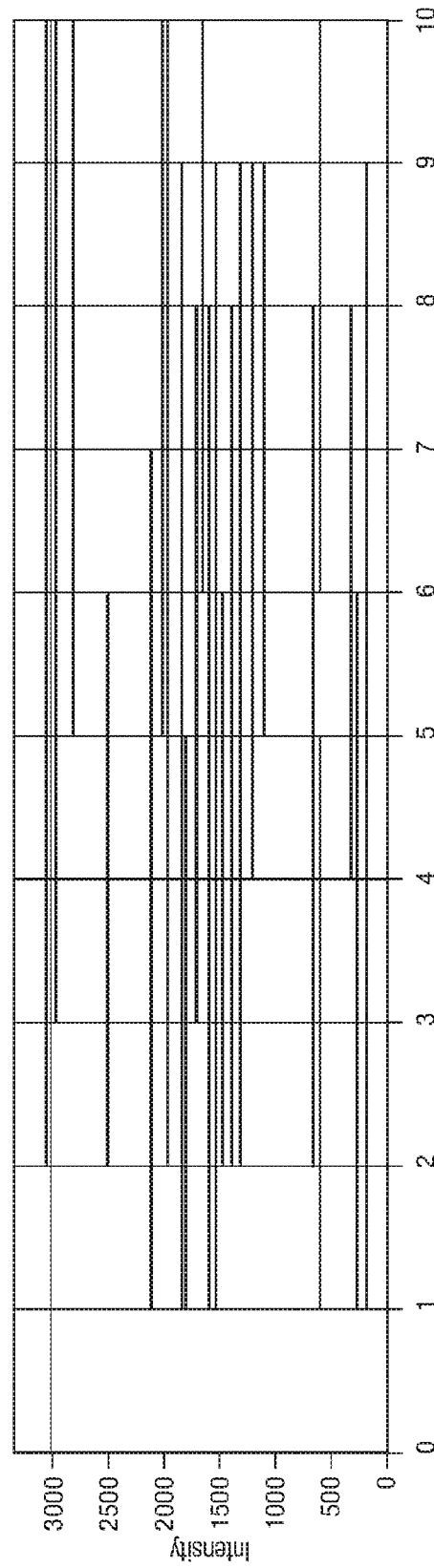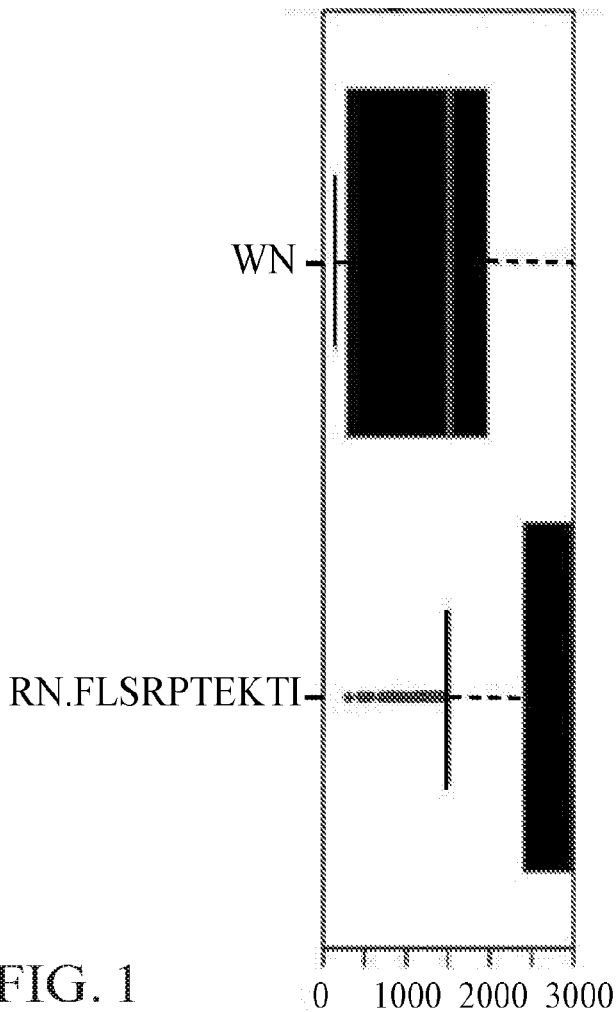
FIG. 1

SUBSTITUTE SHEET (RULE 26)

FIG. 2

FIG. 3

SUBSTITUTE SHEET (RULE 26)

WN

RN.FLSRPTEKTI

0   1000 2000 3000

FIG. 1