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(54) Title: ANTI-IFNAR1 ANTIBODIES FOR TREATING AUTOIMMUNE DISEASES

(57) Abstract: Provided are antibodies or fragments thereof having binding specificity to the human interferon alpha and beta receptor subunit 1 (IFNAR1) protein. In various examples, the antibodies or fragments thereof include a VH and VL CDRs as provided, or variants thereof. Methods of using the antibodies or fragments thereof for treating autoimmune diseases and disorders are also provided.

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ANTI-IFNAR1 ANTIBODIES FOR TREATING AUTOIMMUNE DISEASES

The present invention claims the priority of the PCT/CN2018/106157, filed on September 18, 2018, the contents of which are incorporated herein by its entirety.

BACKGROUND

Systemic lupus erythematosus (SLE), also known simply as lupus, is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue in many parts of the body. Common symptoms include painful and swollen joints, fever, chest pain, hair loss, mouth ulcers, swollen lymph nodes, feeling tired, and a red rash which is most commonly on the face. Often there are periods of illness, called flares, and periods of remission during which there are few symptoms.

The cause of SLE is not clear. The mechanism involves an immune response by autoantibodies against a person's own tissues. These are most commonly anti-nuclear antibodies and they result in inflammation. There is no cure for SLE. Treatments may include NSAIDs, corticosteroids, immunosuppressants, hydroxychloroquine, and methotrexate. SLE significantly increases the risk of cardiovascular disease with this being the most common cause of death.

Type I IFNs, particularly the IFN-αs and IFN-β, have received attention for their roles in the pathogenesis of SLE and other autoimmune and inflammatory syndromes. By signaling through a common receptor (IFNAR), these pleiotropic cytokines affect almost every aspect of innate and adaptive immune responses, including upregulation of MHC and costimulatory molecules, and production of B cell survival factors (BAFF, April) by antigen-presenting cells, culminating in the engagement and expansion of autoreactive T and B cells. Of particular relevance to lupus pathogenesis is the induction of type I IFNs under sterile conditions through the engagement of endosomal Toll-like receptors (TLRs) by self-nucleic acids. There has been extensive interest in creating treatments based on blocking reagents against either the multiple IFN-αs and the single IFN-β, or their common receptor.

SUMMARY

The present disclosure provides antibodies or fragments thereof having binding specificity to the human interferon alpha and beta receptor subunit 1 (IFNAR1) protein, as well as

bispecific antibodies having specificity to IFNAR1 and another antigen such as BAFF. These antibodies and fragments are useful in the treatment of autoimmune diseases such as systemic lupus erythematosus.

One embodiment of the present disclosure provides an antibody or fragment thereof having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, and a light chain variable region comprising light chain complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are selected from the group consisting of: (a) HCDR1: DYYMH (SEQ ID NO: 77), HCDR2: RIDPEDGETKYAPKFQG (SEQ ID NO: 78) or RIDPEDAETKYAPKFQG (SEQ ID NO:79), HCDR3: GGNFYVMDY (SEQ ID NO: 80), LCDR1: KASQNVGTNVV (SEQ ID NO: 81), LCDR2: SASYRVS (SEQ ID NO: 82), and LCDR3: QQKNNYPYT (SEQ ID NO: 83); and (b) HCDR1: DYYIH (SEQ ID NO: 92), HCDR2: RIDPEDGETKYAPKFQG (SEQ ID NO: 93) or RIDPEDAETKYAPKFQG (SEQ ID NO:94), HCDR3: YHGYWALDY (SEQ ID NO: 95), LCDR1: KTSQNVGTNVA (SEQ ID NO: 96), LCDR2: STSYRYS (SEQ ID NO: 97), and LCDR3: HQYFSYPYT (SEQ ID NO: 98).

In some embodiments, the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are HCDR1: DYYMH (SEQ ID NO: 77), HCDR2: RIDPEDAETKYAPKFQG (SEQ ID NO:79), HCDR3: GGNFYVMDY (SEQ ID NO: 80), LCDR1: KASQNVGTNVV (SEQ ID NO: 81), LCDR2: SASYRVS (SEQ ID NO: 82), and LCDR3: QQKNNYPYT (SEQ ID NO: 83).

In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 7, and 84-87 (e.g., SEQ ID NO:85), or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 7, and 84-87 (e.g., SEQ ID NO:85). In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8, and 88-91 (e.g., SEQ ID NO:88), or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 8, and 88-91 (e.g., SEQ ID NO:88). The heavy chain variable region and the light chain variable, as recited here, include the CDR regions as recited above.

In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 85, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 88.

In some embodiments, the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are HCDR1: DYYIH (SEQ ID NO: 92), HCDR2: RIDPEDAETKYAPKFQG (SEQ ID NO:94), HCDR3: YHGYWALDY (SEQ ID NO: 95), LCDR1: KTSQNVGTNVA (SEQ ID NO: 96), LCDR2: STSYRYS (SEQ ID NO: 97), and LCDR3: HQYFSYPYT (SEQ ID NO: 98).

In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 73, and 99-102 (e.g., SEQ ID NO:100), or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 73, and 99-102 (e.g., SEQ ID NO:100).

In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 74, and 103-106 (e.g., SEQ ID NO:105), or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 74, and 103-106 (e.g., SEQ ID NO:105).

In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 100, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105.

In another embodiments, provided is an antibody or fragment thereof having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein, wherein the antibody or fragment thereof can bind to one or more amino acid residues selected from the group consisting of H273, L274, Y275, K276, and K278 of the IFNAR1 protein.

In some embodiments, the antibody or fragment thereof of claim can bind to at least two of the amino acid residues selected from the group, such as K276, and K278. In some embodiments, the antibody or fragment thereof of claim can bind to at least three of the amino acid residues selected from the group, such as H273, K276, and K278; L274, K276, and K278; Y275, K276, and K278; or Y275, K276, and K278. In some embodiments, the

antibody or fragment thereof of claim can bind to at least four, five or all of the amino acid residues selected from the group.

Also provided, in one embodiment, is a bifunctional molecule, comprising a first antigen-binding portion having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein and a second portion having specificity to a second protein, wherein the first antigen-binding portion comprises an antibody fragment of the present disclosure.

In some embodiments, the second portion comprises peptide edratide (hCDR1) or TACI-Ig. In some embodiments, the second portion is an antigen-binding fragment having specificity to a protein selected from the group consisting of BAFF, CD20, CD22, CTLA4, IL6, CXCL13 and C5. In some embodiments, the second portion is an antigen-binding fragment having specificity to a human B-cell-activating factor (BAFF) protein.

In some embodiments, the bifunctional molecule has a format comprising a full antibody fused to two single chain fragments (scFv) or to two Fab fragments (as illustrated as Format 1 herein). In some embodiments, the second portion comprises an antigen-binding fragment of Belimumab.

Uses and methods of the presently disclosed antibodies and fragments are also provided. For instance, the presently disclosed antibodies and fragments can be used for suppressing an immune response or treating an autoimmune disease or disorder in a patient in need thereof.

In some embodiments, the autoimmune disease or disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis (RA), psoriasis/psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus (lupus), inflammatory bowel disease, Addison's disease, Graves' disease, Sjögren's syndrome, Hashimoto's thyroiditis, myasthenia gravis, vasculitis, pernicious anemia, and celiac disease. In some embodiments, the autoimmune disease or disorder is systemic lupus erythematosus.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A-B show that the 8G11H and 485G10H antibodies efficiently blocked IFNα2b-induced reporter gene expression, with potency comparable to that of the corresponding chimeric antibodies.

FIG. 2A-B show that 8G11H and 485G10H antibodies dose-dependently reversed IFNα2b-mediated inhibition of Daudi cell proliferation.

FIG. 3 shows that 8G11H and 485G10H dose-dependently inhibited recombinant IFNα2b-induced secretion of IP-10 by normal PBMC culture.

FIG. 4 shows that the 8G11H and 485G10H antibodies efficiently inhibited the *in vitro* differentiation of dendritic cells as demonstrated by significant reduction of differentiated cell surface markers CD38 and CD86 expression.

FIG. 5 shows that the 8G11H and 485G10H antibodies efficiently inhibited the in vitro differentiation of dendritic cells, which resulted in impaired responses in differentiated cell-mediated MLR as demonstrated by decreased production of IFN-γ by $CD4^+$ T cells.

FIG. 6 shows that humanized monoclonal antibodies 8G11H and 485G10H significantly inhibited the SLE plasma mediated dendritic cell development in *in vitro* system.

FIG. 7 depicts a schematic of designed four formats for the anti-IFNAR/BAFF bispecific antibody (Bi-BFINR).

FIG. 8 shows that the bispecific antibody exhibited similar activity with 8G11 antibody in binding to IFNAR-1 and comparable activity to Belimumab in binding to BAFF.

FIG.9A-B show that Bi-BFINF antibody had comparable activity to chimeric 8G11 in blocking IFNα2b signaling (A); Bi-BFINF antibody exhibited better activity in blockade of BAFF-induced B cell proliferation (B).

FIG. 10A shows time-concentration profiles of 485G10H1L3 in 2 cynomolgus monkeys.

FIG. 10B shows time-concentration profiles of 8G11H2L1 in 2 cynomolgus monkeys.

FIG. 11A shows CD11C, CD38, CD86, MHC Class I, MHC Class II and IFN-alpha R1 expression by FACs after dosing at 24h,48h and 72h.

FIG. 11B shows the serum level of monkey neopterin, beta-2-microglobulin and CRP at 24h,48h and 72h after dosing.

FIG. 11C shows the expression levels of type I IFN-induced gene signatures in monkey PBMCs at 24h,48h and 72h after dosing.

DETAILED DESCRIPTION

Definitions

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

As used herein, an "antibody" or "antigen-binding polypeptide" refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term "antibody" includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

The terms "antibody fragment" or "antigen-binding fragment", as used herein, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term "antibody fragment" includes aptamers, spiegelmers, and diabodies. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

A "single-chain variable fragment" or "scFv" refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ l- γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgG_5, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti- idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or subclass of immunoglobulin molecule.

Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CK) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino- terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CK domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VK domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VK chains (*i.e.* CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3). In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. *See, e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993).

In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive

antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see* "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196:901-917 (1987)).

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

Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be condricthoid in origin (*e.g.*, from sharks).

As used herein, the term "heavy chain constant region" includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a

polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that the heavy chain constant region may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG_1 molecule and a hinge region derived from an IgG_3 molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG_1 molecule and, in part, from an IgG_3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG_1 molecule and, in part, from an IgG_4 molecule.

As used herein, the term “light chain constant region” includes amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

A “light chain-heavy chain pair” refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

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

As used herein the term “CH2 domain” includes the portion of a heavy chain molecule that extends, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional

numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; *see* Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol* 161:4083 (1998)).

As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CK regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

As used herein, the term "chimeric antibody" will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

By "specifically binds" or "has specificity to," it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher

specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

As used herein, the terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

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

As used herein, phrases such as "to a patient in need of treatment" or "a subject in need of treatment" includes subjects, such as mammalian subjects, that would benefit from administration of an antibody or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

Anti-IFNAR1 Antibodies

The present disclosure provides antibodies, including bispecific antibodies and fragments, that have binding specificity to the human interferon alpha and beta receptor subunit 1 (IFNAR1) protein. As demonstrated in the experimental examples, numerous murine anti-human IFNAR1 antibodies were obtained, having high binding affinity to the human IFNAR1 protein. Two of the murine antibody clones, 8G11 and 485G10, were selected for further humanization and characterization. The humanized antibodies dose-dependently reversed IFNα2b-mediated inhibition of Daudi cell proliferation, IFNα2b-induced anti-viral function as well as efficiently inhibited the cell signal elicited by multiple type I IFNs at pM

levels. The IFNs included IFNα 1, IFNα 2a, IFNα 4, IFNα 5, IFNα 6, IFNα 7, IFNα 8, IFNα 10, IFNα 14, IFNα 16, IFNα 17, and IFNα 21.

Additional functional studies showed that these antibodies efficiently inhibited IFNα2b-induced secretion of IP-10 by human PBMCs and inhibited IFNα-dependent or SLE plasma-mediated dendritic cell development as demonstrated by reduced cell surface markers CD38 and CD86 expression, and decreased production of IFN-γ by CD4+ T cells in MLR system.

Various formats of anti-IFNAR1/anti-BAFF bispecific antibodies were also prepared and tested for their binding capabilities and biological functions. These bispecific antibodies exhibited comparable binding activity to monospecific 8G11 in blocking IFNα2b signaling, and even better activity in blockade of BAFF-induced B cell proliferation.

In accordance with one embodiment of the present disclosure, provided are antibodies and fragments thereof that include the heavy chain and light chain variable domains with the CDR regions of the antibodies prepared in the experimental examples. The CDRs are summarized in Table A below.

Table A. CDR Sequences

Antibody chain	**CDR Sequences (CDR1, CDR2, CDR3 in order, for VH or VL)**	**SEQ ID NO:**
4A6-VH	DYYMH	77
	RIDPDDGETKYAPKFQG	107
	GGNYYVMDN	108
4A6-VL	KASQNVGTNVA	109
	TASYRYS	110
	QQYFSYPHT	111
4B12-VH	DSYMH	112
	RIDPEDGETNYAPKFQG	113
	RVSSLYAMDY	114
4B12-VL	KASQNVGTNVA	109
	LASYRYS	115
	QQYNNYPWT	116
4D8-VH	DYYMH	77
	RIDPEDAETKYAPKFQG	79
	GGNFYVMDY	80
4D8-VL	KASQNVGTNVV	81
	SASYRYS	117
	QQKNSYPYT	118
8G11-VH	DYYMH	77
	RIDPEDGETKYAPKFQG	78
	GGNFYVMDY	80
8G11-VL	KASQNVGTNVV	81
	SASYRVS	82
	QQKNNYPYT	83

12G11-VH	DYYMH	77
	RIDPEDGETKYAPKFQG	78
	GGNYYAMDY	119
12G11-VL	KASQNVGTNVA	109
	SASYRYS	117
	QQHNSYTYK	120
17F9-VH	DYYIH	92
	RIDPEDGETKYAPKFQD	121
	YDGYYGFDY	122
17F9-VL	KASQNVGTNVA	109
	STSYRYS	97
	HQYNNYPYT	123
18B6-VH	DYYMC	124
	RIDPEDGETKYAPKFQG	78
	GGNYYAMDY	119
18B6-VL	KASQNVGTNVA	109
	SATYRYS	125
	QQHNSYSYT	126
18C1-VH	DYYMH	77
	RIDPEDGETKYAPKFQG	78
	LGNWVFDY	127
18C1-VL	KASQNVGTNVD	128
	SASYRYS	117
	QQYNTYT	129
19B6-VH	DYYMH	77
	RIDPEDGETKYAPKFQV	130
	GGNFYYFDY	131
19B6-VL	KASQNVGTNVA	109
	SASYRYS	117
	QQCINYPYT	132
20B3-VH	DYYIH	92
	RIDPEDGETKYAPTFQG	133
	YNGYSGFDY	134
20B3-VL	KASQNVGTNVV	81
	SASYRYS	117
	QQYNRYPFT	135
20E10-VH	DYYIH	92
	RIDPEDGETKYAPKFQD	121
	YDGYYGFDY	122
20E10-VL	KASQNVGTNVA	109
	STSYRYS	97
	HQYNNYPYT	123
20E12-VH	DYYMH	77
	RIDPEDGETKYVPKFQG	136
	GGSYYVMDY	137
20E12-VL	KASQNVGTSVA	138
	SASYRYS	117
	QQDNSYPHT	139
21D6-VH	DYYMH	77
	RIDPEDGETKYAPKFQG	78
	LHWSLDS	140
21D6-VL	KASQNVGTAVA	141
	STANRDT	142
	QQYSSYPYT	143
24F6-VH	DYYIH	92
	RVDPEDGETKYVPKFLD	144
	GGNYYAMDY	119
24F6-VL	KASQNVGTNVA	109

	LASYRYS	115
	QQCNNYRLT	145
29E12-VH	DYYIH	92
	RIDPEDGETKYAPKFQD	121
	YDGYYGFDY	122
29E12-VL	KASQNVGTNVA	109
	STSYRYS	97
	HQYNNYPYT	123
30B5-VH	DSYIH	146
	RIDPEDGETKYAPKFQG	78
	WLADYSAMDN	147
30B5-VL	KASEDIYNRLA	148
	GATSLET	149
	QQYWNTLYT	150
30C8-VH	DSYMH	112
	RIDPEDGETKYAPKFQG	78
	RGSSLYAVDY	151
30C8-VL	KASQNVGTSVA	138
	LASYRHR	152
	QQFNIYPWT	153
34H8-VH	DYYLH	154
	RIDPEDGETKYAPKFQG	78
	GGNYDVMDY	155
34H8-VL	KASQNVGTYVV	156
	SASYRYS	117
	QQKNTYPFT	157
36E3-VH	DSYMH	112
	RIDPEDGETKYAPKFQG	78
	RGSSLYAVDY	151
36E3-VL	KASQNVGTSVA	138
	LASYRHR	152
	QQFNIYPWT	153
39F5-VH	DYYIH	92
	RIDPEDGETKYAPKFQV	130
	GGNFYYFDY	131
39F5-VL	KASQNVGTNVA	109
	SASYRYS	117
	QQCINYPYT	132
41F1-VH	DYYMH	77
	RIDPEDGETKYVPKFQG	136
	GGNYYVMDY	158
41F1-VL	KASQNVGTYVA	159
	SASYRYN	160
	QQYNNYPLT	161
46A10-VH	DYYMH	77
	RIDPEDGETKYAPKFQV	130
	GGNFYYFDF	162
46A10-VL	KASQNVGTNVA	109
	SASYRYS	117
	QQCINYPYT	132
47C6-VH	DYYIH	92
	RIDPEDGETKYAPKFQG	78
	GGNFYYFDY	131
47C6-VL	KASQNVGTNVA	109
	SASYRYS	117
	QQCNSYSYT	163
47F5-VH	DYYMH	77
	RIDPEDGETKYVPKFQG	136

	GGSYYVMDY	137
47F5-VL	KASQNVGTSVA	138
	SASYRYS	117
	QQDNSYPHT	139
104A3-VH	DYYIH	92
	RIDPEDGETKYAPKFQG	78
	YDGYYCFDY	164
104A3-VL	KASQNVGTNVA	109
	STSYRYS	97
	QQYNNYPYT	165
106A7-VH	DYYMH	77
	RIDPEDGETKYAPKFQG	78
	DWGHSFDY	166
106A7-VL	KASQNVGTTVA	167
	SASYRYS	117
	QQYNSYT	168
107E12-VH	DYYIH	92
	RIDPEDGETKYAPKFQD	121
	EGSFTGWFPY	169
107E12-VL	KARQSVGTYVA	170
	STSYRYN	171
	QQYHSYPYT	172
124D12-VH	DYYIH	92
	RIDPEDGETKYAPKFQG	78
	YDGYYCFDY	164
124D12-VL	KASQNVGTNVA	109
	STSYRYS	97
	QQYNNYPYT	165
260H8-VH	SDYWN	173
	YISYSGSIYYNPSLKS	174
	SGGMYYFDY	175
260H8-VL	RASGNIHNYLA	176
	NAKTLED	177
	QHFWSIPPT	178
268E9-VH	SDYWN	173
	YISYSGSIYYNPSLKS	174
	SGGMYYFDY	175
268E9-VL	RASGNIHNYLA	176
	NAKTLED	177
	QHFWSIPPT	178
269A7-VH	SDYWN	173
	YISYSGTIYYNPSLKS	179
	SGGMYYFDY	175
269A7-VL	RASGNIHNYLA	176
	NAKTLED	177
	QHFWSIPPT	178
293H10-VH	SDYWN	173
	YISYSGNTDYNPSLKS	180
	SEGMYFFDY	181
293H10-VL	RASGNIHNYLA	176
	NAKTLAD	182
	QHFWSTPPT	183
370E5-VH	DYYIH	92
	RIDPEDGETKYAPKFQG	78
	FGGLTAMDY	184
370E5-VL	KASQNVGTNVA	109
	ATSYRYS	185
	QQYNNYPYT	165

392D6-VH	DYYVH	186
	RIDPEDGETKYAPKFQG	78
	FGGLDAMDY	187
392D6-VL	KASQNVGTNVA	109
	STSYRYN	171
	QQFNRYPYT	188
402G3-VH	SHFIH	189
	WIYPGDDDTEYNHKFNG	190
	RVEYYNGGFAY	191
402G3-VL	KASKNIRNNLG	192
	SGSTLQS	193
	QQYDQYPLT	194
430H6-VH	TYGMGVG	195
	NIWWDDDKYYNPSLKN	196
	HPLPGYKDNYVVDA	197
430H6-VL	RSSQSLEYSDQYTYLE	198
	GVSNRFS	199
	FQATHDPYT	200
485G10-VH	DYYIH	92
	RIDPEDGETKYAPKFQG	78
	YHGYWALDY	95
485G10-VL	KTSQNVGTNVA	96
	STSYRYS	97
	HQYFSYPYT	98
487E3-VH	DCYIH	201
	RIDPEDGETKYAPKFQA	202
	HCNFLYFDY	203
487E3-VL	KASQNVGTIVA	204
	SASYRSS	205
	QQYNNYPVI	206

In some embodiments, the VH CDR1, CDR2, and CDR3 are selected from any set of VH CDR1, CDR2, and CDR3 shown in Table A, and the VL CDR1, CDR2, and CDR3 are selected from any set of VL CDR1, CDR2, and CDR3 shown in Table A. In some embodiments, the VH CDR1, CDR2, and CDR3 and the VL CDR1, CDR2, and CDR3 are selected from those derived from the same antibody in the examples.

In some embodiments, at least one, or two, or three, or four, or five, or six of the VH CDR1, CDR2, and CDR3 and the VL CDR1, CDR2, and CDR3 of the above are modified by one, two or three amino acid additions, deletions, substitutions, or the combinations thereof.

In one embodiment, the anti-IFNAR1 antibody or fragment thereof includes the following CDRs: HCDR1: DYYMH (SEQ ID NO: 77), HCDR2: RIDPEDGETKYAPKFQG (SEQ ID NO: 78), HCDR3: GGNFYVMDY (SEQ ID NO: 80), LCDR1: KASQNVGTNVV (SEQ ID NO: 81), LCDR2: SASYRVS (SEQ ID NO: 82), and LCDR3: QQKNNYPYT (SEQ ID NO: 83).

In one embodiment, one or more of the amino acid residues in the CDRs are substituted with a different amino acid to avoid post-translational modification. An example anti-IFNAR1 antibody or fragment thereof includes the following CDRs: HCDR1: DYYMH (SEQ ID NO: 77), HCDR2: RIDPEDAETKYAPKFQG (SEQ ID NO: 79), HCDR3: GGNFYVMDY (SEQ ID NO: 80), LCDR1: KASQNVGTNVV (SEQ ID NO: 81), LCDR2: SASYRVS (SEQ ID NO: 82), and LCDR3: QQKNNYPYT (SEQ ID NO: 83).

In some embodiments, the antibody is humanized but with one or more of the following back mutations on the heavy chain: 12V, 20L, 24G, 38K, 48I, 68A, 70I, 72A, 79A and 81L, according to Kabat numbering, and combinations thereof. In some embodiments, the antibody is humanized but with one or more of the following back mutations on the light chain: 4M, 13T, 21V, 43S, 46V, 74I, 78V and 87F according to Kabat numbering, and combinations thereof.

Non-limiting examples of heavy chain variable regions include SEQ ID NO: 7, and 84-87. Non-limiting example of light chain variable regions include SEQ ID NO: 8, and 88-91.

In some embodiments, the heavy chain variable region includes SEQ ID NO:85. In some embodiments, the light chain variable region includes SEQ ID NO:88.

In one embodiment, the anti-IFNAR1 antibody or fragment thereof includes the following CDRs: HCDR1: DYYIH (SEQ ID NO: 92), HCDR2: RIDPEDGETKYAPKFQG (SEQ ID NO: 93), HCDR3: YHGYWALDY (SEQ ID NO: 95), LCDR1: KTSQNVGTNVA (SEQ ID NO: 96), LCDR2: STSYRYS (SEQ ID NO: 97), and LCDR3: HQYFSYPYT (SEQ ID NO: C6).

In one embodiment, the anti-IFNAR1 antibody or fragment thereof includes the following CDRs: HCDR1: DYYIH (SEQ ID NO: 92), HCDR2: RIDPEDAETKYAPKFQG (SEQ ID NO:94), HCDR3: YHGYWALDY (SEQ ID NO: 95), LCDR1: KTSQNVGTNVA (SEQ ID NO: 96), LCDR2: STSYRYS (SEQ ID NO: 97), and LCDR3: HQYFSYPYT (SEQ ID NO: C6).

In some embodiments, the antibody is humanized but with one or more of the following back mutations on the heavy chain: 20L, 24G, 38K, 48I, 68A, 70I, 72A, 81L, and 97G, according to Kabat numbering, and combinations thereof. In some embodiments, the antibody is

humanized but with one or more of the following back mutations on the light chain: 13T, 21V, 36Y, 46P, 78V, and 104L according to Kabat numbering, and combinations thereof.

Non-limiting examples of heavy chain variable regions include SEQ ID NO: 73, and 99-102. Non-limiting example of light chain variable regions include SEQ ID NO: 74, and 103-106.

In some embodiments, the heavy chain variable region includes SEQ ID NO:100. In some embodiments, the light chain variable region includes SEQ ID NO:105.

It was an interesting discovery that the presently prepared antibodies target an epitope that is different from known anti-IFNAR1 antibody Anifrolumab. Accordingly, in one embodiment, provided is an antibody or fragment thereof having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein, wherein the antibody or fragment thereof can bind to one or more amino acid residues selected from the group consisting of H273, L274, Y275, K276, and K278 of the IFNAR1 protein.

In some embodiments, the antibody or fragment can bind to at least two of these epitope residues, such as H273 and L274, H273 and Y275, H273 and K276, H273 and K278, L274 and Y275, L274 and K276, L274 and K278, Y275 and K276, Y275 and K278, or K276 and K278.

In some embodiments, the antibody or fragment can bind to at least two of these epitope residues, such as H273, L274, and Y275; H273, L274, and K276; H273, L274, and K278; H273, Y275, and K276; H273, Y275, and K278; H273, K276, and K278; L274, Y275, and K276; L274, Y275, and K278; L274, K276, and K278; and Y275, K276, and K278.

In some embodiments, the antibody or fragment can bind to at least four of these epitope residues, such as L274, Y275, K276, and K278; H273, Y275, K276, and K278; H273, L274, K276, and K278; H273, L274, Y275, and K278; and H273, L274, Y275, and K276.

In some embodiments, the antibody or fragment can bind to all of these epitope residues.

The CDRs, heavy chain variable regions and light chain variable regions of the present disclosure can be further modified. In some embodiments, the modified heavy chain variable region or light chain variable region retains at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity and is still capable of binding to IFNAR1.

In some embodiments, the modification is substitution at no more than one hot spot position from each of the CDRs. In some embodiments, the modification is substitution at one, two or three such hot spot positions. In one embodiment, the modification is substitution at one of the hot spot positions. Such substitutions, in some embodiments, are conservative substitutions.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

Non-limiting examples of conservative amino acid substitutions are provided in the table below, where a similarity score of 0 or higher indicates conservative substitution between the two amino acids.

Amino Acid Similarity Matrix

	C	G	P	S	A	T	D	E	N	Q	H	K	R	V	M	I	L	F	Y	W
W	-8	-7	-6	-2	-6	-5	-7	-7	-4	-5	-3	-3	2	-6	-4	-5	-2	0	0	17
Y	0	-5	-5	-3	-3	-3	-4	-4	-2	-4	0	-4	-5	-2	-2	-1	-1	7	10	
F	-4	-5	-5	-3	-4	-3	-6	-5	-4	-5	-2	-5	-4	-1	0	1	2	9		
L	-6	-4	-3	-3	-2	-2	-4	-3	-3	-2	-2	-3	-3	2	4	2	6			
I	-2	-3	-2	-1	-1	0	-2	-2	-2	-2	-2	-2	-2	4	2	5				
M	-5	-3	-2	-2	-1	-1	-3	-2	0	-1	-2	0	0	2	6					
V	-2	-1	-1	-1	0	0	-2	-2	-2	-2	-2	-2	-2	4						
R	-4	-3	0	0	-2	-1	-1	-1	0	1	2	3	6							
K	-5	-2	-1	0	-1	0	0	0	1	1	0	5								
H	-3	-2	0	-1	-1	-1	1	1	2	3	6									
Q	-5	-1	0	-1	0	-1	2	2	1	4										
N	-4	0	-1	1	0	0	2	1	2											
E	-5	0	-1	0	0	0	3	4												
D	-5	1	-1	0	0	0	4													
T	-2	0	0	1	1	3														
A	-2	1	1	1	2															
S	0	1	1	1																
P	-3	-1	6																	
G	-3	5																		
C	12																			

Conservative Amino Acid Substitutions

For Amino Acid	**Substitution With**
Alanine	D-Ala, Gly, Aib, β-Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser
Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β-Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp

Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

It will also be understood by one of ordinary skill in the art that antibodies as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.,* have a certain percent identity to the starting sequence, *e.g.,* it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

In certain embodiments, the antibody comprises an amino acid sequence or one or more moieties not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, an antibody of the disclosure may comprise a flexible linker sequence, or may be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

Antibodies, variants, or derivatives thereof of the disclosure include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the epitope. For example, but not by way of limitation, the antibodies can be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

In some embodiments, the antibodies may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

The antibodies may be conjugated or fused to a therapeutic agent, which may include detectable labels such as radioactive labels, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic or diagnostic agent, a cytotoxic agent, which may be a drug or a toxin, an ultrasound enhancing agent, a non-radioactive label, a combination thereof and other such agents known in the art.

Bi-functional Molecules

It is contemplated that binding of both the IFNAR1 and another protein involved in the immune system would be advantageous or even synergistic. Accordingly, in some embodiments, bi-functional molecules are provided, such as a bispecific antibody with an anti-IFNAR1 fragment as disclosed herein and another antigen-binding fragment/portion.

The second portion of the bi-functional molecule may be any one of the following, (1) targeting B cells, including targeting B cell growth and survival factor such as anti-BAFF fragment, TACI-Ig; targeting surface molecules of B cells, such as anti-CD20 and anti-CD22 antibody fragments; (2) targeting co-stimulatory molecules, such as CTLA4-Ig and anti-CTLA4 antibody fragment; (3) targeting T cells, such as Edratide (hCDR1); and (4) targeting cytokines and complements, such anti-IL6, anti-CXCL13 and anti-C5 antibody fragments.

In particular it is contemplated that the IFNAR1 and the BAFF (B-cell-activating factor) pathways can have synergistic effect in treating autoimmune diseases and disorders, such as lupus. Various formats of anti-IFNAR1/anti-BAFF bispecific antibodies (illustrated in FIG. 7) were tested for their binding capabilities and biological functions. Surprisingly, these bispecific antibodies exhibited comparable binding activity to monospecific 8G11 in blocking IFNα2b signaling, and even better activity in blockade of BAFF-induced B cell proliferation.

In one embodiment, therefore, provided is a bi-functional molecule having a first antigen-binding portion having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein and a second antigen-binding portion having specificity to a human B-cell-activating factor (BAFF) protein. The anti-IFNAR1 portion can a fragment of any embodiment of the present disclosure. The anti-BAFF portion can be a fragment from Belimumab (see, e.g., Dubey AK, et al., *Journal of Pharmacology & Pharmacotherapeutics*. 2011;2(4):317-319).

The bi-functional molecule can take a format as illustrated in FIG. 7, e.g., having a full antibody fused to two single chain fragments (scFv) or to two Fab fragments. Other formats of bi-functional or bispecific molecules are also provided. In some embodiments, each of the anti-IFNAR1 and anti-BAFF fragments is independently selected from a Fab fragment, a single-chain variable fragment (scFv), or a single-domain antibody. In some embodiments, the bispecific antibody further includes a Fc fragment.

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

The present disclosure also provides isolated polynucleotides or nucleic acid molecules encoding the antibodies, variants or derivatives thereof of the disclosure. The polynucleotides of the present disclosure may encode the entire heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules. Additionally, the polynucleotides of the present disclosure may encode portions of the heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules.

Methods of making antibodies are well known in the art and described herein. In certain embodiments, both the variable and constant regions of the antigen-binding polypeptides of the present disclosure are fully human. Fully human antibodies can be made using techniques described in the art and as described herein. For example, fully human antibodies against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Exemplary techniques that can be used to make such antibodies are described in U.S. patents: 6,150,584; 6,458,592; 6,420,140 which are incorporated by reference in their entireties.

Treatment and Diagnostic Methods

As described herein, the antibodies, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

The present disclosure is further directed to antibody-based therapies which involve administering the antibodies of the disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein. Therapeutic compounds of the disclosure include, but are not limited to, antibodies of the disclosure (including variants and derivatives thereof as described herein) and nucleic acids or polynucleotides encoding antibodies of the disclosure (including variants and derivatives thereof as described herein).

One embodiment provides a method of suppressing an immune response in a patient in need thereof. The method entails administering to the patient an antibody, fragment, or bi-

functional molecule of the present disclosure. In some embodiments, the patient is a tissue or organ transplant recipient.

In some embodiments, a method of treating an autoimmune disease or disorder is provided. Non-limiting examples of autoimmune disease or disorder include type 1 diabetes, rheumatoid arthritis (RA), psoriasis/psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus (lupus), inflammatory bowel disease, Addison's disease, Graves' disease, Sjögren's syndrome, Hashimoto's thyroiditis, myasthenia gravis, vasculitis, pernicious anemia, and celiac disease.

In a particular embodiments, the method is useful for treating systemic lupus erythematosus (lupus).

A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular antibodies, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

Methods of administration of the antibodies, variants or include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray.

The term “parenteral” as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

It may be desirable to administer the antigen-binding polypeptides or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the disclosure, care must be taken to use materials to which the protein does not absorb.

Methods of detecting expression of a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein in a sample are also provided, in some embodiments, comprising contacting the sample with the antibody or fragment thereof, and detecting the binding which indicates expression of IFNAR1 in the sample.

Compositions

The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of an antibody, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

Further, a “pharmaceutically acceptable carrier” will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and

a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1. Generation of mouse monoclonal antibodies against human IFNAR-1

This example shows generation of anti-human-IFNAR-1 mouse monoclonal antibodies using the hybridoma technology.

Immunization

Recombinant human IFNAR-1 proteins containing the entire extracellular region of human IFNAR-1 were used as the immunogen to raise anti-human IFNAR-1 antibodies. C57BL/6, Balb/c，SJL mice or SD rats were first immunized subcutaneously (s.c.). with 50 μg immunogen and then immunized intraperitoneally (i.p.). or s.c. biweekly with 25μg immunogen. Immune response was monitored by retroorbital bleeds. Plasma was screened by ELISA binding assay. In short, His-tagged IFNAR-1 was coated at 0.5μg/ml overnight and then blocked by 5% BSA in PBS. Serial diluted sera were incubated with the coated antigen for 1h at room temperature. The resulting plates were washed with PBS/T and incubated with goat anti-mouse IgG-HRP for 1h at room temperature. The plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. The mice with high titers of anti-IFNAR1 immunoglobulin were selected for fusion and further screening. Three days

prior to sacrifice and removal of the spleens, the mice were final boosted i.p. with 25 μg antigen. The spleens were used for fusion.

Fusion and Hybridoma screening

The mouse splenocytes, isolated from the mice, were fused with a mouse myeloma cell line based upon standard protocols. Single cell suspensions of splenic lymphocytes from immunized mice were fused to one-third the number of SP2/0 non secreting mouse myeloma cells with electrofusion machine .Cells were plated at approximately 1*10E5/well in flat bottom microtiter plate, followed by about 10 days incubation in selective medium containing 1*HAT， 10% fetal bovine serum, in DMEM .After 10 days ,cells were cultured in medium in which the HAT was replaced with HT. Individual wells were then screened by ELISA for mouse anti-IFNAR-1 monoclonal IgG antibodies. The antibody secreting hybridomas were changed medium and screened again after 2 days. If still positive for mouse anti-IFNAR-1 antibodies, hybridoma were sub cloned twice by limiting dilution. The stable sub clones were then cultured in vitro to generate small amount of antibody in tissue culture medium for further characterization with various functional assays.

Clones showing strong blocking ability in IFN-responsive reporter assay were selected for sub cloning. Supernatants of 2-round sub clone were used to confirm ELISA-based human and rhesus IFNAR-1 binding and IFN alpha blocking ability, followed by sequencing and further analysis. After these screenings, 38 clones (4A6, 4B12, 4D8, 8G11, 12G11, 17F9, 18B6, 18C1, 19B6, 20B3, 20E10, 20E12, 21D6, 24F6, 29E12, 30B5, 30C8, 34H8, 36E3, 39F5, 41F1, 46A10, 47C6, 47F5, 104A3, 106A7, 107E12, 124D12, 260H8, 268E9, 269A7,293H10, 370E5, 392D6, 402G3, 430H6, 485G10 and 487E3) were selected. Sequences of these clones are list in Table 1. Chimeric antibodies fused to human IgG1 Fc of these hybridoma were generated for further characterization.

Table 1. Antibody sequences selected from screening

Antibody chain	Sequences* (CDR underlined and bold)	SEQ ID NO:
4A6-VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**<u>DYYMH</u>**	1

	WVKQRTEQGLEWIG**R** **IDPDDGETKYAPKFQG**KATMTADTSSNTAYLQLGS LTSEDAAVYYCAR**GG** **NYYVMDN**WGQGTSVTVSS	
4A6-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKSGQSPKALIY**T** **ASYRYS**GVPDRFTGSGSGTDFTLTISNLQSEDLADYL C**QQYFSYPHT**FGG GTKLEIK	2
4B12-VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DSYMH** WVKERTEQGLEWIG**R** **IDPEDGETNYAPKFQG**KATLTADTSSNTAYLQLSG LTSEDTAVYYCAR**RV** **SSLYAMDY**WGQGTSVTVSS	3
4B12-VL	DIVMTQSQKSMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKPLIY**L** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQYNNYPWT**FGG GTKLEIK	4
4D8-VH	EVRLQQSGAELVQPGASVKLSCTGFGFNIK**DYYMH** WVKQRTEQGLEWIG**R** **IDPEDAETKYAPKFQG**QATITADTSSNTAYVQVSSL SSEDTAVYYCAR**GG**	5

	NFYVMDYWGQGTSVTVSS	
4D8-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVV** WYQQKPGQSPKVLIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLIISNVQSEDLAEYF C**QQKNSYPYT**FGG GTKLEIK	6
8G11-VH	EVQLQQSGAELVKPGASVKLSCTGFGFNIK**DYYMH** WVKQRAEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQVSSL TSEDTAVYYCAR**GG** **NFYVMDY**WGQGTSVTVSS	7
8G11-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVV** WYQQKPGQSPKVLIY**S** **ASYRVS**GVPDRFTGSGSGTDFTLIISNVQSEDLAEYF C**QQKNNYPYT**FGG GTKLEIK	8
12G11-VH	AVQLQQSGAELVKPGASVKLSCTASGFNIK**DYYMH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSL TSEDTAVYYCAR**GG** **NYYAMDY**WGQGTSVTVSS	9
12G11-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF	10

	C**QQHNSYTYK**FGG GTKVEIK	
17F9-VH	EVQLLQSWADLVKPGASVKLSCTASGFNIK**DYYIH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQD**KAAITADTSSNTAYLQLSSL TSEGTAVYYCAR**YD** **GYYGFDY**WGQGTTLTVSS	11
17F9-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKPLIY**S** **TSYRYS**GVPDRFTGSGSGTDFTLTITNVQSEDLAEYF C**HQYNNYPYT**FGG GTKLEIK	12
18B6-VH	EVQLQQSGAKLVKPGASVKLSCTASGFNIK**DYYMC** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTASLQLSSL TSEDTAVYYCAR**GG** **NYYAMDY**WGQGTSVTVSS	13
18B6-VL	DTVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKALIY**S** **ATYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQHNSYSYT**FGG GTKLEIK	14
18C1-VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DYYMH**	15

	WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSL TSEDTAVYYCAR**LG** **NWVFDY**WGQGTTLTVSS	
18C1-VL	DTVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVD** WYQQKSGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQYNTYT**FGGGT KLEIK	16
19B6-VH	EVQLQQFGAELVKPGASVKLSCTASGFNIK**DYYMH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQV**KATITADTSSNTAYLHFSSL TSEDTAVYYCVR**GG** **NFYYFDY**WGQGTTLTVSS	17
19B6-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQCINYPYT**FGG GTKLEIK	18
20B3-VH	EVHLQQSGAELVKPGTSLKLSCTASGFNIK**DYYIH**W VKQRTEQGLEWIG**R** **IDPEDGETKYAPTFQG**KATITADTSSNTAYLQLSSL TSEDTAVYYCAR**YN**	19

	GYSGFDYWGQGTTLTVSS	
20B3-VL	DIVMTQSQKFMSTSEGDRVSVTC**KASQNVGTNVV** WYQQKPGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQYNRYPFT**FGA GTKLELK	20
20E10-VH	EVQLLQSWADLVKPGASVKLSCTASGFNIK**DYYIH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQD**KAAITADTSSNTAYLQLSSL TSEGTAVYYCAR**YD** **GYYGFDY**WGQGTTLTVSS	21
20E10-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKPLIY**S** **TSYRYS**GVPDRFTGSGSGTDFTLTITNVQSEDLAEYF C**HQYNNYPYT**FGG GTKLEIK	22
20E12-VH	EVQLQQSGAEVVKPGASVKLSCTASGFNIK**DYYMH** WVKQRTEQGLECIG**R** **IDPEDGETKYVPKFQG**KATITAETSSNTAYLQLSSL TAEDTAVYYCSR**GG** **SYYVMDY**WGQGTSVTVSS	23
20E12-VL	DVVMTQSRKFMSTSVGDRVSVTC**KASQNVGTSVA** WYQQKLGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF	24

	C**QQDNSYPHT**FGG GTKLEIK	
21D6-VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DYYMH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSL TSEDTAVYYCTS**LH** **WSLDS**WGQGTTLTVSS	25
21D6-VL	DIVMTQSQKFMSTTVGDRVSITC**KASQNVGTAVA**W YQQQPGQSPKPLIY**S** **TANRDT**GVPDRFTGSGSGTDFTLTISNMQSEDLAHY FC**QQYSSYPYT**FGG GTKLEIK	26
24F6-VH	DVQLQQSGAELVKPGASVNLSCTGSGFNIK**DYYIH** WVKQRTEQGLEWIG**R** **VDPEDGETKYVPKFLD**KATITADTSSNTAYLQLSSL TSEDTAVYYCTR**GG** **NYYAMDY**WGQGTSVTVSS	27
24F6-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKSGQSPKALIF**L** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQCNNYRLT**FGS GTKLEIK	28
29E12-VH	EVQLLQSWADLVKPGASVKLSCTASGFNIK**DYYIH**	29

	WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQD**KAAITADTSSNTAYLQLSSL TSEGTAVYYCAR**YD** **GYYGFDY**WGQGTTLTVSS	
29E12-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKPLIY**S** **TSYRYS**GVPDRFTGSGSGTDFTLTITNVQSEDLAEYF C**HQYNNYPYT**FGG GTKLEIK	30
30B5-VH	EAQLQQSGAELVKPGASVKLSCTASGFNIR**DSYIH**W VNQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATMTADTSSNTAYLQLSS LTSEDTAVYYCAS**WL** **ADYSAMDN**WGQGTSVTVSS	31
30B5-VL	DIQMTQSSSSFSVSLGDRVTITC**KASEDIYNRLA**WY QQKPGNAPRLLIS**G** **ATSLET**GVPSRFSGSGSGKDYTLSITSLQTEDVATYY C**QQYWNTLYT**FGG GTKLEMK	32
30C8-VH	EVQLQQSGAELVKPGASVKLSCTSSGFNIK**DSYMH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLNSL TSEDTAVYYCAR**RG**	33

	SSLYAVDYWGQGTSVTVSS	
30C8-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTSVA** WYQQKPGQSPKAVIY**L** **ASYRHR**GVPARFTGSGSGTDFTLTISNVQSEDLAEY FC**QQFNIYPWT**FGG GTKLEIK	34
34H8-VH	EVQLLQSGAELVKPGASVRLSCTASGFNIK**DYYLH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITTDTSSNTAYLQLSSL TSEDTAVYYCTR**GG** **NYDVMDY**WGQGTSVTVSS	35
34H8-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTYVV** WYQQKPGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTIGNVQSEDLAEYF C**QQKNTYPFT**FGG GTKLEIE	36
36E3-VH	EVQLQQSGAELVKPGASVKLSCTSSGFNIK**DSYMH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLNSL TSEDTAVYYCAR**RG** **SSLYAVDY**WGQGTSVTVSS	37
36E3-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTSVA** WYQQKPGQSPKAVIY**L** **ASYRHR**GVPARFTGSGSGTDFTLTISNVQSEDLAEY	38

	FC**QQFNIYPWT**FGG GTKLEIK	
39F5-VH	EVQLQQSGADLVRPGASVKLSCTASGFNIK**DYYIH**W VKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQV**KTTITADTSSNTAYLQFSSL TSEDTAVYYCVR**GG** **NFYYFDY**WGQGSTLTVSS	39
39F5-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQCINYPYT**FGG GTKLEIK	40
41F1-VH	EVQLQQSGAELVKSGASVRLSCTASGFNIK**DYYMH** WVKQRTEKGLEWIG**R** **IDPEDGETKYVPKFQG**KATITADTSSNTVYLQLNSL TSEDTAVYYCVR**GG** **NYYVMDY**WGQGTSVTVSS	41
41F1-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTYVA** WYQQKPGQSPKVVIY**S** **ASYRYN**GVPDRFTGSGSGTDFTLTISNVQPEDLAEYF C**QQYNNYPLT**FGS GTKLEIK	42
46A10-VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DYYMH**	43

	WVKQRTEQGLELIG**R** **IDPEDGETKYAPKFQV**KATITADASSNTAYLQFSSL TSEDAAVYYCVR**GG** **NFYYFDF**WGQGTTLTVSS	
46A10-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKALVY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISDVQSEDLAEYF C**QQCINYPYT**FGG GTKLEIK	44
47C6-VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DYYIH**W VKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLHLSSL TSEDTAVYYCSR**GG** **NFYYFDY**WGQGTSLTVSS	45
47C6-VL	DIVMTQSQKFMSTLVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQCNSYSYT**FGG GTKLEIK	46
47F5-VH	EVQLQQSGAEVVKPGASVKLSCTASGFNIK**DYYMH** WVKQRTEQGLECIG**R** **IDPEDGETKYVPKFQG**KATITAETSSNTAYLQLSSL TAEDTAVYYCSR**GG**	47

	SYYVMDYWGQGTSVTVSS	
47F5-VL	DVVMTQSRKFMSTSVGDRVSVTC**KASQNVGTSVA** WYQQKLGQSPKALIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQDNSYPHT**FGG GTKLEIK	48
104A3-VH	EVQLQQSGAELVKPGASVKVSCTGSGFNIK**DYYIH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITSDTSSNTAYLQLSSL TSGDTAVYFCAR**YD** **GYYCFDY**WGQGTTLTVSS	49
104A3-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKPLIY**S** **TSYRYS**GVPDRFTGSGSGTDFTLTISNVQSADLAAYF C**QQYNNYPYT**FGG GTRLEIK	50
106A7-VH	EVQLQQSGADLVKPGASVKLSCTTSGFNIK**DYYMH** WVNQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSL TSEDTAVYYCAR**DW** **GHSFDY**WGQGTTLTVSS	51
106A7-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTTVA** WYQQKPGQSPKTLIY**S** **ASYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF	52

	C**QQYNSYT**FGGGT KLEMK	
107E12-VH	EVQLQQSGAEFVKPGASVKLSCTASGFNIK**DYYIH**W VTQKTEQGLEWIG**R** **IDPEDGETKYAPKFQD**KATITADSSSNTAYLQLSSL TSVDTAVYFCSR**EG** **SFTGWFPY**WGQGTLVSVSA	53
370E5-VL	DIVMTQSQKFMSTSVGDRVSVTC**KARQSVGTYVA** WYQQKPGQSPKALIY**S** **TSYRYN**GVPDRFTGSGSGTDFTLTISNVQSEDLADY FC**QQYHSYPYT**FGG GTKLEIK	54
124D12-VH	EVQLQQSGAELVKPGASVKVSCTGSGFNIK**DYYIH** WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**RATITSDTSSNTAYLQLSSL TSGDTAVYYCAR**YD** **GYYCFDY**WGQGTTLTVSS	55
124D12-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKPLIY**S** **TSYRYS**GVPDRFTGSGSGTDFTLTISNVQSADLAAYF C**QQYNNYPYT**FGG GTRLEIK	56
260H8-VH	EVQLQESGPGLAKPSQTLSLTCSVTGYSIT**SDYWN**W	57

	IRKFPGNKLEYMG**Y** **ISYSGSIYYNPSLKS**RISITRDTSKNQYYLQLNSVTNEDTATYYCAR**SGG** **MYYFDY**WGQGTTLTVSS	
260H8-VL	DIQMTQSPASLSASVGETVTITC**RASGNIHNYLA**WYQQKQGKSPQLLVY**N** **AKTLED**GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYC**QHFWSIPPT**FGS GTKLEIK	58
268E9-VH	EVQLQESGPGLAKPSQTLSLTCSVTGYSIT**SDYWN**WIRKFPGNKLEYMG**Y** **ISYSGSIYYNPSLKS**RISITRATSKNQYYLQLNSVTNEDTATYYCAR**SGG** **MYYFDY**WGQGTTLTVSS	59
268E9-VL	DIQMTQSPASLSASVGETVTITC**RASGNIHNYLA**WYQQKQGKSPQLLVY**N** **AKTLED**GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYC**QHFWSIPPT**FGS GTKLEIK	60
269A7-VH	EVQLQESGPGLAKPSQTLSLTCSVTGYSIT**SDYWN**WIRKFPGNKLEYMG**Y** **ISYSGTIYYNPSLKS**RISITRDTSKNQYYLQLNSVTNEDTATYYCAR**SGG**	61

	MYYFDYWGQGTTLTVSS	
269A7-VL	DIQMTQSPASLSASVGETVTITC**RASGNIHNYLA**WY QQKQGKSPQLLVY**N** **AKTLED**GVPSRFSGSGSGTQYSLKINSLQPEDFGSYY C**QHFWSIPPT**FGS GTKLEIK	62
293H10-VH	EVQLQESGPGLAKPSQTLSLTCSVTGYSIT**SDYWN**W IRKFPGNKLEYMG**Y** **ISYSGNTDYNPSLKS**RFSITRDTSKNQFYLQLNSVTT EDTATYYCAR**SEG** **MYFFDY**WGQGTTLTVSS	63
293H10-VL	DIQMTQSPASLSASVGETVSITC**RASGNIHNYLA**WY HQKQGKSPQLLVY**N** **AKTLAD**GVPSRFSGSGSGTQYSLKINSLQPEDFGSY YC**QHFWSTPPT**FGS GTKLEIK	64
370E5-VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DYYIH**W VKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSL TSEDTAVYYCAR**FG** **GLTAMDY**WGQGTSVTVSS	65
370E5-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA** WYQQKPGQSPKALIY**A** **TSYRYS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF	66

	C**QQYNNYPYT**FGG GTKLEIK	
392D6-VH	AVQLQQSGTELVKPGASVKLSCSASGFNIK**DYYVH**WVKQKTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATVTADTSSNTAYMQLSSLTSEDTAVYYCTR**FG** **GLDAMDY**WGQGTSVTVSS	67
392D6-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA**WYQQKPGQSPKPLIY**S** **TSYRYN**GVPDRFTGSGSGTEFTLTISNVQSEDLAEYFC**QQFNRYPYT**FGG GTKLEIK	68
402G3-VH	QVQLQQSGAELVKPGSSVKISCKASGFTFT**SHFIH**WIKQQPGNGLKWIG**W** **IYPGDDDTEYNHKFNG**KATLTADKSSSTAYMHLSSLTSEDSAVYFCAR**RV** **EYYNGGFAY**WGQGTLVTVSS	69
402G3-VL	DVQMTQSPSYLAAPPGESVSISC**KASKNIRNNLG**WYQERPGKTPNLLIH**S** **GSTLQS**GAPSRFSGGGSGTDFTLTIRSLESEDSAVYYC**QQYDQYPLT**FGS GTKLEIK	70
430H6-VH	QVTLKESGPGILQPSQTLSLTCTFSGFSLN**TYGMGV**	71

	GWIRQPSGKGLEWL A**NIWWDDDKYYNPSLKN**RLTISKDTSNNQAFLKIT NVDTADTATYFCAR**H** PLPGYKDNYVVDAWGQGASVTVSS	
430H6-VL	DVVLTQTPGSLSVTLGDQASISC**RSSQSLEYSDQYT YLE**WYLQKSGQSPQ LLIY**GVSNRFS**GVPDRFIGSGSGTDFTLKISRVEPEDL GVYYC**FQATHDP** **YT**FGAGTKLELK	72
485G10-VH	EVQLQQSGAELVKPGASVKLSCTGSGFNIK**DYYIH**W VKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSL TSEDTVVYYCGR**YH** **GYWALDY**WGQGTSVTVSS	73
485G10-VL	DIVMTQSQKFMSTSVGDRVSVTC**KTSQNVGTNVA** WYQQKPGQSPKPLIY**S** **TSYRYS**GVPDRFTGSGSGTDFTLIISNVQSEDLAEYF C**HQYFSYPYT**FGG GTLLEIK	74
487E3-VH	EVQLQQSGAELVKPGASVKLSCSASGFNIK**DCYIH**W VKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQA**KATITADTSSNTAYLQLNSL TSEDTAVYYCAR**HC**	75

	NFLYFDYWGQGSTLTVS	
487E3-VL	DIVMTQSQKSMSTSLGDRVTVTC**KASQNVGTIVA**W YQLKPGQSPKTLIY**S** **ASYRSS**GVPDRFTGSGSGTDFTLTISNVQSEDLAEYF C**QQYNNYPVI**FGS GTKLEIR	76

Example 2. Binding properties of anti-IFNAR-1 mouse monoclonal antibodies

This example tested the binding properties of the anti-IFNAR-1 mouse antibodies to the human IFNAR-1 proteins by ELISA assay. In short, His-tagged IFNAR-1 was coated at 0.5μg/ml overnight and then blocked by 5% BSA in PBS. Serial diluted anti-IFNAR-1 antibodies were incubated with the coated antigen for 1h at room temperature. The resulting plates were washed with PBS/T and incubated with goat anti-mouse IgG-HRP for 1h at room temperature. The plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm.The results of the ELISA assays are summarized in **Table 2**, which shows EC_{50} of binding to human IFNAR-1 protein.

Table 2. Binding EC_{50}(nM) on hIFNAR-1 protein.

Antibody	EC_{50} (nM)	Antibody	EC_{50} (nM)
4D8	0.067	269A7	0.476
8G11	0.069	293H10	0.097
34H8	0.064	370 E5	0.052
104A3	0.056	392D6	0.054
106A7	0.099	402G3	0.137
107 E12	0.075	430H6	0.733
124D12	0.062	485G10	0.015
260H8	0.055	487 E3	0.028
268 E9	0.088		

Anti-IFNAR-1 antibody BIACORE Analysis

The binding of the antibodies to recombinant His-tagged human IFNAR-1-ECD protein was examined by Biacore T200 using a capture method. The anti-IFNAR-1 antibodies were captured using anti-human Fc antibody or Protein A which were coated on chip. The serial concentrations of his-tagged human IFNAR-1-ECD protein (0-8nM) were injected over capture antibodies at the flow rate of 30 μl/min. The dissociation phases were 600s or 1200s. The results are shown in **Table 3** below. The Biacore results for the anti-IFNAR-1 antibodies demonstrate that these anti-IFNAR-1 antibodies are high affinity binders to human IFNAR-1.

Table 3. Binding of antibodies to recombinant IFNAR-1 protein

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
4D8	2.31E+06	9.03E-05	3.90E-11
8G11	2.36E+06	6.40E-05	2.71E-11
34H8	2.37E+06	4.41E-05	1.86E-11

Example 3. IFN-responsive reporter assay for screening anti-IFNAR-1 mouse monoclonal antibodies

HEK-Blue™ IFNα/β cells (InvivoGen) are specifically designed to monitor the activation of the JAK-STAT pathway induced by type I IFNs. Upon IFN-α or IFN-β stimulation, these cells activate the JAK-STAT pathway and subsequently the expression of the reporter gene named secreted embryonic alkaline phosphatase (SEAP). SEAP which is secreted in the supernatant is easily detectable by using a SEAP detection reagent QUANTI-Blue™ (InvivoGen). A neutralizing antibody, which blocks interferon binding to its receptor, will inhibit IFN-induced reporter gene expression. This reporter assay was utilized for screening anti-IFNAR-1 mouse monoclonal antibodies.

HEK-Blue™ IFNα/β cells were incubated with 400 U/ml IFNα2b in the presence of serial dilutions of anti-IFNAR-1 mouse monoclonal antibodies overnight. The expression of reporter gene SEAP was determined by using a spectrophotometer at 650 nm. EC_{50} of the tested antibodies are listed in Table 4. Among these antibodies, 4D8, 8G11, 34H8, 485G10 and 487E3 antibodies showed superior effects in blocking IFNα2b signal. Given the analysis on sequence homology, the clones of 8G11 and 485G10 were selected for further humanization and characterization.

Table 4. Reporter assay for the antibodies

Clone ID	EC_{50} (nM)	Clone ID	EC_{50}(nM)
4D8	0.460	286E9	3.871
8G11	0.465	293H10	1.285
34H8	0.560	370E5	2.170
104A3	1.310	392D6	0.824
106A7	3.516	402G3	1.267
107E12	1.207	430H6	1.297
124D12	0.981	485G10	0.286
260H8	5.074	487E3	0.161
269A7	6.713		

Example 4. Anti-IFNAR-1 mAb humanization

A. 8G11

The mouse antibody 8G11 variable region genes were employed to create a humanized MAb. In the first step of this process, the amino acid sequences of the VH and VK of 8G11 were compared against the available database of human Ig gene sequences to identify the overall best-matching human germline Ig gene sequences. For the heavy chain, the closest human match was the *IGHV1-46*01* gene. For the light chain, the best human match was the *IGKV1-9*01* gene.

Humanized variable domain sequences were then designed where the CDR1 (SEQ ID NO:77), 2 (SEQ ID NO:78), and 3 (SEQ ID NO:80) sequences of the 8G11 VH were grafted onto framework sequences of the *IGHV1-46*01* gene and the CDR1 (SEQ ID NO:81), 2 (SEQ ID NO:82) and 3 (SEQ ID NO:83) of the 8G11 light chain were grafted onto framework sequences of the *IGKV1-9*01* gene,. A 3D model was then generated to determine if there were any framework positions where replacing the mouse amino acid to the human amino acid could affect binding and/or CDR conformation. In the case of the heavy chain, K12, V20, A24, R38, M48, V68, M70, R72, V79 and M81 (Kabat numbering) in human framework was identified and subjected to back-mutations to their moue counterpart amino acid i.e.: K12V, V20L, A24G, R38K, M48I, V68A, M70I, R72A, V79A and M81L. In the case of the light chain, L4, A13, I21, A43, L46, T74, L78 and Y87 (Kabat numbering) in human framework was identified and subjected to back-mutation to their moue counterpart amino acid i.e.: L4M, A13T, I21V, A43S, L46V, T74I, L78V and Y87F. At the meantime, G56 A mutation was employed to remove the PTM.

Table 5. 8G11 sequences and CDRs

Antibody chain or domain	Sequences (CDR underlined and bold)	SEQ ID NO:
8G11-VH	EVQLQQSGAELVKPGASVKLSCTGFGFNIK**DYYMH**WVKQRAEQGLEWIG**R IDPEDGETKYAPKFQG**KATITADTSSNTAYLQVSSLTSEDTAVYYCAR**GG NFYVMDY**WGQGTSVTVSS	7
8G11-VL	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVV**WYQQKPGQSPKVLIY**S ASYRVS**GVPDRFTGSGSGTDFTLIISNVQSEDLAEYFC**QQKNNYPYT**FGG GTKLEIK	8
CDRH1	DYYMH	77
CDRH2	RIDPEDGETKYAPKFQG	78
CDRH2 (modified)	RIDPED**A**ETKYAPKFQG	79
CDRH3	GGNFYVMDY	80
CDRL1	KASQNVGTNVV	81
CDRL2	SASYRVS	82
CDRL3	QQKNNYPYT	83

The humanized sequences are listed in **Table 6**: 8G11-VH1, 8G11-VH2, 8G11-VH3, 8G11-VH4, 8G11-VL1, 8G11-VL2, 8G11-VL3, and 8G11-VL4.

Table 6. Humanized sequences

Antibody chain	Sequences (CDR italic; back mutations bold and underlined)	SEQ ID NO:
8G11-VH1	QVQLVQSGAEVKKPGASVKVSCKASGFNIK*DYYMH*WVRQAPGQGLEWMG*R IDPED**A**ETK*YAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR*GG NFYVMDY*WGQGTLVTVSS	84
8G11-VH2	QVQLVQSGAEV**V**KPGASVKVSCK**G**SGFNIK*DYYMH*WVRQAPGQGLEW**I**G*R IDPED**A**ETKYAPKFQG*RVTMT**A**DTSTSTVYMELSSLRSEDTAVYYCAR*GG NFYVMDY*WGQGTLVTVSS	85
8G11-VH3	QVQLVQSGAEV**V**KPGASVKVSCK**G**SGFNIK*DYYMH*WV**K**QAPGQGLEW**I**G*R IDPED**A**ETKYAPKFQG*RVT**I**T**A**DTSTSTVY**L**ELSSLRSEDTAVYYCAR*GG NFYVMDY*WGQGTLVTVSS	86
8G11-VH4	QVQLVQSGAEV**V**KPGASVK**L**SCK**G**SGFNIK*DYYMH*WV**K**QAPGQGLEW**I**G*R IDPED**A**ETKYAPKFQG*R**A**T**I**T**A**DTSTST**A**Y**L**ELSSLRSEDTAVYYCAR*GG NFYVMDY*WGQGTLVTVSS	87
8G11-VL1	DIQLTQSPSFLSASVGDRVTITC*KASQNVGTNVV*WYQQKPGKAPKLLIY*S ASYRVS*GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC*QQKNNYPYT*FGQ GTKLEIK	88
8G11-VL2	DIQLTQSPSFLS**T**SVGDRVTITC*KASQNVGTNVV*WYQQKPGK**S**PK**V**LIY*S ASYRVS*GVPSRFSGSGSGTEFTL**I**ISSLQPEDFATYYC*QQKNNYPYT*FGQ GTKLEIK	89
8G11-VL3	DIQLTQSPSFLS**T**SVGDRVTITC*KASQNVGTNVV*WYQQKPGK**S**PK**V**LIY*S ASYRVS*GVPSRFSGSGSGTEFTL**I**ISS**V**QPEDFATY**F**C*QQKNNYPYT*FGQ	90

	GTKLEIK	
8G11-VL4	DIQ**M**TQSPSFLS**T**SVGDRVT**V**TC*KASQNVGTNVV*WYQQKPGK**S**PK**V**LIY*S* *ASYRVS*GVPSRFSGSGSGTEFTL**I**ISS**V**QPEDFATY**F**C*QQKNNYPYT*FGQ GTKLEIK	91

All the 16 IgGs were expressed in the HEK293 cell line. Express antibodies were performed for affinity ranking. Detailed data of affinity ranking were summarized in **Table 7.**

Table 7. Affinity ranking of humanized antibodies.

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)
VH4+VL3	3.01E+05	8.52E-05	2.84E-10
VH2+VL2	2.87E+05	9.42E-05	3.28E-10
VH4+VL2	2.92E+05	9.69E-05	3.32E-10
VH3+VL3	3.01E+05	1.10E-04	3.64E-10
VH2+VL3	2.65E+05	9.65E-05	3.64E-10
VH4+VL4	2.83E+05	1.05E-04	3.72E-10
VH2+VL4	2.62E+05	1.02E-04	3.88E-10
VH3+VL2	2.84E+05	1.11E-04	3.89E-10
VH3+VL4	2.81E+05	1.13E-04	4.01E-10
VH3+VL1	2.56E+05	1.06E-04	4.13E-10
VH2+VL1	2.02E+05	8.84E-05	4.39E-10
VH4+VL1	2.03E+05	9.94E-05	4.89E-10
VH1+VL1	1.48E+05	9.83E-05	6.62E-10
VH1+VL2	1.67E+05	1.27E-04	7.65E-10
VH1+VL4	1.69E+05	1.41E-04	8.36E-10
VH1+VL3	2.31E+05	2.00E-04	8.65E-10

Based on the affinity ranking results, 4 IgGs (VH4+VL3, VH2+VL2, VH2+VL1, VH1+VL1) were expressed and purified. The purified antibodies were further selected for affinity measurement under different concentrations. Detailed data were summarized in **Table 8**.

Table 8. Affinity of selected humanized antibodies.

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
8G11-H4L3	3.40E+05	7.47E-05	2.20E-10
8G11-H2L2	2.98E+05	1.25E-04	4.19E-10
8G11-H2L1	2.29E+05	9.24E-05	4.03E-10
8G11-H1L1	1.61E+05	2.19E-04	1.36E-09

B. 485G10

The mouse antibody 485G10 variable region genes were employed to create a humanized MAb. In the first step of this process, the amino acid sequences of the VH and VK of 485G10

were compared against the available database of human Ig gene sequences to identify the overall best-matching human germline Ig gene sequences. For the heavy chain, the closest human match was the IGHV1-2*05 gene. For the light chain, the best human match was the IGKV1-16*01 gene.

Humanized variable domain sequences were then designed where the CDR1 (SEQ ID NO:92), 2 (SEQ ID NO:93), and 3 (SEQ ID NO:95) sequences of the 485G10 heavy chain were grafted onto framework sequences of the *IGHV1-2*05* gene and the CDR1 (SEQ ID NO:96), 2 (SEQ ID NO:C5) and 3 (SEQ ID NO:98) of the 485G10 light chain were grafted onto framework sequences of the *IGKV1-16*01* gene,. A 3D model was then generated to determine if there were any framework positions where replacing the mouse amino acid to the human amino acid could affect binding and/or CDR conformation. In the case of the heavy chain, V20, A24, R38, M48, V68, M70, R72, M81, A97 (Kabat numbering) in human framework was identified and subjected to back-mutations to their moue counterpart amino acid i.e.: V20L, A24G, R38K, M48I, V68A, M70I, R72A, M81L, A97G. In the case of the light chain, A13, I21, F36, S46, L78, V104 (Kabat numbering) in human framework was identified and subjected to back-mutation to their moue counterpart amino acid i.e.: A13T, I21V, F36Y, S46P, L78V, V104L. At the meantime, G56 A mutation was employed to remove the PTM.

Table 9. 485G10 sequences and CDRs

Antibody chain or domain	**Sequences (CDR underlined and bold)**	**SEQ ID NO:**
485G10- VH	EVQLQQSGAELVKPGASVKLSCTGSGFNIK**DYYIH**WVKQRTEQGLEWIG**RIDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSLTSEDTVVYYCGR**YHGYWALDY**WGQGTSVTVSS	73
485G10- VL	DIVMTQSQKFMSTSVGDRVSVTC**KTSQNVGTNVA**WYQQKPGQSPKPLIY**STSYRYS**GVPDRFTGSGSGTDFTLIISNVQSEDLAEYFC**HQYFSYPYT**FGGGTLLEIK	74
CDRH1	DYYIH	92
CDRH2	RIDPEDGETKYAPKFQG	93
CDRH2 (modified)	RIDPED**A**ETKYAPKFQG	94
CDRH3	YHGYWALDY	95
CDRL1	KTSQNVGTNVA	96
CDRL2	STSYRYS	97
CDRL3	HQYFSYPYT	98

The humanized sequences are listed in **Table 10:** 485G10-VH0, 485G10-VH1, 485G10-VH2, 485G10-VH3, 485G10-VL1, 485G10-VL2, 485G10-VL3, and 485G10-VL4.

Table 10. Humanized sequences

Antibody chain	Sequences (CDR italic and underlined; back mutations bold)	SEQ ID NO:
485G10-VH0	QVQLVQSGAEVKKPGASVKVSCKASGFNIK*DYYIH*WVRQAPGQGLEWMG*R* *IDPED***A***ETKYAPKFQG*RVTMTRDTSISTAYMELSRLRSDDTVVYYCARYH GYWALDYWGQGTLVTVSS	99
485G10-VH1	QVQLVQSGAEVKKPGASVKVSCKASGFNIK*DYYIH*WVRQAPGQGLEWMG*R* *IDPED***A***ETKYAPKFQG*RVTMT**A**DTSISTAYMELSRLRSDDTVVYYCARYH GYWALDYWGQGTLVTVSS	100
485G10-VH2	QVQLVQSGAEVKKPGASVK**L**SCKASGFNIK*DYYIH*WV**K**QAPGQGLEW**I**G*R* *IDPED***A***ETKYAPKFQG*RVTMT**A**DTSISTAYMELSRLRSDDTVVYYC**G**RYH GYWALDYWGQGTLVTVSS	101
485G10-VH3	QVQLVQSGAEVKKPGASVK**L**SCK**G**SGFNIK*DYYIH*WV**K**QAPGQGLEW**I**G*R* *IDPED***A***ETKYAPKFQG*R**A**T**I**T**A**DTSISTAY**L**ELSRLRSDDTVVYYC**G**RYH GYWALDYWGQGTLVTVSS	102
485G10-VL1	DIQMTQSPSSLSASVGDRVTITC*KTSQNVGTNVA*WFQQKPGKAPKSLIY*S* *TSYRYS*GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC*HQYFSYPYT*FGP GTKVDIK	103
485G10-VL2	DIQMTQSPSSLS**T**SVGDRVTITC*KTSQNVGTNVA*W**Y**QQK**P**GKAPKPLIY*S* *TSYRYS*GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC*HQYFSYPYT*FGP GTKVDIK	104
485G10-VL3	DIQMTQSPSSLS**T**SVGDRVTITC*KTSQNVGTNVA*W**Y**QQK**P**GKAPKPLIY*S* *TSYRYS*GVPSRFSGSGSGTDFTLTISS**V**QPEDFATYYC*HQYFSYPYT*FGP GTK**L**DIK	105
485G10-VL4	DIQMTQSPSSLS**T**SVGDRVI**V**TC*KTSQNVGTNVA*W**Y**QQK**P**GKAPKPLIY*S* *TSYRYS*GVPSRFSGSGSGTDFTLTISS**V**QPEDFATYYC*HQYFSYPYT*FGP GTK**L**DIK	106

All the 16 IgGs could be expressed in the HEK293 cell line. Express antibodies were performed for affinity ranking. Detailed data of affinity ranking were summarized in **table 11.**

Table 11. Affinity ranking of humanized antibodies.

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)
VH1+VL3	3.11E+05	1.01E-04	3.25E-10
VH1+VL2	3.08E+05	2.01E-04	6.52E-10
VH1+VL4	3.27E+05	2.16E-04	6.60E-10
VH2+VL3	2.81E+05	2.38E-04	8.48E-10
VH3+VL2	3.06E+05	2.64E-04	8.64E-10
VH3+VL4	3.54E+05	2.71E-04	7.66E-10
VH3+VL3	3.32E+05	2.79E-04	8.41E-10
VH2+VL2	3.29E+05	2.98E-04	9.06E-10
VH2+VL4	3.03E+05	3.00E-04	9.90E-10
VH0+VL3	2.94E+05	3.16E-04	1.07E-09

VH0+VL4	2.55E+05	3.18E-04	1.25E-09
VH0+VL2	2.79E+05	4.39E-04	1.57E-09
VH1+VL1	3.28E+05	1.03E-03	3.14E-09
VH0+VL1	5.09E+05	4.75E-03	9.33E-09
VH2+VL1	3.23E+05	6.63E-03	2.05E-08
VH3+VL1	9.71E+05	1.85E-02	1.91E-08

Based on the affinity ranking results, 4 IgGs (VH1+VL2, VH1+VL4, VH1+VL3, VH2+VL3,) were expressed and purified. The purified antibodies were further selected for affinity measurement under different concentrations. Detailed data were summarized in **Table 12**.

Table 12. Affinity of selected humanized antibodies.

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
485G10-H1L2	2.07E+05	3.21E-04	1.55E-09
485G10-H1L4	1.96E+05	2.59E-04	1.32E-09
485G10-H1L3	1.87E+05	2.32E-04	1.24E-09
485G10-H2L3	1.86E+05	3.66E-04	1.96E-09

Example 5. Anit-IFNAR-1 Humanized Monoclonal Antibodies Inhibit the Biological Activity of Interferon α2b in IFN-Responsive Reporter Assay

To evaluate the IFNAR-1-blocking function of humanized antibodies, *in vitro* IFN-responsive reporter assay described in Example 3 was used. HEK-Blue™ IFNα/β cells were incubated with 400 U/ml IFNα2b in the presence of serial diluted anti-IFNAR-1 humanized monoclonal antibodies overnight prior to determination by using a spectrophotometer at 650 nM. As shown in **FIG.1,** the 8G11H, and 485G10H antibodies efficiently blocked IFNα2b-induced reporter gene expression. Their potency is comparable to that of the corresponding chimeric antibodies.

Example 6. Anit-IFNAR-1 Humanized Monoclonal Antibodies Inhibit the Biological Activity of Interferon α2b in Daudi Cell Proliferation

The cell line Daudi, derived from a human B-lymphoblast Burkitt's lymphoma, expresses high levels of IFNAR, and the growth of these cells is inhibited by type I interferons. To measure the functional blocking ability of humanized anti-IFNAR-1 antibodies, Daudi cells were cultured with interferon α2b in the presence or absence of a serial diluted anti-IFNAR-1 humanized antibodies. The cell proliferation was measured by Cell Counting Kit-8 (CCK8, DOJINDO Laboratories). As described in **FIG.2**, the 8G11H and 485G10H antibodies dose-

dependently reverse IFNα2b-mediated inhibition of Daudi cell proliferation. Their potency is comparable to that of the corresponding chimeric antibodies.

Example 7. Anti-IFNAR-1 Human Monoclonal Antibodies Inhibit the Biological Activity of Multiple Type I IFNs

To assess the ability of humanized anti-IFNAR-1 antibodies to inhibit the biological activity of multiple type I IFNs, distinct IFN alpha subtypes were tested in the IFN-responsive reporter assay. HEK-Blue™ IFN-α/β cells were incubated with one of the following IFN alpha subtypes: 1, 2a, 4, 5, 6, 7, 8, 10, 14, 16, 17 and 21 in the presence of serial dilutions of the tested antibodies or an isotype control. The reporter signal was determined as described in Example 3. As shown in **Table 13**, the 8G11H and 485G10H antibodies can efficiently inhibit the cell signal elicited by multiple type I IFNs. Their EC_{50} is in the pM level.

Table 13. Reporter assay of multiple Type I IFNs

Clone ID	EC_{50} (nM)		Clone ID	EC_{50} (nM)	
	8G11H	485G10H		8G11H	485G10H
IFNα 1	0.057	0.051	IFNα 8	0.604	0.490
IFNα 2a	0.052	0.048	IFNα 10	0.402	0.550
IFNα 4	0.055	0.054	IFNα 14	0.215	0.153
IFNα 5	0.087	0.098	IFNα 16	0.061	0.079
IFNα 6	0.090	0.072	IFNα 17	0.035	0.035
IFNα 7	0.039	0.047	IFNα 21	0.079	0.065

Example 8. Anti-IFNAR-1 Human Monoclonal Antibodies Inhibit Type I IFN mediated anti-viral function

Type I IFNs were firstly identified by their unique anti-viral function. To evaluate the blocking effects of the anti-IFNAR-1 antibodies on type I IFN-mediated anti-viral function, an IFNα2b-based anti-viral assay was established. Briefly, cell line Huh7 was engineered to contain the full-length HCV genotype 1b replicon (Con1b) which was linked to a firefly luciferase reporter gene. The abundance of reporter gene closely correlated with replication levels of HCV replicon. The activity of anti-IFNAR-1 antibodies in blocking IFNα2b induced anti-viral function can be determined by the restoration of reporter gene levels in IFNα2b-treated Huh7-Con1b cells.

Huh7-Con1b cells were cultured with 400 U/ml IFNα2b in the presence or absence of serial diluted anti-IFNAR-1 humanized monoclonal antibodies for 48 hours. The expression of firefly luciferase reporter gene was measured by addition of Britelite plus reagent and further determined by using a spectrophotometer. As shown in Table 14, anti-IFNAR-1 humanized monoclonal antibodies 8G11H and 485G10H efficiently inhibit IFNα2b-induced anti-viral function, their EC50 is comparable to that of the corresponding chimeric antibodies.

Table 14. IFNα2b-mediated anti-viral assay

Clone ID	**EC_{50} (nM)**
8G11-chimeric	7.733
8G11H	8.067
485G10-chimeric	16.267
485G10H	13.867

Example 9. Inhibition of type I IFN-induced IP-10 secretion by Anti-IFNAR-1 Humanized Monoclonal Antibodies

Upon type I IFNs stimulation, normal peripheral blood mononuclear cells (PBMCs) express a serial of IFN-response genes including IP-10. The activity of anti-IFNAR-1 antibodies was tested for inhibition of IFNα2b-induced secretion of IP-10 by normal PBMCs.

PBMCs were incubated with 400 U/ml IFNα2b in the presence or absence of anti-IFNAR-1 humanized monoclonal antibodies for 48 hours. Supernatants were collected and analyzed for IP-10 concentration by using a quantitative LANCE kit (Cisbio) according to manufacturer recommendations. As shown in FIG.3, anti-IFNAR-1 humanized monoclonal antibodies 8G11H and 485G10H dose-dependently inhibit recombinant IFNα2b-induced secretion of IP-10 by normal PBMC culture.

Example 10. Inhibition of Type I IFN-induced Dendritic Cell Development by Anti-IFNAR-1 Humanized Monoclonal Antibodies

It is well known that type I IFNs induce the development and maturation of dendritic cells, as defined by the expression of specific cell surface markers such as CD38, CD80 and CD86 et al, and the capability to stimulate naïve allogeneic $CD4^+$ T cell proliferation (mixed lymphocyte reaction, MLR). To evaluate the function of anti-IFNAR-1 antibodies in blocking type I IFN signaling on dendritic cells differentiation, IFNα2b-mediated dendritic cells

differentiation assay was established. Monocytes isolated from human PBMCs were cultured with 20 ng/ml GMCSF and 400 U/ml IFNα2b in the presence or absence of anti-IFNAR-1 humanized monoclonal antibodies for 72 hours. Monocyte-derived dendritic cells were collected and further cultured with human naïve allogeneic $CD4^+$ T cells at a ratio of 1:5 for 5 days. The cytokine IFN-γ, reflecting the proliferation status of $CD4^+$ T cells, in the culture supernatants were analyzed by ELISA assay.

As shown in **FIG.4,** the addition of anti-IFNAR-1 humanized monoclonal antibodies, 8G11H or 485G10H, into the differentiation systems results in significant reduction of cell surface markers CD38 and CD86 expression, as well as obvious impaired responses in MLR as demonstrated by decreased production of IFN-γ by CD4+ T cells (**FIG.5**). The results demonstrated that anti-IFNAR-1 humanized monoclonal antibodies, 8G11H and 485G10H, can efficiently block IFNα2b signaling on dendritic cells development.

Example 11. Inhibition of SLE Plasma Mediated Dendritic Cell Development by Anti-IFNAR-1 Humanized Monoclonal Antibodies

The capacity of SLE patients' plasma to induce dendritic cell development correlates with disease activity and depends on the actions of IFNα. In this example, the purified human anti-IFNAR-1 antibodies, 8G11H and 485G10H, were tested for inhibition of SLE plasma-induced dendritic cell development, as assessed by the impaired capability of antibody-treated dendritic cells to stimulate naïve allogeneic $CD4^+$ T cell proliferation (MLR).

Monocytes isolated from human PBMCs were cultured in media containing 25% SLE patient plasma in the presence or absence of anti-IFNAR-1 antibodies (15 μg/ml) for three to five days. The differentiated cells were then collected and co-cultured with human naïve allogeneic $CD4^+$ T cells at a ratio of 1:5 for five days. The cytokine IFN-γ, reflecting the proliferation status of $CD4^+$ T cells, in the culture supernatants were analyzed by ELISA assay. As shown in **FIG.6**, anti-IFNAR-1 humanized monoclonal antibodies 8G11H and 485G10H significantly inhibit the IFNα-dependent dendritic cell development, as demonstrated by impaired capability of differentiated cells to stimulate MLR with marked reduction of the cytokine IFN-γ production by $CD4^+$ T cells.

Example 12. Bispecific antibody to target BAFF and IFNAR-1

This example tested whether certain formats of anti-BAFF/anti-IFNAR1 bispecific antibodies can retain the binding affinities, and inhibitive activities, to both antigens.

The bispecific monoclonal antibody was generated with Belimumab (anti-BAFF) and 8G11 chimeric antibody. **FIG.7** depicts a schematic of designed four formats for the anti-IFNAR/BAFF bispecific antibody (Bi-BFINR). The binding properties of Bi-BFINR to human IFNAR-1 protein and human BAFF protein were detected respectively by ELISA assay. As shown in **FIG.8,** the format 1 of Bi-BFINF exhibited similar activity as 8G11 antibody in binding to IFNAR-1 and comparable activity to Belimumab in binding to BAFF. Furthermore, cell-based assay was performed to characterize the biological function of Bi-BFINF. For anti-IFNAR-1 arm, IFNα2b-based reporter assay was applied. As shown in **FIG.9A,** Bi-BFINF antibody with format 1 has the comparable activity to chimeric 8G11 in blocking IFNα2b signaling. For anti-BAFF arm, BAFF-induced B cell proliferation assay was performed. B cells isolated from Tonsil were cultured with 2 μg/ml recombinant BAFF in the presence or absence of Bi-BFINF for 72 hours. Cell-title glo was added to the cultures to determine B cell numbers. As shown in **FIG.9B**, Bi-BFINF antibody with format 1 exhibits better activity in blockade of BAFF-induced B cell proliferation.

Example 13. Identification of critical amino acids of hIFNAR-1 for 8G11 binding

For epitope-mapping for the 8G11 antibody, a mutation library (alanine scan) of hIFNAR-1(1aa-409aa) was created. Binding of 8G11 Fab to each mutant clone in the alanine scanning library was determined, in duplicate, by high-throughput flow cytometry. For each point, background fluorescence was subtracted from the raw data, which were then normalized to Fab reactivity with WT target protein. For each mutant clone, the mean binding value was plotted as a function of expression (represented by control reactivity). 85288 Mab (R&D) and Anifrolumab were used as control antibody. To identify preliminary primary critical clones a threshold of >70% WT binding to control MAb or 8G11 Fab and <30% WT binding to test 8G11 Fab was applied.

Screening Results of the mutations are listed in **Table 15**. The clone H273A that did not meet the set thresholds but whose decreased binding activity and proximity to critical residues suggested that the mutated residue may be part of the antibody epitope. Critical residues

whose mutation gave the lowest reactivities with specific antibodies are highlighted in bold and underlined. Validated critical residues represent amino acids whose side chains make the highest energetic contributions to the antibody-epitope interaction. Therefore, the highlighted (bold and underlined) residues K276, K278 are likely the major energetic contributors to binding. Results were showed in **Table 16**.

Table 15. Identification of critical residues for 8G11 Fab binding.

Binding Reactivity (%WT)			
Mutation	**8G11 Fab**	**85288 Mab**	**Anifrolumab Mab**
H273A	31.2	95.5	88.1
L274A	22.7	115.1	105.6
Y275A	19.1	86.8	145.5
K276A	1.5	104.4	99.4
K278A	3.9	101.2	105.1

Table 16. Important residues for binding of Fab to the hIFNAR-1 protein.

Antibody Name	Residues
8G11	H273, L274, Y275, **K276, K278**

Competition assays were used to test whether the antibodies 4D8, 8G11, 4B12, 30C8, 30B5, 34H8 and Anifrolumab competed with one another. The results showed that 4D8, 8G11, 4B12, 30C8, 30B5, and 34H8 competed with one another but none competed with Anifrolumab for binding to IFNAR-1. These data show that the newly identified antibodies target the same epitope on IFNAR-1 which is different from that for Anifrolumab.

Example 14. Pharmacokinetics of humanized anti-IFNAR1 antibodies in cynomolgus monkeys

A preliminary pharmacokinetics (PK) study was conducted in cynomolgus monkeys at a dose of 10 mg/kg of 8G11-H2L1 and 485G10-H1L3. Serum samples were collected relative to antibody injection at − 0 (predose), 5Min, 30Min, 1h, 2h, 4h, 8h, 24hr (1 day), 48h (2 days),72hr (3 days), 96hr (4 days), 120hr (5 days), 168hr (7 days), 240hr (10days), 36hr (14days), 504hr (21 days), 672hr (28 days) and 840hr (35 days). The levels of anti-IFNAR1 mAbs were measured by a generic ELISA method using recombinant IFNAR1protein ECD

as the capture antigen. PK parameters were calculated based on statistical moment theory by WinNonlin 6.3 software. Results are shown in Table 17.

Time-concentration profiles indicated that the PK of 8G11-H2L1 and 485G10-H1L3 were more in line with what one would expect for a typical IgG (**FIG. 10A and 10B**),

Table 17. 8G11-H2L1 and 485G10-H1L3 PK parameters in cynomolgus monkeys

i.v. 10 mg/kg		485G10-H1L3	8G11-H2L1
HL_Lambda_z	h	41.9	71.37
T_{max}	h	0.08	0.08
C_{max}	μg/mL	299.05	241.32
AUC_{last}	h*μg/mL	12942.47	15450.93
Vz_F_obs	mL/kg	46.4	65.6
Cl_F_obs	mL/h/kg	0.77	0.64
MRT_{last}	h	62.43	99.49

Example 15. Pharmacodynamics of humanized anti-IFNAR1 antibodies in cynomolgus monkeys

A pharmacodynamic (PD) model was to used study the ability of the anti-IFNAR1 antibodies to inhibit interferon activity in vivo. Cynomolgus monkeys were treated with i.v. infusion of 10mg/kg 8G11-H2L1 and 485G10-H1L3 or vehicle control followed by i.m. dose of human IFN-α2b (3*10E6U/Kg). After treatment and IFN-α2b injection, blood was collected at pre-dose, 24, 72 and 168 h after treatment for PD marker measurement. The expression of CD11C, CD38, CD86, MHC Class I, MHC Class II and IFN-alpha R1 was detected by FACS in total PBMC. As shown in **FIG. 11A**,8G11-H2L1 and 485G10-H1L3 could significantly decrease the HLA-DR expression of CD14+ cell at 24 h after treatment. Meanwhile, 8G11-H2L1 and 485G10-H1L3 were also able to suppress the IFN- alpha R1 expression of CD14+ cell both at 24 h and 72 h after treatment.

The serum levels of monkey neopterin and beta-2-microglobulin were detected by ELISA performed according to the manufacturer's instruction. The level of CRP in peripheral blood was measured by blood biochemistry. As shown in **FIG. 11B**, Neopterin, Beta-2 Microglobulin and CRP in vehicle group were increased rapidly within 24 h. Compared with vehicle control, 8G11-H2L1 and 485G10-H1L3 could suppress the neopterin and beta-2

Microglobulin significantly at 24 h and 72 h after dosing. Also, 8G11-H2L1 and 485G10-H1L3 were able to decrease the CRP significantly at 24 h after dosing.

The expression levels of type I IFN-induced gene signatures in peripheral blood mononuclear cells were detected by real-time PCR. As shown in **FIG. 11C**, the mRNA levels of ISG15, HERC5, IFI27 and IFIT3 were increased rapidly within 24 h in vehicle group. Compared with vehicle control, 8G11-H2L1 and 485G10-H1L3 significantly blocked the induction effects of type I IFN on these genes.

* * *

The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference

CLAIMS

What is claimed is:

1. An antibody or fragment thereof having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, and a light chain variable region comprising light chain complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are selected from the group consisting of:

(a) HCDR1: DYYMH (SEQ ID NO: 77), HCDR2: RIDPEDGETKYAPKFQG (SEQ ID NO: 78) or RIDPEDAETKYAPKFQG (SEQ ID NO:79), HCDR3: GGNFYVMDY (SEQ ID NO: 80), LCDR1: KASQNVGTNVV (SEQ ID NO: 81), LCDR2: SASYRVS (SEQ ID NO: 82), and LCDR3: QQKNNYPYT (SEQ ID NO: 83); and

(b) HCDR1: DYYIH (SEQ ID NO: 92), HCDR2: RIDPEDGETKYAPKFQG (SEQ ID NO: 93) or RIDPEDAETKYAPKFQG (SEQ ID NO:94), HCDR3: YHGYWALDY (SEQ ID NO: 95), LCDR1: KTSQNVGTNVA (SEQ ID NO: 96), LCDR2: STSYRYS (SEQ ID NO: 97), and LCDR3: HQYFSYPYT (SEQ ID NO: 98).

2. The antibody or fragment thereof of claim 1, wherein the antibody is a chimeric antibody or a humanized antibody.

3. The antibody or fragment thereof of claim 1, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are HCDR1: DYYMH (SEQ ID NO: 77), HCDR2: RIDPEDAETKYAPKFQG (SEQ ID NO:79), HCDR3: GGNFYVMDY (SEQ ID NO: 80), LCDR1: KASQNVGTNVV (SEQ ID NO: 81), LCDR2: SASYRVS (SEQ ID NO: 82), and LCDR3: QQKNNYPYT (SEQ ID NO: 83).

4. The antibody or fragment thereof of claim 3, which is humanized and wherein the heavy chain variable region comprises one or more back mutations selected from the group consisting of 12V, 20L, 24G, 38K, 48I, 68A, 70I, 72A, 79A and 81L, according to Kabat numbering, and combinations thereof.

5. The antibody or fragment thereof of claim 3 or 4, which is humanized and wherein the light chain variable region comprises one or more back mutations selected from the group consisting of 4M, 13T, 21V, 43S, 46V, 74I, 78V and 87F according to Kabat numbering, and combinations thereof.

6. The antibody or fragment thereof of claim 3, comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 7, and 84-87, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 7, and 84-87.

7. The antibody or fragment thereof of claim 3 or 6, comprising a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8, and 88-91, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 8, and 88-91.

8. The antibody or fragment thereof of claim 3, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 85 or a peptide having at least 90% sequence identity to SEQ ID NO: 85, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 88 or a peptide having at least 90% sequence identity to SEQ ID NO: 88.

9. The antibody or fragment thereof of claim 3, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 85, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 88.

10. The antibody or fragment thereof of claim 1, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are HCDR1: DYYIH (SEQ ID NO: 92), HCDR2: RIDPEDAETKYAPKFQG (SEQ ID NO:94), HCDR3: YHGYWALDY (SEQ ID NO: 95), LCDR1: KTSQNVGTNVA (SEQ ID NO: 96), LCDR2: STSYRYS (SEQ ID NO: 97), and LCDR3: HQYFSYPYT (SEQ ID NO: 98).

11. The antibody or fragment thereof of claim 10, which is humanized and wherein the heavy chain variable region comprises one or more back mutations selected from the group consisting of 20L, 24G, 38K, 48I, 68A, 70I, 72A, 81L, and 97G, according to Kabat numbering, and combinations thereof.

12. The antibody or fragment thereof of claim 10 or 11, which is humanized and wherein the light chain variable region comprises one or more back mutations selected from the group consisting of 13T, 21V, 36Y, 46P, 78V, and 104L, according to Kabat numbering, and combinations thereof.

13. The antibody or fragment thereof of claim 10, comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 73, and 99-102, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 73, and 99-102.

14. The antibody or fragment thereof of claim 10 or 13, comprising a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:

74, and 103-106, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 74, and 103-106.

15. The antibody or fragment thereof of claim 10, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 100 or a peptide having at least 90% sequence identity to SEQ ID NO: 100, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 or a peptide having at least 90% sequence identity to SEQ ID NO: 105.

16. The antibody or fragment thereof of claim 10, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 100, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105.

17. An antibody or fragment thereof having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, and a light chain variable region comprising light chain complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein:

the set of HCDR1, HCDR2, and HCDR3 is selected from Table A, or CDR sets derived from Table A with one, two, or three amino acid addition, deletion and/or substitution in one or more of the CDRs, and

the set of LCDR1, LCDR2, and LCDR3 are selected from Table A or CDR sets derived from Table A with one, two, or three amino acid addition, deletion and/or substitution in one or more of the CDRs.

18. An antibody or fragment thereof having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein, wherein the antibody or fragment thereof can bind

to one or more amino acid residues selected from the group consisting of H273, L274, Y275, K276, and K278 of the IFNAR1 protein.

19. The antibody or fragment thereof of claim 18, which can bind to at least two of the amino acid residues selected from the group.

20. The antibody or fragment thereof of claim 18, which can bind to at least three of the amino acid residues selected from the group.

21. The antibody or fragment thereof of claim 18, which can bind to all five of the amino acid residues selected from the group.

22. A bifunctional molecule, comprising a first antigen-binding portion having specificity to a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein and a second portion having specificity to a second protein, wherein the first antigen-binding portion comprises an antibody fragment of any one of claims 1-21.

23. The bifunctional molecule of claim 22, wherein the second portion comprises peptide edratide (hCDR1) or TACI-Ig.

24. The bifunctional molecule of claim 22, wherein the second portion is an antigen-binding fragment having specificity to a protein selected from the group consisting of BAFF, CD20, CD22, CTLA4, IL6, CXCL13 and C5.

25. The bifunctional molecule of claim 22, wherein the second portion is an antigen-binding fragment having specificity to a human B-cell-activating factor (BAFF) protein.

26. The bifunctional molecule of claim 25, which has a format comprising a full antibody fused to two single chain fragments (scFv) or to two Fab fragments.

27. The bifunctional molecule of claim 25 or 26, wherein the second portion comprises an antigen-binding fragment of Belimumab.

28. A composition comprising the antibody or fragment thereof of any one of claims 1-21 or the bifunctional molecule of any one of claims 22-27, and a pharmaceutically acceptable carrier.

29. An isolated cell comprising one or more polynucleotide encoding the antibody or fragment thereof of any one of claims 1-21 or the bifunctional molecule of any one of claims 22-27.

30. A polynucleotide encoding one or more chains of the antibody or fragment thereof of any one of claims 1-21 or the bifunctional molecule of any one of claims 22-27.

31. A method of suppressing an immune response or treating an autoimmune disease or disorder in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of any one of claims 1-21 or the bifunctional molecule of any one of claims 22-27.

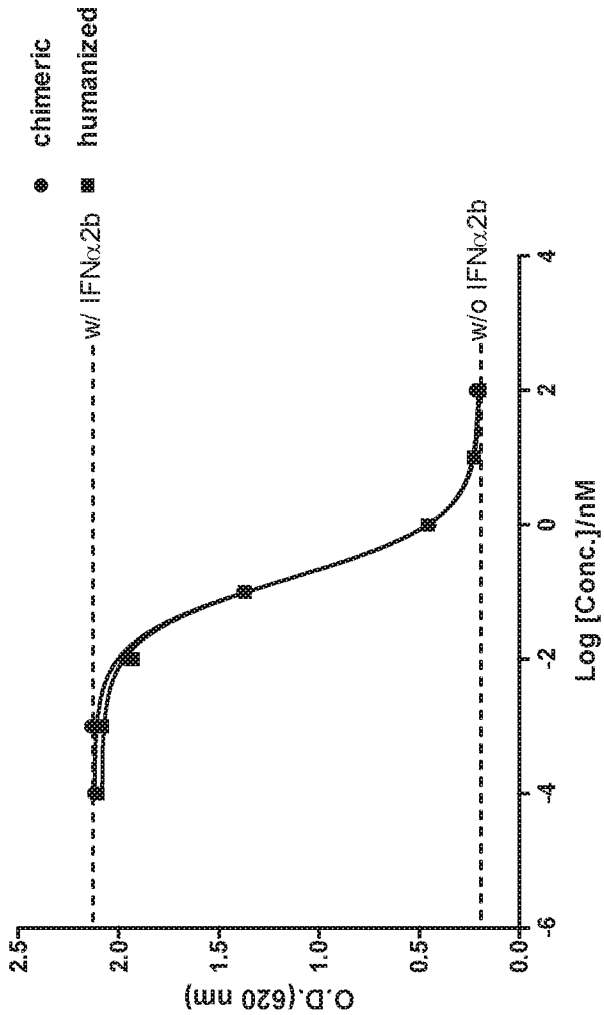
32. Use of the antibody or fragment thereof of any one of claims 1-21 or the bifunctional molecule of any one of claims 22-27 for the preparation of a medicament for suppressing an immune response or treating an autoimmune disease or disorder in a patient in need thereof.

33. The method of claim 31 or the use of claim 32, for treating an autoimmune disease or disorder.

34. The method of claim 31 or the use of claim 32, wherein the autoimmune disease or disorder is selected from the group consisting of type 1 diabetes, rheumatoid arthritis (RA), psoriasis/psoriatic arthritis, multiple sclerosis, systemic lupus erythematosus (lupus), inflammatory bowel disease, Addison's disease, Graves' disease, Sjögren's syndrome, Hashimoto's thyroiditis, myasthenia gravis, vasculitis, pernicious anemia, and celiac disease.

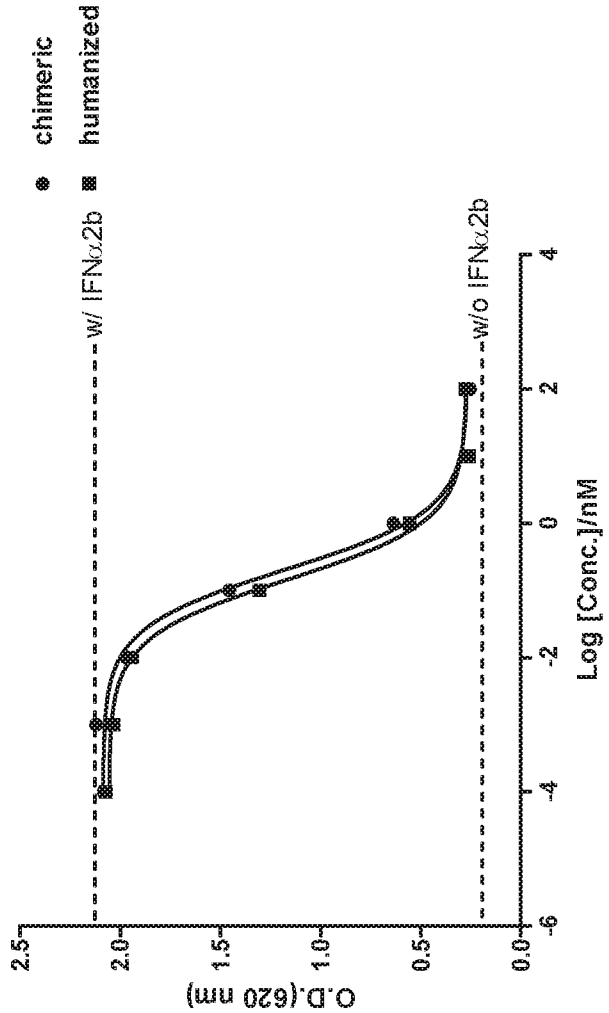
35. The method of claim 31 or the use of claim 32, wherein the autoimmune disease or disorder is systemic lupus erythematosus.

36. A method of detecting expression of a human interferon alpha and beta receptor subunit 1 (IFNAR1) protein in a sample, comprising contacting the sample with the antibody or fragment thereof of any one of claims 1-21, and detecting the binding which indicates expression of IFNAR1 in the sample.



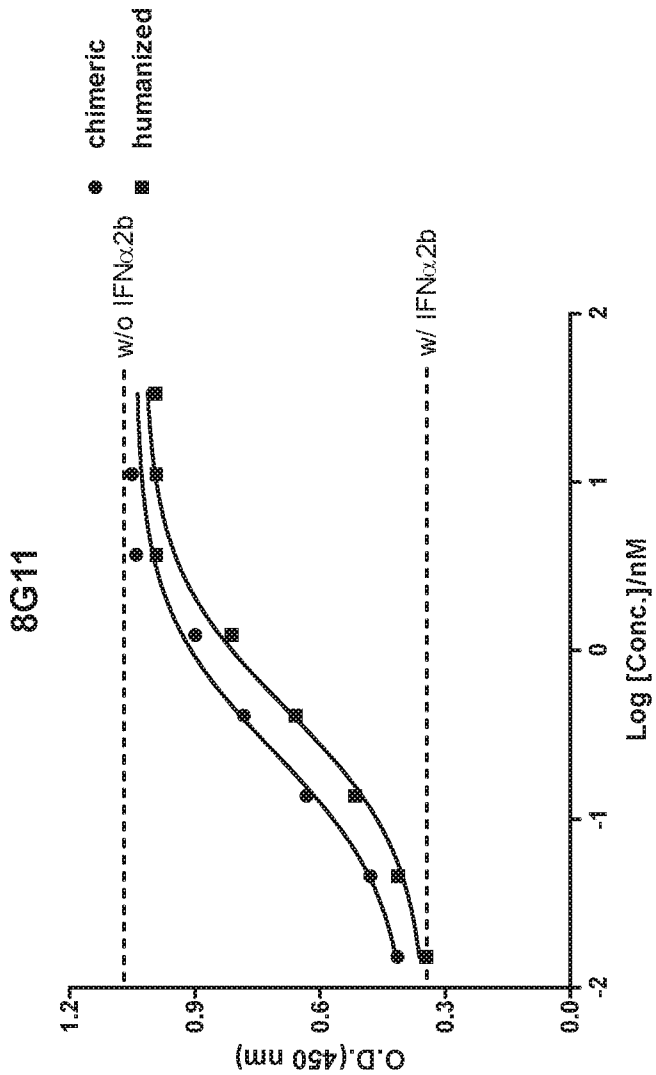
	chimeric	humanized
EC50	0.153	0.161

FIG. 1A



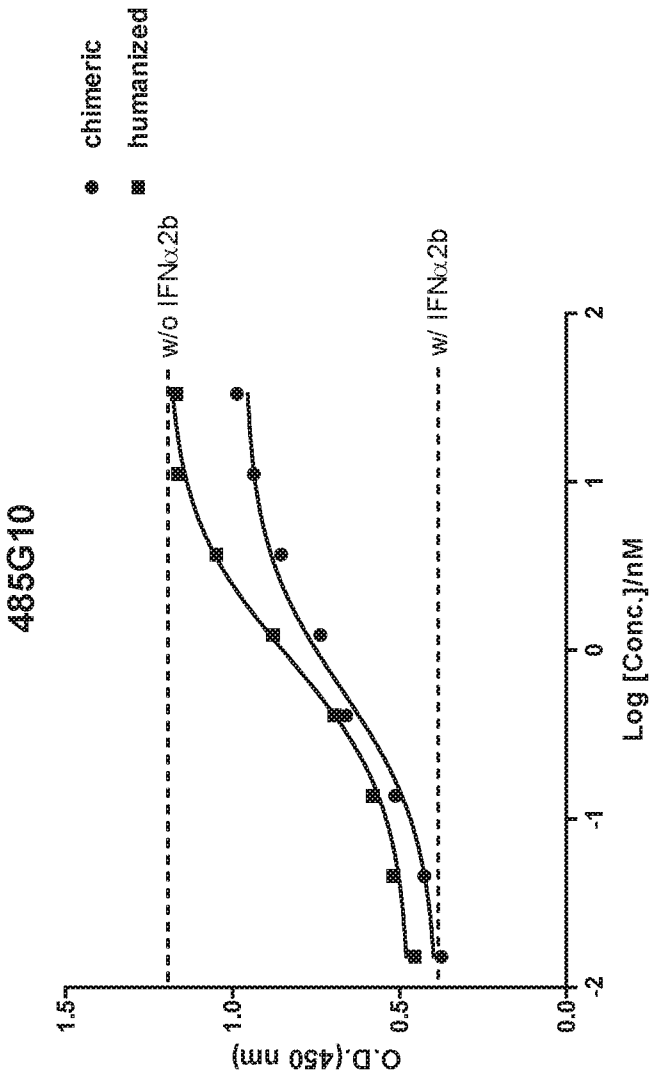
	chimeric	humanized
EC50	0.204	0.145

FIG. 1B



	chimeric	humanized
EC50	0.253	0.449

FIG. 2A



	chimeric	humanized
EC50	0.589	0.915

FIG. 2B

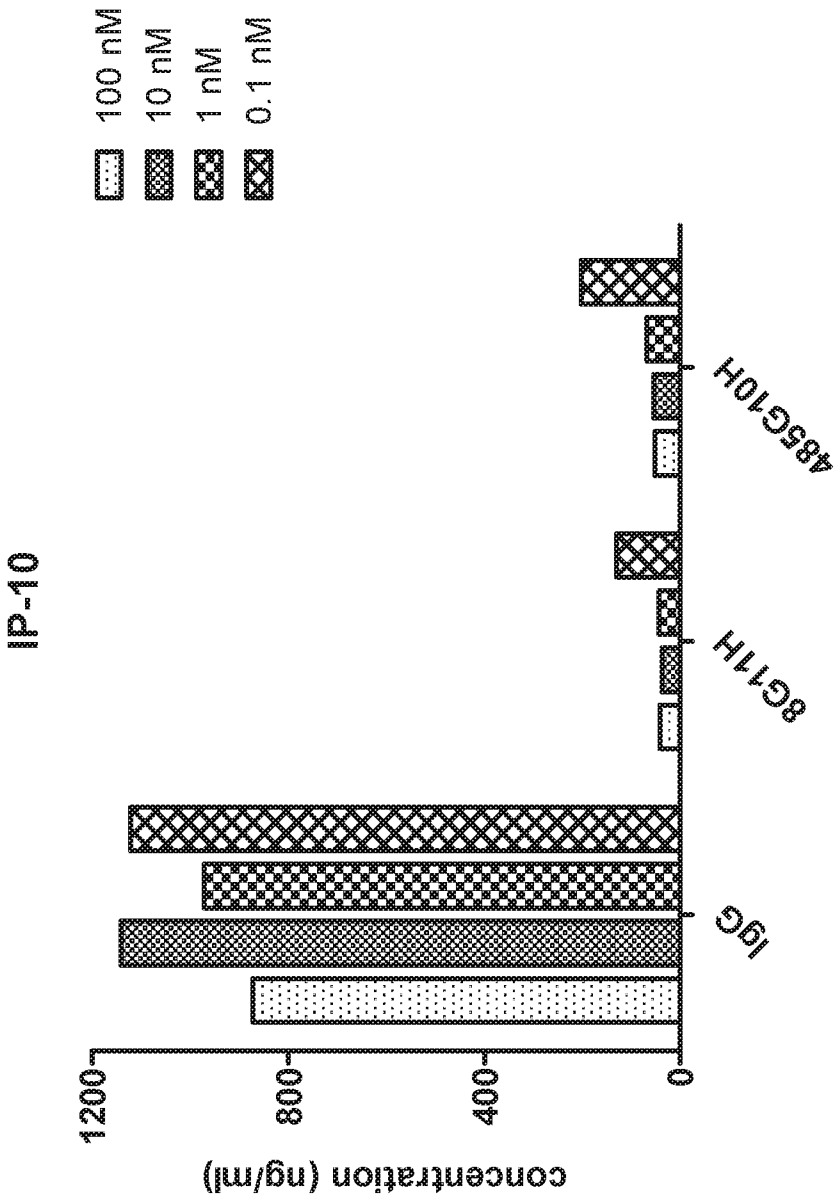

IP-10
100 nM
10 nM
1 nM
0.1 nM
1200
800
400
0
concentration (ng/ml)
IgG
8G11H
485G10H

FIG. 3

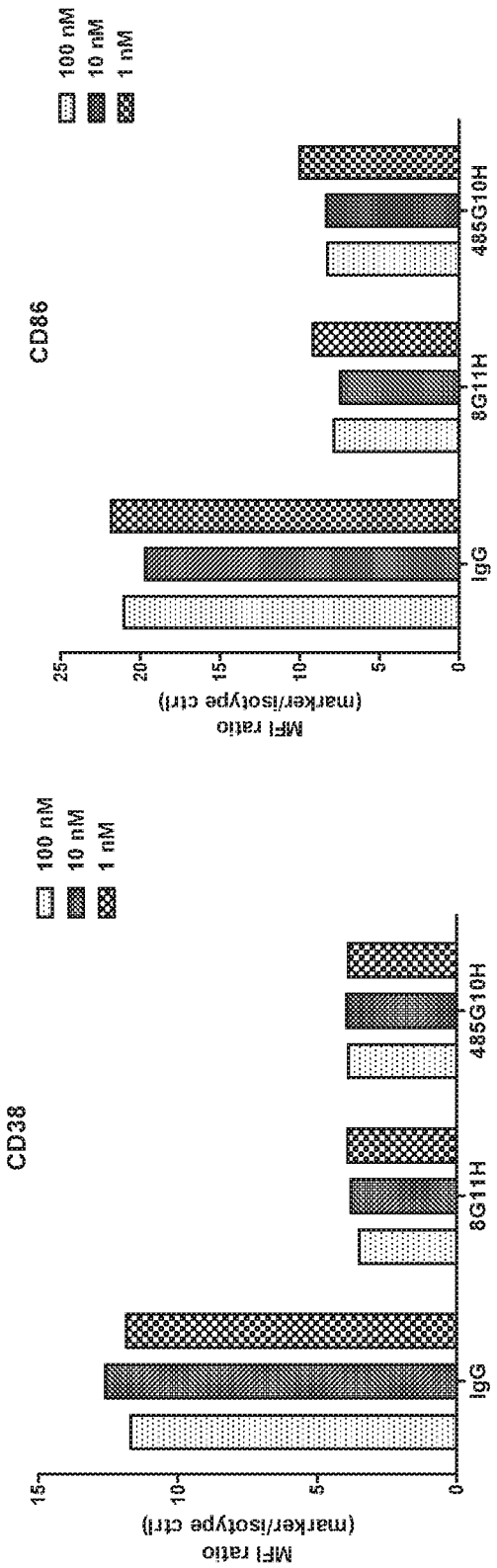
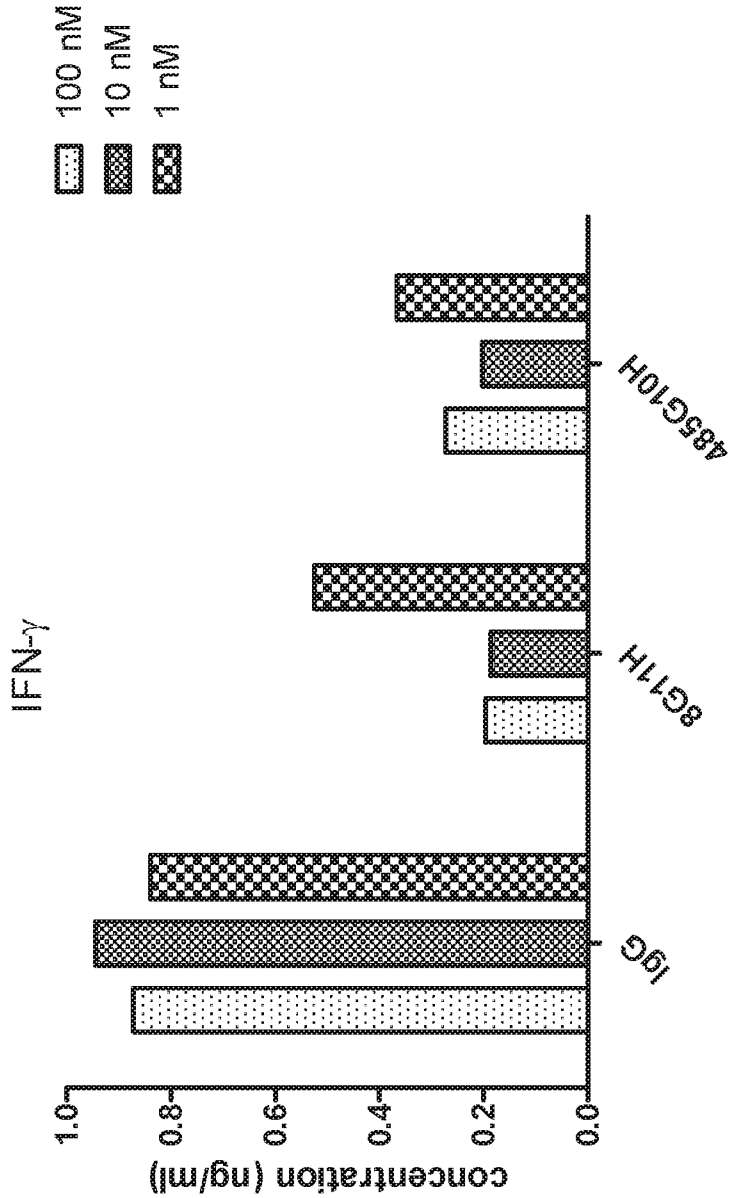

CD38
MFI ratio
(marker/isotype ctrl)
15
10
5
0
IgG
8G11H
485G10H
100 nM
10 nM
1 nM
CD86
MFI ratio
(marker/isotype ctrl)
25
20
15
10
5
0
IgG
8G11H
485G10H
100 nM
10 nM
1 nM

FIG. 4


100 nM
10 nM
1 nM
IFN-γ
concentration (ng/ml)
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0.8
0.6
0.4
0.2
0.0
IgG
8G11H
485G10H

FIG. 5

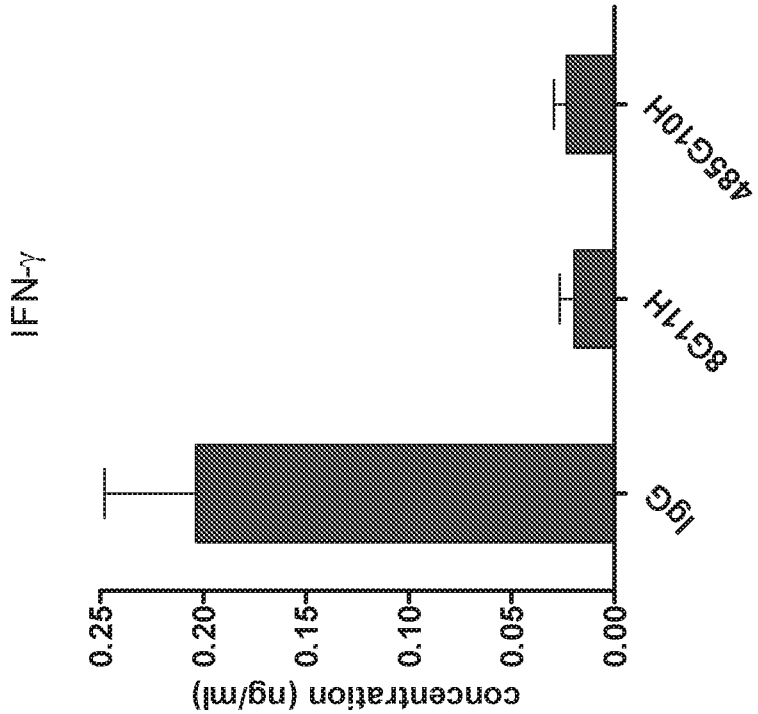

IFN-γ
concentration (ng/ml)
0.25
0.20
0.15
0.10
0.05
0.00
IgG
8G11H
485G10H

FIG. 6

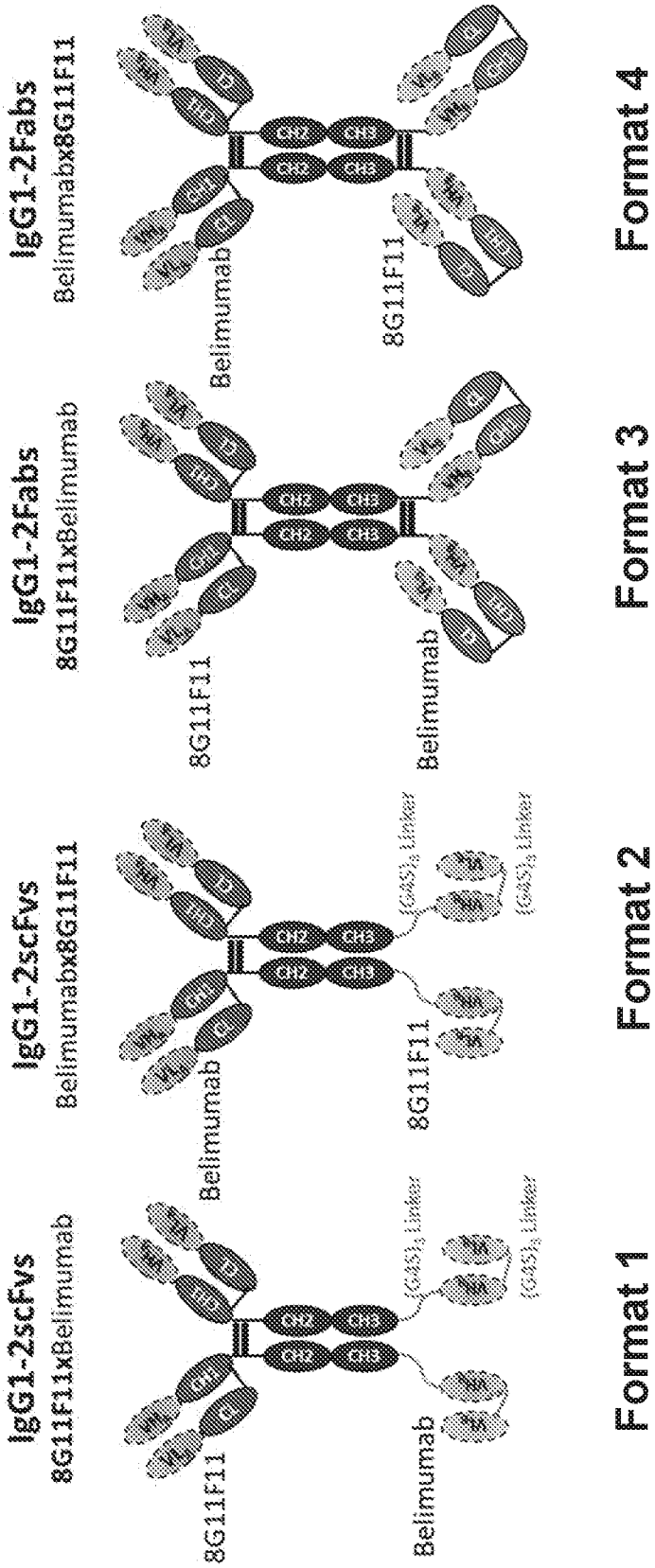

IgG1-2scFvs
8G11F11xBelimumab
8G11F11
Belimumab
Format 1
IgG1-2scFvs
Belimumabx8G11F11
Belimumab
8G11F11
(G4S)3 Linker
(G4S)3 Linker
Format 2
IgG1-2Fabs
8G11F11xBelimumab
8G11F11
Belimumab
Format 3
IgG1-2Fabs
Belimumabx8G11F11
Belimumab
8G11F11
Format 4

FIG. 7

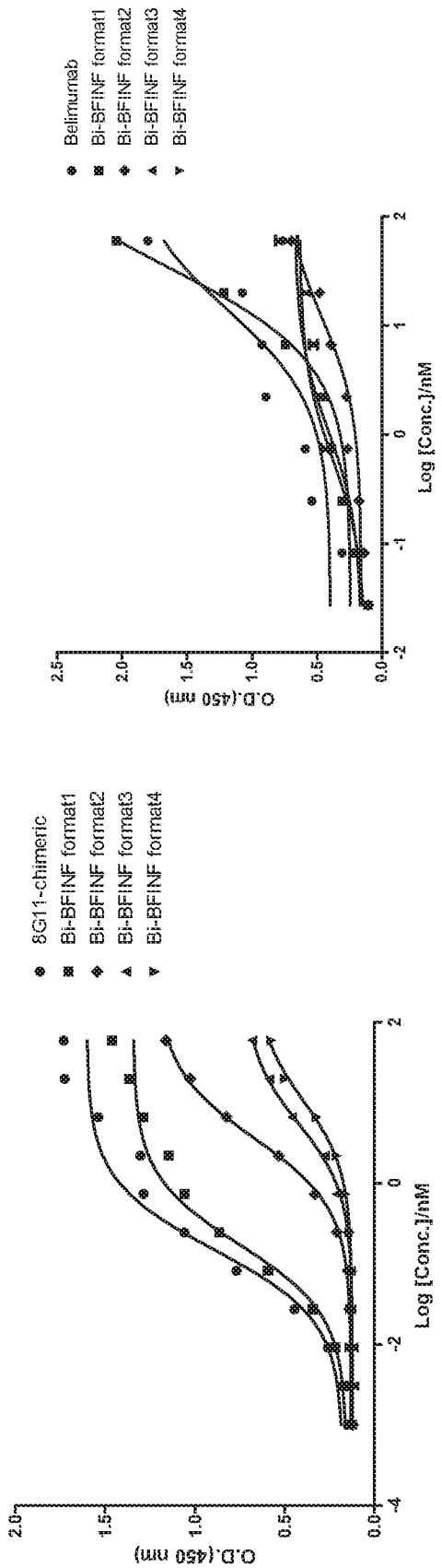

8G11-chimeric
Bi-BFINF format1
Bi-BFINF format2
Bi-BFINF format3
Bi-BFINF format4
O.D.(450 nm)
Log [Conc.]/nM
Belimumab
Bi-BFINF format1
Bi-BFINF format2
Bi-BFINF format3
Bi-BFINF format4
O.D.(450 nm)
Log [Conc.]/nM

FIG. 8

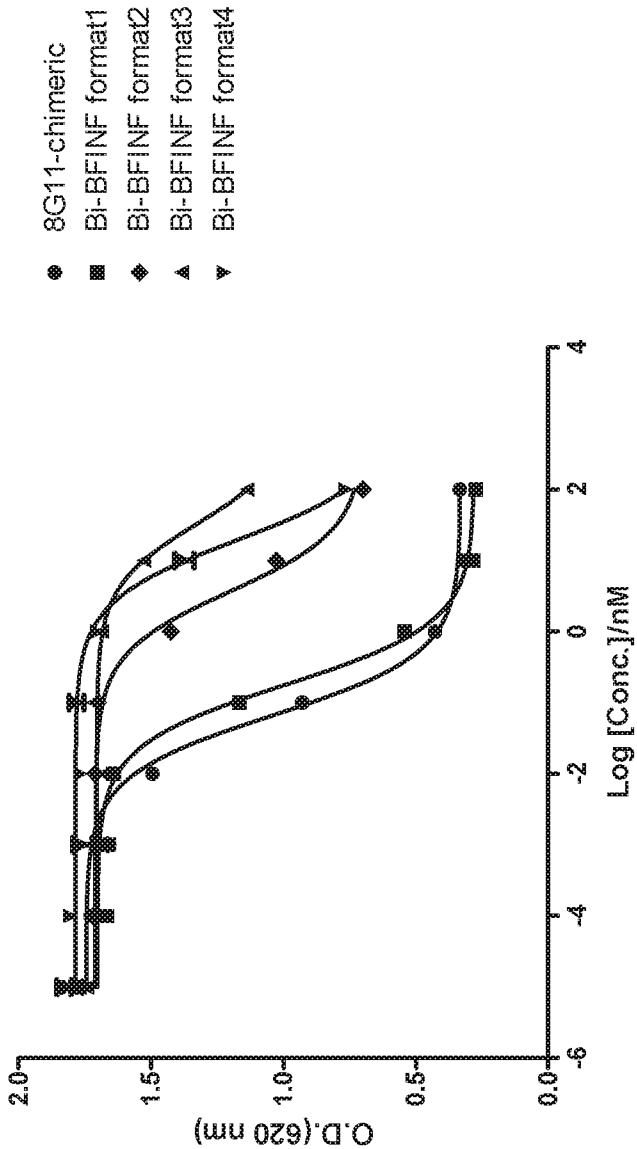

8G11-chimeric
Bi-BFINF format1
Bi-BFINF format2
Bi-BFINF format3
Bi-BFINF format4
O.D.(620 nm)
2.0
1.5
1.0
0.5
0.0
-6
-4
-2
0
2
4
Log [Conc.]/nM

FIG. 9A

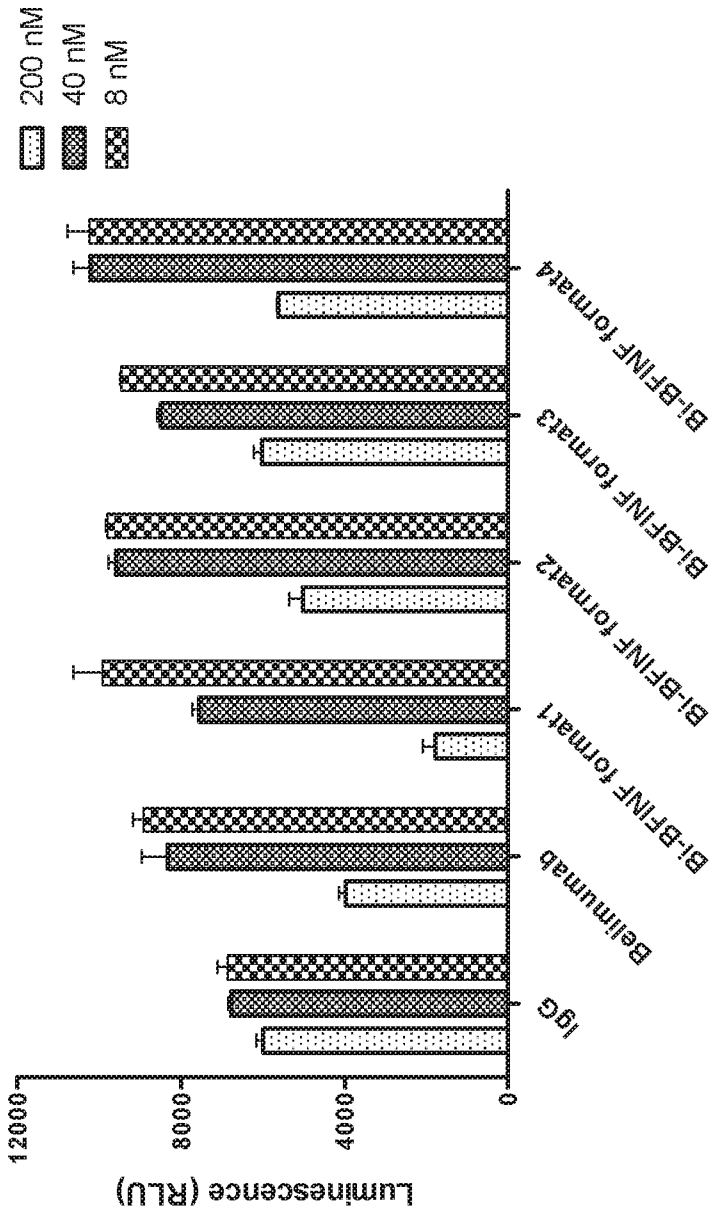

200 nM
40 nM
8 nM
Luminescence (RLU)
12000
8000
4000
0
IgG
Belimumab
BI-BFINF format1
BI-BFINF format2
BI-BFINF format3
BI-BFINF format4

FIG. 9B

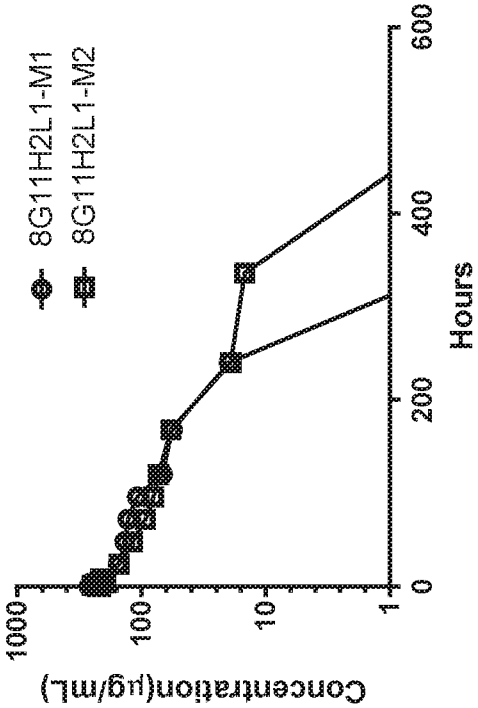


FIG. 10B

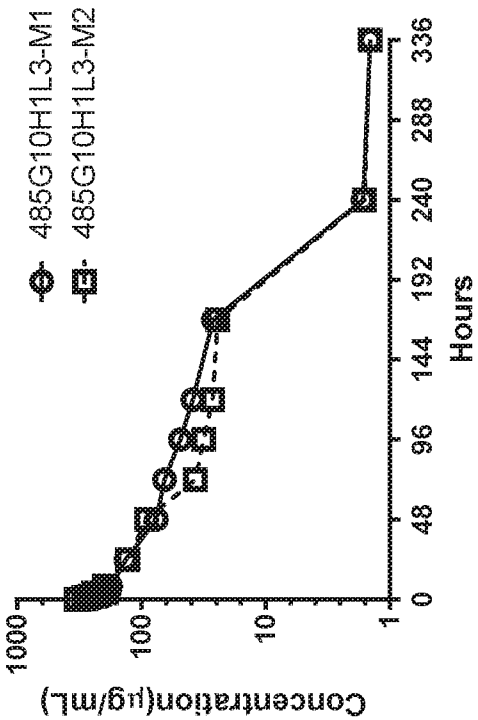
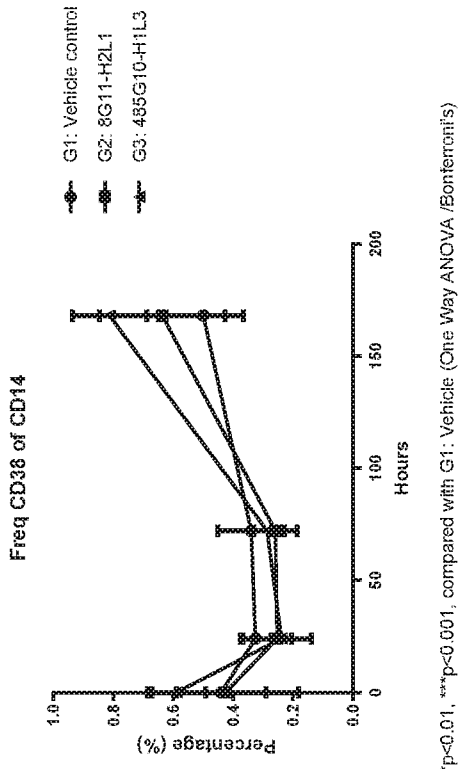
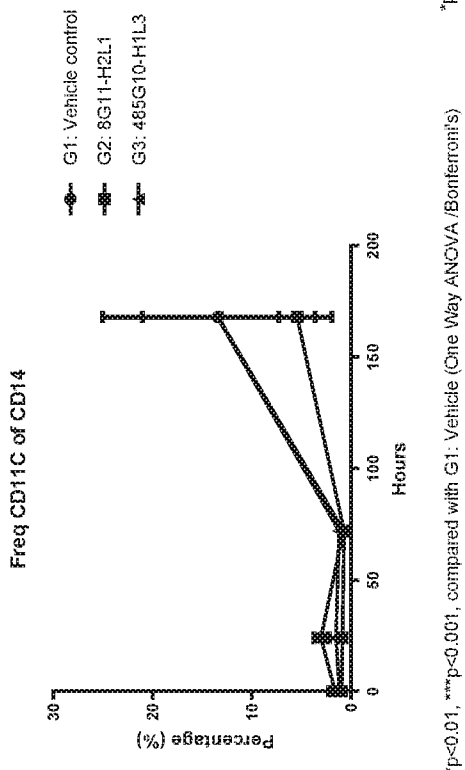


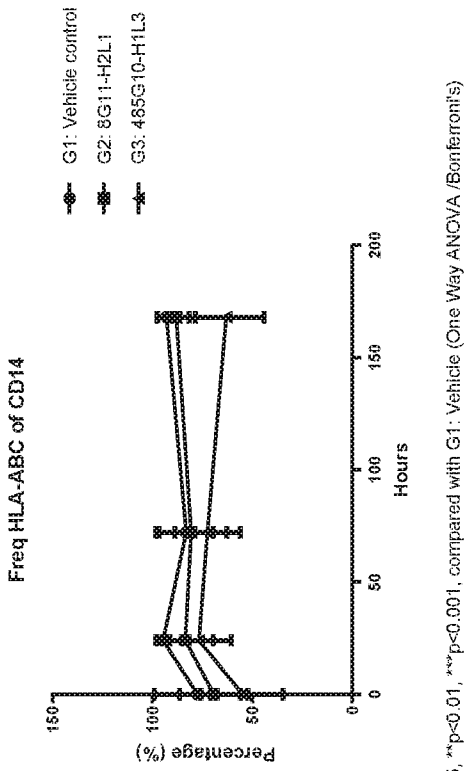
FIG. 10A



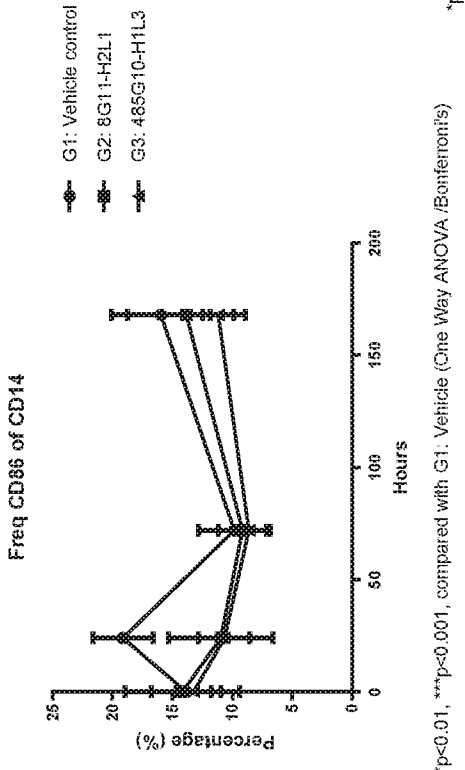
*p<0.05, **p<0.01, ***p<0.001, compared with G1: Vehicle (One Way ANOVA /Bonferroni's)



*p<0.05, **p<0.01, ***p<0.001, compared with G1: Vehicle (One Way ANOVA /Bonferroni's)



*p<0.05, **p<0.01, ***p<0.001, compared with G1: Vehicle (One Way ANOVA /Bonferroni's)



*p<0.05, **p<0.01, ***p<0.001, compared with G1: Vehicle (One Way ANOVA /Bonferroni's)

FIG. 11A

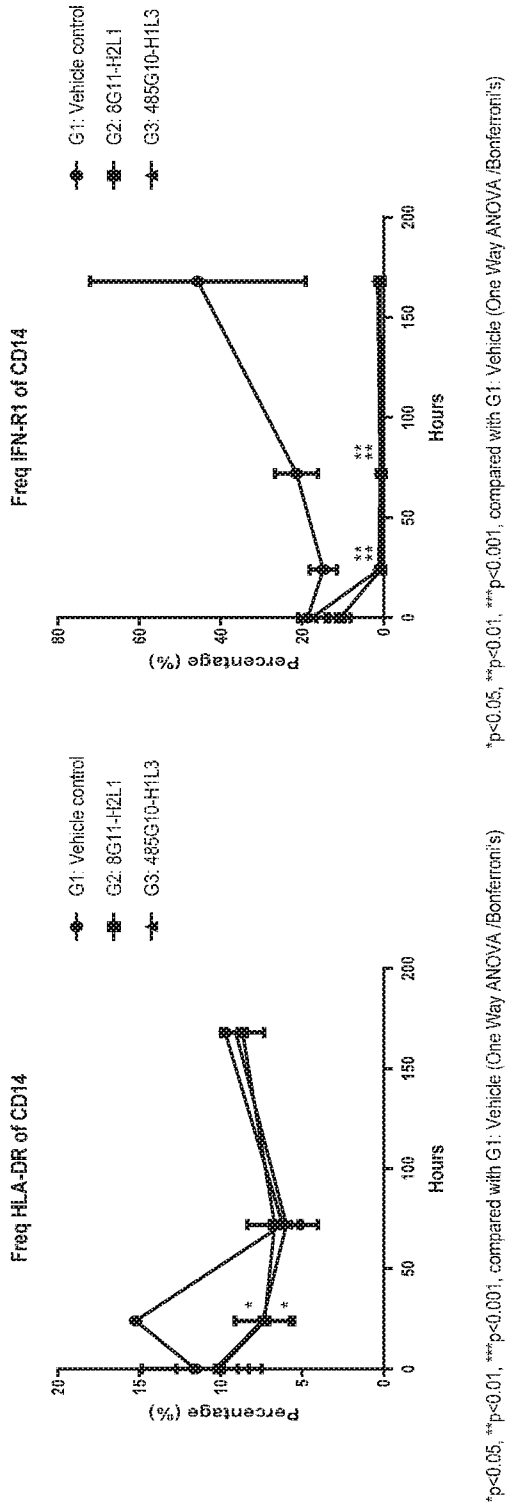
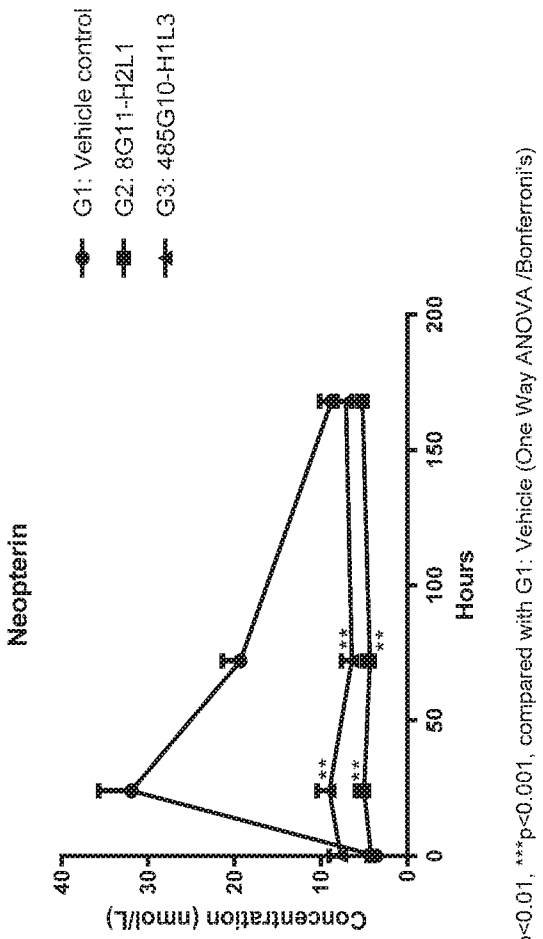


FIG. 11A (cont'd)


Neopterin
G1: Vehicle control
G2: 8G11-H2L1
G3: 485G10-H1L3
Concentration (nmol/L)
40
30
20
10
0
0
50
100
150
200
Hours
**
**
**
*p<0.05, **p<0.01, ***p<0.001, compared with G1: Vehicle (One Way ANOVA /Bonferroni's)

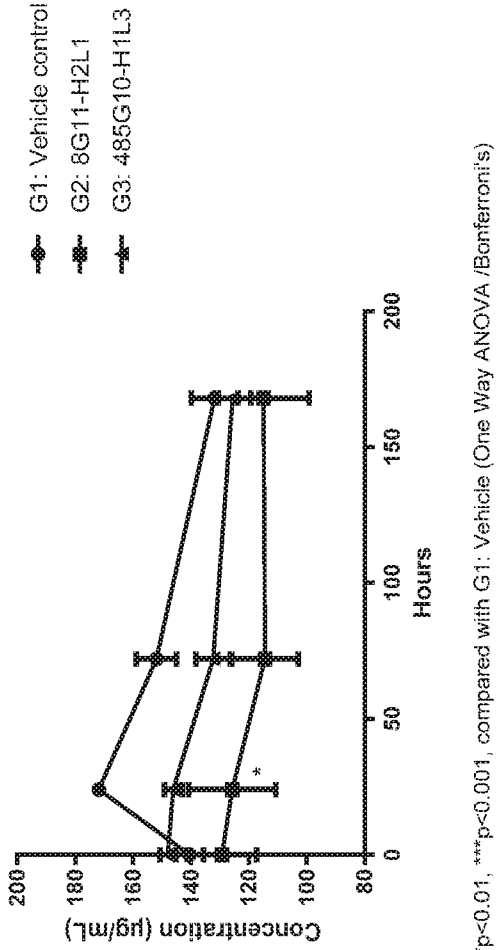
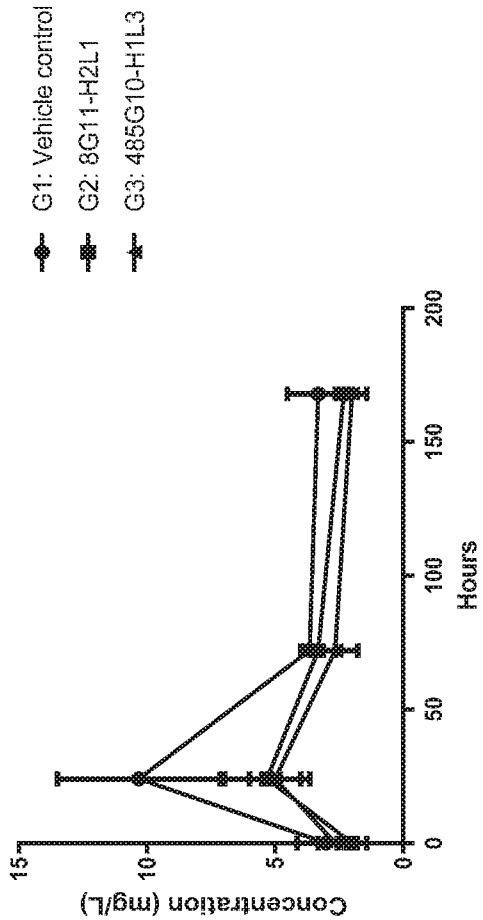

Beta-2 Microglobulin
G1: Vehicle control
G2: 8G11-H2L1
G3: 485G10-H1L3
Concentration (μg/mL)
200
180
160
140
120
100
80
0
50
100
150
200
Hours
*
*p<0.05, **p<0.01, ***p<0.001, compared with G1: Vehicle (One Way ANOVA /Bonferroni's)

FIG. 11B



*p<0.05, **p<0.01, ***p<0.001, compared with G1: Vehicle (One Way ANOVA /Bonferroni's)

FIG. 11B (cont'd)

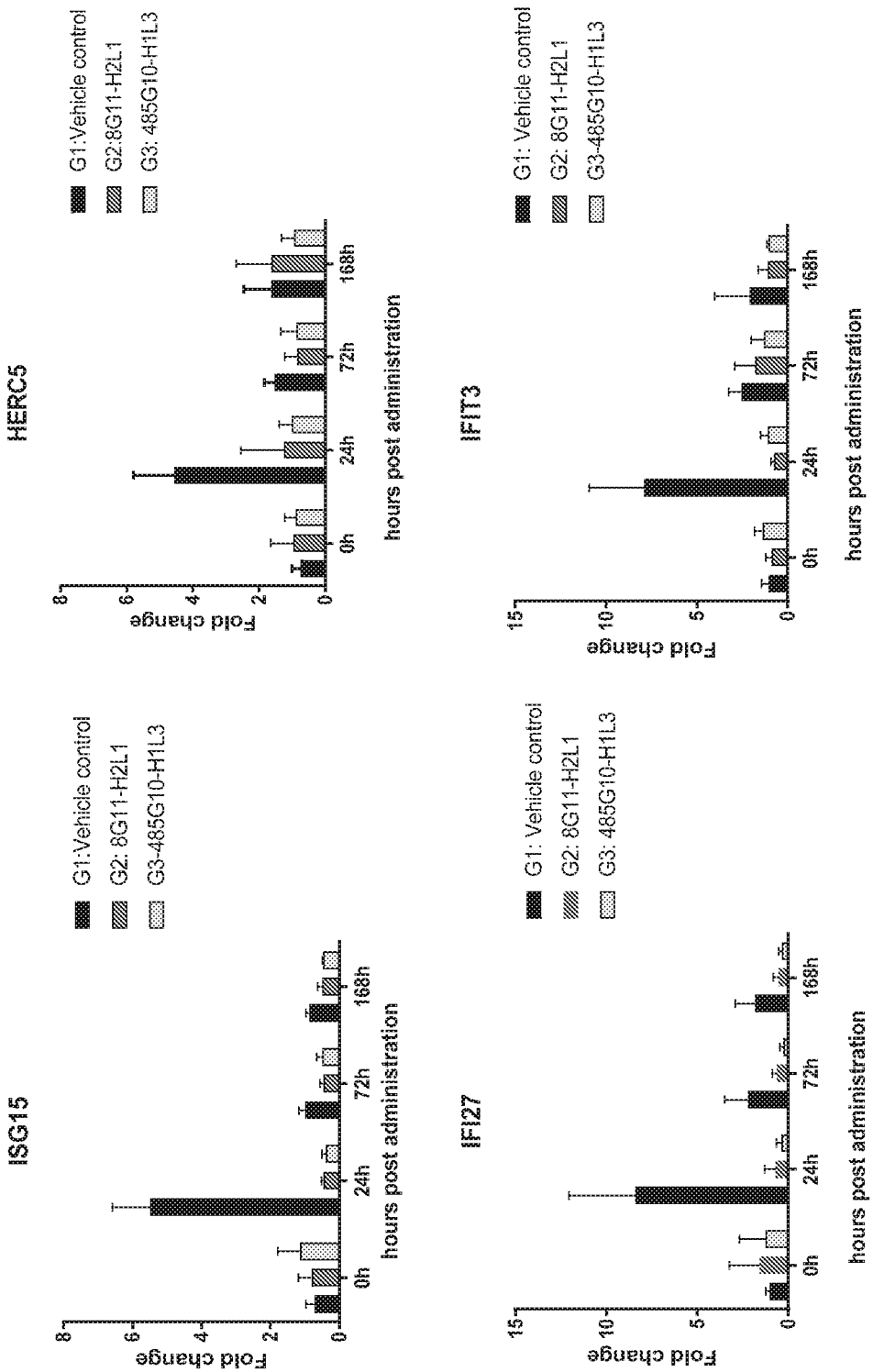

ISG15
HERC5
IFI27
IFIT3
G1:Vehicle control
G2: 8G11-H2L1
G3-485G10-H1L3
G1: Vehicle control
G2: 8G11-H2L1
G3: 485G10-H1L3
Fold change
hours post administration
0h
24h
72h
168h

FIG. 11C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/106412

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/18(2006.01)i; A61K 39/395(2006.01)i; A61P 37/06(2006.01)i; A61P 43/00(2006.01)i; G01N 33/53(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; A61K; A61P; G01N

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

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

TWTXT;GBTXT;SGTXT;DWPI;EPTXT;VEN;USTXT;CHTXT;WOTXT;CNABS;CATXT;ATTXT;KRTXT;LEXTXT; RUTXT;DETXT;JPTXT;CNTXT;PUBMED;ISI WEB OF SCIENCE;CNKI:IFNAR1 , IFNAR-1, INTERFERON ALPHA AND BETA RECEPTOR SUBUNIT 1,IFN RECEPTOR 1,ANTIBODY,ANTIBODIES, SEQ ID NO:77-83, 92-98, AUTOIMMUNE DISEASES, SYSTEMIC LUPUS ERYTHEMATOSUS, LUPUS, EPITOPE,HLYKWK

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008102072 A1 (GENENTECH, INC.) 01 May 2008 (2008-05-01) description paragraphs 15, 47-48, 68, 214-217, 230-231, 247, 261, 274, 292, 296, tables 1-5	18-36
X	LU, J. et al. "Structure-function study of the extracellular domain of the human IFN-α receptor(hIFNAR1) using blocking monoclonal antibodies:the role of domains 1 and 2" *THE JOURNAL OF IMMUNOLOGY*, Vol. 160, 31 December 1998 (1998-12-31), 1784-1787	18-21
A	CN 101001878 A (MEDAREX, INC.) 18 July 2007 (2007-07-18) whole document	1-36
A	CN 106243226 A (BEIJING ZHIREN MEIBO BIOTECHNOLOGY CO., LTD. et al) 21 December 2016 (2016-12-21) whole document	1-36

✓ Further documents are listed in the continuation of Box C.

✓ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 December 2019	**18 December 2019**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing** **100088** **China**	Authorized officer **LI,Ying**
Facsimile No. **(86-10)62019451**	Telephone No. **86-(10)-53961973**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/106412

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RUNKEL, L. et al. "Lupus clinical development:will belimumab's approval catalyse a new paradigm fro SLE drug development" *EXPERT OPINION ON BIOLOGICAL THERAPY,* Vol. 14, 03 February 2014 (2014-02-03), 491-501	1-36
A	KAWANO, Y. et al. "Blocking IFNAR1 inhibits multiple myeloma-driven Treg expansion and immunosuppression" *THE JOURNAL OF CLINICAL INVESTIGATION,* Vol. 128, No. 6, 30 June 2018 (2018-06-30), 2487-2499	1-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/106412

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/106412

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

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

1. ☑ Claims Nos.: **31,33-35**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 31, 33-35 relate to a method for suppressing an immune response or treating an autoimmumune disease in a patient, and therefore do not warrant an international search according to the criteria set out in PCT Rule 39.1(iv). An international search is still carried out on the basis of the antibody or fragment in the manufacture of a medicament for suppressing an immune response or treating an autoimmumune disease in a patient in need thereof.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/106412

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2008102072	A1	01 May 2008	US	6713609	B1	30 March 2004
				US	2006020118	A1	26 January 2006
CN	101001878	A	18 July 2007	AU	2005258077	C1	25 October 2012
				KR	20070047286	A	04 May 2007
				KR	20150041193	A	15 April 2015
				US	8460668	B2	11 June 2013
				ES	2643237	T3	21 November 2017
				CN	102603894	B	30 September 2015
				KR	101335079	B1	12 December 2013
				SI	2662390	T1	30 October 2017
				US	2010143369	A1	10 June 2010
				RU	2010138567	A	27 March 2012
				CN	102603894	A	25 July 2012
				EP	1781705	A4	26 November 2008
				PT	2662390	T	09 October 2017
				EP	2662390	B1	02 August 2017
				CN	102863532	A	09 January 2013
				DK	2662390	T3	06 November 2017
				KR	101541658	B1	07 August 2015
				CY	1115875	T1	25 January 2017
				CA	2570823	A1	05 January 2006
				SI	EP1781705	T1	30 January 2015
				KR	20140033210	A	17 March 2014
				PT	1781705	E	23 December 2014
				MX	2007000023	A	07 March 2007
				RU	2600884	C2	27 October 2016
				JP	2008508864	A	27 March 2008
				JP	5899540	B2	06 April 2016
				CA	2570823	C	24 February 2015
				US	7662381	B2	16 February 2010
				US	2013230534	A1	05 September 2013
				PL	2662390	T3	29 December 2017
				EP	1781705	B1	08 October 2014
				RU	2007102079	A	27 July 2008
				LT	2662390	T	10 October 2017
				HU	E035082	T2	02 May 2018
				HK	1105420	A1	01 March 2013
				AU	2005258077	B2	18 August 2011
				WO	2006002177	A3	22 June 2006
				DK	1781705	T3	12 January 2015
				CN	101001878	B	26 September 2012
				JP	5160887	B2	13 March 2013
				WO	2006002177	A2	05 January 2006
				SI	1781705	T1	30 January 2015
				KR	20130012258	A	01 February 2013
				RU	2412202	C2	20 February 2011
				US	9453077	B2	27 September 2016
				EP	2662390	A1	13 November 2013
				US	2006029601	A1	09 February 2006
				US	10385133	B2	20 August 2019

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/106412

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
		ES 2526194 T3	08 January 2015
CN 106243226 A	21 December 2016	WO 2018023976 A1	08 February 2018
		CN 106243226 B	12 February 2019