

Fc RECEPTOR BASED ASSAYS FOR ANALYZING IgG ANTIBODIES**FIELD OF THE INVENTION**

[0001] The present disclosure is related to a transformed cell, including certain Fc Receptors, and uses thereof, including, methods of characterizing an antibody, selecting a cell clone for use in manufacturing the antibody and/or assessing potency of the antibody. The disclosure also relates to a potency assay, and uses thereof, e.g., in batch release of a pharmaceutical product or composition.

SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in ST.26 XML format. The Sequence Listing is provided as a file titled “30352_US_PRI_SEQUENCE_LISTING.xml” created 04-Nov-2024 and is 31 kilobytes in size. The Sequence Listing information in the ST.26 XML format is incorporated herein by reference in its entirety.

BACKGROUND

[0003] Regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMA), are tasked to ensure the safety and efficacy for various products, including, medicines and biological products. An application for marketing approval of a medicine (or drug product) by such regulatory agencies requires submission of information regarding the drug product including information regarding potency of the drug product and assays to determine such potency.

[0004] Potency assays are a quantitative measure of the biological activity of a drug substance or a drug product. The potency assay needs to be mechanistically relevant to clinical outcome of the therapeutic and is often used to: determine product quality of drug substance and drug product, batch release, comparability studies, or stability testing. Moreover, the assay needs to be quality control (QC) amenable. Potency assays should be sensitive enough to detect differences (e.g., between manufacturing lots) that may impact the mechanism of action (MOA) and function of the product. It is highly desirable for the potency assay to i) have a close functional relationship to the mechanism of action and/or the physiological/pharmacological activity of the product, ii) reliably measure potency

within the product specifications with reproducible results, and iii) provide a highly sensitive readout that detects differences between manufacturing lots that may be of clinical relevance. Additionally, the potency assay should have low intra- and inter-assay variation (to obtain precision needed to support product specifications), sufficient robustness, and be amenable to quality control (QC) and high-throughput analysis. Cell-based potency assays have the added complexity of identifying/engineering an appropriate cell line for use in the assay. In some cases, a further challenge is that the assay must be validated per ICH guidance document Q2(R1) (Validation of Analytical Procedures: Text and Methodology, 2005) so that it can be transferred to testing sites and release sites across the globe.

[0005] The potency of antibodies having a mechanism of action which includes Fc-mediated activity is often measured using potency assays that measure Fc to Fc receptor binding. However, such assays often require primary cells, are unwieldy, and do not replicate results reliably due to variations from cell culture. In some cases, the use of primary cells in potency assays introduces variability because of variations in donor immune status, polymorphisms in expressed genes, and variations in cell-type purity. Therefore, such biological assays are not optimal for batch release purposes.

[0006] The therapeutic utility of an antibody as a drug depends on the ability of the antibody to bind its antigen, and often the antibody's Fc-mediated activities play a role in the antibody's mechanism of action. Therapeutic antibodies rely on two types of functionalities to achieve clinical efficacy: target-specific binding by the Fab (antigen-binding fragment) domain and immune-mediated effector functions – such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC) – via interaction of the Fc domain with Fc receptors (FcR) on various cell types. The Fc portion of a therapeutic antibody thus has an important role in its mechanism of action through its influence on either ADCC, ADCP, or CDC. These FcR present on various cell types can also lead to release of cytokines that help modulate overall immune response and recruit other cells to the site of inflammation.

[0007] Fc γ Receptors (Fc γ R) belong to an immunoglobulin superfamily and include several members further classified as activating and inhibitory Fc γ Rs. Activating Fc γ Rs include, Fc γ RI (CD64), Fc γ RIIa (CD32a), and Fc γ RIIIa (CD16a) while Fc γ RIIb (CD32b) is the inhibitory Fc γ R. Activating Fc γ Rs generate signals within their cells

through an immunoreceptor tyrosine-based activation motif (ITAM) (e.g., phosphorylation of ITAM) present in the intracellular tail of FcγRIIa and induce phagocytosis in macrophages. FcγRI and FcγRIIIa do not have an ITAM but transmit an activating signal by interacting with an adapter protein (also referred to as coreceptor) called Fc Epsilon Receptor Ig (FCER1G), which, like FcγRIIa, contains two YXXL sequences (where X can be any amino acid) that are characteristic of an ITAM (see, e.g., Bournazos et al., “Fcγ Receptor Function and the Design of Vaccination Strategies,” *Immunity* 47.2: 224-233 (2017); Pincetic et al., “Type I and Type II Fc Receptors Regulate Innate and Adaptive Immunity,” *Nature Immunology* 15.8: 707-716 (2014), Sonderrmann et al., “General Mechanism for Modulating Immunoglobulin Effector Function,” *PNAS* 110.24: 9868-9872 (2013); Amigorena, et al., “Tyrosine-containing Motif that Transduces Cell Activation Signals Also Determines Internalization and Antigen Presentation via Type III Receptors for IgG,” *Nature* 358.6384: 337-341 (1992); Swanson and Adam, “The Coordination of Signaling During Fc Receptor-mediated Phagocytosis,” *Journal of Leukocyte Biology* 76.6: 1093-1103 (2004); Takai et al., “FcR γ Chain Deletion Results in Pleiotrophic Effector Cell Defects,” *Cell* 76.3: 519-529 (1994)).

[0008] The activating and inhibitory FcγR have different affinity for different IgG isotypes. FcγRIIIa is the primary driver of ADCC activity and robust quality control (QC) amenable reporter-based assays are validated and available for the same. ADCP activity is mediated by multiple activating FcγRs, such as, FcγRI, FcγRIIa and/or FcγRIIIa and developing assays that analyze antibodies that function via multiple combinations FcγRs is challenging. To the best of applicant's knowledge, only reporter cell lines expressing individual Fcγ receptors are available which is not always ideal. This is because reporter cell lines expressing a single Fcγ receptor in a potency assay may not adequately represent the mechanism of action of an antibody that may require multiple Fcγ receptors. In other words, there is a lack of an appropriate cell line that effectively captures the physiological mode of action of immune cells, such as, macrophages that express a repertoire of FcγR. Also, ADCP is typically monitored using cell lines or primary cells isolated from mouse or human. Such cells, however, may not i) express a full repertoire of human activating FcγRs and/or ii) are not amenable to potency assays. Thus, for the reasons described above, there is a need for fast, replicable, efficient, and sensitive potency assays that are functionally

related to the mechanism of action of the antibody and the pharmacological function of the antibody.

SUMMARY OF THE INVENTION

[0009] Antibodies, with a mechanism of action that needs microglial cell activation, often activate one or more of the repertoires of Fc γ present on microglial cells. Human microglia express three activating receptors Fc γ RI, Fc γ RIIa, and Fc γ RIIIa and inhibitory receptor Fc γ RIIb. Accordingly, for a potency assay to reflect the mechanism of action of the antibody, the assay should ideally use a reporter cell line that expresses at least one of the three activating receptors, at least two of the three activating receptors, or all three activating receptors.

[0010] The present disclosure, among other things, provides a means for overcoming the known deficiencies of potency assays. In one aspect, the present disclosure is related to a method for determining the potency of a drug product comprising an antibody or a fragment thereof, wherein at least one mechanism of action of the antibody or the fragment thereof is mediated through the activation/binding of the antibody or the fragment thereof to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof. In some embodiments, such method includes the following steps: i) determining binding of the antibody or the fragment thereof to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, and/or ii) determining the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, or a variant thereof by the antibody or the fragment thereof.

[0011] One aspect of the present disclosure is related to a transformed cell comprising: i) a nucleic acid encoding a human Fc γ Receptor I α -chain (Fc γ RI α -chain) or a variant thereof; ii) a nucleic acid encoding a human Fc γ RI α -chain or a variant thereof and a nucleic acid encoding a human Fc γ Receptor IIa (Fc γ RIIa) or a variant thereof; iii) a nucleic acid encoding a human Fc γ RI α -chain or a variant thereof and a nucleic acid encoding a human Fc γ Receptor IIIa α -chain (Fc γ RIIIa α -chain) or a variant thereof; iv) a nucleic acid encoding a human Fc γ RIIa or a variant thereof and a nucleic acid encoding a human Fc γ RIIIa α -chain or a variant thereof; v) a nucleic acid encoding a human Fc γ RI α -chain or a variant thereof, a nucleic acid encoding a human Fc γ RIIa or a variant thereof, and a nucleic acid encoding a human Fc γ RIIIa α -chain or a variant thereof; wherein the

transformed cell is transformed/engineered to include the nucleic acids encoding FcγRI α-chain, FcγRIIa, and/or FcγRIIIa α-chain.

[00012] Another aspect of the present disclosure is related to a nucleic acid encoding a human FcγRI α-chain, an ITAM coreceptor, and/or a selection marker (that provides antibiotic resistance) as well as to one or more vectors comprising a nucleic acid encoding for a human FcγRI α-chain, an ITAM coreceptor, and/or a selection marker.

[00013] Another aspect of the present disclosure is related to a nucleic acid encoding a human FcγRIIIa α-chain, an ITAM coreceptor, and/or a selection marker. This aspect is also related to one or more vectors comprising a nucleic acid encoding a human FcγRIIIa α-chain, an ITAM coreceptor, and/or a selection marker.

[00014] Yet another aspect of the present disclosure is related to a nucleic acid encoding a human FcγRIIa and/or a selection marker. This aspect is also related to one or more vectors comprising a nucleic acid encoding a human FcγRIIa and/or a selection marker. Another aspect of the present disclosure is related to a nucleic acid encoding a NFAT response element operably linked to luciferase and/or a selection marker. This aspect is also related to one or more vectors comprising a nucleic acid encoding a NFAT response element operably linked to luciferase and/or a selection marker.

[00015] Another aspect of the present disclosure is related to a process of making a transformed cell including: i) transforming a cell such that it includes a nucleic acid encoding a human FcγRI α-chain or a variant thereof, a nucleic acid encoding a human FcγRIIa or a variant thereof, or a nucleic acid encoding a human FcγRIIIa α-chain or a variant thereof; and ii) selecting a transformed cell that comprises the nucleic acids encoding FcγRI α-chain, FcγRIIa, and/or FcγRIIIa α-chain.

[00016] Another aspect of the present disclosure is related to a method for identifying an antibody or a fragment thereof having at least one mechanism of action mediated through activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof, the method comprising the steps of: i) providing the antibody or a fragment thereof having an Fc receptor binding moiety; ii) contacting the antibody or a fragment thereof with a transformed cell of the present disclosure, optionally, for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof; iii) detecting a signal generated upon contacting the antibody or a fragment thereof with the transformed cell; iv)

analyzing the signal generated in step iii) to determine whether one or more of the receptors of step ii) are activated; and v) identifying the antibody or a fragment thereof as an activator of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof.

[00017] Another aspect of the present disclosure is related to a method for analyzing and selecting at least a batch of a drug product, wherein the batch comprises an antibody or a fragment thereof and at least one mechanism of action of the antibody or the fragment thereof is mediated through activation of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof, the method including the steps of: i) providing a drug product comprising the antibody or the fragment thereof; ii) providing a reference standard antibody or a fragment thereof; iii) contacting the antibody or the fragment thereof with a first transformed cell described herein for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof; iv) contacting the reference standard with a second transformed cell described herein for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof; v) measuring the activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof by the antibody or fragment thereof; vi) measuring the activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof by the reference standard; vii) comparing the activation of the Fc receptors of step v) to the activation of Fc receptors of step vi); and viii) selecting the antibody or fragment thereof that has an acceptable Fc receptor activation as compared to the reference standard. In some embodiments, the Fc receptor activation is a measure of the biological activity of the antibody or fragment thereof. In some embodiments, the first transformed cell includes a nucleic acid that encodes for FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof. In some embodiments, the second transformed cell includes a nucleic acid that encodes for FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof. In some embodiments, the first transformed cell and the second transformed cell have the same repertoire of activating Fc receptors. In some embodiments, the method is used to measure the potency of the drug product. In some embodiments, the method is related to determining acceptability of a drug product's batch based on potency of the drug product.

[00018] Another aspect of the present disclosure is related to a method for assessing an antibody or fragment thereof for a mechanism of action mediated through activation of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof, the method comprising the steps of: i) providing a drug product comprising the antibody or the fragment thereof; ii) providing a reference standard antibody or a fragment thereof; iii) contacting the antibody or the fragment thereof with a first transformed cell described herein for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof; iv) contacting the reference standard with a second transformed cell described herein for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof; v) measuring the activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof by the antibody or fragment thereof; vi) measuring the activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof by the reference standard; vii) comparing the activation of the Fc receptors of step v) to the activation of Fc receptors of step vi); and viii) selecting the antibody or fragment thereof that has an acceptable Fc receptor activation as compared to the reference standard. In some embodiments, the Fc receptor activation is a measure of the biological activity of the antibody or fragment thereof. In some embodiments, the first transformed cell includes a nucleic acid that encodes for FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof. In some embodiments, the second transformed cell includes a nucleic acid that encodes for FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof. In some embodiments, the first transformed cell and the second transformed cell have the same repertoire of activating Fc receptors. In some embodiments, the method is used to assess the potency of the antibody or fragment thereof.

[00019] Another aspect of the present disclosure is related to a method of selecting at least one cell clone for the manufacture of a drug substance, wherein the drug substance is an antibody or a fragment thereof and at least one mechanism of action of the antibody or the fragment thereof is mediated through activation of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof, the method comprising the steps of: i) providing at least one drug substance obtained from at least one cell clone; ii) providing a reference standard antibody or a fragment thereof; iii) contacting the drug

substance with a first transformed cell described herein for a time period sufficient to allow activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof; iv) contacting the reference standard with a second transformed cell described herein for a time period sufficient to allow activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa receptor, a variant thereof, or a combination thereof; v) measuring the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof by the drug substance; vi) measuring the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof by the reference standard; vii) comparing the activation of the Fc receptors of step v) to the activation of Fc receptors of step vi); and viii) selecting the cell clone that has an acceptable Fc receptor activation as compared to the reference standard. In some embodiments, the Fc receptor activation is a measure of the biological activity of the antibody or fragment thereof. In some embodiments, the first transformed cell includes a nucleic acid that encodes for Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof. In some embodiments, the second transformed cell includes a nucleic acid that encodes for Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof. In some embodiments, the first transformed cell and the second transformed cell have the same repertoire of activating Fc receptors.

[00020] The disclosure, features, aspects, and advantages of the present invention, other than those set forth above, will be better understood upon consideration of the following detailed description thereof. The detailed description references the following drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[00021] Figure 1 is an illustration of Fc γ Receptors at a cell's surface.

[00022] Figure 2-4 are graphs showing flow cytometry to assess Fc γ receptor expression at passage 7 (Figure 2A shows Fc γ RI α -chain, Figure 2B shows Fc γ RIIIa α -chain, and Figure 2C shows Fc γ RIIa), passage 11 (Figure 3A shows Fc γ RI α -chain, Figure 3B shows Fc γ RIIIa α -chain, and Figure 3C shows Fc γ RIIa), and passage 38 (Figure 4A shows Fc γ RI α -chain, Figure 4B shows Fc γ RIIIa α -chain, and Figure 4C shows Fc γ RIIa). The graphs show a percentage of the total population of stained cells at that passage. Graphs are normalized to the maximal height (the number of events).

[00023] Figure 5 shows the mean fluorescent intensity (MFI) of cells expressing FcγRI, FcγRIIa, and FcγRIIIa α-chain at cell Passages 7, 11, and 38.

[00024] Figure 6 is a graph showing an exemplary curve plot of the 4-PL equation

$$y = \frac{a - d}{1 + \left(\frac{\text{conc}}{\text{std.c}}\right)^b} + d$$

where y = RLU signal; conc = concentration of test molecule (ng/mL); a = the maximum value of the RLU signal; b = Hill slope of the curve; std.c = EC₅₀ (ng/mL); d = the minimum value of the RLU signal.

[00025] Figure 7 depicts a Bland-Altman Plot comparing the three FcγR assay to the *ex-vivo* mouse microglial assay showing the difference in the bioassay results plotted against the average results for both assays.

DETAILED DESCRIPTION OF THE INVENTION

[00026] The present invention provides a method which is suitable as a potency assay for batch release of a pharmaceutical composition comprising an antibody or fragment thereof capable of binding to the Fc binding region of an Fc receptor, including Fcγ Receptor I, Fcγ Receptor IIa, and Fcγ Receptor IIIa. The present invention provides a method for determining the potency of a drug product comprising an antibody or fragment thereof, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the binding of the antibody or fragment thereof to a Fc receptor, wherein said method comprises determining the binding of the antibody or fragment thereof to an Fc receptor.

[00027] Fc receptors belong to a family of receptors specific for certain amino acids in the constant region of immunoglobulins. Their expression on individual cells depends on the type of receptor. Receptors for almost all immunoglobulin classes have been described. They are referred to as FcγR (for the IgG class), FcαR (for IgA class), FcμR, Fcα/μR, pIgR (for IgM class), IgDR (for IgD class), and FcεR (for IgE class). Multiple FcγRs have been identified which differ in their affinity to bind IgG and relative affinity to bind IgG isotypes (Van Sorge, et al., "FcγR Polymorphisms: Implications for

Function, Disease Susceptibility and Immunotherapy,” *Tissue Antigens* 61.3: 189-202 (2003), which is hereby incorporated by reference in its entirety).

[00028] Fc receptors for use in the present invention may be full-length Fc receptors or fragments thereof wherein the fragment retains the ability to bind an Fc region, for instance the extracellular domain. An Fc receptor for use in the present invention may also be a wildtype Fc receptor of any allotype or a mutant variant thereof, the function of which correlates with the function of an Fc receptor, to which the antibody or fragment thereof binds *in vivo*.

[00029] One aspect of the present disclosure is related to a transformed cell comprising: i) a nucleic acid encoding a human Fcγ Receptor I α-chain (FcγRI α-chain) or a variant thereof; ii) a nucleic acid encoding a human FcγRI α-chain or a variant thereof and a nucleic acid encoding a human Fcγ Receptor IIa (FcγRIIa) or a variant thereof; iii) a nucleic acid encoding a human FcγRI α-chain or a variant thereof and a nucleic acid encoding a human Fcγ Receptor IIIa α-chain (FcγRIIIa α-chain) or a variant thereof; iv) a nucleic acid encoding a human FcγRIIa or a variant thereof and a nucleic acid encoding a human FcγRIIIa α-chain or a variant thereof; v) a nucleic acid encoding a human FcγRI α-chain or a variant thereof, a nucleic acid encoding a human FcγRIIa or a variant thereof, and a nucleic acid encoding a human FcγRIIIa α-chain or a variant thereof; wherein the transformed cell is transformed/engineered to include the nucleic acids encoding FcγRI α-chain, FcγRIIa, and/or FcγRIIIa α-chain.

[00030] In one embodiment, the present disclosure is related to a transformed cell that expresses the human FcγRI α-chain or a variant thereof. In some embodiments, the FcγRI α-chain or the variant thereof is presented on the surface of the transformed cell. One embodiment of the present disclosure is related to a transformed cell that expresses the human FcγRIIa or a variant thereof. In some embodiments, the FcγRIIa or the variant thereof is presented on the surface of the transformed cell. In some embodiments, the present disclosure is related to a transformed cell that expresses the human FcγRIIIa α-chain or a variant thereof. In some embodiments, the FcγRIIIa α-chain or the variant thereof is presented on the surface of the transformed cell.

[00031] In some embodiments, the transformed cell expresses: i) the human FcγRI α-chain or a variant thereof; ii) the human FcγRI α-chain or a variant thereof and the human FcγRIIa or a variant thereof; iii) the human FcγRI α-chain or a variant thereof and

the human FcγRIIIa α-chain or a variant thereof; iv) the human FcγRIIa or a variant thereof and the human FcγRIIIa α-chain or a variant thereof; or v) the human FcγRI α-chain or a variant thereof, the human FcγRIIa or a variant thereof, and the human FcγRIIIa α-chain or a variant thereof. In some embodiments, the transformed cell is such that i) the human FcγRI α-chain or a variant thereof, ii) the human FcγRIIa or a variant thereof, or iii) the human FcγRIIIa α-chain or a variant thereof is presented on the surface of the transformed cell.

[00032] In some embodiments, the transformed cell of the present invention expresses the human FcγRI α-chain having the sequence of SEQ ID NO: 26.

[00033] In some embodiments, the transformed cell of the present invention expresses the human FcγRIIIa α-chain. In some embodiments, the transformed cell of the present invention expresses a V158 variant of the human FcγRIIIa α-chain (SEQ ID NO: 28). In some embodiments, the transformed cell of the present invention expresses a F158 variant of the human FcγRIIIa α-chain.

[00034] In some embodiments, the transformed cell of the present invention expresses the human FcγRIIa. In some embodiments, the transformed cell of the present invention expresses a H131 variant of human FcγRIIa (SEQ ID NO: 25). In some embodiments, the transformed cell of the present invention expresses a R131 variant of human FcγRIIa.

[00035] In one embodiment, the Fc receptor is an FcγRI α-chain or a variant thereof. In the context of the present disclosure, the term variant (which includes fragments thereof) is such that it retains the ability to bind to the Fc region of an antibody or fragment thereof. The term variant also includes chimeric Fc receptors where the FcγRI α-chain or a variant thereof is a part of the chimeric Fc receptor. The FcγRI α-chain is expressed, e.g., on cells of the reticuloendothelial system and mononuclear phagocytes, including monocytes, macrophages and dendritic cells and can be induced on polymorphonuclear neutrophils. Some of the variants of this receptor are described in Geraghty et al., "Effect of FC Receptor Genetic Diversity on HIV-1 Disease Pathogenesis," *Frontiers in Immunology* 10: 970 (2019); Hargreaves et al., "Fcγ Receptors: Genetic Variation, Function, and Disease," *Immunological Reviews* 268.1: 6-24 (2015); Bruhns, Pierre, et al., "Specificity and Affinity of Human Fcγ Receptors and their Polymorphic Variants for Human IgG Subclasses," *Blood, The Journal of the American Society of Hematology*

113.16: 3716-3725 (2009), each of which are hereby incorporated by reference in its entirety.

[00036] In one embodiment, the Fc receptor is an FcγRIIa or a variant thereof. The term variant (which includes fragments thereof) is such that it retains the ability to bind to the Fc region of an antibody or fragment thereof. The term variant also includes chimeric Fc receptors where the FcγRIIa or a variant thereof is a part of the chimeric Fc receptor. The FcγRIIa is one of the several known isoforms of the FcγRII receptor. FcγRIIa is the most widely distributed isotypes and is, *e.g.*, expressed on virtually all myeloid cells, including platelets. Some of the variants of this receptor are described in Geraghty et al., “Effect of FC Receptor Genetic Diversity on HIV-1 Disease Pathogenesis,” *Frontiers in Immunology* 10: 970 (2019); Hargreaves et al., “Fcγ Receptors: Genetic Variation, Function, and Disease,” *Immunological Reviews* 268.1: 6-24 (2015); Bruhns, Pierre, et al., “Specificity and Affinity of Human Fcγ Receptors and their Polymorphic Variants for Human IgG Subclasses,” *Blood, The Journal of the American Society of Hematology* 113.16: 3716-3725 (2009), each of which are hereby incorporated by reference in its entirety.

[00037] In one embodiment, the Fc receptor is an FcγRIIb receptor or a variant thereof. The term variant (which includes fragments thereof) is such that it retains the ability to bind to the Fc region of an antibody or fragment thereof. The term variant also includes chimeric Fc receptors where the FcγRIIb receptor or a variant thereof is a part of the chimeric Fc receptor. Phagocytes and B cells are some examples where the FcγRIIb receptor is expressed. Some of the variants of this receptor are described in Geraghty et al., “Effect of FC Receptor Genetic Diversity on HIV-1 Disease Pathogenesis,” *Frontiers in Immunology* 10: 970 (2019); Hargreaves et al., “Fcγ Receptors: Genetic Variation, Function, and Disease,” *Immunological Reviews* 268.1: 6-24 (2015); Bruhns, Pierre, et al., “Specificity and Affinity of Human Fcγ Receptors and their Polymorphic Variants for Human IgG Subclasses,” *Blood, The Journal of the American Society of Hematology* 113.16: 3716-3725 (2009), each of which are hereby incorporated by reference in its entirety.

[00038] In one embodiment, the Fc receptor is an FcγRIIc receptor or a variant thereof. The term variant (which includes fragments thereof) is such that it retains the ability to bind to the Fc region of an antibody or fragment thereof. The term variant

also includes chimeric Fc receptors where the Fc γ RIIc receptor or a variant thereof is a part of the chimeric Fc receptor. The Fc γ RIIc receptor is, e.g., expressed on NK cells. Some of the variants of this receptor are described in Geraghty et al., “Effect of FC Receptor Genetic Diversity on HIV-1 Disease Pathogenesis,” *Frontiers in Immunology* 10: 970 (2019); Hargreaves et al., “Fc γ Receptors: Genetic Variation, Function, and Disease,” *Immunological Reviews* 268.1: 6-24 (2015); Bruhns, Pierre, et al., “Specificity and Affinity of Human Fc γ Receptors and their Polymorphic Variants for Human IgG Subclasses,” *Blood, The Journal of the American Society of Hematology* 113.16: 3716-3725 (2009), each of which are hereby incorporated by reference in its entirety.

[00039] In one embodiment, the Fc receptor is an Fc γ RIIIa α -chain or a variant thereof. The term variant (which includes fragments thereof) is such that it retains the ability to bind to the Fc region of an antibody or fragment thereof. The term variant also includes chimeric Fc receptors where the Fc γ RIIIa α -chain or a variant thereof is a part of the chimeric Fc receptor. The Fc γ RIIIa α -chain is one of the two known isoforms of the Fc γ RIII receptor. Fc γ RIIIa α -chain is present, e.g., on monocytes, macrophages, NK cells and γ/δ T cells. Some of the variants of this receptor are described in Geraghty et al., “Effect of FC Receptor Genetic Diversity on HIV-1 Disease Pathogenesis,” *Frontiers in Immunology* 10: 970 (2019); Hargreaves et al., “Fc γ Receptors: Genetic Variation, Function, and Disease,” *Immunological Reviews* 268.1: 6-24 (2015); Bruhns, Pierre, et al., “Specificity and Affinity of Human Fc γ Receptors and their Polymorphic Variants for Human IgG Subclasses,” *Blood, The Journal of the American Society of Hematology* 113.16: 3716-3725 (2009), each of which are hereby incorporated by reference in its entirety.

[00040] In some embodiments, the transformed cell also includes a coreceptor nucleic acid that encodes a coreceptor comprising an immunoreceptor tyrosine-based activation motif (ITAM), or a variant thereof. In some embodiments, the coreceptor nucleic acid encodes for a coreceptor comprising the ITAM or a variant thereof selected from CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD79a, CD79b, DAP12 (TYROBP), FcR γ , Fc ϵ Receptor common γ chain (FCER1G), Dectin-1, FcRL1 (CD307a), CLEC-2, and combinations thereof. The term “variant thereof” (which includes fragments thereof) here means that the variant coreceptor retains at least a fraction of its biological activity. In one embodiment,

the coreceptor nucleic acid encodes for Fcε Receptor common γ chain (FCER1G). In some embodiments, the coreceptor FCER1G has the amino acid sequence of SEQ ID NO: 27.

[00041] In some embodiments, the coreceptor is a chimeric coreceptor. The term “chimeric coreceptor” here means that it is a combination of two or more coreceptors or that the chimeric coreceptor comprises a suitable amino acid sequence (or combination of sequences) which confers at least a portion of a first coreceptor’s biological activity to the chimeric coreceptor and at least one amino acid sequence that imparts a detectable biological function and/or characteristic to the chimeric coreceptor that cannot solely be attributed to the first coreceptor. Functional sequences of such chimeric coreceptor may be separated by flexible linker(s). Secondary sequence(s) may also be derived from cytotoxic or apoptotic peptides. Secondary sequences may also confer diagnostic properties.

[00042] In some embodiments, the chimeric coreceptor includes: i) CD3γ intracellular domain, CD3δ intracellular domain, CD3ε intracellular domain, CD3ζ intracellular domain, CLEC-2 intracellular domain, CD79a intracellular domain, CD79b intracellular domain, DAP12 (TYROBP) intracellular domain, Fcε Receptor common γ chain (FCER1G) intracellular domain, or Dectin-1 intracellular domain; and ii) FcγRI extracellular domain, FcγRIIa extracellular domain, FcγRIIb extracellular domain, FcγRIIc extracellular domain, FcγRIIIa extracellular domain, FcγRIIIb extracellular domain, DC-SIGN extracellular domain, TRIM21 extracellular domain, DC-SIGN extracellular domain, FcαRI extracellular domain, or FcεRI extracellular domain.

[00043] In some embodiments, the copy number of the coreceptor within the transformed cell is such that it allows for the activation of Fcγ receptors, including the FcγRI α-chain and the FcγRIIIa receptor. In some embodiments, the transformed cell includes the FcγRI α-chain and a ratio of coreceptor copy number to a FcγRI α-chain copy number is 1:1 or more.

[00044] In some embodiments the FcγRI α-chain associates with an endogenously expressed coreceptor for activation. In some embodiments, the ratio of coreceptor copy number to a FcγRI α-chain copy number is 1:1 or less. In some embodiments, the ratio is about 1:1, 2:1, 3:1, 4:1, 5:1 or 6:1. In some embodiments, the ratio is about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1.

[00045] In some embodiments, the transformed cell comprises the FcγRIIIa α-chain and a ratio of coreceptor copy number to a FcγRIIIa α-chain copy number is 1:1 or

more. In some embodiments, the FcγRIIIa α-chain associates with an endogenously expressed coreceptor. In some embodiments, the ratio of coreceptor copy number to a FcγRIIIa α-chain copy number is 1:1 or less. In some embodiments, the ratio is about 1:1, 2:1, 3:1, 4:1, 5:1 or 6:1. In some embodiments, the ratio is about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1.

[00046] In some embodiments, the transformed cell lacks endogenous human FcγRI α-chain, human FcγRIIa, and human FcγRIIIa α-chain.

[00047] In some embodiments, the transformed cell includes a nucleic acid encoding a reporter. The reporter gene allows to characterize activation of one or more Fcγ receptors or binding of the antibody or fragment thereof to one or more Fcγ receptors. In some embodiments, the reporter is selected from i) reporters derived from Firefly, Renilla, Guassia, or Cypridina or ii) beta-galactosidase, beta-lactamase, red fluorescent protein, green fluorescent protein (GFP), or derivatives thereof. In one embodiment, the reporter is a luminescence reporter. In one embodiment, the luminescence reporter is luciferase.

[00048] In some embodiments, the transformed cell includes a transcription response element operably linked to the nucleic acid encoding the reporter. In some embodiments, the transcription response element is selected from the group consisting of Nuclear Factor of Activated T-cells (NFAT), NF-kB, AP-1, and IL-2 promoter. In one embodiment, the nucleic acid encoding the reporter further encodes NFAT.

[00049] In some embodiments, the transformed cell includes a nucleic acid encoding a selection marker, wherein the selection marker provides the cell with antibiotic resistance. A selection marker is a gene introduced into a cell to confer a trait suitable for artificial selection. Usually, the artificial selection is done using a chemical, such as, an antibiotic.

[00050] In some embodiments, the nucleic acid encoding the selection marker encodes for antibiotic resistance. In some embodiments, the selection marker encodes for resistance to zeocin, puromycin, neomycin, hygromycin, or blasticidin. In some embodiments, puromycin, zeocin, hygromycin B, and blasticidin are added to the cell culture media used to grow transformed cell. In some embodiments, the concentration of the puromycin added to the cell culture media used to grow transformed cell is from about 20 ng/mL to about 2000 ng/mL. In some embodiments, the concentration of the puromycin added to the cell culture media used to grow transformed cell is about 200 ng/mL. In some embodiments, the concentration of the zeocin added to the cell culture media used to grow

transformed cell is from about 10 µg/mL to about 5000 µg/mL. In some embodiments, the concentration of the zeocin added to the cell culture media used to grow transformed cell is about 50 µg/mL. In some embodiments, the concentration of the hygromycin B added to the cell culture media used to grow transformed cell is from about 30 µg/mL to about 3000 µg/mL. In some embodiments, the concentration of the hygromycin B added to the cell culture media used to grow transformed cell is about 300 µg/mL. In some embodiments, the concentration of the blasticidin added to the cell culture media used to grow transformed cell is from about 0.1 µg/mL to about 20 µg/mL. In some embodiments, the concentration of the blasticidin added to the cell culture media used to grow transformed cell is about 2 µg/mL.

[00051] In some embodiments, the nucleic acid encoding the human FcγRI α-chain or a variant thereof further comprises a nucleic acid encoding for a selection marker. In some embodiments, the nucleic acid encoding the human FcγRIIa or a variant thereof further comprises a nucleic acid encoding for a selection marker. In some embodiments, the nucleic acid encoding the human FcγRIIIa α-chain or a variant thereof further comprises a nucleic acid encoding for a selection marker. In some embodiments, the coreceptor nucleic acid further comprises a nucleic acid encoding for a selection marker. In some embodiments, the nucleic acid encoding the selection marker encodes for antibiotic resistance.

[00052] In some embodiments, the transformed cell includes a nucleic acid encoding human FcγRI α-chain or a variant thereof, a Fcε Receptor common gamma chain (FCER1G) or a variant thereof, and a selection marker. In some embodiments, the transformed cell comprises a nucleic acid encoding a human FcγRIIIa α-chain or a variant thereof, a FCER1G or a variant thereof, and a selection marker. In some embodiments, the transformed cell comprises a nucleic acid encoding a human FcγRIIa or a variant thereof and a selection marker. In some embodiments, the transformed cell comprises a nucleic acid encoding NFAT response element operably linked to luciferase and a selection marker.

[00053] In one embodiment, the transformed cell described herein includes i) a nucleic acid encoding human FcγRI α-chain or a variant thereof, a Fcε Receptor Ig (FCER1G), and a selection marker, ii) a nucleic acid encoding a human FcγRIIIa α-chain or a variant thereof, a Fcε Receptor Ig (FCER1G), and a selection marker, iii) a nucleic

acid encoding a human FcγRIIa or a variant thereof and a selection marker, and iv) a nucleic acid encoding NFAT response element operably linked to luciferase and a selection marker.

[00054] In some embodiments, the transformed cell is an animal cell. In some embodiments, the transformed cell is a mammalian cell. In some embodiments, the transformed cell is a Jurkat cell, Raji cell, Wil2s cells, THP-1 cell, U937 cell, COS-1 cell, COS-7 cell, HEK cell, or CHO cell. In some embodiments, the transformed cell is a human cell. In some embodiments, the human cell is a T lymphoblastoid cell. In some embodiments, the transformed cell is a transformed Jurkat cell.

[00055] One aspect of the present disclosure is related to a nucleic acid encoding a human FcγRI α-chain or a variant thereof, an ITAM coreceptor or a variant thereof, and/or a selection marker as well as to one or more vectors comprising a nucleic acid encoding for a human FcγRI α-chain or a variant thereof, an ITAM coreceptor or a variant thereof, and/or a selection marker. In some embodiments, the selection marker encodes for antibiotic resistance to hygromycin. In some embodiments, the ITAM coreceptor is a FCER1G.

[00056] Another aspect of the present disclosure is related to a nucleic acid encoding a human FcγRIIIa α-chain or a variant thereof, an ITAM coreceptor or a variant thereof, and/or a selection marker. This aspect is also related to one or more vectors comprising a nucleic acid encoding a human FcγRIIIa α-chain or a variant thereof, an ITAM coreceptor or a variant thereof, and/or a selection marker. In some embodiments, the selection marker encodes for antibiotic resistance to blasticidin. In some embodiments, the ITAM coreceptor is FCER1G.

[00057] Another aspect of the present disclosure is related to a nucleic acid encoding a human FcγRIIa or a variant thereof and/or a selection marker. This aspect is also related a vector comprising a nucleic acid encoding a human FcγRIIa and/or a selection marker. In some embodiments, the selection marker encodes for antibiotic resistance to Zeocin.

[00058] Another aspect of the present disclosure is related to a nucleic acid encoding a NFAT response element operably linked to luciferase and/or a selection marker. This aspect is also related to a vector comprising a nucleic acid encoding a NFAT response element operably linked to luciferase and/or a selection marker. In some embodiments, the selection marker encodes for antibiotic resistance to puromycin.

[00059] Another aspect of the present disclosure is related to a process of making a transformed cell comprising: i) transforming a cell such that it comprises a nucleic acid encoding a human FcγRI α-chain or a variant thereof; a nucleic acid encoding a human FcγRIIa or a variant thereof, a nucleic acid encoding a human FcγRIIIa α-chain or a variant thereof; ii) selecting a transformed cell that comprises the nucleic acids encoding FcγRI α-chain, FcγRIIa, and/or FcγRIIIa α-chain.

[00060] In some embodiments, the transformed cell further comprises a nucleic acid encoding a reporter and/or a selection marker. In some embodiments, the transformed cell further comprises a coreceptor nucleic acid encoding an immunoreceptor tyrosine-based activation motif (ITAM), or a variant thereof. In some embodiments, the coreceptor nucleic acid encodes for a coreceptor comprising the ITAM or a variant thereof selected from CD3γ, CD3δ, CD3ε, CD3ζ, CD79a, CD79b, DAP12 (TYROBP), FcRγ, Fcε Receptor common γ chain (FCER1G), Dectin-1, FcRL1, CLEC-2, and combinations thereof. The term “variant thereof” (which includes fragments thereof) here means that the variant coreceptor retains at least a fraction of its biological activity.

[00061] In one embodiment, the coreceptor nucleic acid encodes for Fcε Receptor common γ chain (FCER1G). In some embodiments, the chimeric coreceptor includes: i) CD3γ intracellular domain, CD3δ intracellular domain, CD3ε intracellular domain, CD3ζ intracellular domain, CLEC-2 intracellular domain, CD79a intracellular domain, CD79b intracellular domain, DAP12 (TYROBP) intracellular domain, Fcε Receptor common γ chain (FCER1G) intracellular domain, or Dectin-1 intracellular domain; and ii) FcγRI extracellular domain, FcγRIIa extracellular domain, FcγRIIb extracellular domain, FcγRIIc extracellular domain, FcγRIIIa extracellular domain, FcγRIIIb extracellular domain, DC-SIGN extracellular domain, TRIM21 extracellular domain, DC-SIGN extracellular domain, FcαRI extracellular domain, or FcεRI extracellular domain.

[00062] In some embodiments, the transformed cell further comprises a transcription response element operably linked to a nucleic acid encoding the reporter. In some embodiments, the transformed cell is an animal cell. In some embodiments, the transformed cell is a mammalian cell. In some embodiments, the transformed cell is a Jurkat cell, transformed Raji cell, transformed Wil2s cell, transformed THP-1 cell, transformed U937 cell, COS-1 cell, COS-7 cell, HEK cell, or CHO cell. In some

embodiments, the transformed cell is a human cell. In some embodiments, the human cell is a T lymphoblastoid cell. In some embodiments, the transformed cell is a transformed Jurkat cell.

[00063] In some embodiments, the transformed cell comprises the nucleic acid encoding the FcγRI α-chain or the variant thereof; the nucleic acid encoding the FcγRIIa or the variant thereof; and the nucleic acid encoding the FcγRIIIa α-chain or the variant thereof. In some embodiments, the transformed cell further comprises the coreceptor nucleic acid encoding coreceptor comprising the ITAM or the variant thereof.

[00064] One aspect of the present invention is related to a method for identifying an antibody or a fragment thereof as having at least one mechanism of action mediated through activation of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof, the method comprising the steps of: i) providing the antibody or a fragment thereof; ii) contacting the antibody or a fragment thereof with a transformed cell of the present disclosure, optionally, for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof; and iii) detecting a signal generated upon contacting the antibody or a fragment thereof with the transformed cell. In some embodiments, the method further comprises: iv) analyzing the signal generated in step iii) to determine whether one or more of the receptors of step ii) are activated. In some embodiments, the method further comprises: v) identifying the antibody or a fragment thereof as an activator of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof. In one embodiment, the antibody or fragment thereof comprises a Fc binding moiety.

[00065] One aspect of the present invention is related to determining the potency of a drug substance or a drug product comprising an antibody or fragment thereof wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the binding/activation of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof. In one embodiment, this assay is used to determine the potency of the antibody, optionally, by comparing the activation of the one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof by the antibody or fragment thereof with a reference standard. In some embodiments, the method includes determining the binding of the antibody to one or more Fc receptors. In one embodiment, this assay is used to determine the binding efficacy of the antibody to one

of the Fc Receptors, optionally, by comparing the binding of the Fc receptor to i) the antibody or fragment thereof with ii) a reference standard as described herein.

[00066] In some embodiments, the method includes determining the activation of one or more Fc receptors upon coming in contact with the antibody or fragment thereof. In some embodiments, the method includes quantitating the activation of one or more Fc receptors upon coming in contact with the antibody or fragment thereof.

[00067] An aspect of the present invention is related to a method for analyzing and/or selecting at least a batch of a drug product, wherein the batch comprises an antibody or a fragment thereof and at least one mechanism of action of the antibody or the fragment thereof is mediated through activation of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof, the method comprising the steps of: i) providing a drug product comprising the antibody or the fragment thereof; ii) providing a reference standard antibody or a fragment thereof; iii) contacting the antibody or the fragment thereof with a first transformed cell disclosed herein, optionally, for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof; iv) contacting the reference standard with a second transformed cell disclosed herein, optionally, for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof; v) measuring/quantifying the activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof by the antibody or fragment thereof; vi) measuring/quantifying the activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof by the reference standard; vii) comparing the activation of the Fc receptors of step v) to the activation of Fc receptors of step vi); and viii) selecting the antibody or fragment thereof that has an acceptable Fc receptor activation as compared to the reference standard. In some embodiments, the antibody or fragment thereof has equal to or greater activation of at least one Fc receptor as compared to the reference standard. In some embodiments, the first transformed cell includes a nucleic acid that encodes for FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof. In some embodiments, the second transformed cell includes a nucleic acid that encodes for FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof. In some embodiments, the

first transformed cell and the second transformed cell have the same repertoire of activating Fc receptors.

[00068] One aspect of the present disclosure is related to a method of selecting at least one cell clone for the manufacture of a drug substance (or antibody or fragment thereof), wherein the drug substance is an antibody or a fragment thereof and at least one mechanism of action of the antibody or the fragment thereof is mediated through activation of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof, the method comprising the steps of: i) providing at least one drug substance obtained from at least one transformed cell clone; ii) providing a reference standard antibody or a fragment thereof; iii) contacting the drug substance with a first transformed cell disclosed herein, optionally, for a time period sufficient to allow activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof; iv) contacting the reference standard with a second transformed cell disclosed herein, optionally, for a time period sufficient to allow activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof; v) measuring the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof by the drug substance; vi) measuring the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof by the reference standard; vii) comparing the activation of the Fc receptors of step v) to the activation of Fc receptors of step vi); and viii) selecting the cell clone that has an acceptable activation profile as compared to the reference standard. In some embodiments, the first transformed cell includes a nucleic acid that encodes for Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof. In some embodiments, the second transformed cell includes a nucleic acid that encodes for Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof. In some embodiments, the first transformed cell and the second transformed cell have the same repertoire of activating Fc receptors.

[00069] In some embodiments, activation of at least one Fc receptor by the antibody or fragment thereof matches the release specification for the antibody or fragment thereof. In some embodiments, the activation of at least one Fc receptor by the antibody or fragment thereof is acceptable if it matches the release specification for the antibody or fragment thereof.

[00070] In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof is i) from about 50% to about 150% of the reference standard, ii) from about 60% to about 140% of the reference standard, iii) from about 70% to about 130% of the reference standard, iv) from about 80% to about 130% of the reference standard, v) from about 90% to about 110% of the reference standard, or vi) about 100% of the reference standard.

[00071] In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof is acceptable if the activation is i) from about 50% to about 150% of the reference standard, ii) from about 60% to about 140% of the reference standard, iii) from about 70% to about 130% of the reference standard, iv) from about 80% to about 130% of the reference standard, v) from about 90% to about 110% of the reference standard, or vi) about 100% of the reference standard.

[00072] In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof is acceptable if the binding of the antibody or fragment thereof to the receptor is i) from about 50% to about 150% of the reference standard, ii) from about 60% to about 140% of the reference standard, iii) from about 70% to about 130% of the reference standard, iv) from about 80% to about 130% of the reference standard, v) from about 90% to about 110% of the reference standard, or vi) about 100% of the reference standard.

[00073] In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof is from about 80% to about 130% of the reference standard. In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof is from about 60% to about 140% of the reference standard.

[00074] In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof is acceptable if it is from about 80% to about 130% of the reference standard. In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof is acceptable if it is from about 60% to about 140% of the reference standard.

[00075] In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof is acceptable if the binding of the antibody or fragment thereof to the receptor is from about 80% to about 130% of the reference standard. In some embodiments, the activation of at least one Fc receptor by the antibody of fragment thereof

is acceptable if the binding of the antibody or fragment thereof to the receptor is from about 60% to about 140% of the reference standard.

[00076] In some embodiments, the activation of at least one Fc receptor by the antibody or fragment thereof is about 70%, 75%, 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, or 150% of the reference standard. In some embodiments, the activation of at least one Fc receptor by the antibody or fragment thereof acceptable if it is about 70%, 75%, 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, or 150% of the reference standard. In some embodiments, the activation of at least one Fc receptor by the antibody or fragment thereof acceptable if the binding of the antibody or fragment thereof to the receptor is about 70%, 75%, 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, 120%, 125%, 130%, 135%, 140%, 145%, or 150% of the reference standard.

[00077] In some embodiments, the antibody or fragment thereof has equal to or greater activation of at least one Fc receptor as compared to the reference standard. In some embodiments, the activation of at least one Fc receptor by the drug substance matches the release specification for the drug substance. In some embodiments, the activation of at least one Fc receptor by the drug product matches the release specification for the drug product.

[00078] In some embodiments, the reference standard and the antibody or fragment thereof are two different batches (or preparations) of the same antibody or fragment thereof. In some embodiments, the antibody or a fragment thereof and/or the reference standard is contacted with its antigen to form an antibody-antigen complex prior to contacting the antibody or a fragment thereof with the transformed cell.

[00079] In some embodiments, a first signal is generated and detected upon contacting the antibody or the fragment thereof to the first transformed cell and a second signal is generated and detected upon contacting the reference standard to the second transformed cell. In some embodiments, the first signal and/or the second signal is detected using a luminometer. In some embodiments, the signal detection is performed by measuring a reporter activity.

[00080] In some embodiments, the antibody or the fragment thereof binds to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof. In some embodiments, the antibody or the fragment thereof and/or

the reference standard binds to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof.

[00081] In some embodiments, the signal is generated due to binding of the antibody or the fragment thereof to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof. In some embodiments, the signal is generated due to binding of the antibody or the fragment thereof and/or the reference standard to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof. In some embodiments, the signal is generated due to binding of the antibody or the fragment thereof and/or the reference standard to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, a combination thereof, or a coreceptor thereof.

[00082] In some embodiments, the antibody or a fragment thereof is contacted with its antigen to form an antibody-antigen complex prior to contacting the antibody or a fragment thereof with the transformed cell. In some embodiments, the antigen is immobilized on a substrate. In some embodiments, the substrate is selected from the group consisting of a microtiter plate, a coverslip, a slide, a particle, a membrane, a film, and a carbon nanotube.

[00083] In some embodiments, the transformed cell is added at a concentration ranging from about 5,000 cells/mL to about 5,000,000 cells/mL. In some embodiments, the transformed cell is added at a concentration ranging from about 10,000 cells/mL to about 4,000,000 cells/mL. In some embodiments, the transformed cell is added at a concentration ranging from about 10,000 cells/mL to about 3,000,000 cells/mL. In some embodiments, the transformed cell is added at a concentration ranging from about 10,000 cells/mL to about 2,000,000 cells/mL. In some embodiments, the transformed cell is added at a concentration ranging from about 100,000 cells/mL to about 1,000,000 cells/mL. In some embodiments, the transformed cell is added at a concentration ranging from about 100,000 cells/mL to about 300,000 cells/mL. In some embodiments, the transformed cell is added at a concentration of about 100,000 cells/mL, about 200,000 cells/mL, about 300,000 cells/mL, about 400,000 cells/mL, about 500,000 cells/mL, about 600,000 cells/mL, about 700,000 cells/mL, about 800,000 cells/mL, about 900,000 cells/mL.

[00084] In some embodiments, a signal is generated upon activation of one more Fcγ receptors or upon binding of the antibody or fragment thereof to one or more Fcγ receptors. In some embodiments, the signal is detected using a luminometer. In some embodiments, the detection is done by measuring a reporter activity.

[00085] In some embodiments, at least one mechanism of action of the antibody or a fragment thereof is to induce positive signaling via an immunoreceptor tyrosine-based activation motif. In some embodiments, at least one mechanism of action of the antibody or a fragment thereof is mediated i) through the recruitment of Fc bearing cells, ii) by inducing an antibody response, or iii) by inducing phagocytosis. In some embodiments, the recruitment of Fc bearing cells comprises recruitment of platelet, dendritic cell, microglial cell, Kupfer cell, alveolar macrophage, synovial macrophage, Langerhans cells, osteoclasts, tissue-resident macrophage, monocyte, polymorphonuclear cell (PMN), B cell, or natural killer cell. In some embodiments, the reference standard has the same mechanism of action as the antibody or fragment thereof.

[00086] In some embodiments, at least one mechanism of action of the antibody or fragment thereof is mediated through microglial cell. In some embodiments, at least one mechanism of action of the antibody or fragment thereof is to induce clearance of immune complexes. In some embodiments, at least one mechanism of action of the antibody or fragment thereof is mediated through i) Fc mediated cytokine production, ii) antibody dependent cell-mediated cytotoxicity (ADCC), or iii) antibody dependent cell phagocytosis (ADCP). In some embodiments, the reference standard has the same mechanism of action as the antibody or fragment thereof.

[00087] In some embodiments at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of ADCC via polymorphonuclear leukocytes, NK cells, monocytes, or macrophages. In some embodiments at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of ADCP via polymorphonuclear leukocytes, monocytes, or macrophages. In some embodiments, at least one mechanism of action of the antibody or fragment thereof is mediated through Fc mediated cytokine production via polymorphonuclear leukocytes, monocytes, NK cells, macrophages, dendritic cells, B-cells. In some embodiments, the reference standard has the same mechanism of action as the antibody or fragment thereof.

[00088] In some embodiments, at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of PMN degranulation. In some embodiments, the antibody or fragment thereof activates FcγRI α-chain or a variant thereof. In some embodiments, the antibody or fragment thereof activates FcγRIIa or a variant thereof. In some embodiments, the antibody or fragment thereof activates FcγRIIIa α-chain or a variant thereof. In some embodiments, the reference standard has the same mechanism of action as the antibody or fragment thereof.

[00089] In some embodiments, least one mechanism of action of the antibody or fragment thereof is mediated through cross-linking of cells and/or antibodies, immobilized antigens. In some embodiments, a mechanism of action of the antibody or fragment thereof is to induce positive signaling via common γ chain, common β chain, common ζ chain, or a co-receptor comprising an ITAM motif. In some embodiments, the reference standard has the same mechanism of action as the antibody or fragment thereof.

[00090] In some embodiments, a mechanism of action of the antibody or fragment thereof is to induce positive signaling via a chimeric Fc receptor. In some embodiments, at least one mechanism of action of the antibody or fragment thereof is mediated through the recruitment of myeloid cells, Dendritic cells, B-cells, polymorphonuclear leukocytes or natural killer cells. In some embodiments, the reference standard has the same mechanism of action as the antibody or fragment thereof.

[00091] In some embodiments, at least one mechanism of action of the antibody or fragment thereof is to induce negative signaling through an immunoreceptor tyrosine-based inhibition motif. In some embodiments, at least one mechanism of action of the antibody or fragment thereof is mediated through the recruitment of B cells, macrophages, basophil, neutrophils, Dendritic cells, monocytes, and/or monocytes. In some embodiments, the reference standard has the same mechanism of action as the antibody or fragment thereof.

[00092] A drug product is a composition comprising the therapeutically interesting drug, which composition is to be administered to patients in need of treatment with the drug. In one embodiment, the drug product is a drug product comprising an antibody, such as a recombinantly produced antibody drug. In the case of a recombinant antibody drug, the antibody drug product is produced by first producing the antibody in a host cell either generated by cell fusion or recombinant DNA techniques, followed by

harvesting, purification and formulation of the antibody resulting in the drug product. The selection of the cell type for antibody production, co-transfection of modifying enzymes such as carbohydrate transferases and differences in culture and/or process conditions may affect the potency of the resulting antibody as it is well known in the art. Methods for harvesting, purification and formulation of recombinant antibodies are known in the art and may include one or more steps of for instance clarification, concentration, filtration, and chromatography (such as for instance size exclusion and ion-exchange chromatography). The drug product may, in addition to the drug, contain any number of components, which may be added for instance during the process of purification, which components should be acceptable for pharmaceutical use, such as carriers, diluents, adjuvants and excipients. Examples of pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients are Well known in the art and may be such as those disclosed in Remington: The Science and Practice of Pharmacy, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, Pa., 1995.

[00093] In some embodiments, the antibody or fragment thereof and/or the reference standard is a monoclonal antibody, a human antibody, a humanized antibody, or a chimeric antibody. In some embodiments, the antibody or fragment thereof and/or the reference standard is an IgG1 antibody. In some embodiments, the antibody or fragment thereof and/or the reference standard binds to N3pE A β (also referred to as N3pGlu A β), amyloid beta (A β) or A β plaques.

[00094] The term antibody in the context of the present invention refers to an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative of either thereof, which has the ability to specifically bind to an antigen under typical physiological conditions for significant periods of time such as with a half-life of at least about 30 minutes, at least about 45 minutes, at least about one hour, at least about two hours, at least about four hours, at least about 8 hours, at least about 12 hours, about 24 hours or more, about 48 hours or more, about 3, 4, 5, 6, 7 or more days, etc., or any other relevant functionally-defined period (such as a time sufficient to induce, promote, enhance, and/or modulate a physiological response associated with antibody binding to the antigen) and the ability to bind Fc receptors.

[00095] An antibody for use in the present invention may be a bispecific antibody or similar molecule. Indeed, bispecific antibodies and the like may bind any

suitable target in addition to a portion of the original antigen as long as they retain a part capable of binding to an Fc receptor.

[00096] As indicated above, the term antibody herein, unless otherwise stated or clearly contradicted by context, includes fragments, derivatives, variants (including deletion variants) of an antibody that retain the ability to specifically bind to an antigen and to an Fc receptor. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see for instance Bird et al., Science 242, 423-426 (1988) and Huston et al., PNAS USA 85, 5879-5883 (1988), which are hereby incorporated by reference in their entirety). Such single chain antibodies are encompassed within the term antibody unless otherwise noted or clearly indicated by context. Other forms of single chain antibodies, such as molecules described in WO2005037989 (which is hereby incorporated by reference in its entirety), are included within the term antibody.

[00097] The term antibody includes polyclonal antibodies, monoclonal antibodies, such as chimeric antibodies, humanized antibodies, and human antibodies as well as antibody-like polypeptides. An antibody can be of a specific isotype referring to the immunoglobulin class that is encoded by heavy chain constant region genes, for instance IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM. Each isotype has a unique amino acid sequence and possesses a unique set of isotype epitopes distinguishing them from each other.

[00098] In some embodiments, the antibody or fragment thereof is an anti-N3pGlu A β antibody (also referred to as N3pE A β). These anti-N3pE A β antibodies bind human N3pE A β . In an embodiment, the anti-N3pE A β antibodies of the present invention bind an epitope of human N3pE A β (that is only present in amyloid plaques in the brain). Antibodies to N3pE A β are known in the art. For example, U.S. Patent No. 8,679,498; U.S. Patent No. 8,961,972; US Patent No. 10,647,759; US Patent No. 11,312,763, and US Patent No. 11,078,261 (which are hereby incorporated by reference in their entirety) disclose anti-N3pE A β antibodies, method of making the antibodies, antibody formulations, and methods of treating diseases, such as, Alzheimer's disease with such antibodies. In some embodiments, the antibody and/or the reference standard is donanemab. In some

embodiments, the antibody and/or the reference standard is remternetug. Donanemab (disclosed in U.S. Patent No. 8,679,498) is an antibody directed at the pyroglutamate modification of the third amino acid of amyloid beta (N3pE A β) epitope that is present only in brain amyloid deposits. Remternetug (disclosed in U.S. Patent No. US 11,312,763) is another antibody directed at the pyroglutamate modification of the third amino acid of amyloid beta (N3pE A β).

[00099] In some embodiments of the present disclosure, the antibody comprises a heavy chain (HC) and a light chain (LC), wherein the HC comprises a heavy chain variable region (HCVR) and the LC comprises a light chain variable region (LCVR), said HCVR comprising complementarity determining regions (CDRs) HCDR1, HCDR2 and HCDR3 and said LCVR comprising CDRs LCDR1, LCDR2 and LCDR3. In some embodiments, the antibodies of the present invention have the amino acid sequence of LCDR1 given by SEQ ID NO:5, the amino acid sequence of LCDR2 given by SEQ ID NO:6, the amino acid sequence of LCDR3 given by SEQ ID NO:7, the amino acid sequence of HCDR1 given by SEQ ID NO:8, the amino acid sequence of HCDR2 given by SEQ ID NO:9, and the amino acid sequence of HCDR3 given by SEQ ID NO:10.

[000100] In an embodiment, the present invention provides anti-N3pE A β antibody comprising a LCVR and a HCVR, wherein the amino acid sequence of the LCVR is given by SEQ ID NO:1 and the amino acid sequence of the HCVR is given by SEQ ID NO:2. In a further embodiment, the present invention provides anti-N3pE A β antibody comprising a light chain (LC) and a heavy chain (HC), wherein the amino acid sequence of the LC is given by SEQ ID NO:3 and the amino acid sequence of the HC is given by SEQ ID NO:4.

[000101] In some embodiments, the antibodies of the present invention have the amino acid sequence of LCDR1 given by SEQ ID NO:15, the amino acid sequence of LCDR2 given by SEQ ID NO:16, the amino acid sequence of LCDR3 given by SEQ ID NO:17, the amino acid sequence of HCDR1 given by SEQ ID NO:18, the amino acid sequence of HCDR2 given by SEQ ID NO:19, and the amino acid sequence of HCDR3 given by SEQ ID NO:20. In an embodiment, the present invention provides anti-N3pE A β antibody comprising a LCVR and a HCVR, wherein the amino acid sequence of the LCVR is given by SEQ ID NO:11 and the amino acid sequence of the HCVR is given by SEQ ID NO:12. In a further embodiment, the present invention provides an anti-N3pE A β antibody

comprising a light chain (LC) and a heavy chain (HC), wherein the amino acid sequence of the LC is given by SEQ ID NO:13 and the amino acid sequence of the HC is given by SEQ ID NO:14.

[000102] The antibodies of the present invention may be prepared and purified using known methods. For example, cDNA sequences encoding a HC of an anti-N3pE A β antibody and cDNA sequences encoding a LC of the anti-N3pE A β antibody may be cloned and engineered into a GS (glutamine synthetase) expression vector. The engineered immunoglobulin expression vector may then be stably transfected into CHO cells. As one of skill in the art will appreciate, mammalian expression of antibodies will result in glycosylation, typically at highly conserved N-glycosylation sites in the Fc region. Stable clones may be verified for expression of an antibody specifically binding to amyloid deposits or N3pE A β . Positive clones may be expanded into serum-free culture medium for antibody production in bioreactors. Media, into which an antibody has been secreted, may be purified by conventional techniques. For example, the medium may be conveniently applied to a Protein A or G Sepharose FF column that has been equilibrated with a compatible buffer, such as phosphate buffered saline. The column may be washed to remove nonspecific binding components. The bound antibody may be eluted, for example, by pH gradient, and antibody fractions may be detected using techniques such as by SDS-PAGE, and subsequently pooled. The antibody may be concentrated and/or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The product may be immediately frozen, for example at -70°C, or may be lyophilized.

[000103] As used herein, an “antibody” is an immunoglobulin molecule comprising two Heavy Chains (HC) and two Light Chains (LC) interconnected by disulfide bonds. The amino terminal portion of each LC and HC includes a variable region responsible for antigen recognition via the complementarity determining regions (CDRs) contained therein. The CDRs are interspersed with regions that are more conserved, termed framework regions. Assignment of amino acids to CDR domains within the LCVR and HCVR regions of the antibodies of the present invention is based on the following: Kabat numbering convention (Kabat, et al., Ann. NY Acad. Sci. 190:382-93 (1971); Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health

and Human Services, NIH Publication No. 91-3242 (1991)), and North numbering convention (North et al., A New Clustering of Antibody CDR Loop Conformations, *Journal of Molecular Biology*, 406:228-256 (2011)). Following the above methods, the CDRs of the antibodies of the present invention were determined.

[000104] In some embodiments, the anti-N3pE A β antibodies of the present invention include kappa LC and IgG HC. In a particular embodiment, the anti-N3pE A β antibodies of the present invention are of the human IgG1 isotype.

[000105] In some embodiments, the antibodies of the present invention are monoclonal antibodies ("mAbs"). Monoclonal antibodies can be produced, for example, by hybridoma technologies, recombinant technologies, phage display technologies, synthetic technologies, e.g., CDR-grafting, or combinations of such or other technologies known in the art. The monoclonal antibodies of the present invention are human or humanized. Humanized antibodies can be engineered to contain one or more human framework regions (or substantially human framework regions) surrounding CDRs derived from a non-human antibody. Human framework germline sequences can be obtained from ImMunoGeneTics (IMGT[®]) via their website: imgt.org, or from The Immunoglobulin FactsBook by Marie-Paule Lefranc and Gerard Lefranc, Academic 25 Press, 2001, ISBN 012441351. In another embodiment of the present invention, the antibody, or the nucleic acid encoding the same, is provided in isolated form. As used herein, the term "isolated" refers to a protein, peptide or nucleic acid that is not found in nature and is free or substantially free from other macromolecular species found in a cellular environment.

[000106] In particular embodiments of the present invention, the antibodies and fragments thereof (e.g., anti-N3pE A β antibodies), or the nucleic acids encoding same, may be provided in isolated form. As used herein, the term "isolated" refers to a protein, peptide, or nucleic acid that is not found in nature and which is free or substantially free from other macromolecular species found in a cellular environment. "Substantially free" as used herein, means the protein, peptide or nucleic acid of interest that comprises more than 80% (on a molar basis) of the macromolecular species present, preferably more than 90%, and more preferably more than 95%.

[000107] In some embodiments, the antibody or fragments thereof of the present invention is expressed in cell cultures. Following expression and/or secretion of the antibodies and antibody fragments of the present invention, the medium is clarified to

remove cells and the clarified media is purified using any of many commonly used techniques. Purified antibodies and antibody fragments may be formulated into pharmaceutical compositions according to well-known methods for formulating proteins and antibodies for parenteral administration, particularly for subcutaneous, intrathecal, or intravenous administration. The antibodies and antibody fragments may be lyophilized, together with appropriate pharmaceutically acceptable excipients, and later reconstituted with a water-based diluent prior to use. Alternatively, the antibodies and antibody fragments may be formulated in an aqueous solution and stored prior to use. In either case, the stored form, and the injected form of the pharmaceutical compositions of the antibodies and antibody fragments will contain a pharmaceutically acceptable excipient or excipients, which are ingredients other than the antibodies and antibody fragments. Whether an ingredient is pharmaceutically acceptable depends on its effect on the safety and effectiveness or on the safety, purity, and potency of the pharmaceutical composition. If an ingredient is judged to have a sufficiently unfavorable effect on safety or effectiveness (or on safety, purity, or potency) to warrant it not being used in a composition for administration to humans, then it is not pharmaceutically acceptable to be used in a pharmaceutical composition of the antibody and antibody fragments.

[000108] In some embodiments, some of the methods or assay described herein are part of an application for marketing authorization for selling a drug product (including, e.g., a pharmaceutical or pharmaceutical composition). In some embodiments, some of the methods or assays described herein are used as a potency assay for batch release.

[000109] In some embodiments, the present disclosure is related to a drug product comprising the antibody or fragment thereof (as disclosed herein) wherein the drug product is approved for use by a regulatory agency as a pharmaceutical. In some embodiments, the present disclosure is related to a drug product comprising the antibody or fragment thereof (as disclosed herein) wherein the drug product is part of an application for marketing authorization or approved for marketing by at least one regulatory agency.

[000110] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure belongs. Although any methods and materials similar to or

equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and materials are described below.

[000111] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[000112] All headings and sub-headings are used herein for convenience only and should not be construed as limiting the present invention in any way.

[000113] Any combination of the above-described elements in all possible variations thereof is encompassed by the present invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[000114] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (e.g., all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by “about,” where appropriate).

[000115] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context.

[000116] Reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one element is present, unless the context clearly requires that there be one and only one element. The indefinite article “a” or “an” thus usually includes “at least one.” The term “about” means up to $\pm 10\%$.

[000117] As used herein, “nucleic acid”, “polynucleotide”, “nucleic acid molecule” and the like may be used interchangeably and refer to a series of nucleotide bases (also called “nucleotides”) in deoxyribonucleotides (DNA) and ribonucleotides (RNA). The nucleic acid may contain DNA, RNA, and/or their analogs. The term encompasses sequences that include any of the known base analogues of DNA and RNA including 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxymethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-

carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid, oxybutosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, -uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine. The term "nucleic acid" includes, for example, single-stranded and double-stranded molecules. A nucleic acid can be, for example, a gene or gene fragment, exons, introns, a DNA molecule (e.g., cDNA), an RNA molecule (e.g., mRNA), recombinant nucleic acids, plasmids, and other vectors, primers, and probes.

[000118] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression providing peptides, proteins, or antibody portions in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art. See, e.g., Sambrook et al. *MOLECULAR CLONING: LAB. MANUAL* (3rd ed., Cold Spring Harbor Lab. Press, Cold Spring Harbor, N.Y., 2001) and Ausubel et al. *Current Protocols in Molecular Biology* (New York: Greene Publishing Association/Wiley Interscience), 1993.

[000119] The term "recombinant" is used according to its ordinary meaning to mean a molecule that has been created or modified through deliberate human intervention such as by genetic engineering. For example, a recombinant nucleic acid molecule is one having a nucleotide sequence that has been modified to include an artificial nucleotide sequence or to include some other nucleotide sequence that is not present within its native (non-recombinant) form. Further, a recombinant nucleic acid molecule has a

structure that is not identical to that of any naturally occurring nucleic acid molecule or to that of any fragment of a naturally occurring genomic nucleic acid molecule spanning more than one gene. A recombinant nucleic acid molecule also includes, without limitation, a nucleic acid molecule having a sequence of a naturally occurring genomic or extrachromosomal nucleic acid molecule, but which is not flanked by the coding sequences that flank the sequence in its natural position; a nucleic acid molecule incorporated into a construct, expression cassette or vector, or into a host cell's genome such that the resulting polynucleotide is not identical to any naturally occurring vector or genomic DNA; a separate nucleic acid molecule such as a cDNA, a genomic fragment, a fragment produced by amplification methods such as polymerase chain reaction (PCR) and a restriction fragment; and a recombinant nucleic acid molecule having a nucleotide sequence that is part of a hybrid gene (i.e., a gene encoding a fusion protein). As such, a recombinant nucleic acid molecule can be modified (chemically or enzymatically) or unmodified DNA or RNA, whether fully or partially single-stranded or double-stranded or even triple-stranded.

[000120] Methods for synthesizing nucleic acid molecules are well known in the art, such as cloning and digestion of the appropriate sequences, as well as direct chemical synthesis (e.g., ink-jet deposition and electrochemical synthesis). Methods of cloning nucleic acid molecules are described, for example, in Sambrook et al. *MOLECULAR CLONING: LAB. MANUAL* (3rd ed., Cold Spring Harbor Lab. Press, Cold Spring Harbor, N.Y., 2001), Ausubel et al. *Current Protocols in Molecular Biology* (New York: Greene Publishing Association/Wiley Interscience), 1993, and Copeland et al. (2001) *Nat. Rev. Genet.* 2:769-779; PCR Cloning Protocols, 2nd ed. (Chen & Janes eds., Humana Press 2002). Methods of direct chemical synthesis of nucleic acid molecules include the phosphotriester methods of Reese (1978) *Tetrahedron* 34:3143-3179 and Narang et al. (1979) *Methods Enzymol.* 68:90-98; the phosphodiester method of Brown et al. (1979) *Methods Enzymol.* 68:109-151; the diethylphosphoramidate method of Beaucage et al. (1981) *Tetrahedron Lett.* 22:1859-1862; and the solid support methods of Fodor et al. (1991) *Science* 251:767-773; Pease et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:5022-5026; and Singh-Gasson et al. (1999) *Nature Biotechnol.* 17:974-978; as well as US Patent No. 4,485,066. See also, Peattie (1979) *Proc. Natl. Acad. Sci. USA* 76:1760-1764; as well as EP Patent No. 1 721 908; Int'l Patent Application Publication Nos. WO

2004/022770 and WO 2005/082923; US Patent Application Publication No. 2009/0062521; and US Patent Nos. 6,521,427; 6,818,395 and 7,521,178.

[000121] For nucleotide sequences, “variant” refers to a substantially similar nucleotide sequence to a nucleotide sequence of a recombinant nucleic acid molecule as described herein. For nucleotide sequences, a variant comprises a nucleotide sequence having deletions (i.e., truncations) at the 5' and/or 3' end, deletions and/or additions of one or more nucleotides at one or more internal sites compared to the nucleotide sequence of the recombinant nucleic acid molecules as described herein; and/or substitution of one or more nucleotides at one or more sites compared to the nucleotide sequence of the recombinant nucleic acid molecules described herein. One of skill in the art understands that variants are constructed in a manner to maintain the open reading frame.

[000122] Conservative variants include those nucleotide sequences that, because of the degeneracy of the genetic code, result in a functionally active modified protein as described herein. Naturally occurring allelic variants can be identified by using well-known molecular biology techniques such as, for example, polymerase chain reaction (PCR) and hybridization techniques. Variant nucleotide sequences also can include synthetically derived sequences, such as those generated, for example, by site-directed mutagenesis but which still provide a functionally active modified protein. Generally, variants of a nucleotide sequence of the recombinant nucleic acid molecules as described herein will have at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the nucleotide sequence of the recombinant nucleic acid molecules as determined by sequence alignment programs and parameters as described elsewhere herein.

[000123] Variants of the recombinant nucleic acid molecules described herein also can be evaluated by comparing the percent sequence identity between the polypeptide encoded by a variant and the polypeptide encoded by a reference nucleic acid molecule. Thus, for example, an isolated nucleic acid molecule can be one that encodes a polypeptide with a given percent sequence identity to the polypeptide of interest. Percent sequence identity between any two polypeptides can be calculated using sequence alignment programs and parameters described elsewhere herein. Where any given pair of polynucleotides of the present disclosure is evaluated by comparison of the percent sequence identity shared by the two polypeptides they encode, the percent sequence identity

between the two encoded polypeptides can be at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity.

[000124] Determining percent sequence identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms include, but are not limited to, the algorithm of Myers & Miller (1988) CABIOS 4:11-17; the local alignment algorithm of Smith et al. (1981) Adv. Appl. Math. 2:482-489; the global alignment algorithm of Needleman & Wunsch (1970) J. Mol. Biol. 48:443-453; the search-for-local alignment method of Pearson & Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448; the algorithm of Karlin & Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877.

[000125] Compositions of the present disclosure also include nucleic acid constructs, such as expression cassettes or vectors, having promoters operably linked with a nucleic acid molecule that encodes therapeutic proteins for use in the methods described herein. The nucleic acid constructs can be packaged into fecal exosomes and/or nanoparticles and administered to a subject in need thereof as described herein.

[000126] As used herein, “nucleic acid construct” refers to an oligonucleotide or polynucleotide composed of deoxyribonucleotides, ribonucleotides or combinations thereof having incorporated therein the nucleotide sequences described herein.

[000127] As used herein, “expression cassette” refers to a nucleic acid molecule having at least a control sequence operably linked to a coding sequence.

[000128] As used herein, “operably linked” means that the elements of the expression cassette are configured so as to perform their usual function. Thus, control sequences (i.e., promoters) operably linked to a coding sequence are capable of effecting expression of the coding sequence. The control sequences need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated, yet transcribed, sequences can be present between a promoter and a coding sequence, and the promoter sequence still can be considered “operably linked” to the coding sequence.

[000129] As used herein, a “coding sequence” or “coding sequences” refers to a sequence that encodes a particular polypeptide, and is a nucleotide sequence that is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide

in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at a 5' (amino) terminus and a translation stop codon at a 3' (carboxy) terminus. A coding sequence can include viral nucleic acid sequences, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and even synthetic DNA sequences and synthetic RNA sequences (particularly synthetic microRNA sequences). A transcription termination sequence will usually be located 3' to the coding sequence.

[000130] In some embodiments, the nucleic acids or vectors disclosed herein comprise a “promoter.” In some embodiments, the promoter is operably linked to the nucleic acids or vectors disclosed herein. As used herein, a “promoter” refers to a nucleotide region comprising a nucleic acid (i.e., DNA) regulatory sequence, wherein the regulatory sequence is derived from a gene or synthetically created that is capable of binding RNA polymerase and initiating transcription of a downstream (3'-direction) coding sequence. Alternatively, promoters can be selected based upon a desired outcome. Such promoters include “constitutive promoters” (where expression of a polynucleotide sequence operably linked to the promoter is unregulated and therefore continuous), “inducible promoters” (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), and “repressible promoters” (where expression of a polynucleotide sequence operably linked to the promoter is repressed by an analyte, cofactor, regulatory protein, etc.).

[000131] To assist in introducing the nucleotide sequences of interest into the appropriate host cells, the expression cassette can be incorporated or ligated into a vector. As used herein, “vector” refers to a replicon, such as a plasmid, phage, or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. A vector is capable of transferring nucleic acid molecules to the host cells. Bacterial vectors typically can be of plasmid or phage origin. The terms “vector,” “vector construct,” “expression vector,” “gene expression vector,” “gene delivery vector,” “gene transfer vector,” and “expression cassette” all refer to an assembly that is capable of directing the expression of a nucleic acid sequence or gene of interest. Thus, the terms include cloning and expression vehicles. Vectors typically contain one or a small number of restriction endonuclease recognition sites where a nucleic acid molecule of interest can be inserted in a determinable fashion without loss of essential biological function of the

vector, as well as a selectable marker that can be used for identifying and selecting cells transformed with the vector.

[000132] The term “transformed cell” is used according to its ordinary meaning to refer to a cell having a nucleic acid that is introduced/engineered into the cell (or its genome). Transformed cell may then express the protein(s) encoded by the nucleic acid. Methods for introducing such nucleic acid into a cell are known in the art and include chemical-based methods, physical-based methods, and gene editing methods. Such methods include, e.g., calcium phosphate, polymers, FuGENE®, dendrimers, electroporation, biolistic, microinjection, magnetofection, sonoporation, optical transfection, transfection (e.g., lipid-based or chemical based), electroporation, biolistics, gene editing (e.g., zinc finger nuclease, transcription activator-like effector nuclease, CRISPR/Cas gene editing, prime editing, and programmable addition via site-specific targeting elements). Particularly suitable transformed cells of the present disclosure are stably transformed cells.

[000133] The term “cell line” or “cell clone” is used according to its ordinary meaning to refer to a cell culture developed from a single cell and therefore consisting of cells with a uniform genetic makeup. In some examples, the cell clone expresses the antibody or fragment thereof.

[000134] As used herein, “reporter gene,” “reporter protein,” and “reporter,” are all used according to their ordinary meaning as understood by one of ordinary skill in the art to refer to a gene (the “reporter gene”) that encodes a protein (the “reporter protein”) that is attached to a regulatory sequence of another gene of interest to induce detectable or identifiable characteristics in a cell expressing the reporter. Suitable reporters include fluorescent and luminescent proteins. Suitable reporters include, e.g., luc (luciferase enzyme) lacZ (b-galactosidase), GFP (green fluorescent protein), RFP (red fluorescent protein), and other known reporters.

[000135] As used herein, “selection marker” refers a gene that encodes a protein that is attached to a regulatory sequence of another gene of interest to confer resistance to a selection agent in a cell expressing the selection gene. Particularly suitable selection genes include antibiotic-resistance genes. As understood by one of ordinary skill in the art, selection genes are used to select for transformed cells after transfection following contact with the selection agent. When cultured in selective medium including,

for example, an antibiotic, cells that are not transfected or were transiently transfected die, and those that express the antibiotic resistance gene at sufficient levels survive. Suitable antibiotics for use as selection agents include antibiotics such as puromycin, hygromycin, zeocin, blasticidin, neomycin, and other known antibiotics.

[000136] The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the present invention and does not pose a limitation on the scope of the present invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the present invention unless as much is explicitly stated.

[000137] The potency of a drug product is a measure of the activity in a specific assay relative to the activity of a reference standard of the drug product for which therapeutic efficacy may have been assessed. In one embodiment, a method according to the present invention is suitable for use in determining the potency of the drug product as the binding of the antibody to the Fc receptor and this is a direct indication of a mechanism of action of the antibody.

[000138] The citation and incorporation of patent documents herein is done for convenience only and does not reflect any view of the validity, patentability, and/or enforceability of such patent documents.

[000139] The description herein of any embodiment of the present invention using terms such as “comprising”, “having,” “including,” or “containing” with reference to an element or elements is intended to provide support for a similar embodiment of the present invention that “consists of”, “consists essentially of”, or “substantially comprises” that particular element or elements, unless otherwise stated or clearly contradicted by context (e.g., a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

[000140] The present invention includes all modifications and equivalents of the subject matter recited in the embodiments presented herein to the maximum extent permitted by applicable law. All patents, pending patent applications and other publications cited herein are hereby incorporated by reference in their entirety.

[000141] The following Examples further illustrate the present disclosure. It should be understood however, that the Examples are set forth by way of illustration and

not limitation, and that various modifications may be made by one of ordinary skill in the art.

EXAMPLES

Example 1: Vector Construction.

[000142] The nucleotide sequences encoding human FcγRI α-chain (SEQ ID NO: 26; also described in NCBI Reference Sequence: NP_000557.1) and FCER1G (SEQ ID NO: 27; also described in NCBI Reference Sequence: NP_004097.1) were inserted into a mammalian expression vector under CMV and RSV promoters, respectively, with hygromycin selection marker, resulting in a FcγRI α-chain and FCER1G vector expression construct.

[000143] A nucleotide sequence encoding human FcγRIIa, H131 variant (SEQ ID NO: 25; also described in NCBI Reference Sequence: NP_067674.2) was inserted into a mammalian expression vector under CMV promoter with zeocin selection marker, resulting in a FcγRIIa vector expression construct.

[000144] Nucleotide sequences encoding human FcγRIIIa α-chain, V158 variant (SEQ ID NO: 28; also described in NCBI Reference Sequence: AAH17865.1) and FCER1G (SEQ ID NO: 27) were inserted into a mammalian expression vector under CMV and RSV promoters, respectively, with blasticidin selection marker, resulting in a FcγRIIIa α-chain and FCER1G vector expression construct.

[000145] All above mammalian expression constructs contain Transposagen PiggyBacTM sequences for efficient stable line generation.

Example 2: Cell Line Generation for Three FcγR-NFAT Luciferase.

[000146] A parental Jurkat E6-1 cell line (a human T lymphoblastoid cell line derived from an acute T-cell leukemia) was used for cell line generation. Jurkat E6-1 cells were transfected with the NFAT-luc and pPUR vectors using electroporation. Clones were selected for puromycin resistance. The puromycin resistant cell population underwent PMA/ionomycin induction to confirm NFAT-luc expression and ensure that only cells expressing puromycin were preferentially selected and were capable of expressing luciferase. A clone was chosen from the stably transfected Jurkat NFAT-luciferase pool, and this cell line is referred to herein as the “Jurkat NL-4” cell line.

[000147] Once the Jurkat NL-4 cell line was prepared, additional vectors (as described in Example 1) were created to add the Fc γ receptors to the cell line.

[000148] The dual vector carrying the Fc γ RI α -chain (the alpha chain that binds to the Fc portion of the IgG) and FCER1G genes (the common Fc $\epsilon\gamma$ chain containing ITAM motif important for downstream signal transduction) was stably transfected into the Jurkat NL-4 cell line. This cell containing the dual vector is referred to herein as Jurkat Fc γ RI α -chain NFAT-luciferase cell line. This vector also expresses hygromycin resistance providing a capability to select cells that express Fc γ RI α -chain and FCER1G during cell culture. Use of hygromycin and puromycin during cell culture allowed for selection of cells that co-express Fc γ RI α -chain, FCER1G, and NFAT-luciferase protein.

[000149] FCER1G is important for cell-surface expression as well as assembly of functional Fc γ RI α -chain. This dual vector utilized the PiggyBacTM transposase system that is known to insert the vector into the genome with high efficiency. The use of dual vector ensured 1:1 transfection efficiency for both FCER1G and Fc γ RI α -chain and also limited the number of antibiotic selection markers used in cell culture as opposed to the use of two separate vectors.

[000150] Nucleic acids encoding for Fc γ RIIa and Fc γ RIIIa α -chain (α -chain along with FCER1G) receptors (as described in Example 1) were stably transfected into the Jurkat Fc γ RI NFAT-luciferase cell line using the PiggyBacTM transposase system.

[000151] The Fc γ RIIa vector (as described in Example 1) as well as the Fc γ RIIIa α -chain vector (as described in Example 1) were stably transfected into the Jurkat Fc γ RI NFAT-luciferase cell line. The Fc γ RIIa plasmid also expresses resistance to zeocin. The Fc γ RIIIa α -chain vector is a dual vector that carries the Fc γ RIIIa α -chain (the alpha chain which binds to the Fc portion of the IgG) and FCER1G gene (the common Fc $\epsilon\gamma$ chain containing ITAM motif important for downstream signal transduction). The Fc γ RIIIa α -chain vector includes a blasticidin selection marker. Cells transformed with the Fc γ RIIa vector and Fc γ RIIIa α -chain vector were selected using the zeocin and Blasticidin antibiotics. The selected cell line having, all three vectors, are referred to as 3Fc γ R NFAT-luciferase cell line. A single clone from the stable pool of 3Fc γ R NFAT-luciferase cell line was chosen and used for the development of the reporter gene assay.

[000152] A stable pool of the Jurkat 3Fc γ R NFAT-luciferase cell line, frozen at passage 1 was used to create the clonally derived cell line (Jurkat 3Fc γ R NFAT-luciferase

4E8) used in the anti-N3pE Abeta (A β) cell-based assay. Cell sorting was performed for the stable pool of cells, and two clones were identified. Jurkat 3Fc γ R NFAT-luciferase cell line, clone 4E8 was chosen for scale up. The cell line was expanded and passaged once. Twenty vials of cells containing 1 mL at a density of 5×10^6 cells/mL were prepared and transferred to liquid nitrogen.

[000153] The base media for growing the Jurkat 3Fc γ R NFAT-luciferase 4E8 cell line follows the ATCC recommendation of RPMI medium with 10% fetal bovine serum (FBS). The concentration of the selection antibiotics was assessed to ensure that growth of the cell line was maintained along with appropriate selection pressure to retain the inserted genes. The final concentration of selection antibiotics was determined to be 200 ng/mL puromycin, 50 μ g/mL zeocin, 300 μ g/mL hygromycin B, and 2 μ g/mL blasticidin.

[000154] Both a master cell bank and a working cell bank were prepared to support commercial testing of drug substance, drug product, and reference standards. The initial Jurkat 3Fc γ R NFAT-luciferase 4E8 was prepared (representing the first passage), followed by scale up and production of the master cell bank and working cell bank. The Jurkat 3Fc γ R NFAT-luciferase 4E8 cell line was expanded for eight passages to prepare the master cell bank (comprised of 239 vials containing 1.8 mL at 5×10^6 cells/vial), then it was expanded for six passages to prepare the working cell bank (comprised of 241 vials containing 1.8 mL at 5×10^6 cells/vial) for a total of 15 passages from the point of clone selection to the working cell bank.

[000155] Cell line passage stability: One of the challenges overcome by the transfection methods disclosed herein was the instability of Fc γ receptor expression during cell passage. The methods used herein allow for stable expression of all the Fc γ receptors and their coreceptors. Moreover, the coreceptors are expressed such that the ratio between the coreceptor and the receptor was appropriate making the assay functional.

[000156] The Jurkat 3Fc γ R NFAT luciferase 4E8 cell bank was used for method validation. To characterize the Jurkat 3Fc γ R NFAT-luciferase 4E8 working cell banks, Fc γ receptor expression was assessed starting at passage 7 and was monitored until passage 38. The Jurkat 3Fc γ R NFAT-luciferase cells were stained with the PE anti-human CD16 antibody, APC anti-human CD32 antibody and Alexa Fluor[®] 488 anti-human CD64 antibody to measure the expression of Fc γ RIIIa α -chain, Fc γ RIIa and Fc γ RI α -chain, respectively, at three passage intervals. The number of single live cells that were positively

stained is represented as a percentage of total single live cells. The results were normalized to the total number of events. The flow cytometry data, in Figures 2 to 7, show that the clonally derived cell line 4E8 expressed the three Fc γ receptors and the expression was constant through 38 cell line passages. Method performance was assessed from passage 7 to passage 38. Executed assays met all acceptance criteria through passage 38. This data supports utilizing the Jurkat 3Fc γ R NFAT-luciferase 4E8 working cell bank for 38 passages beyond vial thaw.

Example 3: Exemplified Potency Assays.

[000157] A potency assay, e.g., for an antibody like donanemab, needs to reflect the molecule's Fab binding to its antigen and activation of the whole repertoire of microglial activating Fc γ receptors that engage with the Fc portion of an antibody. No known cell line includes all the activating human receptors. Cell lines having a single Fc γ are commercially available, however, assays using a cell line expressing a single receptor is only partially reflective of Fc γ receptor activation. The clonally derived cell line generated in Example 2 solves this problem by providing all of the activating human Fc γ receptors in a single cell line.

[000158] The potency assay of the present disclosure, for use with donanemab, may utilize the clonally derived cell line as described in Example 2. The cells are maintained in RPMI-1640 media which contains 10% FBS, non-essential amino acids (0.1 mM NEAA; Invitrogen), sodium pyruvate (1 mM), L-glutamine (2 mM), puromycin (200 ng/mL), hygromycin (300 μ g/mL), zeocin (50 μ g/mL), and blasticidin (2 μ g/mL). The antibiotic is used to maintain selection pressure that ensures that only cells containing the required receptors and coreceptors remain viable.

[000159] A modified amyloid beta antigen, N3pE amyloid beta (N3pE A β), is immobilized to a 96-well microtiter plate at a concentration of 0.18 μ g/mL in DPBS at 37 °C for 18 to 24 hours. The plate is blocked with 0.5% BSA (blocking solution) at 37 °C for 1 – 2 hours to prevent any non-specific binding. The blocking solution is aspirated from the plate and the plate is washed with DPBS (Dulbecco's PBS). A serial dilution of donanemab drug substance (test sample), drug product (test sample), or reference standard is prepared in RPMI 1640 media containing NEAA (0.1 mM), L-glutamine (2 mM), sodium pyruvate (1 mM) and 1.5% FBS (also referred to herein as assay media).

[000160] The serial dilution is a 3-fold dilution starting at 4,800 ng/mL down to 2.19 ng/mL. The titration of either drug substance, drug product, and/or reference standard is incubated with the bound ligand in the plate for 1.75 – 2.25 hours at 37 °C. At the completion of the incubation, the media is aspirated to remove any excess drug which has not complexed with the ligand.

[000161] Jurkat cells expressing the three activating Fcγ receptors (as described in Example 3) are centrifuged and resuspended in assay media to achieve a cell density 1.5x10⁶ cell/mL. 1.5x10⁵ cells are added to each of the 96 wells with immobilized ligand and incubated at 37 °C, 5% CO₂ for 20 – 28 hours. After incubation the plate is removed from the incubator and Bright-Glo™ reagent (obtained from Promega and used per manufacturer's instructions) is added to each well of the 96-well microtiter plate. The plate is read in a luminescence plate reader and the signal generated is modelled using a 4-parameter fit. In some cases, the Jurkat cells expressing the three activating Fcγ receptors may be added to the 96 well plate in the presence of 10 mg/mL human serum IgG. This approximates the typical IgG concentrations in human plasma (see Herter, S; et al. J. Immunol. 192(5), 2252-2260, 2014).

[000162] The 4PL fit is modelled independently for the sample and the reference standard. Potency is determined by preparing a titration of the reference standard alongside the sample. Relative potency is determined by calculating the ratio of the EC₅₀ of the reference standard to the EC₅₀ of the test sample as shown in Figure 6.

[000163] The general form of the constrained 4-parameter logistic parameterization:

For reference standards

$$y = \frac{a - d}{1 + \left(\frac{\text{conc}}{\text{std} \cdot c} \right)^b} + d,$$

and for test samples

$$y = \frac{a - d}{1 + \left(\frac{\text{conc}}{\text{std} \cdot c} \times \text{rel} \cdot \text{pot} \right)^b} + d,$$

where:

“y” is the luciferase-derived signal (luminescence), measured in Relative Light Units (RLU),

conc is the concentration of drug,
 "a" is the left asymptote,
 "b" is the slope parameter,
 "d" is the right asymptote,
 "std.c" is the EC₅₀ for reference standard,
 "rel.pot" is the relative potency of the sample.

[000164] The general form of the independent 4-parameter logistic parameterization:

For reference standards

$$y = \frac{std.a - std.d}{1 + \left(\frac{conc}{std.c} \right)^{std.b}} + std.d,$$

and for unknown samples

$$y = \frac{unk.a - unk.d}{1 + \left(\frac{conc}{std.c} \times rel.pot \right)^{unk.b}} + unk.d,$$

where

"y" is the luciferase-derived signal (luminescence), measured in Relative Light Units (RLU),
 "conc" is the concentration of drug
 "std.a" and "unk.a" are the left asymptotes for standard and samples, respectively,
 "std.b" and "unk.b" are the slope parameters for standard and samples, respectively,
 "std.d" and "unk.d" are the right asymptotes for standard and samples, respectively,
 "std.c" is the EC₅₀ for reference standard,
 "rel.pot" is the relative potency of the sample.

[000165] The reference standard is prepared from donanemab drug substance material that has defined chemical and physical characteristics. The drug substance batch has been prepared from or is representative of the current manufacturing process. The reference standard can be used for both identity and quantitative standard for the bioassay. The relative potency specification for donanemab is from 80% to 130%.

[000166] Donanemab drug batch testing: Drug batches were tested with the cell-based assay. Drug batches were tested relative to the reference standard and met the relative potency specification for donanemab of 80% to 130%. The relative potency of the acceptable batches is shown in the Table 1 below:

Table 1:

Batch Number	Cell-based Reporter Gene Assay Potency (%)	Batch Number	Cell-based Reporter Gene Assay Potency (%)
D1	90	D11	103
D2	95	D12	102
D3	87	D13	99
D4	82	D14	101
D5	99	D15	103
D6	92	D16	98
D7	108	D17	99
D8	101	D18	101
D9	100	D19	96
D10	95	D20	99

Example 4: Potency Assay with Remternetug.

[000167] The remternetug cell-based assay is run using the same cell line, and analysis methods as described in Example 3, using a reference standard prepared from a remternetug drug substance batch which has been prepared from or is representative of the current manufacturing process, with a relative potency specification of 60-140%.

[000168] The assay procedure is described as follows: A modified amyloid beta antigen (N-pyroglutamate amyloid beta) is immobilized to a microtiter plate at a concentration of 5.25 µg/mL in DPBS at 37 °C for 18 to 24 hours. The plate is blocked with 0.5% BSA at 37 °C for 1–2 hours (blocking solution) to prevent any non-specific binding. The blocking solution is aspirated from the plate and the plate is washed with DPBS. A serial dilution of remternetug drug substance, drug product or reference standard is prepared in RPMI 1640 media containing NEAA (0.1 mM), L-glutamine (2 mM), sodium pyruvate (1 mM), and 10% FBS, (which is referred to as assay media#1).

[000169] Eight non-serial dilutions are prepared starting at 6,000 ng/mL down to 0.732 ng/mL. The titration of either drug substance (test sample), drug product (test sample), and/or reference standard is incubated with the bound ligand in the plate for 1.75

– 2.25 hours at 37 °C. At the completion of the incubation, the media is aspirated, and the plate is washed with DPBS to remove any excess drug which has not complexed with the ligand. Jurkat cells expressing the three activating Fcγ receptors (as described in Example 3) are centrifuged and resuspended in assay media #2 (i.e., assay media #1 containing 1.5% FBS instead of 10% FBS) to achieve a cell density 0.2x10⁶ cell/mL. Cells at a concentration of 0.02x10⁶ added to each of the 96 wells and the plate is incubated at 37 °C, 5% CO₂ for 20 – 28 hours. After incubation the plate is removed from the incubator and BrightGlo™ reagent is added to each well of the 96-well microtiter plate. The plate is read in a luminescence plate reader and the signal generated is modelled using a 4-parameter fit as described above in Example 3.

[000170] In some variations of this method, the Jurkat cells expressing the three activating Fcγ receptors are added to the 96 well plate in the presence of 10 mg/mL human serum IgG. This approximates the typical IgG concentrations in human plasma (see Herter, S; et al. *J. Immunol.* 192(5), 2252-2260, 2014).

[000171] Remternetug drug substance batch testing: Remternetug drug batches were tested with the cell-based assay. Drug batches were tested relative to the reference standard and met the relative potency specification for remternetug of 60% to 140%. The relative potency of the batches is shown in the Table 2 below:

Table 2:

Batch Number	Cell-based Reporter Gene Assay Potency (%)
R1	103
R2	84
R3	100
R4	100
R5	92
R6	95

Example 5: Comparison of Bioactivity of ex-vivo Mouse Microglial Assay and Reporter Gene Assay.

[000172] Known cell-based assays for antibodies possessing Fc-related mechanism of action include an ex-vivo mouse microglial assay. A known disadvantage

of this assay is that it is mechanistically relevant but is not quality control (QC) amenable. Another disadvantage of the microglial assay is that mouse microglial cells express a different set of activating Fc γ receptors than human microglial cells. Additional challenges of this assay are related to the steps involved: the use of transgenic PDAPP mice, preparation of cryostat brain sections, isolation of the primary mouse microglial cells, proper solubilization and dilution of the microglial cell mixture after antibody treatment are all technically difficult to perform. Accordingly, proper and consistent execution of the mouse microglial assay is often technically challenging. Given the level of complexity of assay performance and associated variability, the generation of a full dose response curve that meets parallelism and acceptance criteria per ICH Q6B and USP chapters <1032>, <1033, and <1034> is not always achievable. These challenges of the mouse microglial assays are overcome by the assays of the present disclosure. In one embodiment, the 3Fc γ R assay of the present disclosure provides an assay that captures activity through all three Fc receptors as well as measures the phagocytic activity of antibodies.

[000173] The 3Fc γ R assay of the present disclosure was developed to overcome such challenges. This example compares the ability of the mouse microglial assay and the 3Fc γ R assay to assess biological activity of donanemab and/or the activation of Fc γ R.

[000174] In order to obtain a comparative analysis, drug substance and drug product samples were tested using i) an ex-vivo mouse microglial assay and ii) the Jurkat 3Fc γ R cell-based assay (as described in Examples 2-4). To confirm that the 3Fc γ R assay was mechanistically relevant, in addition to being QC amenable, data was generated using the assay to test the same drug batches tested in ex-vivo mouse microglial assay. The agreement in the results generated between the two assays was determined by assessing the difference between the assay results versus the average result of the assays for a given sample. An agreement in the results of the microglial assay and the 3Fc γ R assay was observed. The results of the comparative analysis are depicted in the Bland-Altman plot in Figure 7.

[000175] The assessment of the data generated and compared for the assays demonstrated that the 3Fc γ R assay is a suitable replacement for the ex-vivo mouse microglial assay.

Example 6: Design of Experiments (DOE) Study with Donanemab and Suitability of assay (QC Amenable).

[000176] Potency assays are a quantitative measure of the biological activity of a drug substance or a drug product. The assay needs to be mechanistically relevant to clinical outcome of the therapeutic and is often used to: determine product quality of drug substance and drug product, batch release, comparability studies, or stability testing. Moreover, the assay needs to be QC amenable. In order to be QC amenable, the assay should be i) at least partially reflective of the mechanism of action of the antibody and ii) use methods that generate data consistently when executed as written. Cell-based assays have the added complexity of identifying/engineering an appropriate cell line for use in the assay. In some cases, a further challenge is that the assay must be validated per ICH guidance document Q2(R1) (Validation of Analytical Procedures: Text and Methodology, 2005) so that it can be transferred to testing sites and release sites across the globe.

[000177] In general, to determine the relative potency of a sample (e.g., for donanemab), a 4-PL curve, that possesses similar activity to the reference standard, must be generated. The first step is to fit the sample and reference standard curves independently. The fit between the sample and reference standard is then assessed for parallelism. Curves that exhibit variability in curve fit (e.g., because of slope, and/or lack of adequate upper or lower anchoring) may fail the parallelism assessment.

[000178] For donanemab, the assay was optimized to produce a suitable 4-PL curve fit that was successfully implemented and validated. The fit for the initial assay exhibited non-specific binding and the last point on the upper asymptote was lower than the previous two points resulting in a “hook.” The challenge with this fit is that the curve might fail the parallelism assessment.

[000179] Other critical factors assessed in assay development were signal-to-noise ratio and slope of the fit. A design of experiments (DOE) approach was used to assess multiple parameters and the interactions of these parameters simultaneously. The data from the initial DOE demonstrated that low numbers of cells per well generated a low luminescent signal and it was revised to increase the number of cells per well in order to increase luminescent signal.

[000180] A second DOE further refined the assay parameters. The table below summarizes the results of the DOE runs and the most significant factors impacting the hook. Signal to noise and slope were also assessed. Parameters assessed are listed below:

- Modified amyloid beta antigen incubation - aBeta inc time
- Modified amyloid beta antigen concentration - aBeta conc
- Aspiration after drug/antigen incubation - aBeta wash
- Number of Jurkat cells/well - cell number
- Total assay incubation time - total inc time

The outcome of the DOE is shown in Table 3.

Table 3:

Screening output	DOE	Factor	Individual p-value
% Hook	1	aBeta inc time	0.0181
% Hook	1	total inc time	0.0248
% Hook	1	aBeta wash	0.0773
% Hook	1	aBeta conc	0.0850
% Hook	1	aBeta inc time * aBeta inc time	0.0357
Signal to Noise	1	total inc time	<.0001
Signal to Noise	1	aBeta wash	0.0355
Signal to Noise	1	total inc time * aBeta inc time	0.0479
Slope	1	aBeta conc	0.0464
% Hook	2	aBeta conc	0.0432
Slope	2	total inc time	0.0010
Slope	2	aBeta inc time	0.0024
Slope	2	total inc time * total inc time	0.0003
Slope	2	total inc time * aBeta inc time	0.0020

[000181] Upon completion of determination of the method conditions, this assay generates a full dose curve that meets parallelism and acceptance criteria per ICH Q6B and U.S. Pharmacopeia (USP) chapters <1032>, <1033>, and <1034> guidelines and

was validated per the guidelines in ICH-Q2(R1) for the use of determining relative potency for drug substance, drug product and reference standards.

Example 7: Selecting Cell Clone for Manufacture of a Drug Substance.

[000182] The assays described in Examples 3 and 4 can be used to select a cell clone that produces an antibody (e.g., donanemab or remternetug) with a desirable bioactivity profile. In one example, material (antibody or drug substance) is obtained from several cell clones expressing the desired antibody using a small scale process. A titration of each small scale process material (sample) is then prepared (either serially or non-serially) and incubated with a 3F γ receptor cell line (e.g., as described in Examples 3 and 4) in a 96-well microtiter plate at 37 °C and 5% CO₂. The Fc portion of the antibody is allowed to interact with any combination of Fc γ receptors expressed on the cell line. In some examples, the Fab interaction is allowed to occur with another cell type, a ligand, or bound to a substrate.

[000183] The incubation time for the Fc interaction to occur is sufficient to activate Fc γ receptor(s) and the downstream NFAT luciferase. After incubation, luciferase reagent such as Bright-Glo™ reagent is added to the 96-well microtiter plate. The amount of signal generated is dependent on the titration of the antibody and could be modelled using a 4-parameter logistic fit.

[000184] To evaluate the biological activity of each small scale process material, a reference material with a known biological activity profile, such as a reference standard, is prepared in the same serial or non-serial manner (as the sample) and run alongside the small scale process material in the assay. The reference material is also modelled using a 4-parameter logistic (4PL) fit. The 4PL fit is modelled independently for the sample and the reference standard. Relative potency is determined by calculating the ratio of the EC₅₀ of the reference standard to the EC₅₀ of the test sample. In some examples, a cell clone or several cell clones are selected if they exhibit an acceptable Fc γ receptor activation profile as compared to the reference standard. In other examples, a cell clone or several cell clones are selected if they exhibit an acceptable biological activity profile as compared to the reference standard.

SEQUENCES

SEQ ID NO:1 LCVR of Donanemab

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSRGKTYLNWLLQKPGQSPQLLIYAV
SKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHYPFTFGQGTKLEI
K

SEQ ID NO:2 HCVR of Donanemab

QVQLVQSGAEVKKPGSSVKVSCKASGYDFTRYINWVRQAPGQGLEWMGWINP
GSGNTKYNEKFKGRVTITADESTSTAYMELSSLRSEDVAVYYCAREGITVYWGQ
GTTVTVSS

SEQ ID NO:3 LC of Donanemab

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSRGKTYLNWLLQKPGQSPQLLIYAV
SKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHYPFTFGQGTKLEI
KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:4 HC of Donanemab

QVQLVQSGAEVKKPGSSVKVSCKASGYDFTRYINWVRQAPGQGLEWMGWINP
GSGNTKYNEKFKGRVTITADESTSTAYMELSSLRSEDVAVYYCAREGITVYWGQ
GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWFYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHY
TQKSLSLSPG

SEQ ID NO:5 LCDR1 of Donanemab

KSSQSLLYSRGKTYLN

SEQ ID NO:6 LCDR2 of Donanemab

AVSKLDS

SEQ ID NO:7 LCDR3 of Donanemab
VQGTHYPFT

SEQUENCED ID NO:8 HCDR1 of Donanemab
GYDFTRYIN

SEQ ID NO:9 HCDR2 of Donanemab
WINPGSGNTKYNEKFKG

SEQ ID NO:10 HCDR3 of Donanemab
EGITVY

SEQ ID NO:11 LCVR of Remternetug
DIQMTQSPSTLSASVGDRVITITCRASQSLGNWLAWYQQKPGKAPKLLIYQASTLE
SGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQHYKGSFWTFGQGTKVEIK

SEQ ID NO:12 HCVR of Remternetug
EVQLLESGLVQPGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAISGS
GGSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAREGGSGSYYN
GFDYWGQGLVTVSS

SEQ ID NO:13 LC of Remternetug
DIQMTQSPSTLSASVGDRVITITCRASQSLGNWLAWYQQKPGKAPKLLIYQASTLE
SGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQHYKGSFWTFGQGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDYSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:14 HC of Remternetug
EVQLLESGLVQPGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAISGS
GGSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAREGGSGSYYN

GF DYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG

SEQ ID NO:15 LCDR1 of Remternetug
RASQSLGNWLA

SEQ ID NO:16 LCDR2 of Remternetug
YQASTLES

SEQ ID NO:17 LCDR3 of Remternetug
QHYKGSFWT

SEQ ID NO:18 HCDR1 of Remternetug
AASGFTFSYPMS

SEQ ID NO:19 HCDR2 of Remternetug
AISGSGGSTYYADSVKG

SEQ ID NO:20 HCDR3 of Remternetug
AREGGSGSYNGFDY

SEQ ID NO:21 LC DNA sequence of Donanemab
gatattgtatgactcagactccactctccctgtccgtcaccctggacagccggcctccatctcctgcaagtcaagtcagagcct
cttatatagtcgcgaaaaacctatttgaattggctcctgcagaagccaggccaatctccacagctcctaatttatgcggtgtctaaa
ctggactctgggggtcccagacagattcagcggcagtggggtcaggcacagatttcacactgaaaatcagcaggggtggaggccga
agatgttggggtttactgcgtgcaaggtacacattacccattcacgtttggccaagggaagctggagatcaaacgaactg
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atcccagagaggccaaagtacagtggaggtggataacgccctccaatcgggtaactcccaggagagtgacacagagcagga
cagcaaggacagcacctacagcctcagcagcacctgacgctgagcaaagcagactacgagaacacaaagtctacgcctgc
gaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgc

SEQ ID NO: 22 HC DNA Sequence of Donanemab

caggctgcagctggctgagctctgggctgaggtgaagaagcctgggtcctcagtgaaggttcctgcaaggcatctgggtacgac
ttcactagatactatataaactgggtgcgacaggccccctggacaagggttgagtgatgggatgattaatcctggaagcggta
atactaagtacaatgagaaattcaagggcagagtcaccattaccgacgaatccacgagcacagcctacatggagctgagc
agcctgagatctgaggacacggcgtgtattactgtgcgagagaaggcatcacgggtctactggggccaaggaccacggctac
cgtctcctcagcctccaccaaggggcccatcggtcttcccgtagcacctcctccaagagcacctctgggggcacagcggccct
gggctgcctggctcaaggactacttcccgaaccgggtgacggtgctggaactcaggcgccctgaccagcggcgtgcacacct
tcccggctgtctacagtcctcaggactctactccctcagcagcgttggtgaccgtgccccccagcagcttgggcacccagacct
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cccaccgtgcccagcacctgaactcctgggggacccgtcagttcttcttccccccaaacccaaggacacctcatgatctcc
cggaccctgaggtcacatgcgtgggtgggtgacgtgagccacgaagaccctgaggtcaagttcaactgtacgtgacggcgt
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caaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggacgagctgaccaagaaccaggtca
gcctgacctgcctggtaaaagcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaaacta
caagaccacgccccctgctggactccgacggctcttcttctctatagcaagctaccgtggacaagagcaggtggcagc
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SEQ ID NO: 23 LC DNA Sequence of Remternetug

gacatccagatgaccagctctcctccaccctgctgcatctgtaggagacagagtcaccatcacttgcggggccagtcagagct
tggttaactgggtggcctggatcagcagaaccagggaagccctaaactcctgatctatcaggcgtctactttagaatctggg
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aagtagcagtggaaggtggataacgccctccaatcgggtaactcccaggagagtgacacagagcaggacagcaagacagca
cctacagcctcagcagcacctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctcggaagtcacccatca
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SEQ ID NO:24 HC DNA Sequence of Remternetug

gagggtgcagctgttgagctctggggaggctgttacagcctgggggtccctgagactctcctgtgcagcctctggattcacct
ttagcagctatcctatgagctgggtccgccaggctccagggaaggggctggagtggtgtcagctattagtggtagtggttag
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cccccatcgagaaaacctctccaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggagc
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gggcagccggagaacaactacaagaccagcccccggtgctggactccgacggctcctctctctatagcaagctaccgtg
gacaagagcaggtggcagcaggggaacgtcttctcatgctcgtgatgcatgaggctctgcacaaccactacacgcagaagag
cctctccctgtctccgggt

SEQ ID NO:25 human FcγRIIa (H131 Variant)

MTMETQMSQNVCPRLWLLQPLTVLLLLASADSQAAPPKAVLKLEPPWINVLQE
DSVTLTCQGARSPESDSIQWFHNGNLIPHTHTQPSYRFKANNNDSGEYTCQTGQTS
LSDPVHLTVLSEWLVLQTPHLEFQEGETIMLRCHSWKDKPLVKVTFQNGKSQK
FSLDPTFSIPQANHSYSGDYHCTGNIGYTLFSSKPVITITVQVPSMGSSSPMGIIA
VVIATAVA AIVAAVVALIYCRKKRISANSTDPVKAAQFEPGRQMI AIRKQLEET
NNDYETADGGYMTLNPRAPTDDDKNIYLTLPNDHVNNSNN

SEQ ID NO:26 human FcγRI α-chain

MWFLTLLLLWVPVDGQVDTTKAVITLQPPWVSVFQEETVTLHCEVLHLPSSST
QWFLNGTATQTSTPSYRITSASVNDSGEYRCQRGLSGRSDPIQLEIHRGWLLQVS
SRVFTEGEPLALRCHAWKDKLVYNVLYYRNGKAFKFFHWNSNLTKTNISHNG
TYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTSPLLEGNLVTLSCETKLLLQ

RPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWCEAATEDGNVLKRSP
ELELQVLGLQLPTPVWFHVLFYLAVGIMFLVNTVLWVTIRKELKRKKKWDLEIS
LDSGHEKKVISSLQEDRHLEEELKCQEKEEQLQEGVHRKEPQGAT

SEQ ID NO:27 human FCER1G
MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLYGIVLTLLYCRLKIQVRKAAITS
YEKSDGVYTGLSTRNQETYETLKHEKPPQ

SEQ ID NO:28 human FcγRIIIa α-chain (V158 Variant)
MWQLLLPTALLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSP
EDNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGW
LLLQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKA
TLKDSGSYFCRGLVGSKNVSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLF
AVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

CLAIMS

1. A transformed cell comprising:
 - i) a nucleic acid encoding a human Fc γ Receptor I α -chain (Fc γ RI α -chain) or a variant thereof;
 - ii) a nucleic acid encoding a human Fc γ RI α -chain or a variant thereof and a nucleic acid encoding a human Fc γ Receptor IIa (Fc γ RIIa) or a variant thereof;
 - iii) a nucleic acid encoding a human Fc γ RI α -chain or a variant thereof and a nucleic acid encoding a human Fc γ Receptor IIIa α -chain (Fc γ RIIIa α -chain) or a variant thereof;
 - iv) a nucleic acid encoding a human Fc γ RIIa or a variant thereof and a nucleic acid encoding a human Fc γ RIIIa α -chain or a variant thereof; or
 - iv) a nucleic acid encoding a human Fc γ RI α -chain or a variant thereof, a nucleic acid encoding a human Fc γ RIIa or a variant thereof, and a nucleic acid encoding a human Fc γ RIIIa α -chain or a variant thereof;wherein the transformed cell is transformed to comprise the nucleic acid encoding Fc γ RI, Fc γ RIIa, and/or the Fc γ RIIIa α -chain.
2. The transformed cell according to claim 1, wherein the transformed cell expresses the human Fc γ RI α -chain or a variant thereof.
3. The transformed cell according to claim 2, wherein human Fc γ RI α -chain or the variant thereof is presented on the surface of the transformed cell.
4. The transformed cell according to any one of claims 1-3, wherein the transformed cell expresses the human Fc γ RIIa or a variant thereof.
5. The transformed cell according to claim 4, wherein Fc γ RIIa or the variant thereof is presented on the surface of the transformed cell.
6. The transformed cell according to any one of claims 1-5, wherein the transformed cell expresses the human Fc γ RIIIa α -chain or a variant thereof.

7. The transformed cell according to claim 6, wherein FcγRIIIa α-chain or the variant thereof is presented on the surface of the transformed cell.

8. The transformed cell according to claim 1, wherein the cell expresses:

- i) the human FcγRI α-chain or a variant thereof;
- ii) the human FcγRI α-chain or a variant thereof and the human FcγRIIa or a variant thereof;
- iii) the human FcγRI α-chain or a variant thereof and the human FcγRIIIa α-chain or a variant thereof;
- iv) the human FcγRIIa or a variant thereof and the human FcγRIIIa α-chain or a variant thereof; or
- v) the human FcγRI α-chain or a variant thereof, the human FcγRIIa or a variant thereof, and the human FcγRIIIa α-chain or a variant thereof.

9. The transformed cell according to claim 8, wherein i) the human FcγRI α-chain or a variant thereof, ii) the human FcγRIIa or a variant thereof, or iii) the human FcγRIIIa α-chain or a variant thereof is presented on the surface of the transformed cell.

10. The transformed cell of any one of claims 1-9, further comprising a nucleic acid encoding a coreceptor wherein the coreceptor comprises an immunoreceptor tyrosine-based activation motif (ITAM) or a variant thereof.

11. The transformed cell according to claim 10, wherein the coreceptor comprising the ITAM or a variant thereof is: CD3γ, CD3δ, CD3ε, CD3ζ, CD79a, CD79b, DAP12 (TYROBP), FcRγ, Fcε Receptor γ chain (FCER1G), Dectin-1, FcRL1, CLEC-2, or combinations thereof.

12. The transformed cell according to claim 11, wherein the coreceptor is Fcε Receptor γ chain (FCER1G).

13. The transformed cell according to claim 10, wherein the coreceptor is a chimeric coreceptor and comprises:

i) CD3 γ intracellular domain, CD3 δ intracellular domain, CD3 ϵ intracellular domain, CD3 ζ intracellular domain, CLEC-2 intracellular domain, CD79a intracellular domain, CD79b intracellular domain, DAP12 (TYROBP) intracellular domain, Fc ϵ Receptor common γ chain (FCER1G) intracellular domain, or Dectin-1 intracellular domain; and

ii) Fc γ RI extracellular domain, Fc γ RIIa extracellular domain, Fc γ RIIb extracellular domain, Fc γ RIIc extracellular domain, Fc γ RIIIa extracellular domain, Fc γ RIIIb extracellular domain, DC-SIGN extracellular domain, TRIM21 extracellular domain, DC-SIGN extracellular domain, Fc α RI extracellular domain, or Fc ϵ RI extracellular domain.

14. The transformed cell of any one of claims 10-13, wherein the transformed cell comprises the Fc γ RI α -chain and a ratio of coreceptor copy number to a Fc γ RI α -chain copy number is 1:1 or more.

15. The transformed cell of claim 14, wherein Fc γ RI α -chain associates with an endogenously expressed coreceptor.

16. The transformed cell of any one of claims 10-13, wherein the transformed cell comprises the Fc γ RIIIa α -chain and a ratio of coreceptor copy number to a Fc γ RIIIa α -chain copy number is 1:1 or more.

17. The transformed cell according to claim 16, wherein Fc γ RIIIa α -chain associates with an endogenously expressed coreceptor.

18. The transformed cell according to any one of claims 1-17, wherein the transformed cell lacks endogenous human Fc γ RI α -chain, human Fc γ RIIa, and human Fc γ RIIIa α -chain.

19. The transformed cell of any one of claims 1-17, further comprising a nucleic acid encoding a reporter.

20. The transformed cell according to claim 19, wherein the reporter is selected from i) reporters derived from Firefly, Renilla, Guassia, or Cypridina or ii) beta-galactosidase, beta-lactamase, red fluorescent protein, green fluorescent protein (GFP), or derivatives thereof.

21. The transformed cell according to claim 20, wherein the reporter is a luminescence reporter.

22. The transformed cell according to claim 21, wherein the luminescence reporter is luciferase.

23. The transformed cell according to claim 19, wherein the cell comprises a transcription response element (TRE) operably linked to the nucleic acid encoding the reporter.

24. The transformed cell according to claim 23, wherein the transcription response element is selected from the group consisting of Nuclear Factor of Activated T-cells (NFAT), NF- κ B, AP-1, and IL-2 promoter.

25. The transformed cell according to claim 19, wherein the nucleic acid encoding the reporter further encodes NFAT.

26. The transformed cell of anyone of claim 1-19, wherein the cell comprises a nucleic acid encoding for a selection marker, wherein the selection marker provides the cell with antibiotic resistance.

27. The transformed cell according to claim 26, wherein the antibiotic resistance is against Zeocin, puromycin, neomycin, hygromycin, or blasticidin.

28. The transformed cell of any one of claims 1-27, wherein the nucleic acid encoding i) the human Fc γ RI α -chain further comprises a nucleic acid encoding for a selection marker; ii) the human Fc γ RIIa further comprises a nucleic acid encoding for a

selection marker; or iii) the human FcγRIIIa α-chain further comprises a nucleic acid encoding for a selection marker.

29. The transformed cell of claim 10, wherein the nucleic acid encoding for the coreceptor further comprises a nucleic acid encoding for a selection marker, wherein the selection marker provides the cell with antibiotic resistance.

30. The transformed cell of any one of claims 1-29, wherein the cell comprises a nucleic acid encoding human FcγRI α-chain, a Fcε Receptor Ig (FCER1G), and a selection marker.

31. The transformed cell of any one of claims 1-30, wherein the cell comprises a nucleic acid encoding a human FcγRIIIa α-chain, a FCER1G, and a selection marker.

32. The transformed cell of any one of claims 1-31, wherein the cell comprises a nucleic acid encoding a human FcγRIIa and a selection marker.

33. The transformed cell of any one of claims 1-32, wherein the cell comprises a nucleic acid encoding NFAT response element operably linked to luciferase and a selection marker.

34. The transformed cell of any one of claims 1-33, wherein the cell comprises:
i) a nucleic acid encoding human FcγRI α-chain, a FCER1G, and a selection marker, ii) a nucleic acid encoding a human FcγRIIIa α-chain, a FCER1G, and a selection marker, iii) a nucleic acid encoding a human FcγRIIa and a selection marker, and iv) a nucleic acid encoding NFAT response element operably linked to luciferase and a selection marker.

35. The transformed cell according to any of claims 1-34, wherein the transformed cell is a human cell.

36. The transformed cell according to claim 35, wherein the human cell is a T lymphoblastoid cell.

37. The transformed cell according to claim 36, wherein the cell is a transformed Jurkat cell, transformed COS-1 cell, transformed COS-7 cell, transformed HEK cell, or transformed CHO cell.

38. A nucleic acid encoding a human FcγRI α-chain, an ITAM coreceptor, and a selection marker.

39. The nucleic acid according to claim 38, wherein the ITAM coreceptor is a FCER1G.

40. The nucleic acid according to claim 38, wherein the human FcγRI α-chain has the polypeptide sequence of SEQ ID NO: 26 and the ITAM coreceptor is FCER1G and has the polypeptide sequence of SEQ ID NO: 27.

41. The nucleic acid according to claim 38, wherein the selection marker encodes for antibiotic resistance to hygromycin.

42. A vector comprising the nucleic acid of any one of claims 38-41.

43. A nucleic acid encoding a human FcγRIIIa α-chain, an ITAM coreceptor, and a selection marker.

44. The nucleic acid according to claim 43, wherein the selection marker encodes for antibiotic resistance to blasticidin.

45. The nucleic acid according to claim 43, wherein the ITAM coreceptor is a FCER1G.

46. The nucleic acid according to claim 43, wherein i) the human FcγRIIIa α-chain is V158 variant and has the polypeptide sequence of SEQ ID NO: 28 and ii) the ITAM coreceptor is FCER1G and has the polypeptide sequence of SEQ ID NO: 27.

47. A vector comprising the nucleic acid of any one of claims 43-46.
48. A nucleic acid encoding a human Fc γ RIIa and a selection marker.
49. The nucleic acid according to claim 48, wherein the selection marker encodes for antibiotic resistance to Zeocin.
50. The nucleic acid according to claim 48, wherein i) the human Fc γ RIIa is H131 variant and has the polypeptide sequence of SEQ ID NO: 25.
51. A vector comprising the nucleic acid of any one of claims 48-51.
52. A nucleic acid encoding NFAT response element operably linked to luciferase and a selection marker.
53. The nucleic acid according to claim 52, wherein the selection marker encodes for antibiotic resistance to puromycin.
54. A vector comprising the nucleic acid of any one of claims 52-53.
55. A process of making a transformed cell comprising:
- i) transforming a cell such that it comprises a nucleic acid encoding a human Fc γ RI α -chain or a variant thereof; a nucleic acid encoding a human Fc γ RIIa or a variant thereof; or a nucleic acid encoding a human Fc γ RIIIa α -chain or a variant thereof; and
 - ii) selecting a transformed cell that comprises the nucleic acids encoding Fc γ RI α -chain, Fc γ RIIa, and Fc γ RIIIa α -chain.
56. The process according to claim 55, wherein the transformed cell further comprises a nucleic acid encoding a reporter and/or a selection marker.

57. The process according to claim 55, wherein the transformed cell further comprises a coreceptor nucleic acid encoding coreceptor comprising an ITAM, or a variant thereof.

58. The process according to claim 57, wherein the coreceptor nucleic acid encodes for FCER1G.

59. The process according to claim 55, wherein the transformed cell further comprises a transcription response element operably linked to a nucleic acid encoding the reporter.

60. The process according to claim 55, wherein the transformed cell is a Jurkat cell, COS-1 cell, COS-7 cell, HEK cell, or CHO cell.

61. The process of claim 55, wherein the cell comprises the nucleic acid encoding the FcγRI or the variant thereof; the nucleic acid encoding the FcγRIIa or the variant thereof; and the nucleic acid encoding the FcγRIIIa α-chain or the variant thereof.

62. The process of claim 61, wherein the cell further comprises the coreceptor nucleic acid encoding coreceptor comprising the ITAM or the variant thereof.

63. A method for identifying an antibody or a fragment thereof having at least one mechanism of action mediated through activation of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof, the method comprising the steps of:

- i) providing the antibody or a fragment thereof;
- ii) contacting the antibody or a fragment thereof with a transformed cell of any one of claims 1-37 for a time period sufficient to allow activation of one or more of FcγRI α-chain, FcγRIIa, FcγRIIIa α-chain, a variant thereof, or a combination thereof;
- iii) detecting a signal generated upon contacting the antibody or a fragment thereof with the transformed cell;

- iv) analyzing the signal generated in step iii) to determine whether one or more of the receptors of step ii) are activated; and
- v) identifying the antibody or a fragment thereof as an activator of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof.

64. The method according to claim 63, wherein the antibody or the fragment thereof binds to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof.

65. The method according to claim 63, wherein the signal is generated due to binding of the antibody or the fragment thereof to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof.

66. The method according to claim 63, wherein the antibody or a fragment thereof is contacted with its antigen to form an antibody-antigen complex prior to contacting the antibody or a fragment thereof with the transformed cell.

67. The method according to claim 66, wherein the antigen is immobilized on a substrate.

68. The method according to claim 67, wherein the substrate is selected from the group consisting of a microtiter plate, a coverslip, a slide, a particle, a membrane, a film, and a carbon nanotube.

69. The method according to claim 63, wherein the transformed cell is added at a concentration ranging from about 10,000 cells/mL to about 5,000,000 cells/mL.

70. The method according to claim 63, wherein the signal is detected using a luminometer.

71. The method according to claim 63, wherein the detecting a signal step is performed by measuring a reporter activity.

72. The method according to claim 63, wherein at least one mechanism of action of the antibody or a fragment thereof is to induce positive signaling via an ITAM.

73. The method according to claim 63, wherein at least one mechanism of action of the antibody or a fragment thereof is mediated i) through the recruitment of Fc bearing cells, ii) by inducing an antibody response, or iii) by inducing phagocytosis.

74. The method according to claim 63, wherein the recruitment of Fc bearing cells comprises recruitment of platelet, dendritic cell, microglial cell, Kupfer cell, alveolar macrophage, monocyte, polymorphonuclear cell (PMN), B cell, or natural killer cell.

75. The method according to claim 63, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through microglial cell.

76. The method according to claim 63, wherein at least one mechanism of action of the antibody or fragment thereof is to induce clearance of immune complexes.

77. The method according to claim 63, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through i) Fc mediated cytokine production, ii) antibody dependent cell-mediated cytotoxicity (ADCC), or iii) antibody dependent cell phagocytosis (ADCP).

78. The method according to claim 77, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of ADCC via polymorphonuclear leukocytes, monocytes, or macrophages.

79. The method according to claim 77, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of ADCP via polymorphonuclear leukocytes, monocytes, or macrophages.

80. The method according to claim 77, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through Fc mediated cytokine production via polymorphonuclear leukocytes.

81. The method according to claim 63, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of PMN degranulation.

82. The method according to claim 63, wherein the antibody or fragment thereof activates FcγRI α-chain or a variant thereof.

83. The method according to claim 63, wherein the antibody or fragment thereof activates FcγRIIa or a variant thereof.

84. The method according to claim 63, wherein the antibody or fragment thereof activates FcγRIIIa α-chain or a variant thereof.

85. The method according to claim 63, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through cross-linking of cells and/or antibodies, immobilized antigens.

86. The method according to claim 63, wherein a mechanism of action of the antibody or fragment thereof is to induce positive signaling via common γ chain, common β chain, common ζ chain, or a coreceptor comprising an ITAM motif.

87. The method according to claim 63, wherein a mechanism of action of the antibody or fragment thereof is to induce positive signaling via a chimeric Fc receptor.

88. The method according to claim 63, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the recruitment of myeloid cells, polymorphonuclear leukocytes or natural killer cells.

89. The method according to claim 63, wherein at least one mechanism of action of the antibody or fragment thereof is to induce negative signaling through an immunoreceptor tyrosine-based inhibition motif.

90. The method according to claim 63, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the recruitment of B cells, macrophages and/or monocytes.

91. The method according to claim 63, wherein the antibody or fragment thereof is a monoclonal antibody, a human antibody, a humanized antibody, or a chimeric antibody.

92. The method according to claim 63, wherein the antibody or fragment thereof is an IgG1 antibody.

93. The method according to claim 63, wherein the antibody or fragment thereof binds to N3pE amyloid beta (N3pE A β), amyloid beta (A β), or A β plaques.

94. The method according to claim 63, wherein the antibody is donanemab.

95. The method according to claim 63, wherein the antibody is remtemetug.

96. A method for assessing an antibody or fragment thereof for a mechanism of action mediated through activation of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof, the method comprising the steps of:

- i) providing a drug product comprising the antibody or the fragment thereof;
- ii) providing a reference standard antibody or a fragment thereof;
- iii) contacting the antibody or the fragment thereof with a first transformed cell of any one of claims 1-37 for a time period sufficient to allow activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof;

- iv) contacting the reference standard with a second transformed cell of any one of claims 1-37 for a time period sufficient to allow activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof;
- v) measuring the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof by the antibody or fragment thereof;
- vi) measuring the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof by the reference standard;
- vii) comparing the activation of the Fc receptors of step v) to the activation of Fc receptors of step vi); and
- viii) selecting the antibody or fragment thereof that has an acceptable Fc receptor activation as compared to the reference standard.

97. The method according to claim 96, wherein the antibody or fragment thereof has equal to or greater activation of at least one Fc receptor as compared to the reference standard.

98. The method according to claim 96, wherein activation of at least one Fc receptor by the antibody or fragment thereof matches the release specification for the antibody or fragment thereof.

99. The method according to claim 96, wherein the activation of at least one Fc receptor by the antibody or fragment thereof is i) from about 50% to about 150% of the reference standard, ii) from about 60% to about 140% of the reference standard, iii) from about 70% to about 130% of the reference standard, iv) from about 80% to about 130% of the reference standard, v) from about 90% to about 110% of the reference standard, or vi) about 100% of the reference standard.

100. The method according to claim 96, wherein the activation of at least one Fc receptor by the antibody or fragment thereof is from about 80% to about 130% of the reference standard.

101. The method according to claim 96, wherein the activation of at least one Fc receptor by the antibody or fragment thereof is from about 60% to about 140% of the reference standard.

102. The method according to claim 96, wherein the reference standard and the antibody or fragment thereof are two different batches of the same antibody or fragment thereof.

103. The method according to claim 96, wherein the antibody or the fragment thereof and/or the reference standard binds to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof.

104. The method according to claim 96, wherein a signal is generated due to binding of the antibody or the fragment thereof and/or the reference standard to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof.

105. The method according to claim 96, wherein the antibody or a fragment thereof and/or the reference standard is contacted with its antigen to form an antibody-antigen complex prior to contacting the antibody or a fragment thereof with the transformed cell.

106. The method according to claim 105, wherein the antigen is immobilized on a substrate.

107. The method according to claim 106, wherein the substrate is selected from the group consisting of a microtiter plate, a coverslip, a slide, a particle, a membrane, a film, and a carbon nanotube.

108. The method according to claim 96, wherein the first and second transformed cells are added at a concentration ranging from about 10,000 cells to about 200,000 cells.

109. The method according to claim 96, wherein a first signal is generated and detected upon contacting the antibody or the fragment thereof to the first transformed cell and a second signal is generated and detected upon contacting the reference standard to the second transformed cell.

110. The method according to claim 109, wherein the first signal and/or the second signal is detected using a luminometer.

111. The method according to claim 110, wherein the signal detection is performed by measuring a reporter activity.

112. The method according to claim 96, wherein at least one mechanism of action of the antibody or a fragment thereof is to induce positive signaling via an immunoreceptor tyrosine-based activation motif.

113. The method according to claim 96, wherein at least one mechanism of action of the antibody or a fragment thereof is mediated i) through the recruitment of Fc bearing cells, ii) by inducing an antibody response, or iii) by inducing phagocytosis.

114. The method according to claim 113, wherein the recruitment of Fc bearing cells comprises recruitment of platelet, dendritic cell, microglial cell, Kupfer cell, alveolar macrophage, monocyte, polymorphonuclear cell (PMN), B cell, or natural killer cell.

115. The method according to claim 96, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through microglial cell.

116. The method according to claim 96, wherein at least one mechanism of action of the antibody or fragment thereof is to induce clearance of immune complexes.

117. The method according to claim 96, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through i) Fc mediated cytokine production, ii) antibody dependent cell-mediated cytotoxicity (ADCC), or iii) antibody dependent cell phagocytosis (ADCP).

118. The method according to claim 117, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of ADCC via polymorphonuclear leukocytes, monocytes, or macrophages.

119. The method according to claim 117, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of ADCP via polymorphonuclear leukocytes, monocytes, or macrophages.

120. The method according to claim 117, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through Fc mediated cytokine production via polymorphonuclear leukocytes.

121. The method according to claim 96, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the induction of PMN degranulation.

122. The method according to claim 96, wherein the antibody or fragment thereof activates Fc γ RI α -chain or a variant thereof.

123. The method according to claim 96, wherein the antibody or fragment thereof activates Fc γ RIIa or a variant thereof.

124. The method according to claim 96, wherein the antibody or fragment thereof activates Fc γ RIIIa α -chain or a variant thereof.

125. The method according to claim 96, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through cross-linking of cells and/or antibodies, immobilized antigens.

126. The method according to claim 96, wherein a mechanism of action of the antibody or fragment thereof is to induce positive signaling via common γ chain, common β chain, common ζ chain, or a coreceptor comprising an ITAM motif.

127. The method according to claim 96, wherein a mechanism of action of the antibody or fragment thereof is to induce positive signaling via a chimeric Fc receptor.

128. The method according to claim 96, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the recruitment of myeloid cells, polymorphonuclear leukocytes or natural killer cells.

129. The method according to claim 96, wherein at least one mechanism of action of the antibody or fragment thereof is to induce negative signaling through an immunoreceptor tyrosine-based inhibition motif.

130. The method according to claim 96, wherein at least one mechanism of action of the antibody or fragment thereof is mediated through the recruitment of B cells, macrophages and/or monocytes.

131. The method according to claim 96, wherein the antibody or fragment thereof and/or the reference standard is a monoclonal antibody, a human antibody, a humanized antibody, or a chimeric antibody.

132. The method according to claim 96, wherein the antibody or fragment thereof and/or the reference standard is an IgG1 antibody.

133. The method according to claim 96, wherein the antibody or fragment thereof and/or the reference standard binds to N3pE A β , amyloid beta (A β), or A β plaques.

134. The method according to claim 96, wherein the antibody and/or the reference standard is donanemab.

135. The method according to claim 96, wherein the antibody and/or the reference standard is remternetug.

136. A method of selecting at least one cell clone for the manufacture of a drug substance, wherein the drug substance is an antibody or a fragment thereof and at least one mechanism of action of the antibody or the fragment thereof is mediated through activation of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof, the method comprising the steps of:

- i) providing at least one drug substance obtained from at least one transformed cell clone;
- ii) providing a reference standard antibody or a fragment thereof;
- iii) contacting the drug substance with a first transformed cell of any one of claims 1-38 for a time period sufficient to allow activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof;
- iv) contacting the reference standard with a second transformed cell of any one of claims 1-37 for a time period sufficient to allow activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof;
- v) measuring the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof by the drug substance;
- vi) measuring the activation of one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof by the reference standard;

- vii) comparing the activation of the Fc receptors of step v) to the activation of Fc receptors of step vi); and
- viii) selecting the cell clone that has an acceptable activation profile as compared to the reference standard.

137. The method according to claim 136, wherein the drug substance has equal to or greater activation of at least one Fc receptor as compared to the reference standard.

138. The method according to claim 136, wherein activation of at least one Fc receptor by the drug substance matches the release specification for the drug substance.

139. The method according to claim 136, wherein the activation of at least one Fc receptor by the drug substance is i) from about 50% to about 150% of the reference standard, ii) from about 60% to about 140% of the reference standard, iii) from about 70% to about 130% of the reference standard, iv) from about 80% to about 130% of the reference standard, v) from about 90% to about 110% of the reference standard, or vi) about 100% of the reference standard.

140. The method according to claim 136, wherein the activation of at least one Fc receptor by the drug substance is from about 80% to about 130% of the reference standard.

141. The method according to claim 136, wherein the activation of at least one Fc receptor by the drug substance is from about 60% to about 140% of the reference standard.

142. The method according to claim 136, wherein the drug substance and/or the reference standard binds to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof.

143. The method according to claim 136, wherein the signal is generated due to binding of the drug substance and/or the reference standard to one or more of Fc γ RI α -chain, Fc γ RIIa, Fc γ RIIIa α -chain, a variant thereof, or a combination thereof.

144. The method according to claim 136, wherein the drug substance is contacted with its antigen to form an antibody-antigen complex prior to contacting the drug substance with the transformed cell.

145. The method according to claim 136, wherein the reference standard is contacted with its antigen to form a complex prior to contacting the reference standard with the transformed cell.