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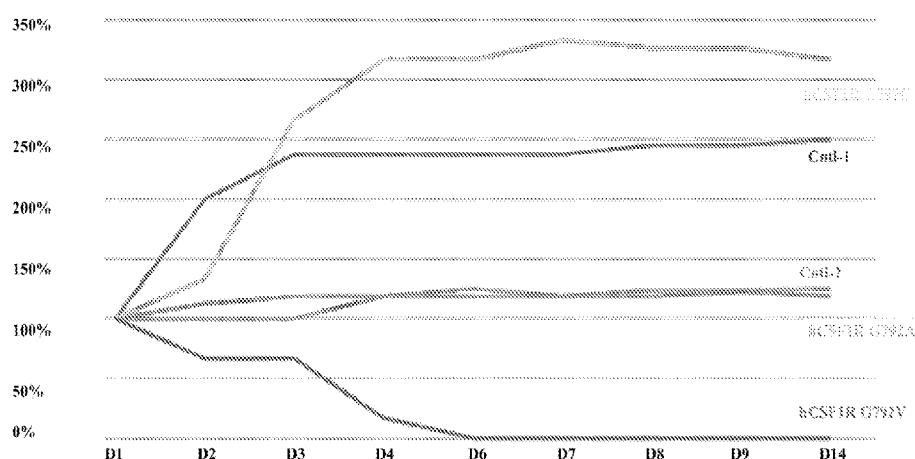
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(54) Title: GENETIC MODIFICATION OF MAMMALIAN CELLS TO CONFER RESISTANCE TO CSF1R ANTAGONISTS

FIG. 1

In Vitro Growth Kinetics of Control and CSF1R mutant Microglia



(57) Abstract: Microglia/monocytes exist within a 'niche' which limits the total number of microglia/monocytes/macrophages that reside within a mammalian central nervous system (CNS). Therefore, methods are needed that can help therapeutically modify microglia, monocytes, and macrophages or the cells that give rise to them to compete with endogenous microglia and partially or completely occupy the CNS niche. The present disclosure features therapeutic microglia, monocytes, or macrophages that have a selective advantage in comparison to endogenous brain resident microglia in their response to CSF1 R inhibitors. Specifically, therapeutic cells developed in the present disclosure do not die at a given dose of CSF1 R inhibitor that is sufficient to kill endogenous microglia. The therapeutic cells described herein can be used to treat neurological diseases.

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GENETIC MODIFICATION OF MAMMALIAN CELLS TO CONFER RESISTANCE TO CSF1R ANTAGONISTS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 63/169,578 filed April 1, 2021, and U.S. Provisional Application No. 63/236,951 filed August 25, 2021, the contents of which is/are incorporated herein in their entirety by reference.

REFERENCE TO A SEQUENCE LISTING

[0002] Applicant asserts that the information recorded in the form of an Annex C/ST.25 text file submitted under Rule 13ter.1(a), entitled >>>UCI 21.03 PCT Sequencing Listing_ST25<<<, is identical to that forming part of the international application as filed. The content of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The present disclosure features methods and compositions for the treatment of neurological diseases; in particular, the methods and compositions feature cells with partial or complete resistance to CSF1R antagonists.

BACKGROUND OF THE DISCLOSURE

[0004] Almost every human neurological disease and injury involves impairments or alterations in the function, gene expression, and activation state of microglia, the resident immune cell of the brain. In some cases, genetic mutations in microglia are the primary cause of neurological disease, whereas, in other diseases, polymorphisms in microglial genes increase the risk of developing the disease. Unfortunately, there are few, if any, effective therapies for many of these diseases and injuries.

[0005] Microglia, monocytes, and macrophages exist within a 'niche' that can limit the total number of microglia, monocytes, and macrophages that reside within the mammalian central nervous system (CNS). Therefore, methods are needed that can help therapeutically modify microglia, monocytes, and macrophages or the cells that give rise to them to compete with endogenous microglia and partially or completely occupy the CNS niche. The goal of the present disclosure is to develop microglial-based therapeutics, including approaches to transplant genetically modified human microglia or related monocyte lineages or progenitor cells into patients.

BRIEF SUMMARY OF THE DISCLOSURE

[0006] It is an objective of the present disclosure to provide compositions and methods that allow for differential resistance to CSF1R antagonists (e.g., partial; complete; increase; decrease) for the treatment of neurological diseases..

[0007] Microglia rely on CSF1R signaling via two ligands (CSF1 and IL-34) for survival, proliferation, and self-renewal. Treatment of mammalian models with various CSF1R antagonists reduces the number of

microglia within the central nervous system. Furthermore, when these compounds are removed, surviving microglia proliferate and rapidly refill the niche. Additionally, it has been found that human stem cell-derived microglia transplanted into the murine brain are also susceptible to and killed by CSF1R inhibition (FIG. 11F, 11G, 11H, 11I, 11J).

[0008] To compete with the endogenous microglia niche, therapeutic microglia, monocytes, macrophages, or their progenitors or precursors would need to have a selective advantage in comparison to endogenous brain resident microglia in their response to CSF1R inhibitors. Specifically, one would need to develop therapeutic cells that do not die at a given dose of CSF1R inhibitor that is sufficient to kill endogenous microglia. In some instances, this selective advantage should be only partial so that a higher dose of CSF1R inhibitors could also kill therapeutic microglia, macrophages, monocytes, or their progenitors or precursors should the need arise for safety purposes.

[0009] In some embodiments, the present disclosure features a cell (e.g., a modified human cell) comprising a nucleic acid encoding a modified CSF1R protein (e.g., a human CSF1R protein) exhibiting differential resistance to a CSF1R antagonist. In other embodiments, the present disclosure features a modified human cell exhibiting differential resistance to a CSF1R antagonist. In further embodiments, the present disclosure features a modified human cell comprising a nucleic acid encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist.

[0010] In some embodiments, the present disclosure may also feature a method of treating a subject. In some embodiments, the method may comprise administering a CSF1R antagonist to the subject in a quantity sufficient to inhibit a CSF1R signal in a cell of the subject and contacting the subject with a modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) having a differential resistance to the CSF1R antagonist. In other embodiments, the method may comprise contacting the subject with a modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) having a differential resistance to a CSF1R antagonist and administering the CSF1R antagonist to the subject in a quantity sufficient to inhibit a CSF1R signal in a cell of the subject.

[0011] In further embodiments, the present disclosure also features a method of treating a subject. The method comprises contacting the subject with a modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) and differentially altering the proliferation or survival of an unmodified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) relative to the modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte).

[0012] In some embodiments, the present disclosure may further feature a nucleic acid composition and vectors encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist as described herein.

[0013] Technical features of the present disclosure include the use of differential resistance to CSF1R

antagonists (e.g., partial, complete, increased, or decreased resistance) to create therapeutic microglia, macrophages, monocytes, or their progenitors or precursors for the treatment of neurological diseases. Without wishing to limit the disclosure to any theory or mechanism, it is believed that the technical feature of the present disclosure advantageously provides for the generation of microglia, monocytes, HSPCs (hematopoietic stem or progenitor cells), or primitive macrophages from pluripotent stem cells with differential resistance to CSF1R antagonists (e.g., CSF1R antagonist resistance). As described herein, without some form of conditioning or selective advantage, transplantation of the aforementioned cells will likely exhibit only partial or limited engraftment into the central nervous system (CNS). The present disclosure develops the currently described approach that will significantly facilitate the therapeutic engraftment of microglia, macrophages, or monocytes or their progenitors (HSPCs, PMPs) in the mammalian brain. None of the presently known prior references or work has the unique, inventive technical feature of the present invention.

[0014] The compositions and methods of this disclosure represent a significant departure from the current paradigm for the use of CSF1R antagonists in medicine. For example, the prior art teaches the use of a CSF1R inhibitor to clear endogenous microglia and facilitate the entry of bone marrow- and/or blood-derived monocytes into the brain. However, bone marrow- and/or blood-derived monocytes that infiltrate the brain remain functionally and transcriptionally distinct from microglia even many months after infiltration. Additionally, the bone marrow- and/or blood-derived monocytes do not have the same sensitivity to CSF1R antagonists as microglia, demonstrating that these cells are not microglia. This *teaches away* from the present disclosure, which facilitates the expansion of therapeutic microglia (e.g., human microglia) comprising a modified CSF1R protein for the treatment of neurological diseases.

[0015] Additionally, most major pharmaceutical companies have developed CSF1R antagonists compounds for the treatment of some forms of cancer. However, endogenous microglia are extremely sensitive to CSF1R antagonists and will die in response to treatments with higher doses of these compounds. This is true of human stem cell-derived microglia transplanted into the murine brain (FIG. 11F, 11G, 11H, 11I, 11J). and cultured human microglia as well (FIG. 4A, 4B, 4C, 4D, and 4E). Paradoxically, the present disclosure utilizes compounds (e.g., CSF1R antagonist) that are toxic to microglia in order to improve the long-term engraftment of transplanted microglia (e.g., transplanted therapeutic microglia; i.e., microglia comprising a modified CSF1R protein). The methods of this disclosure resolve the challenge of achieving widespread engraftment of transplanted microglia (e.g., transplanted therapeutic microglia; i.e., microglia comprising a modified CSF1R protein) in an established microglial niche that would not ordinarily be amenable to such engraftment.

[0016] Furthermore, the technical features of the present disclosure contributed to a surprising result and illustrates the unpredictability of outcomes associated with these mutations. For example, two of the proposed point mutations lead to complete (100%) protection against two CSF1R inhibitors (i.e., CSF1R antagonist) across multiple escalating doses when assessed 24 hours after inhibitor treatment (FIG. 4A,

4B, 4C, 4D, and 4E). In contrast, a third mutation, G795V, prevented the successful differentiation of iPSCs into microglia (FIG. 1). These data demonstrate the importance of the specific DNA and resulting amino acid change that is introduced into CSF1R and that while some changes will achieve the desired resistance to CSF1R inhibitors (i.e., CSF1R antagonist), others may instead compromise the ability for microglial differentiation or survival.

[0017] Thus, the present disclosure features methods and compositions to facilitate the competitive engraftment of human microglia, macrophages, and monocytes and/or their precursor cells (HSPCs, erythromyeloid progenitors (EMPs), or Primitive Macrophage Progenitors (PMPs)). Cells can be derived from a variety of sources, including but not limited to pluripotent stem cells, including embryonic stem cells and induced pluripotent stem cells (iPSCs), bone marrow-derived hematopoietic stem cells, cord blood cells, blood-derived monocytes, fetal yolk sac or tissue macrophages, adult tissue-derived macrophages, or through direct reprogramming of another cell type into a microglia, HSPC, monocyte, or macrophage. Additionally, the present disclosure may feature methods and compositions to facilitate the competitive engraftment of CSF1R expressing cells, including but not limited to cells from the heart, skin, liver, lungs, kidney, eye, prostate, and ovary.

[0018] Any feature or combination of features described herein are included within the scope of the present disclosure provided that the features included in any such combination are not mutually inconsistent as will be apparent from the context, this specification, and the knowledge of one of ordinary skill in the art. Additional advantages and aspects of the present disclosure are apparent in the following detailed description and claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0019] The features and advantages of the present disclosure will become apparent from a consideration of the following detailed description presented in connection with the accompanying drawings in which:

[0020] FIG. 1 shows *in vitro* growth kinetics of control and CSF1R mutant microglia. Isogenic iPSC-derived hematopoietic progenitors were differentiated towards microglia, and cell density was used for the first 14 days. The two wild-type isogenic control lines (Cntl-1 and Cntl-2) demonstrate that some degree of variability is expected in the proliferation rates of iPSC-microglia. Both the hCSF1R G795A and G785C mutants exhibit proliferation rates that are comparable to the two control lines. In contrast, the hCSFR G794V mutant exhibits a rapid reduction in cell density, with no viable cells remaining beyond day 6 of differentiation. These data demonstrate that the specific choice of amino acid substitution is critical for the successful generation of viable microglia that can also exhibit CSF1R antagonist resistance.

[0021] FIG. 2A, 2B, 2C, 2D, 2E, and 2F show selected CSF1R mutations have little effect on transcriptomic signature while simultaneously imparting resistance to PLX5622. FIG. 2A shows a principal component analysis using the top 2,000 genes reveals that the primary source of variation in the dataset

is the WT response to PLX5622. FIG. 2B and FIG. 2C shows a linear regression analysis and calculation of the Pearson correlation coefficient between DMSO-treated MUT1 (i.e., G795A; FIG. 2B) or MUT2 (i.e., G795C; FIG. 2C) and WT microglia confirmed a high degree of concordance when examining the full transcriptome, and the 249 genes core microglial gene signature. FIG. 2D, 2E, and 2F show a 24-hour treatment of WT (FIG. 2D), MUT1 (i.e., G795A; FIG. 2E), and MUT2 (i.e., G795C; FIG. 2F) microglia with PLX5622 induced significant transcriptomic alterations in the WT cells ($FDR \leq 0.05$; $\log_2(FC) \geq \pm 1$) when compared to DMSO-treated cells. However, treatment of both the MUT1 and MUT2 lines failed to significantly alter gene expression, suggesting that these lines are resistant to the effects of PLX5622.

[0022] FIG. 3 shows a distance matrix of CSF1R mutants and control xMGs (microglia). RNA-seq expression counts were normalized and transformed using a variance stabilizing transformation. Pairwise Euclidean distances were calculated between all samples, and samples were hierarchically clustered by distance. These data demonstrate that the transcriptome of G795A mutant microglia is most equivalent to that of WT CSF1R microglia.

[0023] FIG. 4A, 4B, 4C, 4D, and 4E show human stem cell-derived microglia are also susceptible to and killed by CSF1R inhibition *in vitro*. FIG. 4A shows caspase 3/7 levels imaged over 24 hours in culture with complete medium with 0.1%DMSO, 250nm PLX3397, 500nm PLX3397, and 1 μ m PLX 3397. Images captured on Incucyte S3 live-cell imager. FIG. 4B shows caspase 3/7 levels imaged over 24 hours in culture with complete medium with 0.1%DMSO, 250nm PLX5622, 500nm PLX5622, and 1 μ m PLX5622. Images captured on Incucyte S3 live-cell imager. FIG. 4C shows caspase 3/7 levels imaged over 24 hours in culture with complete medium with 0.1%DMSO, 250nm Edicotinib (JNJ-40346527), 500nm Edicotinib, and 1 μ m Edicotinib. Images captured on Incucyte S3 live-cell imager. FIG. 4D shows caspase 3/7 levels imaged over 24 hours in culture with complete medium with 0.1%DMSO, 250nm BLZ945, 500nm BLZ945, and 1 μ m BLZ945. Images captured on Incucyte S3 live-cell imager. FIG. 4E shows representative images of caspase 3/7 fluorescent activity for Wildtype, G795A, and G795C microglia after 24 hrs in culture with PLX3397. For all panels, n=4 images in six independent wells were quantified. Data represented as mean values $\pm$ SEM.

[0024] FIG. 5A and 5B show G795A iPSC-derived microglia are resistant to CSF1R antagonist-induced cell death. As cells can die from processes other than caspase-mediated apoptosis, it is important to determine whether the G795A mutation provides a more broad-based resistance to any form of cell death induced by CSF1R antagonists. Therefore, G795A and wild-type (WT) iPSC-microglia were treated for 24 hours with either 500nM (FIG. 5A) or 1 μ M (FIG. 5B) of DMSO vehicle control or one of four CSF1R antagonists; PLX3397, PLX5622, Edicotinib, and BLZ945. The percent of live versus dead cells was then quantified using a two-color viability/cytotoxicity approach (Thermo L3224; LIVE/DEAD™ Viability/Cytotoxicity Kit). Very few dead microglia are observed 24 hours after treatment with DMSO. In contrast, treatment with CSF1R antagonists induces substantial cell death in wild-type microglia but

little-to-no cell death in G795A microglia. Live cells are denoted with a circle.

[0025] FIG. 6 shows treatment of microglia expressing mutant CSF1R with 25ng/ml Human Macrophage Colony Stimulating Factor (Peprotech #300-25) after 48 hours stimulates tyrosine phosphorylation of CSF-1R/M-CSF-R protein, as detected by PathScan® Phospho-CSF-1R/M-CSF-R (panTyr) Sandwich ELISA Kit n=7. Microglia expressing CSF1R G795A phosphorylate similarly, while microglia expressing CSF1R G795C demonstrate elevated levels of phosphorylation in comparison to WT microglia. These data suggest that whereas both G795A and G795C confer resistance to CSF1R antagonists, the G795C mutation may lead to enhanced CSF1R signaling. Data represented as mean values $\pm$ SEM.

[0026] FIG. 7A, 7B, 7C and 7D show the crystal structure of the CSF1R receptor bound to a CSF1R antagonist. FIG. 7A shows a human CSF1R receptor bound to a PLX5622 CSF1R antagonist, and FIG. 7B shows a CSF1R receptor bound to a PLX3397 CSF1R antagonist. The crystal structure was examined to predict amino acid substitutions that may sterically hinder the binding of CSF1R antagonists while not impairing the normal binding of ATP into the same binding pocket. The crystal structure of the CSF1R antagonist PLX5622 (FIG. 7A) or PLX3397 (FIG. 7B) bound to human CSF1R was previously published. Using this publicly available data, the ATP binding pocket was examined. Through molecular modeling, single amino acid changes were predicted (FIG. 7C) that would likely impair the binding of PLX5622 or PLX3397 or other CSF1R antagonists while not disrupting normal ATP binding to CSF1R. Specifically, this modeling suggests that replacement of amino acid G795 with either an Alanine (A), Valine (V), or Cysteine (C) will increase the steric hindrance of PLX5622 and/or PLX3397 binding without disrupting the normal ability of ATP to bind. Other mutations could also be explored to prevent binding (FIG. 7D).

[0027] FIG. 8 shows DNA chromatograms demonstrate the successful production of isogenic human iPSC cell lines that encode single amino acid point mutations at position 795 within the CSF1R coding sequence. In this particular case, each line was generated via CRISPR-mediated gene editing. However, similar changes could be introduced via various other gene-editing methods, including but not limited to TALENs, traditional homologous recombination, viral gene delivery, or other CRISPR variants.

[0028] FIG. 9A, 9B, 9C, 9D, and 9E show selected CSF1R mutations have little effect on transcriptomic signature *in vivo*. FIG. 9A shows a principal component analysis using the top 2,000 genes reveals that the primary source of variation between G795A, G795C, and WT microglia is attributed to individual animals and not CSF1R mutations. In contrast, when G795V is compared there is some separation based on this mutation, further indicating that G795V may not enable the generation of microglia without substantially altering gene expression. FIG. 9B and 9C show a linear regression analysis and calculation of the Pearson correlation coefficient between G795A (FIG. 9B) or G795C (FIG. 9C) and WT microglia confirmed a high degree of concordance when examining the full transcriptome and the 190 genes core microglial signature. FIG. 9D and 9E show a volcano plot of human microglia explanted 2-months

post-transplantation in MITRG mice exhibit minimal significant transcriptomic alterations in the mutant cells ($\text{FDR} \leq 0.05$; $\log_2(\text{FC}) \geq \pm 1$) when compared to WT cells.

[0029] FIG. 10A and 10B show G795A iPSC-derived microglia exhibit transcriptome signatures following xenotransplantation *in vivo* that are highly similar to that of isogenic wild-type iPSC-derived microglia. Xenotransplantation-compatible mice were engrafted with either wild-type (WT) or G795A iPSC-microglia, and after two months, human cells were isolated and examined via bulk RNA sequencing. FIG. 10A shows a correlation across all detected genes is shown for xMGs isolated from individual WT and G795A engrafted mice. Differences between individual mouse recipients lead to some variability, but WT and G795 xMGs remain highly correlated with R^2 between 0.80 and 0.95. FIG. 10B shows a similar comparison was performed using a more selected microglial-specific gene list. This comparison likewise revealed strong correlations between wild-type and G795A xMGs with R^2 between 0.78 and 0.97. Consistent with prior *in vitro* RNA-sequencing analysis, these results demonstrate that the G795A CSF1R mutation has little to no effect on the underlying transcriptome of human microglia following *in vivo* engraftment and that most of the detected variance is due to differences between individual mouse graft recipients.

[0030] FIG. 11A, 11B, 11C, 11D, 11E, 11F, 11G, 11H, 11I, and 11J show G795A iPSC-derived microglia are resistant to CSF1R agonist treatment and enable robust engraftment of human microglia within the adult mammalian brain. Xenotransplantation-compatible mice received bilateral stereotactic intrahippocampal injections of 500,000 iPSC-derived human microglia (xMGs) at 2-months of age. Mice received xMGs differentiated from either a homozygous CSF1R-resistant G795A human iPSC line or an isogenic unmodified Wild-Type human iPSC. At four months of age, mice were treated with 600mg/kg of PLX3397 in rodent chow *ad libitum* for 4 weeks duration. Mice were then sacrificed, and half-brains were examined by fluorescent immunohistochemistry and confocal microscopy. FIG. 11A-11E shows that over the course of one-month PLX3397 treatment, G795A xMGs proliferate and expand from the initial injection sites within the hippocampus and cortex. Microglia are labeled with IBA-1 (FIG. 11A and 11F), human-specific marker Ku80 (FIG. 11B and 11G) is used to co-label the nuclei of human microglia, and Ki67 (FIG. 11C and 11H) is used to demonstrate 'wavefronts' of proliferative human xMGs migrating toward remaining unoccupied niches within the cortex and thalamus (arrows). FIG. 11E shows a higher power image of the boxed region in FIG. 11D demonstrates that all IBA1+ microglia co-express Ku80. In contrast, only a handful of wild-type human xMGs survive one month of PLX3397 treatment (FIG. 11F - 11I). For example, the arrows in FIG. 11J reveals just four surviving xMGs within the boxed region shown in FIG. 11I.

[0031] FIG. 12 shows quantification of CSF1R-G795A human iPSC-microglial engraftment following transplantation into adult hCSF1 mice. Transplantation of CSF1R-G795A iPSC-microglia into a fully occupied adult microglial niche (without treatment with CSF1R antagonists), produces very limited

engraftment of human microglia (5-10% of total microglia) as measured by co-localization of the microglial marker IBA1 and the human-specific nuclear marker Ku80 (Ku80+/Iba1+). In contrast, when CSF1R-G795A iPSC-microglia transplantation is followed with treatment of mice for 10 days with PLX3397 containing mouse chow (600mg/kg PLX3397 in food), the percent of human microglia increases to almost 25%. After 30 days of PLX3397 treatment (600mg/kg PLX3397 in food), 90-95% of all microglia in the brain are human as evidenced by co-expression of Ku80 and IBA1. By transplanting CSF1R-G795A iPSC-microglia and varying the concentration and duration of CSF1R antagonist treatment either before, during, or after transplantation, one can control the degree of human iPSC-microglial engraftment.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0032] Although embodiments of the disclosure have been described in detail, certain variations and modifications will be apparent to those skilled in the art, including embodiments that do not provide all the features and benefits described herein. It will be understood by those skilled in the art that the present disclosure extends beyond the specifically disclosed embodiments to other alternative or additional embodiments and/or uses and obvious modifications and equivalents thereof. Moreover, while a number of variations have been shown and described in varying detail, other modifications, which are within the scope of the present disclosure, will be readily apparent to those of skill in the art based upon this disclosure. It is also contemplated that various combinations or sub-combinations of the specific features and aspects of the embodiments may be made and still fall within the scope of the present disclosure. Accordingly, it should be understood that various features and aspects of the disclosed embodiments can be combined with or substituted for one another in order to form varying modes of the present disclosure. Thus, it is intended that the scope of the present disclosure herein disclosed should not be limited by the particular disclosed embodiments described herein.

[0033] As used herein, the singular forms “a,” “an,” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including,” “includes,” “having,” “has,” “with,” or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

[0034] TERMS

[0035] As used herein, a “CSF1R expressing cell” may refer to cells that express CSF1R or cells that may be induced to express CSF1R or cells that are differentiated into cells that express CSF1R. Non-limiting examples of CSF1R-expressing cells may include but are not limited to microglia, monocytes, HSPCs (hematopoietic stem or progenitor cells), macrophages, dendritic cells, Langerhans cells, Kupffer cells, Hofbauer cells, Extravillous trophoblasts, phagocytes, or primitive macrophages from pluripotent stem cells. Additionally, a CSF1R expressing cell may refer to microglia that has been directly reprogrammed from iPSCs, monocytes, or fibroblasts via either genetic or small molecule approaches.

[0036] As used herein, “CSF1R expressing phagocyte” may refer to a cell that expresses the CSF1R receptor and has the capacity for phagocytosis; the process by which a cell uses its plasma membrane to

engulf extracellular particles or bacteria, substrates, proteins, lipids, or aggregates and then internalizes this region to form a phagosome. Non-limiting examples of CSF1R-expressing phagocytes may include but are not limited to microglia, macrophages, monocytes, dendritic cells, or other phagocytes. In some embodiments, a CSF1R-expressing phagocyte is a CSF1R-expressing cell.

[0037] As used herein, "differential resistance" may refer to a state occurring in a modified cell in which the inhibition by a CSF1R antagonist of a CSF1R signal exhibits a different dose-response relationship or magnitude of response than is observed in an unmodified cell. In other embodiments, differential resistance may refer to altering the proliferation or survival of an unmodified CSF1R-expressing cell relative to the modified CSF1R-expressing cell. In some embodiments, differential resistance may comprise a partial, complete, increased, or decreased resistance to a CSF1R antagonist.

[0038] As used herein, "CSF1R signal inhibition" may refer to a dose-dependent reduction caused by a CSF1R antagonist in a step of a CSF1R signaling cascade, such as phosphorylation or dephosphorylation events, gene expression, etc.

[0039] As used herein, "partial resistance" refers to modified cells that are still destroyed by higher doses of CSF1R antagonist. Partial resistance could be due to increased binding constant (K_d) or ATP, reduced kinase activity, reduced binding affinity to CSF1 ligand, or reduced signal transduction.

[0040] As used herein, "complete resistance" refers to modified cells in which CSF1R signal inhibition never occurs at any concentration of the antagonist.

[0041] As used herein, an "increased resistance" refers to modified cells in which CSF1R signal inhibition occurs at an increased concentration of the antagonist relative to what is observed in unmodified cells.

[0042] As used herein, a "decreased resistance" refers to modified cells in which CSF1R signal inhibition occurs at a lower concentration of a receptor antagonist relative to what is observed in unmodified cells.

[0043] As used herein, the terms "subject" and "patient" are used interchangeably. As used herein, a subject can be a non-primate (e.g., cows, pigs, horses, cats, dogs, rats, mice, etc.) or a primate (e.g., monkey, ape, and human). In specific embodiments, the subject is a human. In one embodiment, the subject is a mammal (e.g., a human) having a disease, disorder, or condition described herein. In another embodiment, the subject is a mammal (e.g., a human) at risk of developing a disease, disorder or condition described herein. In certain instances, the term patient refers to a human under medical care or animals under veterinary care.

[0044] As used herein, the term "neurological diseases" refers to injuries, trauma, disorders, or diseases that affect the brain as well as the nerves found throughout the body and the spinal cord.

[0045] As used herein, the terms "treat" or "treatment" or "treating" refers to both therapeutic treatment

and prophylactic or preventative measures, wherein the object is to prevent or slow the development of the disease (e.g., a neurological disease), such as slowing down the development of a disorder, or reducing at least one adverse effect or symptom of a condition, disease or disorder, e.g., any disorder characterized by insufficient or undesired organ or tissue function. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced, as that term is defined herein. Alternatively, a treatment is "effective" if the progression of a disease is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or decrease of markers of the disease, but also a cessation or slowing of progress or worsening of a symptom that would be expected in absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of the extent of disease, stabilized (e.g., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" also includes ameliorating a disease, lessening the severity of its complications, preventing it from manifesting, preventing it from recurring, merely preventing it from worsening, mitigating an inflammatory response included therein, or a therapeutic effort to affect any of the aforementioned, even if such therapeutic effort is ultimately unsuccessful.

[0046] Referring now to FIG. 1-12, the present disclosure features methods and compositions for the creation and use of therapeutically designed CSF1R-expressing cells (e.g., therapeutic microglia) that have a differential resistance to CSF1R antagonists for the treatment of neurodegenerative diseases.

[0047] Cells within the central nervous system can exist inside a 'niche' which can limit the total number of cells that can reside within the mammalian CNS. The present disclosure aims to use CSF1R-expressing cells (e.g., CSF1R expressing phagocytes, HSPCs, dendritic cells, erythromyeloid progenitors, microglia, macrophages, or monocytes) to develop new treatments for neurological disease and injury. However, for some diseases, the engraftment and/or maturation of such cells within the CNS will likely be limited by the presence of existing endogenous microglia. For such indications, a method that can provide a selective survival or proliferative advantage to the transplanted mammalian cells would improve engraftment and thus therapeutic activity. Importantly, such an approach must also be safe and not induce uncontrolled proliferation.

[0048] The present disclosure features modified CSF1R-expressing cells or their progenitors or stem cells that are differentiated into cells that express CSF1R, wherein the modified CSF1R-expressing cells have differential resistance to CSF1R antagonists. Also encompassed are methods of making modified cells according to the present disclosure, which may include, in the case of an induced pluripotent stem cells (iPSC)- or hematopoietic stem and progenitor cells (HSPCs)- derived modified cell, introducing a modification into an undifferentiated cell, optionally screening or selecting for the modification, and differentiating the modified cell into a cell or cell lineages such as a monocyte or macrophage lineage.

[0049] The present disclosure features a cell (e.g., a human cell) exhibiting differential resistance to a

CSF1R antagonist. In some embodiments, the present disclosure features a modified human cell exhibiting differential resistance to a CSF1R antagonist. The present disclosure may also feature a cell (e.g., a human cell) comprising a nucleic acid encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist. In some embodiments, the present disclosure features a modified human cell comprising a nucleic acid encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist. In some embodiments, the cell (e.g., a human cell) of the present disclosure expresses CSF1R and may be a CSF1R-expressing cell. In other embodiments, the cell (e.g., a human cell) of the present disclosure can be induced or differentiated to express CSF1R.

[0050] The present disclosure may also feature a modified cell as described herein that is expressing CSF1R. In some embodiments, the present disclosure features a modified human cell as described herein that is expressing CSF1R. The cell may be induced and/or differentiated to express CSF1R and may be selected from a group consisting of pluripotent stem cell, hematopoietic stem cell, an erythromyeloid progenitor, and hematopoietic progenitor cell.

[0051] In some embodiments, CSF1R-expressing cells (e.g., CSF1R-expressing human) cells may include but are not limited to microglia, macrophages, monocytes, or other phagocytes. In other embodiments, cells that can be induced or differentiated to express CSF1R may include but are not limited to pluripotent stem cell, hematopoietic stem cell, an erythromyeloid progenitor, or a hematopoietic progenitor cell. In some embodiments, the modified cell (e.g., the modified human cell) is a precursor cell including but not limited to microglia, monocytes, macrophages, hematopoietic progenitor cells (HPCs) and hematopoietic stem cells (HSCs), erythromyeloid progenitors (EMPs), primitive macrophages, and primitive macrophage progenitors (PMPs) or cord blood hematopoietic stem cells.

[0052] In some embodiments, the modified cells are differentially resistant to CSF1R antagonists. In some embodiments, CSF1R antagonists may include but are not limited to PLX5622, PLX3397 (pexidartinib), BLZ945, Ki20227, JNJ-40346527, JNJ-527 (Edicotinib), cFMS Receptor Inhibitor II, AZ304, ARRY-382, YM-90709, GW2580, PLX108-01, PLX7486, PLX647, ARRY-382, JNJ-40346527, Emactuzumab (RG7155), AMG820, IMC-CS4 (LY3022855), MCS110, BPR1R024, AZD7507, JTE-952, JNJ-28312141, c-FMS-IN-8, or CSF1R-IN-22.

[0053] In other embodiments, a CSF1R antagonist may include any compound that inhibits CSF1R interaction with its cognate ligands (e.g., CSF1 or IL-34), substrates, or downstream effectors. Non-limiting examples of compounds that inhibit CSF1R interaction with its cognate ligands, substrates, or downstream effectors include but are not limited to antibodies or drugs that block the interactions between CSF1R and Phospholipase C-gamma2 (PLCg2), Spleen tyrosine kinase (Syk), and the Grb2/Gab2/Shp2 complex. In some embodiments, differential CSF1R antagonist resistance is conferred by modifying genes downstream of CSF1R. Non-limiting examples may include but are not limited to INPP5D (SHIP1), TREM2, DAP12, PLCG2, PI3K, AKT, PKC, FIMP, JNK, A-SMase, spleen tyrosine kinase (Syk), and the Grb2/Gab2/Shp2 complex. In other embodiments, partial CSF1R antagonist resistance is conferred by

pretreating microglia with agonists or antagonists of CSF1R signaling. In further embodiments, CSF1R antagonists may include any compound that inhibits CSF1R signaling by blocking the ATP binding site (i.e., the ATP binding pocket).

[0054] Table 1 shows a wild type CSF1R sequence and non-limiting examples of modified CSF1R amino acid sequences (i.e., modified CSF1R protein described herein) as well as non-limiting examples of the nucleic acids sequences encoding the modified CSF1R protein described herein. Examples of such nucleic acids include those with one or more silent or conserved nucleic acid substitutions compared to the wild type nucleic acid sequence. **Bolded** nucleic acids (NAs) are examples of where one or more silent or conserved nucleic acid substitutions may be incorporated into the sequence. Potential nucleic acids are highlighted in the description.

Description	Sequence	SEQ ID NO:
Wild Type CSF1R (nucleic acid)	atgggccaggagttctgctgctcctgctggtggccacagcttggcatggcaggaatccagtgatag agcccagtgccctgagctggctgtaagccaggagcaacggtagccttgcgatgtgtggcaatggc agcgtggaatgggatggcccccatcactcactggaccctgtactctgatggctccagcagcatcctca gcaccaacaacgctaccttccaaaacacggggacctatcgctgactgagcctggagaccccctggg aggcagcgccgcatccacctctatgtcaagaccctgcccggccctggaacgtgtagcacaggag gtggtggtgttcgaggaccaggacgactactgccctgtctgtcacagaccgggtgctggaagcaggc gtctcgctggtgctgtgctggccggccccctcatgcccacaccaactactccttctgccttgcatgg cttcaccatccacagggccaagttcattcagagccaggactatcaatgcagtgccctgatgggtggcag gaaggtagtgccatcagcatccggctgaaagtcagaaagtcacccagggccccagccttgacac tgggtgctgcagagctgggtcggtgaggggaggctgccagatcgtgtgctcagccagcagcgttg atgttaactttgatgtcttctccaacacaacaaccaagctcgcaatccctcaacaatctgacttcataa taaccgttacaaaaagtcctgaccccaacctcgatcaagtagattccaacatgccggcaactactcct gcgtggccagcaacgtgcagggaagcactccacctccatgttctccgggtggtagagagtgccactt gaactgagctctgagcagaacctcatccaggagtgaccgtggggaggggctcaacctcaaagtc atggtggaggcctaccaggcctgcaagggtttaactggacctacctgggaccctttctgaccaccagcc tgagcccaagcttgtaatgtctaccaccaaggacacatacaggcacaccttaccctctctgccccgc ctgaagccctctgaggctggccgtactcctcctggccagaaaccaggaggctggagagctctgacg ttgagctcaccctcgataccccccagaggtaagcgtcatatggacattcatcaacggctctggcaccctt ttgtgtgctcctctgggtacccccagcccaacgtgacatggctgcagtgagtgccacactgataggt gtgatgaggcccaagtgctgcaggctggtgatgacctacacctgaggctctgagccaggagcccttcc acaaggtagcggtagagagcctgctgactgttgagaccttagagcacaaccaaactacgagtgacg ggcccaacaacgcgtggggagtggtcctggccttcataccatctctgcaggagcccacacgcac ccccgatgagttccttccacaccagtggtgctgcctgcagtgccatcatggccttctgctgctgctgct cctgctgctattgtacaagataagcagaagcccaagtagcagggtccgctggaagatcatcgagagcta	1

	tgagggcaacagttatacttcatcgacccacgcagctgccttacaacgagaagtgggaggttccccg gaacaacctgcagtttggaagacctcggagctggagccttgggaaggtggaggccacggcctt tggtctgggcaaggaggatgctgtcctgaaggtggctgtgaagatgctgaagtcacggcccatgctgat gagaaggaggccctcatgtccgagctgaagatcatgagccacctgggccagcacgagaacatcgtca acctctgggagcctgtacctatggaggccctgtactggatcacggagtagtctgtctatggcgacctg ctcaacttctgcaaggaaggctgaggccatgctgggacccagcctgagccccggccaggaccccg agggaggcgctgactataagaacatccacctcgagaagaataatgtccgaggagacagtggtcttcc agccagggtgtggacacctatgtggagatgaggcctgtctccacttctcaaatactccttctgagcaa gacctggacaaggaggatggacggccctggagctccgggacctgctcacttctccagccaagtagc ccagggcagtgccctcctcgttccaagaattgcatccaccgggacgtggcagcgcgtaacgtgctgttg accaatggtcatgtggccaagattggggactcgggctggctaggacatcatgaatgactccaactac attgtcaagggaatgccccgctgctgtgaagtggatggccccagagagcatcttgactgtgtctacac gggtcagagcgacgtctggtcctatggcatcctcctctgggagatcttctcacttggtgaatccctacct ggcatcctggtgaacagcaagttctataaactggtgaaggatggataccaaatggccagcctgcattg cccaaagaatatatacagcatcatgcaggcctgctgggcctggagcccaccacagacccacctc cagcagatctgtcctcctcagggagcagggccaagaggacaggagagagcgggactataccaatct gccgagcagcagcagaagcgggtggcagcggcagcagcagcagtgagctggaggaggagagctct agtgagcacctgacctgctgcgagcaaggggatatcgccagcccttgctgcagcccaacaactatca gttctgctga	
Wild Type CSF1R (amino acid)	MGPVLLLLLLVATAWHGQGIPVIEPSVPELVVKPGATVTLRCVGNGSVEWD GPPSPHWTLYSDGSSSILSTNNATFQNTGTYRCTEPGDPLGGSAAIHLVVK DPARPWNVLAQEVVVFEDQDALLPCLLTDPVLEAGVSLVRVRGRPLMRHT NYSFSPWHGFTIHRAKFIQSQDYQCSALMGGRKVMSSIRLKVQKVI PGPP ALTLVPAELVRIRGEAAQIVCSASSVDVNFDFLQHNNTKLAIPQQSDFHNN RYQKVLTLNLDQVDFQHAGNYSVASNVQGHSTSMFFRVVESAYLNLSS EQNLIQEVTVGEGLNLKVMVEAYPGLQGFWNTYLGPFS DHQPEPKLANAT TKD TYRHTFTLSLPRLPSEAGRYSFLARNPGGW RALTFELTLRYPPEVSV IWT FINGSGTLLCAASGYQPNTWLQCSGHTDRCD EAOVLQVWDDPYP EVL SQEPFHKVTVQSLLTVETLEHNQTYECRAHNSVGS GSWAFIPISAGAH THPPDEF LFTPVVACMSIMALLLLLLLLLLLYKYKQKPKYQVRWKIIESYEG NSYTFIDPTQLPYNEKWEFPRNNLQFGKTLGAGAFGKVVEATAFGLGKED AVLK VAVKMLKSTAHAD EKEALMSELKIMSHLGQHENIVNLLGACTHGGPV LVITEYCCYGDLLNFLRRKAEAMLGPSLSPGQDPEGGV DYKNIHLEKKYVR RDSGFSSQGVDTYVEMRPVSTSSNDSFSEQDL DKEDGRPLELRDLLHFS SQVAQGM AFLASKNCIHRDVAARNVLLTNGHVAKIGDFGLARDIMNDSNYI VKGNARLPVKWMAPE SIFDCVYTVQSDVWSYGILLWEIFSLGLNPYPGILV NSKFYKLVK DGYQMAQPAFAPKNIYSIMQACWALEP THRPTFQQICSFLQE	2

	QAQEDRRERDYNLPSSSRSGSGSSSELEEESSSEHLTCCEQGDIAQ PLLOPNNYQFC	
G795A CSF1R (nucleic acid) where NNN may include: GCT, GCC, GCA, and GCG.	atgggccaggagttctgctgctcctgctggtggccacagcttggcatggcagggaaatccagtgatag agcccagtgccctgagctggtcgtgaagccaggagcaacggtgacctgcatgtgtgggaatggc agcgtggaatgggatggcccccatcacctcactggacctgtactctgatggctccagcagcatcctca gcaccaacaacgctaccttcaaaacacggggacctatcgctgactgagcctggagacccccctggg aggcagcgccgcatccacctctatgtcaagacctgcccggccctggaacgtgtagcacaggag gtggtcgtgttcgaggaccaggacgactactgacctgtctgctcacagacctggctggaagcaggc gtctcgtggtgctgtgctggtggccggccccctcatgcccacaccaactactccttctgcccctggcatgg cttcaccatccacagggccaagttcattcagagccaggactatcaatgcagtgccttgatgggtggcag gaaggatggtccatcagcatccggctgaaagtgcagaaagtcatccagggccccagccttgacac tgggtgctgcagagctggtgctgagggagggtgcccagatcgtgtgctcagccagcagcgttg atgttaactttgatgtcttctccaacacaacaaccaagctcgcaatccctcaacaatctgacttcataa taaccgttaccaaaaagtcctgacctcaacctcgatcaagtagatttccaacatgccggcaactactcct gctggtggcagcaacgtgcagggcaagcactccacctcatgttctccgggtggtagagagtgctactt gaactgagctctgagcagaacctcatccaggagtgacctggggaggggctcaacctcaaagtc atggtggaggcctaccaggcctgcaaggttttaactggacctacctgggacctttctgaccaccagcc tgagcccaagcttgctaattgctaccaccaaggacacatacaggcacaccttaccctctctgccccgc ctgaagccctctgaggtggccgtactcctcctggccagaaaccaggaggtgagagctctgacg tttgagctcaccctcgataccccccagaggtaagcgtcatatggacattcatcaacggctctggcaccctt ttgtgtgctgctctgggtacccccagcccaacgtgacatggctgcagtgcagtggccacactgataggt gtgatgaggcccaagtgctgcaggctggtgatgacctacacctgaggtctgagccaggagcccttc acaagggtgacgggtcagagcctgctgactgttgagaccttagagcacaacaaacctacgagtgcag ggcccaacacagcgtggggagtggtcctgggccttcataccatctctgcaggagcccacacgcatac ccccgatgagttccttccacaccagtggtggtcgctgcatgtccatcatggccttgctgctgctgct cctgctgctattgtacaagataagcagaagcccaagtagcaggtccgctggaagatcatcgagagcta tgagggaacaggtatctatctgacccccacgcagctgccttacaacgagaagtgggagttccccg gaacaacctgcagtttgtaagacctcgagctggagcctttggaaggtggtgaggccacggcctt tggctggtggcaaggaggatgctgtcctgaaggtggctgtgaagatgctgaagtcacggcccatgctgat gagaaggaggccctcatgtccgagctgaagatcatgagccacctgggcccagcacgagaacatcgta accttctgggagcctgtacctatggaggccctgtactggtcatcacggagtactgtgctatggcgacctg ctcaactttctgcaaggaaggctgaggccatgctgggacctgagccccggccaggaccccg agggaggcgtcgactataagaacatccacctcgagaagaaatgtccgcagggaagtggtcttcc agccagggtgtggacacctatgtggagatgaggcctgtctccacttctcaaatgactccttctgagcaa gacctggacaaggaggatggacggccccctggagctccgggacctgttctacttctccagccaagtagc ccagggcagtgccctcctcgttccaagaattgcatccaccgggacgtggcagcgtaacgtgctgttg accaatggtcatgtggccaagattNNNgacttcgggctggctagggacatcatgaatgactccaacta	3

	cattgtcaagggcaatgccgcctgcctgtgaagtggatggccccagagagcatcttgactgtgtctaca cggttcagagcgacgtctggtcctatggcatcctcctctgggagatcttctacttgggtgaatccctaccc tggcatcctggtgaacagcaagttctataaaactggtgaaggatggataccaaatggcccagcctgcattt gccccaaagaatatatacagcatcatgcaggcctgctgggcctggagcccacccacagaccacctt ccagcagatctgctccttcctcaggagcaggcccaagaggacaggagagagcgggactataccaat ctgccgagcagcagcagaagcgggtggcagcggcagcagcagcagtgagctggaggaggagagct ctagtgagcacctgacctgctgcgagcaaggggatatgccagccctgctgcagcccaacaactatc agttctgctga	
G795A CSF1R (amino acid)	MGPVLLLLLVATAWHGQGIPVIEPSVPELVVKPGATVTLRCVGNSSVEWD GPPSPHWTLYSDGSSSILSTNNATFQNTGTYRCTEPGDPPLGSSAAIHLVVK DPAFPWNVLAQEVVVFEDQDALLPCLLTDPVLEAGVSLVRVRGRPLMRHT NYSFSPWHGFTIHRAKFIQSQDYQCSALMGGKVMSSISIRLKVQKVIPIGPP ALTLPVPAELVRIRGEAAQIVCSASSVDVNFDFLQHNNTKLAIPQQSDFHNN RYQKVLTLNLDQVDFQHAGNYSVASNVQGHSTSMFFRVVESAYLNLSS EQNLIQEVTVGELNLKVMVEAYPQLQGFWNTYLGPFSDHQPEPKLANAT TKDITYRHTFTLSLPRLKPSEAGRYSFLARNPGGWRALTFELTLRYPPEVSV IWFINGSGTLLCAASGYPPQPNVTWLQCSGHTDRCDQVLAQVWDDPYP EVLSEQEPFHKVTVQSLTETLEHNQTYECRAHNSVSGSGSWAFIPISAGAH THPPDEFLLTPVVACMSIMALLLLLLLLLLLYKYKQKPKYQVRWKIIESYEG NSYTFIDPTQLPYNEKWEFPRNNLQFGKTLGAGAFGKVVEATAFGLKED AVLKVAVKMLKSTAHADKEALMSELKIMSHLGQHENIVNLLGACTHGGPV LVITEYCCYGDLLNFLRRKAEAMLGPSLSPGQDPEGGVDYKNIHLEKKYVR RDSGFSSQGVDTYVEMRPVSTSSNDSFSEQDLKEDGRPLELRDLLHFS SQVAQGMFLASKNCIHRDVAARNVLLTNGHVAKIADFGLARDIMNDSNYI VKGNAFLPVKWMAPESIFDCVYTVQSDVWSYGILLWEIFSLGLNPYPGILV NSKFYKLVKDGYSMAQPAFAPKNIYSIMQACWALEPTHRPTFQQICSFLQE QAQEDRRERDYNLPSSSRSGSGSSSSELEEESSSEHLTCCEQGDIAQ PLLOPNNYQFC	4
G795C CSF1R (nucleic acid): where NNN may include: TGT and TGC.	atgggccaggagttctgctgctcctgctggtggccacagctggcatggtcaggaatccagtgatag agcccagtgccctgagctggtcgtgaagccaggagcaacggtgacctgcatgtgtgggcaatggc agcgtggaatgggatggcccccatcacctcactggaccctgtactctgatggctccagcagcatcctca gcaccaacaacgctaccttccaaaacacggggacctatcgctgactgagcctggagacccccctggg aggcagcgccgccatccacctctatgtcaagaccctgcccggccctggaacgtgtagcacaggag gtggtcgtgttcgaggaccaggacgactactgccctgtctgtcacagaccggtgctggaagcaggc gtctcgtggtgctgtgctgtggccggccctcatgcccacaccaactactcctctgcctggcatgg cttcaccatccacagggccaagttcattcagagccaggactatcaatgcagtgccctgatgggtggcag gaaggtgatgtccatcagcatccggctgaagtgagaaagtcagccaggggccccagccttgacac	5

	tggcgctgcagagctggcgcggttcgaggggaggctgccagatcgtgtgctcagccagcagcgttg atgttaactttgatgtcttctccaacacaacaacaccaagctcgcaatccctcaacaatctgactttcataa taaccgttaccaaaaagtcctgaccctcaacctcgatcaagtagatttccaacatgccggcaactactcct gcgtggccagcaacgtgcagggaagcactccacctccatgttctccgggtggtagagagtgctactt gaacttgagctctgagcagaacctcatccaggaggtagccgtgggggaggggctcaacctcaaagtc atgggtggaggcctaccagggcctgcaagggtttaactggacctacctgggaccttttctgaccaccagcc tgagcccaagcttgctaattgctaccaccaaggacacatacaggcacaccttcacctctctctgcccgc ctgaagccctctgaggctggccgctactccttggccagaaaccaggaggctggagagctctgacg ttgagctcaccttcgataccccccagaggtaagcgtcatatggacattcatcaacggctctggcacctt ttgtgtgctcctctgggtacccccagcccaacgtgacatggctgcagtgcagtggccacactgataggt gtgatgaggcccaagtgtgcaggctctgggatgacctaccctgaggctctgagccaggagcccttcc acaagggtgacgggtcagagcctgtgactgttgagaccttagagcacaaccaaactacgagtgcag ggcccaacaacagcgtggggagtggtcctgggcttcatacccatctctgaggagcccacacgcatc ccccgatgagttcctctcacaccagtggtggtcgcctgcatgtccatcatggccttgctgctgctgct cctgctgctattgtacaagtataagcagaagcccaagtagcaggtccgctggaagatcatcgagagcta tgagggaacagttatactttcatcgacccacgcagctgccttacaacgagaagtgagggttccccg gaacaacctgcagtttgtaagacctcgagctggagcctttgggaaggtggtggaggccacggcctt tggctctgggcaaggaggatgctgtcctgaagggtggctgtgaagatgctgaagtcacggcccatgctgat gagaaggaggccctcatgtccgagctgaagatcatgagccacctgggcccagcacgagaacatcgfca accttctgggagcctgtacctatggaggccctgtactggtcatcacggagtactgttgctatggcgacctg ctcaactttctggaaggaaggctgaggccatgctgggacctgagccccggccaggacccccg agggaggcgtcgactataagaacatccacctcgagaagaaatgtccgagggacagtggttctcc agccagggtgtggacacctatgtggagataggcctgtctccacttctcaaatactcctctctgagcaa gacctggacaaggaggatggacggccctggagctccgggacctgcttacttctccagccaagtagc ccagggcagtgccctctcgtccaagaattgcatccacgggacgtggcagcgcgtaacgtgctgtg accaatggtcatgtggccaagattNNNgacttcgggctggctagggacatcatgaatgactccaacta cattgtcaagggaatgcccgctcctgtgaagtggatggccccagagagcatcttgactgtgtctaca cggttcagagcgacgtctggctctatggcatcctcctctgggagatcttctacttgggctgaatccctaccc tggcatcctggtgaacagcaagttctataaactggtgaaggatggataccaaatggcccagcctgcattt gccccaaagaatatatacagcatcatgcaggcctgctgggcttgagcccaccacagacccacctt ccagcagatctgctccttctcaggagcaggcccaaggagacaggagagagcgggactataccaat ctgccgagcagcagcagaagcgggtggcagcggcagcagcagcagtgagctggaggaggagagct ctagtgagcacctgacctgctgcgagcaaggggatatgccagccctgtctgagcccaacaactatc agttctgctga	
G795C CSF1R (amino acid)	MGPGLVLLLLVATAWHGQGIPVIEPSVPELVVKPGATVTLRCVGNNGSVEWD GPPSPHWTLYSDGSSSILSTNNATFQNTGTYRCTEPGDPLGSSAAIHLVVK DPARPWNVLAEVVFEDQDALLPCLLTDPVLEAGVSLVRVRGRPLMRHT	6

	NYSFSPWHGFTIHRAKFIQSQDYQCSALMGGRKVMSSISIRLKVQKVI PGFP ALTLVPAELVRIRGEAAQIVCSASSVDVNFDFVLQHNNTKLAIPQQSDFHNN RYQKVLTLNLDQVDFQHAGNYSCVASNVQGKHSTSMFFRVVESAYLNLSS EQNLIQEVTVGEGNLKVMVEAYPGLOGFNWYTLGPFSDHQPEPKLANAT TKD TYRHFTLSLPR LKPSEAGRYSFLARNPGGWRALTFELTLRYPPEVSV IWT FINGSGTLLCAASGY PQPNVTWLOCSGHTDRCD E AQVLQVWDDPYP EVLSQEPFHKVTVQSLLT VETLEHNQTYECRAHNSVGSGSWAFIPISAGAH THPPDEF LFTPVVACMSIMALLLLLLLLLLLYKYKQKPKYQVRWKIIESYEG NSYTFIDPTQLPYNEKWEFPRNNLQFGKTLGAGAFGKVVEATAFGLGKED AVLKVAVKMLKSTAHAD E KEALMSELKIMSHLGQHENIVNLLGACTHGGPV LVITEYCCYGDLLNFLRRKAEAMLGPSLSPGQDPEGGV DYKNIHLEKKYVR RDSGFSSQGVDTYVEMRPVSTSSNDSFSEQDLKEDGRPLELRDLLHFS SQVAQGM AFLASKNCIHRDVAARNVLLTNGHVAKICDFGLARDIMNDSNYI VKGNARLPVKWMAPE SIFDCVYTVQSDVWSYGILLWEIFSLGLNPYPGILV NSKFYKLVK DGYQMAQPAFAPKNIYSIMOACWALEP THRPTFQQICSFLQE QAQEDRRERDYTNLPSSSRSGGSGSSSELEEESSSEHLTCCOEQGDIAQ PLLQPNNYQFC	
CSF1R G795V (nucleic acid) where NNN may include: GTT, GTC, GTA, and GTG	atgggccaggagttctgctgctcctgctggtggccacagcttggcatggtcaggaatcccagtgatag agcccagtgccctgagctggtcgtgaagccaggagcaacggtgacctgcatgtgtggcaatggc agcgtggaatgggatggcccccatcactcactggacctgtactctgatggctccagcagcatcctca gcaccaacaacgctaccttcaaaacacggggacctatcgctgactgagcctggagaccccctggg aggcagcgccgcatccacctctatgtcaagacctgcccggccctggaacgtgctagcacaggag gtggtcgtgttcgaggaccaggacgcactactgccctgtctgctcacagaccgggtgctggaagcaggc gtctcgtggtgctgtgctggccggccccctcatgcgccacaccaactactccttctgccttgcatgg cttcaccatccacagggccaagttcattcagagccaggactatcaatgcagtgccctgatgggtggcag gaaggatgtccatcagcatccggctgaaagtcagaaaagtcacccagggccccagccttgacac tggtgctgcagagctggtgcggtatcgaggggaggctgccagatcgtgtgctcagccagcagcgttg atgttaactttgatgtcttctccaacacaacaacaccaagctcgcaatccctcaacaatctgactttcataa taaccgttacaaaaagtcctgacctcaacctcgatcaagtagattccaacatgccggcaactactcct gcgtggccagcaacgtgcagggaagcactccacctcatgttctccgggtggtagagagtgccactt gaactgagctctgagcagaacctcatccaggagtgacctgggggaggggctcaacctcaaagtc atggtggaggcctaccaggcctgcaaggtttaactggacctactgggaccttttctgaccaccagcc tgagcccaagctgctaagtctaccaccaaggacacatacaggcacaccttaccctctctgccccgc ctgaagccctctgaggctggccgctactcctctggccagaaaccaggaggctggagagctctgacg tttagctcaccctcgataccccccagaggtaagcgtcatatggacattcatcaacggctctggcaccctt ttgtgtgctcctctgggtacccccagcccaacgtgacatggctgcagtgagtgccacactgataggt gtgatgaggcccaagtgctgcaggctctgggatgacctacacctgaggtcctgagccaggagcccttcc	7

	acaaggtgacggtgcagagcctgctgactgttgagaccttagagcacaaccaaacctacgagtgcag ggcccacaacagcgtggggagtggtcctgggccttcatacccatctctgcaggagcccacacgcac ccccgatgagttcctcttcacaccagtggtgctgcctgcatgtccatcatggccttgctgctgctgct cctgctgctattgtacaagtataagcagaagcccaagtaccagggtccgctggaagatcatcgagagcta tgagggcaacagttatacttcatcgacccacgcagctgccttacaacgagaagtgggagttccccg gaacaacctgcagtttgtaagacctcggagctggagcctttggaaggtggtggaggccacggcctt tggtctgggcaaggaggatgctgtcctgaaggtggctgtgaagatgctgaagtcacggcccatgctgat gagaaggaggccctcatgtccgagctgaagatcatgagccacctgggccagcacgagaacatcgfca acctctgggagcctgtacccatggaggccctgtactggtcatcacggagtactgtgctatggcgacctg ctcaactttctgcaaggaaggctgaggccatgctgggacccagcctgagccccggccaggacccccg agggaggcgtcgactataagaacatccacctcgagaagaaatgtccgaggggacagtggcttctcc agccagggtgtggacacctatgtggagatgaggcctgtctccacttctcaaatactcctctgagcaa gacctggacaaggaggatggacggccctggagctccgggacctgcttacttctccagccaagtagc ccagggcatggccttctcgctccaagaattgcatccacgggacgtggcagcgcgtaacgtgctgtg accaatggtcatgtggccaagattNNNgacttcgggctggctagggacatcatgaatgactccaacta cattgtcaagggaatgcccgcctgctgtgaagtggatggccccagagagcatcttgactgtgtctaca cgggtcagagcgacgtctggtcctatggcatcctcctctgggagatcttctacttgggctgaatccctacc tgcatcctggtgaacagcaagttctataaactggtgaaggatggataccaaatggccagcctgcattt gccccaaagaatatatacagcatcatgcaggcctgctgggccttggagcccaccacagacccacctt ccagcagatctgctccttctcaggagcaggcccaaggaggacaggagagagcgggactataccaat ctgccgagcagcagcagaagcgggtggcagcggcagcagcagcagcagtgagctggaggaggagagct ctagtgagcacctgacctgctgcgagcaaggggatatgccagccctgtctgcagcccaacaactatc agttctgctga	
CSF1R G795V (amino acid)	MGPVLLLLLVATAWHGQGIPVIEPSVPELVVKPGATVTLRCVGNGSVEWD GPPSPHWTLYSDGSSSILSTNNATFQNTGTYRCTEPGDLGGSAAIHLYVK DPAWPWNVLAQEVVVFEDQDALLPCLLTDPVLEAGVSLVRVRGRPLMRHT NYSFSPWHGFTIHRAKFIQSQDYQCSALMGRKVMSSISIRLKVQKVIPGPP ALTLPVPAELVRIRGEAAQIVCSASSVDVNFDFLQHNNTKLAIPQQSDFHNN RYQKVLTLNLDQVDFQHAGNYSVASNVQGHSTSMFFRVVESAYLNLSS EQNLIQEVTVGEGLNLKVMVEAYPGLQGFWNTYLGPFS DHQPEPKLANAT TKD TYRHTFTLSL PRLKPSEAGRYSFLARNPGGWRALTFELTLRYPPEVSV I WTFINGSGTLLCAASGYQP NVTWLQCSGHTDRCD EAVLQVWDDPYP EVL SQEPFHKVTVQSLLTVETLEHNQTYECRAHNSVSGSWAFIPISAGAH THPPDEF LFTPVVVACMSIMALLLLLLLLLLLYKYKQPKYQVRWKIIESYEG NSYTFIDPTQLPYNEKWEFPRNNLQFGKTLGAGAFGKVVEATAFGLKED AVLK VAVKMLKSTAHAD EKEALMSELKIMSHLGQHENIVNLLGACTHGGPV LVITEYCCYGDLLNFLRRKAEAMLGPSLS PGQDPEGGV DYNHLEKKYVR	8

	RDSGFSSQGVDTYVEMRPVSTSSNDSFSEQDLKDGRPLELRDLLHFS SQVAQGMAFLASKNCIHRDVAARNVLLTNGHVAKIVDFGLARDIMNDSNYI VKGNAARLPVKWMAPEIFDCVYTVQSDVWSYGILLWEIFSLGLNPYPGILV NSKFYKLVKDGYSMAQPAFAPKNIYSIMQACWALEPTHRPTFQQICSFLLQE QAQEDRRERDYNLPSSSRSGSGSSSSELEEESSSEHLTCCEQGDIAQ PLLQPNNYQFC	
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[0055] In some embodiments, the cell (e.g., the modified human cell) comprises one or more genetic modifications in a gene for a CSF1R receptor (i.e., CSF1R protein). In some embodiments, the cell (e.g., the modified human cell) comprises one or more genetic modifications in a CSF1R protein (i.e., a CSF1R receptor).

[0056] In some embodiments, the one or more genetic modifications in the CSF1R receptor (i.e., CSF1R protein) do not induce constitutively active CSF1R signaling. The modified CSF1R receptor (i.e., CSF1R protein) may still be activated (e.g., phosphorylated) with a ligand of the CSF1R protein. In some embodiment, the ligand of the CSF1R protein may include but is not limited to CSF1, IL-34, a CSF1R agonist or activating antibody, or a combination thereof. In some embodiments, the modified CSF1R protein is activated by a CSF1 ligand. In some embodiments, the CSF1 ligand induces phosphorylation of the modified CSF1R protein. In other embodiments, the modified CSF1R protein is activated by an IL-34 ligand. In some embodiments, the IL-34 ligand induces phosphorylation of the modified CSF1R protein.

[0057] In some embodiments, the one or more genetic modifications result in a modified ATP binding pocket. The modified ATP binding pocket of the CSF1R receptor (i.e., CSF1R protein) may have a reduced binding space (compared to a wild type CSF1R protein). In some embodiments, the modified ATP binding pocket cannot bind a drug (e.g., a CSF1R antagonist or agonist). In other embodiments, the modified ATP binding pocket can bind ATP. In some embodiments, the one or more genetic modifications in the CSF1R receptor do not interfere with the ATP binding activity of the CSF1R protein. In some embodiments, the one or more genetic modifications in the CSF1R receptor lack a pocket for a drug (e.g., a CSF1R antagonist or agonist) to bind but do not interfere with the normal ATP binding activity of the CSF1R protein.

[0058] In some embodiments, the one or more genetic modifications is a point mutation. In some embodiments, the one or more genetic modifications result in a single amino acid substitution. In other embodiments, the one or more genetic modifications result in single amino acid insertion. In further embodiments, the one or more genetic modifications result in single amino acid deletion.

[0059] In some embodiments, the one or more genetic modifications result in a change in the amino acid sequence of the CSF1R protein. The change in the amino acid sequence may optionally comprise the

substitution of an amino acid residue selected from G795, L785, M637, E633, and V647. In some embodiments, the one or more genetic modifications result in an amino acid substitution. In some embodiments, the one or more genetic modifications result in a single amino acid substitution. The single amino acid point mutations may be selected from a group consisting of G795A, G795V, and G795C.

[0060] In some embodiments, the CSF1R gene is modified to confer partial or complete resistance to the CSF1R antagonist in the modified cells. In some embodiments, the CSF1R gene is modified with a single point mutation. In other embodiments, single point mutations of the CSF1R gene may include but are not limited to these single amino acid substitutions at positions G795, L785, M637, E633, and/or V647. As non-limiting examples, the substitutions may comprise G795A, G795V, or G795C, or G795D, or G795E, or G795F, or G795G, or G795H, or G795I, or G795K, or G795L, or G795M, or G795N, or G795P, or G795Q, or G795R or G795S, or G795T, or G795W, or G795Y.

[0061] Without wishing to be bound by any theories or mechanisms, the substitution of these residues or any other amino acid residue that is in direct contact with small molecule inhibitors bound to the ATP binding site would be predicted to alter their binding. Amino acid substitutions comprising slightly larger functional groups: serine; threonine; cysteine; valine; leucine; isoleucine; and methionine are expected to introduce bulky substituents that clash with plexikon binding yet still permit ATP binding for CSF1R function. Much larger amino acid substitutions: phenylalanine, tyrosine, lysine, arginine, and tryptophan, are expected to sterically clash with both inhibitors and ATP thereby inhibiting normal CSF1R function. By contrast, substitutions with amino acids with less bulky side chains such as glycine or alanine may improve binding to antagonist species that are only weakly bound by the native sequence due to steric hindrance by slightly larger or incompatibly polar side chains. Those of skill in the art will appreciate, then, that these and other residues that define binding sites for allosteric antagonists may be subject to modification in accordance with the object of this disclosure.

[0062] In other embodiments, the one or more genetic modifications result in a change in a plurality of amino acids of the CSF1R protein. In some embodiments, the change in a plurality of amino acids are to adjacent amino acids. In other embodiments, the change in a plurality of amino acids are to non-adjacent amino acids.

[0063] In some embodiments, the one or more genetic modifications (e.g., point mutations) are within the ATP-binding pocket of the CSF1R protein (i.e., a CSF1R receptor). In other embodiments, the one or more genetic modifications (e.g., point mutations) are outside the ATP-binding pocket of the CSF1R protein (i.e., a CSF1R receptor). Without wishing to limit the present disclosure to any theories or mechanisms, it is believed that mutations outside the ATP-binding pocket of the CSF1R protein may work through allostery to inhibit antagonist binding.

[0064] In some embodiments, the one or more genetic modifications are introduced *ex vivo*. In other embodiments, the one or more genetic modifications are induced by transfecting or introducing into a cell

(e.g., a human cell) a nucleic acid encoding a transgene. In further embodiments, the one or more genetic modifications are introduced by transduction or introduction of a targeted nuclease, nickase, or base-editing effector or ribonucleoprotein complex. In some embodiments, introducing the nucleic acid encoding a transgene in a cell comprises contacting the cell (e.g., the human cell) with a viral vector.

[0065] In some embodiments, the one or more genetic modifications (e.g., point mutations) in the CSF1R gene are generated via site-directed or random mutagenesis methods known in the art, including, without limitation, CRISPR-Cas, TALEN, and ZFN genome editing systems. Alternatively, or additionally, modified CSF1R transgenes may be introduced into cells (e.g., human cells) by, e.g., DNA or RNA vectors such as naked nucleic acids, liposome or other encapsulated nucleic acid vectors, artificial chromosomes, and/or viral vectors such as lentiviral and adeno-associated viral vectors.

[0066] In some embodiments, cells (e.g., human cells) comprising a modified CSF1R protein as described herein retain a gene expression profile of a cell (e.g., a human cell) comprising a wild-type CSF1R protein. In some embodiments, *in vitro* cells (e.g., *in vitro* human cells) comprising a modified CSF1R protein have a similar gene expression profile as *in vitro* cells (e.g., *in vitro* human cells) comprising a wild-type CSF1R protein. In other embodiments, cells (e.g., human cells) comprising a modified CSF1R protein that are transplanted *in vivo* have a similar gene expression profile of a transplanted cell (e.g., human cell) that does not comprise a modified CSF1R protein. In further embodiments, modified CSF1R-expressing cells (e.g., CSF1R-expressing phagocytes) as described herein retain a gene expression profile of wild-type CSF1R-expressing cells (e.g., CSF1R-expressing phagocytes).

[0067] The present disclosure may also feature a composition comprising a CSF1R-expressing cell as described herein. The present disclosure may also feature a composition comprising a CSF1R-expressing phagocyte as described herein. In some embodiments, the composition comprises a plurality of cells. In some embodiments, at least 0.1%, at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 99% or at least 99.5% of the plurality of cells are CSF1R-expressing cells having differential resistance to a CSF1R antagonist. In other embodiments, at least 0.1%, at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 99% or at least 99.5% of the plurality of cells are CSF1R-expressing phagocytes having differential resistance to a CSF1R antagonist.

[0068] The present disclosure features a method of treating a subject. The method comprises administering a CSF1R antagonist to the subject in a quantity sufficient to inhibit a CSF1R signal in a cell of the subject and contacting the subject with a modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) having a differential resistance to a CSF1R antagonist. In other embodiments, the method comprises contacting the subject with a modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) having a differential resistance to a CSF1R antagonist and

administering the CSF1R antagonist to the subject in a quantity sufficient to inhibit a CSF1R signal in a cell of the subject. In some embodiments, differential resistance to the CSF1R antagonist is partial resistance or complete resistance to the CSF1R antagonist.

[0069] The modified CSF1R-expressing cells (e.g., modified human CSF1R-expressing cells) described herein may further comprise one or more modifications (e.g., one or more genetic modifications in another gene (e.g., outside of the CSF1R gene). In some embodiments, the modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) expresses a gene product useful to treat, cure, ameliorate, prevent or palliate a neurological disease. In some embodiments, the gene product is not otherwise expressed by the CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte). In other embodiments, the modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) expresses other genetic modifications useful to treat, cure, ameliorate, prevent or palliate a neurological disease. In other embodiments, other genetic modifications (outside of the CSF1R gene) are used to correct a mutated gene to treat a disease caused by mutations in that other gene.

[0070] In some embodiments, the CSF1R signal that is inhibited by the CSF1R antagonist is the proliferation or survival of a CSF1R-expressing cell (e.g., a CSF1R-expressing phagocyte) that is endogenous to the subject. In other embodiments, the CSF1R antagonist is administered to the subject in a quantity sufficient to reduce the proliferation or survival of the endogenous CSF1R-expressing cell (e.g., a CSF1R-expressing phagocyte) of the subject relative to the modified CSF1R-expressing cell (e.g., a modified CSF1R-express phagocyte).

[0071] The present disclosure may also feature a method of treating a subject. The method may comprise contacting the subject with a modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) and differentially altering the proliferation or survival of an unmodified CSF1R-expressing cell (e.g., an unmodified CSF1R-expressing phagocyte) relative to the modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte). In some embodiments, the modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte) is partially resistant to a CSF1R antagonist. In some embodiments, the step of differentially altering the proliferation or survival of the unmodified CSF1R-expressing cell (e.g., an unmodified CSF1R-expressing phagocyte) comprises administering the CSF1R antagonist to the subject in a quantity sufficient to reduce proliferation or survival of the unmodified CSF1R-expressing cell (e.g., an unmodified CSF1R-expressing phagocyte) relative to the modified CSF1R-expressing cell (e.g., a modified CSF1R-expressing phagocyte).

[0072] The methods described herein may kill about 0.1%, or about 1%, or about 5%, or about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 99%, or about 99.5% of endogenous CSF1R-expressing phagocyte within the CNS of the subject. In other embodiments, the methods described herein may kill all the endogenous CSF1R-expressing phagocytes within the CNS of the subject.

[0073] The methods described herein may further allow for the engraftment of about 0.1%, or about 1%, or about 5%, or about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 99%, or about 99.5% of the modified CSF1R-expressing phagocyte within the CNS of the subject. In some embodiments, the methods described herein allow for complete engraftment of the modified CSF1R-expressing phagocyte within the CNS of the subject.

[0074] In some embodiments, the methods described herein are directed towards treating, preventing, palliating, or ameliorating a neurological disease by contacting a subject with a modified cell or vector of this disclosure. In some embodiments, a neurological disease comprises a disease of the nervous system. Non-limiting examples of neurological diseases may include but are not limited to dementia, a neurodegenerative disease, a genetic disease, a spinal cord injury, a traumatic brain injury, a disease caused or worsened by exposure to a chemical or other agent, etc.

[0075] The present disclosure may also feature a composition for the treatment of neurological disease, the composition comprising a plurality of modified cells having differential (e.g., increased, decreased, partial or complete) resistance to CSF1R antagonists. In some embodiments, the modified cells are CSF1R-expressing cells.

[0076] The present disclosure may further feature nucleic acid compositions and vectors encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist as described herein. In some embodiments, the nucleic acid compositions and vectors may include a payload including but not limited to a transgene, a marker or reporter gene, etc. In some embodiments, the expression vector comprises a nucleic acid composition as described herein operably linked to an expression control sequence.

[0077] In some embodiments, the nucleic acid composition comprises a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist. In some embodiments, the differential resistance to the CSF1R antagonist is partial resistance, complete, increased, or decreased resistance to the CSF1R antagonist.

[0078] In other embodiments, the nucleic acid composition comprises a modified CSF1R protein comprising one or more genetic modifications. In some embodiments, the one or more genetic modifications result in an amino acid substitution which is selected from a group consisting of G795A, G795V, G795C, L785, M637, E633, and V647.

[0079] In some embodiments, the present disclosure features an expression vector comprising a nucleic acid composition comprising a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist.

[0080] In some embodiments, the expression vectors described herein may be used to modify cultured

or endogenous cells. In some embodiments, the expression vectors described herein are used to transfect a cultured cell, such that the culture cell or the progeny of said cell express the polypeptide. In other embodiments, the expression vectors described herein are used to modify an endogenous CSF1R-expressing cell by transducing the nucleic acid (e.g., by infecting with the viral vector to create a CSF1R-antagonist resistant cell *in situ*).

[0081] Methods and compositions of the present disclosure may advantageously be used in human or veterinary applications whereby a modified cell or a vector or genome-editing-system (e.g., a CRISPR-Cas, TALEN, or ZFN system) configured to introduce a mutation of the instant disclosure and thereby create a modified cell, is used in the prevention, palliation, amelioration or cure of a disease or disorder. In general, these methods will comprise the steps of contacting a subject with a modified cell or a vector or genome editing system configured to generate such a cell and contacting the subject with a CSF1R antagonist.

[0082] Turning first to subjects for whom the methods of this disclosure may be suitable, any subject may be a candidate for treatment according to these methods, but it may be advantageous to treat those subjects who suffer from, or are predicted or predisposed to suffering from, a disease such as a neurological disease that can be prevented, palliated, ameliorated or cured by contacting the subject with a cell of the present disclosure characterized by a differential response to CSF1R antagonists relative to a native or endogenous cell of the subject. Without limiting the foregoing, a neurological disease amenable to treatment by methods of this disclosure may comprise dementia, a neurodegenerative disease, a demyelinating disease, a mood or personality disorder, a traumatic brain injury, a genetic disease, a malignant or benign tumor, a metastatic tumor, or growth, etc.

[0083] A subject can be contacted with a modified cell or a vector of the disclosure at any suitable time and by any suitable route of administration, including without limitation by intravenous, intramuscular, intraperitoneal, intraparenchymal, intrathecal, intercranial, interosseus or other injection or infusion, transdermal administration (for vectors) and/or by surgical implantation or transplantation. Any suitable number of cells or titer of the vector may be administered, e.g., 10^1 , 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , etc. cells, and/or 10^{14} , 10^{13} , 10^{12} , 10^{11} , 10^{10} , 10^9 , or fewer virions or viral genomes. With respect to modified cells, the cells with which the subject is contacted may be derived from the subject's own cells (autologous cells) or they may be from another donor (allogeneic cells).

[0084] Those of skill in the art will appreciate that the differential sensitivity of modified cells of this disclosure to CSF1R antagonists may be most clearly manifest in the presence of a CSF1R antagonist. Therefore, while the subject may be contacted at any time with a CSF1R antagonist (including without limitation as pre-treatment or conditioning treatment prior to contacting a subject with a modified cell of this disclosure), some embodiments of this disclosure comprise administering a CSF1R antagonist to a subject simultaneously with and/or following the step of contacting the subject with the modified cell or vector. The CSF1R antagonist may be administered continuously or discontinuously, and the initiation or

discontinuation of the administration may be scheduled or pre-programmed, or it may be in response to a physiological readout from the subject such as a biomarker concentration, a serum or tissue concentration of a biomarker or a small molecule active ingredient or metabolite thereof, an imaging signal or sign, etc.

[0085] As one non-limiting example, in the case of a modified cell that exhibits reduced sensitivity (i.e., partial or complete resistance) to a CSF1R antagonist, said CSF1R antagonist may be administered to a subject to reduce the proliferation or survival of native cells within a niche occupied by such cells, thereby imparting a selective advantage to modified cells and optionally increasing a rate of engraftment or other behavior of the modified cells relative to that observed in the absence of the CSF1R antagonist. This administration may be initiated – and optionally terminated – prior to, simultaneously, or subsequently to the step of contacting the subject with the modified cell or vector. Without limiting the foregoing, in the case where the modified cell is a microglial cell or microglial progenitor cell, a CSF1R antagonist may be administered to a subject prior to, coincident with, and/or subsequent to the transplantation of modified microglial-lineage cells to limit the proliferation and/or survival of endogenous microglia, thereby imparting an advantage to the modified microglia which may result in a greater degree of engraftment and/or a greater spatial distribution of the modified microglia than might otherwise be observed.

[0086] Alternatively, or additionally, a modified cell of this disclosure may exhibit increased sensitivity to CSF1R antagonists relative to an unmodified cell. In this instance, the CSF1R antagonist is generally (but not necessarily) withheld before and during the step of contacting the subject with the modified cell, and instead is administered after the subject has been contacted with the modified cell or vector, for instance, to limit the proliferation or survival of the modified cells at the conclusion of a course of treatment, in response to a signal from a physiological or pharmacological measurement, and/or in response to an adverse event or a reduction in the efficacy of a therapeutic intervention utilizing the modified cells.

[0087] A modified cell of this disclosure optionally comprises one or more additional modifications relative to a native cell, of the same type. These modifications may include, without limitation, one or more transgenes, or one or more genetic modifications that correct a mutation or reduce or increase the expression of a gene product. Without limiting the foregoing, this disclosure includes a microglial cell engineered to express a CSF1R allele with reduced sensitivity to a CSF1R antagonist, and a secreted transgene such as an enzyme or a binding protein, an RNA such as antisense, miRNA, or siRNA, a cell surface protein such as a chimeric antigen receptor, a membrane-bound immunoglobulin or fragment thereof, an aptamer, etc. The disclosure also comprises vectors and methods of making such cells substantially as described above, and a method of treating a subject using a cell or vector to treat the subject.

[0088] Without wishing to limit the present disclosure to any theory or mechanism, it is believed that a variety of methods could be used to achieve the presently claimed cells that have a differential resistance to a CSF1R antagonist. Described herein are non-limiting methodologies/examples that accomplish this

goal. Equivalents or substitutes are within the scope of the present disclosure.

[0089] EXAMPLE

[0090] The following is a non-limiting example of the present invention. It is to be understood that said example is not intended to limit the present invention in any way. Equivalents or substitutes are within the scope of the present invention.

[0091] To facilitate the competitive engraftment of human microglia, macrophages, and monocytes and/or their precursor cells (hematopoietic progenitor cells (HPCs) and hematopoietic stem cells (HSCs), erythromyeloid progenitors (EMPs), or Primitive Macrophage Progenitors (PMPs), several approaches could be used independently or in combination. 1) using the previously published crystal structure of the CSF1R receptor bound to an antagonist (PMID: 31434879) to predict amino acid substitutions that may sterically hinder the binding of CSF1R antagonists while not impairing the normal binding of ATP into the same binding pocket. 2) Using PCR-mediated random mutagenesis to introduce amino acid changes followed by exposure of iPS-microglia to CSF1R antagonists and then sequencing of surviving mutants to identify mutations that confer resistance to CSF1R antagonists, but which do not concurrently induce constitutively active CSF1R signaling. 3) Modifying genes downstream of CSF1R, for example, delete or transiently reduce expression of SHIP1 (INPP5D) to enhance CSF1R signal transduction. 4) Pretreat microglia being transplanted with agonists of CSF1R signaling, could also use a siRNA or microRNA gene delivery approach to modulate and transiently increase CSF1R signaling in transplanted cells to promote resistance to CSF1R antagonist treatments. Below are provided additional details and data related to the first two strategies.

[0092] **Strategy 1:** A crystal structure of the CSF1R receptor bound to an antagonist (PMID: 31434879) to predict amino acid substitutions that may sterically hinder the binding of CSF1R antagonists while not impairing the normal binding of ATP into the same binding pocket. The crystal structure of PLX5622 bound to human CSF1R was obtained and the ATP binding pocket was examined (FIG. 7A). Through molecular modeling single amino acid changes were predicted that would likely impair the binding of PLX5622 or PLX3397 or other CSF1R antagonists while not disrupting normal ATP binding to CSF1R. Specifically, this modeling suggests that replacement of amino acid G795 with either an Alanine (A), Valine (V), or Cysteine (C) will increase the steric hindrance of PLX5622 and/or PLX3397 binding without disrupting the normal ability of ATP to bind (FIG. 7A and 7B). Other mutations could also be explored to prevent binding (FIG. 7D). A CRISPR targeting strategy was designed to generate human iPSCs carrying each of these single amino acid substitutions; G795A, G795V, G795C, and successfully produced these modified human iPSC lines (Sanger Sequencing Chromatograms demonstrating the edited sequences are shown in FIG. 8). The resulting iPSCs were then differentiated into microglia and exposed to varying concentrations of either PLX3397 or PLX5622. To detect microglial cell death (apoptosis) a caspase-3/7 fluorogenic reporter was used. FIG. 4A demonstrates the dose-dependent induction of cell death in wild-type (WT) unedited human iPSC-microglia versus the G795A and G795C CSF1R mutant lines in

response to PLX3397 and FIG. 4B shows the response to PLX5622. Within 24 hours of PLX compound treatment WT human microglia exhibit a robust dose-dependent induction of apoptosis in response to either compound. In contrast, both the G795A and G795C mutant lines show complete resistance to cell death. FIG. 4F provided representative images of cell density (phase-contrast microscopy, 1st column) and fluorescent Caspase-3/7 activity (2nd column) across each dose of PLX3397 and PLX5622.

[0093] Further inspection of the crystal structure also presents other positions which, if mutated, could result in a reduction of PLX5622 and/or PLX3397 binding. Examples include L785, M637, E633, and V647 (FIG. 4D). These positions are all within close contact to PLX5622 and/or PLX3397 binding sites and may prevent binding when mutated.

[0094] **Strategy 2:** PCR-mediated random mutagenesis will be used to introduce amino acid changes followed by exposure of iPS-microglia to CSF1R antagonists and then sequencing of surviving mutants to identify mutations that confer resistance to CSF1R antagonists, but which do not concurrently induce constitutively active or suppressed CSF1R signaling. A pool of iPSC or monocyte cell lines will be generated that carry random mutations in the CSF1R gene. This pool will then be differentiated into microglia and the survival of cells monitored in response to either CSF1/IL-34 withdrawal or CSF1R antagonist treatments. Cells that survive for longer under these conditions will be examined via PCR and sequencing to identify CSF1R mutations that may confer resistance to CSF1R antagonists. Counter screens will then be performed to identify those CSF1R mutations that do not also lead to hyperproliferation or significant loss in proliferation. Once optimal mutations are identified iPSC lines carrying these mutations will be expanded and microglia derived from these lines will be examined *in vivo* in chimeric models to determine resistance to CSF1R antagonists *in vivo*.

[0095] Resistance to each of the CSF1R inhibitors tested was quantified using a fluorogenic caspase 3/7 detector (Essen BioScience) and time-lapse imaging over 24 hours. WT microglia exhibited significant levels of increased caspase to increasing concentrations of PLX3397 (MedChemExpress), PLX5622 (MedChemExpress), Edicotinib, and BLZ945, as compared to DMSO control treatment over 24 hours of imaging, signifying an increased cell death response to inhibiting the macrophage-colony stimulating factor/colony-stimulating factor 1 receptor (CSF1R). On the other hand, G795A and G795C iPSC-microglia exhibited no significant increase in caspase levels to increasing concentrations of either PLX3397, PLX5622, Edicotinib, or BLZ945, as compared to DMSO control treatment. These results indicate an acquired resistance to PLX treatment as a result of the genetically modified CSF1 receptor (see FIG. 4A, 4B, 4C, 4D, and 4E).

[0096] **Cell death assay:** iPS-microglia were plated at 70K cells per 96-well plate (6 wells per line per condition). At time 0, all microglia were treated with IncuCyte Caspase-3/7 Green Apoptosis Assay Reagent 1:1000. Cells were maintained in the described medium: fresh complete medium + 0.1%DMSO, complete media + 250nM PLX3397, complete medium + 500nM PLX3397, complete medium + 1µM PLX3397, complete medium + 250nM PLX5622, complete medium + 500nM PLX5622, complete medium

+ 1 μ m PLX5622, complete medium + 250nM Edicotinib, complete medium + 500nM Edicotinib, complete medium + 1 μ m Edicotinib, complete medium + 250nM BLZ945, complete medium + 500nM BLZ945, complete medium + 1 μ m BLZ945. Four 20 $\times$ images per well were collected every hour for 24 hours. Using IncuCyte 2020B software, image masks for phase confluence (visually gated out apoptotic cells) as well as caspase 3/7 signal (green) were generated. Graphs display caspase normalized to phase confluence, completed with 3 lines: WT, G795A, G795C (see FIG. 4A, 4B, 4C, 4D, and 4E).

[0097] **EMBODIMENTS**

[0098] The following embodiments are intended to be illustrative only and not to be limiting in any way.

[0099] Embodiment 1: A modified human cell exhibiting differential resistance to a CSF1R antagonist.

[00100] Embodiment 2: The cell of embodiment 1, comprising a nucleic acid encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist.

[00101] Embodiment 3: A modified human cell comprising a nucleic acid encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist.

[00102] Embodiment 4: The cell of any one of embodiments 1-3, comprising one or more genetic modifications in the CSF1R protein.

[00103] Embodiment 5: The cell of embodiment 4, wherein the one or more genetic modifications results in a change in the amino acid sequence of CSF1R, the change optionally comprising the substitution of an amino acid residue selected from G795, L785, M637, E633, and V647.

[00104] Embodiment 6: The cell of any one of embodiments 1-5, wherein the one or more genetic modifications results in a modified ATP binding pocket.

[00105] Embodiment 7: The cell of embodiment 6, wherein the modified ATP binding pocket has reduced binding space.

[00106] Embodiment 8: The cell of embodiment 7, wherein the modified ATP binding pocket cannot bind a CSF1R antagonist.

[00107] Embodiment 9: The cell of embodiment 6, wherein the modified ATP binding pocket can bind ATP.

[00108] Embodiment 10: The cell of any one of embodiments 1-9, wherein the one or more genetic modifications in the CSF1R protein do not interfere with ATP binding activity of the CSF1R protein.

[00109] Embodiment 11: The cell of any one of embodiments 1-10, wherein the one or more genetic modifications in the CSF1R protein do not induce constitutively active CSF1R signaling.

[00110] Embodiment 12: The cell of embodiment 11, wherein the modified CSF1R protein is activated by

a CSF1 ligand.

[00111] Embodiment 13: The cell of embodiment 12, wherein the CSF1 ligand induces phosphorylation of the modified CSF1R protein.

[00112] Embodiment 14: The cell of embodiment 11, wherein the modified CSF1R protein is activated by an IL-34 ligand.

[00113] Embodiment 15: The cell of embodiment 14, wherein the IL-34 ligand induces phosphorylation of the modified CSF1R protein.

[00114] Embodiment 16: The cell of embodiment 4, wherein the one or more genetic modifications are introduced *ex vivo*.

[00115] Embodiment 17: The cell of embodiment 4, wherein the one or more genetic modifications are induced by transfecting or introducing into the cell a nucleic acid encoding a transgene, or by transduction or introduction of a targeted nuclease, nickase or base-editing effector or ribonucleoprotein complex.

[00116] Embodiment 18: The cell of embodiment 17, wherein introducing the nucleic acid encoding a transgene in a cell comprises contacting the cell with a viral vector.

[00117] Embodiment 19: The cell of any one of embodiments 1-18, wherein the cell comprising a modified CSF1R protein retains a gene expression profile of a cell comprising a wild-type CSF1R protein.

[00118] Embodiment 20: The cell of any one of embodiments 1-19, further comprising one or more modifications in another gene.

[00119] Embodiment 21: A modified human cell according to any one of embodiments 1-20 that is expressing CSF1R.

[00120] Embodiment 22: The cell of embodiment 21, wherein the cell is induced and differentiated to express CSF1R and is selected from a group consisting of pluripotent stem cell, hematopoietic stem cell, an erythromyeloid progenitor, and hematopoietic progenitor cell.

[00121] Embodiment 23: The cell of embodiment 22, wherein the cell is a CSF1R-expressing cell and is selected from a group consisting of microglia, macrophages, monocytes, or other phagocytes.

[00122] Embodiment 24: A composition comprising a CSF1R-expressing cell according to any one of embodiments 20-23.

[00123] Embodiment 25: The composition of embodiment 24, wherein the CSF1R-expressing cell is a CSF1R-expressing cell.

[00124] Embodiment 26: The composition of embodiment 25, comprising a plurality of cells, wherein at least 0.1%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the cells are CSF1R expressing cells having differential resistance to a CSF1R antagonist.

[00125] Embodiment 27: A method of treating a subject, the method comprising: (a) administering a CSF1R antagonist to the subject in a quantity sufficient to inhibit a CSF1R signal in a cell of the subject; and (b) contacting the subject with a modified CSF1R-expressing cell having a differential resistance to the CSF1R antagonist.

[00126] Embodiment 28: A method of treating a subject, the method comprising: (a) contacting the subject with a modified CSF1R-expressing cell having a differential resistance to a CSF1R antagonist; and (b) administering the CSF1R antagonist to the subject in a quantity sufficient to inhibit a CSF1R signal in a cell of the subject.

[00127] Embodiment 29: The method of embodiment 27 and embodiment 28, wherein the CSF1R-expressing cell is a CSF1R-expressing cell.

[00128] Embodiment 30: The method of any one of embodiments 27-29, wherein the differential resistance to the CSF1R antagonist is partial resistance or complete resistance to the CSF1R antagonist.

[00129] Embodiment 31: The method of any one of embodiments 27-30, wherein the modified CSF1R-expressing cell retains a gene expression profile of a wild-type CSF1R-expressing cell.

[00130] Embodiment 32: The method of any one of embodiments 27-31, wherein the modified CSF1R-expressing cell expresses a gene product or other genetic modification useful to treat, cure, ameliorate, prevent or palliate a neurological disease.

[00131] Embodiment 33: The method of embodiment 32, wherein the gene product is a transgene that is not otherwise expressed by the CSF1R-expressing cell.

[00132] Embodiment 34: The method of embodiment 33, wherein the CSF1R signal that is inhibited by the CSF1R antagonist is proliferation or survival of a CSF1R-expressing cell that is endogenous to the subject.

[00133] Embodiment 35: The method of any one of embodiments 27-34, wherein the CSF1R antagonist is administered to the subject in a quantity sufficient to reduce the proliferation and/or survival of the endogenous CSF1R-expressing cell relative to the modified CSF1R-expressing cell.

[00134] Embodiment 36: A method of treating a subject, the method comprising: (a) contacting the subject with a modified CSF1R-expressing cell, and (b) differentially altering the proliferation or survival of an unmodified CSF1R-expressing cell relative to the modified CSF1R-expressing cell.

[00135] Embodiment 37: The method of embodiment 36, wherein the modified CSF1R-expressing cell is a CSF1R-expressing phagocyte.

[00136] Embodiment 38: The method of embodiment 36 and embodiment 37, wherein the modified CSF1R-expressing cell is partially resistant to a CSF1R antagonist.

[00137] Embodiment 39: The method of embodiment 36, wherein the step of differentially altering the proliferation or survival of the unmodified CSF1R-expressing cell comprising administering the CSF1R antagonist to the subject in a quantity sufficient to reduce proliferation and/or survival of the unmodified CSF1R-expressing cell relative to the modified CSF1R-expressing cell.

[00138] Embodiment 40: A nucleic acid composition encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist.

[00139] Embodiment 41: The composition of embodiment 40, wherein the differential resistance to the CSF1R antagonist is partial resistance or complete resistance to the CSF1R antagonist.

[00140] Embodiment 42: The composition of embodiment 40, wherein the differential resistance to the CSF1R antagonist is an increased resistance to the CSF1R antagonist.

[00141] Embodiment 43: The composition of embodiment 40, wherein the differential resistance to the CSF1R antagonist is a decreased resistance to the CSF1R antagonist.

[00142] Embodiment 44: The composition of any one embodiments 40-43, wherein the modified CSF1R protein comprises one or more genetic modifications.

[00143] Embodiment 45: The composition of embodiment 44, wherein the one or more genetic modifications results in a change in the amino acid sequence of CSF1R, the change optionally comprising the substitution of an amino acid residue selected from G795, L785, M637, E633, and V647.

[00144] Embodiment 46: The composition of embodiment 44, wherein the one or more genetic modifications results in a modified ATP binding pocket.

[00145] Embodiment 47: The composition of embodiment 46, wherein the modified ATP binding pocket has reduced binding space.

[00146] Embodiment 48: The composition of embodiment 47, wherein the modified ATP binding pocket cannot bind a CSF1R antagonist.

[00147] Embodiment 49: The composition of embodiment 46, wherein the modified ATP binding pocket can bind ATP.

[00148] Embodiment 50: The composition of embodiment 44, wherein the one or more genetic

modifications in the CSF1R protein do not interfere with ATP binding activity of the CSF1R protein.

[00149] Embodiment 51: The composition of embodiment 44, wherein the one or more genetic modifications in the CSF1R protein do not induce constitutively active CSF1R signaling.

[00150] Embodiment 52: The composition of embodiment 51, wherein the modified CSF1R protein is activated by a CSF1 ligand.

[00151] Embodiment 53: The composition of embodiment 52, wherein the CSF1 ligand induces phosphorylation of the modified CSF1R protein.

[00152] Embodiment 54: The composition of embodiment 51, wherein the modified CSF1R protein is activated by an IL-34 ligand.

[00153] Embodiment 55: The cell of embodiment 54, wherein the IL-34 ligand induces phosphorylation of the modified CSF1R protein.

[00154] Embodiment 56: An expression vector comprising the nucleic acid composition of any one of embodiments 40-55 operably linked to an expression control sequence.

[00155] Embodiment 57: A cultured cell transfected with the vector of embodiment 56, or a progeny of said cell, wherein the cultured cells express the polypeptide.

[00156] Embodiment 58: An endogenous cell infected with the vector of embodiment 56, wherein the endogenous cells express the polypeptide.

[00157] As used herein, the term "about" refers to plus or minus 20% of the referenced number.

[00158] Although there has been shown and described the preferred embodiment of the present disclosure, it will be readily apparent to those skilled in the art that modifications may be made thereto which do not exceed the scope of the appended claims. Therefore, the scope of the disclosure is only to be limited by the following claims. In some embodiments, the figures presented in this patent application are drawn to scale, including the angles, ratios of dimensions, etc. In some embodiments, the figures are representative only and the claims are not limited by the dimensions of the figures. In some embodiments, descriptions of the disclosure described herein using the phrase "comprising" includes embodiments that could be described as "consisting essentially of" or "consisting of", and as such the written description requirement for claiming one or more embodiments of the present disclosure using the phrase "consisting essentially of" or "consisting of" is met.

WHAT IS CLAIMED IS:

1. A modified human cell exhibiting differential resistance to a CSF1R antagonist.
2. The cell of claim 1, comprising a nucleic acid encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist.
3. A modified human cell comprising a nucleic acid encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist.
4. The cell of any one of claims 1-3, comprising one or more genetic modifications in the CSF1R protein.
5. The cell of claim 4, wherein the one or more genetic modifications results in a change in the amino acid sequence of CSF1R, the change optionally comprising the substitution of an amino acid residue selected from G795, L785, M637, E633, and V647.
6. The cell of any one of claims 1-5, wherein the one or more genetic modifications results in a modified ATP binding pocket.
7. The cell of claim 6, wherein the modified ATP binding pocket has reduced binding space.
8. The cell of claim 7, wherein the modified ATP binding pocket cannot bind a CSF1R antagonist.
9. The cell of claim 6, wherein the modified ATP binding pocket can bind ATP.
10. The cell of any one of claims 1-9, wherein the one or more genetic modifications in the CSF1R protein do not interfere with ATP binding activity of the CSF1R protein.
11. The cell of any one of claims 1-10, wherein the one or more genetic modifications in the CSF1R protein do not induce constitutively active CSF1R signaling.
12. The cell of claim 11, wherein the modified CSF1R protein is activated by a CSF1 ligand.
13. The cell of claim 12, wherein the CSF1 ligand induces phosphorylation of the modified CSF1R protein.
14. The cell of claim 11, wherein the modified CSF1R protein is activated by an IL-34 ligand.
15. The cell of claim 14, wherein the IL-34 ligand induces phosphorylation of the modified CSF1R protein.
16. The cell of claim 4, wherein the one or more genetic modifications are introduced *ex vivo*.
17. The cell of claim 4, wherein the one or more genetic modifications are induced by transfecting or introducing into the cell a nucleic acid encoding a transgene, or by transduction or introduction of a targeted nuclease, nickase or base-editing effector or ribonucleoprotein complex.
18. The cell of claim 17, wherein introducing the nucleic acid encoding a transgene in a cell comprises contacting the cell with a viral vector.
19. The cell of any one of claims 1-18, wherein the cell comprising a modified CSF1R protein retains a gene expression profile of a cell comprising a wild-type CSF1R protein.
20. The cell of any one of claims 1-19, further comprising one or more modifications in another gene.
21. A modified human cell according to any one of claims 1-20 that is expressing CSF1R.
22. The cell of claim 21, wherein the cell is induced and differentiated to express CSF1R and is

- selected from a group consisting of pluripotent stem cell, hematopoietic stem cell, an erythromyeloid progenitor, and hematopoietic progenitor cell.
23. The cell of claim 22, wherein the cell is a CSF1R-expressing cell and is selected from a group consisting of microglia, macrophages, monocytes, or other phagocytes.
24. A composition comprising a CSF1R-expressing cell according to any one of claims 20-23.
25. The composition of claim 24, wherein the CSF1R-expressing cell is a CSF1R-expressing cell.
26. The composition of claim 25, comprising a plurality of cells, wherein at least 0.1%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the cells are CSF1R expressing cells having differential resistance to a CSF1R antagonist.
27. A method of treating a subject, the method comprising:
- a) administering a CSF1R antagonist to the subject in a quantity sufficient to inhibit a CSF1R signal in a cell of the subject; and
 - b) contacting the subject with a modified CSF1R-expressing cell having a differential resistance to the CSF1R antagonist.
28. A method of treating a subject, the method comprising:
- a) contacting the subject with a modified CSF1R-expressing cell having a differential resistance to a CSF1R antagonist; and
 - b) administering the CSF1R antagonist to the subject in a quantity sufficient to inhibit a CSF1R signal in a cell of the subject,
29. The method of claim 27 and claim 28, wherein the CSF1R-expressing cell is a CSF1R-expressing cell.
30. The method of any one of claims 27-29, wherein the differential resistance to the CSF1R antagonist is partial resistance or complete resistance to the CSF1R antagonist.
31. The method of any one of claims 27-30, wherein the modified CSF1R-expressing cell retains a gene expression profile of a wild-type CSF1R-expressing cell.
32. The method of any one of claims 27-31, wherein the modified CSF1R-expressing cell expresses a gene product or other genetic modification useful to treat, cure, ameliorate, prevent or palliate a neurological disease.
33. The method of claim 32, wherein the gene product is a transgene that is not otherwise expressed by the CSF1R-expressing cell.
34. The method of claim 33, wherein the CSF1R signal that is inhibited by the CSF1R antagonist is proliferation or survival of a CSF1R-expressing cell that is endogenous to the subject.
35. The method of any one of claims 27-34, wherein the CSF1R antagonist is administered to the subject in a quantity sufficient to reduce the proliferation and/or survival of the endogenous CSF1R-expressing cell relative to the modified CSF1R-expressing cell.

36. A method of treating a subject, the method comprising:
- a) contacting the subject with a modified CSF1R-expressing cell, and
 - b) differentially altering the proliferation or survival of an unmodified CSF1R-expressing cell relative to the modified CSF1R-expressing cell.
37. The method of claim 36, wherein the modified CSF1R-expressing cell is a CSF1R-expressing phagocyte.
38. The method of claim 36 and claim 37, wherein the modified CSF1R-expressing cell is partially resistant to a CSF1R antagonist.
39. The method of claim 36, wherein the step of differentially altering the proliferation or survival of the unmodified CSF1R-expressing cell comprising administering the CSF1R antagonist to the subject in a quantity sufficient to reduce proliferation and/or survival of the unmodified CSF1R-expressing cell relative to the modified CSF1R-expressing cell.
40. A nucleic acid composition encoding a modified CSF1R protein exhibiting differential resistance to a CSF1R antagonist.
41. The composition of claim 40, wherein the differential resistance to the CSF1R antagonist is partial resistance or complete resistance to the CSF1R antagonist.
42. The composition of claim 40, wherein the differential resistance to the CSF1R antagonist is an increased resistance to the CSF1R antagonist.
43. The composition of claim 40, wherein the differential resistance to the CSF1R antagonist is a decreased resistance to the CSF1R antagonist.
44. The composition of any one claims 40-43, wherein the modified CSF1R protein comprises one or more genetic modifications.
45. The composition of claim 44, wherein the one or more genetic modifications results in a change in the amino acid sequence of CSF1R, the change optionally comprising the substitution of an amino acid residue selected from G795, L785, M637, E633, and V647.
46. The composition of claim 44, wherein the one or more genetic modifications results in a modified ATP binding pocket.
47. The composition of claim 46, wherein the modified ATP binding pocket has reduced binding space.
48. The composition of claim 47, wherein the modified ATP binding pocket cannot bind a CSF1R antagonist.
49. The composition of claim 46, wherein the modified ATP binding pocket can bind ATP.
50. The composition of claim 44, wherein the one or more genetic modifications in the CSF1R protein do not interfere with ATP binding activity of the CSF1R protein.
51. The composition of claim 44, wherein the one or more genetic modifications in the CSF1R protein do not induce constitutively active CSF1R signaling.
52. The composition of claim 51, wherein the modified CSF1R protein is activated by a CSF1 ligand.

53. The composition of claim 52, wherein the CSF1 ligand induces phosphorylation of the modified CSF1R protein.
54. The composition of claim 51, wherein the modified CSF1R protein is activated by an IL-34 ligand.
55. The cell of claim 54, wherein the IL-34 ligand induces phosphorylation of the modified CSF1R protein.
56. An expression vector comprising the nucleic acid composition of any one of claims 40-55 operably linked to an expression control sequence.
57. A cultured cell transfected with the vector of claim 56, or a progeny of said cell, wherein the cultured cells express the polypeptide.
58. An endogenous cell infected with the vector of claim 56, wherein the endogenous cells express the polypeptide.

FIG. 1

In Vitro Growth Kinetics of Control and CSF1R mutant Microglia

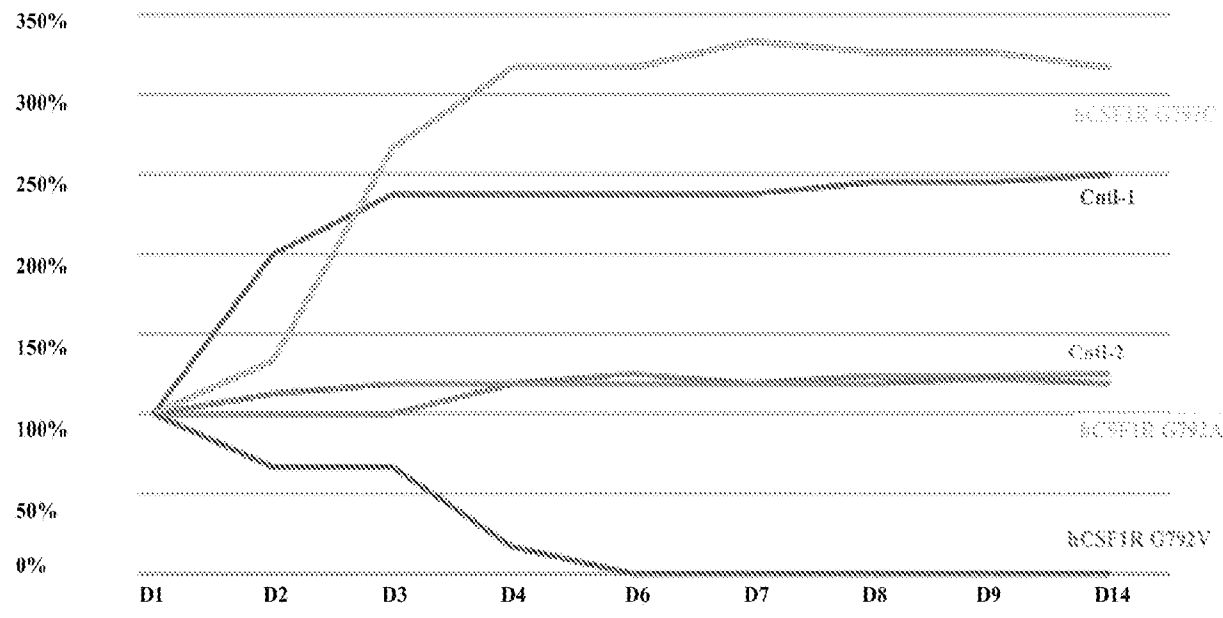


FIG. 2B

FIG. 2A

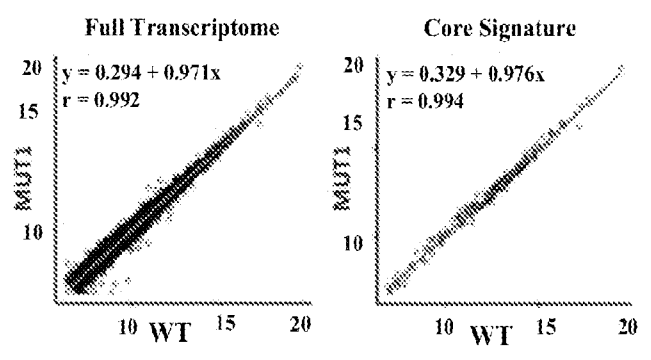
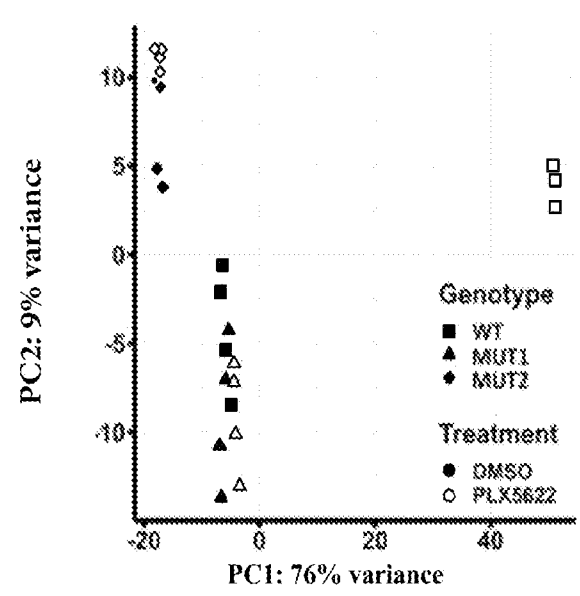


FIG. 2C

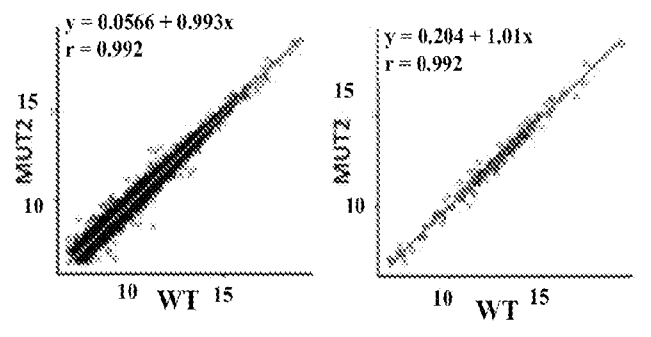


FIG. 2D

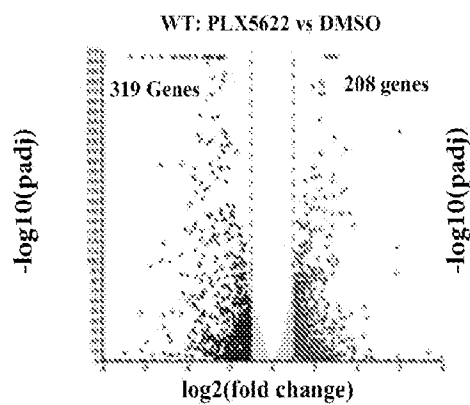


FIG. 2E

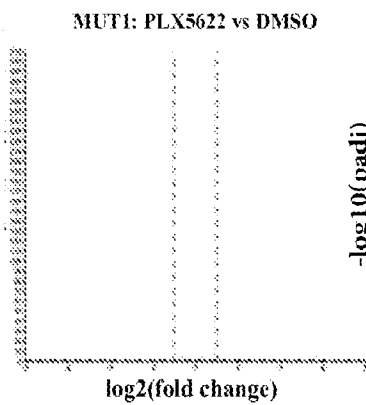


FIG. 2F

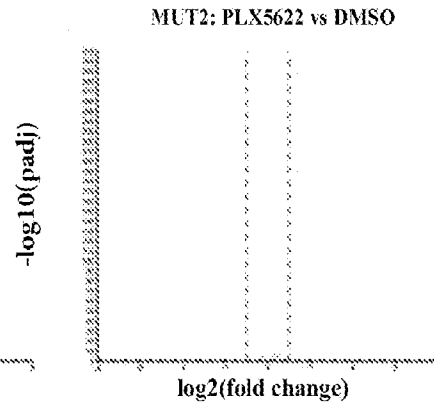


FIG. 3

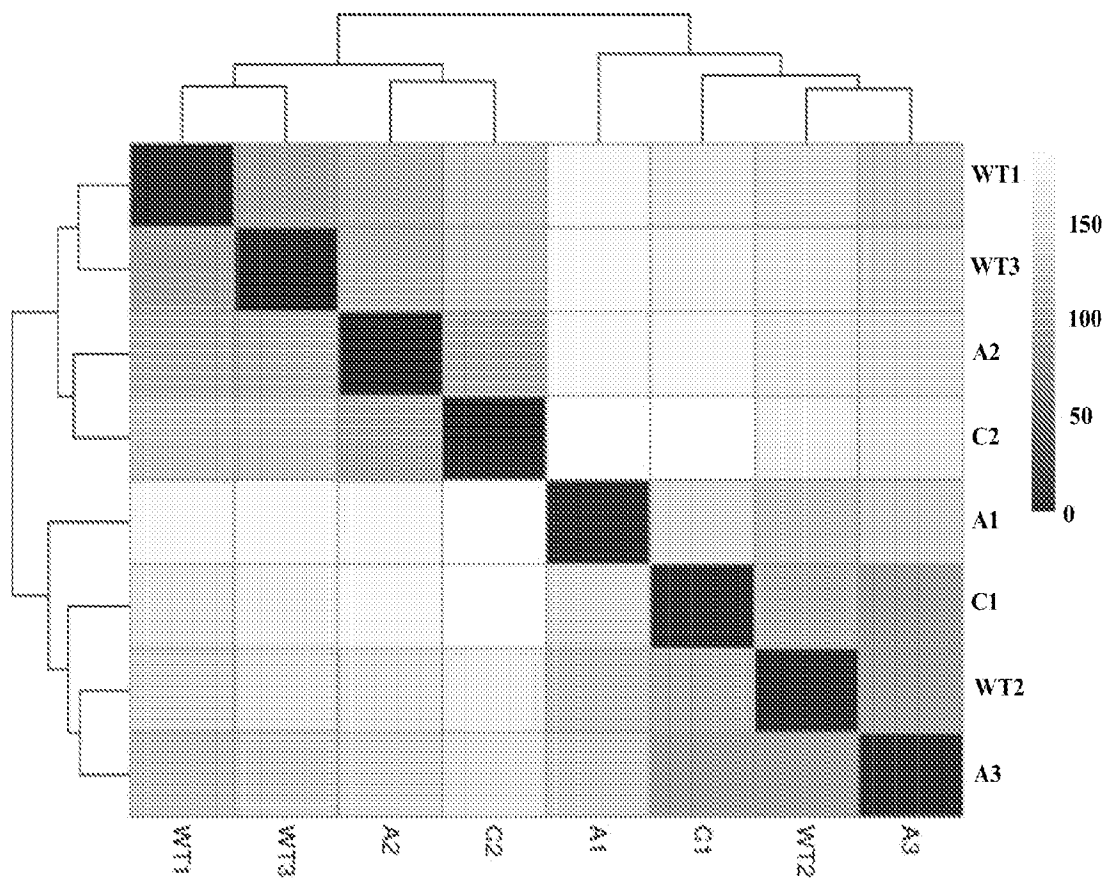


FIG. 4A

Plexxikon 3397

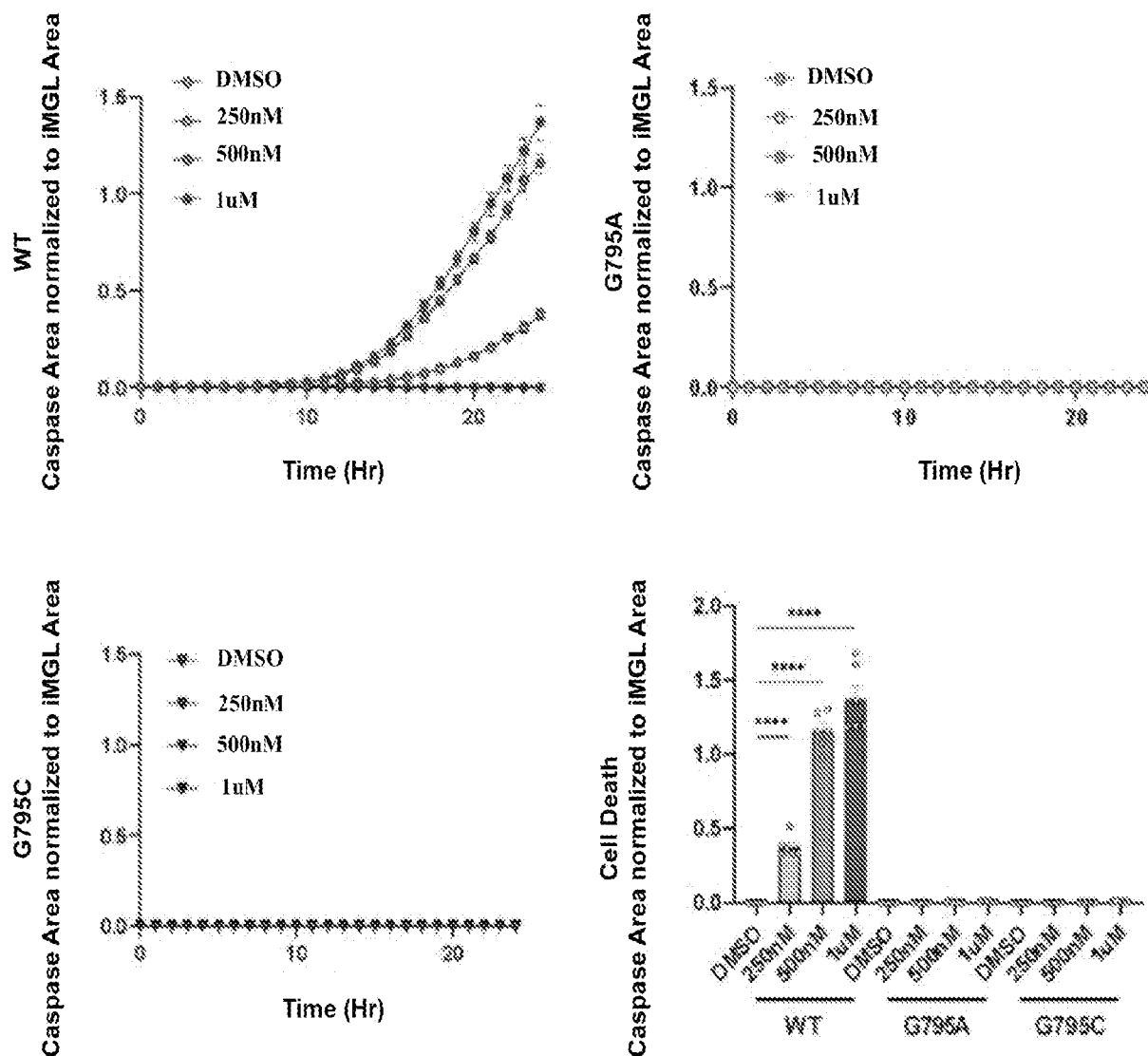


FIG. 4B

Plexxikon 5622

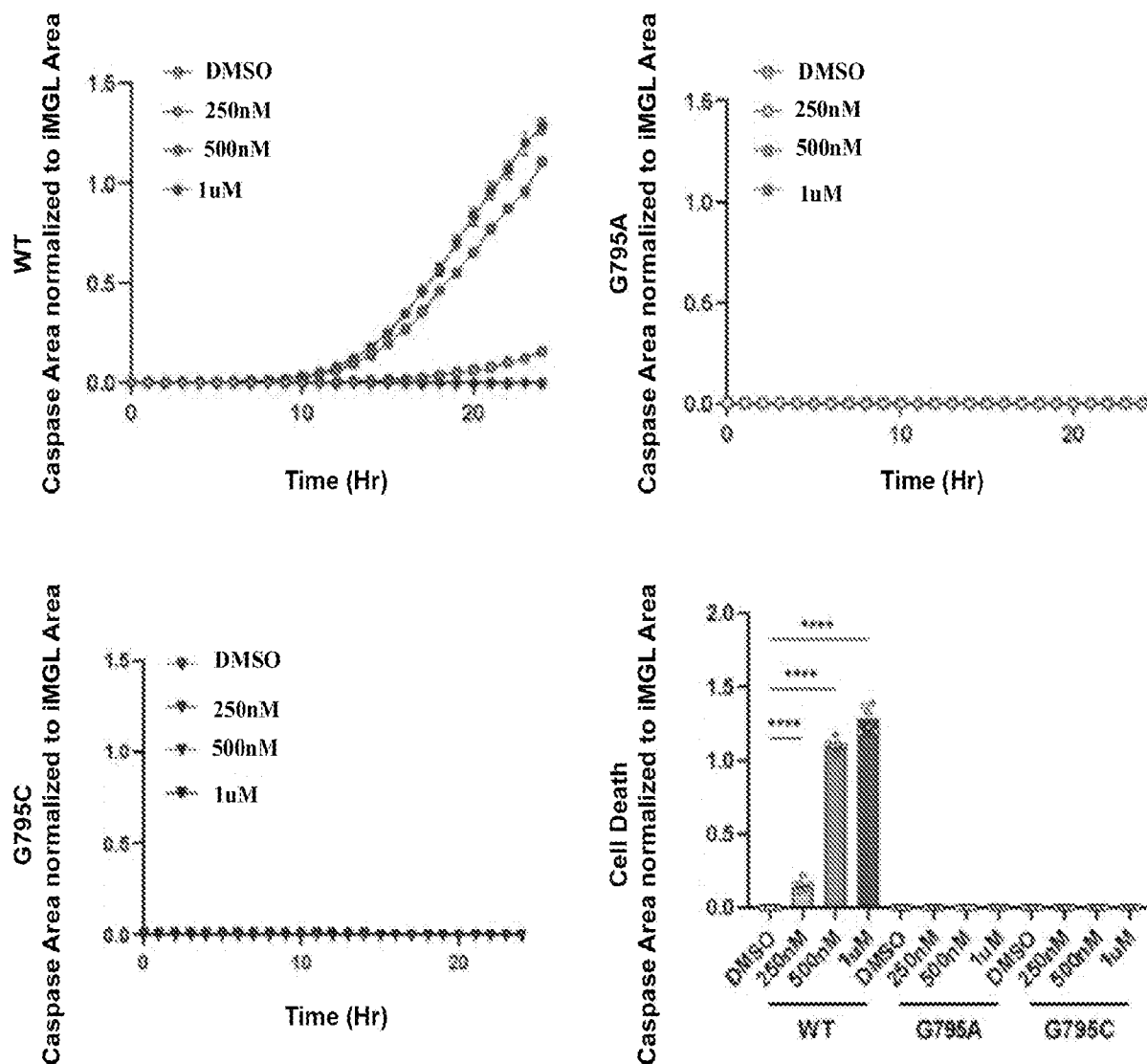


FIG. 4C

Edicotinib

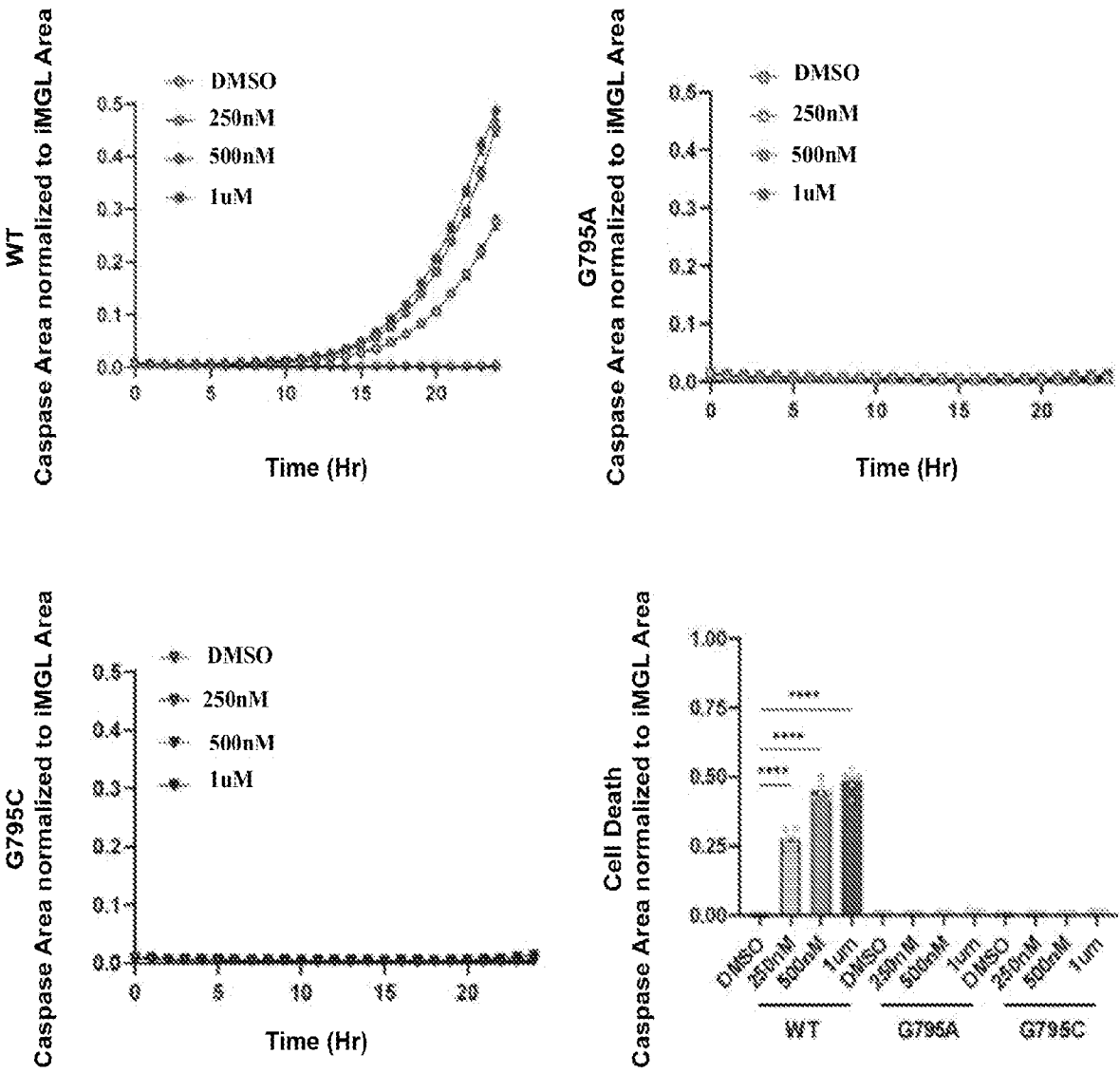


FIG. 4D

BLZ945

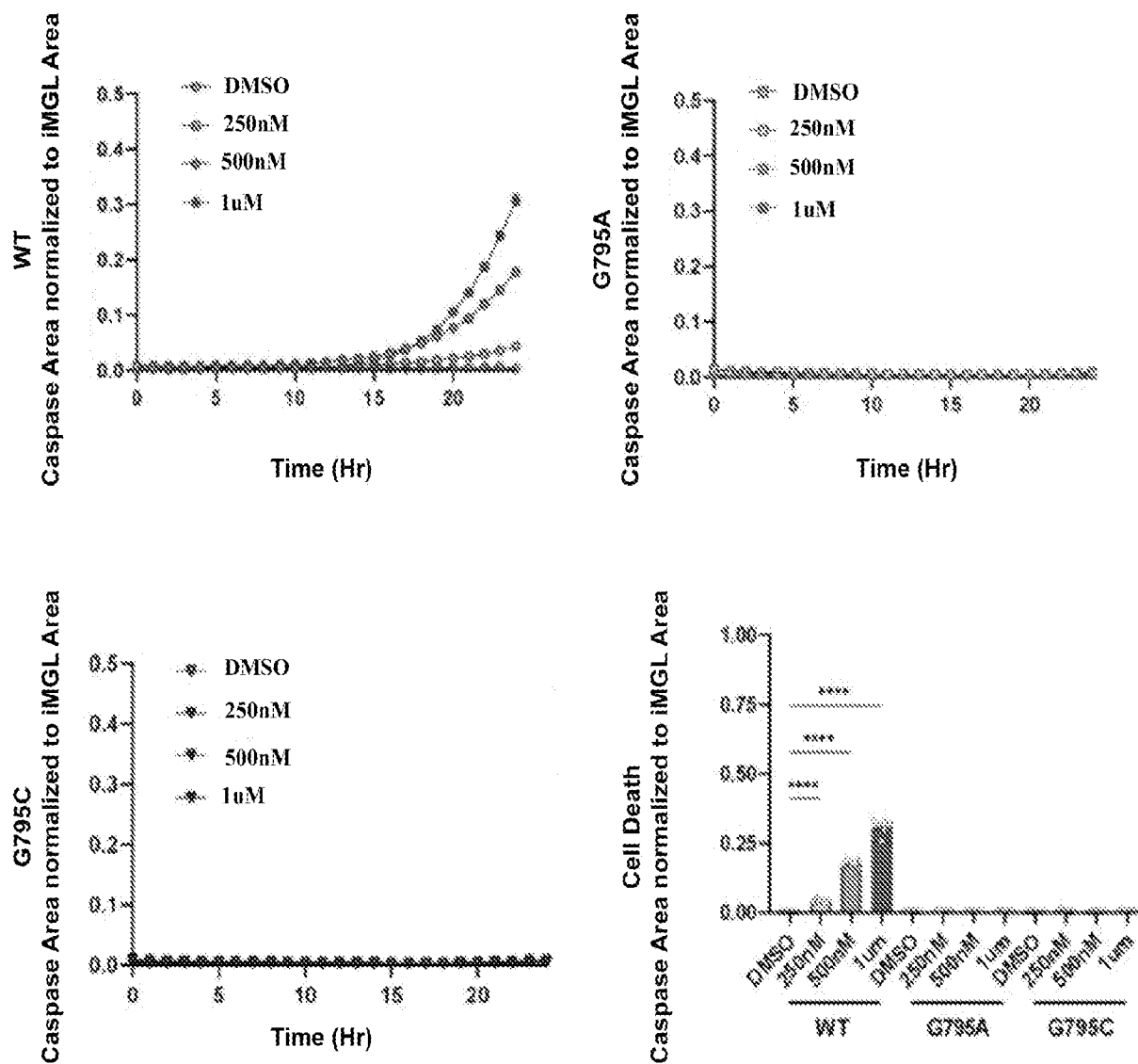
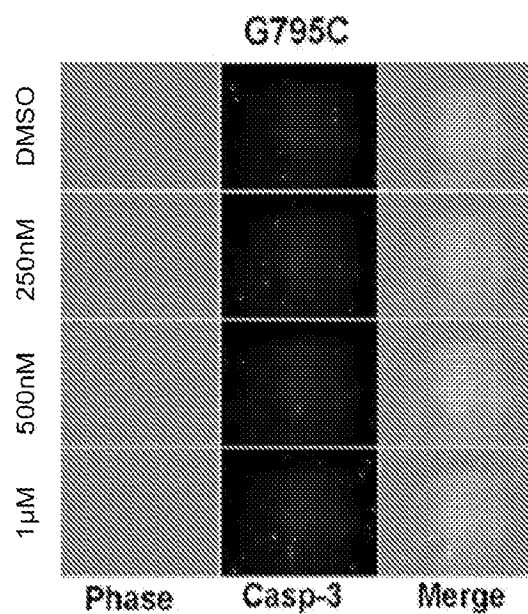
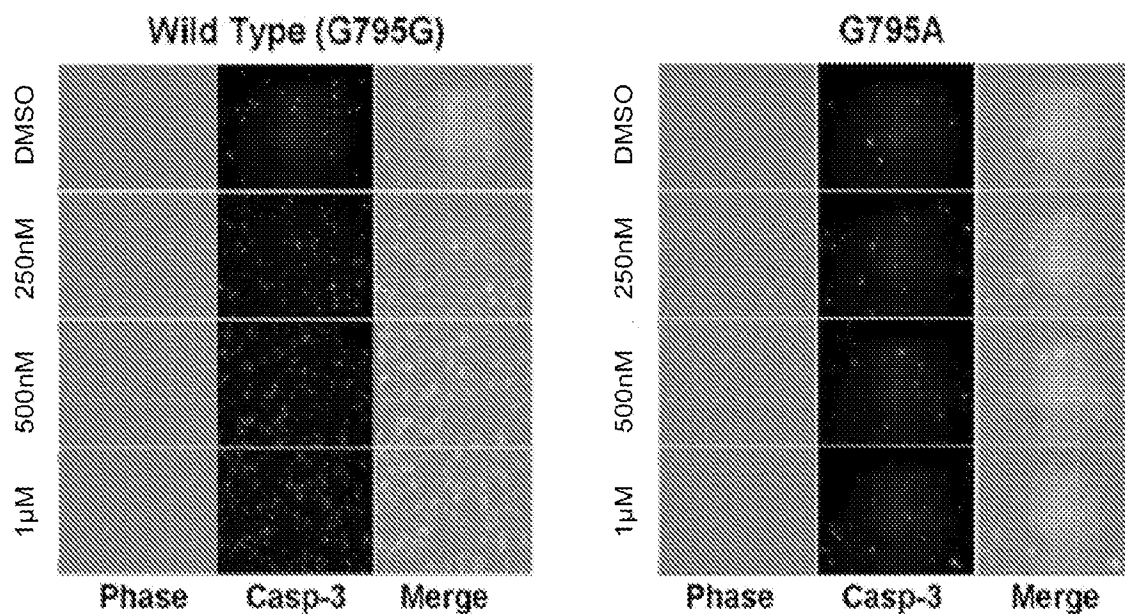


FIG. 4E
Plexxikon 3397



Percent Live vs Dead after 24hrs (500nM)

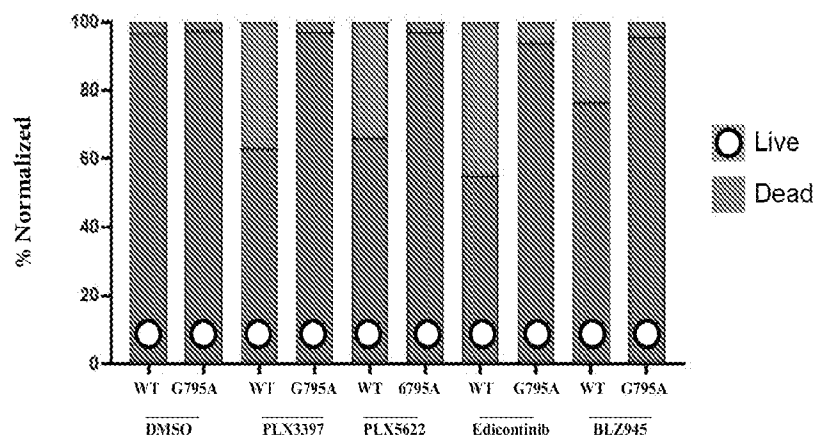


FIG. 5B

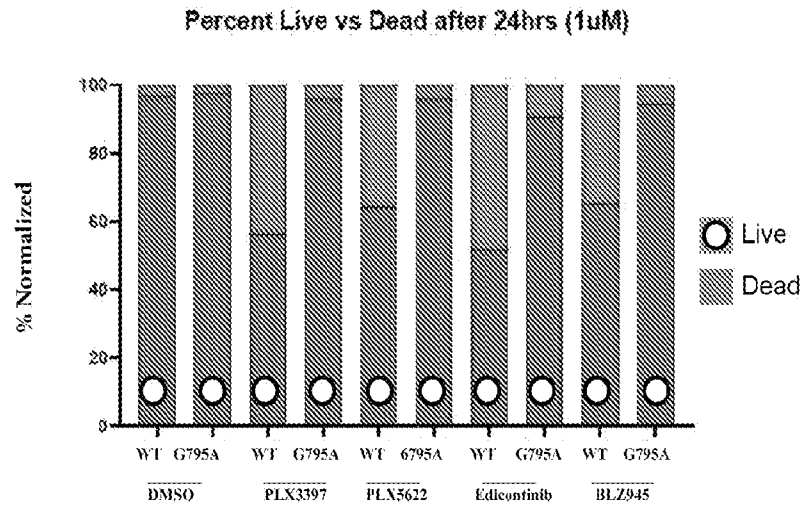


FIG. 6

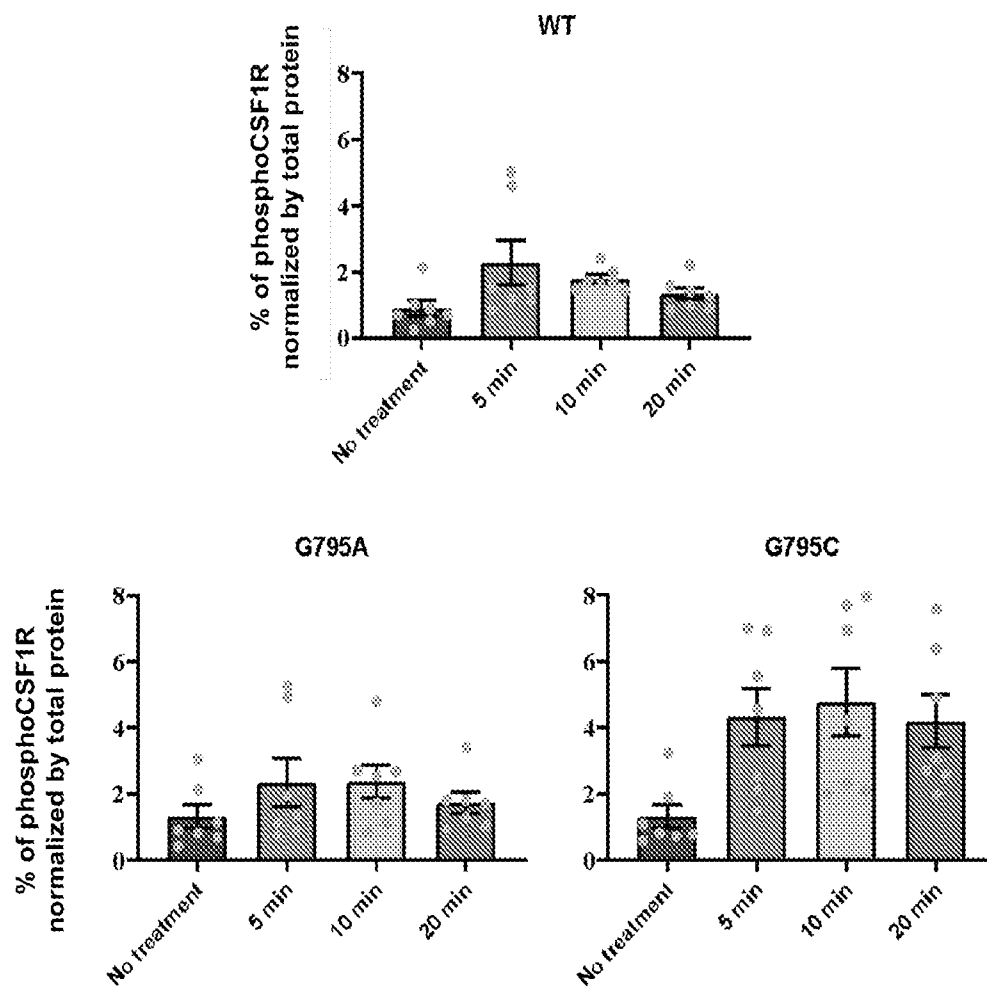


FIG. 7A

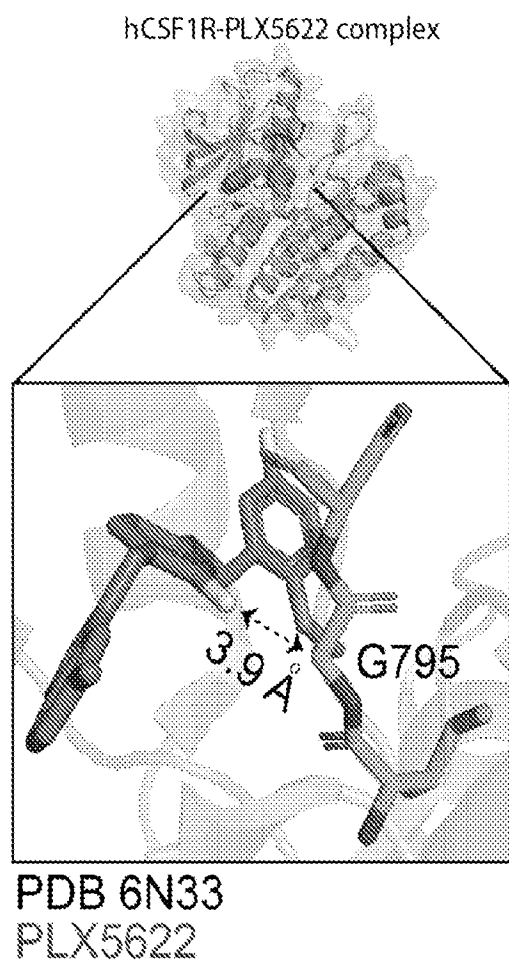


FIG. 7B

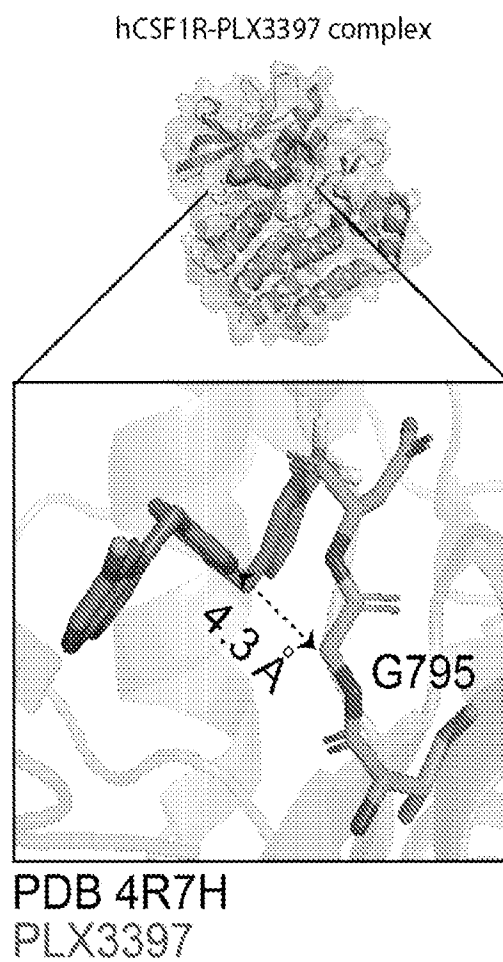


FIG. 7C

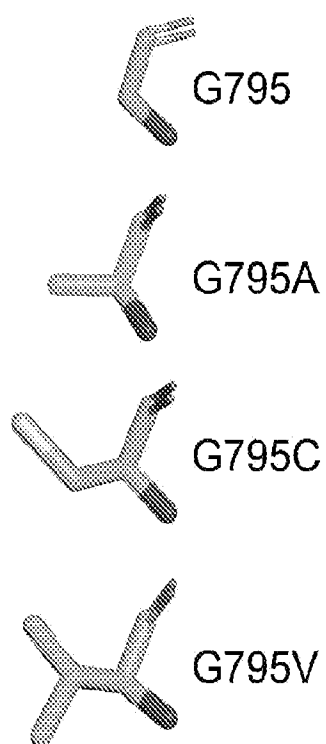


FIG. 7D

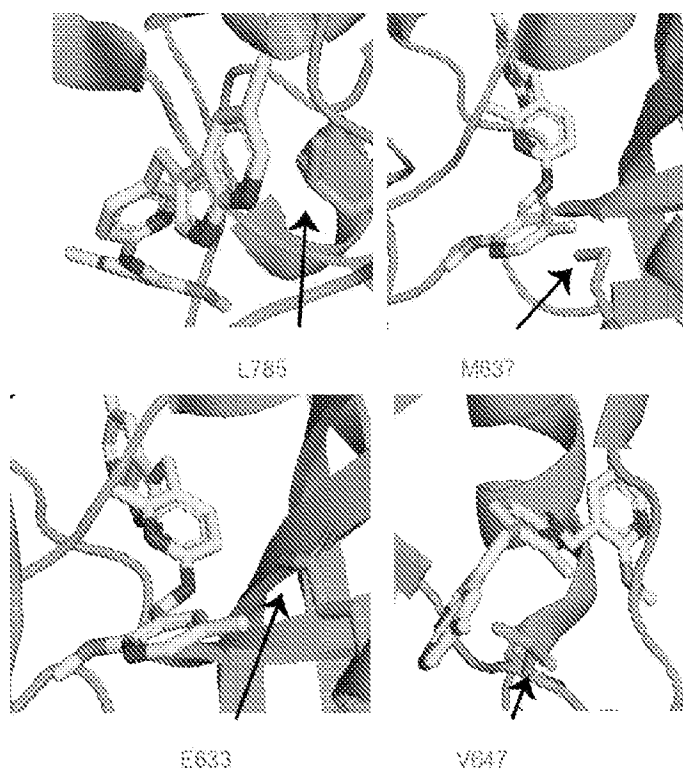


FIG. 8

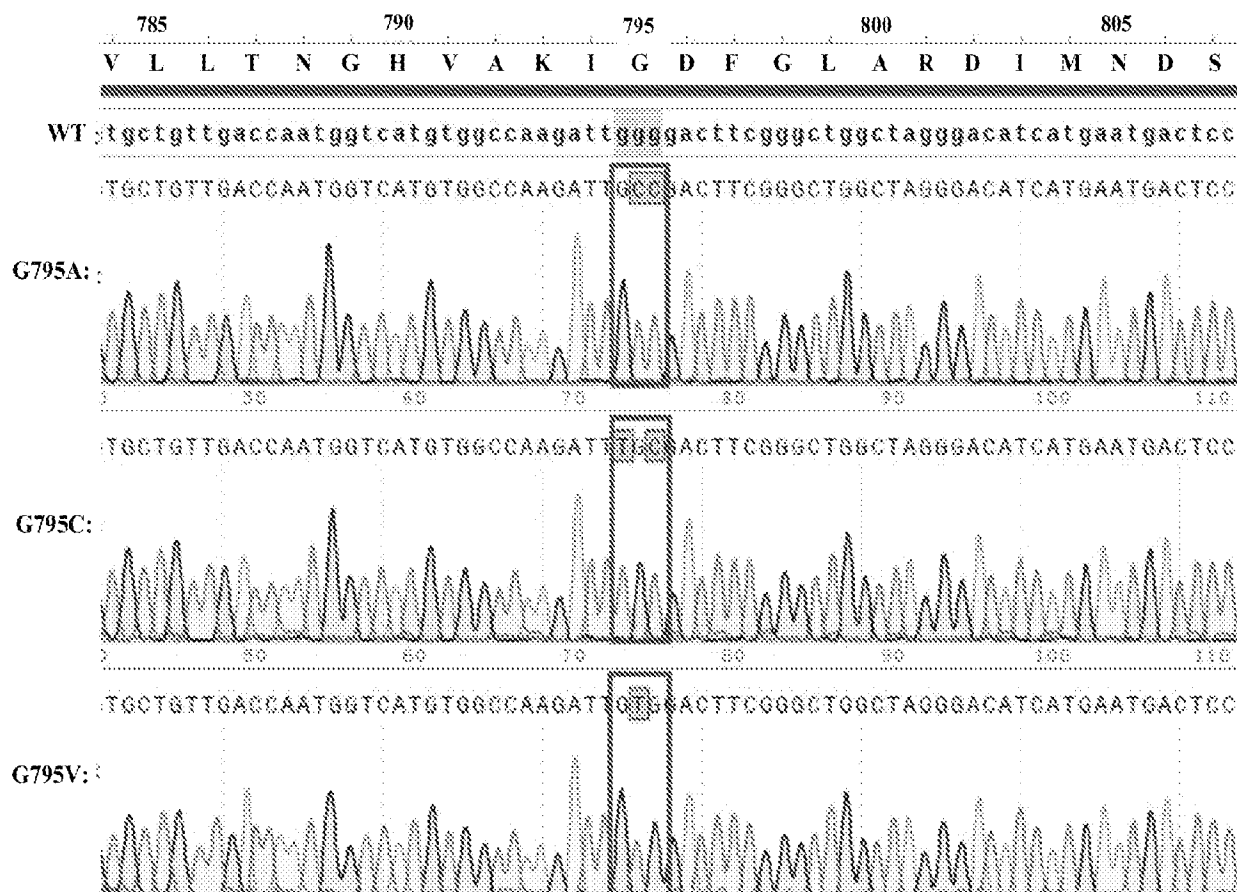


FIG. 9A

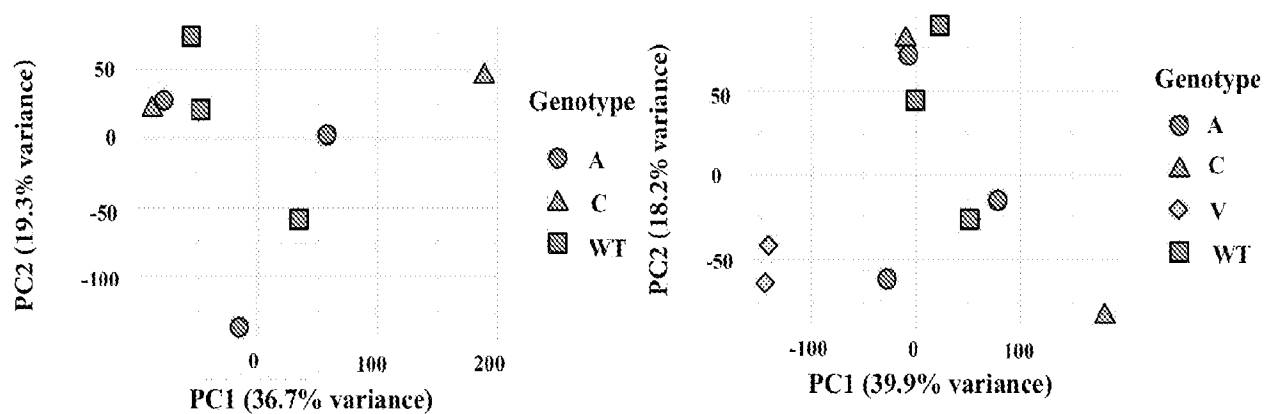


FIG. 9B

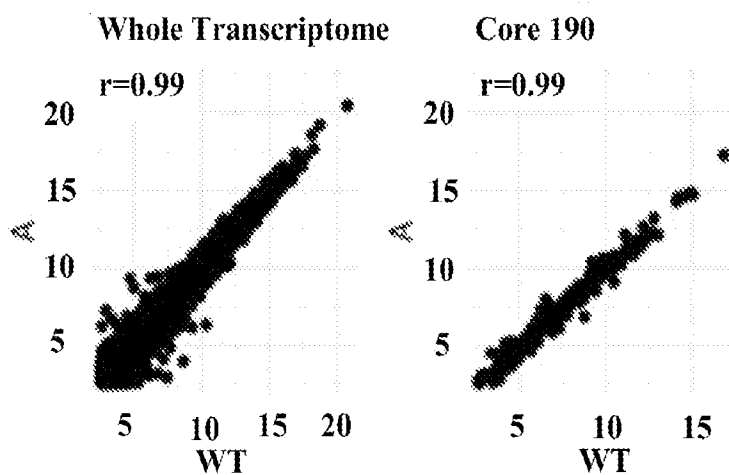


FIG. 9C

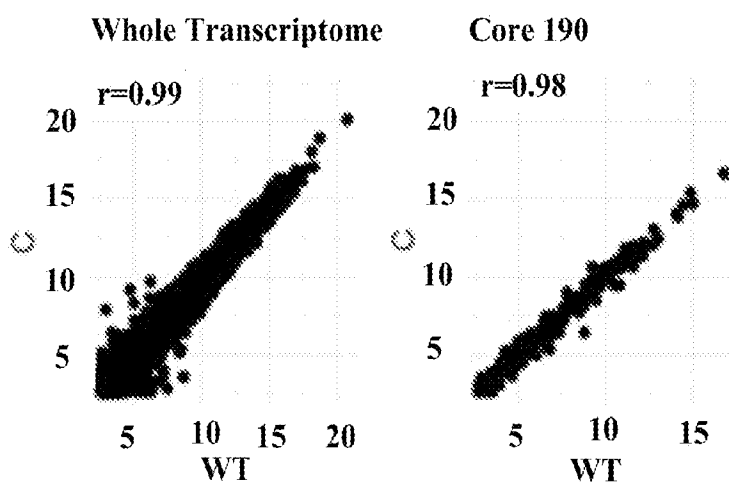


FIG. 9D

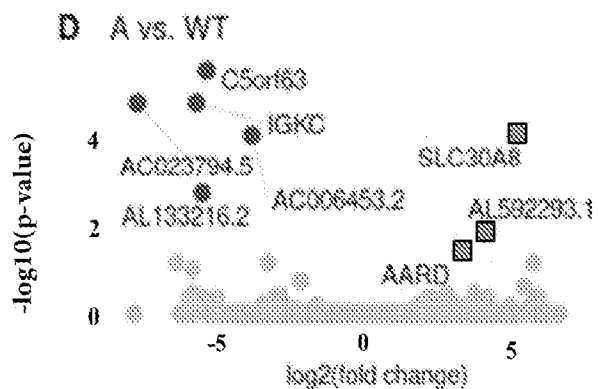


FIG. 9E

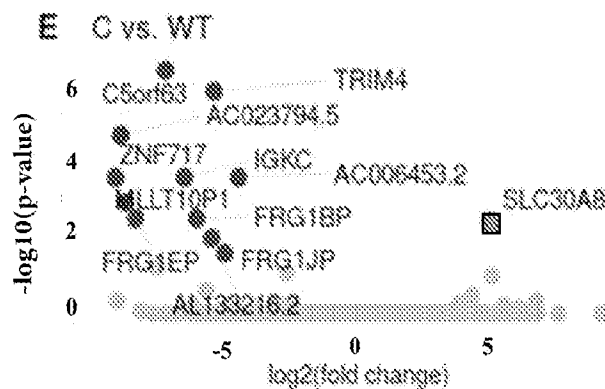


FIG. 10A

WT_vs_G795A Cell Line Correlation Plots

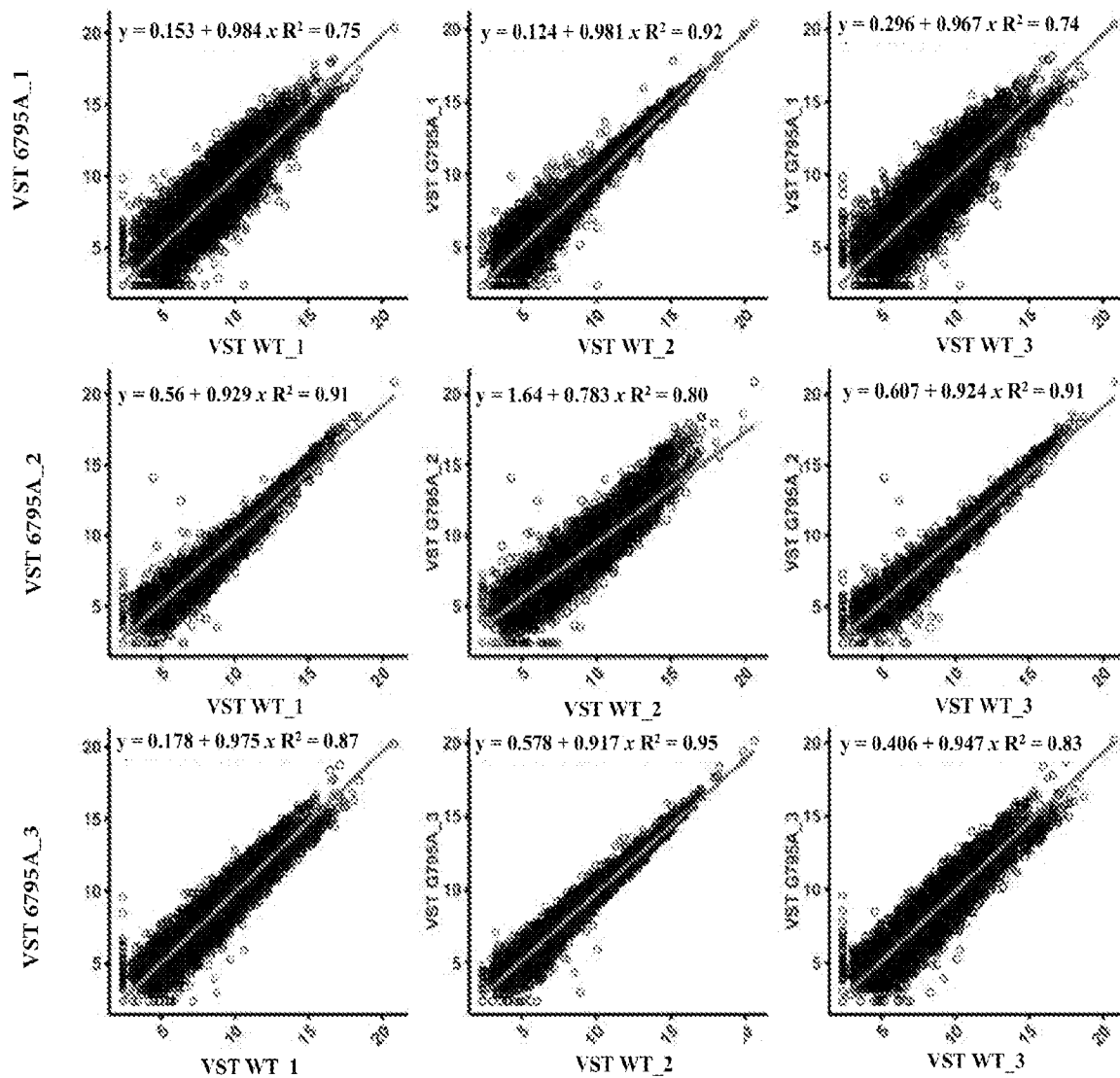


FIG. 10B

WT_vs_G795A Freeman Signature Cell Line Correlation Plots

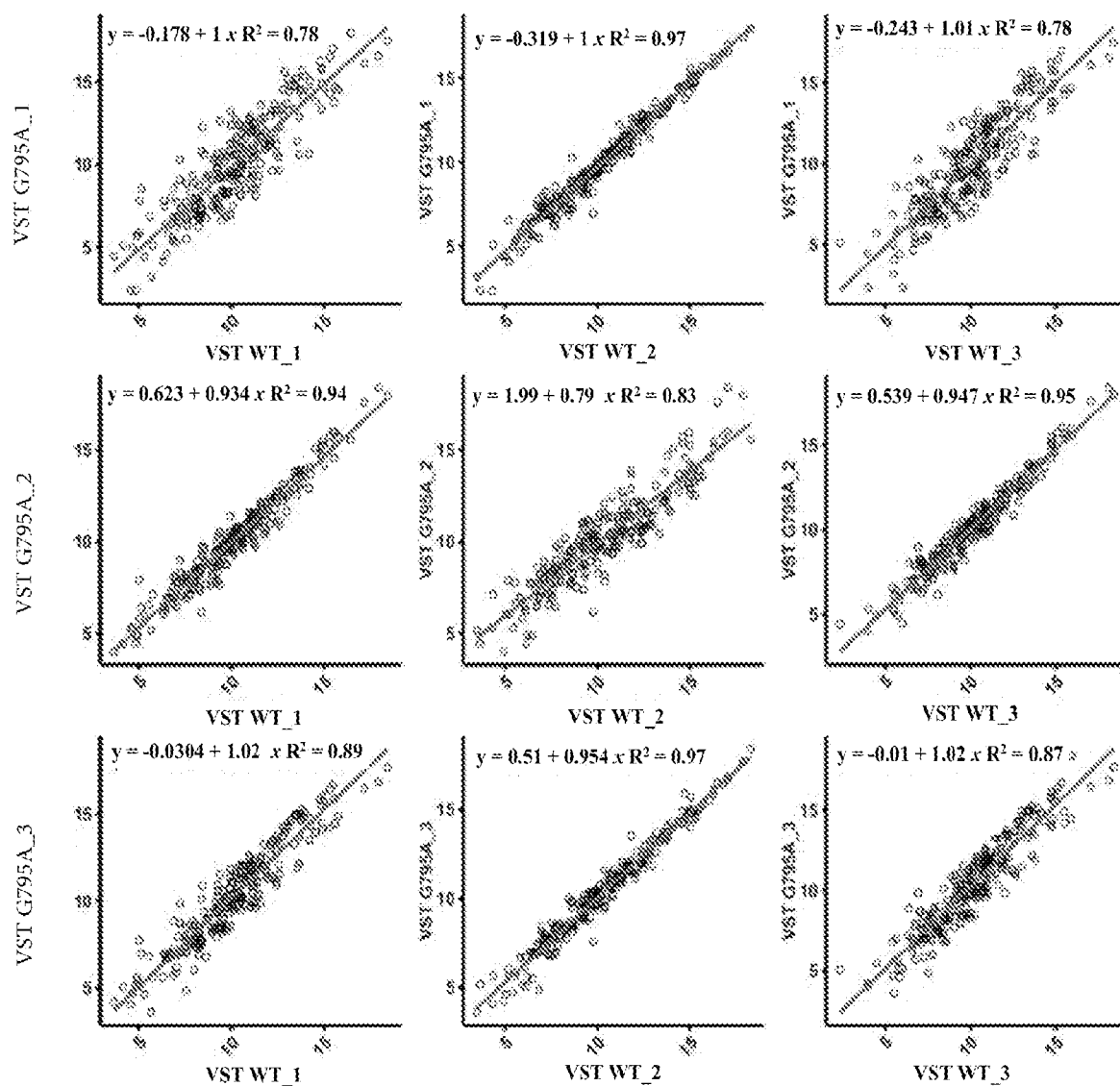


FIG. 11A

IBA-1

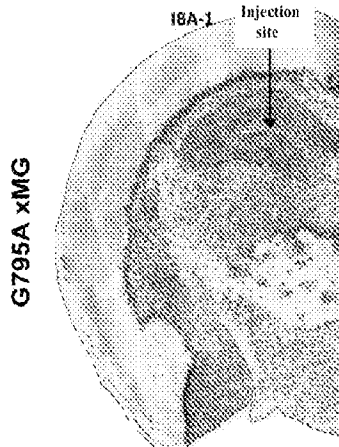


FIG. 11B

Human Nuclei (Ku80)

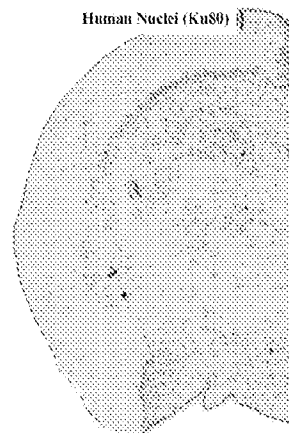


FIG. 11C

Ki67 (mitotic nuclei)

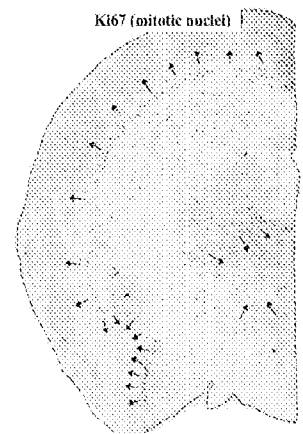


FIG. 11D

IBA-1/Ku80/Ki67

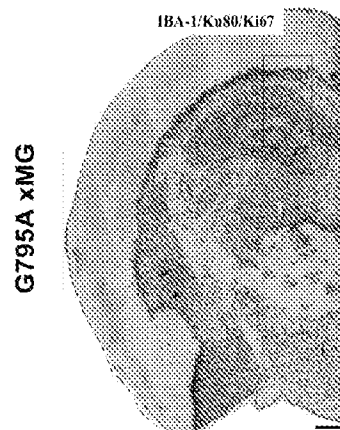
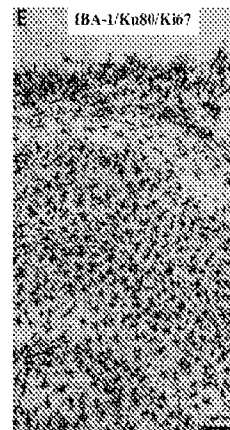
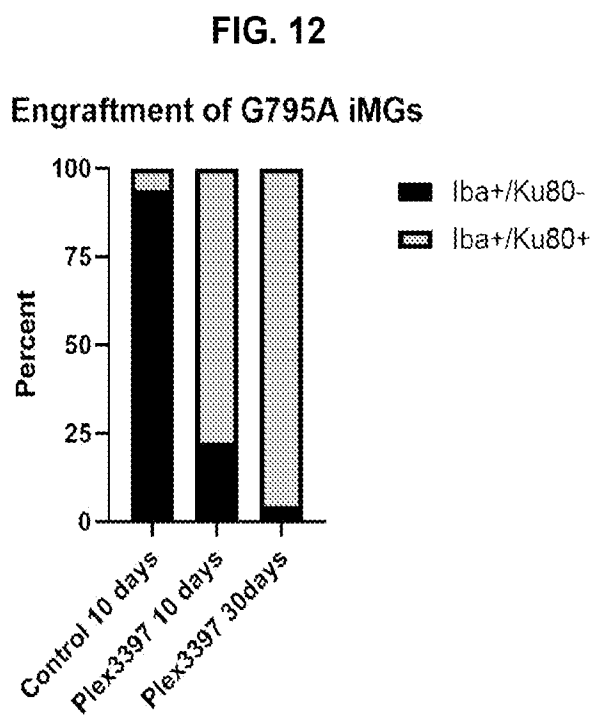
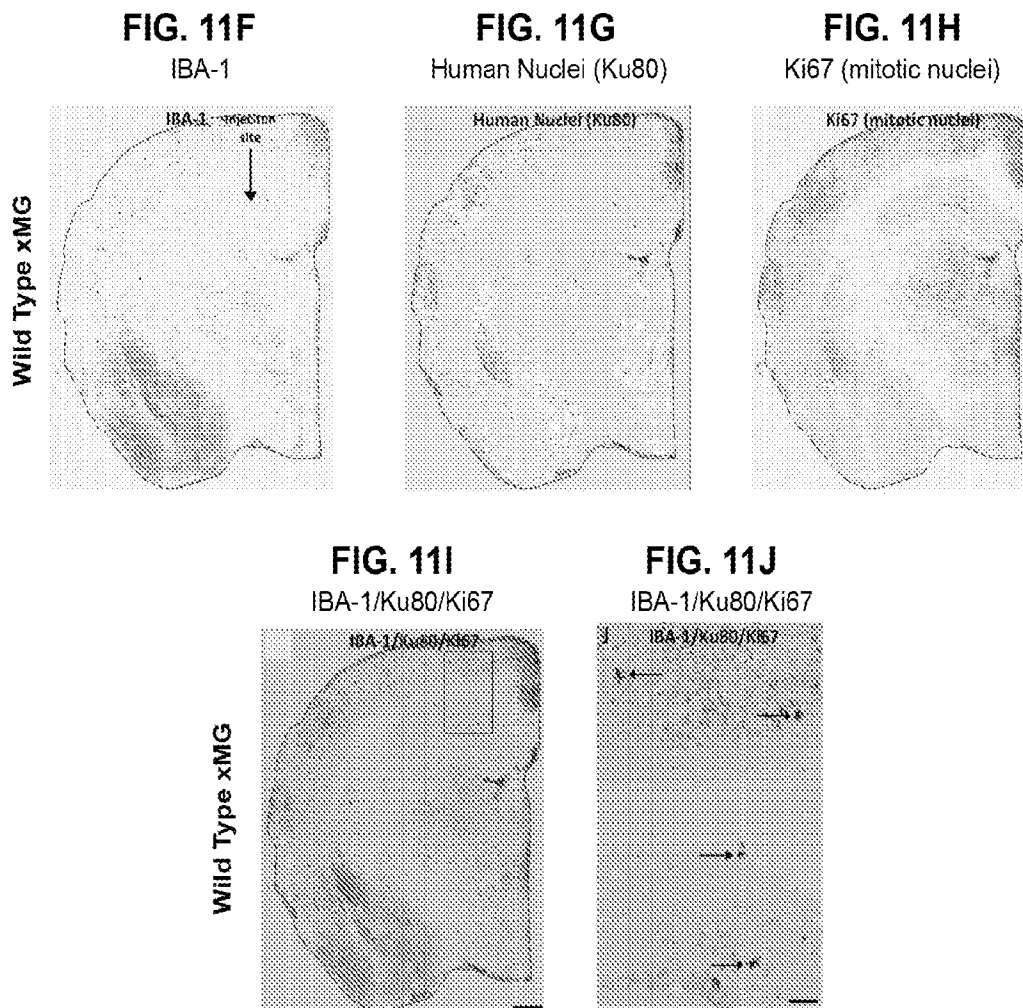


FIG. 11E

IBA-1/Ku80/Ki67





INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/23139

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07H 21/04; C12N 15/113; A61K 48/00 (2022.01)

ADD. A61K 39/12; A61K 39/395 (2022.01)

CPC - INV. C07H 21/04; C12N 15/113; A61K 48/00

ADD. A61K 39/12; A61K 39/395; C07K 2317/76

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2020/033791 A1 (NOVOBRANTSEVA TATIANA I) 13 February 2020; page 2, lines 20-21; page 10, lines 29-30; page 12, lines 16-17; page 13 lines , 6-7 and 22-24 and 28; page 16, lines 1-2; page 20, line 28; page 29, lines 6-8; page 52, line 19-20; page 53, lines 28-29 ; page 64, lines 11-12; page 79, line 7; page 105, lines 8-9 ;page 110, line 2 ; page 166, lines 8-10; page 163, lines 32-33; page 164, line 1; page 167, lines 15-16; page 188, line 13-14	1-5, 16-18, 27-28, 40-45. 51-55 ---
Y	STANLEY. "CSF-1 Receptor Signaling in Myeloid Cells". Cold Spring Harbor Perspectives in Biology. Web. 2014; page 2, figure 1a; page 7, left column, top and middle paragraph; DOI: 10.1101/cshperspect.a021857	46-50

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

31 July 2022 (31.07.2022)

Date of mailing of the international search report

AUG 30 2022

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/23139

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

3. ☒ Claims Nos.: 6-15, 19-26, 29-35, 38, 56-58
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-5, 16-18, 27-28, 40-55;
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/23139

-Continued From Box No. III: Observations where unity of invention is lacking-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-5, 16-18, 27-28, and 40-55 is directed to modified human cells exhibiting differential resistance to a CSF1R antagonist, a nucleic acid composition encoding a CSF1R protein exhibiting differential resistance to a CSF1R antagonist, and methods of treating a subject comprising administering said modified human cells and a CSF1R antagonist to the subject.

Group II, claims 36-37 and 39 is directed to a method of treating a subject involving differentially altering the proliferation or survival of unmodified vs. modified cells in the subject.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include modified human cells exhibiting differential resistance to a CSF1R antagonist, not present in Group II; and the special technical features of Group II include differentially altering the proliferation or survival of an unmodified CSF1R-expressing cell relative to a modified CSF1R-expressing cell, not present in Group I.

Groups I and II share the technical features including: a method of treating a subject comprising contacting the subject with modified CSF1R-expressing cells.

However, these shared technical features are previously disclosed by the publication entitled "Genetically modified macrophages accomplish targeted gene delivery to the inflamed brain in transgenic Parkin Q311X(A) mice: importance of administration routes" by Haney, et al. (hereinafter "Haney").

Haney discloses a method of treating a subject (systemically administered macrophages to delivery therapeutics; abstract) comprising contacting the subject with modified CSF1R-expressing cells (administering modified macrophages to a mouse model of Parkinson's disease, wherein macrophages are CSF1R-expressing cells as stipulated by the instant PCT application (paragraph [0035]); abstract).

Since none of the special technical features of the Groups I-II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Haney reference, unity of invention is lacking.