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(71) Applicants: INSTITUT NATIONAL DE LA SANTE
ET DE LA RECHERCHE MEDICALE (INSERM)
[FR/FR]; 101, Rue de Tolbiac, 75013 PARIS (FR).
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
[FR/FR]; 3, rue Michel Ange, 75016 PARIS
(FR). UNIVERSITE COTE D'AZUR [FR/FR]; Grand
Château 28 avenue de Valrose, 06100 NICE (FR).

(72) Inventors: BULAVIN, Dmitry; Oncogenesis ans stress
response / Bulavin Team / Institute for Research on Cancer
and Aging, Nice Centre Antoine Lacassagne 33 Avenue de
Valombrose, 06100 NICE (FR). MARTINEZ
TRIANA, Francisco; Oncogenesis ans stress response /
Bulavin Team / Institute for Research on Cancer and Ag-
ing, Nice Centre Antoine Lacassagne 33 Avenue de Valom-
brose, 06100 NICE (FR).

(74) Agent: HABASQUE, Etienne et al.; LAVOIX, 2, place
d'Estienne d'Orves, 75441 PARIS CEDEX 09 (FR).

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(54) Title: COMPOUNDS FOR TREATING A DISEASE ASSOCIATED WITH MACROPHAGE SENESENCE

(57) Abstract: The present invention relates to the use of a compound which is chosen from inhibitors of the mammalian target of rapamycin (mTOR) and PI3K, NNMT inhibitors, Janus kinase 2 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, AOX inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations, serotonin-norepinephrine reuptake inhibitors and phosphatidylinositol 3-kinase (PI3K) inhibitors, in treating a disease associated with macrophage senescence.



WO 2022/106579 A1

Compounds for treating a disease associated with macrophage senescence

FIELD OF THE INVENTION

The invention relates to the use of a specific compound for treating a disease associated with macrophage senescence.

BACKGROUND OF THE INVENTION

The ongoing COVID-19 pandemic has devastated the world economies putting multiple lives at risk with yet uncertain future and the possibility of a second wave both in Europe and around the world. The current knowledge of the determinants of severe COVID-19 is primarily observational and is characterized by cytokine storm, acute respiratory distress syndrome (ARDS), and in some cases by systemic inflammation-related athology. Significant efforts have been made to identify compounds and drugs that would lower the impact of the virus on human body to reduce morbidity and mortality. In parallel, numerous companies and government institutions are developing vaccines with the goal to create herd immunity in the population. While the hope is that such vaccines will be effective, several concerns still remain :

First, it is not clear how efficient such vaccines will be if the virus mutates.

Second, the most affected population is elderly, who in turn are very poor responders in developing an immune response to vaccination. In that case, if the population will develop herd immunity and the virus spread will be largely blocked, the vulnerability of older people still will be an important issue to consider.

Because of all the above, in parallel to the efforts to develop efficient vaccines against COVID-19, there is a great need for efficient drugs for lowering the clinical symptoms in patients who develop an advanced form of the disease.

COVID-19 also represents a perfect example of an age-related disease – the most vulnerable people are of age of 60 and older. Early in the COVID-19 outbreak, it was noted that older adults accounted for a disproportionate number of severe cases and deaths, and this has been corroborated by a number of epidemiological and observational studies. Advanced age is now considered as the principal risk factor for COVID-19 complications. While the underlying reasons for age-specific sensitivity remain a major unresolved question, accumulation of senescent cells has been proposed as an important driver of numerous age-related diseases.

Senescence, which is a form of terminal cell cycle arrest, is characterized by increased production of multiple factors, called Senescence-Associated Secretory Phenotype

(SASP), many of which are powerful inducers of inflammation. Furthermore, senescent cells in older organisms have a lower potential to be eliminated due to downregulation of some ligands and receptors on their surface that are required for recognition by T lymphocytes and NK cells. Thus, senescent cells can continue persisting in the aging organism producing SASP, thereby driving and exacerbating local and systemic inflammation. An enhanced response to COVID-19 infection in elderly people could be largely amplified due to the presence of senescent cells, which are absent in young individuals.

The morbidity and mortality seen in COVID-19 is associated with excessive inflammation, and/or with the development of ARDS. Among cells, macrophages could contribute to viral spread, excessive inflammation and activation-induced lymphocytic cell death during the infection. Thus, dysregulated activation of the macrophages may contribute to COVID-19-associated hyper-inflammation. In addition, a subset of macrophages has been described in patients with severe COVID-19, that is enriched in genes associated with tissue repair and promotes fibrosis generation, such as in liver cirrhosis. This suggests that the pathogenicity of infiltrating macrophages could extend beyond the promotion of acute inflammation and is also in line with the fibrotic complications observed in patients under mechanical ventilation.

There is thus a need for an efficient way for reducing the life-threatening impact of COVID-19 in older people, and much generally, an efficient way for treating age-related diseases. There is also a need for lowering the number of senescent cells, especially a subset of senescent cells, in older organisms through prevention of their accumulation or through their elimination.

SUMMARY OF THE INVENTION

The inventors have surprisingly discovered that the majority of macrophages in an old animal carry the markers of senescence. They speculate that an ageing-induced senescence is an important contributor to macrophage hyper-reactivity in COVID-19 patients. Thus, lowering the level of senescence induction or removing a fraction of senescent macrophages could contribute to reducing COVID-19-associated hyper-inflammation. This could in turn lower the morbidity and mortality seen in COVID-19 patients, that is associated with excessive inflammation.

The inventors also showed that specific compounds are able to attenuate age-induced senescence in macrophages.

Moreover, the inventors have discovered that not only macrophages, but other immune cells such as T cells (including exhausted T cells and Treg cells), are concerned by senescence. As shown in the Examples, the global picture is that the nicotinamide N-methyltransferase (NNMT) pathway is at the center of senescent state of macrophages (p-Macs). Indeed, the role of NNMT in the regulation of senescence and immunosuppression in old macrophages is key :

Activation of NNMT is linked to senescent-like phenotype in p16^{High} macrophages.

Thus, inhibition of this enzyme through methylated nicotinamide (mNAM) (inhibitor towards NNMT), CD38 inhibitors (CD38 is an upstream donor of NAM that is required for the NNMT-dependent senescent-like phenotype in p16^{High} macrophages), and/or AOX inhibitors (which would result in accumulation of methylated NAM - an inhibitor of NNMT) could be viable options to reduce macrophage senescence and immunosuppression linked with it.

The inventors have also discovered that in the context of this pathway, many compounds may be used to reach this purpose.

Thus, the present invention relates to the use of at least one compound chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors, Janus kinase 2 inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, aldehyde oxydase (AOX) inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations and serotonin-norepinephrine reuptake inhibitors, for treating a disease associated with macrophage senescence.

Preferably, the present invention relates to the use of at least one compound chosen from BGT226 and its salts, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib, Voxelisib, Ruxolitinib, NNMT inhibitors including mNAM, Pacritinib, Berzosertib, CD38 inhibitor 78c, apigenin, Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene and its salts, Butacaine, Terfenadine, Valnemulin and its salts, Clomifene and its salts, Cetrimonium and its salts, and Duloxetine and its salts for treating a disease associated with macrophage senescence.

DETAILED DESCRIPTION OF THE INVENTION

The inventors show in the examples that the majority of macrophages in an old animal carry the markers of senescence, and that among all the tested compounds, only a fraction of specific compounds are able to attenuate age-induced senescence in

macrophages. In this fraction, the compounds are specifically chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors, Janus kinase 2 inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, aldehyde oxydase (AOX) inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations and serotonin-norepinephrine reuptake inhibitors.

Thus, a first object of the present invention relates to the use of at least one compound chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors, Janus kinase 2 inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, aldehyde oxydase (AOX) inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations and serotonin-norepinephrine reuptake inhibitors, in treating a disease associated with macrophage senescence.

Preferably, the present invention relates to the use of at least one compound chosen from BGT226 and its salts, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib, Voxelisib, Ruxolitinib, Pacritinib, Berzosertib, NNMT inhibitors including mNAM, CD38 inhibitor 78c, apigenin, Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene and its salts, Butacaine, Terfenadine, Valnemulin and its salts, Clomifene and its salts, Cetrimeronum and its salts, and Duloxetine and its salts in treating a disease associated with macrophage senescence.

As used herein, the « macrophage » is a type of white blood cell that ingests foreign particles, infectious microorganisms, cellular debris, cancer cells, and damaged or dying cells by a process called phagocytosis. A macrophage is capable of cell division, proliferation and tissue motility. Macrophages are professional phagocytes found in essentially all tissues where they take various forms (with various names, e.g., histiocytes, Kupffer cells, alveolar macrophages, microglia and others), but all are part of the mononuclear phagocyte system. Besides phagocytosis, they play a critical role in nonspecific defense (innate immunity) and also help initiate specific defense mechanisms (adaptive immunity) by recruiting other immune cells, such as lymphocytes, and through antigen presentation (e.g. to T lymphocytes). Human macrophages are about 21 micrometers in diameter. They can be identified using flow cytometry or immunohistochemical staining by their specific expression of proteins such as CD14,

CD40, CD11b, CD64, F4/80 (mice)/EMR1 (human), lysozyme M, MAC-1/MAC-3 and CD68.

The term « senescence » or « senescent » is used herein in reference to cells which are incapable of further cell division. Cellular senescence is characterized by growth cycle arrest in the G1 phase, absence of S phase and lifespan control by multiple dominant genes. Commonly used criteria to establish cell senescence include cellular enlargement and flattening, cessation of proliferation as measured by the failure to increase the number of cells in culture over a two-week period, failure to subculture or form colonies at clonal density, and lack of significant incorporation of [³H] thymidine. Although senescent cells (SCs) remain viable for long time periods, they cannot be stimulated to enter the S phase of the cell cycle by any combination of growth factors or physiological mitogens. Cells which are normally capable of proliferation *in vitro* can be epigenetically reprogrammed by exposure to genotoxic (i.e. irradiation, chemotherapeutic drugs...) or oncogenic (activation of dominant oncogenes) stresses and such cells are characterized by permanent cell cycle arrest, unresolved constitutive DNA damage response and constitutive activation of NF-κB that drives the expression and production of a series of bioactive, largely proinflammatory factors (SASP). Senescent cells may remain viable for long time periods (many months), during which RNA and protein are synthesized. SCs express the p16(Ink4a) gene and exhibit acidic β-galactosidase activity detectable at a more neutral pH (β-gal^{pH6}).

The « senescent macrophages » define a subset of macrophages which express the p16(Ink4a) gene and exhibit acidic β-galactosidase activity detectable at a more neutral pH (β-gal^{pH6}), and which accumulate with age. p16(Ink4a)/β-gal^{pH6}-positive macrophages are capable of cell division and phagocytosis. Induction of the senescent phenotype may represent a specific type of macrophage activation or differentiation. Thus, p16(Ink4a)/β-gal^{pH6}-positive macrophages accumulating in tissues of old subjects may not necessarily be activated by senescent cells, but are found within aged (i.e. senescent) organisms. Senescent macrophages are removed from a population of mixed cells which includes these cells and senescent cells, by treatment of the population with a delivery vehicle that comprises a toxin such as clodronate, because senescent macrophages phagocytose the toxin whereas senescent cells do not. A senescent macrophage expresses cell surface markers characteristic of a macrophage, including CD11b and F4/80.

As shown in figure 2, senescent macrophages show a decreased expression, as compared to p16⁻ cells, of at least one of, preferably all of the following genes : *Ccnb1*,

Ccnb2, *Ccna2*, *Ccne2*, *Cdk1*, *Plk1* and *Aurkb*. By decreased expression, it is meant a significantly lower expression, for example of at least 2-times, preferably 3-times.

As used herein, the term « subject » or « patient » means a mammal, preferably a human.

5 The subject according to the invention is adult. Preferably, the subject is an old subject, i.e. of at least 55 years old, preferably at least 60 years old, preferably at least 65 years old, preferably of at least 70 years old. Preferably, it is an old human.

10 As used herein, the term « treatment » or « treating » refers to an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total),
15 whether detectable or undetectable. Within the context of the invention, the term « treatment » or « treating » may also mean prolonging survival as compared to expected survival if not receiving treatment.

20 The term « disease associated with macrophage senescence » refers to a disease in an adult subject which is caused by and/or correlated to the presence of senescent macrophages. Said disease is preferably chosen from age-related diseases, viral infections such as COVID-19 and acute respiratory distress syndrome (ARDS).

25 The term « age-related disease » refers to a disease in an adult subject such as cancer, a metabolic disease, diseases of the eye such as an age-related and diabetic retinopathies, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, Alzheimer's disease, osteoarthritis, cardiac diastolic dysfunction, benign prostatic hypertrophy, aortic aneurysm or emphysema. Cancer includes but is not limited to prostate cancer, colon cancer, lung cancer, squamous cell cancer of the head and neck,
30 esophageal cancer, hepatocellular carcinoma, gastric cancer, pancreatic cancer, ovarian cancer or breast cancer. In the case of cancer treatment, the use of a compound according to the invention to lower macrophages senescence would be especially efficient in combination with other types of treatment such as immune-checkpoint therapy, irradiation or/and chemotherapy.

The term « age-related disease » in addition refers to a condition but not a disease that is abnormal in old subjects. One example of such condition is immunosenescence which manifests in overall reduction in the ability to develop a proper immune response in older individuals. Among such attenuated responses is a low efficiency of vaccination in older people, a very well established fact for vaccinations against flu viruses and now COVID19 vaccines in older people. As such, the compounds of the invention could significantly improve the overall vaccination efficiency in response to different vaccines including COVID19 and/or flu, in said old subjects.

Viral infections include infections due to a virus, and which can lead to an acute respiratory distress syndrome.

Viral infections preferably include viral respiratory infections, notably due to influenzaviruses (A, B, C or D), respiratory syncytial virus (RSV), rhinoviruses, coronaviruses, adenoviruses or parainfluenza viruses. Preferably, the respiratory viruses are responsible for severe pneumonia or severe flu.

Among the coronaviruses, especially three human coronaviruses produce symptoms that are potentially severe, which are Middle East respiratory syndrome-related coronavirus (MERS-CoV), severe acute respiratory syndrome-related coronavirus (SARS-CoV) and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2).

Preferably, the viral infection is an infection due to a coronavirus, preferably SARS-CoV-2 infection.

Preferably, the viral infection is COVID-19, which is the disease due to an infection with SARS-CoV-2 virus.

Preferably, the use of the present invention aims to treat severe COVID-19 forms. Severe COVID-19 forms are characterized by cytokine storm and/or by acute respiratory distress syndrome (ARDS). Cytokine storm is an abnormally and uncontrolled strong pro-inflammatory response occurring after infection (hyperinflammation), and which may lead to the occurrence of ARDS. More specifically, the cytokine storm results from a sudden acute increase in circulating levels of different pro-inflammatory cytokines including IL-6, IL-1, TNF- α and interferon. This increase in cytokines results in influx of various immune cells, such as macrophages, neutrophils, and T cells from the circulation into the site of infection with destructive effects on human tissue resulting from destabilization of endothelial cell to cell interactions, damage of vascular barrier, capillary damage, diffuse alveolar damage, multiorgan failure, and ultimately death. Lung injury is one consequence of the cytokine storm that can progress into acute lung injury or its more severe form ARDS.

Thus, the use of the compound of the invention could help reducing COVID-19-associated hyperinflammation. Typically, the subject may be of any age. By « reducing COVID-19-associated hyperinflammation », it is meant significantly reducing the hyperinflammation induced by SARS-Cov-2 infection, notably reducing the levels of pro-inflammatory cytokines chosen from IL-6, IL-1, TNF- α , interferon and their mixtures. By « level » of a given cytokine, it is meant the concentration of said cytokine. Said concentration(s) may be measured in a sample of the subject, for example a blood sample, a plasma sample or a serum sample.

By reducing COVID-19-associated hyperinflammation, the use of the compound of the invention helps treating severe COVID-19 forms.

ARDS is a type of respiratory failure characterized by rapid onset of widespread inflammation in the lungs. Severe shortness of breath is the main symptom of ARDS. It may be the consequence of viral infections and especially severe pneumonia or SARS-CoV-2 infection, or of infections mostly caused by bacteria and especially of sepsis, severe sepsis or septic shock.

The present invention relates to the use of at least one compound chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors including mNAM, Janus kinase 2 inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, aldehyde oxidase (AOX) inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations and serotonin-norepinephrine reuptake inhibitors, for treating a disease associated with macrophage senescence.

The present invention also relates to the use of methylated nicotinanide (mNAM) for treating a disease associated with macrophage senescence. Indeed, said mNAM may be used orally, for example as a dietary supplement to lower NNMT activity and thus treat a disease associated with macrophage senescence.

The present invention also relates to a product comprising a compound according to the invention and an immune-checkpoint therapy, irradiation or/and chemotherapy, as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

Preferably said compound according to the invention is chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors including mNAM, Janus kinase 2 inhibitors, CD38 inhibitors, inhibitors of the

enzyme ataxia telangiectasia and Rad3 related, aldehyde oxydase (AOX) inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations and serotonin-norepinephrine reuptake inhibitors.

Preferably the immune-checkpoint therapy is chosen from antibodies directed against immune checkpoints ; preferably chosen from PD1 and PDL1 antibodies ; preferably from nivolumab and pembrolizumab.

Preferably chemotherapy is a classical chemotherapy, known in the art.

Preferably, the present invention relates to the use of at least one compound which is chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors includin mNAM, Janus kinase 2 inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, aldehyde oxydase (AOX) inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations, and serotonin-norepinephrine reuptake inhibitors for treating age-related diseases, viral infections, such as severe COVID-19 forms, or acute respiratory distress syndrome (ARDS).

The compound of the invention is chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors includin mNAM, Janus kinase 2 (JAK2) inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related (ATR), aldehyde oxydase (AOX) inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations, and serotonin-norepinephrine reuptake inhibitors.

More preferably, the compound of the invention is chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors includin methylated NAM, Janus kinase 2 (JAK2) inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related (ATR) and aldehyde oxydase (AOX) inhibitors.

As used herein, the term "inhibitor" of a given target has its general meaning in the art and refers to any compound, natural or synthetic, that blocks, suppresses, or reduces (including significantly) said target.

mTOR inhibitors are a well-known class of drugs that inhibit mTOR. mTOR is a serine/threonine-specific protein kinase which regulates cellular metabolism, growth and

proliferation. The most established mTOR inhibitors are so-called rapalogs, and include rapamycin and its analogs.

PI3K inhibitors are a well-known class of drugs that inhibit one or more PI3K enzymes. PI3K enzymes are part of the PI3K/AKT/mTOR pathway, which is involved in cell growth, metabolism and translation initiation. PI3K includes different isoforms, classified into classes I to IV PI3Ks based on primary structure, regulation, and *in vitro* lipid substrate specificity.

Preferably, the PI3K inhibitor is a specific class I PI3K inhibitor. Class I PI3Ks have a catalytic subunit called p110, which includes four isoforms : p110 alpha, p110 beta, p110 gamma and p110 delta. Preferably, the PI3K inhibitor is specific for p110 alpha.

By « dual inhibitor of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K) », it is meant a drug that inhibit both mTOR and PI3K.

By « salt » or « pharmaceutically acceptable salt » in the present invention, it is meant any salt of the compound with an acid or a base. The pharmaceutically acceptable salt may be the hydrochloride salt, the dihydrochloride salt, the maleate salt, the citrate salt, or the chloride salt.

Preferably, the dual PI3K/mTOR inhibitor is chosen from BGT226 and its salts, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib and Voxtalib. These compounds are described below.

Preferably, the dual PI3K/mTOR inhibitor is chosen from BGT226, BGT226 maleate, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib and Voxtalib.

Inhibitors of NNMT are a class of drugs that inhibit the activity of nicotinamide N-methyl transferase (NNMT). Preferably, they include mNAM, NNMTi, JBSNF-000088 and Trigonelline and its salts. These compounds are described below.

JAK2 inhibitors are a well-known class of drugs that inhibit the activity of the Janus kinase 2 enzyme (JAK2), thereby interfering with the JAK-STAT pathway. Preferably, the JAK2 inhibitor is Pacritinib or Ruxolitinib. These compounds are described below.

CD38 inhibitors are a class of drugs that inhibit CD38 (ADP ribosyl cyclase/hydrolase). Preferably, the CD38 inhibitor is CD38 inhibitor 78c or apigenin. These compounds are described below.

Inhibitors of the enzyme ataxia telangiectasia and Rad3 related (ATR) are a class of drugs that inhibit the ATR enzyme. ATR is a DNA repair enzyme, and is activated in response to single strand breaks. Preferably, the ATR inhibitor is Berzosertib. This compound is described below.

5

Aldehyde oxydase (AOX) inhibitors are a class of drugs which are common with selective estrogen receptor modulators. Preferably, the aldehyde oxydase (AOX) inhibitor shows a triphenylethylene or an indole core. Preferably, the aldehyde oxydase (AOX) inhibitor is chosen from Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene and its salts, and
10 Clomifene and its salts. More preferably, the AOX inhibitor is chosen from Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene, Bazedoxifene acetate, Clomifene and Clomifene citrate. These compounds are described below.

15

Anesthetic agents are well-known drugs used to induce anesthesia. Preferably the anesthetic agent is a local anesthetic agent, more preferably butacaine. This compound is described below.

20

Antagonists of the histamine H1 receptor are well-known drugs used to treat allergies. They block the action of histamine at the H1 receptor, thereby helping to relieve allergic reactions. Preferably the antagonist of the histamine H1 receptor is Terfenadine. This compound is described below.

25

Pleuromutilin and its derivatives are antibacterial drugs that inhibit protein synthesis in bacteria by binding to the peptidyl transferase component of the 50S subunit of ribosomes. Preferably, the compound is Valnemulin or its salts, more preferably Valnemulin or Valnemulin hydrochloride. This compound is described below.

30

Topical antiseptic cations are chemical compounds which comprise a hydrocarbon backbone and a quaternary amine (as it is a cation, i.e. positively charged). Preferably, the topical antiseptic cation is Cetrimonium or its salts, more preferably cetrimonium chloride or cetrimonium bromide. These compounds are described below.

35

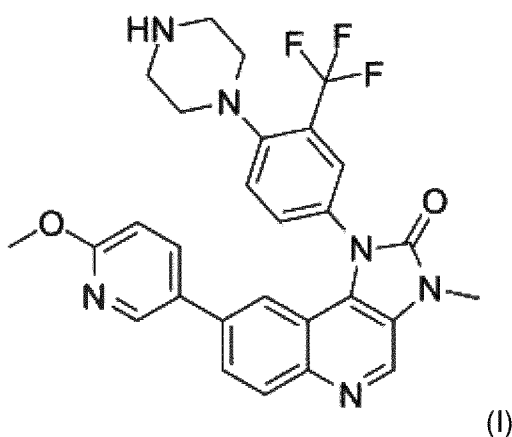
Serotonin-norepinephrine reuptake inhibitors are a a well-known class of antidepressant drugs. Preferably, the serotonin-norepinephrine reuptake inhibitor is Duloxetine or its salts, preferably Duloxetine or Duloxetine hydrochloride. This compound is described below.

Preferably, the present invention relates to the use of at least one compound chosen from BGT226 and its salts, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib, Voxtalisib, Ruxolitinib, Pacritinib, NNMT inhibitors including mNAM, Berzosertib, Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene and its salts, Butacaine, Terfenadine, Valnemulin and its salts, Clomifene and its salts, Cetrimonium and its salts, Duloxetine and its salts, for treating a disease associated with macrophage senescence.

More preferably, the present invention relates to the use of at least one compound chosen from BGT226 and its salts, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib, Voxtalisib, Ruxolitinib, Pacritinib, NNMT inhibitors including mNAM, Berzosertib, Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene and its salts, Terfenadine, Valnemulin and its salts, and Clomifene and its salts, for treating a disease associated with macrophage senescence.

The use of the invention also includes using mixtures of two or more of the compounds of the invention.

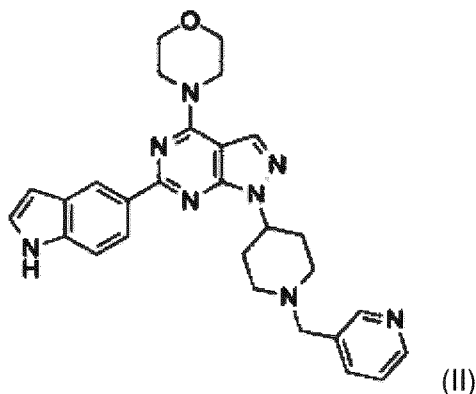
BGT226 is the molecule of the following structure (I) (chemical name: 8-(6-methoxypyridin-3-yl)-3-methyl-1-[4-piperazin-1-yl-3-(trifluoromethyl)phenyl]imidazo[4,5-c]quinolin-2-one); it has the CAS number 915020-55-2:



It may be used as such (as a free base), or in a salt form such as maleate. The maleate salt is called NVP-BGT226, and has the CAS number 1245537-68-1.

BGT226 and its salts are dual inhibitors of phosphatidylinositol 3-kinase (PI3K) and the mammalian target of rapamycin (mTOR).

WAY600 is the molecule of the following structure (II) (chemical name : 4-[6-(1H-indol-5-yl)-1-[1-(pyridin-3-ylmethyl)piperidin-4-yl]pyrazolo[3,4-d]pyrimidin-4-yl]morpholine) ; it has the CAS number 1062159-35-6 :

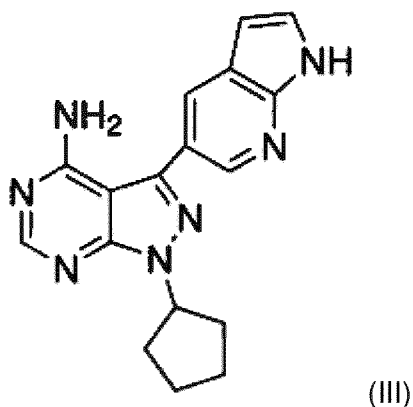


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WAY600 is a dual inhibitor of the mammalian target of rapamycin (mTOR) and PI3K.

PP121 is the molecule of the following structure (III) (chemical name : 1-cyclopentyl-3-(1H-pyrrolo[2,3-b]pyridin-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine) ; it has the CAS number 1092788-83-4 :

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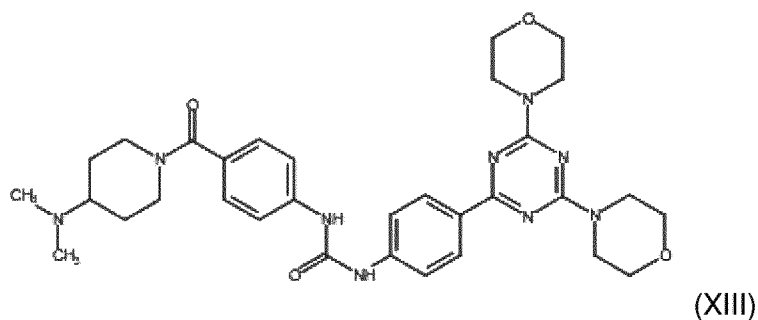


PP121 inhibits c-Abl (IC_{50} of 18 nM), PI3K (IC_{50} of < 0.06 μ M), PDGFR (IC_{50} of 2 nM), Hck (IC_{50} of 8 nM), mTOR (IC_{50} of 10 nM), SRC (IC_{50} of 14 nM), and DNA-PK (IC_{50} of < 0.06 μ M).

15

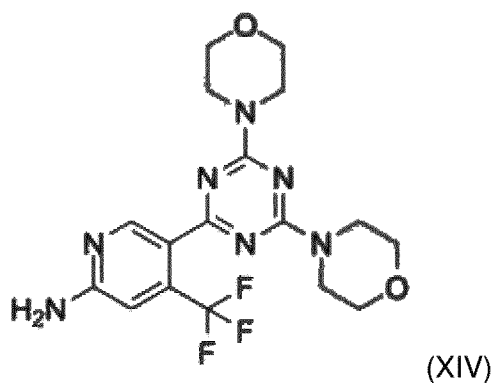
Gedatolisib is the molecule of the following structure (XIII) (chemical name : *N*-[4-[[4-(Dimethylamino)-1-piperidinyl]carbonyl]phenyl]-*N'*-[4-(4,6-di-4-morpholinyl-1,3,5-triazin-2-yl)phenyl]urea) ; it has the CAS number: 1197160-78-3 :

14



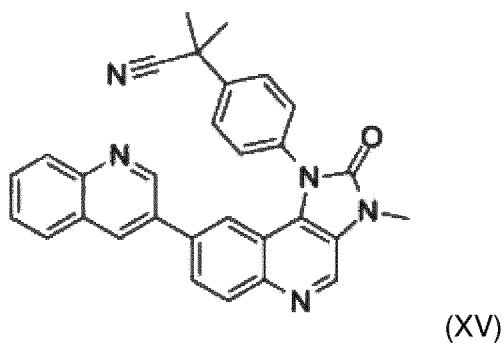
It is a dual inhibitor of mTOR/PI3K.

Bimiralisib is the molecule of the following structure (XIV) (chemical name : 5-(4,6-dimorpholin-4-yl-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-amine) ; it has the CAS number: 1225037-39-7 :



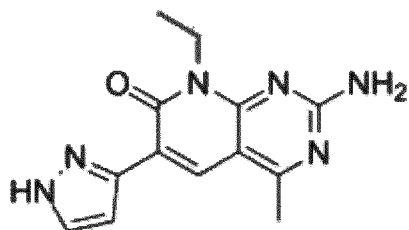
It is a dual inhibitor of mTOR/PI3K.

Dactolisib is the molecule of the following structure (XV) (chemical name : 2-methyl-2-[4-(3-methyl-2-oxo-8-quinolin-3-ylimidazo[4,5-c]quinolin-1-yl)phenyl]propanenitrile) ; it has the CAS number: 915019-65-7 :



It is a dual inhibitor of mTOR/PI3K.

Voxtalisib is the molecule of the following structure (XVI) (chemical name : 2-amino-8-ethyl-4-methyl-6-(1*H*-pyrazol-5-yl)pyrido[2,3-*d*]pyrimidin-7-one); it has the CAS number: 934493-76-2 :

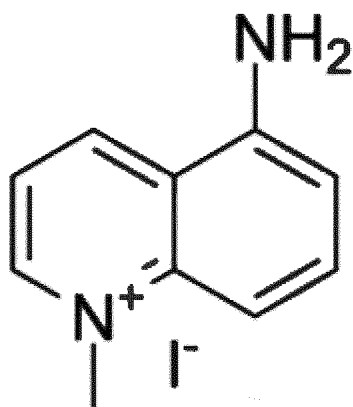


(XVI)

- 5 It is a dual inhibitor of mTOR/PI3K.

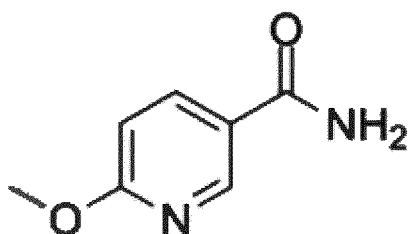
mNAM is methylated nicotinamide.

NNMTi (CAS number 42464-96-0) is the following compound :

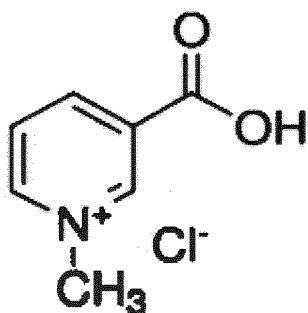


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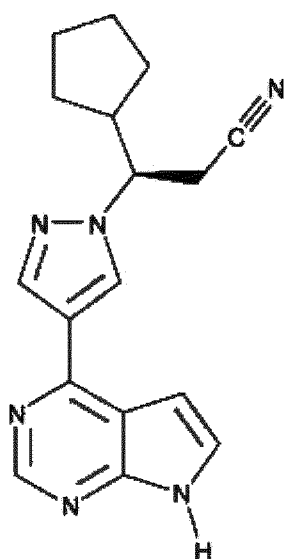
JBSNF-000088 (6-methoxynicotinamide ; CAS number 7150-23-4) is the following compound :



- 15 Trigonelline is 1-Methylpyridin-1-ium-3-carboxylate (CAS number 535-83-1). Its hydrochloride salt is the following compound :



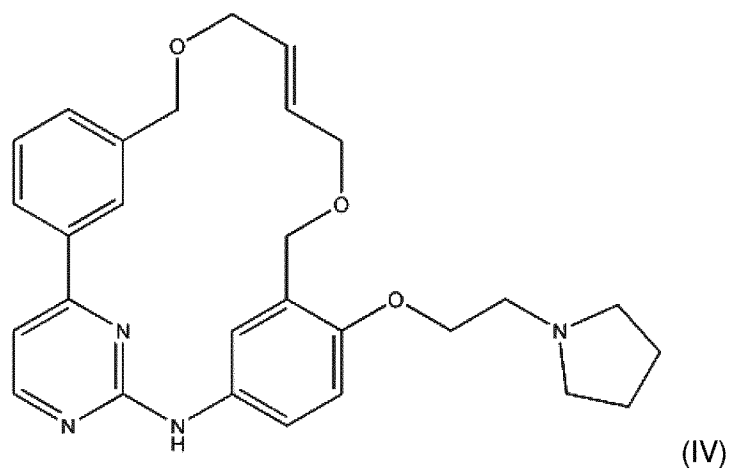
Ruxolitinib is the molecule of the following structure (XVII) (chemical name : (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)pyrazol-1-yl]propanenitrile ; it has the CAS number: 941678-49-5 :



(XVII)

Ruxolitinib is a macrocyclic Janus kinase inhibitor, and inhibits Janus kinase 1 (JAK1) and Janus kinase 2 (JAK2).

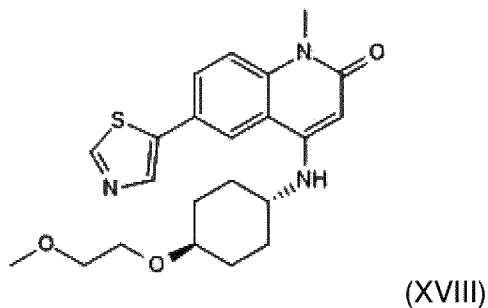
Pacritinib is the molecule of the following structure (IV) ; it has the CAS number 937272-79-2 :



Pacritinib is a macrocyclic Janus kinase inhibitor, and inhibits Janus kinase 2 (JAK2) and Fms-like tyrosine kinase 3 (FLT3).

5

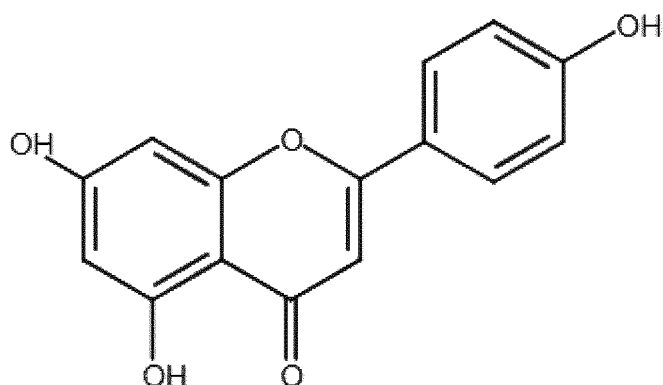
CD38 inhibitor 78c is the molecule of the following structure (XVIII) (chemical name : 4-[[*trans*-4-(2-Methoxyethoxy)cyclohexyl]amino]-1-methyl-6-(5-thiazolyl)-2(1*H*)-quinolinone; it has the CAS number: 1700-637-55-3 :



10 CD38 inhibitor 78c is a CD38 inhibitor.

Apigenin is the molecule of the following structure (XIX) (chemical name : 5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one; it has the CAS number: 520-36-5 :

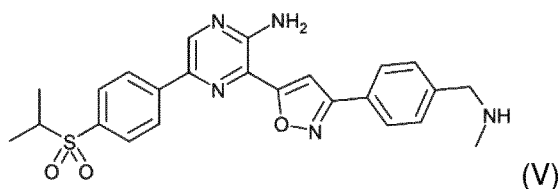
18



(XIX)

It is a CD38 inhibitor.

Berzosertib is the molecule of the following structure (V) ; it has the CAS number 1232416-25-9:



(V)

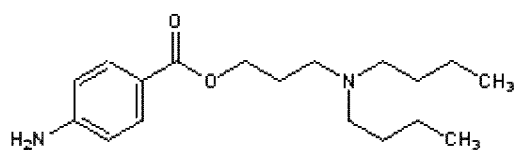
Berzosertib is an inhibitor of the enzyme ataxia telangiectasia and Rad3 related (ATR), and with a lower potency as an inhibitor of ATM serine/threonine kinase (ATM).

Tamoxifen and 4-Hydroxytamoxifen (also called afimoxifene) are AOX inhibitors. Their respective CAS numbers are 10540-29-1 and 68392-35-8.

Raloxifene is an AOX inhibitor. Its CAS number is 84449-90-1.

Bazedoxifene is also an AOX inhibitor,. Its CAS number is 198481-32-2. It may be used as such (as a free base), or in a salt form such as the acetate salt.

Butacaine is the molecule of the following structure (VI) ; it has the CAS number 149-16-6:

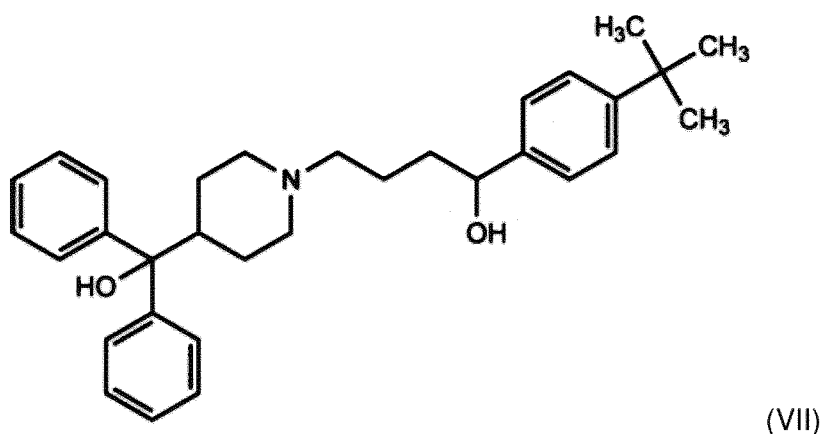


(VI)

It is a local anesthetic.

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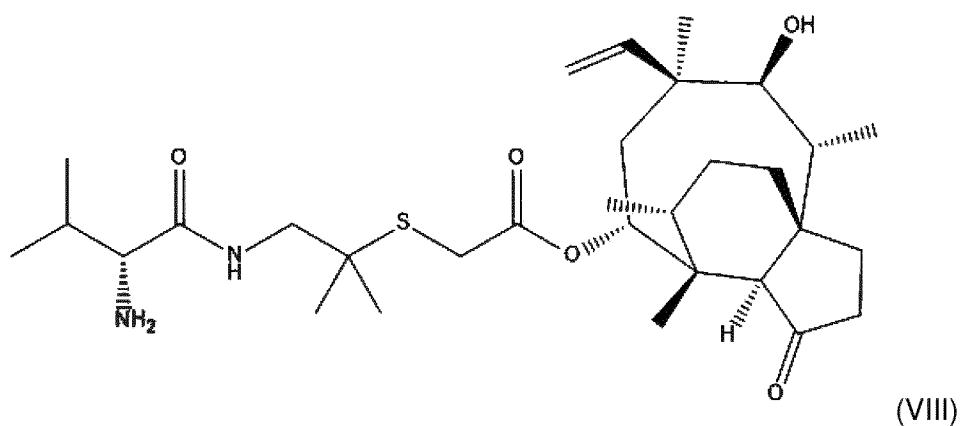
Terfenadine is the molecule of the following structure (VII) ; it has the CAS number 50679-08-8:



It is an antagonist of the histamine H1 receptor.

5

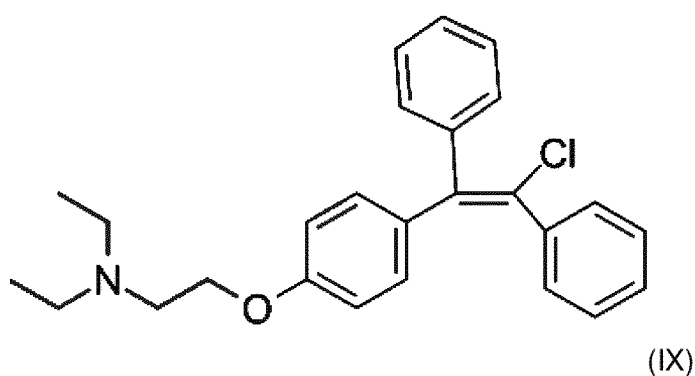
Valnemulin is the molecule of the following structure (VIII) ; it has the CAS number 101312-92-9:



It is an antibiotic of the class of pleuromutilin and its derivatives. It may be used as such (as a free base), or in a salt form such as the hydrochloride salt.

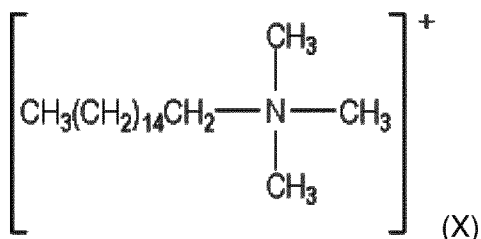
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Clomifene is the molecule of the following structure (IX) ; it has the CAS number 911-45-5:



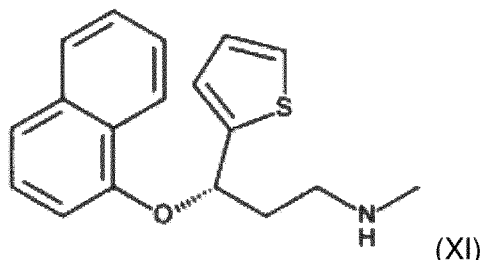
It is an AOX inhibitor. It may be used as such (as a free base), or in a salt form such as the citrate salt.

Cetrimonium (or cetyltrimethylammonium) is the following cation (X) ; it has the CAS number 6899-10-1:



It is usually used in the form of a salt, i.e. with a monovalent anion. Typically, the salt is the chloride or the bromide salt. Preferably, cetrimonium salt is cetrimonium chloride, which is a topical antiseptic and surfactant.

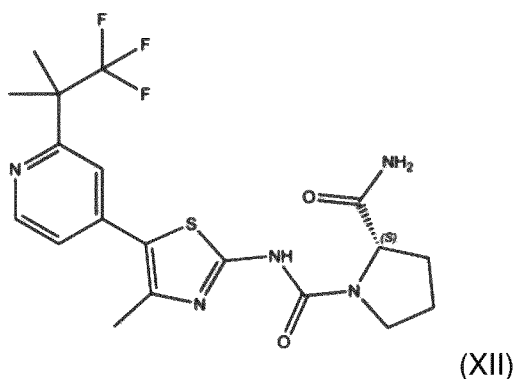
Duloxetine is the molecule of the following formula (XI) ; it has the CAS number 116539-59-4:



It may be used as such (as a free base), or in a salt form such as the hydrochloride salt.

It is a serotonin-norepinephrine reuptake inhibitor.

Alpelisib (also called BYL719) is the molecule of the following formula (XII) ; it has the CAS number 1217486-61-7:



Its chemical name is (2S)-1-N-{4-Methyl-5-[2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridin-4-yl]-1,3-thiazol-2-yl}pyrrolidine-1,2-dicarboxamide. It is an alpha-specific PI3K inhibitor.

Preferably, the compound of the invention is chosen from dual mTOR/PI3K inhibitors.

Preferably, the compound of the invention is chosen from BGT226 and its salts, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib and Voxtalib.

Preferably, the compound of the invention is chosen from dual inhibitors of phosphatidylinositol 3-kinase (PI3K) and of mTOR. Preferably, the compound of the invention is chosen from BGT226 and its salts, PP121, Gedatolisib, Bimiralisib, Dactolisib and Voxtalib.

More preferably, the compound of the invention is chosen from dual inhibitors of phosphatidylinositol 3-kinase and of mTOR, and more preferably from BGT226 and its salts, PP121, Gedatolisib, Bimiralisib, Dactolisib and Voxtalib ; Janus kinase 2 inhibitors, and more preferably from Ruxolitinib and Pacritinib ; AOX inhibitors, and more preferably from Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, and Bazedoxifene and its salts ; CD38 inhibitors, and more preferably CD38 inhibitor 78c and apigenin ; and NNMT inhibitors, and more preferably mNAM, NNMTi, JBSNF-000088 and Trigonelline and its salts.

More preferably, the compound of the invention is chosen from BGT226 and its salts, PP121, Gedatolisib, Bimiralisib, Dactolisib, Voxtalib, Ruxolitinib, Pacritinib, mNAM, NNMTi, JBSNF-000088, Trigonelline and its salts, Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene and its salts, CD38 inhibitor 78c and apigenin.

More preferably, the compound of the invention is chosen from BGT226, PP121, Gedatolisib, Bimiralisib, Dactolisib and Voxtalib.

Preferably, the compound of the invention is chosen from JAK inhibitors. Preferably, the compound of the invention is chosen from Ruxolitinib and Pacritinib.

Preferably, the invention relates to the use of at least one compound which is chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and PI3K, Janus kinase 2 inhibitors, NNMT inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, AOX inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations, and serotonin-norepinephrine reuptake inhibitors, as mentioned above, in treating a disease associated with macrophage senescence in an old subject.

The present invention also relates to a method for treating a disease associated with macrophage senescence in a subject in need thereof, preferably in an old subject, comprising administering to said subject at least one compound which is chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and PI3K, Janus kinase 2 inhibitors, NNMT inhibitors including mNAM, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, AOX inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations, and serotonin-norepinephrine reuptake inhibitors, as mentioned above.

Typically the compounds according to the invention as described above are administered to the subject in a therapeutically effective amount.

By a "therapeutically effective amount" of the compound of the present invention is meant a sufficient amount of said compound for treating the disease associated with macrophage senescence at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the compound of the present invention for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the compound of the present invention, preferably from 1 mg to about 100 mg. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In a particular embodiment, the compound according to the invention may be used in a concentration between 0.01 mM and 20 mM, particularly, the compound of the invention may be used in a concentration of 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0 mM.

5 According to the invention, the compound of the present invention is administered to the subject in the form of a pharmaceutical composition. Typically, the compound of the present invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to
10 molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In the pharmaceutical compositions of the present invention for oral, sublingual,
15 subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions,
20 sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular
25 isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions;
30 formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as
35 bacteria and fungi. Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a

surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The compound of the present invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized agent of the present inventions into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the typical methods of preparation are vacuum drying and freeze-drying techniques which yield a powder of the compound of the present invention plus any additional desired ingredient from a previously sterile- filtered solution thereof. The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as

the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The invention will be further illustrated by the following examples and figures.

Description of the figures

The figures listed in the following examples are as follows :

Figure 1. Determining the percentage of p16^{High} cells (representative of senescence) in F4/80-positive macrophages obtained from peritoneal cavity, spleen, stromal-vascular fraction of abdominal fat (SVF), liver and bone marrow of 2-, 12- and 18-month-old p16-Cre/R26-mTmG mice (left panel). Statistical significance was calculated using ANOVA plus a Dunnet test. Data are mean $\pm$ standard deviation (SD), *** p<0.001. Percentage of p16^{High} cells (representative of senescence) in F4/80-positive macrophages obtained from peritoneal cavity and liver in a model of inflammatory colitis induced by dextran sulfate sodium (DSS) (central panel). P-values were calculated with t-test. Data are mean $\pm$ standard deviation (SD), *** p<0.001 and * p<0.05. Percentage of p16^{High} cells (representative of senescence) in F4/80-positive intratumoral macrophages (right panel). MC38 colorectal cancer cells were implanted subcutaneously. Tumors were analyzed 10 days after implantation. Data are mean $\pm$ standard deviation (SD).

Figure 2. Analysis of mRNA expression based on RNA-Seq (presented as Fpkms (Fragments per kilobase of exon model per million reads mapped)) for genes related to cell cycle progression: *Ccnb1*, *Ccnb2*, *Ccna2*, *Ccne2*, *Cdk1*, *Plk1*, *Aurkb* and *Cdkn2a* genes in 1-year-old p16^{High} versus p16^{Low} peritoneal macrophages (p-Macs). A t-test statistical significance was calculated and is shown in the graph. Data are mean $\pm$ standard deviation (SD).

Figure 3. Proliferation was analyzed by EdU incorporation in p-Macs isolated from 12-month-old p16-Cre/R26-mTmG mouse. Percent of EdU-positive cells in p16^{High} EGFP-positive versus p16^{Low} EGFP-negative fractions was calculated. Statistical significance was calculated using a t-test. Data are mean $\pm$ SD; **p<0.01.

Figure 4. Analysis of mRNA expression based on RNA-Seq (Fpkms) of immunosuppressive factors: *Arg1*, *Vsig4*, *Timd4*, *Clec4g*, *Gpnmb*, *Il10*. Statistical meaning was calculated using a t-test and is shown in plots. Data are mean $\pm$ SD.

Figure 5. Analysis of senescence-associated β -galactosidase (SA β -gal) positive peritoneal macrophages (p-Macs) obtained from young (2-month old) and old (24-month old) mice. Mean of % of SA β -gal positive cells was plotted. Error bars correspond to standard deviation (s.d). t-test statistical significance was calculated and is shown in the graph.

Figure 6. Expression of different pro-inflammatory (top panels) and immunosuppressive (low panels) factors in p-Macs from young and old mice was analyzed by RT-PCR. Statistical significance was calculated using a t-test and is expressed in plots. Data are mean $\pm$ SD.

Figure 7. Immunostaining for senescence markers in p-Macs. p-Macs from wild-type 2-month-old and 24-month-old mice were isolated and stained for H3K9me2 and MacroH2A, two histone markers related to senescence. In the same way, p-Macs from 12-month-old p16-Cre/R26-mTmG mice were isolated and stained for H3K9me2 marker. Statistical significance was calculated using a t-test and is expressed in plots. Data represent mean $\pm$ SD.

Figure 8. Activity of all Kinase Inhibitors library compounds over the % of SA β -gal positive cells normalized relative to the only-vehicle treated p-Macs. The compounds were analyzed after 24 h at 1 μ M. The threshold line as shown (as determined by at least 50% of activity) was used for the compounds selection for the secondary confirmatory assay.

Figure 9. Activity of all FDA-approved drugs library compounds over the % of SA β -gal positive cells normalized relative to the only-vehicle treated p-Macs. The compounds were

analyzed after 24 h at 1 μ M. The threshold line as shown (as determined by at least 50% of activity) was used for the compounds selection for the secondary confirmatory assay.

Figure 10. Activity of the compounds selected to perform the secondary confirmatory assay from the Kinase Inhibitors library. Black points represent the mean of the relative % of SA β -gal positive p-Macs and gray squares represent the mean of the relative % of total p-Macs. Error bars correspond to standard deviation. Black arrows indicate the compounds selected to test their potency in subsequent IC₅₀ screen.

Figure 11. Activity of the compounds selected to perform the secondary confirmatory assay from the FDA-approved drugs library. Black points represent the mean of the relative % of SA β -gal positive p-Macs and gray squares represent the mean of the relative % of total p-Macs. Error bars correspond to standard deviation. Black arrows indicate the compounds selected to test their potency in subsequent IC₅₀ screen.

Figure 12. Activity of the 7 most active compounds selected after confirmatory screen of the Kinase Inhibitors library. The IC₅₀ values were calculated for the relative % of SA β -gal positive (black points and lines) and the relative % of total p-Macs (gray squares and lines) for each compound. The first 4 graphs correspond to the best active compounds. The X-axis values are showed on a logarithmic scale. The concentration used to calculate the IC₅₀ values corresponds to micromolar (μ M). Note that dual mTOR/PI3k inhibitors BGT226 and Way600 showed the strongest activity towards inhibiting senescence in peritoneal macrophages.

Figure 13. Activity of the 12 most active compounds selected after confirmatory assay from the FDA-approved drugs library. The IC₅₀ values were calculated for the relative % of SA β -gal positive (black points and lines) and the relative % of total p-Macs (gray squares and lines) for each compound. The X-axis values are showed on a logarithmic scale. The concentration used to calculate the IC₅₀ values corresponds to micromolar (μ M).

Figure 14. A relative % of SA β -gal positive (black points and lines) cells, and the relative % of total p-Macs (gray squares and lines) of the 5 compounds with higher potency from a kinase inhibitor library screen within 5 days after a 24h treatment and medium replacement.

Figure 15. A relative % of SA β -gal positive (black points and lines) and for the relative % of total p-Macs (gray squares and lines) of the 6 compounds from an FDA approved drug library screen within 5 days after a 24h treatment and medium replacement.

Figure 16. Analysis of Hits expansion. Different members of the same family of compounds with higher activity were tested (single PI3k and mTOR inhibitors, dual PI3k/mTOR inhibitors, JAK and AOX inhibitors) in p-Macs from 12-month-old mice at 5 different concentrations. The IC_{50} values were calculated for the relative percent of SA- β -gal-positive (black points and lines) and the relative percent of total cells (gray squares and lines) for each compound. The X-axis values are showed in a logarithmic scale. The concentrations used to calculate the IC_{50} values correspond to micromolar (μ M). Note that dual PI3k/mTOR and AOX inhibitors show strongest activity towards inhibiting senescence in peritoneal macrophages.

Figure 17. Effect of the most active drugs on the expression of different inflammatory and immunosuppressive factors in p-Macs obtained from 2-month-old and 12-month-old mice. Cells were treated with Way600, Bgt226 at 400 nM and Raloxifene at 1 μ M during 24 h. Statistical significance was calculated using a t-test and is shown in plots relative to non-treated conditions for different age. Statistical significance within the same age group was determined using ANOVA plus Dunnet test against their respective non-treated samples. Data are mean $\pm$ SD. P value when significant is show in the graphs.

Figure 18. Effect of control old p-Macs and drug-treated old p-Macs on T cell proliferation and different T cell subpopulation. **A.** Proliferation of T cells based on CFSE fluorescence was analyzed after a co-culture with young (2-months-old) and old (2-years-old) p-Macs in the presence of different proliferative stimuli (PMA+Ionomycin (left panel) or CD3/CD25 activating beads (right panel)). Statistical significance was calculated using a t-test and is shown in plots. **B.** Effect of the drugs on macrophage ability to regulate T cell proliferation. Macrophages were pre-treated with Way600, Bgt226 (both at 400 nM), and Raloxifene (1 μ M) for 24h, drugs were removed by extensive washing with PBS and macrophages were co-cultured with CFSE-stained T cells for 7 days. Statistical significance within the same age group was determined using ANOVA plus Dunnet test against p+Macs in non-treated group. **C.** Effect of the drugs on macrophage ability to regulate T cell Tregs (defined as CD4⁺ FoxP3⁺) (left panel) and exhausted (defined as CD4⁺ PD-1⁺) (right panel) T cell populations. Macrophages were pre- treated with Way600, Bgt226 (both at 400 nM), and Raloxifene (1 μ M), drugs were removed by extensive washing with PBS and

macrophages were co-cultured with T cells (in a 1:2 ratio) for 7 days in the presence of CD3/CD25 beads (1:1 ratio for beads/T cells). Statistical significance within the same age group was determined using ANOVA plus Dunnet test against p-Macs in non-treated condition. Data are mean $\pm$ SD. p value when significant is shown in the graphs.

Figure 19. *Nnmt* is overexpressed in $p16^{\text{high}}$ and aged p-Macs and promotes senescent-like state. **A.** Overexpression of *Nnmt* in $p16^{\text{High}}$ macrophages. Analysis of *Nnmt* mRNA in $p16^{\text{High}}$ and $p16^{\text{Low}}$ peritoneal macrophages based on RNA-Seq (shown in FpkM). Differences and P-value was determined with t-test. **B.** Effect of different NNMT inhibitors on the percent of SA- β -gal-positive cells. p-Macs isolated from 12-month-old mice were treated with JBSNF-000088 (JBSNF)(4 μ M) and NNMT inhibitor (NNMTi) (1 μ M) for 24 h, and methylated Nicotinamide (mNAM) during 72 h (0.1 mM). **C.** Effect of Way600, Bgt226 (both at 400nM), and Raloxifene (1 μ M) on the expression of *Nnmt* mRNA. p-Macs isolated from 2-month-old and 12-month-old mice were treated for 24h and RNA was isolated and analyzed by qPCR. Differences were determined using ANOVA plus Dunnet post-hoc test against non-treated condition for each age group. Statistical significance between non-treated conditions of different age groups was determined by the t-test. P-Value is shown in the plots. **D.** Effect of JBSNF-000088 on the expression of pro-inflammatory and immunosuppressive factors. Difference between conditions was analyzed by t-test. p-values are shown in each plot. Data are mean $\pm$ SD.

Figure 20. In vitro analysis of activity of available NNMT inhibitors. IC_{50} of NNMT inhibition was determined in a free-cell assay. Values were determined as follows: NNMTi (IC_{50} =2.7 μ M), JBSNF-000088 (IC_{50} =17.72 μ M), Trigonelline (IC_{50} =139.15 μ M), and mNAM (IC_{50} =48.4 μ M). X-axis values in the graphs are represented in logarithmic scale.

Figure 21. CD38 is an upstream donor of NAM that is required for the *Nnmt*-dependent senescent-like phenotype in $p16^{\text{High}}$ macrophages. **A.** Percentage of cells positive for Cd38 and $p16^{\text{High}}$ -EGFP in different tissues was analyzed in 18-month-old $p16\text{-Cre/R26-mTmG}$ mice. **B.** Effect of Way600, Bgt226 (both at 400nM), and Raloxifene (1 μ M) on the expression of Cd38 mRNA. p-Macs isolated from 2-month-old and 12-month-old mice were treated for 24h, *Cd38* mRNA was analyzed by qPCR. Difference was determined using ANOVA plus Dunnet post-hoc tests versus non-treated conditions for each age group. Statistical significance between non-treated conditions for different age groups was determined by t-test. P-Value is shown in the plots. **C.** Effect of different CD38 inhibitors on the percent of SA- β -gal-positive cells. p-Macs isolated from 12-month-old

mice were treated with CD38 inhibitors, Compound-78c (C-78c) and apigenin at 1 μ M for 24h. Differences were determined using ANOVA plus Dunnet post-hoc tests versus non-treated conditions. p-values are shown in the plot. **D.** Effect of C-78c on the mRNA expression of pro-inflammatory and immunosuppressive factors. Differences between conditions were analyzed by t-test. p-values are shown in each plot. Data are mean $\pm$ SD.

Examples

Example 1 : Levels of p16^{High} macrophages in different tissues at different age and in pathological conditions

Two-month-old, 12-month-old and 18-month-old C57BL/6 p16-Cre/R26-mTmG knock-in mice were used for the analysis of senescence based on the number of EGFP-p16^{High} cells (Figure 1, left panel). Cells from peritoneal cavity were collected by lavage with cold DMEM. Liver and abdominal fat were dissociated by incubating with 1 mg/ml collagenase type A at 37°C for 45min and filtered through a 30 μ m strainer to obtain single cell suspensions and in the case of the abdominal fat to obtain stromal-vascular fraction (SVF). Spleens were mashed and filtered through a 75 μ m and 30 μ m strainers to obtain single cell suspensions. For bone marrow purification, muscles were removed from both legs and an incision was made between lesser and greater trochanter. Bones then were centrifuged at 10,000g for 1min. Bone marrow was collected, re-suspended in HBSS and filtered through a 30 μ m strainer to obtain single cell suspension. Blood cells were removed using a lysis buffer. In all cases, cells were blocked (HBSS 1% BSA, 4% FBS, 2 mM EDTA) and stained with fluorescent conjugated anti-F4/80 antibody. Samples were analyzed by flow cytometry using Cytotflex system (Beckman-Coulter). Next, 2 months mice were treated with 3% dextran sulfate sodium (DSS) dissolved in the drinking water for 7 days to induce intestinal inflammation and colitis. On day 7, DSS was removed and mice were analyzed 2 days later. Cells from peritoneal cavity and liver (after digestion) were stained with fluorescent conjugated antibody against F4/80. Percentage of p16^{High} in F4/80⁺ fraction was determined by flow cytometry (Figure 1, central panel). Differences were 27.02 ($\pm$ 2.272 SEM) for peritoneal cavity and 1.007 ($\pm$ 0.3575 SEM) for liver. Finally, 5X10⁵ murine colorectal syngeneic tumor cells MC38 were injected subcutaneously into 2-month-old mice. Tumors were analyzed on day 10 after dissociation into a single-cell suspension (using 1 mg/ml collagenase type A at 37°C for 45min and filtered through a 30 μ m strainer). Cell suspension was stained with fluorescent conjugated antibody against

F4/80. Percentage of p16^{High} in F4/80⁺ fraction was determined by flow cytometry (Figure 1, right panel). The inventors observed a significant (more than 40%) increase in the percentage of p16^{High} macrophages after 12 months, especially in peritoneum, SVF and liver (Figure 1, left panel). In addition, the inventors observed a significant increase in the number of senescent p16^{High} macrophages in young mice (2-month-old) in response to inflammation (Figure 1, central panel) and during tumor growth (Figure 1, left panel).

Example 2 : Level of senescence markers in primary peritoneal macrophages (p-Macs) in old and young mice

Primary peritoneal macrophages were obtained from 2-month-old and 24-month-old C57BL/6 wild-type mice. 3×10^4 cells were seeded in 96 wells plates and cultured at 37°C with 5% of CO₂. The next day, cells were washed to eliminate the non-adherent populations and to determine senescence level on p-Macs, the cells were stained for senescence-associated β -galactosidase activity (SA β -gal). For that, cells were washed with PBS and fixed for 4 min at room temperature in 4% PFA, washed 3 times with DPBS and stained overnight at 37°C in staining solution: 40mM Citrate-sodium phosphate pH 6; 5mM K₃[Fe(CN)₆]; 5mM K₄[Fe(CN)₆]; 2mM MgCl₂; 150mM NaCl; 1mg/ml X-gal. Stained cells were washed with PBS and nuclei were tagged with DAPI. 10X pictures were made using a bright field and fluorescence microscope. Pictures from 2 and 24 month-old p-Macs and controls were made and then analyzed using ImageJ software through recorded macros. The inventors found that at 2 months, the mean of SA β -gal positive p-Macs was 8.7 % (± 1.7 s.d); and 84.77 % (± 10.77 s.d) for 24 months p-Macs (Figure 5, left panel). The average percent of SA- β -gal-positive cells was determined as follows: percent of SA- β -gal-positive cells = SA- β -gal-positive cells/ total cell number. To further confirm senescence, total RNA from F4/80⁺ p-Macs obtained from 2- and 24-month-old C57BL/6 wild type mice was isolated and analyzed to determinate the expression of *p16* mRNA by quantitative real-time PCR (RT-PCR) (Figure 5, right panel). The inventors observed an increase of p16 mRNA expression in 2- year-old mice. Difference between means was 32.65 (± 3.9 SEM). Thus, analysis of 2 senescent markers further confirmed that mouse peritoneal macrophages undergo senescence with age.

Example 3: Proliferation-associated genes are downregulated in senescent primary peritoneal macrophages

Primary peritoneal macrophages were obtained from 1-year-old C57BL/6 GFP-reporter p16-Cre/R26-mTmG knock-in mice. Senescent p-Macs were purified using an F4/80 column and p16^{High} and p16^{Low} cells were separated by FACS-sorting based on EGFP analysis. RNA from GFP-positive and -negative cells was purified and analyzed using RNA-sequencing. HTSeq v0.6.1 was used to count the reads numbers mapped to each gene. The FPKM for each gene was calculated based on the length of the gene and reads count mapped to this gene. FPKM, expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced, considers the effect of sequencing depth and gene length for the reads count at the same time, and is currently the most commonly used method for estimating gene expression levels. Using DAVID bioinformatics resources v6.8, the inventors found a functional cluster of downregulated genes related with cell cycle and proliferation composed by 61 genes (p-Value= 1.4E-72). Some of the most downregulated genes are shown in the Figure 2 and includes to *Ccnb1*, *Ccnb2*, *Ccna2*, *Ccne2*, *Cdk1*, *Plk1* and *Aurkb*. Additionally, the inventors checked the levels of *Cdkn2a*, which was overexpressed as expected. This data highlights the deep engagement of the senescent p-Macs with the cell cycle arrest typical for senescent cells.

Example 4 : Analysis of proliferative capacity of p16^{High} p-Macs

Primary p-Macs were isolated from 12-month-old p16-Cre/mTmG mice by peritoneal lavage. Cells were plated and cultured in RPMI medium (10% FBS heat-inactivated, 1% penicillin /streptomycin) in the presence of fluorescent 5-Ethynyl-2'-deoxyuridine (EdU) for 24h. Cells were subsequently fixed and stained for EdU. Nuclei were counterstained with DAPI. Pictures were obtained with the EVOS fluorescence microscope (Thermofisher) and analyzed using an ImageJ software. Percent of p16^{High} and p16^{Low} EdU positive cells was determined (Figure 3). The inventors found that p16^{High} cells show no EdU staining suggesting that they are not proliferative – a strong feature of senescent cells.

Example 5 : Overexpression of Immunosuppressive genes in p16^{High} peritoneal macrophages

p-Macs were obtained from 1-year-old p16-Cre/mTmG mice and purified using an F4/80 column, p16^{High} and p16^{Low} cells were subsequently separated by FACS based on the EGFP expression. These 2 fractions were used for RNA-Seq analysis. HTSeq v0.6.1 was used to count the reads numbers mapped to each gene. Using DAVID bioinformatics resources v6.8, the inventors found a functional cluster of downregulated genes related to

immunosuppressive genes (p-Value= 4.26E-05). Cluster is composed by *Arg1*, *Vsig4*, *Timd4*, *Clec4g*, *Gpnmb* (Figure 4). Also *Il10* was manually analyzed and showed the same pattern of expression. Thus, p16^{High}p-Macs possess a much high expression level of immunosuppressive genes suggesting that they are in fact anti-inflammatory.

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Example 6 : Analysis of expression of pro-inflammatory and immunosuppressive genes in primary peritoneal macrophages from old and young mice

F4/80⁺ p-Macs were isolated from 2- and 24-month-old mice using magnetic beads (Miltenyi Biotec). Expression of various pro-inflammatory genes, and previously identified in p16^{High} p-Macs immunosuppressive genes (Figure 4) was determined in total RNA by RT-PCR. The inventors observed an overexpression of all genes analyzed (Figure 6). The statistical significance was carried out using a t-test and is show for each plot. Differences between means were for *Cxcl13* 3.2 ± 0.38 ; *Il1a* $4,220 \pm 0,53$; *Il1b* 2.692 ± 0.2974 ; *Il6* 4.179 ± 0.6690 ; *Il10* 29.42 ± 2.575 ; *Arg1* 3.535 ± 0.4451 ; *Vsig4* 2.202 ± 0.3881 ; *Timd4* 0.7547 ± 0.1712 were $\pm$ represent SEM. These data confirmed that old macrophages express higher level of both pro-inflammatory and immunosuppressive genes.

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Example 7 : p-Macs obtained from 24-month-old mice and p16^{High} p-Macs from 1-year-old mice exhibit several senescence markers

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p-Macs were isolated from 2- and 24-month-old wild-type mice and 12-month-old p16-Cre/mTmG mice. p-Macs were stained with antibodies against senescence nuclear markers H3K9me2 and MacroH2A (Figure 7, upper panels). In addition, p-Macs obtained from 1-year-old p16-Cre/mTmG mice were stained for H3k9me2 marker (Figure 7, low panels). Pictures were captured using confocal Zeiss LSM 880 microscope (Zeiss). Cells were counted using ImageJ. The inventors observed an increase in the number of cells expressing senescent markers in both old and p16^{High} p-Macs. The statistical significance was analyzed using a t-test and is show for each plot. Differences between means were for H3K9me2 (WT) $13.89 (\pm 4.408 \text{ SEM})$, Macro-H2A $7.466 (\pm 2.226 \text{ SEM})$ and, H3K9me2 (p16-Cre/mTmG) $12.48 (\pm 4.396 \text{ SEM})$. Thus, analysis of 2 additional senescent markers further confirmed that mouse peritoneal macrophages undergo senescence with age.

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Example 8 : Senescence detection on murine peritoneal macrophages (p-Macs)

Primary peritoneal macrophages were obtained from 1-year-old wild type mice. Mice were sacrificed by cervical dislocation; skin from the abdominal area was removed. 8 mL of Roswell Park Memorial Institute 1640 medium (RPMI) supplemented with 1X antibiotic-antifungal (Gibco) containing 100 units/mL of penicillin, and 100 µg/mL of streptomycin was injected into the abdominal cavity. The medium contained p-Macs were collected again and centrifuged at 1500 rpm by 8 min. Pellet was resuspended in the RPMI medium supplemented with 1X antibiotic and 5% fetal bovine serum (heat-inactivated) (FBS)(Gibco). 3×10^4 cells were seeded in 96 wells plates and cultured at 37°C with 5% of CO₂. The next day, cells were washed to eliminate the non-adherent populations. To determine senescence cells were stained for senescence-associated β-galactosidase activity (SA β-gal). For that, cells were washed with PBS and fixed for 4 min at room temperature in 4% PFA, washed 3 times with DPBS and stained overnight at 37 °C in staining solution: 40mM Citrate-sodium phosphate pH 6; 5mM K₃[Fe(CN)₆]; 5mM K₄[Fe(CN)₆]; 2mM MgCl₂; 150mM NaCl; 1mg/ml X-gal. Stained cells were washed with PBS and nuclei were tagged with DAPI. 10X pictures were made using a bright field and fluorescence microscope. Total cell number and SA β-gal positive cells were counted using ImageJ software. The average of % SA β-gal cells was determined (% SA β-gal= SA β-gal positive cells/ total cell) in 36.8 (± 8.9 s.d).

Example 9 : Pharmacological primary screening for reducing % SA β-gal cells using Kinase inhibitor library

To find new molecules with the ability to reduce the % SA β-gal positive cells, 3×10^4 isolated p-Macs were seeded by well in a 96-wells plates with RPMI medium supplemented with 5% FBS and cultured at 37°C with 5% of CO₂. p-Macs were treated with the Kinase Inhibitor chemical library (KIL) from Sellekchem (Catalog No.L1200) identified with the Order No. Z77740. 355 small molecules distributed in 4 96-wells plates compose the KIL. p-Macs were incubated in the presence of compounds for 24 h at 1 µM. The inventors used as negative control the same concentration of the vehicle (Vh). After treatment, cells were washed, fixed, and stained for SA β-gal and nuclear tagged as described previously. Pictures from all compounds and controls were made and then analyzed using ImageJ software using recorded macros. The activity of each molecule over p-Macs was referred to as % SA β-gal positive cells relativized to the % SA β-gal positive cells determined in the Vh treated cells (Figure 8). The inventors picked 39 compounds for further validation using a limit threshold the 50% of activity in reducing the

relative % of SA β -gal positive cells. These compounds were used for subsequent secondary confirmation.

Example 10 : Pharmacological primary screening for reducing % SA β -gal cells using FDA-approved drugs library

To find new molecules with the ability to reduce the % SA β -gal positive cells, 3×10^4 isolated p-Macs were seeded by well in a 96-wells plates with RPMI medium supplemented with 5% FBS and cultured at 37°C with 5% of CO₂. p-Macs were treated with the FDA-approved drugs library (FDA-AL) from SelleckChem (Catalog No.L1300) identified with the order No. Z77740. 1163 small molecules distributed in 14 96-wells plates compose the KIL. p-Macs were incubated in the presence of compounds for 24 h at 1 μ M. The inventors used as negative control the same concentration of the vehicle (Vh). After treatment, cells were washed, fixed, and stained for SA β -gal and nuclear tagged as described previously. Pictures from all compounds and controls were made and then analyzed using ImageJ software using recorded macros. The activity of each molecule over p-Macs was referred to as % SA β -gal positive cells relativized to the % SA β -gal positive cells determined in the Vh-treated cells (Figure 9). The inventors picked 61 compounds for further validation using a limit threshold the 40% of activity in reducing the relative % of SA β -gal positive cells. These compounds were used for subsequent secondary confirmation.

Example 11 : Confirmatory assay from KIL picked compounds

To confirm the ability to attenuate SA β -gal activity, the 39 compounds picked from KIL were re-tested in duplicate. 3×10^4 isolated p-Macs were seeded in a 96-wells plates with RPMI medium supplemented with 5% FBS and cultured at 37°C with 5% of CO₂. Cells were treated with the selected compounds at 1 μ M or with Vh for 24 h. After treatment, cells were washed, fixed, and stained for SA β -gal and nuclear tagged as described previously. Pictures from all compounds and controls were made and then analyzed using ImageJ software using recorded macros. The activity of each molecule over p-Macs was referred to as % SA β -gal positive cells relativized to the % SA β -gal positive cells determined in the Vh-treated cells. Additionally, the inventors evaluated the variation on the total cell number to predict specific effects over senescent p-Macs or general toxicity. After screening the inventors confirmed 7 compounds with activity to reduce the levels of % SA β -gal with respect to the Vh (Figure 10).

Example 12 : Confirmatory assay from FDA-AL picked compounds

To confirm the ability to attenuate SA β -gal activity, the 61 compounds picked from FDA-AL were re-tested in duplicate. 3×10^4 isolated p-Macs were seeded in a 96-wells plates with RPMI medium supplemented with 5% FBS and cultured at 37°C with 5% of CO₂. Cells were treated with the selected compounds at 1 μ M or with Vh for 24 h. After treatment, cells were washed, fixed, and stained for SA β -gal and nuclear tagged as described previously. Pictures from all compounds and controls were made and then analyzed using ImageJ software using recorded macros. The activity of each molecule over p-Macs was referred to as % SA β -gal positive cells relativized to the % SA β -gal positive cells determined in the Vh treated cells. Additionally, the inventors evaluated the variation on the total cell number to predict specific effects over senescent p-Macs or general toxicity. After screening, the inventors confirmed 11 compounds with activity to reduce the levels of % SA β -gal with respect to the Vh (Figure 11).

Example 13 : IC₅₀ analysis of KIL compounds

To determinate the IC₅₀ of the compounds identified from the KIL, 3×10^4 isolated p-Macs were seeded in a 96-wells plates with RPMI medium supplemented with 5% FBS and cultured at 37°C with 5% of CO₂. Cells were treated for 24 h with the selected compounds at 6 different concentrations (3, 1, 0.33, 0.11, 0.37 μ M, and Vh only) by duplicate. The selected compounds include AZD8055, Berzosertib, MK2206, BGT226, Pacritinib, PP121 and WAY600. After treatment, cells were washed, fixed, and stained for SA β -gal and nuclear tagged as described previously. Pictures from all compounds and controls were made and then analyzed using ImageJ software using recorded macros. The activity of each molecule over p-Macs was referred to as relative % SA β -gal positive cells and the relative % of total cells. With these data, the inventors calculated the IC₅₀ for each compound using Prism GraphPad software (Figure 12). After analysis, the inventors found that the most potent compounds are BGT226 (IC₅₀<0.1 μ M), WAY600 (IC₅₀=0.29 μ M), PP121 (IC₅₀=0.3 μ M), and Pacritinib (IC₅₀<0.43 μ M).

Example 14 : IC₅₀ analysis of the compounds confirmed from the FDA-AL

To determinate the IC₅₀ of the compounds identified from the FDA-AL, 3×10^4 isolated p-Macs were seeded by well in a 96-wells plates with RPMI medium supplemented with 5%

FBS and cultured at 37°C with 5% of CO₂. Cells were treated for 24 h with the selected compounds at 6 different concentrations (3, 1, 0.33, 0.11, 0.37 µM, and Vh only) by duplicate. The compounds selected include Tamoxifen, Bazedoxifene, Butacaine, Terfenadine, Valnemulin, Clomifene, Cetrimonium, and Duloxetine. As this list includes 3 AOX inhibitors in the top rank, the inventors also included 4-Hydroxytamoxifen (the active metabolite of Tamoxifen). After treatment, cells were washed, fixed, and stained for SA β-gal and nuclear tagged as described previously. Pictures from all compounds and controls were taken and then analyzed using ImageJ software using recorded macros. The activity of each molecule over p-Macs was referred to as relative % SA β-gal positive cells and the relative % of total cells. With these data, the inventors calculated the IC₅₀ for each compound using Prism GraphPad software (Figure 13). After analysis, the inventors found that the most potent compounds are 4-Hydroxytamoxifen (IC₅₀=0.02 µM), Bazedoxifene (IC₅₀=0.6 µM), Valnemulin (IC₅₀=0.9 µM), Tamoxifen (IC₅₀=1 µM), Clomifene (IC₅₀=1.2 µM) and Terfenadine (IC₅₀=1.22 µM).

Example 15 : Extended impact of the KIL compounds on macrophage senescence

To determine if the effect observed in a 24h assay is transitory and thus reversible, 3X10⁴ isolated p-Macs were seeded by well in a 96-wells plates with RPMI medium supplemented with 5% FBS and cultured at 37°C with 5% of CO₂. Cells were treated for 24 h with BGT226, PP121, WAY600, Pacritinib or Berzosertib at 1 µM. After that, compounds were withdrawn. The % SA β-gal positive cells and the % of total cell number (as survival parameter) were determined for 5 days. Data from all compounds were relativized again Vh treated cells on day 1 (Figure 14). The data indicates that the compounds with the persistent effect are BGT226 and PP121. They also are compounds with less toxicity.

Example 16 : Extended impact of the FDA-AL compounds on macrophage senescence

To determine if the effect observed in a 24h assay is transitory and thus reversible, 3X10⁴ isolated p-Macs were seeded by well in a 96-wells plates with RPMI medium supplemented with 5% FBS and cultured at 37°C with 5% of CO₂. Cells were treated for 24 h with Tamoxifen, Bazedoxifene, Clomifene, Valnemulin, Terfenadine and 4-Hydroxytamoxifen at 1 µM. After that, compounds were withdrawn from medium. The % SA β-gal positive cells and the % of total cell number (as survival parameter) were

determined at day 1, 3 and 5. Data from all compounds were relativized again Vh treated cells on day 1 (Figure 15). The data corroborate the previous effects showing a reduction of the % of SA β -Gal-positive macrophages more than 50% relative to the Vh-treated cells.

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Example 17 : Hit expansion and IC₅₀ analysis of additional senescence –reducing compounds

To expand identified compounds, the inventors next tested compounds from the same families. 3X10⁴ p-Macs were seeded per well in 96-well plates with RPMI medium supplemented with 5% FBS and cultured at 37 °C with 5% CO₂. Cells were treated for 24h with 2 different single PI3K and mTOR inhibitors, 4 other PI3K/mTOR dual inhibitors, 1 JAK inhibitor and another AOX inhibitor as shown in Figure 16. The percent of SA- β -gal-positive cells and a total cell number were determined. The inventors confirmed that the significant effect in blocking macrophage senescence could be achieved with dual PI3K/mTOR but not single PI3K or single mTOR inhibitors (Figure 16). The best newly identified compounds after the hit expansion included the dual PI3K/mTOR inhibitors Gedatolisib (IC₅₀=0.04 μ M), and Dactolisib (IC₅₀=0.05 μ M); as well as AOX inhibitor Raloxifene (IC₅₀=0.35 μ M). These inhibitors also displayed low cell toxicity. These results confirmed that the most efficient classes of inhibitors to block macrophage senescence are dual PI3k/mTor and AOX inhibitors.

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Example 18 : Effect of most active compounds on pro-inflammatory and immunosuppressive gene expression

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1X10⁶ p-Macs isolated from 2- and 12-month –old wild-type mice were seeded and cultured in 6-well plates with RPMI medium supplemented with 5% FBS. Cells were treated with Way600, Bgt226 (both at 400 nM) and Raloxifene (1 μ M) for 24 h. Total RNA was isolated and analyzed for the expression of pro-inflammatory (including *Il1a*, *Il1b*, *Il6*, *Il15*) (Figure 17, upper panels), and immunosuppressive factors (*Arg1*, *Il10*, *Timd4* and *Vsig4*) (Figure 17, low panels). As expected, different drugs had a variable but consistent attenuating effect on the expression of immunosuppressive factors with Bgt226 being most active.

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Example 19 : Effect of old p-Macs on T cell proliferation and subtypes

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p-Macs were isolated from 2- and 24-month-old mice and co-cultured with freshly isolated T cell obtained from spleens of 2-month-old mice. T cells were isolated using untouched T-cells kit based on magnetic beads separation (Miltenyi Biotec). 1×10^5 T cells stained with FITC-carboxyfluorescein succinimidyl ester (CFSE) were co-cultured for 7 days with young or old macrophages (ratio 1:2) in RPMI medium supplemented with 10% FBS in the presence of 2 ng/mL of IL2 with or without a T cell PMA+Ionomycin activating cocktail (Biolegend) (Figure 18A, left panel) or with CD3/CD25 T-cell activating beads (Gibco) at a 1:1 ratio (Figure 18A, right panel). Proliferation was measured by flow cytometry after determining the intensity of CFSE fluorescence in CD3⁺ population. The inventors found that young macrophages induced proliferation of T cells, but this effect was lost when T cell were co-cultured with old macrophages (Figure 18A). Next, using the same experimental set-up the inventors tested the effect of drug-treated old macrophages on T cell proliferation. Old macrophages were treated for 24h with Way600 and Bgt226 (both at 400 nM), and raloxifene at 1 μ M. Next day, drugs were removed by extensive PBS washing and the experiments continued for another 6 days. The inventors found that Way600 increased and Bgt226 and Raloxifene reduced the percentage of proliferative T cells (Figure 18B). Next, the inventors evaluated the ability of drugs to reduce the percentage of Tregs (defined as CD4⁺ FoxP3⁺) and exhausted (defined as CD4⁺ PD-1⁺) T cell populations. The inventors found that old macrophages significantly induced formation of Tregs while 24h pre-treatment with all drugs significantly reduced this activity (Figure 18C). In addition, Way600 and Bgt226 reduced the fraction of exhausted T cells. Thus, senescence macrophages can induce immune-suppressive T cell subtypes while their pre-treatment with identified drugs significantly blocked this activity.

Example 20 : NNMT pathway is at the center of senescent state of p-Macs

p-Macs were obtained from 1-year-old p16-Cre/mTmG mice. p-Macs were purified using an F4/80 column and p16^{High} and p16^{Low} cells were separated by FACS based on the EGFP expression. These 2 cell fractions were subsequently used for RNA-Seq analysis. HTSeq v0.6.1 was used to count the read numbers mapped to each gene. Using this analysis, the inventors identify that one of the most overexpressed gene in p16^{High} p-Macs was Nicotinamide N-methyltransferase (*Nnmt*) (Figure 19A) with difference between fkm means of 15.77 ($\pm$ 2.097 SEM).

p-Macs were isolated from 12-month-old mice and were treated with different NNMT inhibitors for 24 h, including JBSNF-000088 (4 μ M) and NNMT inhibitor (NNMTi) (1 μ M), and methylated Nicotinamide (mNAM) (72 h at 0.1 mM). The inventors found that all 3

NNMT inhibitors were able to reduce the percent of SA- β -gal-positive macrophages (Figure 19B).

1X10⁶ p-Macs isolated from 2- and 12-month -old wild-type mice were seeded and cultured in 6-well plates with RPMI medium supplemented with 5% FBS. Cells were treated with Way600, Bgt226 (both at 400 nM) and Raloxifene (1 μ M) for 24 h. Total RNA was isolated and analyzed to determine the expression of *Nnmt*. The inventors found that Way600 and Bgt226 significantly reduced the expression of *Nnmt* mRNA both in young and old p-Macs (Figure 19C).

Similarly, the inventors treated p-Macs obtained from 12-month-old mice with JBSNF-000088 (4 μ M) for 24h and analyzed the expression of pro-inflammatory and immunosuppressive factors. Using a t-test, the inventors found the differences in Il1a, Il1b, Cxcl13 (pro-inflammatory factors) (left panels), and Timd4 and Il10 (immunosuppressive factors, right panels) after the treatment.

The inventors found a significant reduction in expression of all analyzed genes (Figure 19C) suggesting the key role of NNMT in regulation of senescence and immunosuppression in old macrophages.

Example 21 : In vitro analysis of activity of available NNMT inhibitors

Several NNMT inhibitors are currently available yet all have been reported to have low potency. To analyze these compounds, the inventors performed a cell-free inhibition in vitro assay for NNMT activity with different compounds using a commercial kit (Promokine, Promocell). The inventors evaluated the potency of 3 compounds and 1 metabolite by calculating the IC₅₀. Tested compounds and identified IC₅₀ were: NNMTi - IC₅₀=2.7 μ M, JBSNF-000088 -IC₅₀=17.72 μ M, Trigonelline- IC₅₀=139.15 μ M, and mNAM - IC₅₀=48.4 μ M (Figure 20). This data shows that methylated NAM (mNAM) has an inhibitory activity towards NNMT and thus can be used in vivo including as a dietary supplement to lower NNMT activity.

Example 22 : CD38 is an upstream donor of NAM that is required for the Nnmt-dependent senescent-like phenotype in p16^{High} macrophages

While the inventors identified several NNMT inhibitors (as shown in Figure 20), none of them were capable of inhibiting Nnmt in a nanomolar range of concentrations. In order for NNMT to work efficiently, the present of NAM is required. In turn, NAM could be provided by the enzyme called CD38. Next, the inventors evaluated whether CD38 could be a

viable target to reduce senescence in macrophages. CD38 protein is highly expressed in many immune cells and catalyzes the conversion of NAD to NAM. Since NAM and SAM are the substrates of NNMT, NAM availability is a key factor to control NNMT activity. First, the inventors evaluated the expression of CD38 in p16^{High} cells present in different tissues. Single cell suspensions from peritoneal cavity, spleen, liver, abdominal fat (SVF), bone marrow and peripheral blood of 18-month-old mice were prepared and CD38 was analyzed with a fluorescent conjugated antibody by flow cytometry (Figure 21A). The inventors observed that in many tissues a high percent of p16^{High} cells were also positive for CD38. Next, they evaluated the effect of Way600, Bgt226 and raloxifene on CD38 expression. p-Macs were purified from 2- and 12-month-old mice, treated for 24h with indicated drugs and total RNA was isolated and analyzed by RT-PCR. They found a reduction in *Cd38* mRNA expression in 12-month-old p-Macs after treatment with all 3 drugs (Figure 21B). Next, the inventors tested the ability of 2 different previously reported CD38 inhibitors, C-78c and Apigenin, to reduce SA- β -gal activity in peritoneal macrophages. They found that after 24h treatment, both compounds reduced the percent of β -gal positive cells (Figure 21C). Next, the inventors tested the effect of CD38 inhibition on the expression of pro-inflammatory (Figure 21D, upper panels) and immunosuppressive genes (Figure 21D, low panels). *Il1a* was upregulated after treatment, while *Il1b*, *Il6* and *Cxcl13* were downregulated. In the same way, *Nnmt*, *Arg1*, *Il10*, *Timd4* and *Vsig4* were downregulated after C-78c treatment. Thus, these results confirmed that inhibition of CD38 could be a viable option to reduce macrophage senescence and immunosuppression linked with it.

CLAIMS

1. Compound which is chosen from dual inhibitors of the mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (PI3K), NNMT inhibitors,
5 Janus kinase 2 inhibitors, CD38 inhibitors, inhibitors of the enzyme ataxia telangiectasia and Rad3 related, aldehyde oxydase (AOX) inhibitors, anesthetic agents, antagonists of the histamine H1 receptor, pleuromutilin and its derivatives, topical antiseptic cations and serotonin-norepinephrine reuptake inhibitors, for use in treating a disease associated with macrophage senescence.
10
2. Compound for use according to claim 1, wherein the disease associated with macrophage senescence is chosen from age-related diseases, viral infections, and acute respiratory distress syndrome.
- 15 3. Compound for use according to any one of claims 1 to 2, wherein it is chosen from BGT226 and its salts, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib, Voxelisib, Ruxolitinib, Pacritinib, Berzosertib, Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene and its salts, NNMT inhibitors including mNAM, NNMTi, JBSNF-000088 and Trigonelline and its salts, CD38 inhibitor 78c, apigenin,
20 Butacaine, Terfenadine, Valnemulin and its salts, Clomifene and its salts, Cetrimonium and its salts, and Duloxetine and its salts.
- 25 4. Compound for use according to any one of claims 1 to 3, wherein it is chosen from BGT226, BGT226 maleate, WAY600, PP121, Gedatolisib, Bimiralisib, Dactolisib, Voxelisib, Ruxolitinib, Pacritinib, Berzosertib, Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, Bazedoxifene, Bazedoxifene acetate, NNMT inhibitors including mNAM, CD38 inhibitor 78c, apigenin, Butacaine, Terfenadine, Valnemulin, Valnemulin hydrochloride, Clomifene and Clomifene citrate, Cetrimonium, Cetrimonium chloride, Cetrimonium bromide, Duloxetine and Duloxetine
30 hydrochloride.
5. Compound for use according to any one of claims 2 to 4, wherein the age-related disease is chosen from cancer, metabolic disease, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, Alzheimer's disease,

osteoarthritis, cardiac diastolic dysfunction, benign prostatic hypertrophy, aortic aneurysm and emphysema, preferably cancer.

5 6. Compound for use according to any one of claims 2 to 4, wherein the viral infection is chosen from infections due to a virus which can lead to an acute respiratory distress syndrome, preferably the viral infection is chosen from viral respiratory infections, notably due to influenza viruses (A, B, C or D), respiratory syncytial virus, rhinoviruses, coronaviruses, adenoviruses or parainfluenza viruses.

10 7. Compound for use according to any one of claims 2 to 4 or 6, wherein the viral infection is chosen from an infection with Middle East respiratory syndrome-related coronavirus (MERS-CoV), an infection with severe acute respiratory syndrome-related coronavirus (SARS-CoV) and an infection with severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) also called COVID-19.

15 8. Compound for use according to any one of claims 2 to 4 or 6 to 7, wherein the viral infection is chosen from severe COVID-19 forms.

20 9. Compound for use according to any one of claims 2 to 4 or 6 to 8, wherein the acute respiratory distress syndrome is the consequence of viral infections, and especially of severe pneumonia or SARS-CoV-2 infection, or of infections mostly caused by bacteria and especially of sepsis, severe sepsis or septic shock.

25 10. Compound for use according to any one of claims 1 to 9, in treating a disease associated with macrophage senescence in an old subject, i.e. of at least 55 years old.

30 11. Compound for use according to any one of claims 1 to 10, for reducing COVID-19-associated hyperinflammation.

12. Compound for use according to claim 11, for reducing the levels of pro-inflammatory cytokines chosen from IL-6, IL-1, TNF- α , interferon and their mixtures.

35 13. Compound for use according to any one of claims 1 to 12, wherein it is chosen from dual inhibitors of phosphatidylinositol 3-kinase and of mTOR, and more preferably from BGT226 and its salts, PP121, Gedatolisib, Bimiralisib, Dactolisib

and Voxtalisib ; NNMT inhibitors including mNAM ; Janus kinase 2 inhibitors, and more preferably from Ruxolitinib and Pacritinib ; AOX inhibitors, and more preferably from Tamoxifen, 4-Hydroxytamoxifen, Raloxifene, and Bazedoxifene and its salts ; and CD38 inhibitors, and more preferably from CD38 inhibitor 78c and apigenin.

14. Methylated nicotimanide for use for treating a disease associated with macrophage senescence, preferably orally.

15. Product comprising a compound according to any one of claims 1 to 13, and an immune-checkpoint therapy, irradiation or/and chemotherapy, as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

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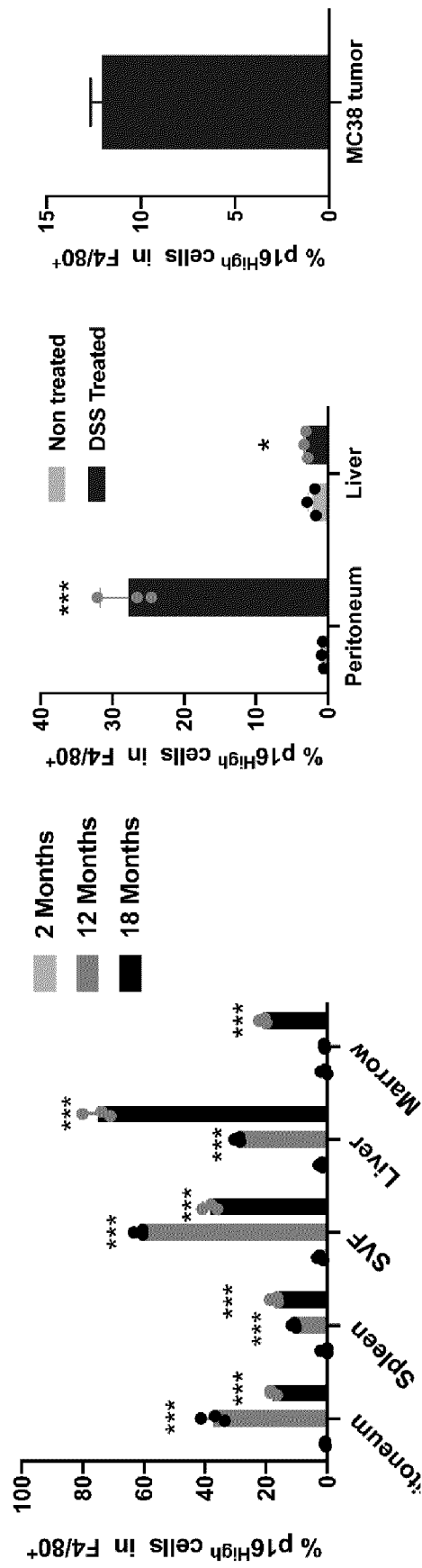


FIG.1

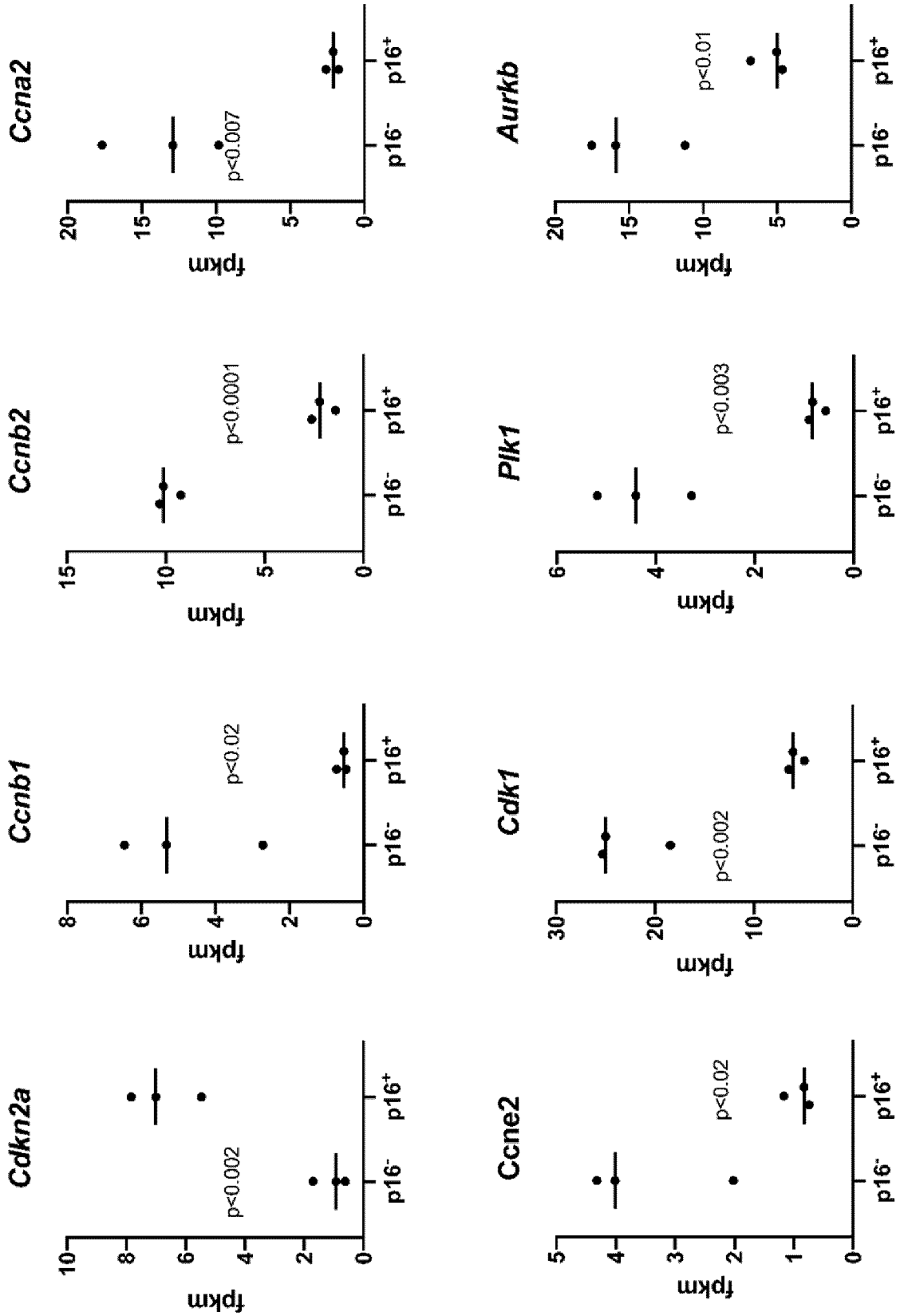
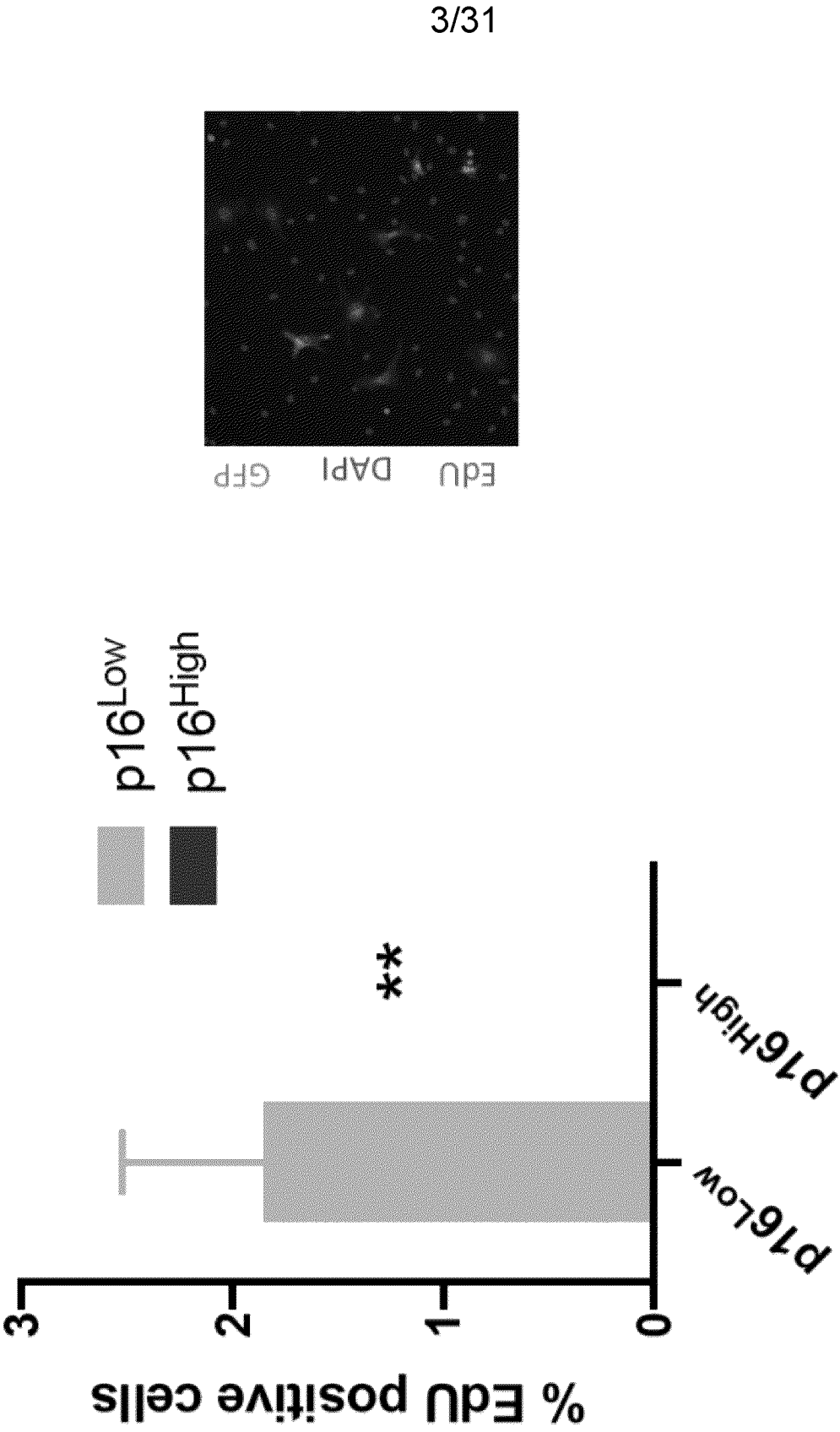


FIG.2



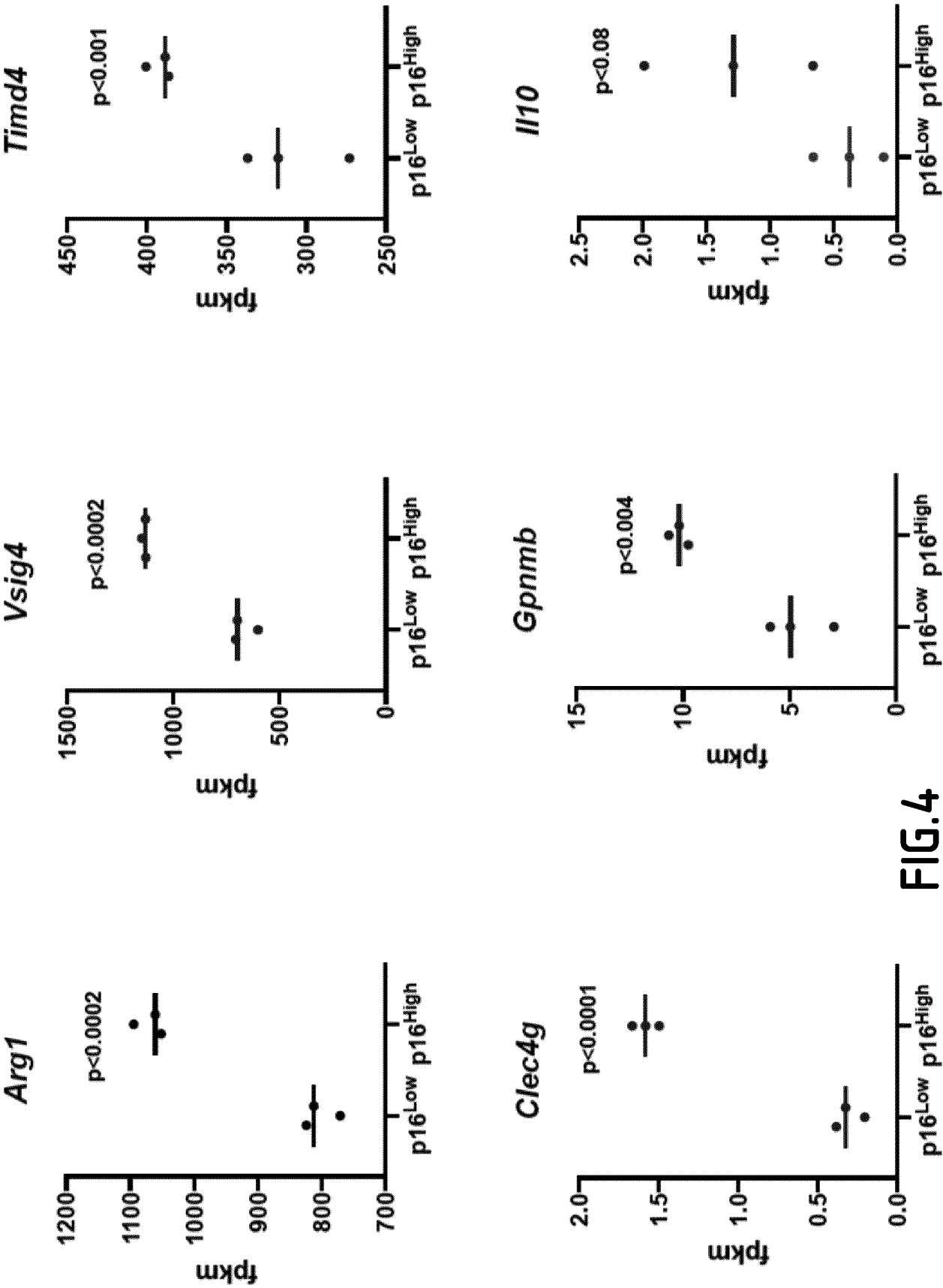
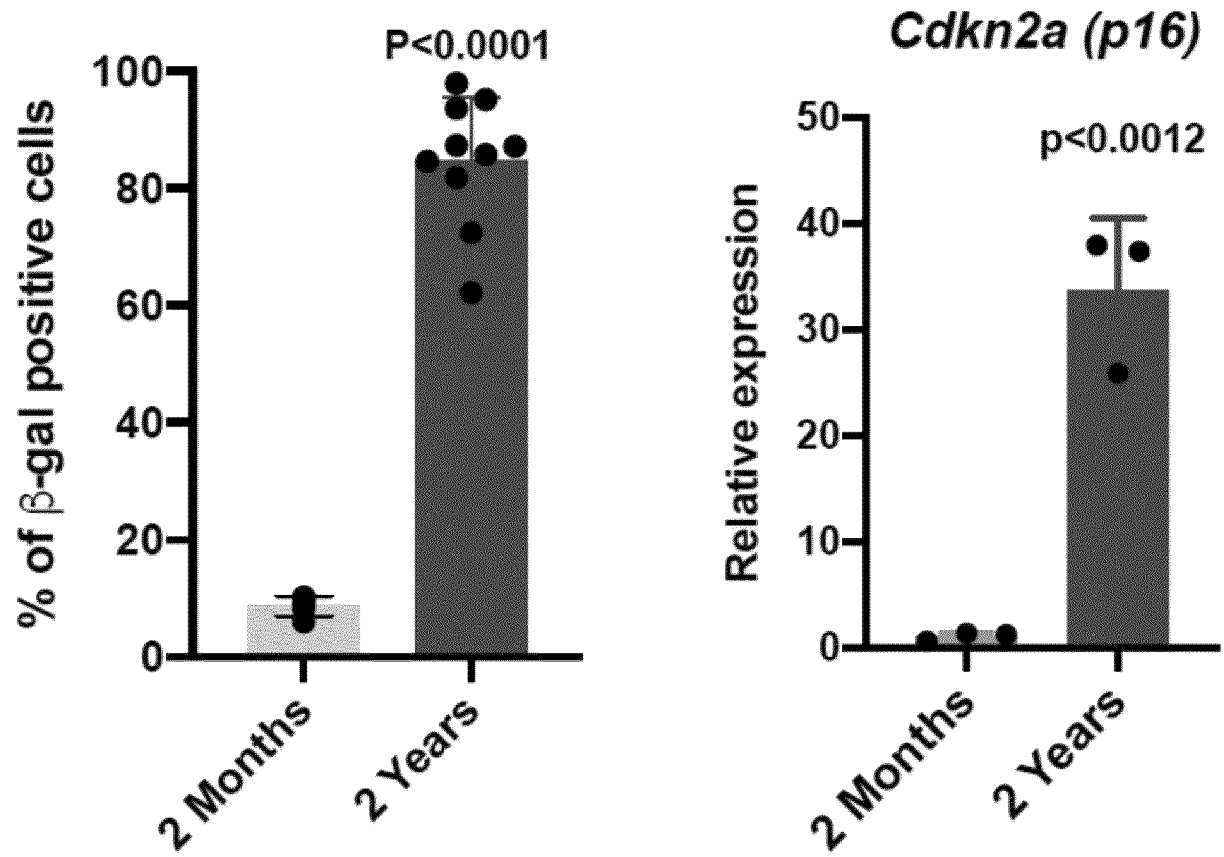


FIG.4

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FIG.5

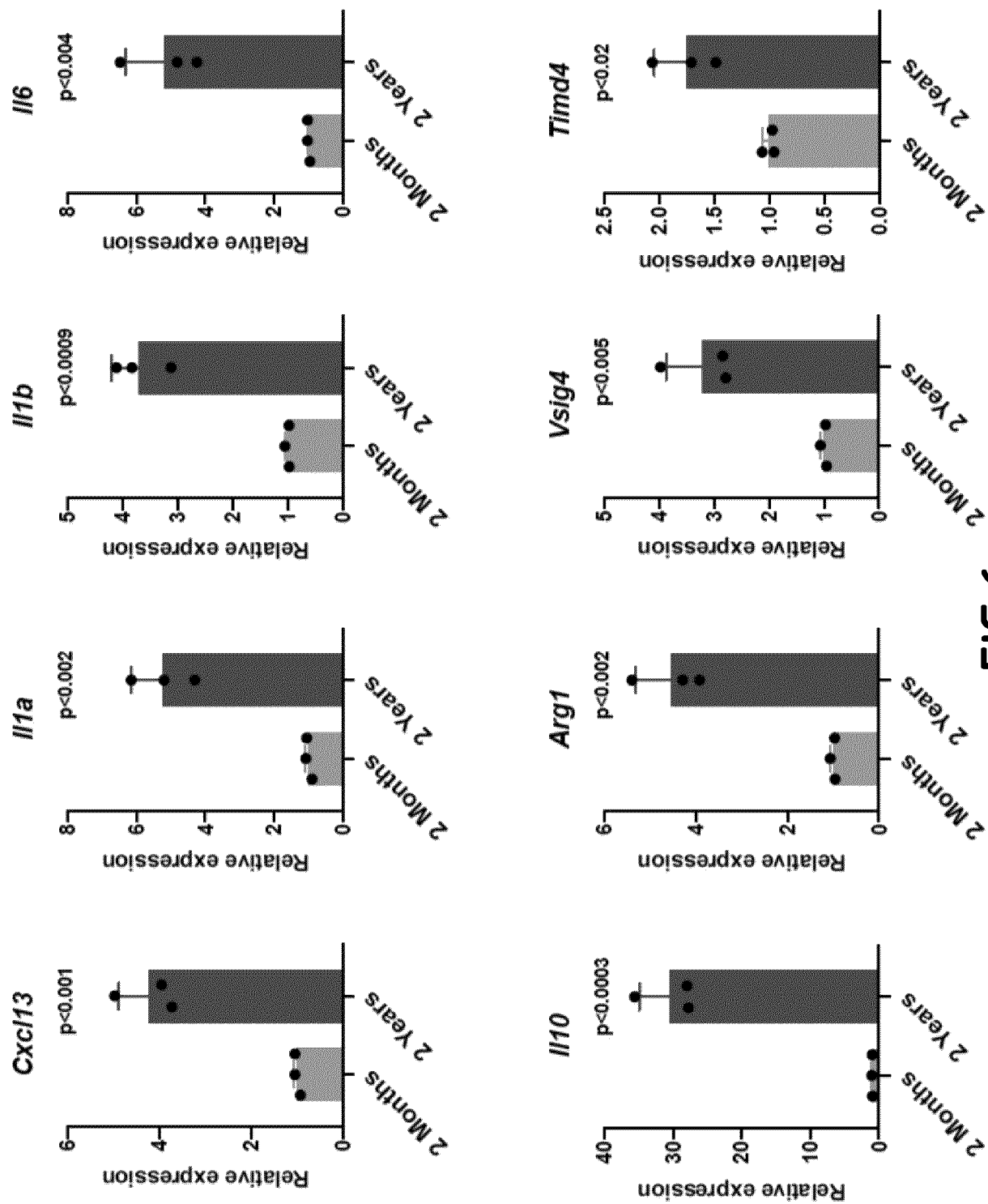


FIG.6

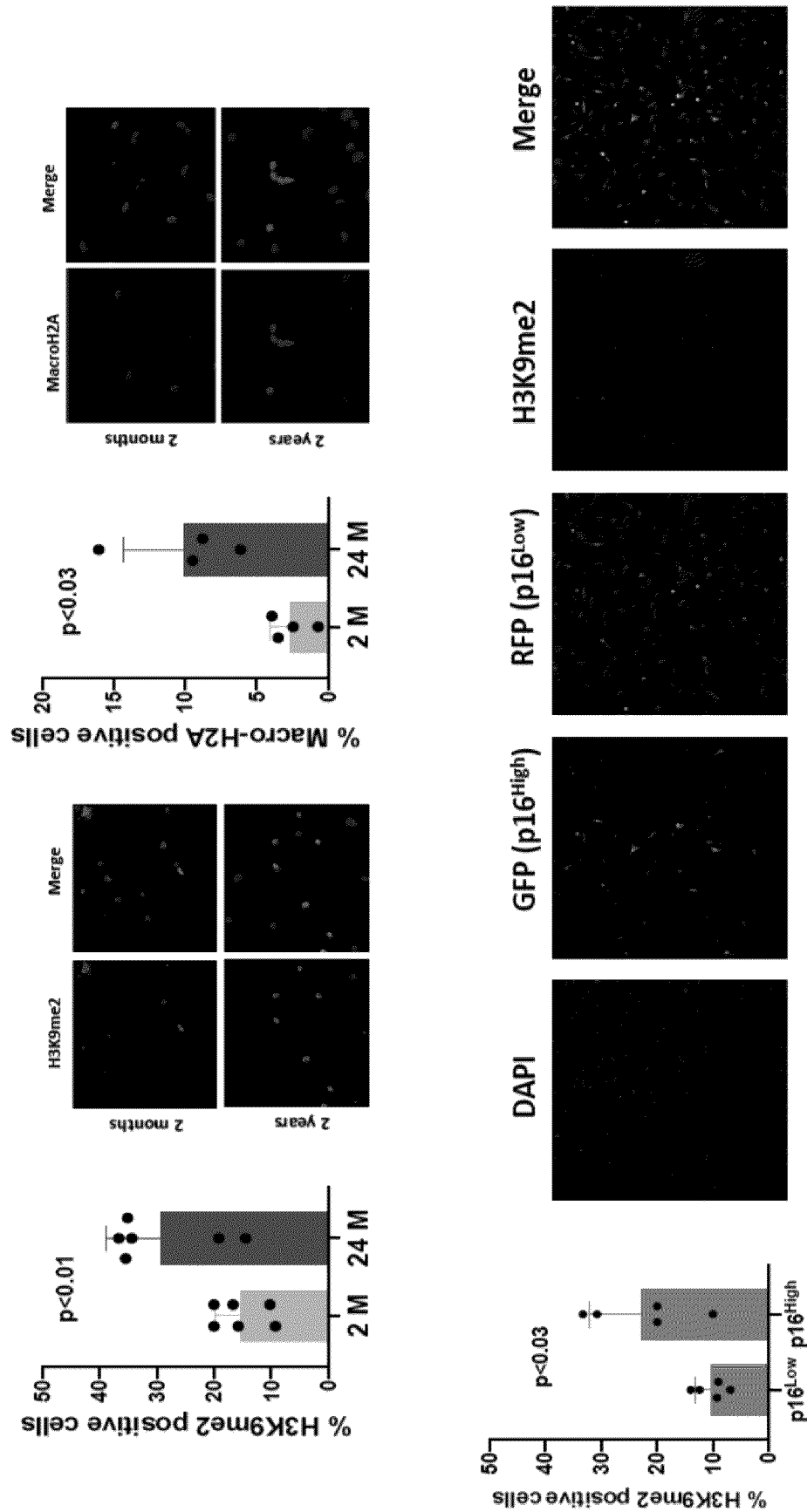


FIG. 7

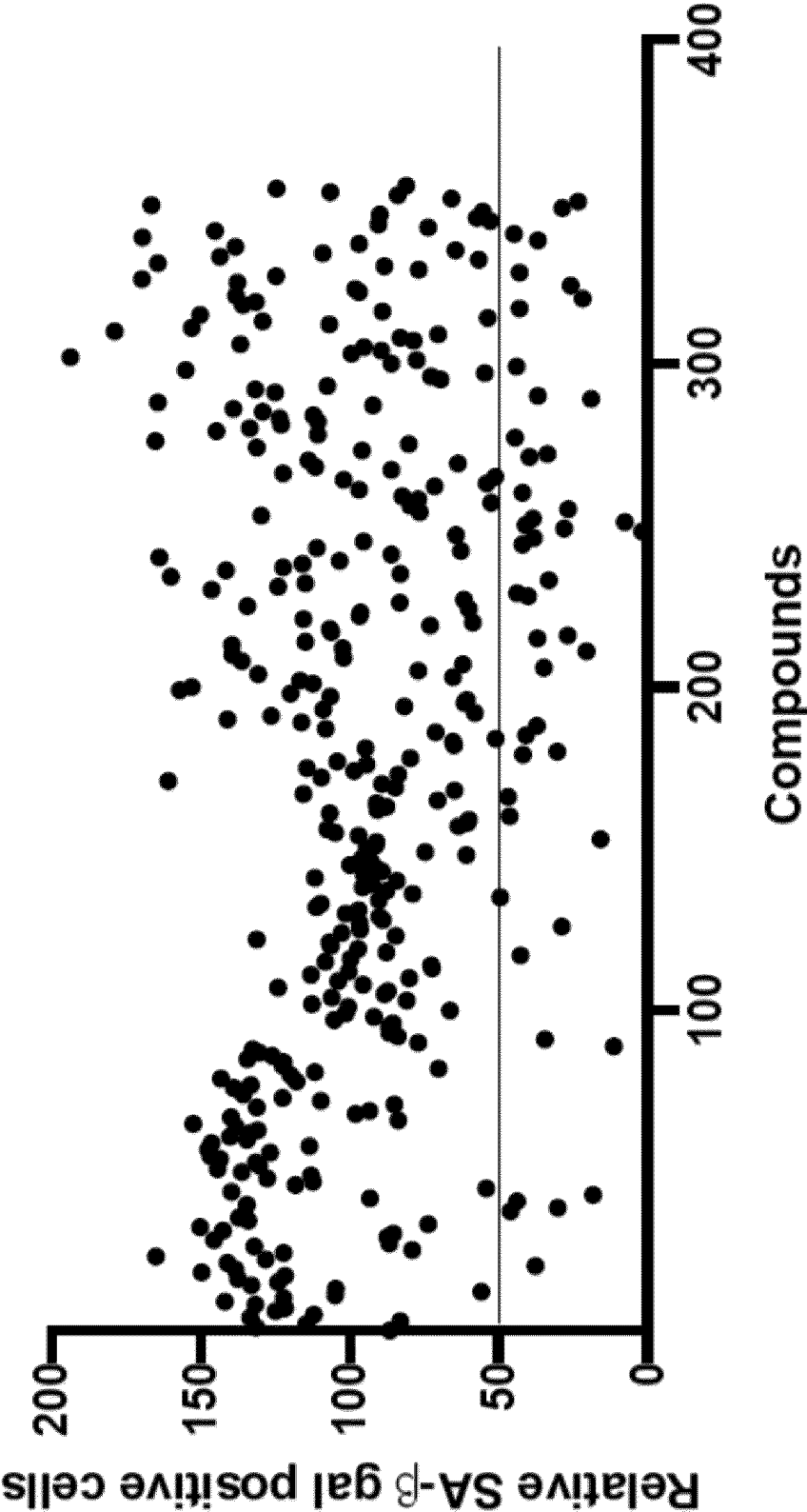


FIG.8

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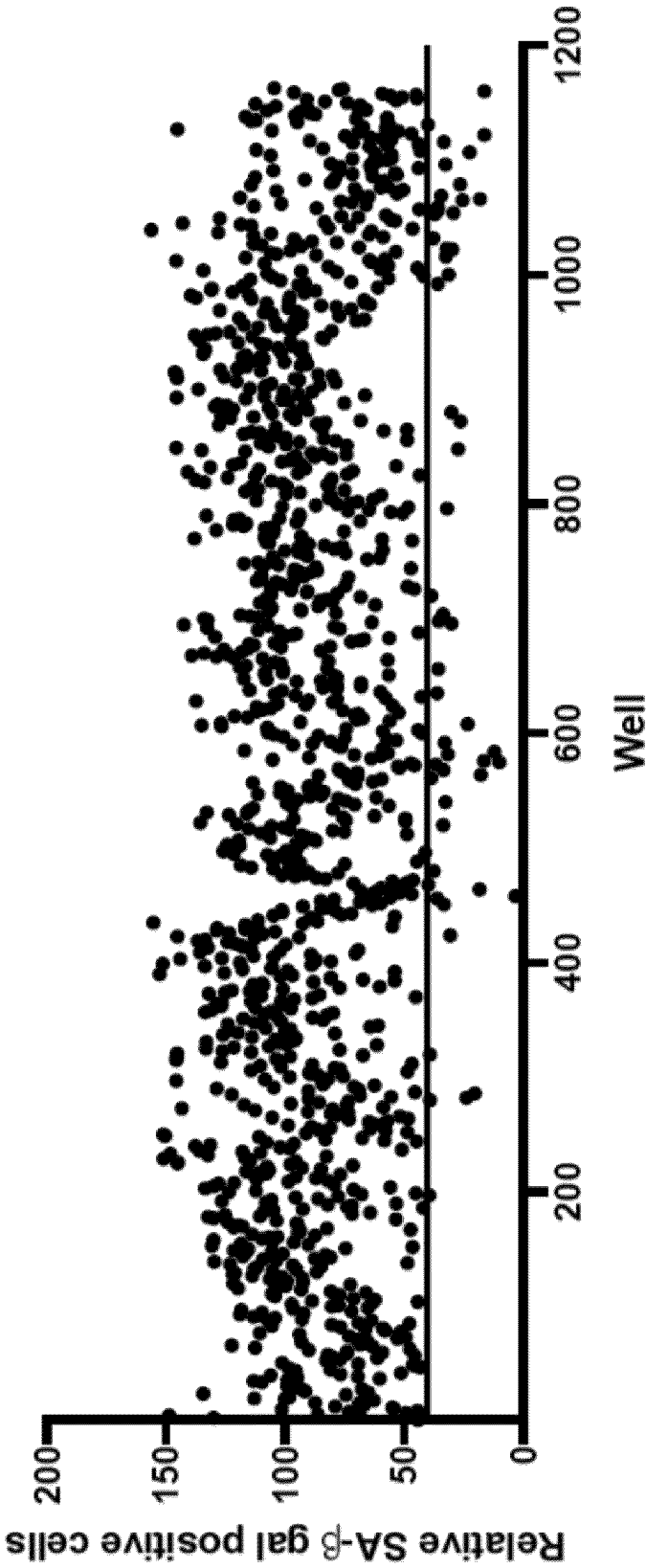


FIG.9

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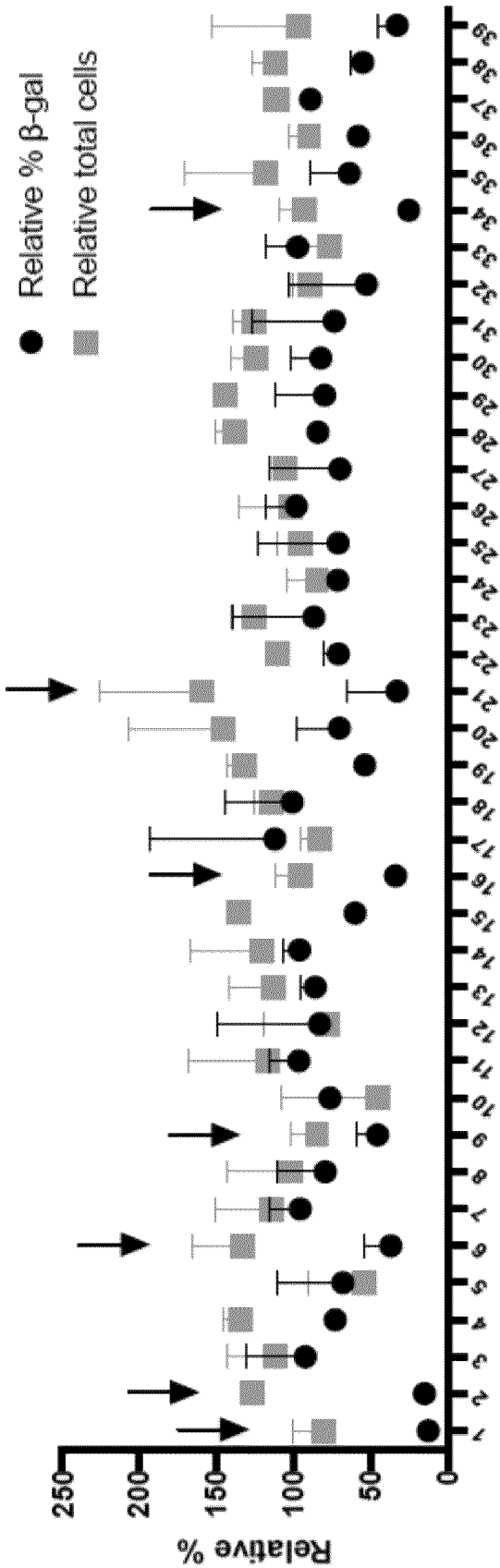


FIG.10

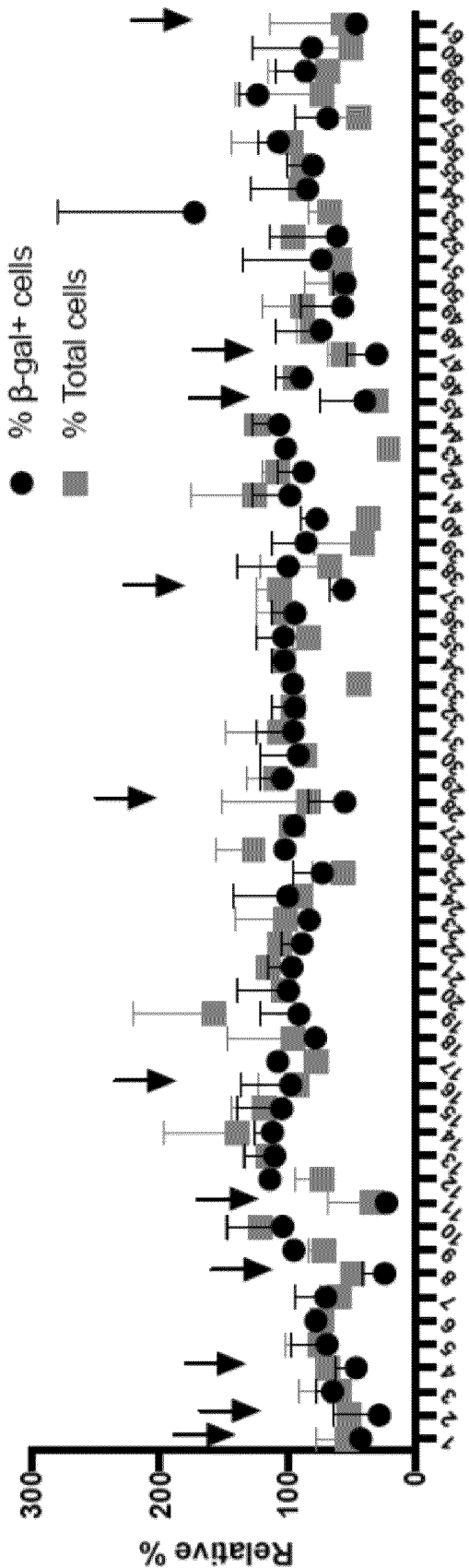
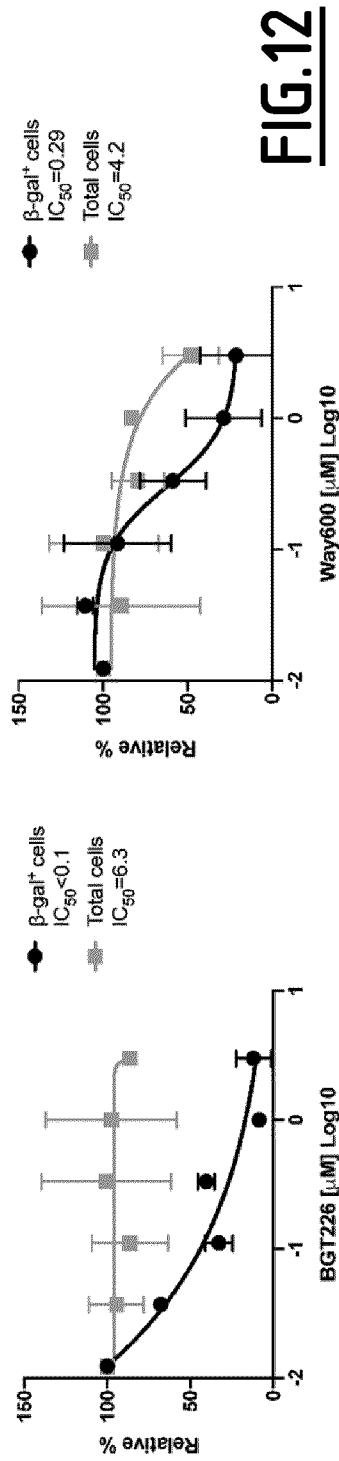
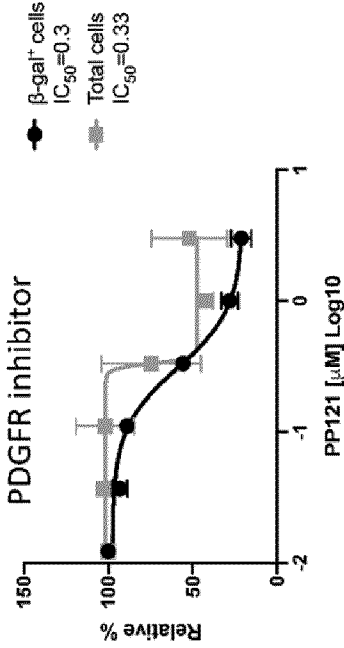


FIG.11

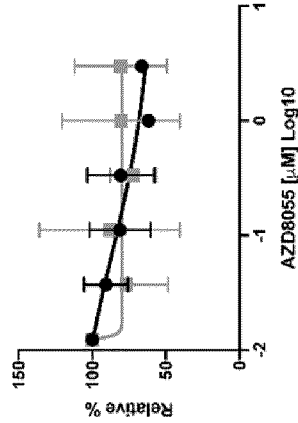
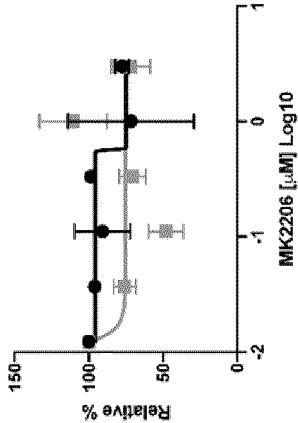
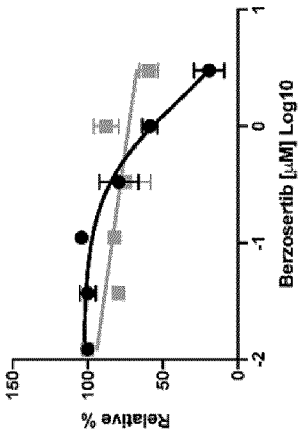
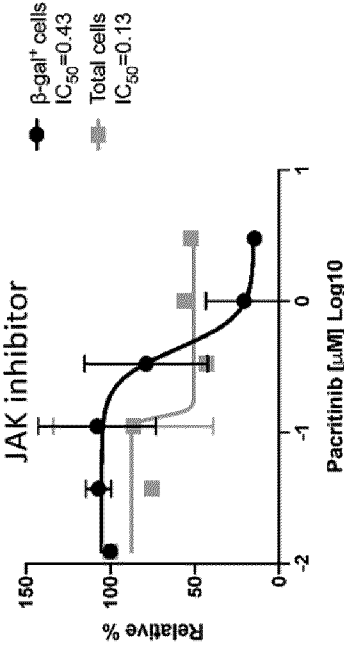
PI3k/mTOR dual inhibitors



PDGFR inhibitor



JAK inhibitor



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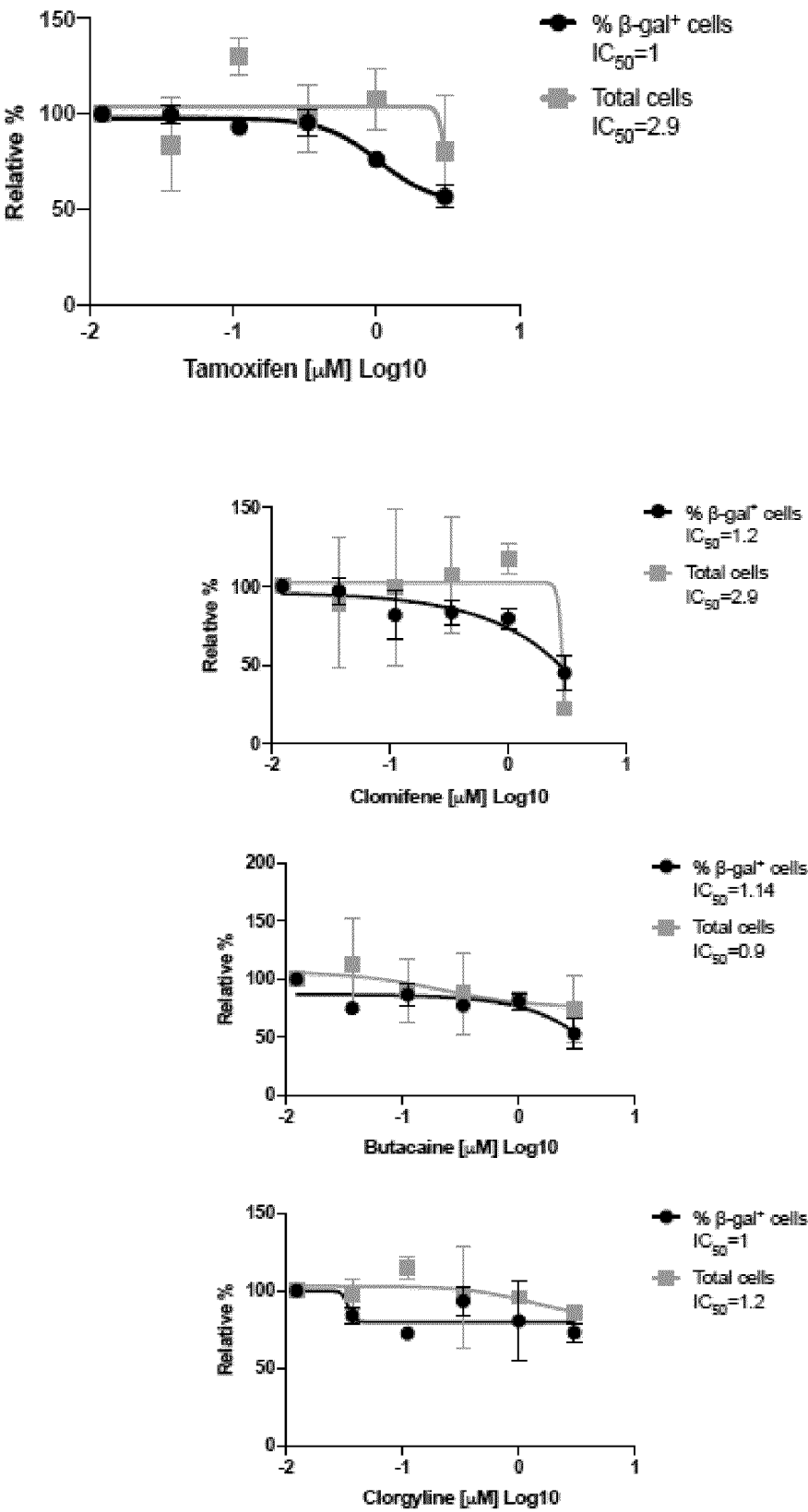


FIG.13

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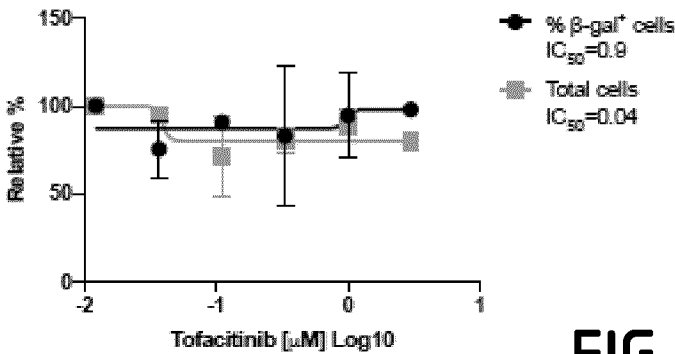
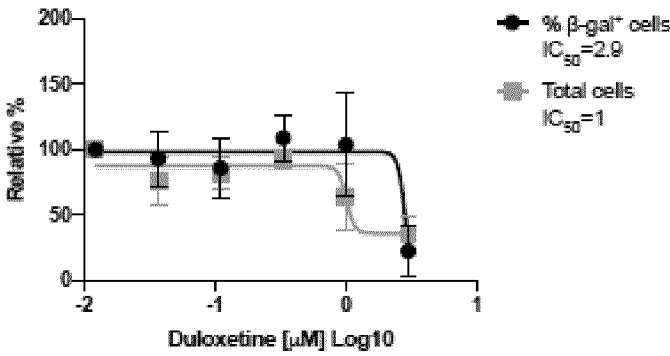
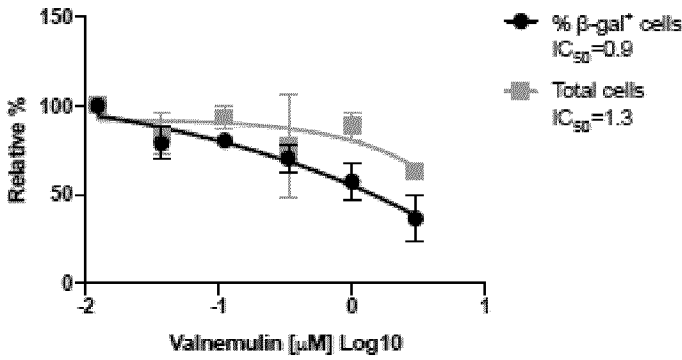
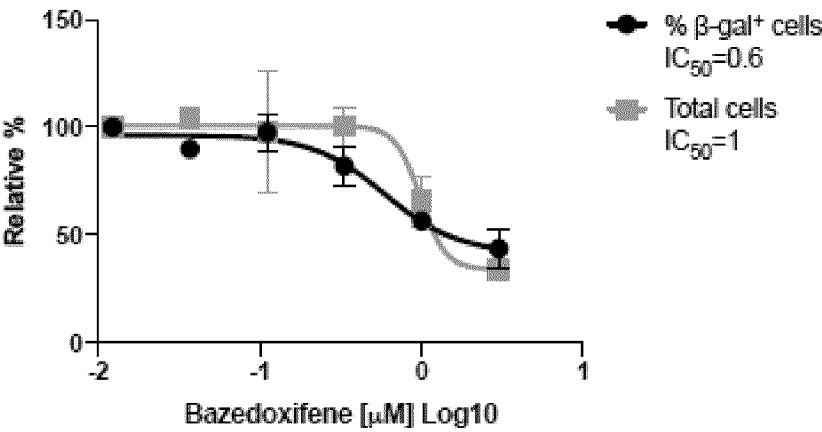


FIG.13 Continued

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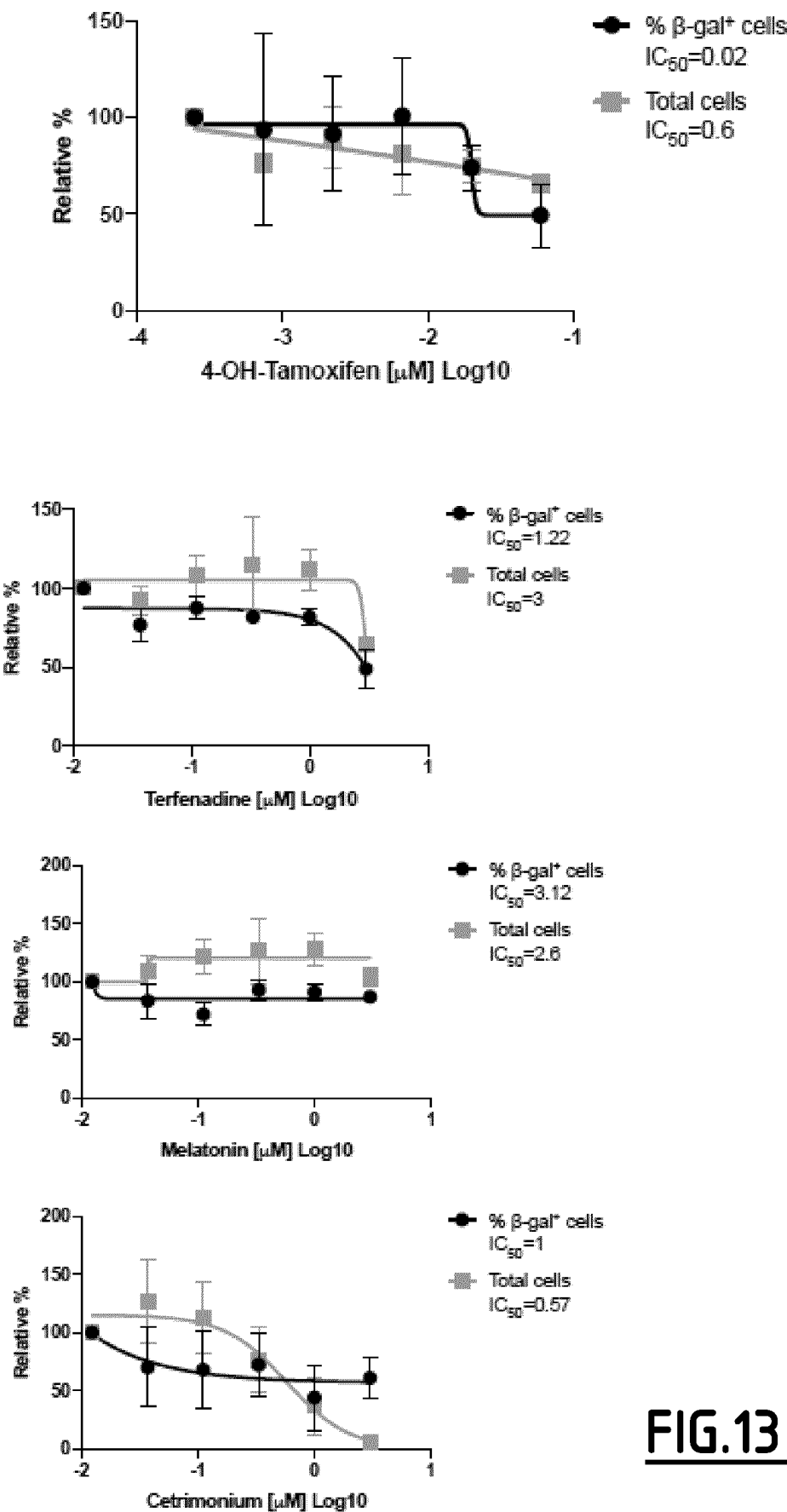


FIG.13 End

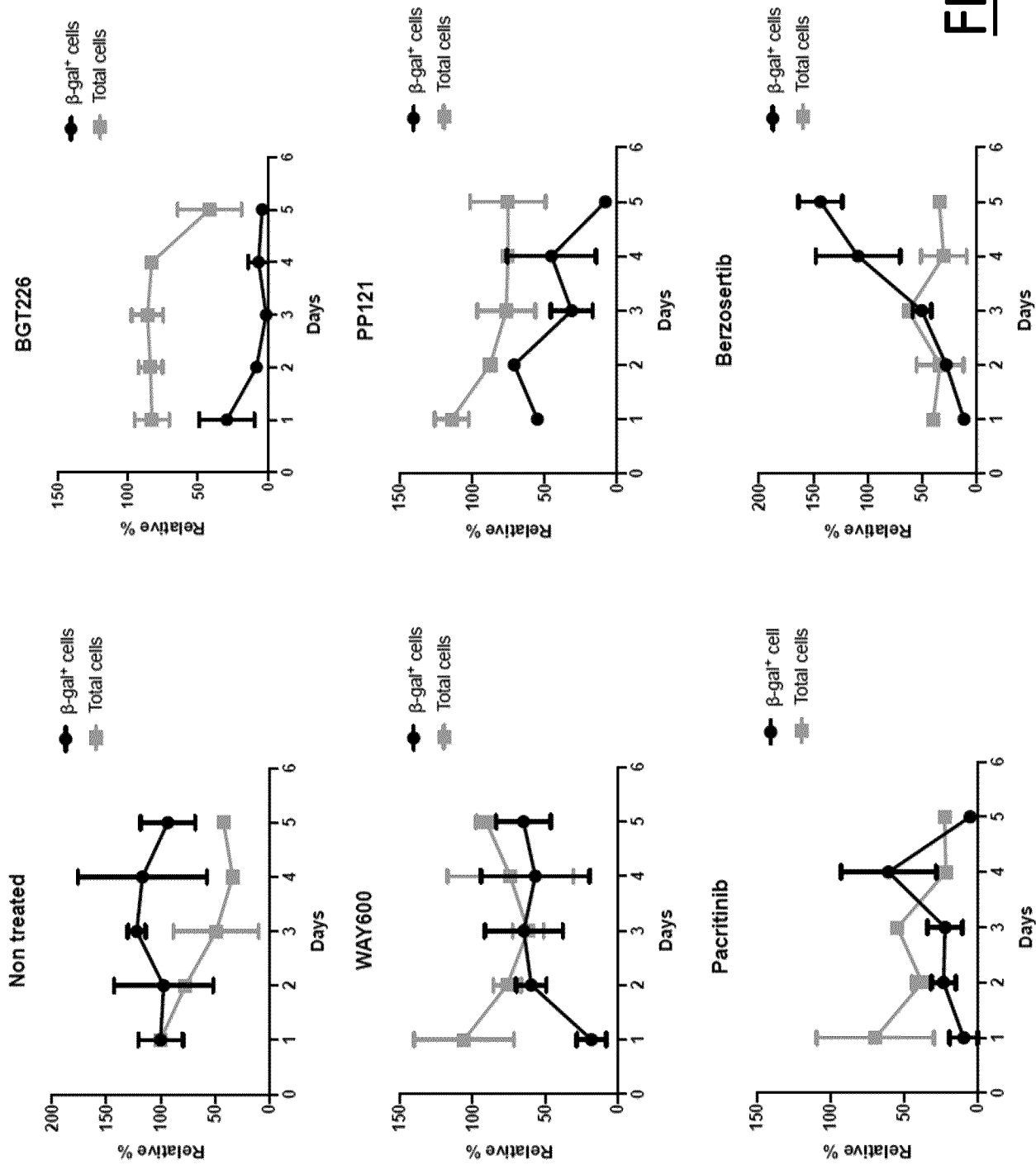


FIG.14

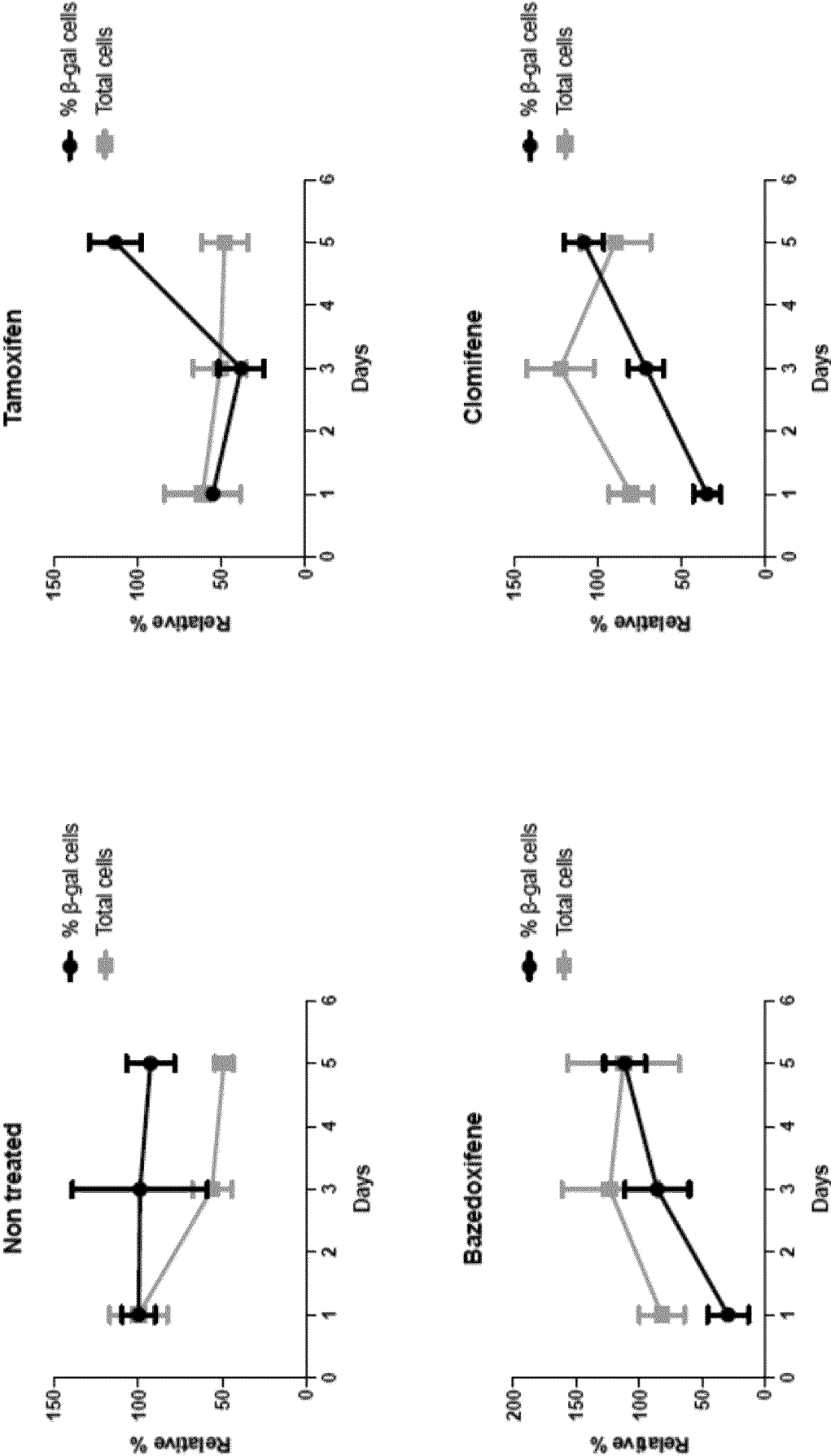


FIG.15

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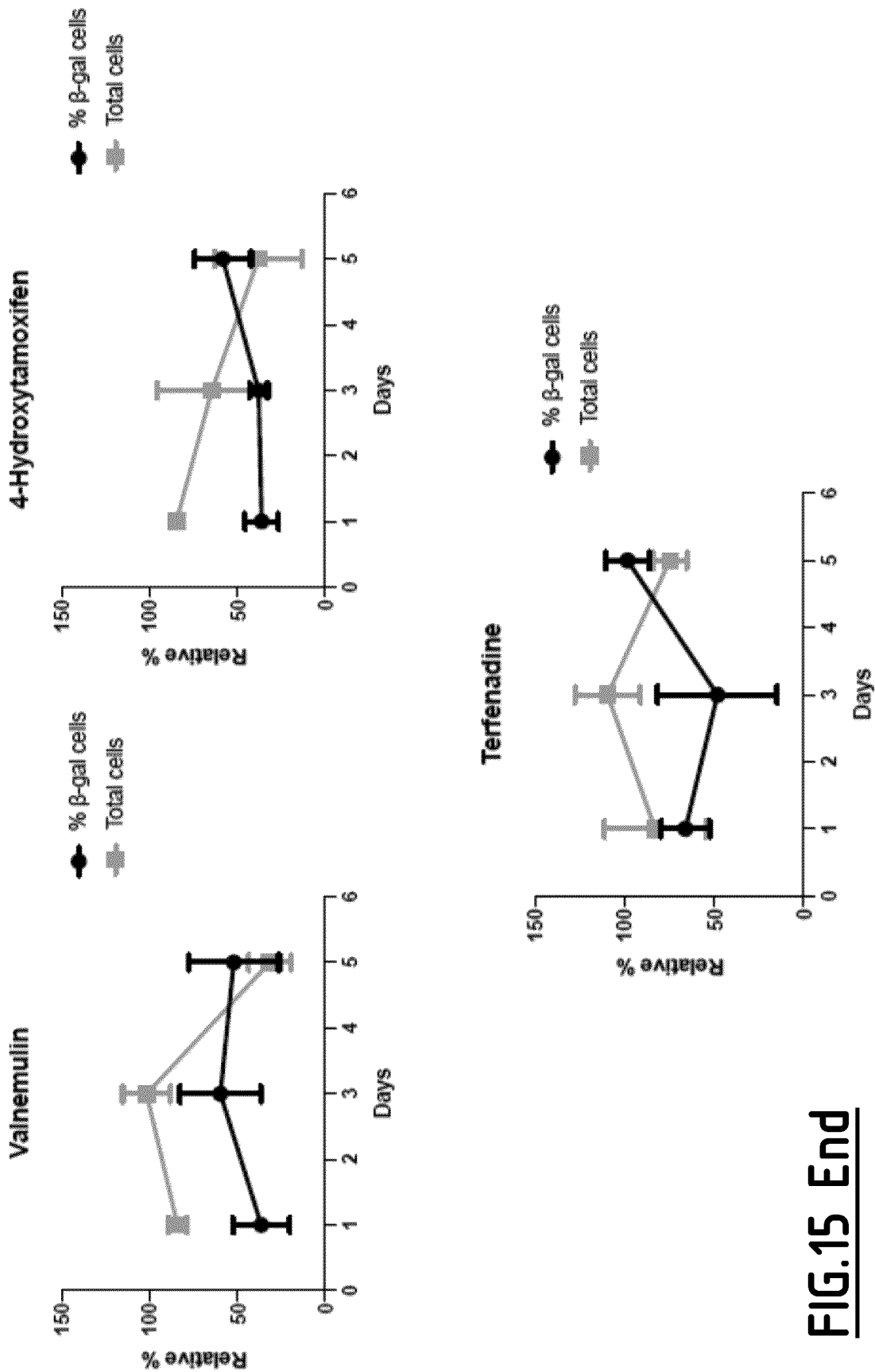
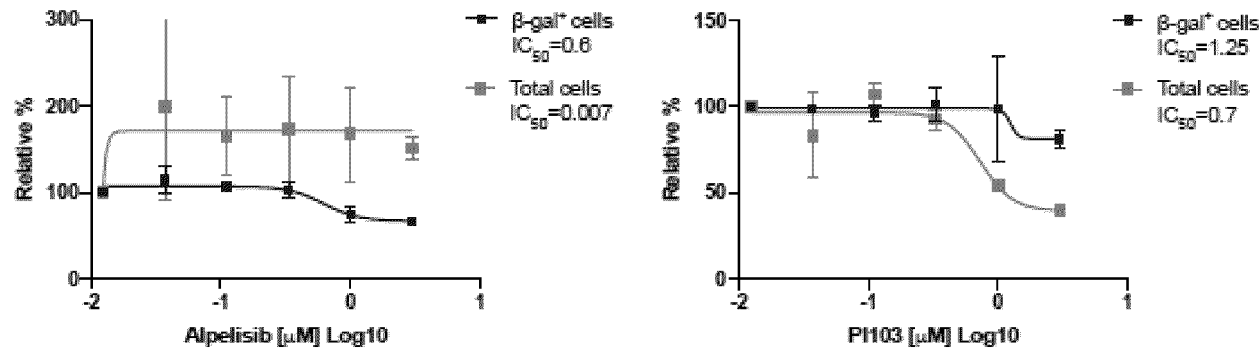


FIG.15 End

PI3K Inhibitors



mTOR Inhibitors

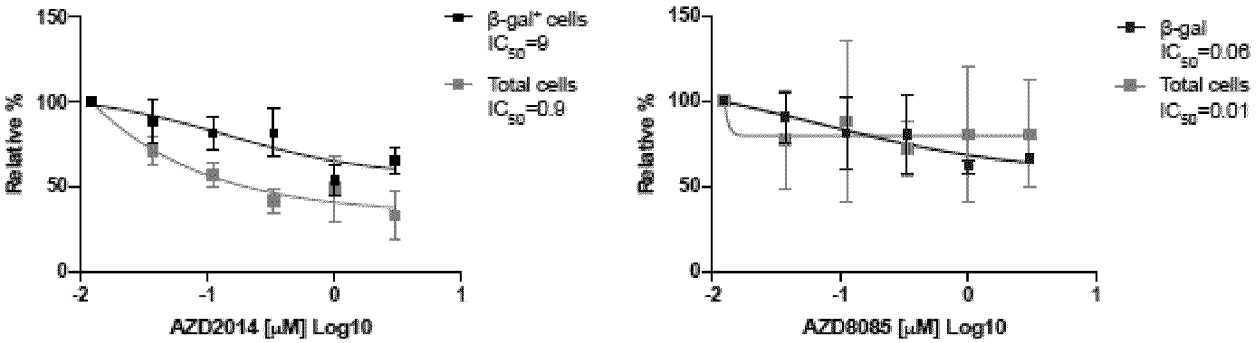
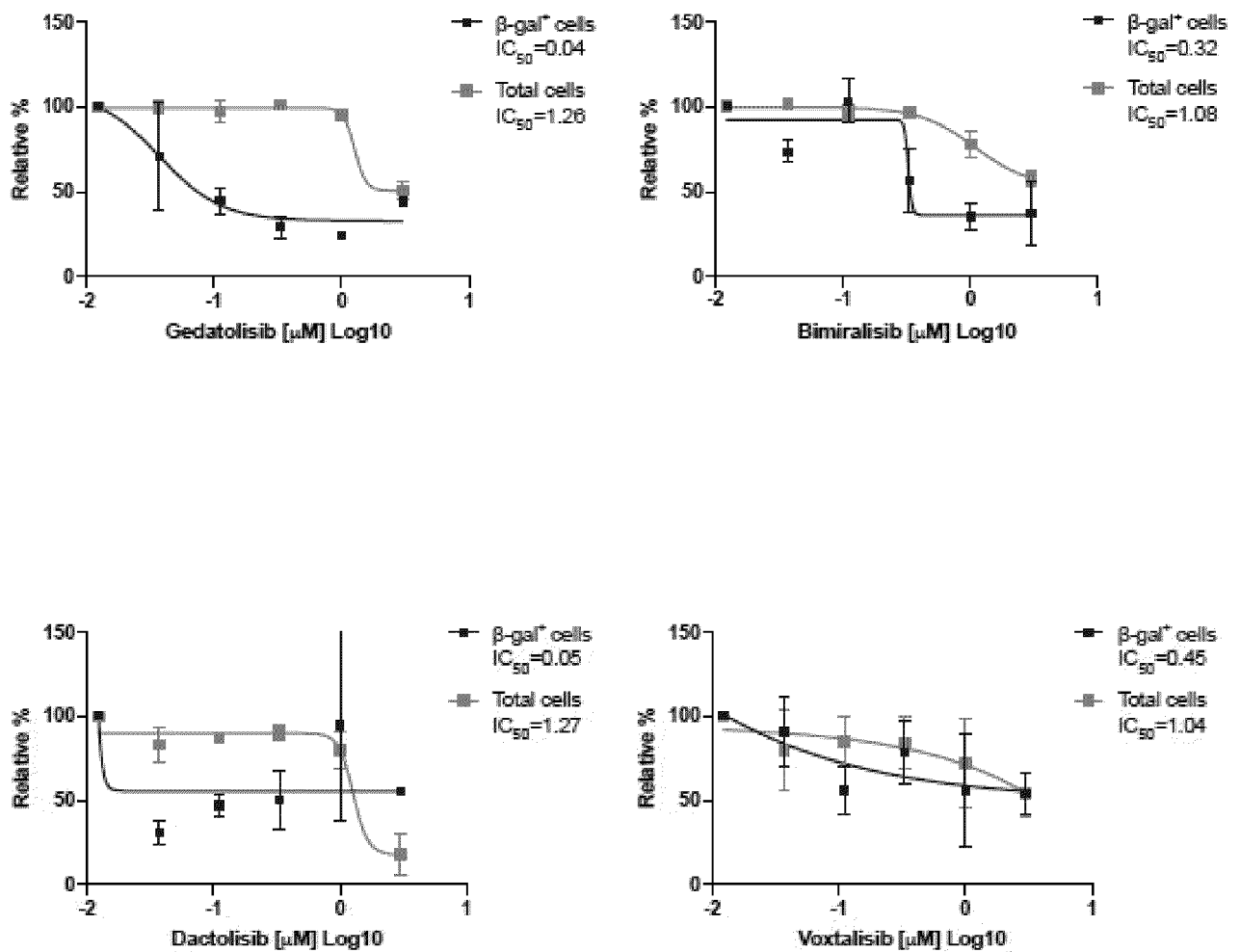


FIG.16

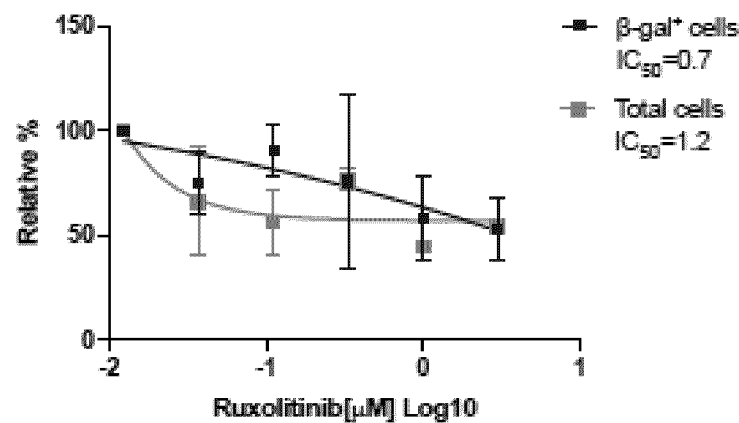
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PI3K/mTOR dual Inhibitors

**FIG.16 Continued**

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JAKs Inhibitor



AOX Inhibitor

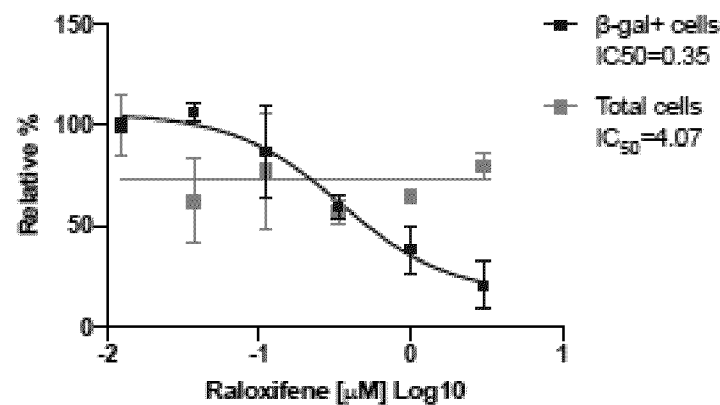


FIG.16 End

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Pro-inflammatory factors

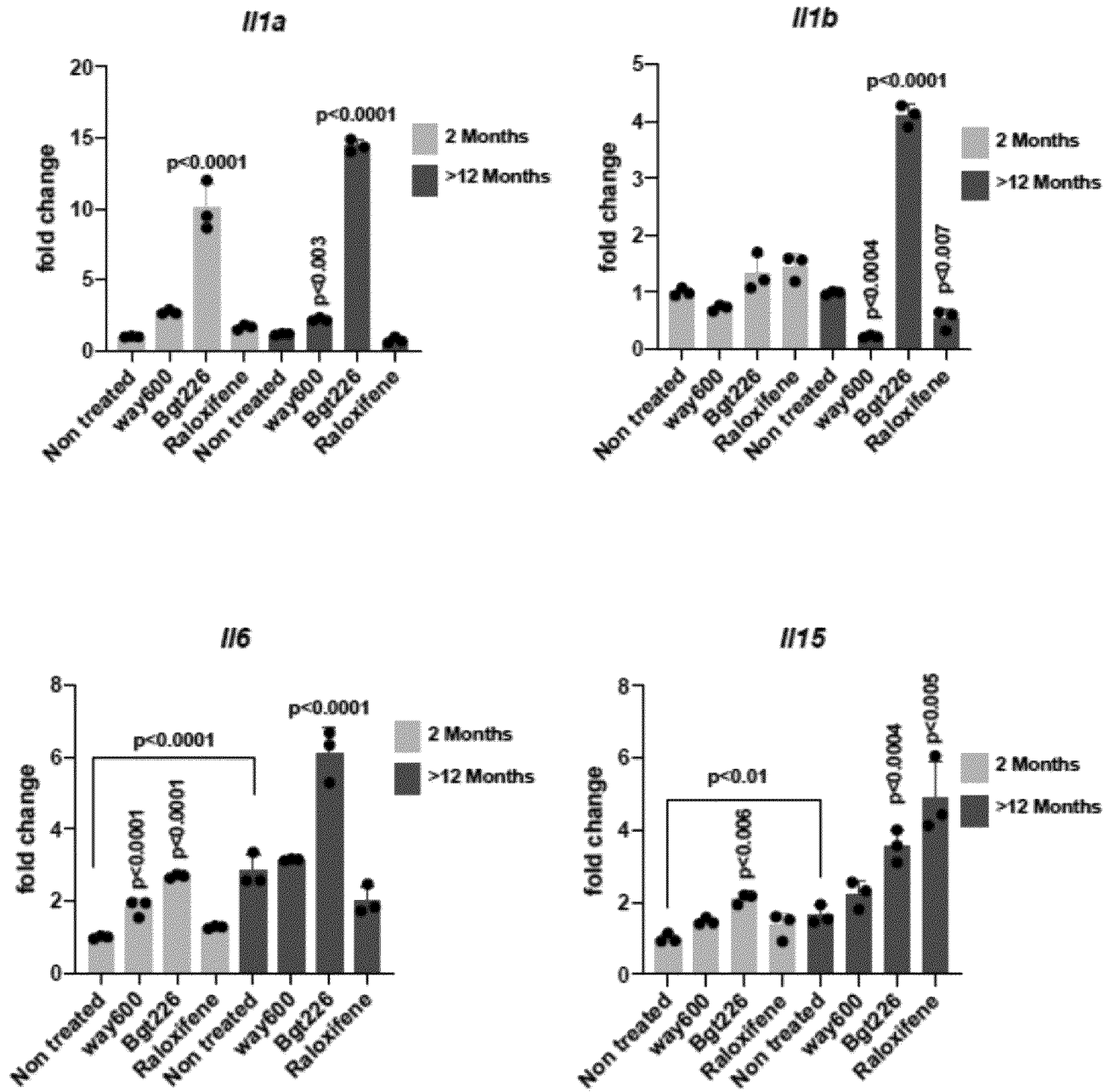


FIG.17

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Immunosuppressive factors

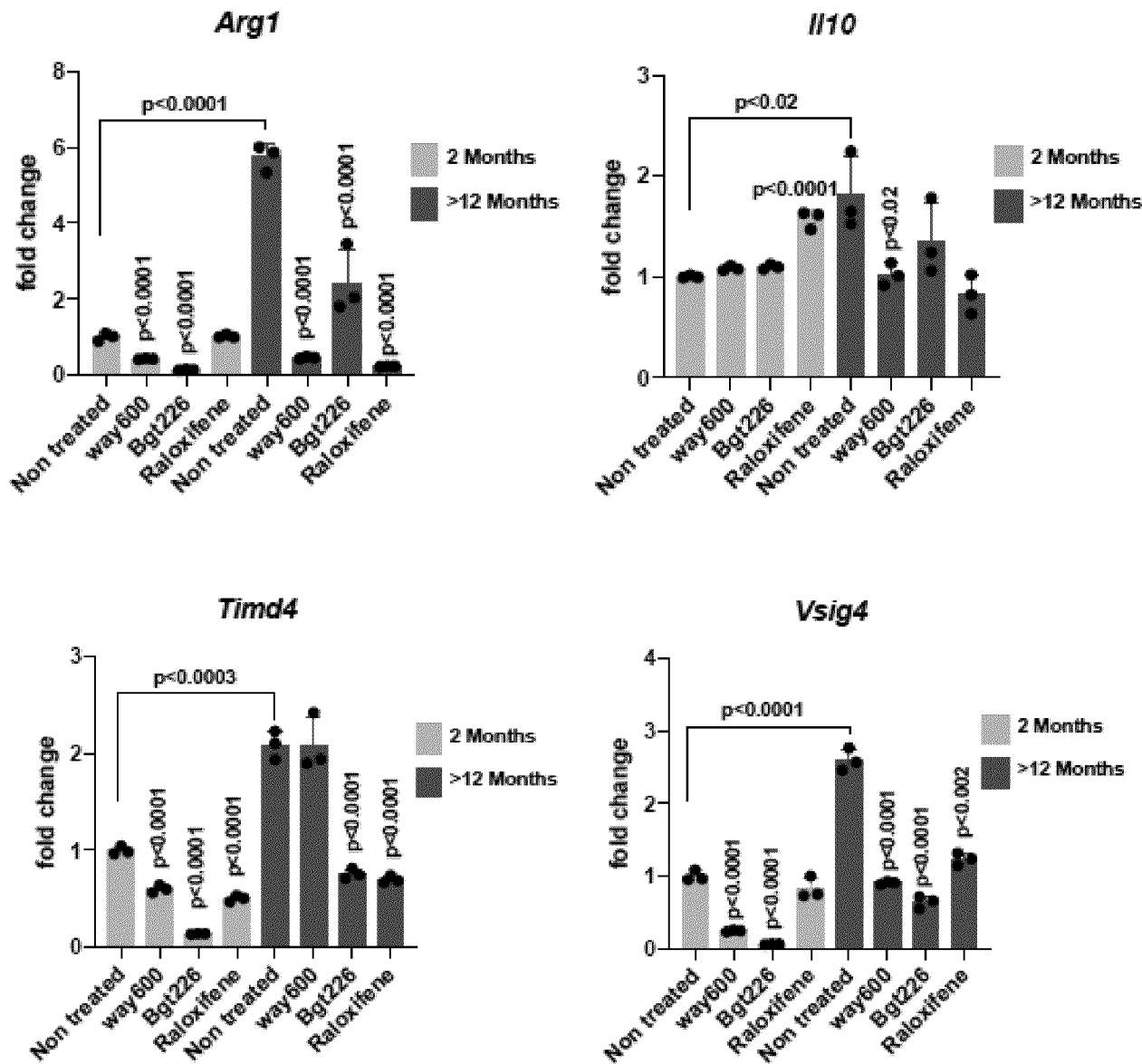


FIG.17 End

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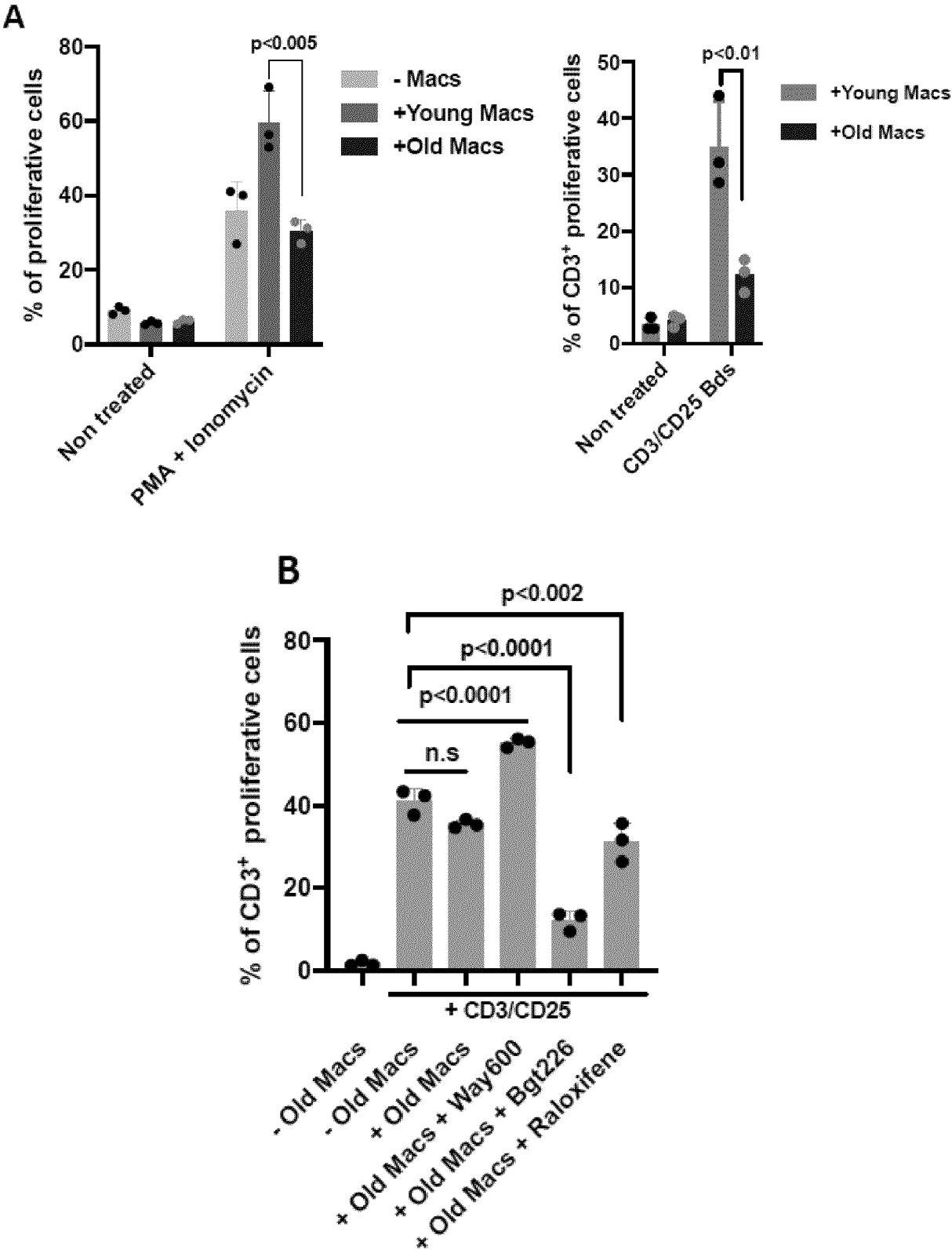


FIG.18

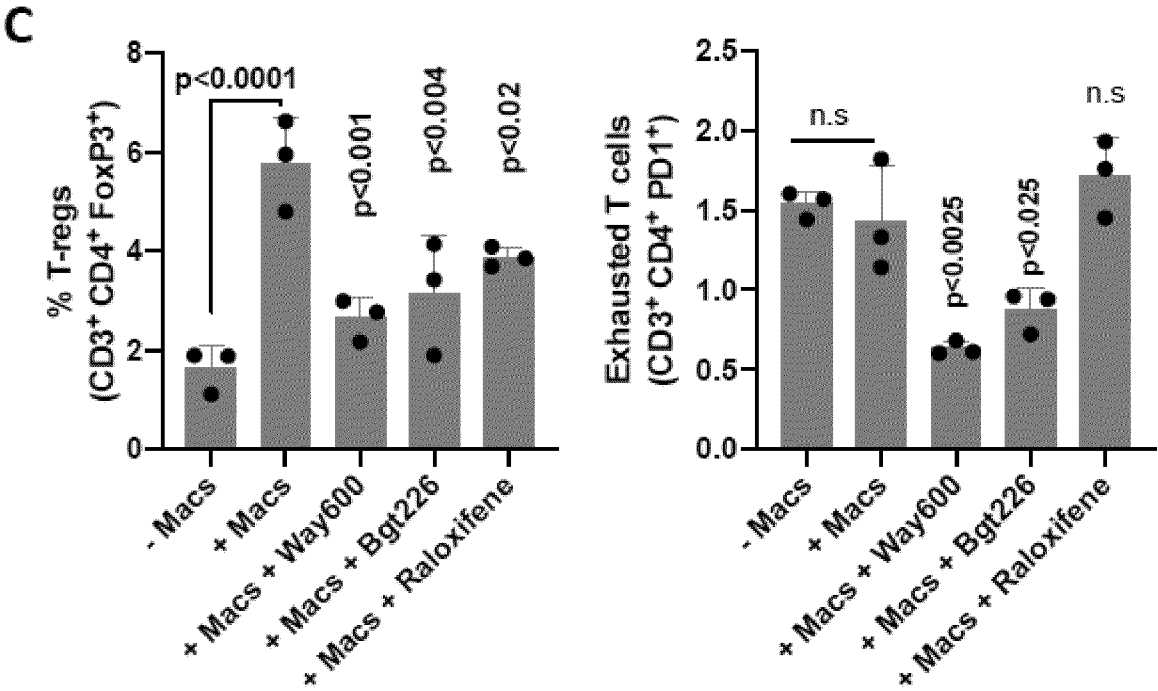


FIG.18 End

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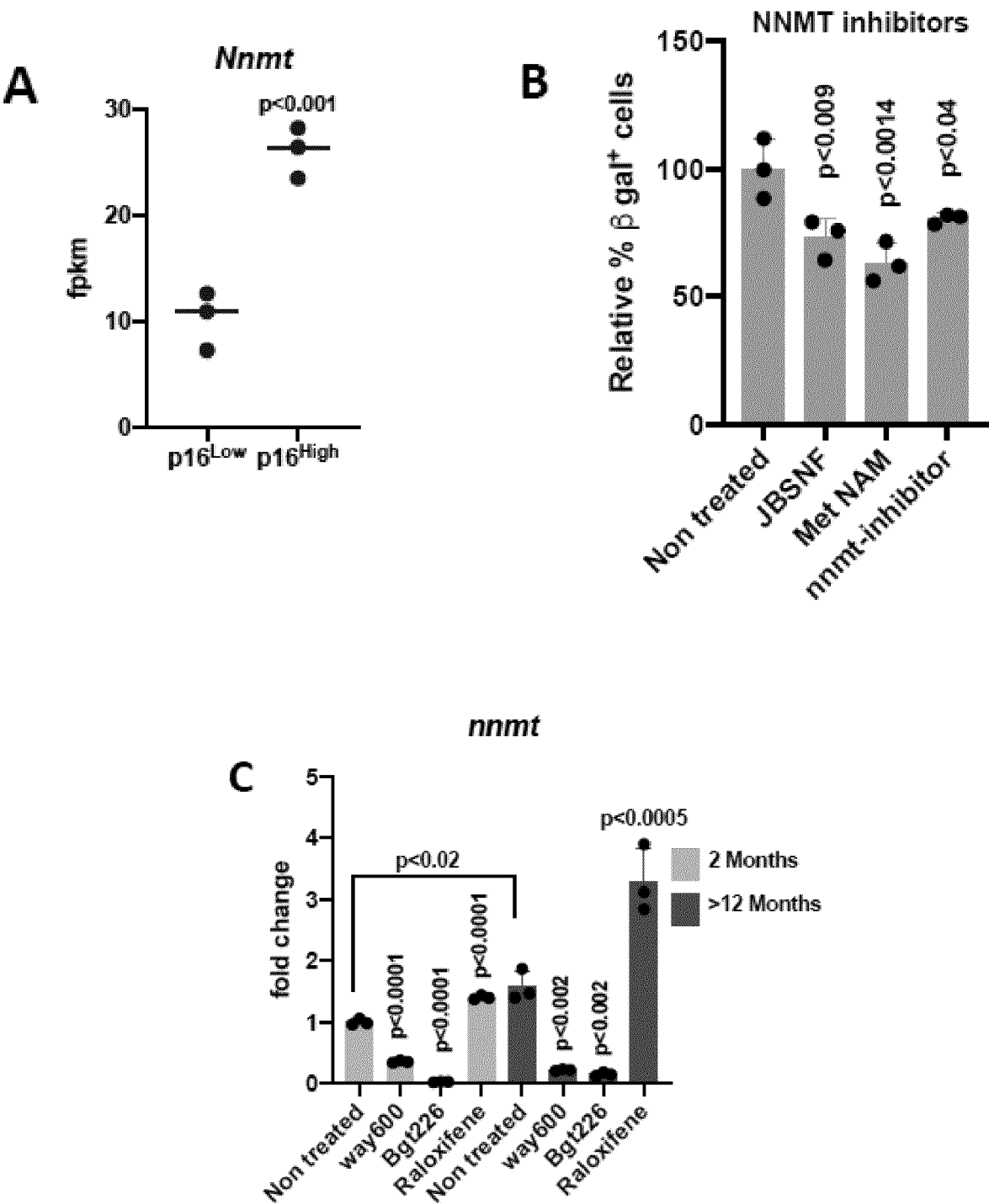


FIG.19

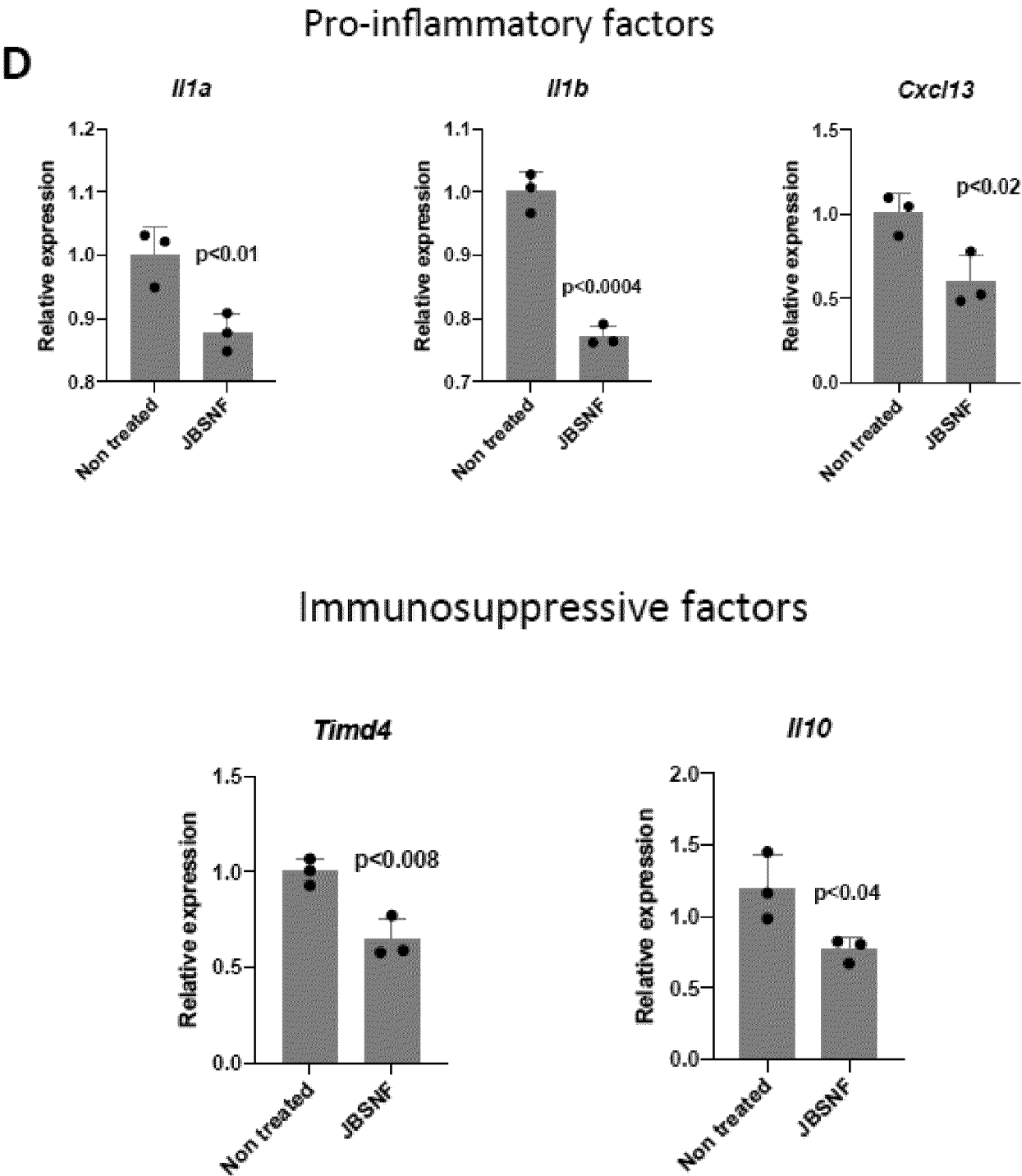


FIG.19 end

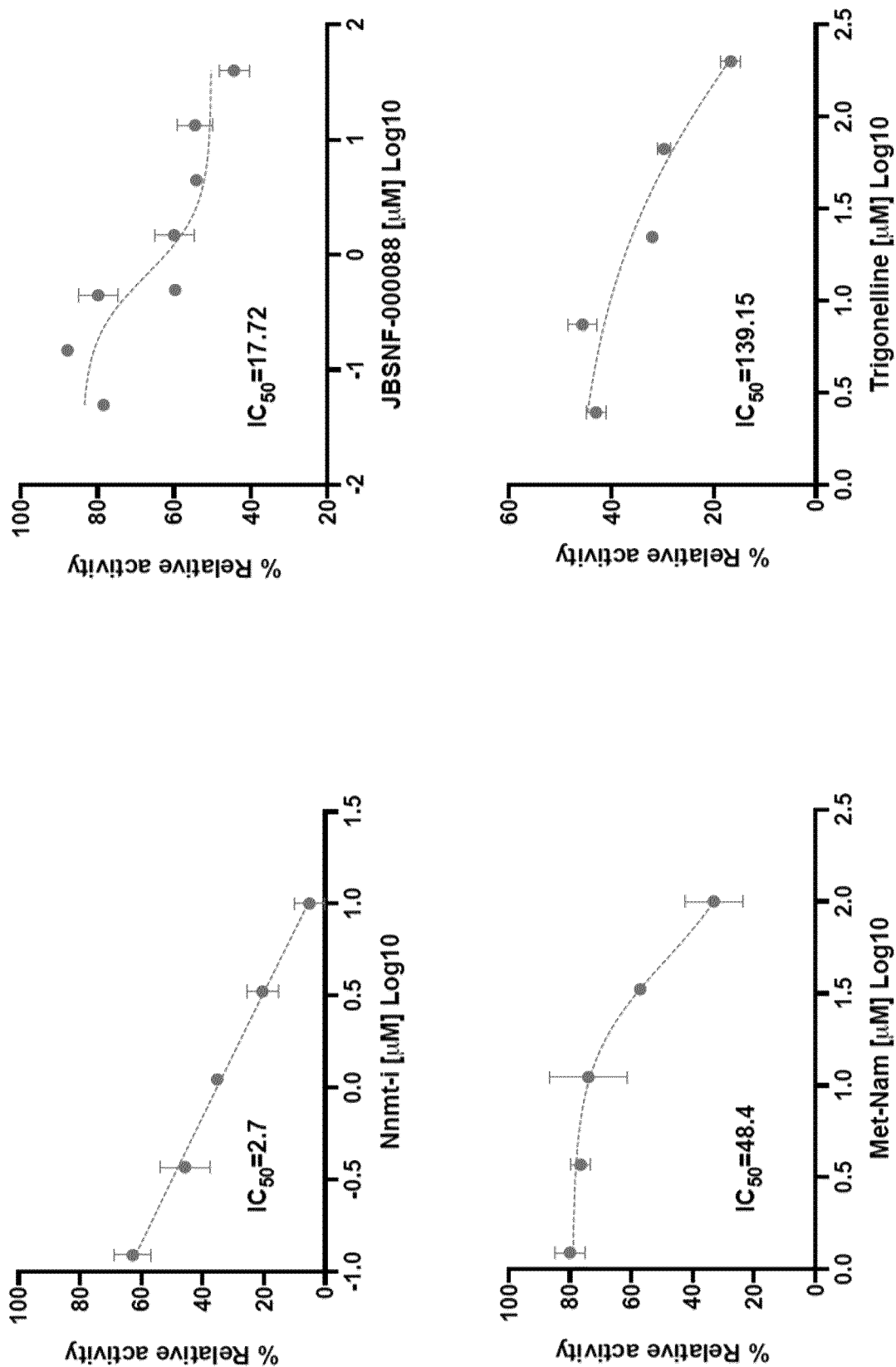


FIG.20

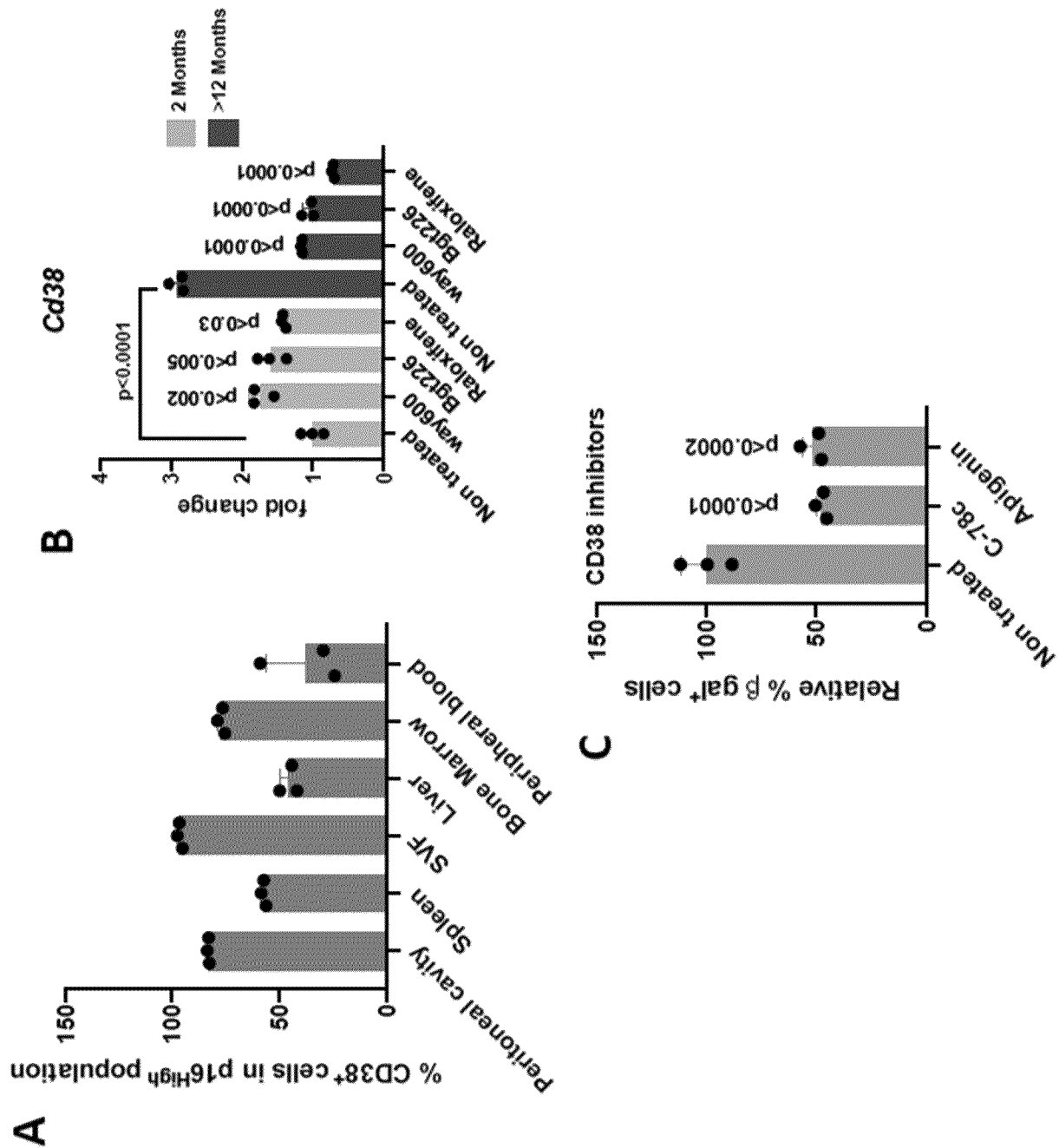


FIG.21

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Pro-inflammatory factors

D

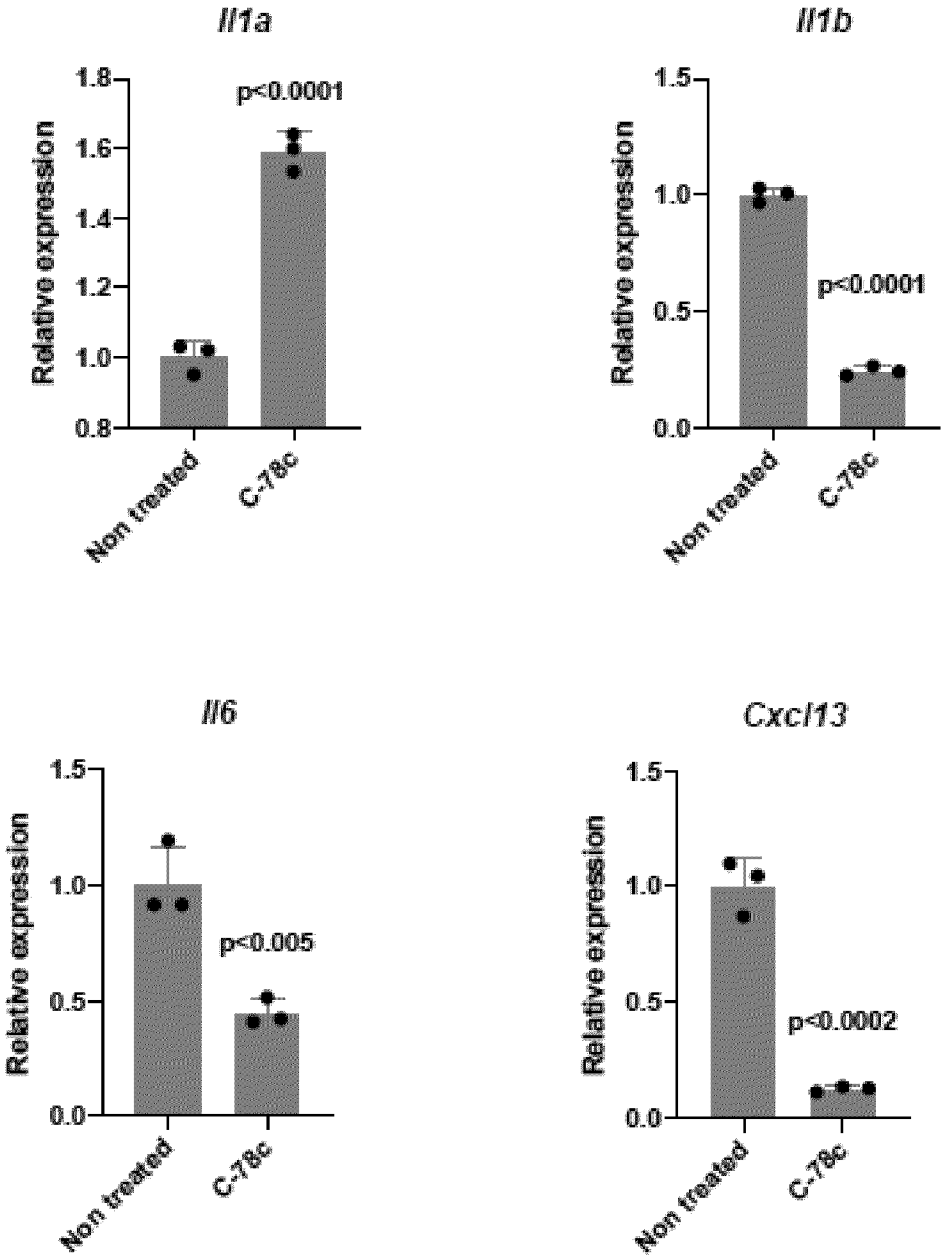


FIG.21 Continued

Immunosuppressive factors

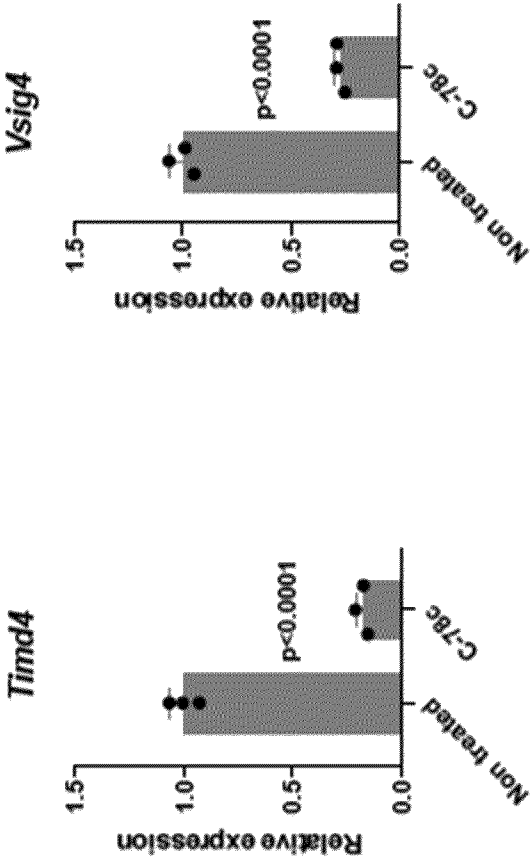
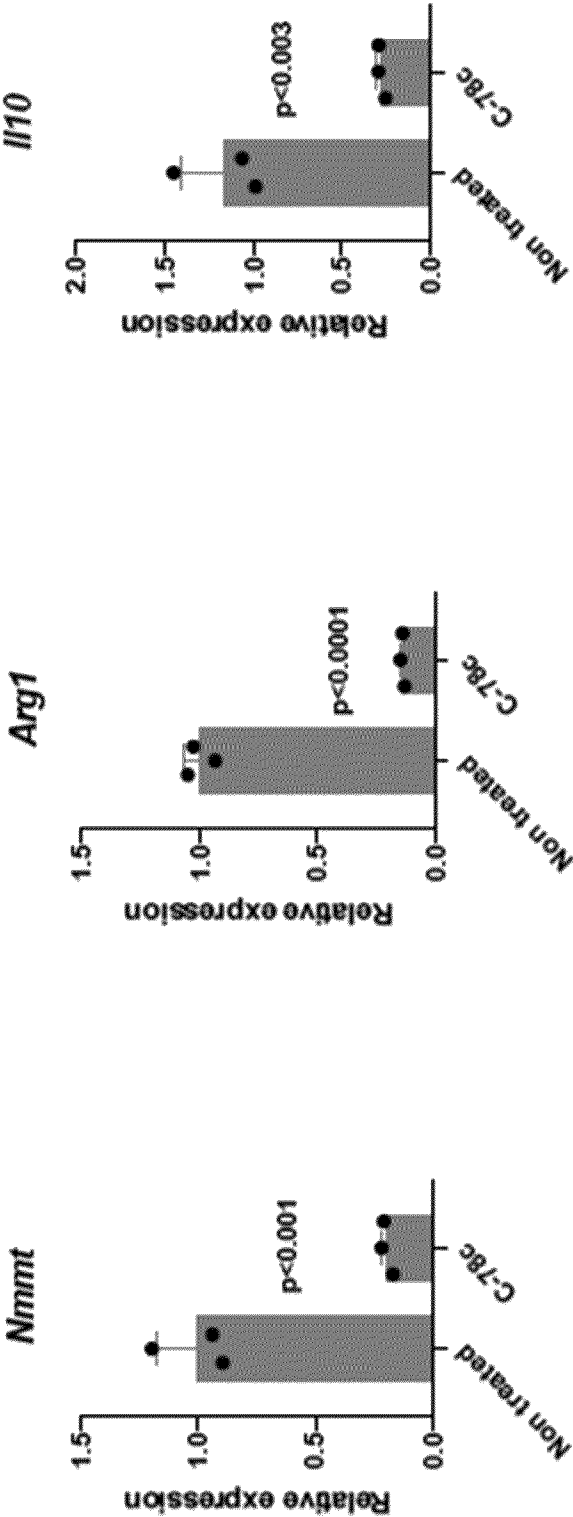


FIG.21 End

International application No
PCT/EP2021/082221

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2021/082221

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 2013/192367 A1 (NOVARTIS AG [CH]; HIRAWAT SAMIT [US]; RIVA ALESSANDRO [US]) 27 December 2013 (2013-12-27)	1-5, 10, 12, 13, 15
Y	claim 1-5, 11; p. 26, l. 1-3 -----	1-13, 15
A	WO 2020/019107 A1 (FOSUN ORINOVE PHARMATECH INC [CN]) 30 January 2020 (2020-01-30) [0001], [0077], [0250], [0060], [0063], [0285], Fig. 18, Fig. 16, example 16, [0294] -----	1-13, 15
X	WO 2016/100882 A1 (NOVARTIS AG [CH]; CAO ZHU ALEXANDER [US]) 23 June 2016 (2016-06-23)	1-5, 10, 12, 13, 15
Y	p. 96, l. 13-24; p. 98, middle; p. 19, last , in particular lines 23-31 -----	1-13, 15
X	WO 2018/237173 A1 (NOVARTIS AG [CH]; CREMASCO VIVIANA [US]) 27 December 2018 (2018-12-27)	1-5, 10, 12, 13, 15
Y	first of summary; p. 116, second ; p. 118, 4th ; p. 144, compound A41; p. 133, last ; p. 150, first -----	1-13, 15
X	US 2015/218274 A1 (SABATOS-PEYTON CATHERINE ANNE [US] ET AL.) 6 August 2015 (2015-08-06)	1-5, 10, 12, 13, 15
Y	[0659] -----	1-13, 15
X	US 2018/371093 A1 (BILIC SANELA [US] ET AL.) 27 December 2018 (2018-12-27)	1-5, 10, 12, 13, 15
Y	[919], [0828], [1222] -----	1-13, 15
X	CN 107 750 164 A (GENENTECH INC) 2 March 2018 (2018-03-02)	1-5, 10, 12, 13, 15
Y	[0634], [0006]; p. 1, first -----	1-13, 15
X	US 2018/043011 A1 (MCNEEL DOUGLAS G [US] ET AL.) 15 February 2018 (2018-02-15)	1-5, 10, 12, 13, 15
Y	claims 1, 14, 23; claim 24, [0041], [0023], [0011] -----	1-13, 15
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International application No

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X	DATABASE Embase [Online] 1 January 2019 (2019-01-01), Mannick J ET AL: "Oral TORC1 inhibitor dactolisib enhances immune function and reduces the incidence of respiratory tract infections in elderly subjects with asthma", XP055798976, Database accession no. EMB-630351882	1, 2, 6, 7, 10, 12, 13
Y	abstract	1-13, 15
X	----- WO 2012/118978 A1 (UNIV COLORADO REGENTS [US]; ONCOTHYREON INC [US] ET AL.) 7 September 2012 (2012-09-07)	1-5, 10, 12, 13
Y	claims 1-2, 6-7	1-13, 15
X	----- WO 2013/147649 A2 (KHOLIN MAXIM NIKOLAEVICH [RU]; FEDICHEV PETR OLEGOVICH [RU] ET AL.) 3 October 2013 (2013-10-03) [0246], claims 1-2, 7	1-4, 6, 7, 9, 10, 13
X	----- WO 2014/137946 A1 (JACKSON H M FOUND MILITARY MED [US]) 12 September 2014 (2014-09-12)	1-5, 10-13
Y	claims 1, 11; [0046]	1-13, 15
X	----- WO 2012/103524 A2 (UNIV PRINCETON [US]; KADMOR CORP LLC [US] ET AL.) 2 August 2012 (2012-08-02)	1-4, 6, 7, 10, 12, 13
Y	[0007], [0052]-0053; 6, 304; claims	1-13, 15
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Y,P	title, abstract	1-13
	----- -/--	

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2021/082221

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHENG YUNFENG ET AL: "Immunoregulation with mTOR inhibitors to prevent COVID-19 severity: A novel intervention strategy beyond vaccines and specific antiviral medicines", JOURNAL OF MEDICAL VIROLOGY , vol. 92, no. 9 1 September 2020 (2020-09-01), pages 1495-1500, XP055798984, US ISSN: 0146-6615, DOI: 10.1002/jmv.26009 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7272823/pdf/JMV-9999-na.pdf title, abstract; Fig. 1; p. 4 -----	1-4, 6-13
X	HUSAIN AMJAD ET AL: "Rapamycin as a potential repurpose drug candidate for the treatment of COVID-19", CHEMICO-BIOLOGICAL INTERACTIONS, ELSEVIER SCIENCE IRLAND, IR, vol. 331, 6 October 2020 (2020-10-06), XP086316596, ISSN: 0009-2797, DOI: 10.1016/J.CBI.2020.109282 [retrieved on 2020-10-06] title, abstract; p. 4, first -----	1-4, 6-13
X	LIAO CHEN-YU ET AL: "Rapamycin Reverses Metabolic Deficits in Lamin A/C-Deficient Mice", CELL REPORTS, vol. 17, no. 10, 1 December 2016 (2016-12-01), pages 2542-2552, XP055798263, US ISSN: 2211-1247, DOI: 10.1016/j.celrep.2016.10.040 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6594831/pdf/nihms-824881.pdf> title, abstract, Fig. on p. 2 ----- -/--	1-4, 6-13

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2021/082221

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Anonymous >: "Asana BioSciences Announces Positive Topline Results from Phase 2b Study of Oral JAK/SYK JAK Inhibitor Gusacitinib (ASN002) in Patients with Chronic Hand Eczema: Rapid and Significant Improvement Demonstrated 12:00 UTC", , 2 June 2020 (2020-06-02), XP055891391, Retrieved from the Internet: URL:https://www.biospace.com/article/releases/asana-biosciences-announces-positive-topline-results-from-phase-2b-study-of-oral-jak-syk-inhibitor-gusacitinib-asn002-in-patients-with-chronic-hand-eczema-rapid-and-significant-improvement-demonstrated-/ [retrieved on 2022-02-14] p. 3, last -----	1-13, 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2021/082221

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.:
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:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1-13, 15 (all partially)

Remark on Protest

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

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13, 15(all partially)

directed to a dual inhibitor of mTOR and PI3K for use in treating a disease associated with macrophage senescence and/or cancer, wherein the dual inhibitor is BGT226 or its salts or dactolisib.

2. claims: 1-13, 15(all partially)

directed to a dual inhibitor of mTOR and PI3K for use in treating a disease associated with macrophage senescence and/or cancer, wherein the dual inhibitor is WAY-600 or PP121.

3. claims: 1-13, 15(all partially)

directed to a dual inhibitor of mTOR and PI3K for use in treating a disease associated with macrophage senescence and/or cancer, wherein the dual inhibitor is gedatolisib or bimiralisib.

4. claims: 1-13, 15(all partially)

directed to a dual inhibitor of mTOR and PI3K for use in treating a disease associated with macrophage senescence and/or cancer, wherein the dual inhibitor is voxalisib.

5. claims: 14(completely); 1-13, 15(partially)

directed to an NNMT inhibitor, including methylated nicotinamide for use in treating a disease associated with macrophage senescence and/or cancer.

6. claims: 1-13, 15(all partially)

directed to a JAK2 inhibitor for use in treating a disease associated with macrophage senescence or cancer, wherein the JAK2 inhibitor is Ruxolitinib

7. claims: 1-13, 15(all partially)

directed to a JAK2 inhibitor for use in treating a disease associated with macrophage senescence, wherein the JAK2 inhibitor is Pacritinib.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. claims: 1-13, 15(all partially)

directed to a CD38 inhibitor for use in treating a disease associated with macrophage senescence and/or cancer.

9. claims: 1-12, 15(all partially)

directed to an inhibitor of the enzyme ataxia telangiectasia and Rad3-related, for use in treating a disease associated with macrophage senescence and/or cancer.

10. claims: 1-13, 15(all partially)

directed to a AOX inhibitors for use in treating a disease associated with macrophage senescence and/or cancer, wherein the AOX inhibitor is tamoxifen.

11. claims: 1-13, 15(all partially)

directed to a AOX inhibitors for use in treating a disease associated with macrophage senescence and/or cancer, wherein the AOX inhibitor is 4-hydroxytamoxifen or clomifen.

12. claims: 1-13, 15(all partially)

directed to a AOX inhibitors for use in treating a disease associated with macrophage senescence and/or cancer, wherein the AOX inhibitor is raloxifen or bazedoxifen

13. claims: 1-12, 15(all partially)

directed to an anesthetic agent for use in treating a disease associated with macrophage senescence and/or cancer.

14. claims: 1-12, 15(all partially)

directed to an antagonist of the histamine H1 receptor for use in treating a disease associated with macrophage senescence and/or cancer.

15. claims: 1-12, 15(all partially)

directed to a pleuromutilin and its derivatives for use in treating a disease associated with macrophage senescence and/or cancer.

16. claims: 1-12, 15(all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

directed to a topical antiseptic cation for use in treating
a disease associated with macrophage senescence and/or
cancer.

17. claims: 1-12, 15(all partially)

directed to a topical antiseptic cation for use in treating
a disease associated with macrophage senescence and/or
cancer.

18. claims: 1-12, 15(all partially)

directed to any further compound covered by the claims, i.e.
a compound not covered by inventions I to XVII for use in
treating a disease associated with macrophage senescence
and/or cancer.

INTERNATIONAL SEARCH REPORT

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International application No

PCT/EP2021/082221

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