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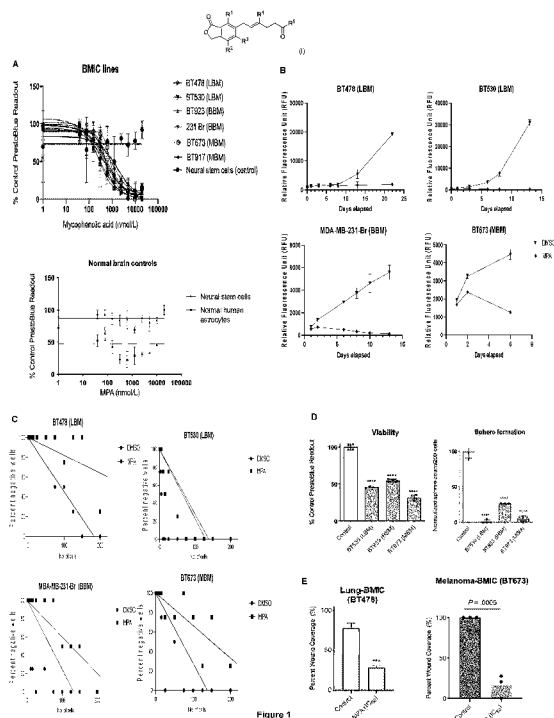
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(54) Title: INOSINE MONOPHOSPHATE DEHYDROGENASE (IMPDH) INHIBITORS FOR THE TREATMENT AND PREVEN-
TION OF BRAIN METASTASIS OF A CANCER

(57) Abstract: The present application relates to inosine monophosphate dehydrogenase (IMPDH) inhibitors for treating or preventing brain metastasis of a cancer. For example, the IMPDH inhibitors are compounds of Formula I.

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TITLE: INOSINE MONOPHOSPHATE DEHYDROGENASE (IMPDH) INHIBITORS FOR
THE TREATMENT AND PREVENTION OF BRAIN METASTASIS OF A CANCER

RELATED APPLICATIONS

[0001] The present application claims the benefit of priority of co-pending United States provisional patent application no. 63/471,558 filed on June 7, 2023 the contents of which are incorporated herein by reference in their entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] A computer readable form of the Sequence Listing "3244-P70574PC00__SequenceListing" (3,753 bytes) was created on June 4, 2024, is filed herewith by electronic submission and is incorporated by reference herein.

FIELD

[0003] The present application relates to inosine monophosphate dehydrogenase (IMPDH) inhibitors for treating or preventing brain metastasis of a cancer. For example, the IMPDH inhibitors are small molecules, such as mycophenolate acid derivatives.

[0004] Brain metastases (BM) are ten times more frequent than primary brain tumors (*Cancer Treat. Rev.* 2003; 29(6):533-540) and patients diagnosed with BM face a 90% mortality rate within 4-12 months of their diagnosis (*Brain Metastases* Vol 5.; 2011). Lung cancer, breast cancer, and melanoma account for 85% of the primary cancers that metastasize to the brain (*Cancer Treat. Rev.* 2003; 29(6):533-540). While the current standard of care treatment for BM comprises of surgical resection and/or radiation therapy, such therapeutic strategies are palliative, and BM remains ostensibly incurable. Moreover, the incidence of BM is increasing due to better systemic treatment options for primary cancers. While significant progress has been made in understanding the genetic landscape (*Front. Oncol.* 2017; 7:230; *Cancer Discov.* 5(11):1164-1177; & *Nat. Genet.* March 2020: 1-7) in secondary brain tumor formation, there remains a lack of clinically relevant models that can identify therapeutically suitable targets for the treatment of BM.

[0005] The bulk of cells within a primary tumor vary in their proliferative, differentiation, and self-renewal capacities, as well as their metastatic capacity. Only 0.01% of metastasizing primary tumor cells are capable of initiating and sustaining a secondary tumor (*Am. J. Pathol.* 1998;153(3):865-873). This cell population is theorized to have inherent stem-like and tumor-initiating properties that drive malignant tumor progression and contribute to drug resistance and relapse (*J. Biomed. Sci.* 2018;25(20):1-18; *Cell Cycle* 2008; 7(2); *J. Cell Biol.* 2012;198(3):281-293 & *Genome Biol.* 2010; 1(5): R52.). A stem-

like cell population, termed brain-metastasis initiating cells (BMICs), was isolated and characterized from patient-derived lung (*Acta. Neuropathol.* 2017;134(6):923-940; & *Cancer Res.* 2018;78(17):5124-5134), breast- and melanoma-BM (Bassey-Archibong et al., *Proc Natl Acad Sci USA* 2023 120(8):e2205247120). BMICs evade conventional therapies and migrate away from their primary tumors to the brain to form BM (*Cancer Res.* 2018; 78(17):5124-5134). Therefore, developing therapeutic strategies to prophylactically eradicate BMICs may be a more effective approach than treating existing BM (*Cell Cycle* 2008; 7(2)).

SUMMARY

[0006] Mycophenolic acid (MPA) and MPA derivatives have been shown to inhibit or block inosine monophosphate dehydrogenase (IMPDH). MPA and MPA derivatives have also been shown to inhibit growth and/or survival of brain metastatic initiating cells (BMICs), and thereby to inhibit the progression of metastatic cancer to the brain, and inhibit brain metastasis.

[0007] The present application includes a method of treating or preventing brain metastasis of a cancer comprising administering a therapeutically effective amount of an inosine monophosphate dehydrogenase (IMPDH) inhibitor to a subject in need thereof.

[0008] The present application also includes a method of inhibiting or delaying brain metastasis of a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor.

[0009] The present application also includes a method of inhibiting brain-metastasis initiating cell (BMIC) activity comprising administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof.

[0010] The present application also includes a method of reducing the risk of brain metastasis of a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof.

[0011] The present application also includes a method of lengthening the period of survival of a subject having a cancer or a subject that has had a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof.

[0012] The present application further includes a method of producing an anti-cancer effect in subject having a cancer or a subject that has had a cancer comprising

administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof.

[0013] In some embodiments, the IMPDH inhibitor is any agent that inhibits expression of IMPDH gene or protein, that induces IMPDH protein degradation or that inhibits IMPDH protein activity.

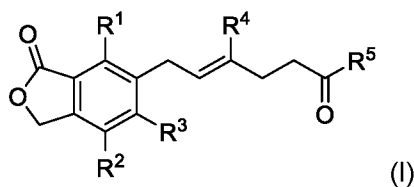
[0014] In some embodiments, the agent that inhibits expression of IMPDH gene or protein and inhibiting expression of IMPDH gene or protein is by IMPDH gene knockdown, IMPDH gene knockout or by IMPDP gene editing.

[0015] In some embodiments, the agent that inhibits expression of IMPDH gene or protein is an antisense oligonucleotide complementary to an IMPDH DNA or RNA sequence or a variant or fragment thereof; and/or a nucleic acid such as a small interfering RNA (siRNA), dicer substrate DNA, hairpin RNA, microRNA (miRNA), RNAi and splice-regulating oligonucleotides and/or gene editing system such as a clustered regularly interspaced short palindromic repeat (CRISPR)-Cas system (CRISPR system); zinc finger nuclease (ZFN) system, or transcription activator-like effector-based nuclease (TALEN) system.

[0016] In some embodiments, the agent inhibits expression of IMPDH protein or inhibits IMPDH protein activity and the agent is an antibody that specifically binds IMPDH or an antigen binding fragment thereof.

[0017] In some embodiments, the agent that that induces IMPDH protein degradation is a targeted IMPDH protein degrader such as IMPDH targeting proteolysis targeting chimera (PROTAC), an IMPDH targeting molecular glue degrader, a selective estrogen receptor degrader (SERD), an IMPDH targeting monoclonal antibody or an IMPDH targeting antibody-drug conjugate.

[0018] In some embodiments, the IMPDH inhibitor is a small molecule inhibitor of IMPDH protein activity such as a compound of Formula I or a pharmaceutically acceptable salt, prodrug and/or solvate thereof,



wherein

R¹ is selected from OH, halo, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R² is selected from H, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R³ is selected from H, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R⁴ is selected from H, C₁₋₄alkyl and C₁₋₄haloalkyl;

R⁵ is selected from R⁶, OR⁶, NR⁶R⁷, OC₁₋₆alkenyleneNR⁶R⁷ and NR⁸C₁₋₆alkenyleneNR⁶R⁷;

R⁶ is selected from H, C₁₋₆alkyl, C₁₋₆haloalkyl, C₃₋₁₀cycloalkyl, C₃₋₁₀heterocycloalkyl, C₁₋₆alkyleneC₃₋₁₀cycloalkyl, and C₁₋₆alkyleneC₃₋₁₀heterocycloalkyl, the latter six groups being optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR⁹R¹⁰, OR⁹, CO₂R⁹ and C(O)NR⁹R¹⁰;

R⁷ is selected H, C₁₋₆alkyl and C₁₋₆haloalkyl; or

R⁶ and R⁷ are joined to form, together with the nitrogen atom therebetween, a 3- to 12-membered heterocycloalkyl optionally containing one or two additional heteromoiety selected from N, NR¹¹, O, S, S(O), and SO₂ and optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR¹²R¹³, OR¹², CO₂R¹² and C(O)NR¹²R¹³;

R⁸, R⁹ and R¹⁰ are independently selected H, C₁₋₆alkyl and C₁₋₆haloalkyl;

R¹¹ is selected from H, C₁₋₆alkyl, C₁₋₆haloalkyl, CO₂C₁₋₆alkyl and CO₂C₁₋₆haloalkyl; and

R¹² and R¹³ are independently selected H, C₁₋₆alkyl and C₁₋₆haloalkyl.

[0019] Other features and advantages of the present application will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating embodiments of the application, are given by way of illustration only and the scope of the claims should not be limited by these embodiments, but should be given the broadest interpretation consistent with the description as a whole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The present application will be described in greater detail with reference to the attached drawings and Tables in which:

[0021] Figure 1 are graphs showing (A) the exemplary compound MPA inhibits the cell viability of multiple patient-derived lung-BM (LBM), breast-BM (BBM), and melanoma-BM (MBM) cells while not effecting normal neural stem cells or normal human astrocytes at the same concentrations. (B) the assessment of cell viability of patient derived BMICs

treated with exemplary compound MPA (I-7) or its vehicle. PrestoBlue® readout is normalized to vehicle-treated cells, $P < 0.0001$. (C) Limiting dilution analysis regression curves of patient-derived BMICs after a 6-day treatment with exemplary compound MPA (I-7) or its vehicle: plotted using GraphPad Prism. (D) Representative bar graphs depicting the normalized fluorescence unit and normalized number sphere count per 200 cells with MPA treatment at IC_{80} vs. vehicle control. (E) MPA slows lung- and melanoma- brain-metastasis initiating cells (BMIC) migration in a wound healing assay. Data is expressed as an average of replicates ($n=4$).

[0022] Figure 2 shows that exemplary compound MPA slows BM progression in mice following a short exposure to BMICs prior to engraftment. (A) Shows the timeline where BMICs or primary lung cancer cells were treated with MPA *ex vivo* for four days followed by either intracranial or intrathoracic engraftment into immunocompromised mice, respectively. CRUK0748 lung tumor cells were treated with MPA at IC_{80} for four days *ex vivo* prior to being orthotopically engrafted into the thoracic cavity of mic. (B and D) Mice injected with MPA-treated cells had significantly reduced brain tumor burden. (C and E) Mice injected with MPA-treated cells had significantly increased survival advantage compared to vehicle treated mice. (F) Mice injected with MPA (I-7)-treated cells had a significantly increased survival benefit, (G) fewer human cells detected in their brains by flow cytometry following humane endpoint, compared to mice injected with vehicle control cells and (H) secondary sphere formation assay showed that MPA-treated BMICs do not regain sphere forming capability following MPA removal from the culture media.

[0023] Figure 3 shows patient derived xenograft (PDX) models mimic patient data. CRUK0748, a patient- derived lung tumor cell line that metastasized to the brain of the patient it was retrieved from, was confirmed to metastasize to the brains of mice in animal models following intrathoracic injection. CRUK0733, a patient-derived lung tumor cell line that has not metastasized to the brain of the patient it was retrieved from, did not metastasize in the animal model. In the graph shown in this figure, exemplary compound MPA demonstrates a dose-response anti-proliferative effect on BMICs isolated from the brains of mice following CRUK0748 metastasis to the brain.

[0024] Figure 4 shows that when the exemplary compound MPA crosses the blood brain barrier the progression of BM is effectively slowed over time. (A) Shows the timeline where mice were either orthotopically (intrathoracically) or intracardiac injected with lung BMICs, followed by daily oral gavage administration of MPA (100 mg/kg) or placebo for 14 days. (B) Following intracardiac-injection and subsequent treatment, mice treated with MPA

showed a significant decrease in brain tumor burden seven days post-injection (left panel), no significant reduction in tumor burden following treatment completion (middle panel), and did not experience a survival benefit compared to placebo (right panel). (C) Following orthotopic injection and subsequent treatment as described in (A), mice treated with MPA showed a significant increase in survival compared to placebo (left panel), but had the same number of human cells detected in their brains following humane endpoint (right panel). n.s. = not significant.

[0025] Figure 5 shows increasing the brain penetration of exemplary compounds enhances anti-BM phenotype *in vivo*. (A) exemplary compound MPA (I-7) and analogue exemplary compound I-3 dose-response curves against a lung-BMIC line (BT478), breast-BMIC line (MBA-MD-231 Br), melanoma-BMIC line (BT673) and normal brain controls. Exemplary compound I-3 inhibits lung-, breast-, and melanoma-BMICs similarly to MPA. (B) Brain penetrance was evaluated *in vitro* using the PAMPA assay. PAMPA scored > 6 indicate low CNS permeability, whereas PAMPA scores <6 indicate high CNS permeability. Caffeine was used as a BBB-permeable control. (C) An intracranial *in vivo* model with oral gavage treatment demonstrated (D) that treatment with exemplary compound I-3 (brain-penetrant) had an increased survival benefit relative to MPA (non-brain-penetrant) and placebo.

[0026] Figure 6 shows the exemplary IMPDH inhibitor compounds of the application target the *de novo* GTP synthesis pathway in BMICs. (A) Schematic of the *de novo* GTP synthesis pathway, illustrating that IMPDH is not involved in the salvage GTP synthesis pathway. (B) GDP and GTP levels are significantly higher in BMIC lines compared to normal human astrocytes (NHA) and reduced with both exemplary compound MPA and exemplary compound I-3-treatment. N.s. = not significant, **** = $p < 0.0001$. (C) AICAR levels are significantly higher when IMPDH is pharmacologically inhibited. (D) Exogenous guanine supplementation into cell culture media (12 μ M) rescues BMICs from exemplary compound MPA and exemplary compound I-3 inhibition. (E-F) CRISPR knockout IMPDH stops lung-BMIC proliferation and (G) sphere formation.

[0027] Figure 7 are graphs showing mechanistic experiments. (A) Exemplary compound MPA inhibits BMIC viability similarly to merimepodib, a selective-IMPDH inhibitor. (B) Exemplary MPA and compound I-3 treatment did not influence the levels of dihydroorotic acid, a key metabolite in pyrimidine synthesis.

DETAILED DESCRIPTION

I. Definitions

[0028] Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present application herein described for which they are suitable as would be understood by a person skilled in the art.

[0029] All features disclosed in the specification, including the claims, abstract, and drawings, and all the steps in any method or process disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. Each feature disclosed in the specification, including the claims, abstract, and drawings, can be replaced by alternative features serving the same, equivalent, or similar purpose, unless expressly stated otherwise.

[0030] The term “compound of the application” or “compound of the present application” and the like as used herein refers to any inosine monophosphate dehydrogenase (IMPDH) inhibitor, including compounds of Formula I and including pharmaceutically acceptable salts, solvates and/or prodrugs thereof.

[0031] The term “composition of the application” or “composition of the present application” and the like as used herein refers to a composition comprising a compound of the application and at least one additional ingredient.

[0032] The term “and/or” as used herein means that the listed items are present, or used, individually or in combination. In effect, this term means that “at least one of” or “one or more” of the listed items is used or present. The term “and/or” with respect to pharmaceutically acceptable salts and/or solvates thereof means that the compounds of the application exist as individual salts and hydrates, as well as a combination of, for example, a solvate of a salt of a compound of the application.

[0033] As used in the present application, the singular forms “a”, “an” and “the” include plural references unless the content clearly dictates otherwise. For example, an embodiment including “a compound” should be understood to present certain aspects with one compound, or two or more additional compounds.

[0034] In embodiments comprising an “additional” or “second” component, such as an additional or second compound, the second component as used herein is chemically different from the other components or first component. A “third” component is different from the other, first, and second components, and further enumerated or “additional” components are similarly different.

[0035] As used herein, the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “include” and “includes”) or “containing” (and any form of containing, such as “contain” and “contains”), are inclusive or open-ended and do not exclude additional, unrecited elements or process/method steps.

[0036] As used herein, the word “consisting” and its derivatives, are intended to be close ended terms that specify the presence of stated features, elements, components, groups, integers, and/or steps, and also exclude the presence of other unstated features, elements, components, groups, integers and/or steps.

[0037] The term “consisting essentially of”, as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of these features, elements, components, groups, integers, and/or steps.

[0038] Terms of degree such as “substantially”, “about” and “approximately” as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least $\pm 5\%$ of the modified term if this deviation would not negate the meaning of the word it modifies.

[0039] The term “suitable” as used herein means that the selection of the particular compound or conditions would depend on the specific synthetic manipulation to be performed, the identity of the molecule(s) to be transformed and/or the specific use for the compound, but the selection would be well within the skill of a person trained in the art. All process/method steps described herein are to be conducted under conditions sufficient to provide the product shown. A person skilled in the art would understand that all reaction conditions, including, for example, reaction solvent, reaction time, reaction temperature, reaction pressure, reactant ratio and whether or not the reaction should be performed under an anhydrous or inert atmosphere, can be varied to optimize the yield of the desired product and it is within their skill to do so.

[0040] The present application refers to a number of chemical terms and abbreviations used by those skilled in the art. Nevertheless, definitions of selected terms are provided for clarity and consistency.

[0041] The term “protecting group” or “PG” and the like as used herein refers to a chemical moiety which protects or masks a reactive portion of a molecule to prevent side

reactions in those reactive portions of the molecule, while manipulating or reacting a different portion of the molecule. After the manipulation or reaction is complete, the protecting group is removed under conditions that do not degrade or decompose the remaining portions of the molecule. The selection of a suitable protecting group can be made by a person skilled in the art. Many conventional protecting groups are known in the art, for example as described in "Protective Groups in Organic Chemistry" McOmie, J.F.W. Ed., Plenum Press, 1973, in Greene, T.W. and Wuts, P.G.M., "Protective Groups in Organic Synthesis", John Wiley & Sons, 3rd Edition, 1999 and in Kocienski, P. Protecting Groups, 3rd Edition, 2003, Georg Thieme Verlag (The Americas).

[0042] The term "cell" as used herein refers to a single cell or a plurality of cells and includes a cell either in a cell culture or in a subject.

[0043] The term "subject" as used herein includes all members of the animal kingdom including mammals. Thus, the methods and uses of the present application are applicable to both human therapy and veterinary applications.

[0044] The term "pharmaceutically acceptable" means compatible with the treatment of subjects.

[0045] The term "pharmaceutically acceptable carrier" means a non-toxic solvent, dispersant, excipient, adjuvant or other material which is mixed with an active ingredient (for example, a compound of the application) to permit the formation of a pharmaceutical composition, i.e., a dosage form capable of administration to a subject.

[0046] The term "pharmaceutically acceptable salt" means either an acid addition salt or a base addition salt which is suitable for, or compatible with the treatment of subjects.

[0047] An acid addition salt suitable for, or compatible with, the treatment of subjects is any non-toxic organic or inorganic acid addition salt of any basic compound.

[0048] A base addition salt suitable for, or compatible with, the treatment of subjects is any non-toxic organic or inorganic base addition salt of any acidic compound.

[0049] The term "prodrug" as used herein means a compound, or salt and/or solvate of a compound, that, after administration, is converted into an active drug.

[0050] The term "solvate" as used herein means a compound, or a salt or prodrug of a compound, wherein molecules of a suitable solvent are incorporated in the crystal lattice.

[0051] The term “inert organic solvent” as used herein refers to a solvent that is generally considered as non-reactive with the functional groups that are present in the compounds to be combined together in any given reaction so that it does not interfere with or inhibit the desired synthetic transformation. Organic solvents are typically non-polar and dissolve compounds that are nonsoluble in aqueous solutions.

[0052] The term “alkyl” as used herein, whether it is used alone or as part of another group, means straight or branched chain, saturated alkyl groups. The number of carbon atoms that are possible in the referenced alkyl group are indicated by the prefix “C_{n1-n2}”. For example, the term C₁₋₁₀alkyl means an alkyl group having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms.

[0053] The term “alkylene”, whether it is used alone or as part of another group, means straight or branched chain, saturated alkylene group, that is, a saturated carbon chain that contains substituents on two of its ends. The number of carbon atoms that are possible in the referenced alkylene group are indicated by the prefix “C_{n1-n2}”. For example, the term C₁₋₁₀alkylene means an alkylene group having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms. All alkyl groups are optionally fluorosubstituted unless otherwise indicated.

[0054] The term “cycloalkyl,” as used herein, whether it is used alone or as part of another group, refers to cyclic groups containing from 3 to 20 atoms and at least one carbocyclic non aromatic ring. Cycloalkyl groups are either saturated or unsaturated (i.e., contain one or more double bonds). The number of carbon atoms that are possible in the referenced cycloalkyl group are indicated by the numerical prefix “C_{n1-n2}”. For example, the term C₃₋₁₀cycloalkyl means a cycloalkyl group having 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms..

[0055] The term “heterocycloalkyl” as used herein, whether it is used alone or as part of another group, refers to cyclic groups containing at least one non-aromatic ring containing from 3 to 20 atoms in which one or more of the atoms are a heteroatom selected from O, S, SO, SO₂, N, NH and substituted N and the remaining atoms are C. Heterocycloalkyl groups are either saturated or unsaturated (i.e., contain one or more double bonds). When a heterocycloalkyl group contains the prefix C_{n1-n2} this prefix indicates the number of carbon atoms in the corresponding carbocyclic group, in which one or more, suitably 1 to 5, of the ring atoms is replaced with a heteroatom as selected from O, S, SO, SO₂, N, NH and substituted N and the remaining atoms are C. Heterocycloalkyl groups are optionally benzofused. When part of another group, “heterocycloalkyl” also refers to cyclic groups containing at least one heterocycloalkyl group fused to one or more

cyclic groups (e.g. heterocycloalkyl groups are optionally fused to aryl, heteroaryl, heterocycloalkyl and cycloalkyl groups as defined herein).

[0056] The term “aryl” as used herein, whether it is used alone or as part of another group, refers to cyclic groups containing from 6 to 20 atoms and at least one carbocyclic aromatic ring.

[0057] The term “heteroaryl” as used herein, whether it is used alone or as part of another group, refers to cyclic groups containing at least one heteroaromatic ring containing 5-20 atoms in which one or more of the atoms are a heteroatom selected from O, S, N, NH and substituted N and the remaining atoms are C. When a heteroaryl group contains the prefix C_{n1-n2} this prefix indicates the number of carbon atoms in the corresponding carbocyclic group, in which one or more, suitably 1 to 5, of the ring atoms is replaced with a heteroatom as defined above. Heteroaryl groups are optionally benzofused.

[0058] All cyclic groups, including aryl, heteroaryl, heterocyclo and cycloalkyl groups, contain one (i.e. are monocyclic) or more than one ring (i.e. are polycyclic). When a cyclic group contains more than one ring, the rings may be fused, bridged or spirofused.

[0059] The term “benzofused” as used herein refers to a polycyclic group in which a benzene ring is fused with another ring.

[0060] A first ring being “fused” with a second ring means the first ring and the second ring share two adjacent atoms there between.

[0061] A first ring being “bridged” with a second ring means the first ring and the second ring share two non-adjacent atoms there between.

[0062] A first ring being “spirofused” with a second ring means the first ring and the second ring share one atom there between.

[0063] The terms “halo” or “halogen” as used herein, whether it is used alone or as part of another group, refers to a halogen atom and includes fluoro, chloro, bromo and iodo.

[0064] The term “haloalkyl” as used herein refers to an alkyl group as defined above in which one or more of the available hydrogen atoms have been replaced with a halogen atom. Thus, for example, “ C_{1-6} haloalkyl” (or “ C_1 - C_6 haloalkyl”) refers to a C_1 to C_6 linear or branched alkyl group as defined above with one or more halogen substituents.

[0065] The term “chloroalkyl” as used herein refers to an haloalkyl group as defined above wherein the halogen atom is chloro.

[0066] The term “fluoroalkyl” as used herein refers to an haloalkyl group as defined above wherein the halogen atom is fluoro.

[0067] As used herein, the term “alkoxy” as used herein, alone or in combination, includes an alkyl group connected to an oxygen connecting atom.

[0068] The term “haloalkoxy” as used herein refers to an alkoxy group as defined above in which one or more of the available hydrogen atoms have been replaced with a halogen atom. Thus, for example, “C₁₋₆ haloalkoxy” (or “C₁-C₆ haloalkoxy”) refers to a C₁ to C₆ linear or branched alkoxy group as defined above with one or more halogen substituents.

[0069] The term “available”, as in “available hydrogen atoms” or “available atoms” refers to atoms that would be known to a person skilled in the art to be capable of replacement by another atom or group.

[0070] The term “optionally substituted” as used herein means that the referenced group is unsubstituted or substituted.

[0071] When a group is substituted with one or more substituents, it understood that the selection of those substituents is independent of each other. That is, the one or more substituents may be the same or different.

[0072] The symbol  when drawn perpendicularly across a bond indicates a point of covalent attachment of a chemical group.

[0073] The term “treating” or “treatment” as used herein and as is well understood in the art, means 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 a disease, disorder or condition, stabilized (i.e. not worsening) state of a disease, disorder or condition, preventing spread of a disease, disorder or condition, delay or slowing of a disease, disorder or condition progression, amelioration or palliation of a disease, disorder or condition state, diminishment of the reoccurrence of a disease, disorder or condition, and remission (whether partial or total), whether detectable or undetectable. “Treating” and “treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. “Treating” and “treatment” as used herein also include prophylactic treatment. For example, a subject with cancer is treated to prevent progression, or alternatively a subject who has had cancer is treated to prevent recurrence.

[0074] "Palliating" a disease, disorder or condition means that the extent and/or undesirable clinical manifestations of the disease, disorder or condition are lessened and/or time course of the progression is slowed or lengthened, as compared to not treating the disease, disorder or condition.

[0075] The term "preventing", "prevention" or "prophylaxis", or synonym thereto, as used herein refers to a reduction in the risk or probability of a subject becoming afflicted with a disease, disorder or condition or manifesting a symptom associated with a disease, disorder or condition, and includes blocking the onset of the disease, disorder or condition, and/or to a reduction in the risk or probability of re-occurrence of the disease, disorder or condition in a subject that has had the disease, disorder or condition.

[0076] The term "disease, disorder or condition" as used herein refers to a disease, disorder or condition that is treated by inhibiting inosine-monophosphate dehydrogenase (IMPDH).

[0077] The term "treated by inhibition of " as used herein means that the disease, disorder or condition to be treated is affected by, modulated by and/or has some biological basis, either direct or indirect, that includes the presence in a cell of inosine-monophosphate dehydrogenase (IMPDH).

[0078] The term "disease, disorder or condition that is treated by inhibition of inosine-monophosphate dehydrogenase (IMPDH)" means that the disease, disorder or condition to be treated is affected by, modulated by and/or has some biological basis, either direct or indirect, that includes IMPDH activity. These diseases respond favourably when IMPDH activity associated with the disease, disorder or condition is inhibited by the compounds or compositions of the application.

[0079] The term "IMPDH inhibitor" or "inhibitor of IMPDH" as used herein means any agent or composition that detectably inhibits, slows and/or disrupts IMPDH activity when in the presence of the agent compared to otherwise the same conditions, except for in the absence in the agent.

[0080] As used herein, the term "effective amount" or "therapeutically effective amount" means an amount of a compound or any agent that is an IMPDH inhibitor, or one or more compounds or any agents that are IMPDH inhibitors, or a pharmaceutically acceptable salt, prodrug and/or solvate thereof, or compositions comprising a compound or any agent that is an IMPDH inhibitor or a pharmaceutically acceptable salt, prodrug

and/or solvate thereof, that is effective, at dosages and for periods of time necessary to achieve the desired result.

[0081] By “inhibiting” it is meant any detectable inhibition, slowing and/or disruption in the presence of a compound or any agent that is an IMPDH inhibitor compared to otherwise the same conditions, except for in the absence in the compound.

[0082] By “inhibiting or delaying brain metastasis of a cancer” means any reduction of cancer metastasis in the brain compared to otherwise the same conditions, except for in the absence in the compound or any agent that is an IMPDH inhibitor, and includes slowing in the rate of cancer growth and metastasis.

[0083] The term “delaying brain metastasis” as used herein refers to any delay or slowing of brain metastasis of a cancer, in the presence of a compound or any agent that is an IMPDH inhibitor compared to a control, (for example, otherwise identical conditions except in the absence of the compound or any agent that is an IMPDH inhibitor).

[0084] The term “lengthening the period of survival” as used herein refers to any increase in the survival of a subject having a cancer, or a subject that has had a cancer, in the presence of a compound or any agent that is an IMPDH inhibitor, (for example, otherwise identical conditions except in the absence of the compound or any agent that is an IMPDH inhibitor).

[0085] The term “brain metastasis,” as used herein, refers to cancer cells that have migrated from the original location of the cancer (e.g., primary cancer) to the brain.

[0086] The term “metastasis,” as used herein, refers to the presence of one or more cancer cells at a location that is not physically contiguous with the original location of the cancer (e.g., primary cancer).

[0087] The term “primary cancer” or “primary tumor” as used herein, refers to an original or first cancer or tumor in a subject that is the source of metastasis.

[0088] The term “brain-metastasis initiating cells” or “BMICs” as used herein refers to primary tumor cells capable of initiating and sustaining a metastasis in the brain.

[0089] The term “premetastatic brain-metastasis initiating cells” or “premetastatic BMICs” are BMICs that have not initiated or formed a metastasis in the brain that is detectable.

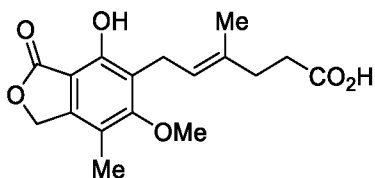
[0090] The term “detectable metastasis” or “metastasis that is detectable” as used herein refers to a cluster of cells that may be identifiable by standard imaging techniques used to identify metastasis.

[0091] The term “anti-cancer effect” as used herein refers to the prevention and/or inhibition of brain metastasis of a cancer in a subject.

[0092] The term “reducing the risk of brain metastasis of a cancer” as used herein refers to any decrease in the risk of formation of brain metastasis of a cancer in the presence of a compound or any agent that is an IMPDH inhibitor compared to a control, (for example, otherwise identical conditions except in the absence of the compound or any agent that is an IMPDH inhibitor).

[0093] The term “administered” as used herein means administration of a therapeutically effective amount of a compound or any agent that is an IMPDH inhibitor, or one or more compounds or any agents that are IMPDH inhibitors, or a composition to a cell or a subject.

[0094] The term “mycophenolic acid” or “MPA” as used herein refers to a compound having the chemical name: (4E)-6-(4-Hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoic acid and having the chemical formula:



[0095] The term “cancer” as used herein refers to a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body (i.e. metastasize).

[0096] The term “PROTAC” as used herein refers to proteolysis targeting chimeras.

[0097] The term “IMPDH” as used herein refers to inosine-monophosphate dehydrogenase.

[0098] The term “LCMS” as used herein refers to liquid chromatography-mass spectrometry.

[0099] The term “NMR” as used herein refers to nuclear magnetic resonance.

- [00100] The term "aq." as used herein refers to aqueous.
- [00101] The term "N" as used herein, for example in "4N", refers to the unit symbol of normality to denote "eq/L".
- [00102] The term "M" as used herein, for example in 4M, refers to the unit symbol of molarity to denote "moles/L".
- [00103] The term "DIPEA" as used herein refers to N,N-diisopropyl ethylamine.
- [00104] The term "DMF" as used herein refers to dimethylformamide.
- [00105] The term "THF" as used herein refers to tetrahydrofuran.
- [00106] The term "DMSO" as used herein refers to dimethylsulfoxide.
- [00107] The term "EtOAc" as used herein refers to ethyl acetate.
- [00108] The term "MeOH" as used herein refers to methanol.
- [00109] The term "EtOH" as used herein refers to ethanol.
- [00110] The term "MeCN" or "ACN" as used herein refers to acetonitrile.
- [00111] The term "HCl" as used herein refers to hydrochloric acid.
- [00112] The term "TFA" as used herein refers to trifluoroacetic acid.
- [00113] The term "Hex" as used herein refers to hexanes.
- [00114] The term "PBS" as used herein refers to phosphate-based buffer.
- [00115] The term "IPA" as used herein refers to isopropyl alcohol.
- [00116] The term "dppf" as used herein refers to 1,1'-bis(diphenylphosphino)ferrocene.
- [00117] The term "RT" as used herein refers to room temperature.
- [00118] The term "HPLC" as used herein refers to high-performance liquid chromatography.
- [00119] The term "PPA" as used herein refers to polyphosphoric acid.
- [00120] The term "TEA" or "Et₃N" as used herein refer to triethylamine.
- [00121] The term "EDTA" as used herein refers to ethylenediaminetetraacetic acid.
- [00122] The term "ATP" as used herein refers to adenosine triphosphate.
- [00123] The term "FBS" as used herein refers to fetal bovine serum.

- [00124] The term “FBF” as used herein refers to fibroblast growth factor.
- [00125] The term “EGF” as used herein refers to epidermal growth factor.
- [00126] The term “BSA” as used herein refers to bovine serum albumin.
- [00127] The term “DMEM” as used herein refers to Dulbecco’s Modified Eagle Medium.
- [00128] The term “MEM” as used herein refers to Minimum Essential Medium.
- [00129] The term “BBB” as used herein refers to blood brain barrier.
- [00130] The term “BM” as used herein refers to brain metastases.
- [00131] The term “MPA” as used herein refers to mycophenolic acid.
- [00132] The term “GTP” as used herein refers to guanosine triphosphate.
- [00133] The term “CMap” as used herein refers to connectivity map.
- [00134] The term “SDS” as used herein refers to “sodium dodecyl sulfate”.
- [00135] The term “PVDF” as used herein refers to “polyvinylidene difluoride”.
- [00136] The term “PAMPA” as used herein refers to “parallel artificial membrane permeability assay”.
- [00137] The term “TBS” as used herein refers to “tris-buffered saline”.
- [00138] The term “TBA” as used herein refers to “tributylamine”.
- [00139] The term “EDC” as used herein refers to 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.
- [00140] The term “HOBt” as used herein refers to “hydroxybenzotriazole”.
- [00141] The term “AICA” as used herein refers to aminoimidazole carboxamide ribonucleotide.
- [00142] The term “UHPLC-MS” as used herein refers to “ultra-high performance liquid chromatography-mass spectrometry”.

II. Methods and Uses of the Application

- [00143] Using established lung-to-brain metastases models (*Acta. Neuropathol.* 2017;134(6):923-940; & *Cancer Res.* 2018;78(17):5124-5134), the Applicant has captured a population of lung- brain metastases initiating cells (BMICs) that are newly arrived in the brain. These lung-BMICs were undetectable by immunochemistry and would not be

expected to be detectable by standard imaging techniques, and termed pre-metastatic BMICs (*Cancer Res.* 2018; 78(17):5124-5134). A Connectivity Map (CMap) computational analysis (*Science* 2006; 313(5795):1929-1935) was applied to the transcriptome of premetastatic lung-BMICs and apomorphine was identified as a candidate compound to block the formation of lung-BM (*Cancer Res.* 2018; 78(17):5124-5134). The model was then expanded to include breast- and melanoma-premetastatic BMICs and a shared gene signature of premetastatic BMICs common to all three cohorts was identified (Bassey-Archibong et al., *Proc Natl Acad Sci USA* 2023 120(8):e2205247120).

[00144] This shared premetastatic BMIC signature was utilized to uncover targetable therapeutic vulnerabilities in lung-, breast-, and melanoma BMICs by applying CMap analysis. The Applicant surprisingly identified mycophenolic acid (MPA), an FDA-approved immunosuppressant and other agents as inhibitors of inosine-monophosphate dehydrogenase (IMPDH), as potent suppressors of BMIC activity.

[00145] Mechanistically, MPA was found to inhibit IMPDH, the rate-limiting enzyme in the de novo GTP synthesis pathway. Further, metabolomic analyses revealed that MPA acts on-target to disrupt de novo GTP synthesis.

[00146] Accordingly, the Applicant has identified IMPDH as a key regulator of cancer metastasis to the brain and purine synthesis as a metabolic vulnerability in BM. The Applicant has shown that genetic perturbation of IMPDH and pharmacological inhibition using IMPDH inhibitors, such as small molecule inhibitors (e.g., compounds of the application) and CRISPR gene knockout studies, prevents BMICs proliferation *in vitro*. Pharmacological inhibition of IMPDH slows BM outgrowth *in vivo* independent of primary cancer type.

[00147] Due to the predicted low blood brain barrier (BBB) permeability of MPA, the Applicant further developed and synthesized a library of BBB permeable derivatives of MPA. The derivatives, for example, exemplary compound I-3 were found to possess *in vivo* efficacy against brain metastasis (BM) compared to MPA and to significantly extend survival. Small molecule IMPDH inhibitors, such as MPA and MPA derivatives have been shown to inhibit or block inosine monophosphate dehydrogenase (IMPDH). MPA and MPA derivatives have also been shown to inhibit growth and/or survival of brain metastatic initiating cells (BMICs), and thereby to inhibit the progression of metastatic cancer to the brain, and inhibit brain metastasis.

[00148] Accordingly, the present application includes a method of treating or preventing brain metastasis of a cancer comprising administering a therapeutically effective

amount of an inosine monophosphate dehydrogenase (IMPDH) inhibitor to a subject in need thereof.

[00149] The present application also includes a use of an IMPDH inhibitor for treating or preventing brain metastasis of a cancer as well as a use of an IMPDH inhibitor for the preparation of a medicament for treating or preventing brain metastasis of a cancer. The application further includes an IMPDH inhibitor for use to treat or prevent brain metastasis of a cancer.

[00150] In some embodiments, the IMPDH inhibitor is administered or used for preventing brain metastasis of a cancer in a subject in need thereof. Therefore, the present application includes a method of preventing brain metastasis of a cancer in a subject in need thereof by administering or using a therapeutically effective amount of an inosine monophosphate dehydrogenase (IMPDH) inhibitor to a subject in need thereof.

[00151] In some embodiments, the IMPDH inhibitor delays brain metastasis of a cancer and the treating brain metastasis of a cancer is by inhibiting or delaying brain metastasis of a cancer. Therefore, in some embodiments, the IMPDH inhibitor is administered or used for inhibiting or delaying brain metastasis of a cancer in a subject.

[00152] Accordingly, the present application also includes a method of inhibiting or delaying brain metastasis of a cancer comprising administering a therapeutically effective amount an IMPDH inhibitor. The present application also includes a use of an IMPDH inhibitor for inhibiting or delaying brain metastasis of a cancer as well as a use of an IMPDH inhibitor for the preparation of a medicament for inhibiting or delaying brain metastasis of a cancer. The application further includes an IMPDH inhibitor for use in inhibiting or delaying brain metastasis of a cancer.

[00153] In some embodiments, the IMPDH inhibitor is administered or used for inhibiting brain metastasis of a cancer. In some embodiments, the IMPDH inhibitor is administered or used for delaying brain metastasis of a cancer.

[00154] In some embodiments, the brain metastasis of a cancer is delayed by about 1 month, about 2 months, about 3 months, about 4 months, about 6 months, about 8 months, about 10 months, about 12 months, about 14 months, about 18 months, about 20 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years or more. In some embodiments, brain metastasis of a cancer is delayed by about 1 month to about 10 years, about 1 month to about 8 years, about 1 month to about 5 years, about 1 month

to about 3 years, about 1 month to about 1 year, about 1 month to about 8 months, or about 1 month to about 6 months.

[00155] In some embodiments, the treating or preventing brain metastasis of a cancer is by inhibiting brain-metastasis initiating cell (BMIC) activity. Therefore, in some embodiments, an IMPDH inhibitor is administered or used for inhibiting brain-metastasis initiating cell (BMIC) activity in a subject. Accordingly, in some embodiments, the present application also includes a method of inhibiting brain-metastasis initiating cell (BMIC) activity comprising administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof. The present application also includes a use of an IMPDH inhibitor for inhibiting BMIC activity as well as a use of an IMPDH inhibitor for the preparation of a medicament for inhibiting BMIC activity. The application further includes an IMPDH inhibitor for use in inhibiting BMIC activity.

[00156] In some embodiments, inhibiting BMIC activity inhibits the metastatic progression of BMICs to the brain and/or in the brain. Therefore, in some embodiments, treating or preventing brain metastasis of a cancer is by inhibiting the metastatic progression of BMICs to the brain and/or in the brain. Accordingly, in some embodiments, an IMPDH inhibitor is administered or used for inhibiting metastatic progression of BMICs to the brain and/or in the brain in a subject.

[00157] In some embodiments, inhibiting metastatic progression of BMICs to the brain inhibits the infiltration of cancer cells in the brain.

[00158] In some embodiments, inhibiting BMIC activity inhibits the migration of brain-metastasis initiating cells (BMICs) to the brain. Therefore, in some embodiments, treating or preventing brain metastasis of a cancer is by inhibiting migration of brain-metastasis initiating cells (BMICs) to the brain. Accordingly, in some embodiments, an IMPDH inhibitor is administered or used for inhibiting the migration of brain-metastasis initiating cells (BMICs) to the brain in a subject in need thereof.

[00159] In some embodiments, inhibiting BMIC activity inhibits growth and survival of BMICs in the brain. Therefore, in some embodiments, treating or preventing brain metastasis of a cancer is by inhibiting growth and survival of brain-metastasis initiating cells (BMICs) in the brain. Accordingly, in some embodiments, an IMPDH inhibitor is administered or used for inhibiting growth and survival of brain-metastasis initiating cells (BMICs) in the brain by use or administration of a compound of the application to a subject in need thereof.

[00160] In some embodiments, inhibiting BMIC activity reduces the risk of brain metastasis of a cancer. Therefore, in some embodiments, the present application also includes a method of reducing the risk of brain metastasis of a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof. The present application also includes a use of an IMPDH inhibitor for reducing the risk of brain metastasis of a cancer as well as a use of an IMPDH inhibitor for the preparation of a medicament for reducing the risk of brain metastasis of a cancer. The application further includes an IMPDH inhibitor for use to reduce the risk of brain metastasis of a cancer.

[00161] In some embodiments, inhibiting BMIC activity lengthens the period of survival of a subject having the cancer or a subject that has had the cancer. Therefore, in some embodiments, the present application also includes a method of lengthening the period of survival of a subject having a cancer or a subject that has had a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof. The present application also includes a use of an IMPDH inhibitor for lengthening the period of survival of a subject having a cancer or a subject that has had a cancer as well as a use of a compound of the application for the preparation of a medicament for lengthening the period of survival of a subject having a cancer or a subject that has had a cancer. The application further includes an IMPDH inhibitor for use to lengthen the period of survival of a subject having a cancer or a subject that has had a cancer.

[00162] In some embodiments, the survival period is lengthened by about 1 month, about 2 months, about 3 months, about 4 months, about 6 months, about 8 months, about 10 months, about 12 months, about 14 months, about 18 months, about 20 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years or more. In some embodiments, survival period is lengthened by about 1 month to about 10 years, about 1 month to about 8 years, about 1 month to about 5 years, about 1 month to about 3 years, about 1 month to about 1 year, about 1 month to about 8 months, or about 1 month to about 6 months.

[00163] In some embodiments, the BMICs are premetastatic BMICs. Therefore, in some embodiments, an IMPDH inhibitor is administered or used for inhibiting premetastatic BMIC activity, inhibiting metastatic progression of premetastatic BMICs to the brain, inhibiting migration of premetastatic BMICs to the brain and/or inhibiting growth and survival of BMICs in the brain.

[00164] In some embodiments, the cancer is selected from lung cancer, breast cancer, melanoma, colon cancer, kidney cancer, renal cell carcinoma, mesothelioma, ovarian cancer, pancreatic cancer, sarcoma, leukemia, lymphoma, urothelial cancer, head and neck cancer, osteosarcoma and bladder cancer. In some embodiments, the cancer selected from lung cancer, breast cancer and melanoma. In some embodiments, the cancer is lung cancer.

[00165] In some embodiments, the cancer is a primary cancer.

[00166] In an embodiment, the subject is a mammal. In another embodiment, the subject is human.

[00167] In some embodiments, the “subject in need thereof” is a subject having a cancer or a subject that has had a cancer. In some embodiments, the subject in need thereof is a subject having a cancer. In some embodiments, the subject in need thereof is a subject that has had a cancer and is not known to have brain metastasis of the cancer prior to treatment with or use of an IMPDH inhibitor. In some embodiments, the subject in need thereof is a subject having the cancer and the cancer is in remission. In some embodiments, the subject in need thereof is a subject that is cancer-free after having had the cancer. In some embodiments, the “subject in need thereof” is a subject having a cancer and the cancer is refractory to a chemotherapy or radiotherapy.

[00168] The present application further includes a method of producing an anti-cancer effect in subject having a cancer or a subject that has had a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof. The present application also includes a use of an IMPDH inhibitor for producing an anti-cancer effect in subject having a cancer or a subject that has had a cancer as well as a use of an IMPDH inhibitor for the preparation of a medicament for producing an anti-cancer effect in subject having a cancer or a subject that has had a cancer. The application further includes an IMPDH inhibitor for use to produce an anti-cancer effect in subject having a cancer or a subject that has had a cancer.

[00169] In some embodiments, the present application also includes a method of treating or preventing a brain metastasis of a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor in combination with another known agent useful for treating or preventing a brain metastasis of a cancer and/or in combination with another known agent for treating cancer in a subject in need thereof. The present application also includes a use of an IMPDH inhibitor in combination with a known agent useful for treating or preventing a brain metastasis of a cancer in combination with another

known agent useful for treating or preventing a brain metastasis of a cancer and/or in combination with another known agent for treating cancer in a subject in need thereof as well as a use of an IMPDH inhibitor for the preparation of a medicament treating or preventing a brain metastasis of a cancer in combination with another known agent useful for treating or preventing a brain metastasis of a cancer and/or in combination with another known agent for treating cancer in a subject in need thereof. The application further includes an IMPDH inhibitor for use treating or preventing a brain metastasis of a cancer in combination with another known agent useful for treating or preventing a brain metastasis of a cancer and/or in combination with another known agent for treating cancer in a subject in need thereof.

[00170] In some embodiments, the another agent useful for treating or preventing a brain metastasis of a cancer and/or the another known agent for treating cancer in a subject in need thereof is a cancer treatment. In some embodiments, the cancer treatment is selected from one or more radiotherapy, chemotherapy, targeted therapies such as antibody therapies (including anti-PD1 and/or anti-PD-L1 antibodies) and small molecule therapies such as tyrosine-kinase inhibitors therapies, glutaminase inhibitors (e.g., glutaminase-1 (GLS1) inhibitors), and asparagine synthetase (ASNS) inhibitors, immunotherapy, hormonal therapy and anti-angiogenic therapies.

[00171] In some embodiments, the chemotherapy is a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is cisplatin. In some embodiments, the chemotherapeutic agent is L-asparaginase (L-ASNase).

[00172] In some embodiments, the small molecule therapy is a glutaminase (e.g., glutaminase-1, (GLS1)) inhibitor or an asparagine synthetase (ASNS) inhibitor.

[00173] A person skilled in the art would appreciate that metastases are commonly detected through the sole or combined use of magnetic resonance imaging (MRI) scans, computed tomography (CT) scans, positron emission tomography (PET), blood and platelet counts, liver function studies, chest X-rays and bone scans in addition to the monitoring of specific symptoms.

[00174] In some embodiments, the IMPDH inhibitor is administered or used as soon as possible after a cancer diagnosis. In an embodiment, the IMPDH inhibitor is administered or used as soon as possible after a subject that has had cancer is in remission or is cancer-free.

[00175] In some embodiments, the IMPDH inhibitor is any agent that inhibits expression of IMPDH gene or protein, that induces IMPDH protein degradation or that inhibits IMPDH protein activity.

[00176] In some embodiments, the IMPDH inhibitor is any agent that inhibits expression of IMPDH gene or protein. In some embodiments, inhibiting expression of IMPDH gene or protein is by changing the content of DNA or degrading mRNA. In some embodiments, inhibiting expression of IMPDH gene or protein is by IMPDH gene knockdown, IMPDH gene knockout or by IMPDP gene editing.

[00177] In some embodiments, the agent that inhibits expression of IMPDH gene or protein is a nucleic acid. In some embodiments, the nucleic acid is an antisense oligonucleotide. In some embodiments, the antisense oligonucleotide is complementary to an IMPDH DNA or RNA sequence or a variant or fragment thereof.

[00178] In some embodiments, the agent that inhibits expression of IMPDH gene or protein is a nucleic acid selected from small interfering RNA (siRNA), dicer substrate DNA, hairpin RNA, microRNA (miRNA), RNAi and splice-regulating oligonucleotides. In some embodiments, the agent that inhibits expression of IMPDH gene or protein is selected from small interfering RNA (siRNA) and microRNA (miRNA).

[00179] In some embodiments, the agent that inhibits expression of IMPDH gene or protein is a gene editing system. In some embodiments, the gene editing system is a clustered regularly interspaced short palindromic repeat (CRISPR)-Cas system (CRISPR system); zinc finger nuclease (ZFN) system, or transcription activator-like effector-based nuclease (TALEN) system.

[00180] In some embodiments, the agent that inhibits expression of IMPDH gene or protein is a CRISPR system. In some embodiments, the CRISPR system is as described herein under "Mechanistic studies suggest IMPDH activity as a targetable vulnerability in BMICs".

[00181] In some embodiments, the agent that inhibits expression of IMPDH protein or inhibits IMPDH protein activity is an antibody that specifically binds IMPDH or an antigen binding fragment thereof.

[00182] As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen (for example, IMPDH). Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single

chain, Fab, Fab' and F(ab')₂ fragments, an Fab expression library, single-chain antibody molecules (e.g., scFv), bispecific antibodies and antibody-drug conjugates.

[00183] As used herein, the expression “specifically binds” means that the antibody reacts with one or more antigenic determinants of the desired antigen and does not bind other polypeptides or binds other polypeptides at much lower affinity ($K_d > 10^{-6}$).

[00184] In some embodiments, the IMPDH inhibitor is any agent that induces IMPDH protein degradation.

[00185] In some embodiments, the agent that induces IMPDH protein degradation (optionally IMPDH inhibitor) is a targeted IMPDH protein degrader. In some embodiments, the targeted IMPDH protein degrader is selected from an IMPDH targeting proteolysis targeting chimera (PROTAC), an IMPDH targeting molecular glue degrader, a selective estrogen receptor degrader (SERD), an IMPDH targeting monoclonal antibody or an IMPDH targeting antibody-drug conjugate.

[00186] In some embodiments, the targeted protein degrader is a small molecule targeted protein degrader selected from an IMPDH targeting proteolysis targeting chimera (PROTAC), an IMPDH targeting molecular glue degrader and a selective estrogen receptor degrader (SERD). In some embodiments, the targeted protein degrader is selected from an IMPDH targeting proteolysis targeting chimera (PROTAC), and an IMPDH targeting molecular glue degraders. In some embodiments, the targeted protein degrader is an IMPDH targeting monoclonal antibody or an IMPDH targeting antibody-drug conjugate. In some embodiments, the antibody-drug conjugate is antibody-PROTAC conjugate.

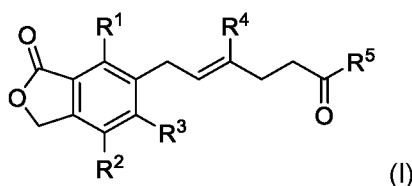
[00187] In some embodiments, PROTACs are bifunctional molecules that comprise a ligase binding group and a target protein binding group (e.g. an IMPDH binding group) which are joined together by a linker. By binding with high affinity to a target protein in the cell while binding with high affinity to the ligase, PROTACs function to recruit proteins (e.g., enzymes such as IMPDH) to a ligase which are then degraded and/or otherwise inhibited by the bifunctional compounds. In exemplary embodiments, the ubiquitination ligase binding group is a Von Hippel-Lindau E3 ubiquitin ligase (VHL) binding group, a cereblon E3 ubiquitin ligase binding group, or mouse double minute 2 homolog (MDM2 or HDM2) E3 ubiquitin ligase binding group, or IAP E3 ubiquitin ligase binding group. Many examples of PROTACs are now known in the art for targets including kinases, hormone receptors, and proteases (see for example, Tsai, J., et al. Nat Rev Mol Cell Biol (2024), Lai et al., Nature Reviews Drug Discovery volume 16, pages101–114 (2017).

[00188] In some embodiments, molecular glue degraders are monovalent bifunctional molecules comprising a ligase binding group and a target protein binding group but which do not comprise a linker. In some embodiments, molecular glue degraders bind the target protein and ligase through cooperative binding and reshape protein surface to enhance the affinity of the target protein and ligase for each other and/or promote novel protein-protein interactions. In some embodiments, the molecular glue degraders are naturally occurring protein degrader such Zinc²⁺ ions, viral peptides, auxins, RNA or hormones. In some embodiments, the molecular glue degraders are selected from thalidomide, lenolidamide, pomalidomine, and further thalidomide-based analogues such as CC-122, CC-220, CC-90009, CC-92480, ZXH-1-161, and SJ6986, dCeMM1-4, NRX-252114 and NRX-252262 and CR8 (see for example, Tsai, J., et al. Nat Rev Mol Cell Biol (2024), Sasso J, et al., Biochemistry. 2023 Feb 7; 62(3): 601–623).

[00189] In some embodiments, selective estrogen receptor degraders (SERDs) bind to the estrogen receptor (ER) and induce a conformational change that results in the degradation and/or downregulation of the receptor. In some embodiments, the degradation of the ER prevents ER-mediated signaling and inhibits the growth and survival of ER-expressing cancer cells (see for example, Tsai, J., et al. Nat Rev Mol Cell Biol (2024)).

[00190] In some embodiments, the IMPDH inhibitor is any agent that inhibits IMPDH protein activity. In some embodiments, the agent that inhibits IMPDH protein activity is a small molecule inhibitor of IMPDH protein activity.

[00191] In some embodiments, the small molecule inhibitor of IMPDH protein activity (optionally, the IMPDH inhibitor) is a compound of Formula I or a pharmaceutically acceptable salt, prodrug and/or solvate thereof,



wherein

R¹ is selected from OH, halo, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R² is selected from H, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R³ is selected from H, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R^4 is selected from H, C_{1-4} alkyl and C_{1-4} haloalkyl;

R^5 is selected from R^6 , OR^6 , NR^6R^7 , OC_{1-6} alkenylene NR^6R^7 and NR^8C_{1-6} alkenylene NR^6R^7 ;

R^6 is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{1-6} alkylene C_{3-10} cycloalkyl, and C_{1-6} alkylene C_{3-10} heterocycloalkyl, the latter six groups being optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$;

R^7 is selected H, C_{1-6} alkyl and C_{1-6} haloalkyl; or

R^6 and R^7 are joined to form, together with the nitrogen atom therebetween, a 3- to 12-membered heterocycloalkyl optionally containing one or two additional heteromoiety selected from N, NR^{11} , O, S, S(O), and SO_2 and optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, $NR^{12}R^{13}$, OR^{12} , CO_2R^{12} and $C(O)NR^{12}R^{13}$;

R^8 , R^9 and R^{10} are independently selected H, C_{1-6} alkyl and C_{1-6} haloalkyl;

R^{11} is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, CO_2C_{1-6} alkyl and CO_2C_{1-6} haloalkyl; and

R^{12} and R^{13} are independently selected H, C_{1-6} alkyl and C_{1-6} haloalkyl.

[00192] In some embodiments, R^1 is selected from OH, F, Cl, C_{1-4} alkoxy and C_{1-4} haloalkoxy. In some embodiments, R^1 is selected from OH, F, Cl, C_{1-4} alkoxy, C_{1-4} chloroalkoxy and C_{1-4} fluoroalkoxy. In some embodiments, R^1 is selected from OH, F, Cl, OCH_3 , OCH_2CH_3 , $OCH_2CH_2CH_3$, $OCH(CH_3)_2$, $OCH(CH_3)CH_2CH_3$, $OC(CH_3)_3$, OCF_3 , $OCFH_2$, $OCHF_2$, OCH_2CF_2H , OCH_2CF_3 and OCH_2CFH_2 . In some embodiments, R^1 is selected from OH, OCH_3 , OCH_2CH_3 , OCF_3 , $OCFH_2$ and $OCHF_2$. In some embodiments, R^1 is OH.

[00193] In some embodiments, R^2 is selected from H, C_{1-4} alkyl, C_{1-4} chloroalkyl, C_{1-4} fluoroalkyl, C_{1-4} alkoxy, C_{1-4} chloroalkoxy and C_{1-4} fluoroalkoxy. In some embodiments, R^2 is selected from H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $C(CH_3)_3$, CF_3 , CFH_2 , CHF_2 , CH_2CF_2H , CH_2CF_3 , CH_2CFH_2 , $C(CF_3)_3$, OCH_3 , OCH_2CH_3 , $OCH_2CH_2CH_3$, $OCH(CH_3)_2$, $OCH(CH_3)CH_2CH_3$, $OC(CH_3)_3$, OCF_3 , $OCFH_2$, $OCHF_2$, OCH_2CF_2H , OCH_2CF_3 , OCH_2CFH_2 , and $OC(CF_3)_3$. In some embodiments, R^2 is selected from H, C_{1-4} alkyl and C_{1-4} fluoroalkyl. In some embodiments, R^2 is selected from H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $C(CH_3)_3$, CF_3 , CFH_2 , CHF_2 , CH_2CF_2H , CH_2CF_3 , CH_2CFH_2 and $C(CF_3)_3$. In some embodiments, R^2 is selected from H and CH_3 . In some embodiments, R^2 is CH_3 .

[00194] In some embodiments, R^3 is selected from H, C_{1-4} alkyl, C_{1-4} chloroalkyl, C_{1-4} fluoroalkyl, C_{1-4} alkoxy, C_{1-4} chloroalkoxy and C_{1-4} fluoroalkoxy. In some embodiments, R^3 is selected from H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $C(CH_3)_3$, CF_3 , CFH_2 , CHF_2 , CH_2CF_2H , CH_2CF_3 , CH_2CFH_2 , $C(CF_3)_3$, OCH_3 , OCH_2CH_3 , $OCH_2CH_2CH_3$, $OCH(CH_3)_2$, $OCH(CH_3)CH_2CH_3$, $OC(CH_3)_3$, OCF_3 , $OCFH_2$, $OCHF_2$, OCH_2CF_2H , OCH_2CF_3 , OCH_2CFH_2 , and $OC(CF_3)_3$. In some embodiments, R^3 is selected from H, C_{1-4} alkoxy and C_{1-4} fluoroalkoxy. In some embodiments, R^3 is selected from H, OCH_3 , OCH_2CH_3 , $OCH_2CH_2CH_3$, $OCH(CH_3)_2$, $OCH(CH_3)CH_2CH_3$, $OC(CH_3)_3$, OCF_3 , $OCFH_2$, $OCHF_2$, OCH_2CF_2H , OCH_2CF_3 , OCH_2CFH_2 and $OC(CF_3)_3$. In some embodiments, R^3 is OCH_3 .

[00195] In some embodiments, R^4 is selected from H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $C(CH_3)_3$, CF_3 , CFH_2 , CHF_2 , CH_2CF_2H , CH_2CF_3 , CH_2CFH_2 and $C(CF_3)_3$. In some embodiments, R^4 is selected from H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $C(CH_3)_3$. In some embodiments, R^4 is selected from H and CH_3 . In some embodiments, R^4 is CH_3 .

[00196] In some embodiments, R^5 is selected from R^6 , OR^6 , NR^6R^7 , OC_{1-5} alkenylene NR^6R^7 and NR^8C_{1-4} alkenylene NR^6R^7 . In some embodiments, R^5 is R^6 . In some embodiments, R^5 is selected from OR^6 , NR^6R^7 , OC_{1-5} alkenylene NR^6R^7 and NR^8C_{1-4} alkenylene NR^6R^7 . In some embodiments, R^5 is OR^6 . In some embodiments, R^5 is selected from NR^6R^7 , OC_{1-5} alkenylene NR^6R^7 and NR^8C_{1-4} alkenylene NR^6R^7 . In some embodiments, R^5 is selected from NR^6R^7 and NR^8C_{1-4} alkenylene NR^6R^7 .

[00197] In some embodiments, R^6 is selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{3-10} heterocycloalkyl, C_{1-4} alkylene C_{3-10} cycloalkyl and C_{1-4} alkylene C_{3-10} heterocycloalkyl, the latter six groups being optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$.

[00198] In some embodiments, R^6 is selected from H, C_{1-6} alkyl, C_{1-6} fluoroalkyl and C_{1-6} chloroalkyl, the latter three groups being optionally substituted with one to four substituents selected from OH, CN, NO_2 , CHO, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$.

[00199] In some embodiments, R^6 is selected from C_{3-10} cycloalkyl and C_{1-4} alkylene C_{3-10} cycloalkyl optionally substituted with one to three substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} fluoroalkyl, C_{1-6} chloroalkyl, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$. In some embodiments, the C_{3-10} cycloalkyl in R^6 is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and indanyl. In some embodiments, R^6 is selected from C_{3-8} cycloalkyl and C_{1-4} alkylene C_{3-8} cycloalkyl.

optionally substituted with one to three substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆fluoroalkyl, C₁₋₆chloroalkyl, NR⁹R¹⁰, OR⁹, CO₂R⁹ and C(O)NR⁹R¹⁰. In some embodiments, C₃₋₈cycloalkyl in R⁶ is selected from cyclobutyl, cyclopentyl and cyclohexyl. In some embodiments, the C₃₋₈cycloalkyl in R⁶ is optionally substituted with one to three substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₄alkyl, C₁₋₄fluoroalkyl, C₁₋₄chloroalkyl, NR⁹R¹⁰, OR⁹, CO₂R⁹ and C(O)NR⁹R¹⁰.

[00200] In some embodiments, R⁶ is selected from C₃₋₁₀heterocycloalkyl and C₁₋₄alkyleneC₃₋₁₀heterocycloalkyl, optionally substituted with one to three substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆fluoroalkyl, C₁₋₆chloroalkyl, NR⁹R¹⁰, OR⁹, CO₂R⁹ and C(O)NR⁹R¹⁰. In some embodiments, C₃₋₁₀heterocycloalkyl in R⁶ is selected from aziridinyl, oxiranyl, thiiranyl, oxaxiridinyl, dioxiranyl, azetidiny, oxetanyl, 1,3-dioxolanyl, theitanyl, diazetidinyl, dioxetanyl, dithietanyl, tetrahydrofuranyl, tetrahydrothiophenyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, 2-oxopiperazinyl, 2-oxopiperdiny, 2-oxopyrrolidinyl, isoxthiolidinyl, thiazolidinyl, isothiazolidinyl, dioxolanyl, dithiolanyl, piperidinyl, triazolyl, furazanyl, oxadiazolyl, thiadiazolyl, dioxazolyl, dithiazolyl, tetrazolyl, oxatetrazolyl, tetrahydropyranyl, piperazinyl, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, dioxanyl, dithianyl, tetrahydrofuryl, azepanyl, oxepanyl, thiepanyl, diazepanyl, tetrahydroquinolinyl and tetrahydroisoquinolinyl. In some embodiments, C₃₋₁₀heterocycloalkyl in R⁶ is selected from azetidiny, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperazinyl, piperidinyl and morpholinyl. In some embodiments, C₃₋₁₀heterocycloalkyl in R⁶ is selected from azetidiny, pyrrolidinyl, piperazinyl, piperidinyl and morpholinyl. In some embodiments, C₃₋₁₀heterocycloalkyl in R⁶ is optionally substituted with one to three substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₄alkyl, C₁₋₄fluoroalkyl, C₁₋₄chloroalkyl, NR⁹R¹⁰, OR⁹, CO₂R⁹ and C(O)NR⁹R¹⁰. In some embodiments, C₃₋₁₀heterocycloalkyl in R⁶ is optionally substituted with one or two substituents selected from C₁₋₄alkyl, C₁₋₄fluoroalkyl, C₁₋₄chloroalkyl and CO₂R⁹.

[00201] In some embodiments, R⁸, R⁹ and R¹⁰ are independently selected from H, C₁₋₄alkyl and C₁₋₄haloalkyl. In some embodiments, R⁸, R⁹ and R¹⁰ are independently selected from H, C₁₋₄alkyl, C₁₋₄fluoroalkyl and C₁₋₄chloroalkyl. In some embodiments, R⁸, R⁹ and R¹⁰ are independently selected from H, CH₃, CH₂CH₃, CH₂CH₂CH₃, CH(CH₃)₂, CH(CH₃)CH₂CH₃, C(CH₃)₃, CF₃, CFH₂, CHF₂, CH₂CF₂H, CH₂CF₃, CH₂CFH₂, CCl₃, CH₂CClH₂, CCl₂H, CClH₂, CH₂CCl₂H and CH₂CCl₃.

[00202] In some embodiments, R⁸ and R¹⁰ are independently selected from H, CH₃, CF₃, CFH₂, CHF₂, CCl₃, CCl₂H and CClH₂. In some embodiments, R⁸ and R¹⁰ are

independently selected from H, CH₃ and CF₃. In some embodiments, R⁹ is selected from H, CH₃, CH₂CH₃, CH₂CH₂CH₃, CH(CH₃)₂, and C(CH₃)₃. In some embodiments, R⁹ is C(CH₃)₃.

[00203] In some embodiments, R⁷ is selected from H, C₁₋₄alkyl and C₁₋₄haloalkyl. In some embodiments, R⁷ is selected from H, C₁₋₄alkyl, C₁₋₄fluoroalkyl and C₁₋₄chloroalkyl. In some embodiments, R⁷ is selected from H, CH₃, CH₂CH₃, CH₂CH₂CH₃, CH(CH₃)₂, CH(CH₃)CH₂CH₃, C(CH₃)₃, CF₃, CFH₂, CHF₂, CH₂CF₂H, CH₂CF₃, CH₂CFH₂, CCl₃, CH₂CClH₂, CCl₂H, CClH₂, CH₂CCl₂H and CH₂CCl₃. In some embodiments, R⁷ is selected from selected from H, CH₃ and CF₃.

[00204] In some embodiments, R⁶ and R⁷ are joined to form, together with the nitrogen atom therebetween, a 3- to 12-membered heterocycloalkyl ring optionally containing one or two additional heteromoieties selected from N, NR¹¹, O, S, S(O), and SO₂ and optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR¹²R¹³, OR¹², CO₂R¹² and C(O)NR¹²R¹³. In some embodiments, R⁶ and R⁷ are joined to form, together with the nitrogen atom therebetween, a 3- to 10-membered heterocycloalkyl ring optionally containing one or two additional heteromoieties selected from N, NR¹¹, O, S, S(O), and SO₂ and optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR¹²R¹³, OR¹², CO₂R¹² and C(O)NR¹²R¹³.

[00205] In some embodiments, R⁶ and R⁷ are joined to form, together, together with the nitrogen atom therebetween, a 4- to 10-membered heterocycloalkyl ring optionally containing one or two additional heteromoieties selected from NR¹¹, O, S, S(O), and SO₂ and optionally substituted with one or two substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR¹²R¹³, OR¹², CO₂R¹² and C(O)NR¹²R¹³.

[00206] In some embodiments, R⁶ and R⁷ are joined to form, together with the nitrogen atom therebetween, a 8- to 10-membered heterocycloalkyl ring optionally containing one or two additional heteromoieties selected from NR¹¹, O, S, S(O), and SO₂ and optionally substituted with one or two substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR¹²R¹³, OR¹², CO₂R¹² and C(O)NR¹²R¹³. In some embodiments, the 8- to 10-membered heterocycloalkyl ring is selected from indoliny, tetrahydroquinoliny and tetrahydroisoquinoliny. In some embodiments, the 8- to 10-membered heterocycloalkyl is optionally substituted with one substituent selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₄alkyl, C₁₋₄haloalkyl and CO₂R¹². In some embodiments, the 8- to 10-membered heterocycloalkyl is unsubstituted.

[00207] In some embodiments, R^6 and R^7 are joined to form, together with the nitrogen atom therebetween, a 4- to 7-membered heterocycloalkyl ring optionally containing one or two additional heteromoiety selected from NR^{11} , O, S, S(O), and SO_2 and optionally substituted with one or two substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, $NR^{12}R^{13}$, OR^{12} , CO_2R^{12} and $C(O)NR^{12}R^{13}$. In some embodiments, the 4- to 7-membered heterocycloalkyl ring is selected from azetidiny, pyrrolidiny, pyrazolidiny, imidazolidiny, pyrazoliny, imidazoliny, morpholiny, thiomorpholiny, thiomorpholiny sulfoxide, piperidiny and piperaziny. In some embodiments, the 4- to 7-membered heterocycloalkyl ring is selected from azetidiny, pyrrolidiny, morpholiny and piperaziny. In some embodiments, the 4- to 7-membered heterocycloalkyl ring is pyrrolidiny. In some embodiments, the 4- to 7-membered heterocycloalkyl is optionally substituted with one or two substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} fluoroalkyl, C_{1-6} chloroalkyl, $NR^{12}R^{13}$, OR^{12} , CO_2R^{12} and $C(O)NR^{12}R^{13}$. In some embodiments, the 4- to 7-membered heterocycloalkyl is optionally substituted with one or two substituents selected from OH, F, Cl, C_{1-6} alkyl, C_{1-6} fluoroalkyl, C_{1-6} chloroalkyl, OR^{12} and CO_2R^{12} . In some embodiments, the 4- to 7-membered heterocycloalkyl is optionally substituted with one or two substituents selected from C_{1-6} alkyl, C_{1-6} fluoroalkyl, C_{1-6} chloroalkyl and CO_2R^{12} .

[00208] In some embodiments, the 4- to 7-membered heterocycloalkyl is unsubstituted.

[00209] In some embodiments, R^6 and R^7 are joined to form, together with the nitrogen atom therebetween, a 5- to 6-membered heterocycloalkyl ring containing one additional NR^{11} heteromoiety and optionally substituted with one or two substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, $NR^{12}R^{13}$, OR^{12} and CO_2R^{12} , $C(O)NR^{12}R^{13}$. In some embodiments, the 5- to 6-membered heterocycloalkyl ring containing one additional NR^{11} heteromoiety is selected from pyrazolidiny, imidazolidiny, pyrazoliny, imidazoliny, and piperaziny. In some embodiments, the 5- to 6-membered heterocycloalkyl ring containing one additional NR^{11} heteromoiety is piperaziny.

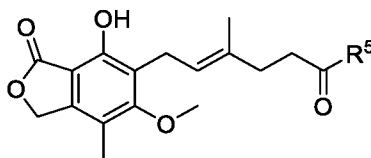
[00210] In some embodiments, R^5 is R^6 and R^6 is not H.

[00211] In some embodiments, R^{11} is selected from H, C_{1-4} alkyl, C_{1-4} haloalkyl, CO_2C_{1-4} alkyl and CO_2C_{1-4} haloalkyl. In some embodiments, R^{11} is selected from H, C_{1-4} alkyl, C_{1-4} fluoroalkyl, C_{1-4} chloroalkyl, CO_2C_{1-4} alkyl, CO_2C_{1-4} fluoroalkyl and CO_2C_{1-4} chloroalkyl. In some embodiments, R^{11} is selected from H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $C(CH_3)_3$, CF_3 , CFH_2 , CHF_2 , CH_2CF_2H , CH_2CF_3 , CH_2CFH_2 , CCl_3 ,

CH₂CClH₂, CCl₂H, CClH₂, CH₂CCl₂H, CH₂CCl₃, CO₂CH₃, CO₂CH₃, CO₂CH₂CH₃, CO₂CH₂CH₂CH₃, CO₂CH(CH₃)₂, CO₂CH(CH₃)CH₂CH₃, CO₂C(CH₃)₃, CO₂CF₃, CO₂CFH₂, CO₂CHF₂, CO₂CH₂CF₂H, CO₂CH₂CF₃, CO₂CH₂CFH₂, CO₂CCl₃, CO₂CH₂CClH₂, CO₂CCl₂H, CO₂CClH₂, CO₂CH₂CCl₂H, CO₂CH₂CCl₃. In some embodiments, R¹¹ is selected from selected from H, CH₃, CF₃ and CO₂C(CH₃)₃.

[00212] In some embodiments, R¹² and R¹³ are independently selected from H, C₁₋₄alkyl, C₁₋₄chloroalkyl and C₁₋₄fluoroalkyl. In some embodiments, R¹² and R¹³ are independently selected from H, CH₃, CH₂CH₃, CH₂CH₂CH₃, CH(CH₃)₂, CH(CH₃)CH₂CH₃, C(CH₃)₃, CF₃, CFH₂, CHF₂, CH₂CF₂H, CH₂CF₃, CH₂CFH₂, C(CF₃)₃, CCl₃, CClH₂, CHCl₂, CH₂CCl₂H, CH₂CCl₃, CH₂CClH₂, C(CCl₃)₃. In some embodiments, R¹² and R¹³ are independently selected from H, C₁₋₄alkyl and C₁₋₄fluoroalkyl. In some embodiments, R¹² and R¹³ are independently selected from H, CH₃, CH₂CH₃, CH₂CH₂CH₃, CH(CH₃)₂, CH(CH₃)CH₂CH₃, C(CH₃)₃, CF₃, CFH₂, CHF₂, CH₂CF₂H, CH₂CF₃, CH₂CFH₂ and C(CF₃)₃. In some embodiments, R¹² and R¹³ are independently selected from H, CH₃, CH₂CH₃, , CH(CH₃)₂, C(CH₃)₃, CF₃, CFH₂ and CHF₂.

[00213] In some embodiments, the compound Formula I, or a pharmaceutically acceptable salt, prodrug and/or solvate thereof is defined as follows:



wherein

R⁵ is selected from OR⁶, NR⁶R⁷, OC₁₋₆alkenyleneNR⁶R⁷ and NR⁸C₁₋₆alkenyleneNR⁶R⁷;

R⁶ is selected from H, C₁₋₆alkyl, C₁₋₆haloalkyl, C₃₋₁₀cycloalkyl, C₃₋₁₀heterocycloalkyl, C₁₋₆alkyleneC₃₋₁₀cycloalkyl, and C₁₋₆alkyleneC₃₋₁₀heterocycloalkyl, the latter six groups being optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR⁹R¹⁰, OR⁹, CO₂R⁹ and C(O)NR⁹R¹⁰;

R⁷ is selected H, C₁₋₆alkyl and C₁₋₆haloalkyl; or

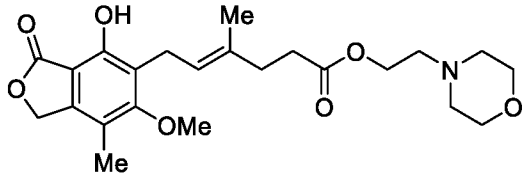
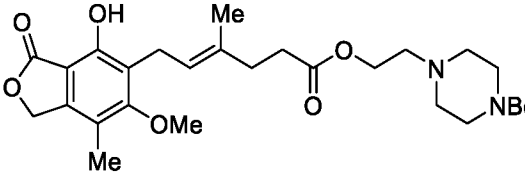
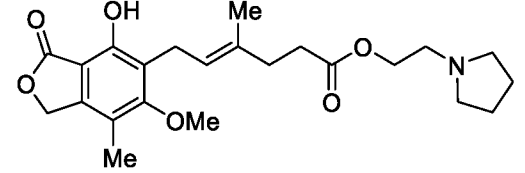
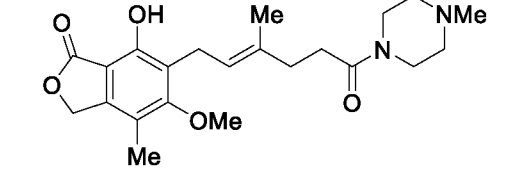
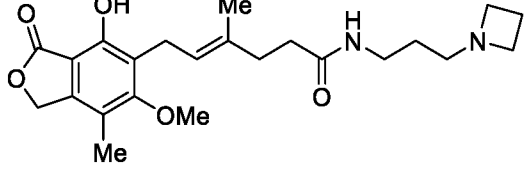
R⁶ and R⁷ are joined to form, together with the nitrogen atom therebetween, a 3- to 12-membered heterocycloalkyl optionally containing one or two additional heteromoiety selected from N, NR¹¹, O, S, S(O), and SO₂ and optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR¹²R¹³, OR¹², CO₂R¹² and C(O)NR¹²R¹³;

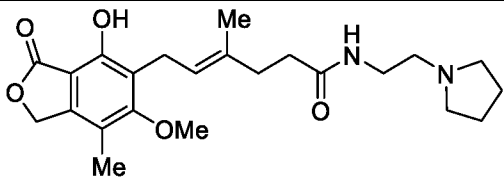
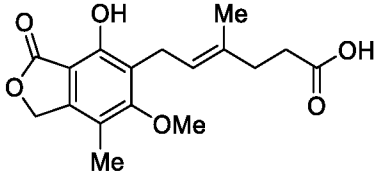
R⁸, R⁹ and R¹⁰ are independently selected H, C₁₋₆alkyl and C₁₋₆haloalkyl;

R¹¹ is selected from H, C₁₋₆alkyl, C₁₋₆haloalkyl, CO₂C₁₋₆alkyl and CO₂C₁₋₆haloalkyl; and

R¹² and R¹³ are independently selected H, C₁₋₆alkyl and C₁₋₆haloalkyl.

[00214] In some embodiments, the compounds of Formula (I) are selected from:

Compound I.D.	Structure	Chemical Name
I-1		tert-butyl (E)-4-(2-((6-(4-((tert-butyldimethylsilyl)oxy)-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoyl)oxy)ethyl)piperazine-1-carboxylate
I-2		tert-butyl (E)-4-(2-((6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoyl)oxy)ethyl)piperazine-1-carboxylate
I-3		2-(pyrrolidin-1-yl)ethyl (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate
I-4		(E)-7-hydroxy-5-methoxy-4-methyl-6-(3-methyl-6-(4-methylpiperazin-1-yl)-6-oxohex-2-en-1-yl)isobenzofuran-1(3H)-one
I-5		(E)-N-(3-(azetidin-1-yl)propyl)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enamide
		(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-

I-6		dihydroisobenzofuran-5-yl)-4-methyl-N-(2-(pyrrolidin-1-yl)ethyl)hex-4-enamide
I-7		(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoic acid

or a pharmaceutically acceptable salt, prodrug and/or solvate thereof.

[00215] An IMPDH inhibitor is either used alone or in combination with other known agents useful for treating or preventing brain metastasis and/or for treating cancer. When used in combination with other agents useful in treating or preventing brain metastasis and/or for treating cancer, it is an embodiment that the IMPDH inhibitor is administered contemporaneously with those agents. As used herein, “contemporaneous administration” of two substances to a subject means providing each of the two substances so that they are both biologically active in the individual at the same time. The exact details of the administration will depend on the pharmacokinetics of the two substances in the presence of each other and can include administering the two substances within a few hours of each other, or even administering one substance within 24 hours of administration of the other, if the pharmacokinetics are suitable. Design of suitable dosing regimens is routine for one skilled in the art. In particular embodiments, two substances will be administered substantially simultaneously, i.e., within minutes of each other, or in a single composition that contains both substances. It is a further embodiment of the present application that a combination of agents is administered to a subject in a non-contemporaneous fashion. In some embodiments, compounds of the present application are administered with another therapeutic agent simultaneously or sequentially in separate unit dosage forms or together in a single unit dosage form. Accordingly, the present application provides a single unit dosage form comprising an IMPDH inhibitor (e.g. a compound of Formula I), an additional therapeutic agent, and a pharmaceutically acceptable carrier.

[00216] Treatment methods comprise administering to a subject a therapeutically effective amount of an IMPDH inhibitor and optionally consist of a single administration, or alternatively comprise a series of administrations, and optionally comprise concurrent administration or use of one or more other therapeutic agents. For example, in some embodiments, an IMPDH inhibitor may be administered at least once a week. In some

embodiments, the IMPDH inhibitor may be administered to the subject from about one time per two or three weeks, or about one time per week to about once daily for a given treatment. In another embodiment, the compounds are administered 2, 3, 4, 5 or 6 times daily. The length of the treatment period depends on a variety of factors, such as the severity of the disease, disorder or condition, the age of the subject, the concentration and/or the activity of the IMPDH inhibitor, and/or a combination thereof. It will also be appreciated that the effective dosage of the IMPDH inhibitor used for the treatment may increase or decrease over the course of a particular treatment regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the IMPDH inhibitor is administered to the subject in an amount and for duration sufficient to treat the subject. In some embodiments treatment comprise prophylactic treatment. For example, a subject with early cancer can be treated to prevent progression, or alternatively a subject in remission can be treated with a compound or composition of the application to prevent recurrence.

[00217] The dosage of an IMPDH inhibitor varies depending on many factors such as the pharmacodynamic properties of the compound, the mode of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the frequency of the treatment and the type of concurrent treatment, if any, and the clearance rate of the IMPDH inhibitor in the subject to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. An IMPDH inhibitor may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response. Dosages will generally be selected to maintain a serum level of an IMPDH inhibitor from about 0.01 µg/cc to about 1000 µg/cc, or about 0.1 µg/cc to about 100 µg/cc. As a representative example, oral dosages of a compound of the application will range between about 0.05 mg per day to about 3000 mg per day for an adult, suitably about 1 mg per day to about 2000 mg per day, more suitably about 5 mg per day to about 1000 mg per day. For parenteral administration, a representative amount is from about 0.001 mg/kg to about 10 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.01 mg/kg to about 1 mg/kg or about 0.1 mg/kg to about 1 mg/kg will be administered. For oral administration, a representative amount is from about 0.001 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.01 mg/kg to about 1 mg/kg or about 0.1 mg/kg to about 1 mg/kg. For administration in suppository form, a representative amount is from about 0.1 mg/kg to about 10 mg/kg or about 0.1 mg/kg to about 1 mg/kg. An IMPDH inhibitor may be administered in a single daily, weekly or monthly dose or the total daily dose may be divided into two, three or four daily doses.

[00218] In an embodiment, effective amounts vary according to factors such as the disease state, age, sex and/or weight of the subject. In a further embodiment, the amount of a given compound or compounds that will correspond to an effective amount will vary depending upon factors, such as the given drug(s) or compound(s), the pharmaceutical formulation, the route of administration, the type of condition, disease or disorder, the identity of the subject being treated, and the like, but can nevertheless be routinely determined by one skilled in the art.

[00219] In an embodiment the pharmaceutically acceptable salt is an acid addition salt or a base addition salt. The selection of a suitable salt may be made by a person skilled in the art (see, for example, S. M. Berge, et al., "Pharmaceutical Salts," J. Pharm. Sci. 1977, 66, 1-19).

[00220] An acid addition salt suitable for, or compatible with, the treatment of subjects is any non-toxic organic or inorganic acid addition salt of any basic compound. Basic compounds that form an acid addition salt include, for example, compounds comprising an amine group. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric, nitric and phosphoric acids, as well as acidic metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids which form suitable salts include mono-, di- and tricarboxylic acids. Illustrative of such organic acids are, for example, acetic, trifluoroacetic, propionic, glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, mandelic, salicylic, 2-phenoxybenzoic, p-toluenesulfonic acid and other sulfonic acids such as methanesulfonic acid, ethanesulfonic acid and 2-hydroxyethanesulfonic acid. In an embodiment, the mono- or di-acid salts are formed, and such salts exist in either a hydrated, solvated or substantially anhydrous form. In general, acid addition salts are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection criteria for the appropriate salt will be known to one skilled in the art. Other non-pharmaceutically acceptable salts such as but not limited to oxalates may be used, for example in the isolation of an IMPDH inhibitor for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

[00221] A base addition salt suitable for, or compatible with, the treatment of subjects is any non-toxic organic or inorganic base addition salt of any acidic compound. Acidic compounds that form a basic addition salt include, for example, compounds

comprising a carboxylic acid group. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium or barium hydroxide as well as ammonia. Illustrative organic bases which form suitable salts include aliphatic, alicyclic or aromatic organic amines such as isopropylamine, methylamine, trimethylamine, picoline, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins, and the like. Exemplary organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline, and caffeine. The selection of the appropriate salt may be useful, for example, so that an ester functionality, if any, elsewhere in a compound is not hydrolyzed. The selection criteria for the appropriate salt will be known to one skilled in the art.

[00222] Solvates of an IMPDH inhibitor include, for example, those made with solvents that are pharmaceutically acceptable. Examples of such solvents include water (resulting solvate is called a hydrate) and ethanol and the like. Suitable solvents are physiologically tolerable at the dosage administered.

[00223] In embodiments of the present application, the IMPDH inhibitors described herein may have at least one asymmetric center. Where compounds possess more than one asymmetric center, they may exist as diastereomers. It is to be understood that all such isomers and mixtures thereof in any proportion are encompassed within the scope of the present application. It is to be further understood that while the stereochemistry of the compounds may be as shown in any given compound listed herein, such compounds may also contain certain amounts (for example, less than 20%, suitably less than 10%, more suitably less than 5%) of compounds of the present application having an alternate stereochemistry. It is intended that any optical isomers, as separated, pure or partially purified optical isomers or racemic mixtures thereof are included within the scope of the present application.

[00224] The IMPDH inhibitors may also exist in different tautomeric forms and it is intended that any tautomeric forms which the compounds form, as well as mixtures thereof, are included within the scope of the present application.

[00225] The IMPDH inhibitors may further exist in varying polymorphic forms and it is contemplated that any polymorphs, or mixtures thereof, which form are included within the scope of the present application.

[00226] The IMPDH inhibitors may further be radiolabeled and accordingly all radiolabeled versions of IMPDH inhibitors are included within the scope of the present application. The IMPDH inhibitors also include those in which one or more radioactive atoms are incorporated within their structure.

[00227] To be clear, in the above, the term “a compound” also includes embodiments wherein a compound are referenced. Likewise, the term “compounds of the application” also includes embodiments wherein only one compound is referenced.

[00228] The IMPDH inhibitors are suitably formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. Accordingly, the present application further includes a pharmaceutical composition comprising an effective amount of an IMPDH inhibitor and a pharmaceutically acceptable carrier wherein the IMPDH inhibitor is present in amount effective to treat or prevent brain metastasis of a cancer.

[00229] In some embodiments, the treating or preventing brain metastasis of a cancer is by inhibiting or delaying brain metastasis of a cancer, inhibiting BMIC activity, inhibiting metastatic progression of BMICs to the brain, inhibiting migration of BMICs to the brain and/or inhibiting growth and survival of BMICs in the brain.

[00230] The present application further includes a pharmaceutical composition comprising an effective amount of an IMPDH inhibitor and a pharmaceutically acceptable carrier wherein the IMPDH inhibitor is present in amount effective to reduce the risk of brain metastasis of a cancer.

[00231] The present application further includes a pharmaceutical composition comprising an effective amount of an IMPDH inhibitor and a pharmaceutically acceptable carrier wherein the IMPDH inhibitor is present in an amount effective to lengthen the period of survival of a subject having a cancer.

[00232] The present application further includes a pharmaceutical composition comprising an effective amount of an IMPDH inhibitor and a pharmaceutically acceptable carrier wherein the IMPDH inhibitor is present in amount effective to produce an anti-cancer effect.

[00233] The IMPDH inhibitors are administered to a subject in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. For example, an IMPDH inhibitor is administered by oral, inhalation, parenteral, buccal, sublingual, nasal, rectal, vaginal, patch, pump, minipump, topical or transdermal

administration and the pharmaceutical compositions formulated accordingly. In some embodiments, administration is by means of a pump for periodic or continuous delivery. Conventional procedures and ingredients for the selection and preparation of suitable compositions are described, for example, in Remington's Pharmaceutical Sciences (2000 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

[00234] Parenteral administration includes systemic delivery routes other than the gastrointestinal (GI) tract, and includes, for example intravenous, intra-arterial, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary (for example, by use of an aerosol), intrathecal, rectal and topical (including the use of a patch or other transdermal delivery device) modes of administration. Parenteral administration may be by continuous infusion over a selected period of time.

[00235] In some embodiments, an IMPDH inhibitor is orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it is enclosed in hard or soft shell gelatin capsules, or it is compressed into tablets, or it is incorporated directly with the food of the diet. In some embodiments, the compound is incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, caplets, pellets, granules, lozenges, chewing gum, powders, syrups, elixirs, wafers, aqueous solutions and suspensions, and the like. In the case of tablets, carriers that are used include lactose, corn starch, sodium citrate and salts of phosphoric acid. Pharmaceutically acceptable excipients include binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). In embodiments, the tablets are coated by methods well known in the art. In the case of tablets, capsules, caplets, pellets or granules for oral administration, pH sensitive enteric coatings, such as Eudragits™ designed to control the release of active ingredients are optionally used. Oral dosage forms also include modified release, for example immediate release and timed-release, formulations. Examples of modified-release formulations include, for example, sustained-release (SR), extended-release (ER, XR, or XL), time-release or timed-release, controlled-release (CR), or continuous-release (CR or Contin), employed, for example, in the form of a coated tablet, an osmotic delivery device, a coated capsule, a microencapsulated microsphere, an agglomerated particle, e.g., as of molecular sieving type particles, or, a fine hollow permeable fiber bundle, or chopped hollow permeable fibers, agglomerated or held in a

fibrous packet. Timed-release compositions are formulated, for example as liposomes or those wherein the active compound is protected with differentially degradable coatings, such as by microencapsulation, multiple coatings, etc. Liposome delivery systems include, for example, small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. In some embodiments, liposomes are formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines. For oral administration in a capsule form, useful carriers or diluents include lactose and dried corn starch.

[00236] In some embodiments, liquid preparations for oral administration take the form of, for example, solutions, syrups or suspensions, or they are suitably presented as a dry product for constitution with water or other suitable vehicle before use. When aqueous suspensions and/or emulsions are administered orally, the compound of the application is suitably suspended or dissolved in an oily phase that is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents are added. Such liquid preparations for oral administration are prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); and preservatives (e.g., methyl or propyl p-hydroxybenzoates or sorbic acid). Useful diluents include lactose and high molecular weight polyethylene glycols.

[00237] It is also possible to freeze-dry an IMPDH inhibitor and use the lyophilizates obtained, for example, for the preparation of products for injection.

[00238] In some embodiments, an IMPDH inhibitor is administered parenterally. For example, solutions of a compound of the application are prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. In some embodiments, dispersions are prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. A person skilled in the art would know how to prepare suitable formulations. For parenteral administration, sterile solutions of the IMPDH inhibitor are usually prepared, and the pH's of the solutions are suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic. For ocular administration, ointments or droppable liquids are delivered, for example, by ocular delivery systems known to the art such as applicators or eye droppers. In some embodiment, such compositions include mucomimetics such as hyaluronic acid, chondroitin sulfate, hydroxypropyl methylcellulose

or polyvinyl alcohol, preservatives such as sorbic acid, EDTA or benzyl chromium chloride, and the usual quantities of diluents or carriers. For pulmonary administration, diluents or carriers will be selected to be appropriate to allow the formation of an aerosol.

[00239] In some embodiments, an IMPDH inhibitor is formulated for parenteral administration by injection, including using conventional catheterization techniques or infusion. Formulations for injection are, for example, presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. In some embodiments, the compositions take such forms as sterile suspensions, solutions or emulsions in oily or aqueous vehicles, and contain formulating agents such as suspending, stabilizing and/or dispersing agents. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. Alternatively, the IMPDH inhibitors are suitably in a sterile powder form for reconstitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[00240] In some embodiments, compositions for nasal administration are conveniently formulated as aerosols, drops, gels and powders. For intranasal administration or administration by inhalation, the IMPDH inhibitors are conveniently delivered in the form of a solution, dry powder formulation or suspension from a pump spray container that is squeezed or pumped by the patient or as an aerosol spray presentation from a pressurized container or a nebulizer. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which, for example, take the form of a cartridge or refill for use with an atomising device. Alternatively, the sealed container is a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form comprises an aerosol dispenser, it will contain a propellant which is, for example, a compressed gas such as compressed air or an organic propellant such as fluorochlorohydrocarbon. Suitable propellants include but are not limited to dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, heptafluoroalkanes, carbon dioxide or another suitable gas. In the case of a pressurized aerosol, the dosage unit is suitably determined by providing a valve to deliver a metered amount. In some embodiments, the pressurized container or nebulizer contains a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator are, for example, formulated containing a powder mix of an IMPDH inhibitor and a suitable powder base such as lactose or starch. The aerosol dosage forms can also take the form of a pump-atomizer.

[00241] Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, wherein a compound of the application is formulated with a carrier such as sugar, acacia, tragacanth, or gelatin and glycerine. Compositions for rectal administration are conveniently in the form of suppositories containing a conventional suppository base such as cocoa butter.

[00242] Suppository forms of the IMPDH inhibitors are useful for vaginal, urethral and rectal administrations. Such suppositories will generally be constructed of a mixture of substances that is solid at room temperature but melts at body temperature. The substances commonly used to create such vehicles include but are not limited to theobroma oil (also known as cocoa butter), glycerinated gelatin, other glycerides, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol. See, for example: Remington's Pharmaceutical Sciences, 16th Ed., Mack Publishing, Easton, PA, 1980, pp. 1530-1533 for further discussion of suppository dosage forms.

[00243] In some embodiments an IMPDH inhibitor is coupled with soluble polymers as targetable drug carriers. Such polymers include, for example, polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide-phenol, polyhydroxyethylaspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, in some embodiments, an IMPDH inhibitor is coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and crosslinked or amphipathic block copolymers of hydrogels.

[00244] In some embodiments, an IMPDH inhibitor may be coupled with viral, non-viral or other vectors. Viral vectors may include retrovirus, lentivirus, adenovirus, herpesvirus, poxvirus, alphavirus, vaccinia virus or adeno-associated viruses. Non-viral vectors may include nanoparticles, cationic lipids, cationic polymers, metallic nanoparticles, nanorods, liposomes, micelles, microbubbles, cell-penetrating peptides, or lipospheres. Nanoparticles may include silica, lipid, carbohydrate, or other pharmaceutically acceptable polymers.

[00245] An IMPDH inhibitor including pharmaceutically acceptable salts and/or solvates thereof is suitably used on their own but will generally be administered in the form of a pharmaceutical composition in which the IMPDH inhibitor (the active ingredient) is in association with a pharmaceutically acceptable carrier. Depending on the mode of

administration, the pharmaceutical composition will comprise from about 0.05 wt% to about 99 wt% or about 0.10 wt% to about 70 wt%, of the active ingredient, and from about 1 wt% to about 99.95 wt% or about 30 wt% to about 99.90 wt% of a pharmaceutically acceptable carrier, all percentages by weight being based on the total composition.

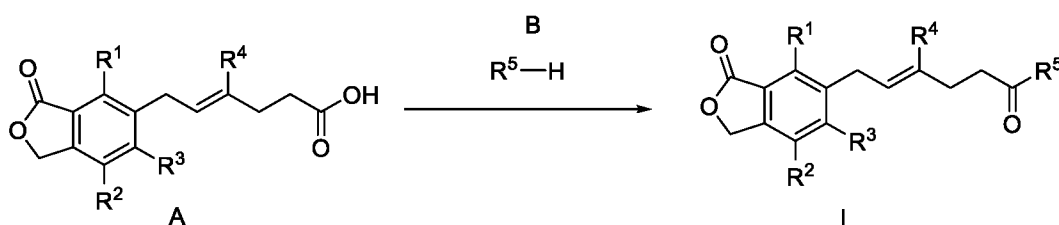
III. Methods of Preparation of Compounds of the Application

[00246] Compounds of Formula I can be prepared by various synthetic processes. The choice of particular structural features and/or substituents may influence the selection of one process over another. The selection of a particular process to prepare a given compound of Formula I is within the purview of the person of skill in the art. Some starting materials for preparing compounds of the present application are available from commercial chemical sources. Other starting materials, for example as described below, are readily prepared from available precursors using straightforward transformations that are well known in the art.

[00247] Mycophenolic acid (MPA, I-7) and mycophenolate mofetil (MMF, I-1) are both available from commercial sources or can be prepared using methods known in the art. For example, MPA is available from AmBeed (Arlington Heights, Illinois) and MMF is available from Aaron Chemicals (San Diego, California).

[00248] The compounds of Formula I generally can be prepared according to the processes illustrated in the Schemes below. In the structural formulae shown below the variables are as defined in Formula I unless otherwise stated. A person skilled in the art would appreciate that many of the reactions depicted in the Schemes below would be sensitive to oxygen and water and would know to perform the reaction under an anhydrous, inert atmosphere if needed. Reaction temperatures and times are presented for illustrative purposes only and may be varied to optimize yield as would be understood by a person skilled in the art.

[00249] Accordingly, in some embodiments, the compounds of Formula I, wherein R^5 is selected from OR^6 , NR^6R^7 , $OC_{1-6}alkenyleneNR^6R^7$ and $NR^8C_{1-6}alkenyleneNR^6R^7$ are prepared as shown in Scheme 1. Therefore, a carboxylic acid compound of Formula A is coupled with a compound of Formula B, R^5-H under suitable coupling conditions such as in the presence of a coupling reagent (e.g., 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, EDC) and a base (e.g., DMAP) in a suitable solvent to provide a compound of Formula I.



Scheme 1

[00250] Generally, the reactions described above are performed in a suitable inert organic solvent and at temperatures and for times that will optimize the yield of the desired compounds. Examples of suitable inert organic solvents include, but are not limited to, 2-propanol, dimethylformamide (DMF), 1,4-dioxane, methylene chloride (CH_2Cl_2), chloroform (CHCl_3), tetrahydrofuran (THF), toluene (PhMe), and the like.

[00251] The formation of a desired compound salt is achieved using standard techniques. For example, the neutral compound is treated with an acid or base in a suitable solvent and the formed salt is isolated by filtration, extraction or any other suitable method.

[00252] The formation of solvates will vary depending on the compound and the solvate. In general, solvates are formed by dissolving the compound in the appropriate solvent and isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions. The selection of suitable conditions to form a particular solvate can be made by a person skilled in the art. Examples of suitable solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a "hydrate".

[00253] Prodrugs of the compounds of the present application may be, for example, conventional esters formed with available hydroxy, thiol, amino or carboxyl groups. For example, available hydroxy or amino groups may be acylated using an activated acid in the presence of a base, and optionally, in inert solvent (e.g., an acid chloride in pyridine).

[00254] Throughout the processes described herein it is to be understood that, where appropriate, suitable protecting groups will be added to, and subsequently removed from, the various reactants and intermediates in a manner that will be readily understood by one skilled in the art. Conventional procedures for using such protecting groups as well as examples of suitable protecting groups are described, for example, in "*Protective Groups in Organic Synthesis*", T.W. Green, P.G.M. Wuts, Wiley-Interscience, New York, (1999). It is also to be understood that a transformation of a group or substituent into another group or substituent by chemical manipulation can be conducted on any intermediate or final

product on the synthetic path toward the final product, in which the possible type of transformation is limited only by inherent incompatibility of other functionalities carried by the molecule at that stage to the conditions or reagents employed in the transformation. Such inherent incompatibilities, and ways to circumvent them by carrying out appropriate transformations and synthetic steps in a suitable order, will be readily understood to one skilled in the art. Examples of transformations are given herein, and it is to be understood that the described transformations are not limited only to the generic groups or substituents for which the transformations are exemplified. References and descriptions of other suitable transformations are given in "Comprehensive Organic Transformations – A Guide to Functional Group Preparations" R.C. Larock, VHC Publishers, Inc. (1989). References and descriptions of other suitable reactions are described in textbooks of organic chemistry, for example, "Advanced Organic Chemistry", March, 4th ed. McGraw Hill (1992) or, "Organic Synthesis", Smith, McGraw Hill, (1994). Techniques for purification of intermediates and final products include, for example, straight and reversed phase chromatography on column or rotating plate, recrystallisation, distillation and liquid-liquid or solid-liquid extraction, which will be readily understood by one skilled in the art.

[00255] The products of the processes of the application may be isolated according to known methods, for example, the compounds may be isolated by evaporation of the solvent, by filtration, centrifugation, chromatography or other suitable method.

[00256] One skilled in the art will recognize that where a reaction step of the present application is carried out in a variety of solvents or solvent systems, said reaction step may also be carried out in a mixture of the suitable solvents or solvent systems

EXAMPLES

[00257] The following non-limiting examples are illustrative of the present application:

A. SYNTHESIS AND CHARACTERIZATION OF EXEMPLARY COMPOUNDS OF THE APPLICATION

General Methods

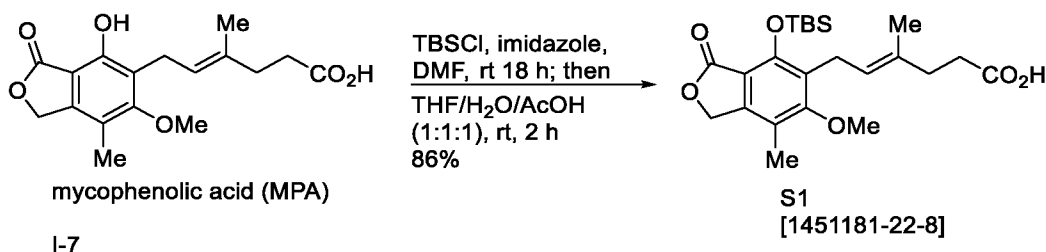
[00258] Chemical shifts in ^1H NMR and ^{13}C NMR spectra are reported in parts per million (ppm) relative to tetramethylsilane (TMS), with calibration to TMS (δ_{H} , δ_{C} 0.0) or the residual solvent peaks according to values reported by Gottlieb et al. (chloroform: δ_{H} 7.26, δ_{C} 77.16) (*J. Org. Chem.* 1997, 62, 7512-7515). When peak multiplicities are given, the

following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; sept., septet; dd, doublet of doublets; m, multiplet; br, broad; app., apparent; *gem*, geminal. ^1H NMR spectra were acquired at 400 or 700 MHz with a default digital resolution (Brüker parameter: FIDRES) of 0.22 and 0.15 Hz/point, respectively. Coupling constants reported herein therefore have uncertainties of ± 0.4 Hz and ± 0.3 Hz, respectively. All assignments of protons and carbons relied on data from 2-dimensional NMR experiments including COSY, HMQC, and HMBC. The ^{13}C NMR spectra provided herein ($^{13}\text{C}\{^1\text{H}\}$ DEPTQ-135; Brüker pulse program deptqgppsp) show CH and CH_3 carbon signals below the baseline and C and CH_2 carbons above the baseline. Melting points (mp) are uncorrected. Reactions were carried out at room temperature (rt) if temperature is not specified. Compounds purified by normal-phase flash chromatography (*J. Org. Chem.* 1978, 43, 2923-2925) used Teledyne CombiFlash Rf+ and NextGen 300+ purification systems with pre-packed silica cartridges (either 40–63 μM or 20–40 μM particle size). High-resolution mass spectrometry (HRMS) data was obtained using a Brüker micrOTOF II system with electrospray ionization (ESI) and paired with an Agilent HPLC and UV detector.

[00259] Exemplary compound mycophenolic acid (MPA, [24280-93-1], I-7) was purchased from AmBeed (Arlington Heights, Illinois). Mycophenolate mofetil (MMF, [128794-94-5]) and 2-aminoethylpyrrolidine were purchased from Aaron Chemicals (San Diego, California, www.aaronchem.com). *tert*-Butyldimethylchlorosilane (TBS-Cl) and *N*-methylpiperazine were purchased from Oakwood Chemical (Estill, South Carolina). 1-Boc-4-(2-hydroxyethyl)piperazine, *N*-(2-hydroxyethyl)pyrrolidine, 4-dimethylaminopyridine (DMAP), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) were purchased from AK Scientific (Union City, California). *N*-(3-Aminopropyl)azetidine was purchased from Enamine (Kyiv, Ukraine). Tetrabutylammonium fluoride (TBAF) was purchased as a solution in THF from Sigma-Aldrich (St. Louis, Missouri, U.S.A.). Imidazole was purchased from Fisher Scientific (Hampton, New Hampshire, U.S.A.)

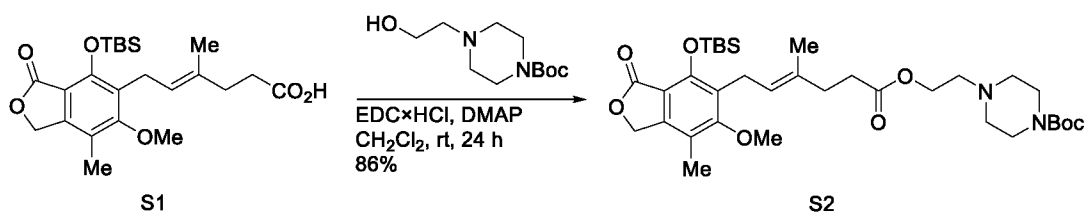
Example 1: Synthesis of I-2

(E)-6-(4-*tert*-Butyldimethylsilyloxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoic acid (S1)



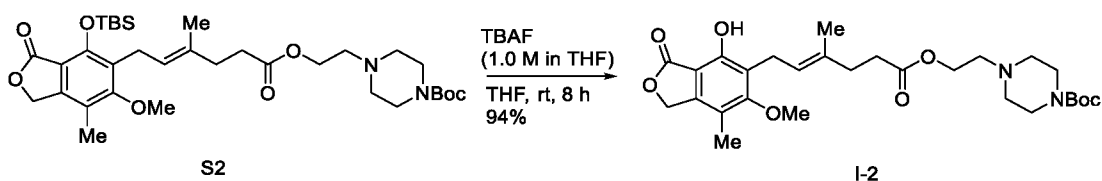
[00260] As a variation of the procedure developed by (*J. Enzyme Inhib. Med. Chem.* 2016,31, 974-982) DMF (20 mL, 0.3 M) was added under an atmosphere of argon to a 100 mL round bottom flask containing mycophenolic acid (I-7) (2.0 g, 6.24 mmol, 1 equiv). To the solution was added imidazole (2.76 g, 40.6 mmol, 6.5 equiv) followed by TBSCl (3.76 g, 24.96 mmol, 4.0 equiv) and the reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was diluted with water (42 mL) and EtOAc (30 mL). The layers were separated and the aqueous was extracted with ethyl acetate (30 mL). The combined organics were washed once with 1% HCl and three times with water, dried with Na₂SO₄, and concentrated under reduced pressure to obtain a colourless oil. The oil was then dissolved in a 1:1:1 solvent mixture of THF:H₂O:AcOH (30 mL) and stirred at room temperature for two hours. The reaction was diluted with water and extracted with ethyl acetate. The combined organics were washed with water twice, dried with Na₂SO₄, and concentrated under reduced pressure to obtain a colourless oil which solidified under vacuum. The white solids were vacuum filtered and washed excessively with hexanes to obtain TBS-protected mycophenolic acid S1 (2.34 g, 5.38 mmol, 86%) as a white solid without the need for further purification. *R*_f = 0.66 (60:39:1 EtOAc/Hex/AcOH). Mp 129–131 °C (lit. 94–96 °C (*Enzyme Inhib. Med. Chem.* 2016, 31, 974-982)). ¹H NMR (400 MHz, CDCl₃): δ 5.20 (tq, *J* = 6.4, 1.3 Hz, 1H), 5.06 (s, 2H), 3.73 (s, 3H), 3.38 (d, *J* = 6.4 Hz, 2H), 2.44–2.36 (m, 2H), 2.33–2.24 (m, 2H), 2.15 (s, 3H), 1.75 (d, *J* = 1.3 Hz, 3H), 1.02 (s, 9H), 0.24 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 179.1, 169.4, 163.3, 151.9, 146.2, 133.5, 127.7, 124.0, 118.1, 111.8, 67.8, 60.9, 34.2, 32.8, 26.2 (3C), 23.8, 18.9, 16.5, 11.6, –3.4 (2C). LCMS (ESI) *m/z*: 457.2017 calcd for C₂₃H₃₄O₆SiNa⁺ [M + Na]⁺; Found 457.2038. Spectral data were consistent with those previously reported (*Enzyme Inhib. Med. Chem.* 2016, 31, 974-982).

2-(4-Boc-piperazino)ethyl (E)-6-(4-tert-Butyldimethylsilyloxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate (S2)



[00261] To a 10 mL round bottom flask containing TBS-protected mycophenolic acid S1 (0.200 g, 0.46 mmol, 1 equiv) under an atmosphere of argon was added CH_2Cl_2 (2 mL, 0.23 M). To the solution was added 4-(2-hydroxyethyl)-1-Boc-piperazine (0.12 g, 0.55 mmol, 1.2 equiv), DMAP (0.005 g, 0.046 mmol, 0.1 equiv), and EDC·HCl (0.10 g, 0.55 mmol, 1.2 equiv) and the reaction was stirred for 17 hours. The reaction mixture was concentrated under reduced pressure and diluted with water ethyl acetate. The layers were separated and the aqueous was extracted with ethyl acetate. The combined organics were washed twice with water and brine, dried with Na_2SO_4 , and concentrated under reduced pressure. The product was purified by column chromatography (85% EtOAc/Hexanes) providing the ester S2 as a colourless oil (0.26 g, 0.4 mmol, 86%). $R_f = 0.25$ (70% EtOAc/Hex). ^1H NMR (400 MHz, CDCl_3): δ 5.18 (tq, $J = 6.4, 1.4$ Hz, 1H), 5.07 (s, 2H), 4.15 (t, $J = 5.9$ Hz, 2H), 3.74 (s, 3H), 3.42–3.37 (m, 6H), 2.58 (t, $J = 5.9$ Hz, 2H), 2.45–2.34 (m, 6H), 2.31–2.24 (m, 2H), 2.15 (s, 3H), 1.75 (d, $J = 1.4$ Hz, 3H), 1.45 (s, 9H), 1.03 (s, 9H), 0.24 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3): δ 173.2, 169.2, 163.2, 154.7, 151.7, 146.1, 133.7, 127.6, 123.6, 118.0, 111.6, 79.6, 67.6, 61.6, 60.7, 56.7, 53.1 (2C), 44.1 (br), 43.0 (br), 34.4, 33.0, 28.4 (3C), 26.1 (3C), 18.7, 16.4, 11.4, –3.5 (2C). LCMS (ESI) m/z : 647.3722 calcd for $\text{C}_{34}\text{H}_{55}\text{N}_2\text{O}_8\text{Si}^+ [\text{M} + \text{H}]^+$; Found 647.3696.

Mycophenolic acid, 2-(4-Boc-piperazino)ethyl ester (I-2)

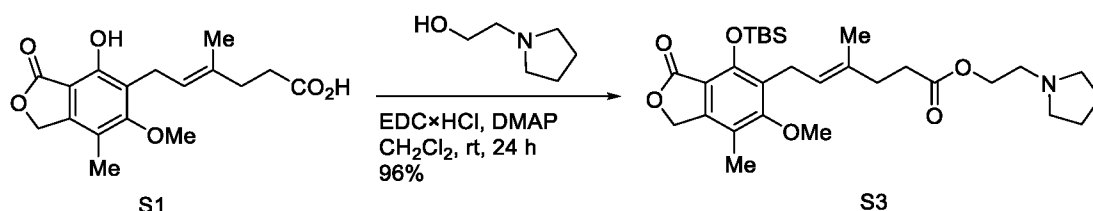


[00262] To a 5 mL round bottom flask containing S2 (0.075 g, 0.12 mmol, 1 equiv) under an atmosphere of argon was added THF (0.6 mL, 0.23 M). TBAF as a 1.0 M solution in THF (0.14 mL, 0.14 mmol, 1.2 equiv) was added and the reaction stirred at room temperature for eight hours. The reaction was diluted with water and extracted with ethyl acetate. The combined organics were dried with Na_2SO_4 and concentrated under reduced pressure. The product was purified by column chromatography eluting with 85% EtOAc/Hexanes providing the free phenol I-2 as a colourless oil (0.058 g, 0.1 mmol, 94%).

R_f = 0.25 (70% EtOAc/Hex). ^1H NMR (400 MHz, CDCl_3): δ 7.96 (brs, 1H), 5.0 (dq, J = 6.9, 1.4 Hz, 1H), 5.17 (s, 2H), 4.12 (t, J = 5.8 Hz, 2H), 3.75 (s, 3H), 3.44–3.38 (m, 4H), 3.36 (d, J = 6.9 Hz, 2H), 2.58 (t, J = 5.8 Hz, 2H), 2.47–2.37 (m, 6H), 2.31–2.24 (m, 2H), 2.13 (s, 3H), 1.78 (d, J = 1.4 Hz, 3H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, CDCl_3): δ 173.3, 172.9, 163.7, 154.8, 153.8, 144.2, 134.1, 123.0, 122.3, 116.7, 106.5, 79.7, 70.1, 62.0, 61.1, 56.8, 53.4 (2C), 44.2 (br), 43.0 (br), 34.8, 33.1, 28.5 (3C), 22.8, 16.2, 11.7. LCMS (ESI) m/z : 533.2857 calcd for $\text{C}_{28}\text{H}_{41}\text{N}_2\text{O}_8^+$ [$\text{M} + \text{H}$] $^+$; Found 533.2870.

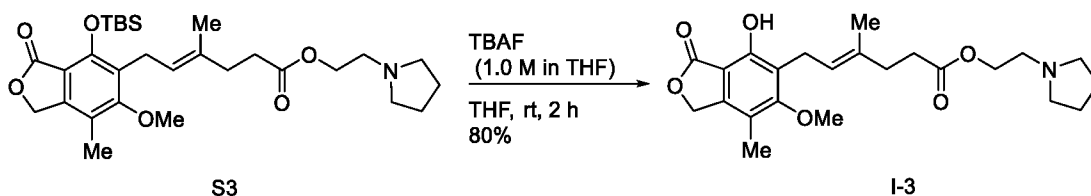
Example 2: Synthesis of I-3

2-(1-Pyrrolidinyl)ethyl E-6-(4-tert-butyldimethylsilyloxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate (S3)



[00263] To a 100 mL round bottom flask containing TBS-protected mycophenolic acid **S1** (2.29 g, 5.28 mmol, 1 equiv) under an atmosphere of argon was added CH_2Cl_2 (23 mL, 0.23 M). To the solution, was added *N*-(2-hydroxyethyl)pyrrolidine (0.74 mL, 6.34 mmol, 1.2 equiv), DMAP (0.064 g, 0.53 mmol, 0.1 equiv), and EDC·HCl (1.21 g, 6.34 mmol, 1.2 equiv) and the reaction was stirred for 24 hours. The reaction mixture was concentrated under reduced pressure and diluted with 70 mL water and 70 mL ethyl acetate. The layers were separated and the aqueous was extracted with ethyl acetate. The combined organics were washed with water (2 × 40 mL), brine (2 × 40 mL), dried with Na_2SO_4 , and concentrated under reduced pressure providing ester **S3** as a colourless oil (2.70 g, 5.07 mmol, 96%) that was used without any further purification. R_f = 0.23 (4% MeOH/ CH_2Cl_2). ^1H NMR (400 MHz, CDCl_3): δ 5.16 (tq, J = 5.1, 1.4 Hz, 1H), 5.04 (s, 2H), 4.15 (t, J = 5.9 Hz, 2H), 3.71 (s, 3H), 3.35 (d, J = 6.3 Hz, 2H), 2.71 (t, J = 5.9 Hz, 2H), 2.60–2.57 (brs, 4H), 2.40–2.36 (m, 2H), 2.28–2.24 (m, 2H), 2.13 (s, 3H), 1.82–1.76 (m, 4H), 1.73 (brs, 3H), 1.00 (s, 9H), 0.21 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3): δ 173.3, 169.2, 163.2, 151.7, 146.1, 133.7, 127.7, 123.6, 118.0, 111.7, 67.7, 62.9, 60.8, 54.6, 54.5, 34.4, 33.0, 26.1, 23.7, 23.5, 18.8, 16.4, 11.5, –3.4 (2C). LCMS (ESI) m/z : 532.3089 calcd for $\text{C}_{29}\text{H}_{46}\text{N}_2\text{O}_6\text{Si}^+$ [$\text{M} + \text{H}$] $^+$; Found 532.3111.

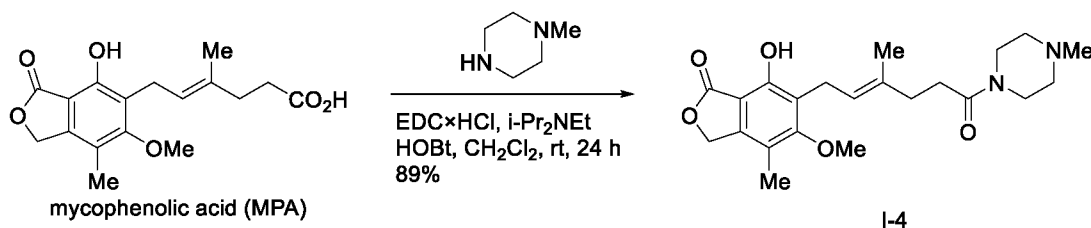
Mycophenolic acid, 2-(1-pyrrolidinyl)ethyl ester (I-3)



[00264] To a 100 mL round bottom flask containing S3 (2.66 g, 5.0 mmol, 1 equiv) under an atmosphere of argon was added THF (22 mL, 0.23 M). TBAF as a 1.0 M solution in THF (6 mL, 6 mmol, 1.2 equiv) was added and the reaction stirred at room temperature for two hours. The reaction was diluted with water and extracted with ethyl acetate. The combined organics were dried with Na₂SO₄ and concentrated under reduced pressure. The product was purified by column chromatography eluting with 6% MeOH/DCM providing the free phenol I-3 as a white solid (1.67 g, 4.0 mmol, 80%). *R*_f = 0.32 (6% MeOH/CH₂Cl₂). Mp 82–83 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.7–6.7 (brs, 1H), 5.21 (tq, *J* = 6.9, 1.3 Hz, 1H), 5.17 (s, 2H), 4.12 (t, *J* = 5.9 Hz, 2H), 3.75 (s, 3H), 3.37 (d, *J* = 6.9 Hz, 2H), 2.71 (t, *J* = 5.9 Hz, 2H), 2.63–2.54 (m, 4H), 2.44–2.38 (m, 2H), 2.33–2.26 (m, 2H), 2.13 (s, 3H), 1.82–1.75 (m, 7H). ¹³C NMR (100 MHz, CDCl₃): δ 173.5, 172.9, 163.7, 154.2, 144.2, 134.0, 123.2, 122.6, 116.4, 106.6, 70.0, 63.6, 61.1, 54.9 (2C), 54.6, 34.8, 33.2, 23.6 (2C), 22.8, 16.2, 11.6. LCMS (ESI) *m/z*: 418.2224 calcd for C₂₃H₃₂NO₆⁺ [*M* + *H*]⁺; Found 418.2234.

Example 3: Synthesis of I-4

Mycophenolic acid, 4-methylpiperazine amide (I-4)

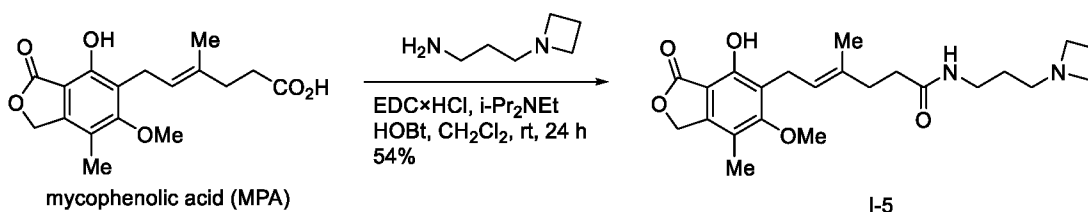


[00265] Diisopropylethylamine (165 μL, 0.95 mmol) was added to a suspension of mycophenolic acid (100 mg, 0.31 mmol, I-7), EDC·HCl (90 mg, 0.47 mmol), HOBT (63 mg, 0.47 mmol), and N-methylpiperidine (38 mg, 0.38 mmol) in CH₂Cl₂ (6 mL). After stirring the reaction mixture overnight at rt for 24 h, it was diluted with CH₂Cl₂ (20 mL), washed with saturated Na₂CO₃ (20 mL), dried over Na₂SO₄, and concentrated under reduced pressure. Flash chromatography (0→15% MeOH/CH₂Cl₂) provided amide I-4 as a pale yellow oil (112 mg, 0.28 mmol, 89%). ¹H NMR (400 MHz, CDCl₃): δ 5.22 (dd, *J* = 6.8, 1.3 Hz, 2H), 5.20 (s, 2H), 3.76 (s, 3H), 3.63–3.56 (m, 2H), 3.48–3.42 (m, 2H), 3.39 (d, *J* = 6.8 Hz, 2H), 2.43–2.26 (m, 8H), 2.29 (s, 3H), 2.15 (s, 3H), 1.82 (d, *J* = 1.3 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ

173.1, 171.3, 163.8, 153.8, 144.1, 135.0, 122.5, 122.4, 116.9, 106.6, 70.2, 61.2, 55.3, 54.9, 46.2, 45.6, 41.6, 35.2, 32.1, 22.8, 16.6, 11.7.

Example 4: Synthesis of I-5

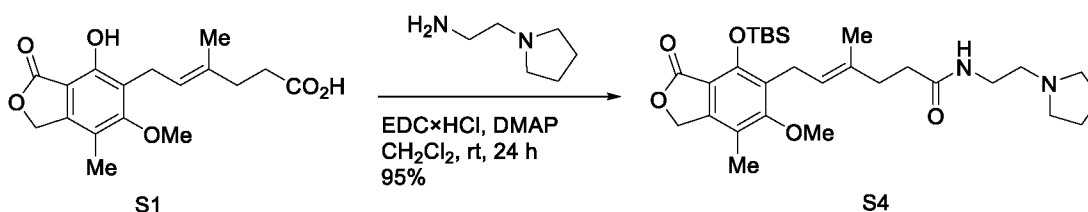
Mycophenolic acid, 3-(azetidin-1-yl)propyl amide (I-5)



[00266] Diisopropylethylamine (165 μ L, 0.95 mmol) was added to a suspension of mycophenolic acid (100 mg, 0.31 mmol), EDC·HCl (90 mg, 0.47 mmol), HOBT (63 mg, 0.47 mmol), and 3-(azetidin-1-yl)propylamine (42 mg, 0.37 mmol) in CH₂Cl₂ (6 mL). After stirring the reaction mixture overnight at rt for 24 h, it was diluted with CH₂Cl₂ (20 mL), washed with saturated Na₂CO₃ (20 mL), dried over Na₂SO₄, and concentrated under reduced pressure. Flash chromatography (0→10% MeOH/CH₂Cl₂) provided amide I-5 as a pale yellow oil (70 mg, 0.17 mmol, 54%). ¹H NMR (400 MHz, CDCl₃): δ 7.20 (t, *J* = 6.1 Hz, 1H), 5.21 (tq, *J* = 6.9, 1.5 Hz, 1H), 5.19 (s, 2H), 4.00 (appt, *J* = 7.3 Hz, 4H), 3.75 (s, 3H), 3.37 (d, *J* = 6.9 Hz, 2H), 3.28 (appq, *J* = 6.1 Hz, 2H), 3.06 (appt, *J* = 6.7 Hz, 2H), 2.52 (app pent, *J* = 8.0 Hz, 2H), 2.34–2.24 (m, 4H), 2.14 (s, 3H), 1.84 (pent, *J* = 6.5 Hz, 2H), 1.80 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 173.8, 173.0, 163.8, 153.9, 144.3, 134.8, 122.9, 122.4, 116.8, 106.6, 70.1, 61.2, 53.9 (2C), 52.8, 35.9, 35.3, 35.0, 24.2, 22.8, 16.4, 16.2, 11.7.

Example 5: Synthesis of I-6

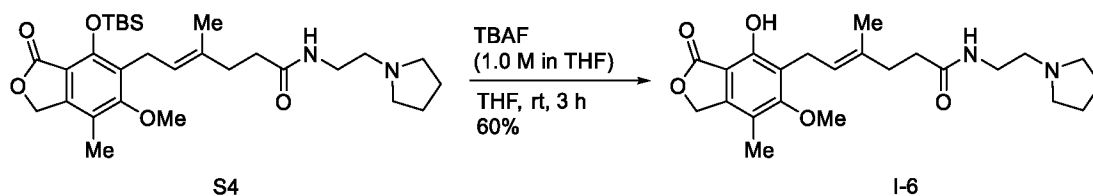
2-(1-Pyrrolidinyl)ethyl (E)-6-(4-tert-butyldimethylsilyloxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate (S4)



[00267] To a 10 mL round bottom flask containing TBS-protected mycophenolic acid S1 (0.20 g, 0.46 mmol, 1 equiv, I-7) under an atmosphere of argon was added CH₂Cl₂ (2 mL, 0.23 M). To the solution was added 2-(pyrrolidin-1-yl)ethylamine (0.063 g, 0.55 mmol, 1.2 equiv), DMAP (0.005 g, 0.046 mmol, 0.1 equiv), and EDC·HCl (0.10 g, 0.55 mmol, 1.2

equiv) and the reaction was stirred for 17 hours. The reaction mixture was concentrated under reduced pressure and diluted with water ethyl acetate. The layers were separated and the aqueous was extracted with ethyl acetate. The combined organics were washed twice with water and brine, dried with Na₂SO₄, and concentrated under reduced pressure. The crude was purified by column chromatography (15% MeOH/CH₂Cl₂) providing amide S4 a yellow oil (0.23 g, 0.43 mmol, 95%). *R*_f = 0.38 (20% MeOH/CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃): δ 7.04 (brs, 1H), 5.17 (brt, *J* = 6.4 Hz, 1H), 5.06 (s, 2H), 3.73 (s, 3H), 3.46 (appq, *J* = 5.5 Hz, 2H), 3.36 (d, *J* = 6.4 Hz, 2H), 3.03–2.92 (m, 4H), 2.89 (appt, *J* = 5.6 Hz, 2H), 2.35–2.26 (brs, 4H), 2.14 (s, 3H), 2.02–1.92 (m, 4H), 1.75 (d, *J* = 1.4 Hz, 3H), 1.01 (s, 9H), 0.22 (s, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 173.4, 169.4, 163.4, 151.8, 146.2, 134.3, 127.8, 123.5, 118.1, 111.7, 67.8, 60.9, 55.5, 54.3 (2C), 36.6, 35.2 (2C), 26.2 (3C), 23.8, 23.5 (2C), 18.9, 16.5, 11.6, –3.4 (2C). LCMS (ESI) *m/z*: 531.3249 calcd for C₂₉H₄₇N₂O₅Si⁺ [M + H]⁺; Found 531.3244.

Mycophenolic acid, 2-(1-pyrrolidinyl)ethyl amide (I-6)



[00268] To a 5 mL round bottom flask containing TBS-protected phenol S4 (0.10 g, 0.19 mmol, 1 equiv) under an atmosphere of argon was added THF (0.94 mL, 0.23 M). TBAF as a 1.0 M solution in THF (0.23 mL, 0.23 mmol, 1.2 equiv) was added and the reaction stirred at room temperature for three hours. The reaction was diluted with water and extracted with ethyl acetate. The combined organics were dried with Na₂SO₄ and concentrated under reduced pressure. The product was purified by column chromatography eluting with 15% MeOH/CH₂Cl₂ providing the free phenol I-6 as a white solid (0.047 g, 0.11 mmol, 60%). *R*_f = 0.38 (6% MeOH/CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃): δ 7.60 (brs, 1H), 5.23 (tq, *J* = 6.9, 1.4 Hz, 1H), 5.19 (s, 2H), 3.75 (s, 3H), 3.58 (appq, *J* = 5.5 Hz, 2H), 3.37 (d, *J* = 6.9 Hz, 2H), 3.33–3.10 (brs, 4H), 3.12 (appt, *J* = 5.5 Hz, 2H), 2.40–2.27 (m, 4H), 2.14 (s, 3H), 2.13–2.05 (m, 4H), 1.81 (d, *J* = 1.4 Hz, 3H). ¹³C NMR (175 MHz, CDCl₃): δ 173.8, 173.0, 163.8, 154.2, 144.3, 134.7, 123.1, 122.5, 116.6, 106.6, 70.1, 61.2, 55.9, 54.5 (2C), 36.0, 35.3, 35.0, 23.4 (2C), 22.8, 16.3, 11.7. LCMS (ESI) *m/z*: 417.2384 calcd for C₂₃H₃₃N₂O₅⁺ [M + H]⁺; Found 417.2391.

B. BIOLOGY

Cell Culture

[0100] BM cell lines from primary lung (BT478, BT530), breast (BT923, BT930) and melanoma (BT673, BT917) cancers were derived from primary patient samples with written consent from the patients and approved by the Hamilton Health Sciences McMaster Health Sciences Research Ethics Board (REB #07366), in compliance with Canada's Tri-Council Policy Statement on the Ethical Conduct for Research Involving Humans and International Ethical guidelines for Biomedical Research Involving Human Subjects. MDA-MB-231 was purchased from American Type Culture Collection and used to generate a brain metastasis derivate following injection into the mammary fat pad and isolation from the brain at humane endpoint. LBM cell lines were cultured in NeuroCult™ Complete (NCC) media consisting of NeuroCult™ NS-A Basal Medium (Stemcell™ technology #05750) and supplemented with 50 mL of NeuroCult™ Supplement, 20ng/mL epidermal growth factor (EGF), 10ng/mL fibroblast growth factor (FGF), 0.1% heparin and 1% penicillin-streptomycin. Alternatively, cell lines were maintained in NeuroCult Complete (NCC) media consisting of NeuroCult™ NS-A Basal Medium (Stemcell™ technology #05750) and supplemented with 50 mL of NeuroCult™ Supplement, 20ng/mL epidermal growth factor (EGF), 10ng/mL fibroblast growth factor (FGF), 2 µg/mL heparin and 1% penicillin-streptomycin. BBM and MBM cell lines were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12, Thermofisher Scientific #10565018) supplemented with 10% fetal bovine serum (FBS, Wisent Bio Products) and 1% penicillin-streptomycin. Human fetal neural stem cells (hNSCs) were isolated using a previously described protocol (Venugopal C., et al., J. Neurooncol. 2012;109(3):457-466 & Suk, Y., et al., STAR Protoc. 2022;3(3):101628) and were cultured in NCC. Normal human astrocytes were purchased from American Type Culture Collection and cultured in NCC. All cell lines were grown as tumour spheres or cultured adherently and maintained at 37°C with a humidified atmosphere of 5% CO₂.

Generation of lung-BM lines from patient samples

[00269] Two patient-derived lung adenocarcinoma samples were obtained as gifts; one that was derived from a patient who developed BM following their primary lung tumor diagnosis (CRUK0748) and one that was derived from a patient who, to date, has not developed BM (CRUK0733). Cell lines were derived from both samples, tagged with firefly luciferase, and used in an orthotopic lung-BM animal model to anticipate BM formation (or lack thereof) as was seen in the human patients from which the samples were biopsied from. Following orthotopic injection of cells, mice succumb to primary tumor burden before

their brain tumors can grow to a fatal size (Singh, M., et al., *Acta Neuropathol.* 2017;134(6):923-940; & Singh, M., et al., *Front Oncol.* 2017;7). Nonetheless, *ex vivo* bioluminescent imaging was used to confirm that the mice orthotopically injected with (metastatic) CRUK0748 cells developed BM prior to succumbing to their primary lung tumor burden, while the mice injected with (non-metastatic) CRUK0733 cells did not.

In vivo preclinical evaluation studies

[00270] All animal experiments were performed in accordance with the Canadian Council on Animal Care (CCAC) under animal utilization protocol (19-01-01) approved by the Animal Research Ethics Board (AREB). Human tissues were isolated using protocols approved by the Human Integrated Research Ethics Board (HIREB).

[00271] Sex considerations were factored into the research design and analysis. To the best of the Applicant's knowledge, no studies have found any sex-related differences pertaining to response to an IMPDH inhibitor treatment. However, apart from breast cancer, significantly higher rates of brain metastases have been reported in males compared to females in nearly all primary cancer types. In this study, both male and female mice have been included.

[00272] All experimental procedures involving animal work has been reviewed and approved by McMaster University Animal Research Ethics Board. Non-obese diabetic-severe combined immunodeficient IL2r γ ^{null} (NSG) mice were used for all experiments. Equal numbers of male and female mice were used for all experiments. Healthy mice were 6-8 weeks at time of use. Mice were anesthetized by gas anesthesia using isoflurane (4% induction, 2.5% maintenance) before procedure. Cells were engineered to express firefly luciferase and were injected intracardially, orthotopically, or intracranially. BMICs were injected intracardially (ICa) as previously described (Singh, M., et al., *Acta Neuropathol.* 2017;134(6):923-940). In addition to ICa injections, lung BMICs, breast BMICs, and melanoma BMICs were injected intracranially, followed by treatment with drug (100 mg/kg) or vehicle (n=6 per cohort). MPA and exemplary compound I-3 were administered by oral gavage (100 mg/kg). Mice were monitored weekly for signs of illness, and upon reaching endpoint, brains were harvested and split into two separate analyses: hematoxylin and eosin staining and *in vitro* culture and expansion.

In vivo imaging

[00273] Bioluminescent imaging was performed using an IVIS Spectrum In Vivo Imaging System (PerkinElmer) as per the manufacturer's instructions. Imaging and quantification of signals is controlled by the analysis software Living Image® (Xenogen).

Mice are weighed and injected intraperitoneally with 10 μ L/g of 15mg/mL solution of D-Luciferin firefly solution (PerkinElmer) in phosphate buffered saline (Invitrogen) 10 minutes before being imaged, and anesthetized (4% induction, 2.5% maintenance isoflurane). Mice were then placed onto a warmed stage inside the instrument and imaged for a maximum of 3 minutes depending on the tumor size. Regions of interest are quantified as radiance (p/sec/cm²/sr) using Living Image software for a standardized comparison between images.

Fluorescence-activated cell sorting

[00274] BMICs were dissociated into single cell suspensions and resuspended in phosphate buffered saline (PBS, Wisent Bio) with 2 mM EDTA. Cells were stained with APC-conjugated anti-human TRA-1-85 (CD147; Cat # 130-128-900, Miltenyi® Biotec®) and incubated for 15 minutes at room temperature. The viability dye 7-Aminoactinomycin D (7-AAD; Cat # 00-6993-50, eBioscience) is used to exclude dead cells; incubation with 7-AAD allows for penetration of compromised membranes and binding to DNA (Arndt-Jovin, D.J., and Jovin, T.M., *Methods Cell Biol.* 1989;30:417-448). Live cells were analyzed using Summit 5.4 software on MoFlo® XDP cell sorter (Beckman Coulter) to confirm human BMIC metastasis to the brain.

ii. Method Details

Connectivity Map Analysis

[00275] The Broad Institute's original CMap was used to identify possible drug candidates that could affect the expression of the deregulated genes revealed by transcriptome analyses of premetastatic BMICs. Over 1200 small molecules were assessed in this analysis using Bioconductor package PharmacoGx (Smirnov P. et al., *Bioinformatics* 2016;32(8):1244-1246). The analysis revealed 380 drugs with the ability to affect one or more of the 3951 deregulated genes in lung-, breast-, and melanoma-BMICs. Drugs were filtered by resulting connectivity score (connectivity score < 0, to denote their ability to revert gene deregulation) and associated significance (P < 0.01). The 48 drugs that fit these criteria were sourced and assayed in a preliminary drug screen to determine which drugs were effective in inhibiting BMIC proliferation. The lung-BMIC RNA-Seq data used for this analysis has been previously described and accessible through GEO Series accession number GSE110495 (Singh, M et al. (2017) *Acta Neuropathol.* 134, 923–940). The breast- and melanoma-BMIC RNA-Seq data used for this analysis has also been previously described and is accessible through GEO Series accession number GSE220156 (Bassey-Archibong, B., et al. (2023) *PNAS* 120).

Manual Cell Plating

[00276] In preparation for *in vitro* assessment of dose-response, proliferation, and sphere formation in the presence of candidate drug(s), BMICs were dissociated into single cell suspensions using Liberase® (Roche, for cells grown in suspension) or TrypLE® (ThermoFisher, for cells grown adherently) and resuspended in serum-free media; LBM BMICs are resuspended in NCC while BBM and MBM BMICs are resuspended in Stem Cell Media (SCM, 75% DMEM GlutaMAX™ and 25% F12 GlutaMAX™ supplemented with 0.2% 50× B27 supplement, 20ng/mL epidermal growth factor (EGF), 10ng/mL fibroblast growth factor (FGF), 0.1% heparin and 1% penicillin-streptomycin). Live cells were counted using the Countess trypan blue exclusion assay and manually plated into wells at the appropriate density (Seyfrid, M., *Humana Press* 2011:7-12).

In vitro functional assays

[00277] For preliminary drug screening, drugs were plated at a concentration of 10 µM in a 96-well plate, in triplicates at a density of 1000 cells/well, and incubated at 37°C with a humidified atmosphere of 5% CO₂ for three days. Vehicle controls for cell death were used in each functional experiment. Following treatment, PrestoBlue® (20 µL, Invitrogen), a resazurin-based cell viability reagent and fluorescence indicator of cell metabolism, was added to each well to estimate proliferation approximately two hours prior to measuring fluorescence intensity via FLUOstar™ Omega Fluorescence 556 Microplate reader (BMG LABTECH) at an excitation and emission wavelength of 540 nm and 590 nm, respectively. Results were analyzed using Omega analysis software.

[00278] Dose-response assays were conducted using the same protocol apart from drugs being plated using two-fold serial dilutions (20 µM - 39 nM) as previously (Adile, A.A., et al., *Methods Mol Biol.* 2019;189-196). The half maximal inhibitory concentration (IC₅₀) was determined by plotting percent cell viability by the logarithmic concentration of drug. IC₈₀ concentrations will be used for subsequent functional assays (cell proliferation, sphere formation), as determined by the following formula:

$$IC_F = \frac{100 - F^{\frac{1}{H}}}{F} \times IC_{50}$$

where F = fraction of maximal response and H = hill slope

[00279] Cell proliferation assays were conducted using the same protocol, except for drugs being plated at their IC₈₀ for a four-day incubation period. All results were illustrated and analyzed for significance using GraphPad Prism™ 8 software.

Clonogenic Sphere formation assay

[00280] To assess tumor sphere forming capacity under clonogenic conditions, neurospheres were dissociated into single cells and plated at a low density of 200 cells per well in low-binding culture treated 96-well plates to prevent cell adhesion. Cells are incubated at 37°C with a humidified atmosphere of 5% CO₂ with drug at IC₈₀ for seven days. Drug vehicle was used as a control. The number of spheres per well is manually counted at the four-day and seven-day incubation time points. Results were illustrated and analyzed for significance using GraphPad Prism™ 8 software.

Limiting Dilution Assays

[00281] In the limiting dilution assay, cells were plated at a range of different cell concentrations (200 cells/well- 1 cell/well) in a low-binding 96-well plate in triplicates. The plate was incubated at 37°C with a humidified atmosphere of 5% CO₂. Seven days later, the number of wells per condition that contained sphere-colonies under 10x magnification were counted. The frequency of BMICs within a given cell population was determined by linear regression analysis. Data was displayed as a scatter plot graph and the corresponding trend line; on the Y-axis the percentage of wells without detectable spheres and on the X-axis the number of seeded cells per well. Based on the Poisson distribution, the frequency of BMICs in the sample is the value corresponding to 37% of wells without detectable spheres (Seyfrid, M., et al., (2019). In (Humana Press, New York, NY), pp. 79–84; Hu, Y., and Smyth, G.K. (2009) J. Immunol. Methods 347, 70–78).

Migration assay

[00282] Cells were plated at a density of 15,000 to 25,000 cells (depending upon the cell line) per 70 µL media supplemented with 10% FBS into two separate wells of a bi-silicon structure within a 48-well plate. The cells were allowed to adhere for 24 hours at 37°C within a humidified atmosphere of 5% CO₂ to form a monolayer of cells. After 24 hours the silicon inserts were detached from the 48-well plate, leaving behind two monolayers of cells that were separated by an empty 'wound' and enabling of cell migration into the exclusion zone. The media is then removed from the well and the cells were washed with 500 µL of pre-warmed PBS and replenished with 1 mL of media containing 250 nm SYTOX™ green, 2.5% FBS and drug at IC₈₀ or vehicle (DMSO) control. The plate was inserted into the Incucyte® *in vitro* imaging system where the 'wound' was imaged periodically over time. The Incucyte® scan type chosen was 300ms adherent cell-by-cell alongside phase and green imaging channels within a 10x objective. The images were

uploaded to ImageJ where the wound area was calculated to determine the wound coverage percentage:

$$\frac{(\text{Wound area of selective time point/Wound area of Time 0})}{100} = \text{Wound Coverage Percentage} \quad \times$$

Compounds

[00283] Mycophenolic acid was purchased from Tocris (catalogue # 1505, Bristol, United Kingdom) and the remaining Connectivity Map compounds were sourced from the Centre for Microbial Chemical Biology at McMaster University.

Parallel artificial membrane permeability (PAMPA) assay

[00284] The permeability of the compounds was evaluated using the PAMPA assay (Lee, G. et al., (2001). Functional Expression of P-glycoprotein in Rat Brain Microglia, J Pharmacol Exp Ther. Oct;299(1):204-12). The PAMPA assembly consisted of the acceptor plate (MultiScreen IP Filter Plate, Millipore Sigma, Canada) and the donor plate (96 well Collection Plate, Millipore Sigma, Canada). The artificial membrane solution was prepared as 15 mg/mL of polar brain lipid from porcine (Sigma Aldrich, Oakville, Canada) in a solution of 60% chloroform / 40% dodecane. 7 µL of the mixture was pipetted into each acceptor plate well (top compartment). Thereafter, 300 µL of PBS (1 × PBS, pH 7.4, 5% DMSO) solution was added to each well of the acceptor plate and 300 µL of drug-containing donor solutions (50 µM compounds in 1 × PBS, pH 7.4, 5% DMSO) was added to each well of the donor plate (bottom compartment) in triplicate. The acceptor plate was placed into the donor plate and the assembly was incubated at room temperature for 16 hours. After incubation, aliquots of 10 µL from each well of acceptor and donor plate were transferred into a 96-well plate and 190 µL of acetonitrile (containing IS: 300 nM Dexamethasone, 100 nM Phenacetin), was added into each well. The plate was vortexed at 750 rpm for 2 min and was centrifuged at 7,000 g for 10 minutes. The concentration of the compounds was determined by LC/MS/MS.

[00285] The effective permeability (P_e), in units of centimeter per second, was calculated using the following equation:

$$\text{Log } P_e = \text{Log} \left[C \times \left[-LH \left(1 - \frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{donor}}} \right) \right] \right]$$

Where: $C = VD \times VA / [(VD + VA) \times t \times A]$; VD = volume of donor compartment (0.30 mL); VA = volume of acceptor compartment (0.30 mL); A = filter area (0.24 cm² for Multi-Screen Permeability Filter plate); and t = incubation time (in seconds).

MDR1-MDCKII Assays

[00286] MDR1-MDCKII permeability assays were performed by Wuxi AppTec Co. Briefly, MDR1-MDCK1 cells were seeded onto polycarbonate membranes in 96-well insert system plates and allowed to develop into monolayers. Exemplary compound I-7 (MPA) and exemplary compound I-3 (2 µM in 10 mM HEPES pH 7.4, 1% DMSO) were applied to either the apical or basolateral side of the monolayer. The plate was incubated for 2.5 h (37 °C, 5% CO₂) and the media was sampled on either side and the compound present was quantified by LC-MS/MS and the concentrations were used to calculate the efflux ratio. Digoxin (10 µM), nadolol (2 µM), and metoprolol (2 µM) were used as controls.

Brain Tissue Binding Assay

[00287] Brain homogenate binding assays were performed by Wuxi AppTec Co. Briefly, CD-1 pooled mouse brain homogenate (Cat: MSE00BRAINYZA) was obtained and treated with exemplary compound I-7 (MPA) or exemplary compound I-3 at a final concentration of 2 µM. The samples were applied to a dialysis well plate and sealed with dialysis membrane. Dialysis buffer (100 mM sodium phosphate pH 7.4, 150 mM NaCl) was applied to opposite side of the membrane (receiver well of the plate), and samples were incubated at 37°C with 5% CO₂ for 4 h. Following completion of incubation period, samples were recovered from each side of the dialysis membrane and processed by protein precipitation, and analysis by LC-MS/MS for compound concentration. A corresponding set of samples was also prepared, and stop solution (acetonitrile containing 200 ng/mL tolbutamide and 200 ng/mL labetalol) was immediately added, and samples were obtained from each side of the dialysis plate for mass spectrometric analysis.

Western Blot

[00288] 30-60 µg of denatured protein was loaded and resolved on sodium dodecyl sulfate (SDS) polyacrylamide gel followed by electro-transfer onto a polyvinylidene difluoride (PVDF) membrane. Protein concentrations were quantified using the Bradford Assay (BioRad). Membranes were blocked with 3% bovine serum albumin (BSA) in 1 × Tris-buffered saline (TBS) for phosphorylated proteins and 3% skim dry milk in 1 × TBS-Tween for non-phosphorylated proteins, followed by an incubation step with respective primary antibodies as well as a loading control of interest at 4°C overnight. Membranes

were then washed and hybridized with secondary antibodies for one hour at room temperature followed by band visualized using LuminataTM Forte Western HRP Substrate (Millipore) and protein detection using Chemidoc®. Immunoblots were quantified with ImageJ software and protein levels were normalized to the loading control. Alternatively, Membranes were blocked with 100% methanol for 30 seconds, allowed to dry at room temperature, and incubated with the respective antibody overnight at 4°C. Mouse monoclonal anti-IMPDH (Santa Cruz Biotechnology; catalogue # sc:166551) and mouse monoclonal anti-GAPDH (Abcam; catalogue #ab8245) were used at a 1:1000 dilution. For development, the anti-IMPDH antibody was used with ThermoFisher SuperSignal® West Femto Maximum Sensitivity Substrate while the anti-GAPDH antibody was used with RioRad® Clarify ECL reagents. Immunoblots were visualized with ImageLab software.

Firefly-luciferase lentivirus generation

[00289] A lentiviral vector expressing Firefly Luciferase (Addgene, RRID:Addgene_118017) was used for this study. Replication-incompetent lentivirus is produced by co-transfection of the Firefly Luciferase vector and packing vectors pMD2G and psPAX2 in HEK293T cells at ~80% confluency using Lipofectamine 3000 reagent (ThermoFisher) as per manufacturer's instructions. Viral supernatant is harvested every 24 hours for a total of three days and concentrated by PEGit (System Biosciences) as per manufacturer's instructions. The viral pellet is resuspended in 1.0 mL of DMEM, aliquoted, and stored at -80°C. BMIC lines are transduced with lentiviral vectors and treated with puromycin after 48 hours of transduction as a selection marker to develop stable cell lines.

Metabolomics mass spectrometry

[00290] For metabolomics profiling, 106 cells (BT478, BT530, NHAs) were cultured in the presence of MPA (I-7), exemplary compound I-3 (IC₈₀) or vehicle control, for 6 hours, and then collected, washed with PBS, and flash frozen in liquid nitrogen. Metabolites were extracted with a solution of cold acetonitrile/methanol/water (2:2:1) from the cell pellets and protein precipitation was performed by three cycles of freeze/thawing and sonication. The LC-MS metabolomics analysis was performed as previously described (cite DHODH paper). Briefly, a UHPLC-MS system consisting of an Agilent 6550 qToF coupled to an Agilent 1290 binary pump UHPLC system was used. The source parameters were as follows: Gas temperature, 150°C at 14 L/min and 45 psig; Sheath gas temperature, 325°C at 12 L/min; Capillary and nozzle voltages were set to -2.0 kV. iFunnel conditions were changed from default to -30 V DC, High pressure funnel drop -100 V and RF voltage of 110 V, low pressure funnel drop -50 V and RF voltage of 60 V. Chromatographic separation

was achieved by ion-paired chromatography. In brief, 2 mL of each sample was injected onto Agilent ZORBAX® Extend-C18 (150 mm 3 2.1 mm i.d.; 1.8 mm) column using tributylamine (TBA) as an ion pairing agent (solvent A: 3% methanol, 97% water 10 mM TBA, 15 mM Acetic acid, solvent B: 100% methanol). The linear gradient employed was as follows: 0–2.5 min 99% A, 2.5–7.5 min decrease to 80% A, 7.5–13 min to 55% B and finally 13–15 min to 99% B and held for 1 min. The column was re-generated for 2 min at 1% B. The flow rate was set to 250 mL/min. The column temperature was maintained at 25°C. Skyline was used for data processing of metabolites in a library of standards using known retention times and MS/MS spectra. Integrated peak areas for the metabolites were exported for further statistical and metabolite enrichment analysis by using online MetaboAnalyst®.

Generation of IMPDH knockout lines

[00291] Guide RNAs (gRNAs) targeting AAVS1 (5'-GGGGCCACTAGGGACAGGAT-3', SEQ I.D. No. 1) and IMPDH1 B: (5'-ACCGCGGTGTGTAACACAGCCA-3') and IMPDH2 A: (5'-aCCGTCCATGGGAGAGGAAACCAG-3' SEQ I.D. No. 2) were obtained from TKOv3 (Hart T. et al, G3 Genes/Genomes/Genetics. 2017; 7 (8):2719–2727 and cloned into a single-gRNA lentiCRISPRv2 construct (Addgene 52961). Sequences were verified using Sanger sequencing. Each plasmid was packaged independently into lentivirus using second-generation packaging constructs as described previously (Hart. T. et al., Cell. 2015; 163(6):1515-26. BMICs were infected with lentivirus containing single-gRNA lentiCRISPRv2 constructs targeting AAVS1 or IMPDH1 or IMPDH2 (three gRNAs). Twenty-four hours post-infection, virus-containing media was replaced with fresh media containing puromycin (1-2 µg/mL) (ThermoFisher, Cat#A1113803) for 48-72 hours. The knockout efficiency was validated by Western Blotting for evaluation of IMPDH protein expression.

Statistical analysis

[00292] Replicates from at least three samples were used for all applicable experiments. Data collected from respective *in vitro* experiments were represented using GraphPad Prism 6 software. Student t tests and two-way ANOVA analyses were conducted using the same software, with a p-value < 0.05 deemed as statistically significant. For *in vivo* studies, medium survival differences were measured using Kaplan-Meier survival curves and significance determined by the Log rank test. Kaplan-Meier survival curves were plotted and analyzed using GraphPad Prism software.

iii. Results

Connectivity Map analysis of pre-metastatic BMIC gene expression profile reveals mycophenolic acid as a selective BMIC inhibitor *in vitro*.

[00293] Preclinical models of lung-, breast-, and melanoma-BM using three different injection routes: (i) orthotopic, (ii) intracardiac, and (iii) intracranial have been established (Singh, M., et al. (2017). *Acta Neuropathol.* 134, 923–940; Singh, M., et al. (2018) *Cancer Res* 78, 5124–5134; Bassey-Archibong, B., et al. (2023). *PNAS* 120; Singh, M., et al. (2019), *In Vivo Murine Models of Brain Metastasis*. In, pp. 231–238). In brief, BM tumors surgically removed from BM patients are processed and cultured in tumorsphere-enriching media to establish BMIC lines; BMIC lines are subsequently injected into NSG mice *via* orthotopic (lung, fat pad, or subcutaneous for lung-, breast-, and melanoma-BM, respectively), intracardiac, or intracranial routes. These orthotopic models of lung-, breast-, and melanoma-BM are able to capture BMICs at their early or “pre-metastatic” stage of BM development where BMICs have seeded the brain but have not yet formed metastatic lesions that are visible by immunohistochemistry. The transcriptomic profiles of premetastatic lung-, breast-, and melanoma-BMICs were studied by RNA sequencing and found that they are distinct from their BMIC line counterparts.

[00294] To identify potential druggable targets for BM, the transcriptomes of pre-metastatic lung-, breast-, and melanoma- BMICs were first characterized (Bassey-Archibong et al., *Proc Natl Acad Sci USA* 2023 120(8):e2205247120) (*Cancer Res.* 2018;78(17):5124-5134). 3,951 genes that are commonly differentially expressed in the pre-metastatic cohort of cells compared to those isolated from established patient tumors were identified (Bassey-Archibong et al., *Proc Natl Acad Sci USA* 2023 120(8):e2205247120). The shared gene signature of pre-metastatic BMICs (defined by the commonly shared gene signature from BMICs of all three primary tumor cohorts) were inputted as a query signature for computational CMap analysis to identify compounds that can evoke opposing transcriptional changes. The goal of using CMap was to generate testable hypotheses about drugs that have not yet been characterized in certain disease contexts, where a query gene signature (i.e., pre-metastatic gene signature) is compared against a reference database containing signatures representing a change in cellular state in response to a drug, gene, disease, or other perturbation. 380 compounds were suggested by CMap to affect the pre-metastatic signature, of which 194 were predicted to have an ‘opposing’ effect. Only, 48 candidate compounds were identified whose activity oppose a premetastatic transcriptomic query signature (*Science* 2006;313(5795):1929-1935.) . To validate these findings, all 48 compounds were screened for their capacity to affect the viability of a patient-derived lung BMIC line (BT478) at a concentration of 10μM.

Several (e.g., seven) compounds were identified belonging to diverse chemical families that significantly inhibited BMIC viability. One such compound, apomorphine, has previously been reported to block lung-BM (*Cancer Res.* 2018;78(17):5124-5134), thus, validating this target discovery pipeline.

[00295] The identified compounds were fully evaluated based on the criteria of novelty in brain metastasis treatment, potential toxicity for their current indication(s). One criteria of interest in selecting a lead compound was synthetic tractability since evidence of blood-brain barrier (BBB) permeability was not found. The compounds would need to be chemically modified to enhance BBB penetration. The natural product MPA (I-7) was selected as the lead compound for further study because it possessed a relatively high anti-BMIC activity against multiple patient-derived BMIC lines from lung-, breast-, and melanoma-BM below its clinically relevant concentration of 10 μ M (Neumann, I., et al., *Nephrol. Dial. Transplant.* 2008;23(11):3514-3520), which is nontoxic to neural stem cells at the same concentrations (Figure 1 A). Furthermore, exemplary compound MPA (I-7) also inhibited BMIC proliferation over time (Figure 1 B) and significantly reduces the frequency of stemness in BMIC lines in both limiting dilution assays (Figure 1 C) and s clonogenic sphere formation, which are *in vitro* surrogate measure for stem cell self-renewal (Figure 1 D). This suggests that MPA is targeting the stem-like properties of BMICs that are presumed to drive their tumor-initiating properties, which allow them to evade conventional therapies (Singh, M., et al. (2014). *Int. J. Mol. Sci.* 15, 9117–9133). Finally, using an *in vitro* wound healing assay it was determined that exemplary compound MPA (I-7) inhibited the migration of patient derived BMICs (Figure 1 E), suggesting that exemplary compound MPA (I-7) targets phenotypes relevant to the metastatic tumor initiation cascade (Lopez T. et al., *Biomedicines.* 2022;10(11):2784; Flier J.S., et al. *N Engl J Med.* 1986;315(26):1650-1659).

[00296] Clinically, exemplary compound MPA (I-7) is an approved immunosuppressant used for the prophylaxis of organ rejection in transplant patients (*Immunopharmacology* 2000;47(2-3):85-118 & *Cancers (Basel)* 2019;11(9)). However, to the best of the Applicant's knowledge, neither MPA or its target, IMPDH, have been previously implicated in BM. Exemplary compound MPA (I-7) is predicted to not penetrate the BBB effectively and has not been previously considered for preventing brain cancer. Nonetheless, due to its selectivity toward BMIC inhibition over normal brain cell controls *in vitro* and the lack of targeted therapies for BM that can extend patient survival, exemplary compound MPA (I-7) was utilized as a starting point to uncover new targets for BM research

while developing BBB permeable analogues of MPA to confirm the targets therapeutic relevance preclinically.

Exemplary compound mycophenolic acid (I-7) slows BM progression in mice in an *ex vivo* treatment PDX model

[00297] It was next determined whether exemplary compound MPA (I-7) can impact the ability BMICs to recapitulate BM *in vivo* using an established lung- and melanoma-BM patient derived xenografts (PDX) models (Bassey-Archibong et al., Proc Natl Acad Sci USA 2023 120(8):e2205247120). To assess whether exemplary compound MPA (I-7) treatment would affect brain tumor formation, BMICs were treated *in vivo* with either exemplary compound MPA (I-7) (at its 80% maximal inhibitory concentration; IC₈₀) or the placebo. A clonogenic secondary sphere formation assay showed that MPA (I-7)-treated BMICs did not regain sphere forming capability following MPA (I-7) removal from the culture media. While not being bound by theory, this suggests that MPA (I-7)'s effect on BMICs is either irreversible or that MPA (I-7) is targeting an important pathway for BM formation. Thereafter, following *in vitro* pretreatment of BMICs with MPA (I-7) or DMSO control, equal numbers of viable tumor cells were orthotopically engrafted into immune-compromised mice (Figure 2 A). Mice engrafted with exemplary compound MPA (I-7) -treated BMICs showed a significantly reduced brain tumor burden two weeks post-injection and survived significantly longer than mice injected with placebo-treated BMICs. This phenotype was recapitulated in both lung- and melanoma-BM PDX models (Figure 2 B-E).

[00298] To obtain a clinically relevant correlate to the data above, two patient-derived lung adenocarcinoma samples were acquired; one that was derived from a patient who developed BM following their primary lung tumor diagnosis (CRUK0748) and one that was derived from a patient who, to date, has not developed BM (CRUK0733). BM initiating capacity was confirmed in the PDX models. The tagged CRUK0748 cells from the brains of mice were harvested and cultured in stem cell-enrichment media conditions to derive a CRUK0748-BMIC line. MPA was tested against CRUK0748-BMICs in a dose response assay, which confirmed that MPA inhibits BMIC within the primary CRUK0748 lung sample (Figure 3).

[00299] Once it was observed that exemplary compound MPA (I-7) effected BM initiation in the brain following *in vivo* treatment (see Figure 2 B-E), exemplary compound MPA (I-7) was examined to determine whether it could inhibit metastasis. To determine whether exemplary compound MPA (I-7) slows the spread of metastasizing BMICs from a primary tumor to the brain, primary CRUK0748 lung tumor cells were treated *ex vivo* with

either exemplary compound MPA (I-7) or placebo control before injecting the cells into the intrathoracic cavity of mice (see Figure 2 B). Mice orthotopically injected with MPA-pretreated cells experienced a significant increase in median survival time of six days compared to the mice injected with placebo-treated cells (fewer TRA-1-85-positive human cells) (Figure 2 F). Since mice succumb to their primary tumor burden, their brains were sorted for the human cell marker TRA-1-85 by flow cytometry to assess MPA activity toward metastasis. Mice bearing tumors treated with MPA had significantly fewer TRA-1-85-positive cells detected in their brains (Figure 2 G). Taken together, the data obtained from these *in vivo* studies demonstrate that metastatic brain tumor formation is significantly slowed following a three-day *ex vivo* treatment of BMICs with exemplary compound MPA (I-7). Furthermore, a secondary sphere formation assay showed that MPA-treated BMICs do not regain sphere forming capability following MPA removal from the culture media. While not being bound by theory, this suggests that exemplary compound MPA's (I-7) effect on BMICs is either irreversible or that exemplary compound MPA (I-7) is targeting an important pathway for BM formation (Figure 2 H).

Blood-brain-barrier penetrance and BM preventative therapy.

[00300] It was next considered whether exemplary compound MPA (I-7) could target premetastatic BMICs in the circulation in a more clinically relevant *in vivo* treatment model. To this end, mice were intracardiac-injected with patient-derived lung BMICs and began treating them daily with either exemplary compound MPA (I-7) or placebo by oral gavage (Figure 4 A). MPA-treated mice had a significantly reduced brain tumor burden seven days post injection (Figure 4 B), suggesting that the BMICs were being effectively targeted outside of the brain cavity. However, a difference in brain tumor burden was no longer observed 14 days post-injection, and all mice reached humane endpoint at a similar timepoint, regardless of treatment. While not being bound by theory, these results suggest that exemplary compound MPA (I-7) targets peripheral, but not central, BMICs due to its limitation of crossing the BBB.

[00301] Next, mice were injected orthotopically with the primary lung CRUK0748 cell line and treated daily as described above. In this model, MPA-treated mice survived significantly longer than their placebo-treated counterparts, suggesting that exemplary compound MPA (I-7) slowed the growth of their primary lung tumors. Once humane endpoint was reached, there was no significant difference in the number of human cells detected in mouse brains as determined by flow cytometry (Figure 4 C). While this contradicts results from the *ex vivo* orthotopic treatment model, not wishing to be bound by

theory, it is speculated that BMICs can escape exemplary compound MPA (I-7) treatment in the circulation once they penetrate the BBB to seed the brain. These data suggest that BBB-penetrance may be a limitation to exemplary compound MPA's (I-7) ability to slow BM and therefore a good property of a potential anti-BM therapy.

Design and synthesis of BBB-permeable MPA derivatives.

[00302] It was hypothesized that the poor BBB penetration of exemplary compound MPA (I-7) is likely due at least in part to the carboxylic acid functionality ($pK_a \sim 5$). since CNS-active drugs are generally neutral or basic (pK_a 7.5–10.5) (*J. Med. Chem.* 2021;64(18):13152-13173 & *NeuroRx*. 2005;2(4):541-553). The permeability of carboxylic acids across membranes may be improved by masking the charge through ester prodrugs which may provide improved lipophilicity and which can be cleaved by cellular esterases *in vivo* (*Nat. Rev. Drug Discov.* 2008;7(3):255-270.). The 2-morpholinoethyl ester of exemplary compound MPA (I-7), which is called mycophenolate mofetil or MMF (I-1), was developed to improve the oral availability of exemplary compound MPA (I-7) and has been in clinical use since 1995 (*Transpl. Proc.* 1990;22:1659–1662; US4753935A; *Lancet* 1996;348(9038):1357-1359). Here, a series of MPA derivatives was developed and synthesized, and tested for their BBB permeability, with sidechains bearing tertiary amines of the appropriate basicity to improve both permeability and solubility. The activities of the esterase-cleavable esters (I-1 – I-3) were compared with more metabolically stable amide derivatives (I-4 – I-6), since MPA amides have been reported as IMPDH inhibitors previously (*Bioorg. Med. Chem. Lett.* 2020;30(24):127543 & *J. Enzyme Inhib. Med. Chem.* 2018;33(1):972-977) albeit never designed to prioritize BBB permeability.

[00303] Each analogue's selectivity for BMICs was evaluated using patient derived BMIC lines and neural stem cells as a normal brain cell control. A summary of each analogue's activity in the dose-response assays is provided in Table 1.

Table 1

Compound I. D.	Lung-BM IC ₅₀ (μM)	Neural stem cell IC ₅₀ (μM)
MPA (I-7)	3.61	N/A
MMF (I-1)	48.0	31.2
I-2	17.1	353
I-3	15.3	N/A
I-4	N/A	493

I-5	N/A	182
I-6	24.7	70.8

[00304] One of the analogues, exemplary compound I-3, was selected for *in vivo* preclinical study because it retained exemplary compound MPA's (I-7) therapeutic window in its selectivity for BMICs compared to normal brain cells (Figure 5 A) and was suggested to be BBB-penetrant using the *in vitro* parallel artificial membrane permeability assay (PAMPA, Figure 5 B).

[00305] To explore active transport/efflux mechanisms, a MDCK-MDR1 cell monolayer model was used to show that both exemplary compounds MPA (I-7) and I-3 have a high permeability for entering the brain and are not significant substrates for P-glycoprotein (ABCB1), which is known to play a key role in limiting small molecules from entering the brain (Table 2).

Table 2

	Mean P_{app} (10^{-6} cm/s) A to B	Mean P_{app} (10^{-6} cm/s) B to A	Efflux Ratio	Rank of P_{app}
Mycophenolic Acid (MPA, I-7)	26.0	10.0	0.384	High
I-3	14.8	12.5	0.848	High
Digoxin (P-gp substrate)	0.556	11.8	21.2	Low
Nadolol (Low permeability)	0.198	Not Detected	-	Low
Metoprolol (High Permeability)	29.5	Not Detected	-	High

[00306] Additionally, the similar efflux ratio results between exemplary compounds MPA (I-7) and I-3 in MDCK-MDR1 assays suggests that the ester linkage does not substantially alter the active transport mechanisms. Finally, to explore if there is differential partitioning of either exemplary compounds MPA (I-7) and I-3 in brain homogenate, the free and bound drug concentrations were explored. The composition of plasma and brain are quite different (>20-fold more lipids in the brain, >2 fold more protein in the plasma) and mouse brain homogenate was treated with each compound followed by equilibrium dialysis and mass spectrometry to determine the fraction of bound (65.9%) and un-bound drug (34.1%) for MPA (I-7)(Table 3).

Table 3

	% Unbound	% Bound
Mycophenolic Acid (MPA, I-7)	34.1 ± 3.0	65.9 ± 3.8
I-3	Not Recovered	Not Recovered
Propranolol (Control Compound with High Binding)	2.3 ± 0.0	97.6 ± 0.3

[00307] This is a relatively high fraction of un-bound drug, consistent with the efficacy of the MPA (I-7) observed. However, the mass of exemplar compound I-3 could not be detected following completion of the assay, and a proper bound/unbound fraction could not be determined. This suggests that compound I-3 is metabolized within the brain homogenate, and subsequently, likely abides by the bound-/unbound- ratios determined for MPA (I-7).

A BBB-permeable analogue of mycophenolic acid slows BM *in vivo*.

[00308] To examine whether exemplary compound I-3 increased BBB-permeability leads to improved anti-tumor activity compared to exemplary compound MPA (I-7), mice were intracranially injected with lung-BMICs and treated daily by oral gavage with either vehicle, exemplary compound MPA (I-7), or exemplary compound I-3 (Figure 5 C). The cells were injected intracranially to ensure that any survival benefit would be due to the compounds crossing the BBB and targeting BMICs in the brain. Mice treated with exemplary compound I-3 showed a significant survival advantage in this model compared to both exemplary compound MPA (I-7) and vehicle treated groups, whereas there was no survival advantage for MPA-treated mice compared to vehicle (Figure 5 D). This suggested that enhancing the BBB-permeability of exemplary compound MPA (I-7) can be done without sacrificing its biological selectivity towards BMICs over normal brain cells, and indicated that a compound that can still reach the tumor cells after brain colonization is critical for longer lasting effects.

Mechanistic studies suggest IMPDH activity as a targetable vulnerability in BMICs.

[00309] In parallel to the preclinical studies described above, it was examined whether exemplary compound MPA's (I-7) known target, IMPDH, is the relevant target in the context of its anticancer mechanism of action. IMPDH is the first rate-limiting enzyme in *de novo* GTP synthesis (Figure 6 A). It is an established druggable target known to be upregulated in highly proliferating cells, such as T cells and B cells (*Cancers (Basel)*. 2019;11(9)). To confirm whether IMPDH is relevant in exemplary compound MPA's (I-7)

efficacy against BMICs, it was first confirmed that a structurally distinct and selective IMPDH-inhibitor, merimepodib (*Org. Process Res. Dev.* 2008;12(4):666-673.), displayed a similar dose-response effect against the patient-derived lung BMICs (Figure 7 A).

[00310] Highly proliferative cells – like T cells and B cells – rely on *de novo* GTP synthesis to keep up with their high metabolic demands, whereas BMICs, similarly to other cancer stem-like cells, are characterized by a slower rate of proliferation. Therefore, to understand why slowly proliferating BMICs are vulnerable to perturbations in *de novo* GTP synthesis, their metabolic landscape was considered. LCMS-based metabolomics profiling were used to map the differential polar metabolome of vehicle- or MPA or exemplary compound I-3-treated BM cells in comparison to normal human astrocytes. Consistent with on-target IMPDH inhibition, GDP and GTP levels were significantly reduced with both exemplary compound MPA (I-7) and exemplary compound I-3 treatment, (Figure 6 B) (*Nat. Commun.* 2020;11(1):3811), while aminoimidazole carboxamide ribonucleotide (AICAR), an intermediate in the *de novo* purine synthesis pathway upstream of IMPDH, was shown to accumulate with drug treatment (Figure 6 C). This suggests that exemplary compound I-3 acts on target and has the same metabolic profile as exemplary compound MPA (I-7) for both cell lines tested, and further suggests that BMICs cannot rely on the salvage pathway (see Figure 6 A) to sufficiently fulfill their GTP pools. Notably, MPA and exemplary compound I-3 treatment did not influence the levels of dihydroorotic acid (Figure 7 B), a key metabolite in pyrimidine biosynthesis, suggesting that the drug's effect is specific to the purine biosynthesis pathway. Taken together, it has been shown that *de novo* GTP synthesis is a critical vulnerability in metastatic brain tumors and that IMPDH is a tractable target within this pathway.

[00311] It was reasoned that if IMPDH is indeed the target of MPA in this biological context, then exogenous guanine supplementation (i.e., upregulating the nucleotide salvage GTP synthesis pathway, which IMPDH is not involved in, see Figure 6 A) should fully rescue exemplary compound MPA (I-7) and exemplary compound I-3's anti-BMIC phenotype by stimulating the purine nucleotide salvage pathway. As expected, exogenous guanine supplementation to cell culture media (12 μ M) rescued BMIC viability after chemical IMPDH perturbation with both exemplary compound MPA (I-7) and exemplary compound I-3 (Figure 6 D). This suggests that BMICs may be reliant on *de novo* GTP synthesis, potentially due to a purine salvage deficiency and/or high glucose availability in the brain (see Figure 6 D) (*Physiol. Rev.* 2019;99(1):949-1045).

[00312] To confirm on-target activity of MPA and exemplary compound I-3 against IMPDH, two patient-derived lung-BMIC cell lines, BT478 and BT530 were used, to generate *IMPDH* knockout lines. In parallel, the safe-harbor locus, *AAVS1*, was knocked out as a control cell line. Knockouts were validated by western blot (Figure 6 E). Loss of *IMPDH* led to tumor cell death (Figure 6 F). Furthermore, *IMPDH* knockout cell lines exhibited a significantly reduced sphere formation capacity compared to *AAVS1* control cell lines (Figure 6 G). Taken together, these data provide direct mechanistic evidence that IMPDH is the target responsible for exemplary compound MPA (I-7) and exemplary compound I-3's anti-tumor phenotypes and that it is an important driver of BMIC proliferation.

iv. Discussion

[00313] BM remains the most common adult brain tumor and the most understudied due to its dismal prognosis and lack of clinically relevant experimental models (Patchell, R.A., *Cancer Treat. Rev.* 2003; 29(6):533-540 & Gupta, G.P., and Massagué, J. *Cell* 2006;127(4):679-695). Current therapies are mainly palliative, highlighting the urgent need for new therapeutic strategies. To address this unmet clinical need, a phenotypic drug screening strategy was employed to identify tool compounds that can be used to unravel promising new targets for BM research. Unlike target-based drug screening, phenotypic drug screening intends to identify compounds capable of evoking a desired pharmacological effect (i.e., a compound that kills BM-initiating cells (BMICs) without affecting noncancerous brain cell controls) (Kiriiri, G.K., et al., *Futur. J. Pharm. Sci.* 2020;6(1):27). This mode of drug discovery establishes therapeutic relevance earlier in the drug discovery pipeline and enhances the chances of serendipitous discoveries because it does not require prior knowledge of the mechanism of action; this latter point greatly reduces the otherwise common problem of drug off-target effects, which often occurs with target-based drug screening (Lin, A., et al., *Sci. Transl. Med.* 2019;11(509)).

[00314] Here, exemplary compound MPA (I-7) was used as a starting point to reveal IMPDH as a therapeutically tractable target for BM research. A BBB-permeable analogues of exemplary compound MPA (I-7) were synthesized. Using an *in vivo* BM model, it was found that exemplary compound I-3 increased survival relative to both control and exemplary compound MPA (I-7). In this study, it was shown that IMPDH inhibition in BM acts on-target to inhibit *de novo* GTP biosynthesis leading to purine nucleotide depletion (Zhou, W., et al., *Nat. Commun.* 2020; 11(1):3811). Complete phenotypic rescue with the addition of guanine supports this mechanism of action.

[00315] Altered metabolism is a hallmark of cancer (Hanahan, D., and Weinberg, R.A., *Cell* 2011; 144(5):646-674) and the level of biology closest to phenotype (Zhou, W., and Wahl, D.R., *Mol. Cell Oncol.* 2020; 7(6):1834902), making it an attractive process to target in regulating cancer cell growth. Stem-like glioma cells have been recently reported to reprogram their metabolism to aid in self-renewal, implicating *de novo* GTP synthesis as a cancer dependency in primary brain tumors (Bao, S., et al., *Nature* 2006; 444(7120):756-760). The purine nucleotide GTP can be biosynthesized by the salvage pathway or by the *de novo* pathway in cells (Naffouje, R., et al., *Cancers (Basel)* 2019; 11(9).). Whereas the salvage pathway uses available purine nucleosides to produce purine mononucleotides, *de novo* biosynthesis is an energy-demanding process that is upregulated in many types of cancers; proliferating B and T lymphocytes are solely dependent on the *de novo* pathway for purine biosynthesis. Further, the normal adult brain has lower demands for GTP synthesis and preferentially favors purine salvage (Wen, P.Y., et al., *Neuro Oncol.* 2012; 14(7):819-829 & Allsop, J., and Watts, R.W.E., Purine Synthesis and Salvage in Brain and Liver. In: Springer, Boston, MA; 1984:21-26), which suggests that the salvage pathway is defective or insufficient in BM thus creating a vulnerability through targeting the *de novo* pathway, rendering IMPDH inhibition as a tractable and nontoxic target for BM with a potentially high therapeutic window.

[00316] Purines are the building blocks of DNA and are involved in many cellular processes. It has been shown that exemplary compound MPA (I-7) treatment and subsequent IMPDH inhibition interfering with various steps of the cell cycle, which ultimately suppresses cell proliferation (Benjanuwattra, J., et al., *Eur. J. Pharmacol.* 2020; 887:173580). MPA has been implicated as an anti-tumor drug through the suppression of *de novo* purine synthesis which has also been shown to contribute to the aggressive nature of the primary brain tumors. Elevated rates of *de novo* purine synthesis have been shown to maintain the tumorigenic capacity of glioma-initiating cells and contribute to enhanced DNA repair in radiation-resistance glioblastoma. A similar dependency for GTP was seen in the examples herein, showing that targeting IMPDH could be beneficial for metastatic brain tumors.

[00317] In other cancers, MPA treatment and subsequent IMPDH inhibition has been shown to interfere with various steps of the cell cycle, which ultimately suppresses cell proliferation. In some cancers, exemplary compound MPA (I-7) induces differentiation and senescence, with evidence of interfering with cell binding to human umbilical vein endothelial cells, migration into an endothelial cell monolayer, and decreased angiogenesis in the context of vasculitis (Huang, Y., et al., *Int. Immunopharmacol* 2005; 5(6):1029-1039).

[00318] Collectively, the present studies have shown *de novo* purine synthesis to be implicated as a metabolic vulnerability in BM that is targetable through its rate-limiting enzyme, IMPDH. IMPDH inhibitors are already an FDA-approved class of drugs used clinically as clinically, making the barrier to clinical translation low^{43,58}, which is particularly important in this patient population, whose median survival remains at 4-12 months (Zhou, W., et al., *Nat. Commun.* 2020; 11(1):3811 & Wang, X., et al., *Nat. Neurosci.* 2017; 20(5):661-673). Furthermore, the notion that IMPDH inhibitors are selective toward BMICs but seemingly nontoxic to normal brain tissue has warranted further research into the metabolic profiles of BMICs and the optimization of brain-penetrant IMPDH-inhibitors for clinical translation. If effective, targeting *de novo* GTP synthesis in BMICs could slow their metastatic ability and serve as a first-in-class anti-BM therapy, while translating to the future development of other anti-cancer therapies for tumors with the same metabolic dependencies.

[00319] While the present application has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the application is not limited to the disclosed examples. To the contrary, the present application is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[00320] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present application is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

FULL CITATIONS FOR DOCUMENTS REFERRED TO IN THE SPECIFICATION

[00321] A number of publications are cited herein. Full citations for these references are provided below. Each of these references is incorporated herein by reference in its entirety into the present disclosure, to the same extent as if each individual reference was specifically and individually indicated to be incorporated by reference.

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CLAIMS:

1. A method of treating or preventing brain metastasis of a cancer comprising administering a therapeutically effective amount of an inosine monophosphate dehydrogenase (IMPDH) inhibitor to a subject in need thereof.
2. The method of claim 1, wherein the method is for preventing brain metastasis of the cancer in a subject in need thereof.
3. The method of claim 1, wherein the treating or preventing brain metastasis of the cancer is by inhibiting or delaying the brain metastasis of the cancer.
4. The method of claim 3, wherein the brain metastasis of the cancer is delayed by about 1 month, about 2 months, about 3 months, about 4 months, about 6 months, about 8 months, about 10 months, about 12 months, about 14 months, about 18 months, about 20 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years or more.
5. The method of claim 1, wherein the treating or preventing brain metastasis of a cancer is by inhibiting brain-metastasis initiating cell (BMIC) activity.
6. The method of claim 5, wherein inhibiting BMIC activity inhibits the metastatic progression of BMICs to the brain and/or in the brain.
7. The method of claim 6, wherein inhibiting metastatic progression of BMICs to the brain inhibits the infiltration of cancer cells in the brain.
8. The method of claim 5, wherein inhibiting BMIC activity inhibits the migration of brain-metastasis initiating cells (BMICs) to the brain.
9. The method of claim 5, wherein inhibiting BMIC activity inhibits growth and survival of BMICs in the brain.
10. The method of claim 5, wherein inhibiting BMIC activity reduces the risk of brain metastasis of a cancer.
11. The method of claim 5, wherein inhibiting BMIC activity lengthens the period of survival of a subject having the cancer or a subject that has had the cancer.
12. The method of claim 11, wherein the survival period is lengthened by about 1 month, about 2 months, about 3 months, about 4 months, about 6 months, about 8 months, about 10 months, about 12 months, about 14 months, about 18 months, about 20 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years or more.

13. The method of any one of claims 5 to 12, wherein the BMICs are premetastatic BMICs.
14. The method of any one of claims 1 to 13, wherein the cancer is selected from lung cancer, breast cancer, melanoma, colon cancer, kidney cancer, renal cell carcinoma, mesothelioma, ovarian cancer, pancreatic cancer, sarcoma, leukemia, lymphoma, urothelial cancer, head and neck cancer, osteosarcoma and bladder cancer.
15. The method of claim 14, wherein the cancer is selected from lung cancer, breast cancer and melanoma.
16. The method of any one of claims 1 to 15, wherein the cancer is a primary cancer.
17. The method of any one of claims 1 to 16, wherein the subject in need thereof is a subject having the cancer or a subject that has had the cancer.
18. A method of producing an anti-cancer effect in subject having a cancer or a subject that has had a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor to a subject in need thereof.
19. A method of treating or preventing a brain metastasis of a cancer comprising administering a therapeutically effective amount of an IMPDH inhibitor in combination with another known agent useful for treating or preventing a brain metastasis of a cancer and/or in combination with another known agent for treating cancer in a subject in need thereof.
20. The method of any one of claims 1 to 19, wherein the IMPDH inhibitor is any agent that inhibits expression of IMPDH gene or protein, that induces IMPDH protein degradation or that inhibits IMPDH protein activity.
21. The method of claim 20, wherein the agent inhibits expression of IMPDH gene or protein and inhibiting expression of IMPDH gene or protein is by IMPDH gene knockdown, IMPDH gene knockout or by IMPDP gene editing.
22. The method of claim 20, wherein the agent inhibits expression of IMPDH gene or protein and the agent is an antisense oligonucleotide complementary to an IMPDH DNA or RNA sequence or a variant or fragment thereof.
23. The method of claim 20, wherein the agent inhibits expression of IMPDH gene or protein and the agent is a nucleic acid selected from small interfering RNA (siRNA), dicer substrate DNA, hairpin RNA, microRNA (miRNA), RNAi and splice-regulating oligonucleotides.
24. The method of claim 20, wherein the agent inhibits expression of IMPDH gene or protein and the agent is a gene editing system.

25. The method of claim 24, wherein gene editing system is a clustered regularly interspaced short palindromic repeat (CRISPR)-Cas system (CRISPR system); zinc finger nuclease (ZFN) system, or transcription activator-like effector-based nuclease (TALEN) system.

26. The method of claim 20, wherein the agent inhibits expression of IMPDH protein or inhibits IMPDH protein activity and the agent is an antibody that specifically binds IMPDH or an antigen binding fragment thereof.

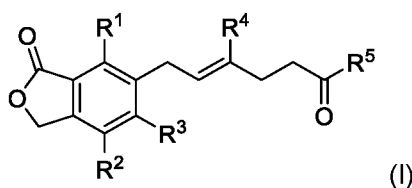
27. The method of claim 20, wherein the IMPDH inhibitor is any agent that induces IMPDH protein degradation.

28. The method of claim 27, wherein the agent that induces IMPDH protein degradation is a targeted IMPDH protein degrader.

29. The method of claim 28, wherein the targeted IMPDH protein degrader is selected from an IMPDH targeting proteolysis targeting chimera (PROTAC), an IMPDH targeting molecular glue degrader, a selective estrogen receptor degrader (SERD), an IMPDH targeting monoclonal antibody or an IMPDH targeting antibody-drug conjugate.

30. The method of claim 20, wherein the IMPDH inhibitor is any agent that inhibits IMPDH protein activity and the agent that inhibits IMPDH protein activity is a small molecule inhibitor of IMPDH protein activity.

31. The method of claim 30, wherein the small molecule inhibitor of IMPDH protein activity is a compound of Formula I or a pharmaceutically acceptable salt, prodrug and/or solvate thereof,



wherein

R¹ is selected from OH, halo, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R² is selected from H, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R³ is selected from H, C₁₋₄alkyl, C₁₋₄haloalkyl, C₁₋₄alkoxy and C₁₋₄haloalkoxy;

R⁴ is selected from H, C₁₋₄alkyl and C₁₋₄haloalkyl;

R^5 is selected from R^6 , OR^6 , NR^6R^7 , $OC_{1-6}alkenyleneNR^6R^7$ and $NR^8C_{1-6}alkenyleneNR^6R^7$;

R^6 is selected from H, $C_{1-6}alkyl$, $C_{1-6}haloalkyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{1-6}alkyleneC_{3-10}cycloalkyl$, and $C_{1-6}alkyleneC_{3-10}heterocycloalkyl$, the latter six groups being optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO_2 , CHO, $C_{1-6}alkyl$, $C_{1-6}haloalkyl$, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$;

R^7 is selected H, $C_{1-6}alkyl$ and $C_{1-6}haloalkyl$; or

R^6 and R^7 are joined to form, together with the nitrogen atom therebetween, a 3- to 12-membered heterocycloalkyl optionally containing one or two additional heteromoiety selected from N, NR^{11} , O, S, S(O), and SO_2 and optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO_2 , CHO, $C_{1-6}alkyl$, $C_{1-6}haloalkyl$, $NR^{12}R^{13}$, OR^{12} , CO_2R^{12} and $C(O)NR^{12}R^{13}$;

R^8 , R^9 and R^{10} are independently selected H, $C_{1-6}alkyl$ and $C_{1-6}haloalkyl$;

R^{11} is selected from H, $C_{1-6}alkyl$, $C_{1-6}haloalkyl$, $CO_2C_{1-6}alkyl$ and $CO_2C_{1-6}haloalkyl$; and

R^{12} and R^{13} are independently selected H, $C_{1-6}alkyl$ and $C_{1-6}haloalkyl$.

32. The method of claim 31, wherein R^1 is selected from OH, F, Cl, $C_{1-4}alkoxy$ and $C_{1-4}haloalkoxy$.

33. The method of claim 32, wherein R^1 is OH.

34. The method of any one of claims 31 to 33, wherein R^2 is selected from H, $C_{1-4}alkyl$, $C_{1-4}chloroalkyl$, $C_{1-4}fluoroalkyl$, $C_{1-4}alkoxy$, $C_{1-4}chloroalkoxy$ and $C_{1-4}fluoroalkoxy$.

35. The method of claim 34, wherein R^2 is CH_3 .

36. The method of any one of claims 31 to 35, wherein R^3 is selected from H, $C_{1-4}alkyl$, $C_{1-4}chloroalkyl$, $C_{1-4}fluoroalkyl$, $C_{1-4}alkoxy$, $C_{1-4}chloroalkoxy$ and $C_{1-4}fluoroalkoxy$.

37. The method of claim 36, wherein R^3 is OCH_3 .

38. The method of any one of claims 31 to 37, wherein R^4 is selected from H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $C(CH_3)_3$, CF_3 , CFH_2 , CHF_2 , CH_2CF_2H , CH_2CF_3 , CH_2CFH_2 and $C(CF_3)_3$.

39. The method of claim 38, wherein R^4 is CH_3 .

40. The method of any one of claims 31 to 39, wherein R^5 is selected from R^6 , OR^6 , NR^6R^7 , $OC_{1-5}alkenyleneNR^6R^7$ and $NR^8C_{1-4}alkenyleneNR^6R^7$.

41. The method of claim 40, wherein R^5 is R^6 .

42. The method of claim 40, wherein R^5 is selected from OR^6 , NR^6R^7 , $OC_{1-5}alkenyleneNR^6R^7$ and $NR^8C_{1-4}alkenyleneNR^6R^7$.

43. The method of claim 40, wherein R^5 is selected from NR^6R^7 , $OC_{1-5}alkenyleneNR^6R^7$ and $NR^8C_{1-4}alkenyleneNR^6R^7$.

44. The method of any one of claims 40 to 43, wherein R^6 is selected from H, $C_{1-6}alkyl$, $C_{1-6}haloalkyl$, $C_{3-10}cycloalkyl$, $C_{3-10}heterocycloalkyl$, $C_{1-4}alkyleneC_{3-10}cycloalkyl$ and $C_{1-4}alkyleneC_{3-10}heterocycloalkyl$, the latter six groups being optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO_2 , CHO, $C_{1-6}alkyl$, $C_{1-6}haloalkyl$, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$.

45. The method of claim 44, wherein R^6 is selected from H, $C_{1-6}alkyl$, $C_{1-6}fluoroalkyl$ and $C_{1-6}chloroalkyl$, the latter three groups being optionally substituted with one to four substituents selected from OH, CN, NO_2 , CHO, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$.

46. The method of claim 44, wherein R^6 is selected from $C_{3-8}cycloalkyl$ and $C_{1-4}alkyleneC_{3-8}cycloalkyl$ optionally substituted with one to three substituents selected from OH, F, Cl, CN, NO_2 , CHO, $C_{1-6}alkyl$, $C_{1-6}fluoroalkyl$, $C_{1-6}chloroalkyl$, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$.

47. The method of claim 44, wherein R^6 is selected from $C_{3-10}heterocycloalkyl$ and $C_{1-4}alkyleneC_{3-10}heterocycloalkyl$, optionally substituted with one to three substituents selected from OH, F, Cl, CN, NO_2 , CHO, $C_{1-6}alkyl$, $C_{1-6}fluoroalkyl$, $C_{1-6}chloroalkyl$, NR^9R^{10} , OR^9 , CO_2R^9 and $C(O)NR^9R^{10}$.

48. The method of claim 47, wherein the $C_{3-10}heterocycloalkyl$ in R^6 is selected from azetidiny, pyrrolidiny, imidazolidiny, pyrazolidiny, piperaziny, piperidiny and morpholiny.

49. The method of claim 47 or claim 48, wherein the $C_{3-10}heterocycloalkyl$ in R^6 is optionally substituted with one or two substituents selected from $C_{1-4}alkyl$, $C_{1-4}fluoroalkyl$, $C_{1-4}chloroalkyl$ and CO_2R^9 .

50. The method of any one of claims 31 to 49, wherein R^8 , R^9 and R^{10} are independently selected from H, $C_{1-4}alkyl$ and $C_{1-4}haloalkyl$.

51. The method of claim 50, wherein R^8 and R^{10} are independently selected from H, CH_3 and CF_3 .

52. The method of claim 50, wherein R^9 is selected from H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, $CH(CH_3)_2$, and $C(CH_3)_3$.

53. The method of any one of claims 31 to 49, wherein R^7 is selected from H, C_{1-4} alkyl and C_{1-4} haloalkyl.

54. The method of claim 53, wherein R^7 is selected from H, C_{1-4} alkyl, C_{1-4} fluoroalkyl and C_{1-4} chloroalkyl.

55. The method of any one of claims 31 to 43, wherein R^6 and R^7 are joined to form, together with the nitrogen atom therebetween, a 3- to 12-membered heterocycloalkyl ring optionally containing one or two additional heteromoiety selected from N, NR^{11} , O, S, $S(O)$, and SO_2 and optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, $NR^{12}R^{13}$, OR^{12} , CO_2R^{12} and $C(O)NR^{12}R^{13}$.

56. The method of claim 55, wherein R^6 and R^7 are joined to form, together with the nitrogen atom therebetween, a 8- to 10-membered heterocycloalkyl ring optionally containing one or two additional heteromoiety selected from NR^{11} , O, S, $S(O)$, and SO_2 and optionally substituted with one or two substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, $NR^{12}R^{13}$, OR^{12} , CO_2R^{12} and $C(O)NR^{12}R^{13}$.

57. The method of claim 55, wherein R^6 and R^7 are joined to form, together with the nitrogen atom therebetween, a 4- to 7-membered heterocycloalkyl ring optionally containing one or two additional heteromoiety selected from NR^{11} , O, S, $S(O)$, and SO_2 and optionally substituted with one or two substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, $NR^{12}R^{13}$, OR^{12} , CO_2R^{12} and $C(O)NR^{12}R^{13}$.

58. The method of claim 57, wherein the 4- to 7-membered heterocycloalkyl ring is selected from azetidiny, pyrrolidinyl, morpholinyl and piperazinyl.

59. The method of claim 57 or claim 58, wherein the 4- to 7-membered heterocycloalkyl is optionally substituted with one or two substituents selected from C_{1-6} alkyl, C_{1-6} fluoroalkyl, C_{1-6} chloroalkyl and CO_2R^{12} .

60. The method of claim 57 or claim 58, wherein the 4- to 7-membered heterocycloalkyl is unsubstituted.

61. The method of claim 55, wherein R^6 and R^7 are joined to form, together with the nitrogen atom therebetween, a 5- to 6-membered heterocycloalkyl ring containing one additional NR^{11} heteromoiety and optionally substituted with one or two substituents selected from OH, F, Cl, CN, NO_2 , CHO, C_{1-6} alkyl, C_{1-6} haloalkyl, $NR^{12}R^{13}$, OR^{12} and CO_2R^{12} , $C(O)NR^{12}R^{13}$.

62. The method of claim 61, wherein the 5- to 6-membered heterocycloalkyl ring containing one additional NR¹¹ heteromoiety is selected from pyrazolidinyl, imidazolidinyl, pyrazolinyl, imidazolinyl and piperazinyl.

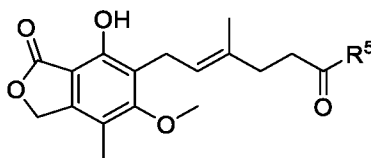
63. The method of any one of claims 31 to 62, wherein R¹¹ is selected from H, C₁₋₄alkyl, C₁₋₄fluoroalkyl, C₁₋₄chloroalkyl, CO₂C₁₋₄alkyl, CO₂C₁₋₄fluoroalkyl and CO₂C₁₋₄chloroalkyl.

64. The method of claim 63, wherein R¹¹ is selected from selected from H, CH₃, CF₃ and CO₂C(CH₃)₃.

65. The method of any one of claims 31 to 62, wherein R¹² and R¹³ are independently selected from H, C₁₋₄alkyl, C₁₋₄chloroalkyl and C₁₋₄fluoroalkyl.

66. The method of claim 65, wherein, R¹² and R¹³ are independently selected from H, CH₃, CH₂CH₃, CH₂CH₂CH₃, CH(CH₃)₂, CH(CH₃)CH₂CH₃, C(CH₃)₃, CF₃, CFH₂, CHF₂, CH₂CF₂H, CH₂CF₃, CH₂CFH₂ and C(CF₃)₃.

67. The method of claim 31, wherein the compound Formula I, or a pharmaceutically acceptable salt, prodrug and/or solvate thereof is defined as follows:



wherein

R⁵ is selected from OR⁶, NR⁶R⁷, OC₁₋₆alkenyleneNR⁶R⁷ and NR⁸C₁₋₆alkenyleneNR⁶R⁷;

R⁶ is selected from H, C₁₋₆alkyl, C₁₋₆haloalkyl, C₃₋₁₀cycloalkyl, C₃₋₁₀heterocycloalkyl, C₁₋₆alkyleneC₃₋₁₀cycloalkyl, and C₁₋₆alkyleneC₃₋₁₀heterocycloalkyl, the latter six groups being optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR⁹R¹⁰, OR⁹, CO₂R⁹ and C(O)NR⁹R¹⁰;

R⁷ is selected H, C₁₋₆alkyl and C₁₋₆haloalkyl; or

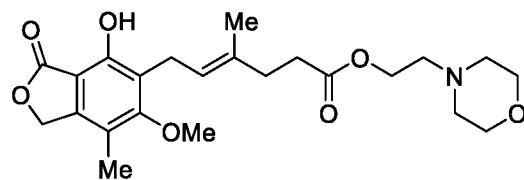
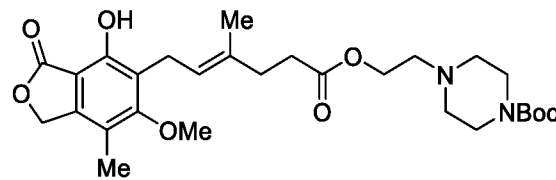
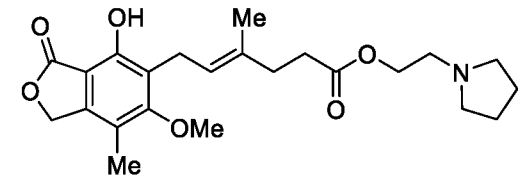
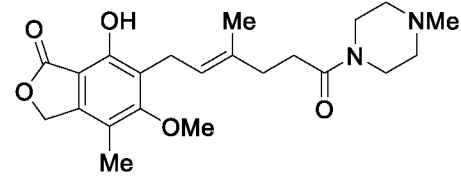
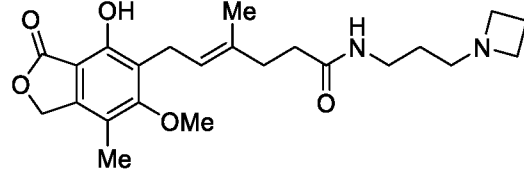
R⁶ and R⁷ are joined to form, together with the nitrogen atom therebetween, a 3- to 12-membered heterocycloalkyl optionally containing one or two additional heteromoieties selected from N, NR¹¹, O, S, S(O), and SO₂ and optionally substituted with one to four substituents selected from OH, F, Cl, CN, NO₂, CHO, C₁₋₆alkyl, C₁₋₆haloalkyl, NR¹²R¹³, OR¹², CO₂R¹² and C(O)NR¹²R¹³;

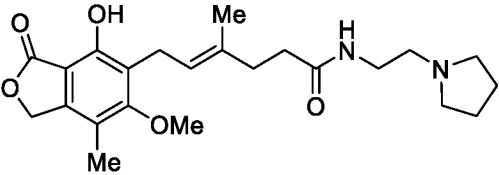
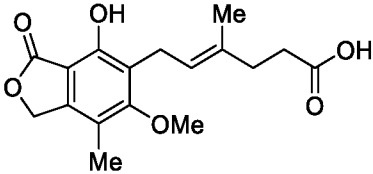
R⁸, R⁹ and R¹⁰ are independently selected H, C₁₋₆alkyl and C₁₋₆haloalkyl;

R¹¹ is selected from H, C₁₋₆alkyl, C₁₋₆haloalkyl, CO₂C₁₋₆alkyl and CO₂C₁₋₆haloalkyl; and

R¹² and R¹³ are independently selected from H, C₁₋₆alkyl and C₁₋₆haloalkyl.

68. The method of claim 31, wherein the compounds of Formula (I) are selected from:

Compound I.D.	Structure
I-1	
I-2	
I-3	
I-4	
I-5	
I-6	

	 and
I-7	

or a pharmaceutically acceptable salt, prodrug and/or solvate thereof.

Figure 1

A

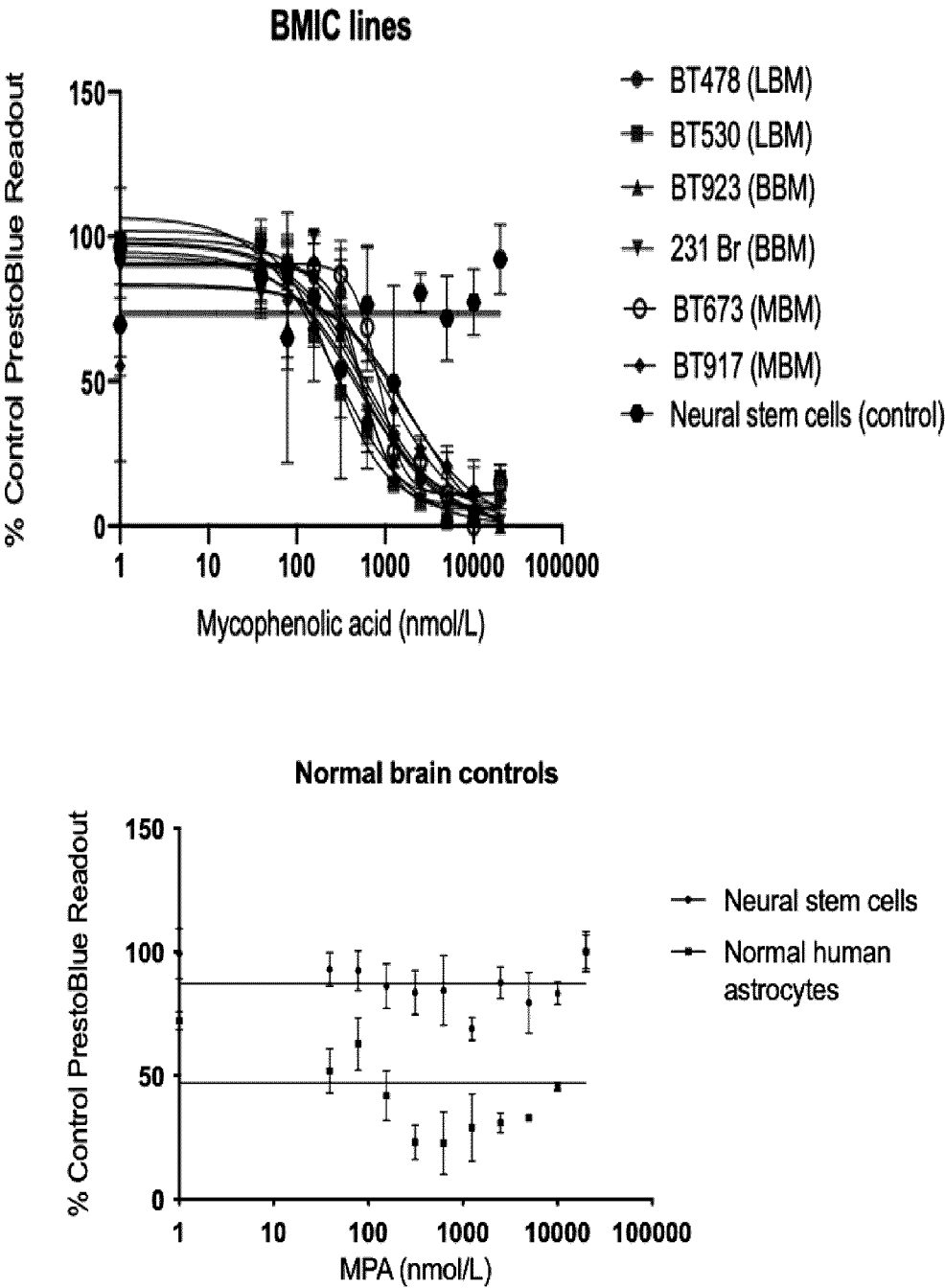


Figure 1 continued

B

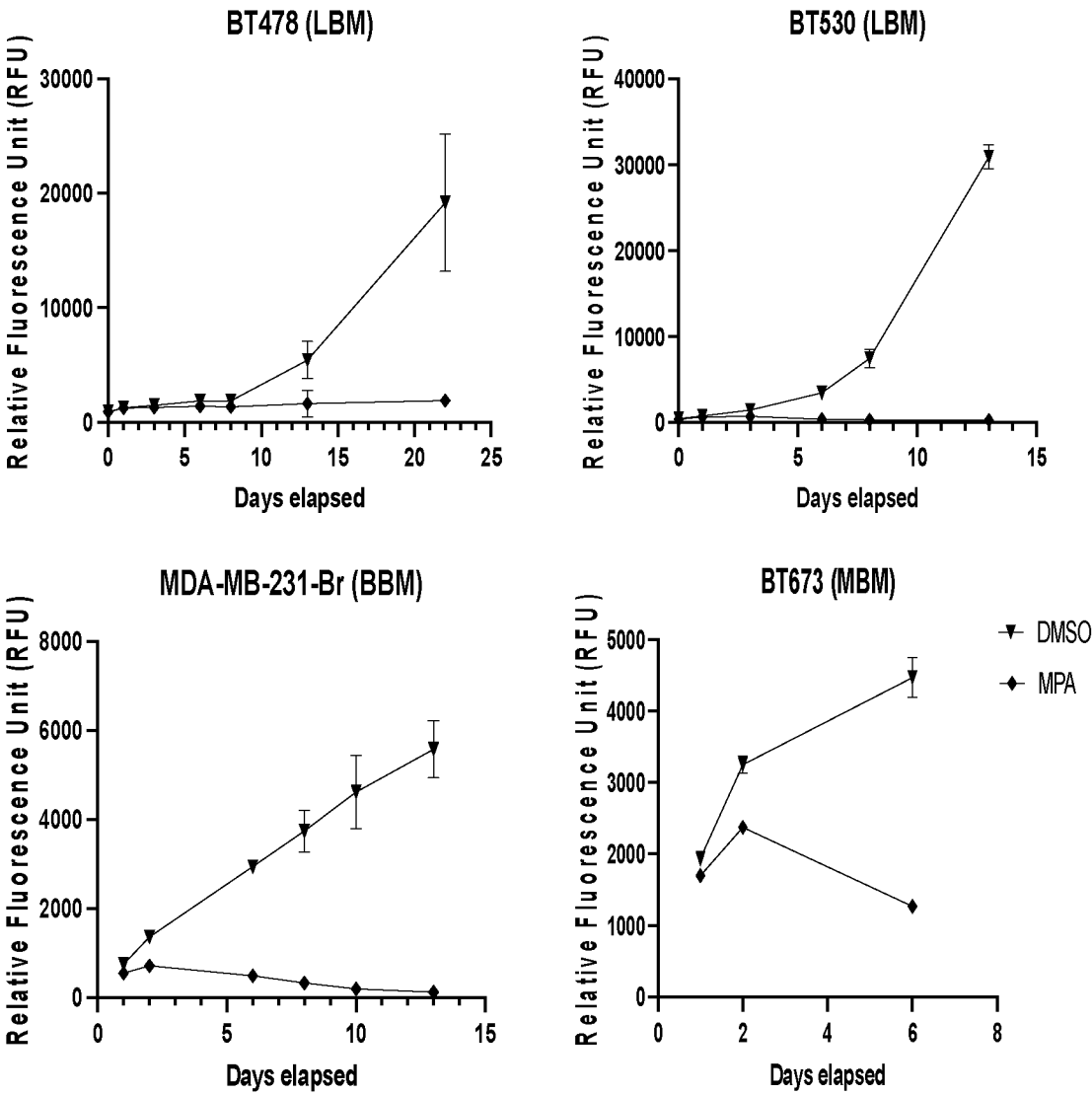


Figure 1 continued

C

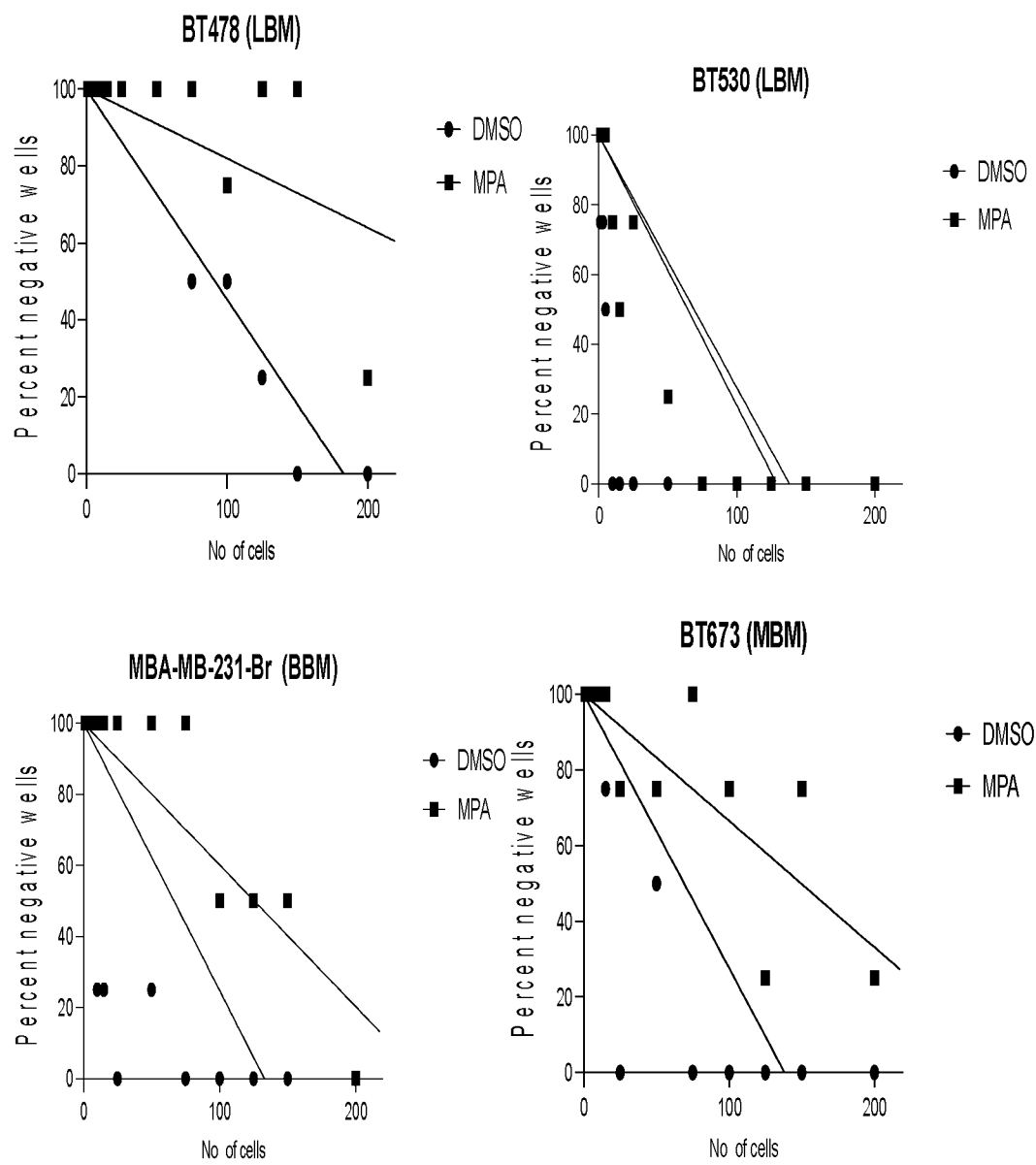
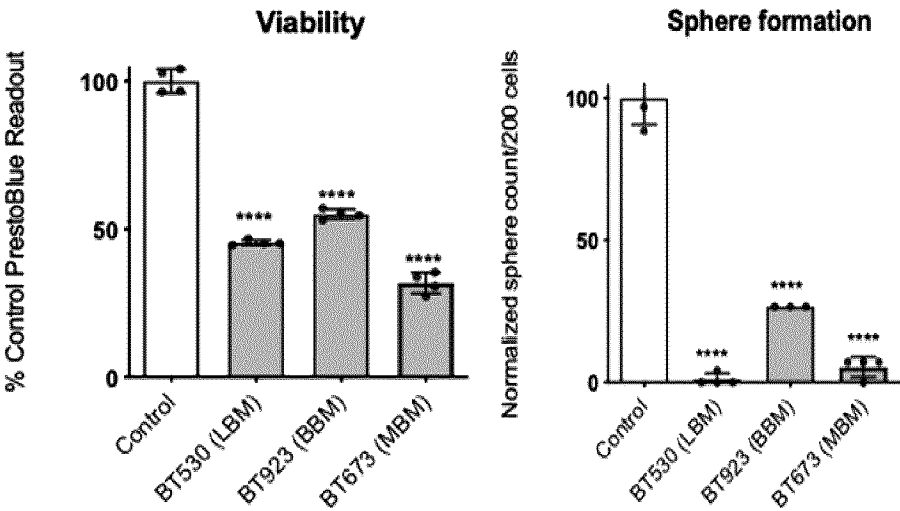


Figure 1 continued

D



E

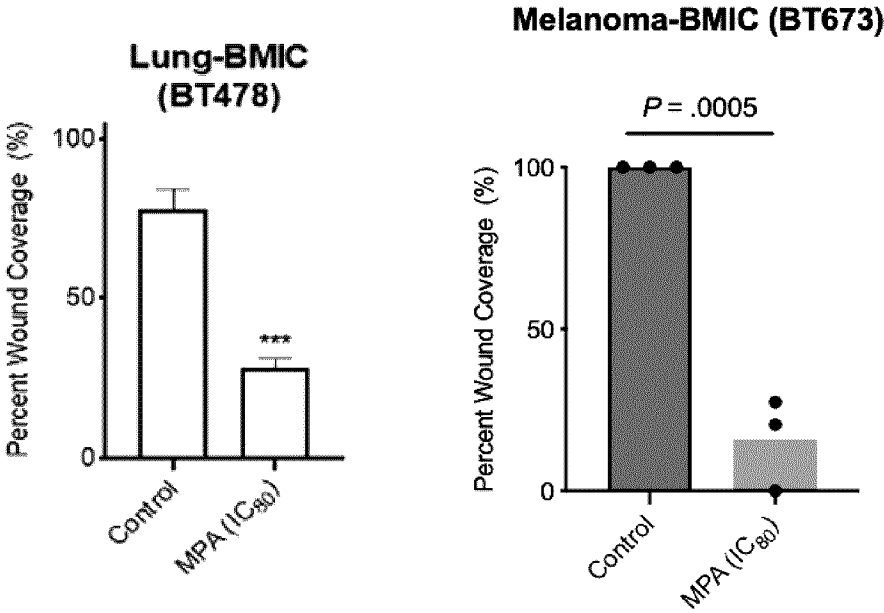


Figure 2

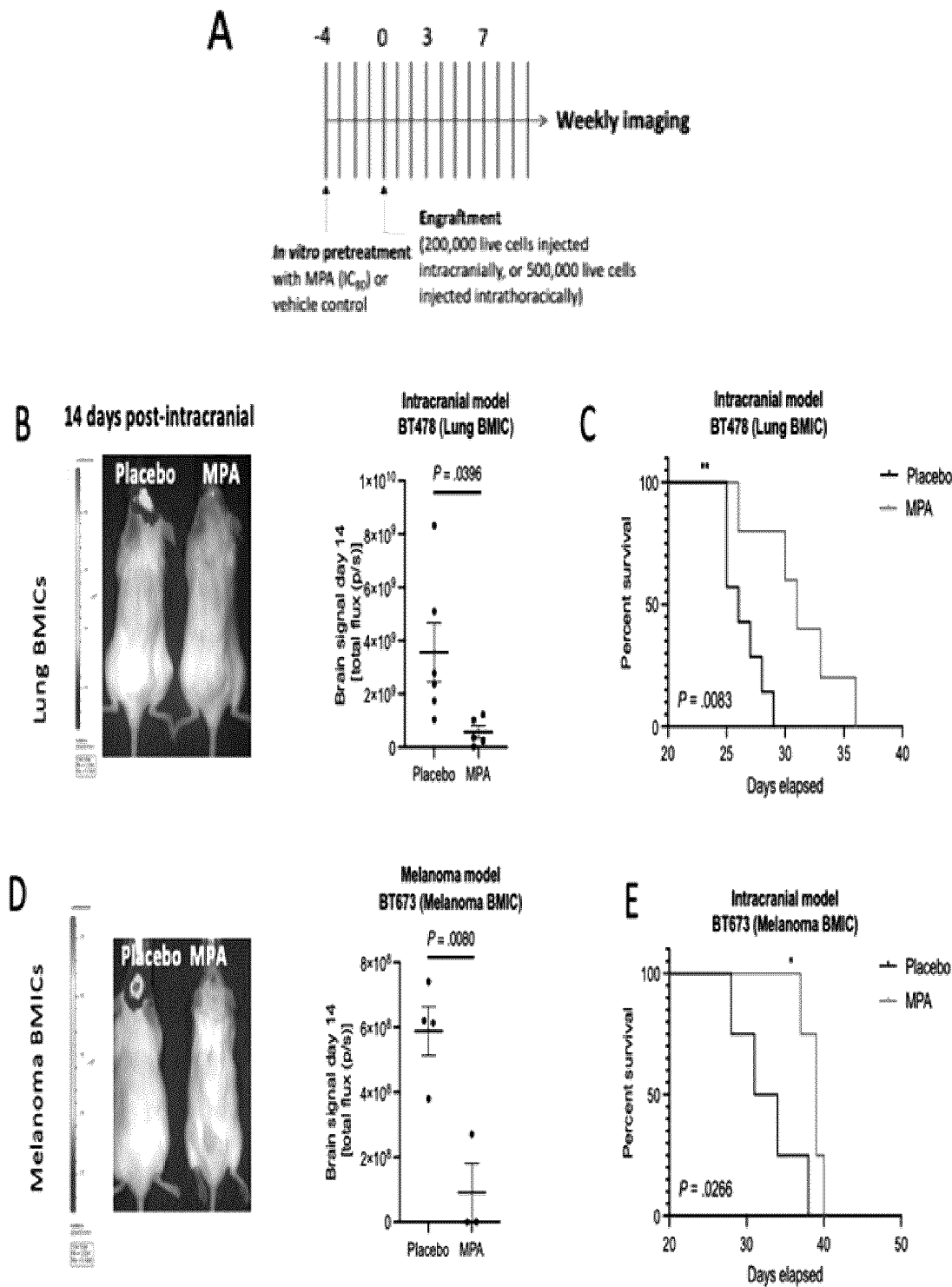


Figure 2 continued

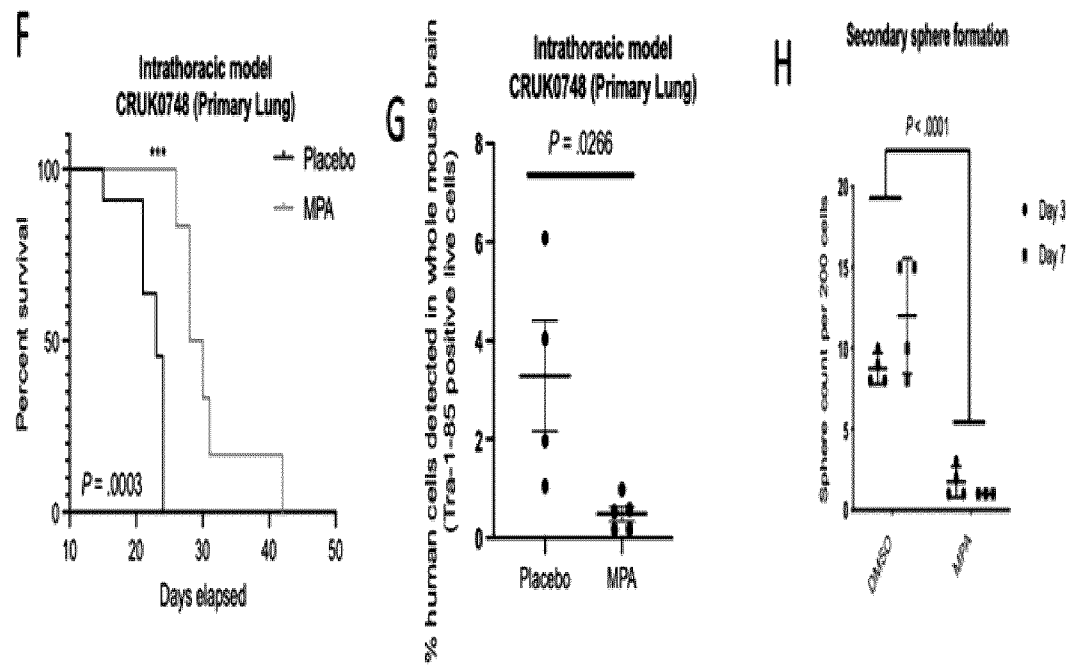


Figure 3

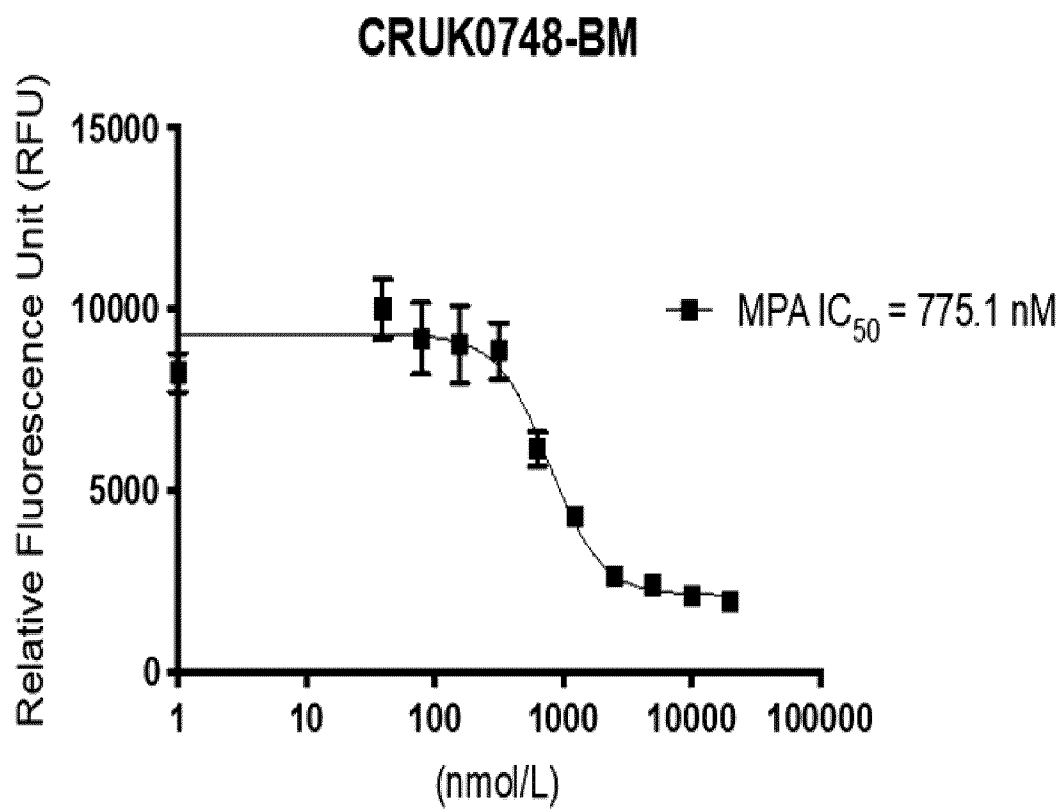


Figure 4

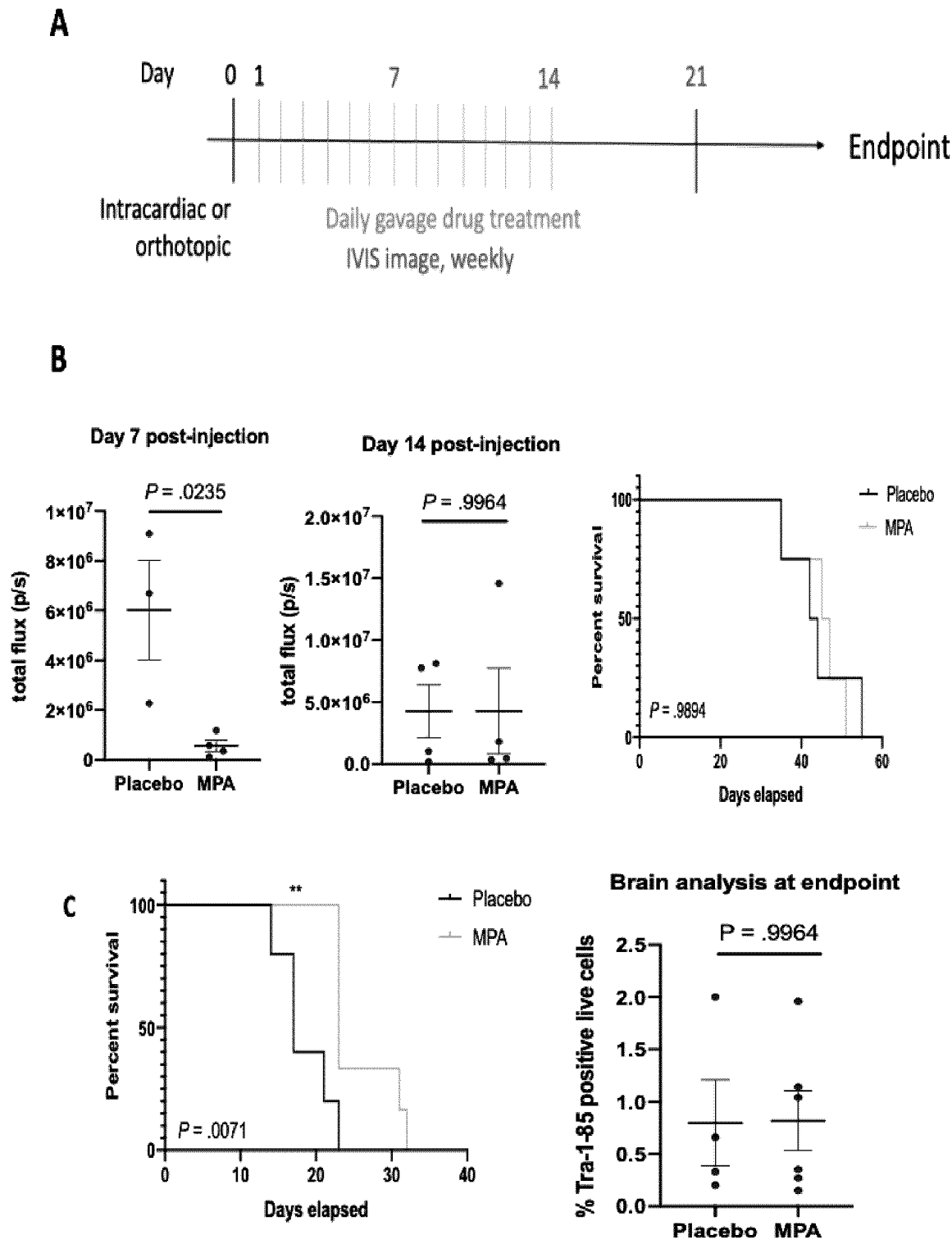


Figure 5

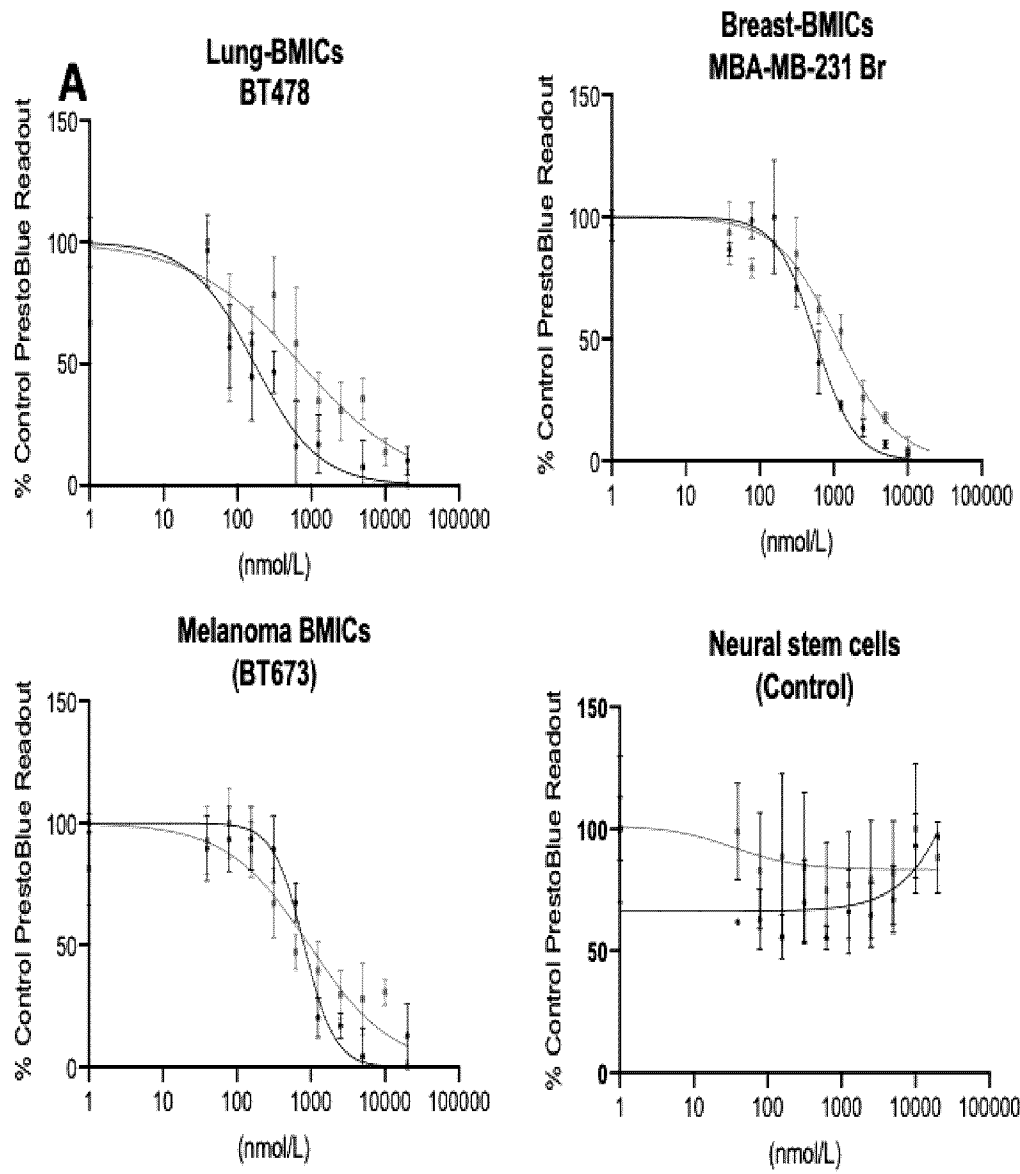
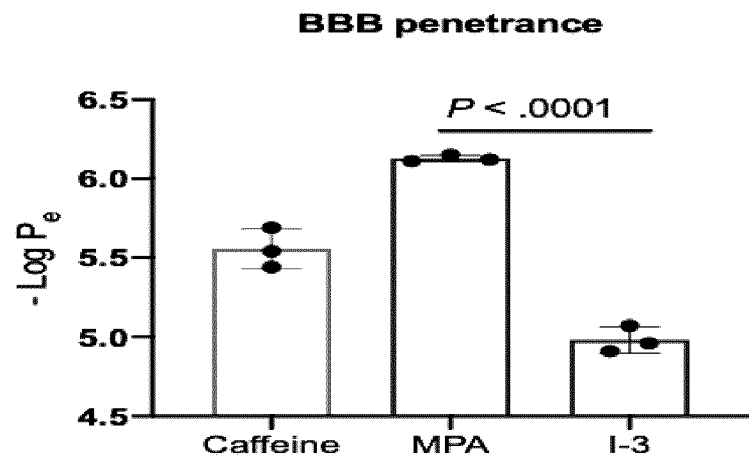
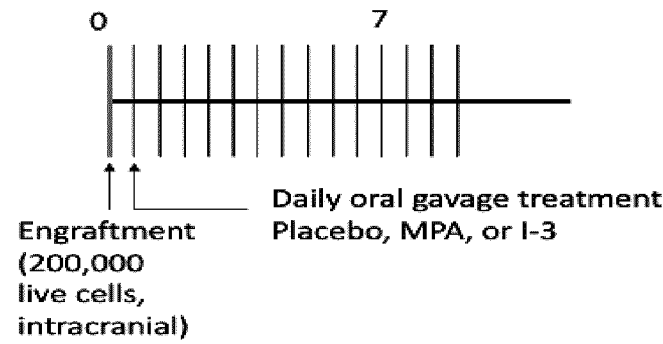


Figure 5

B



C



D

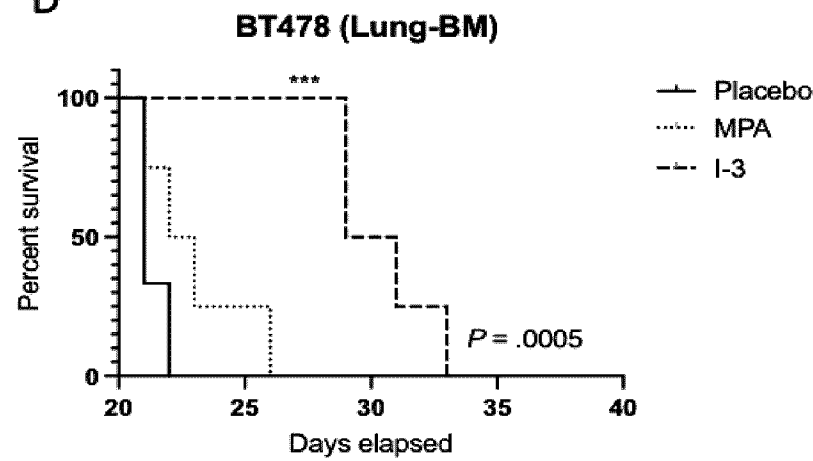


Figure 6

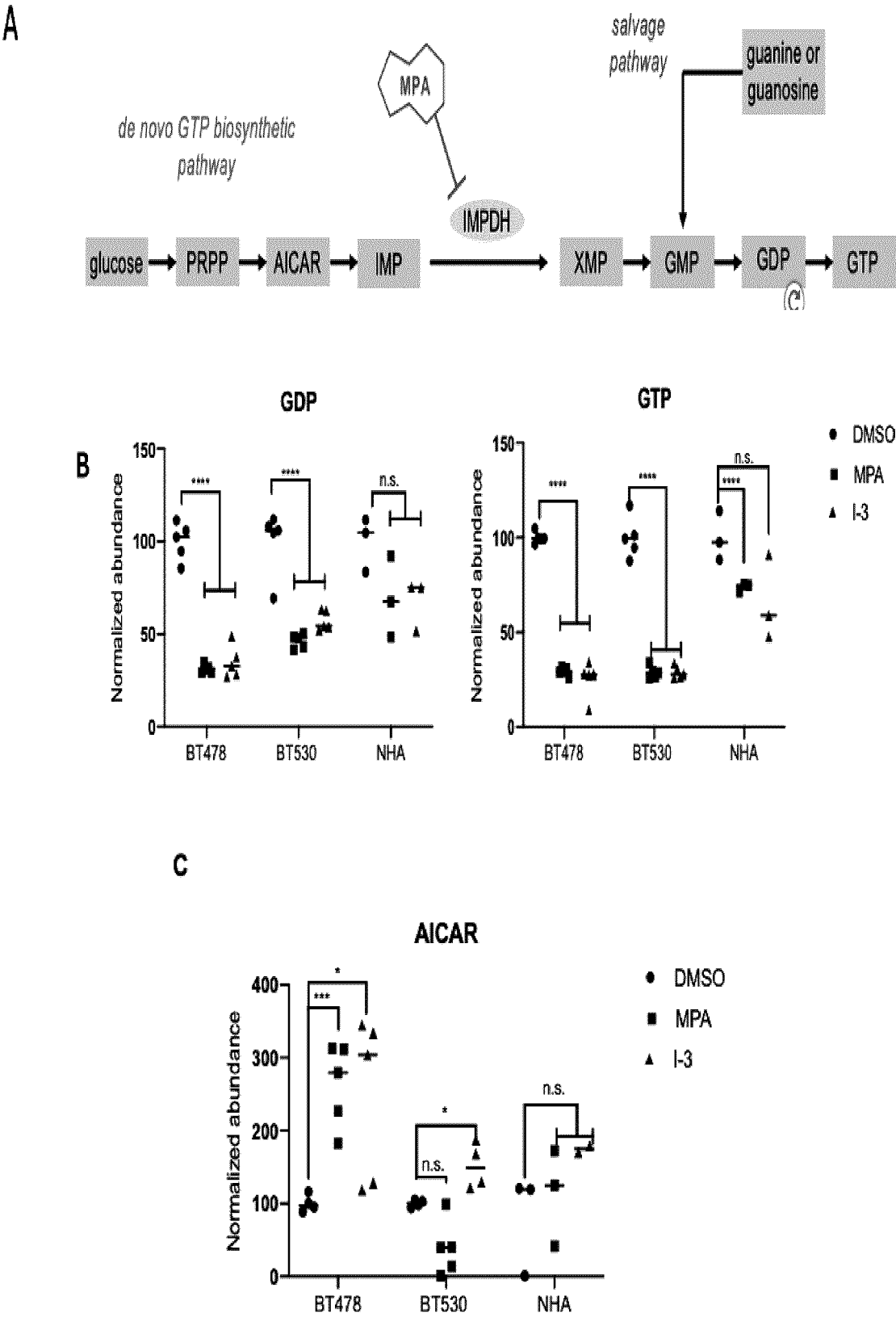


Figure 6 continued

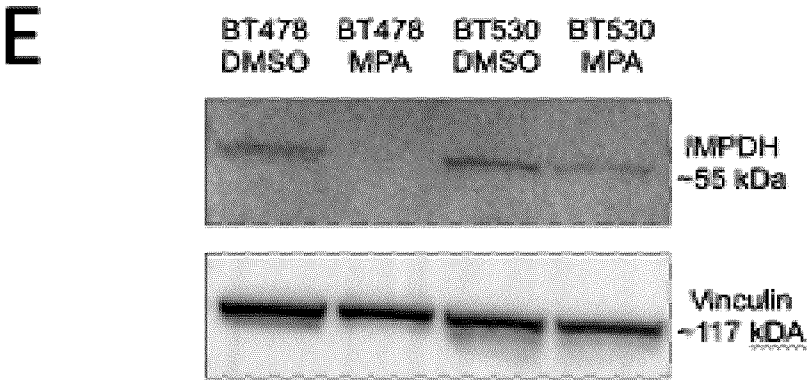
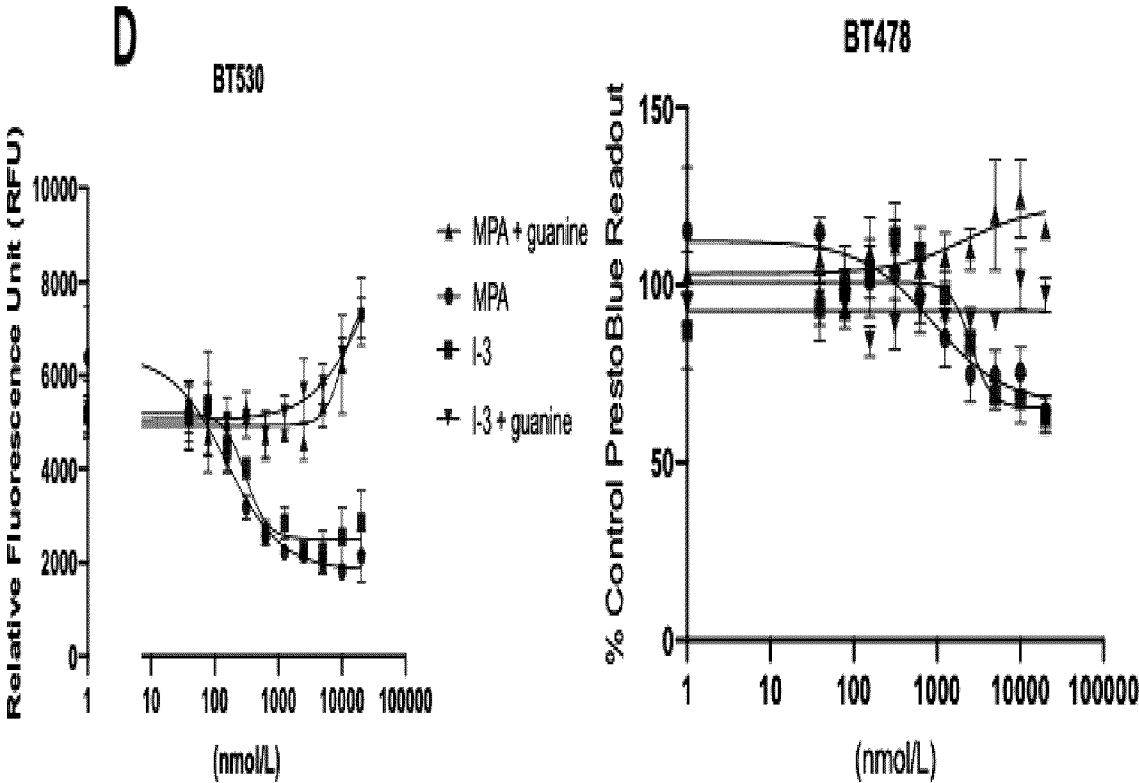


Figure 6 continued

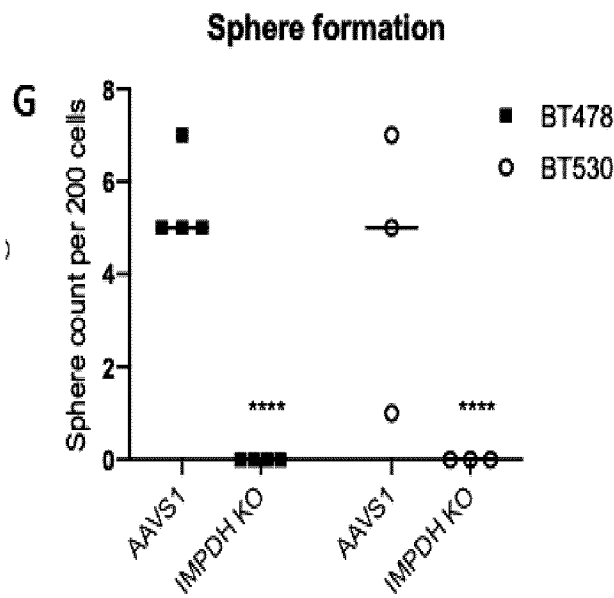
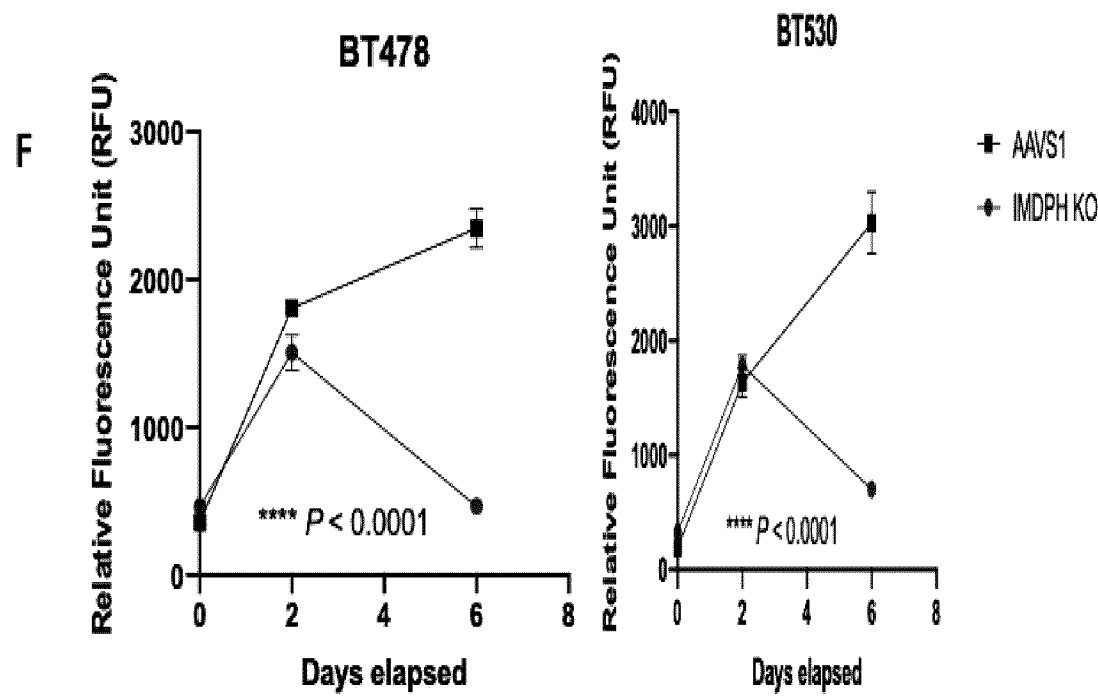
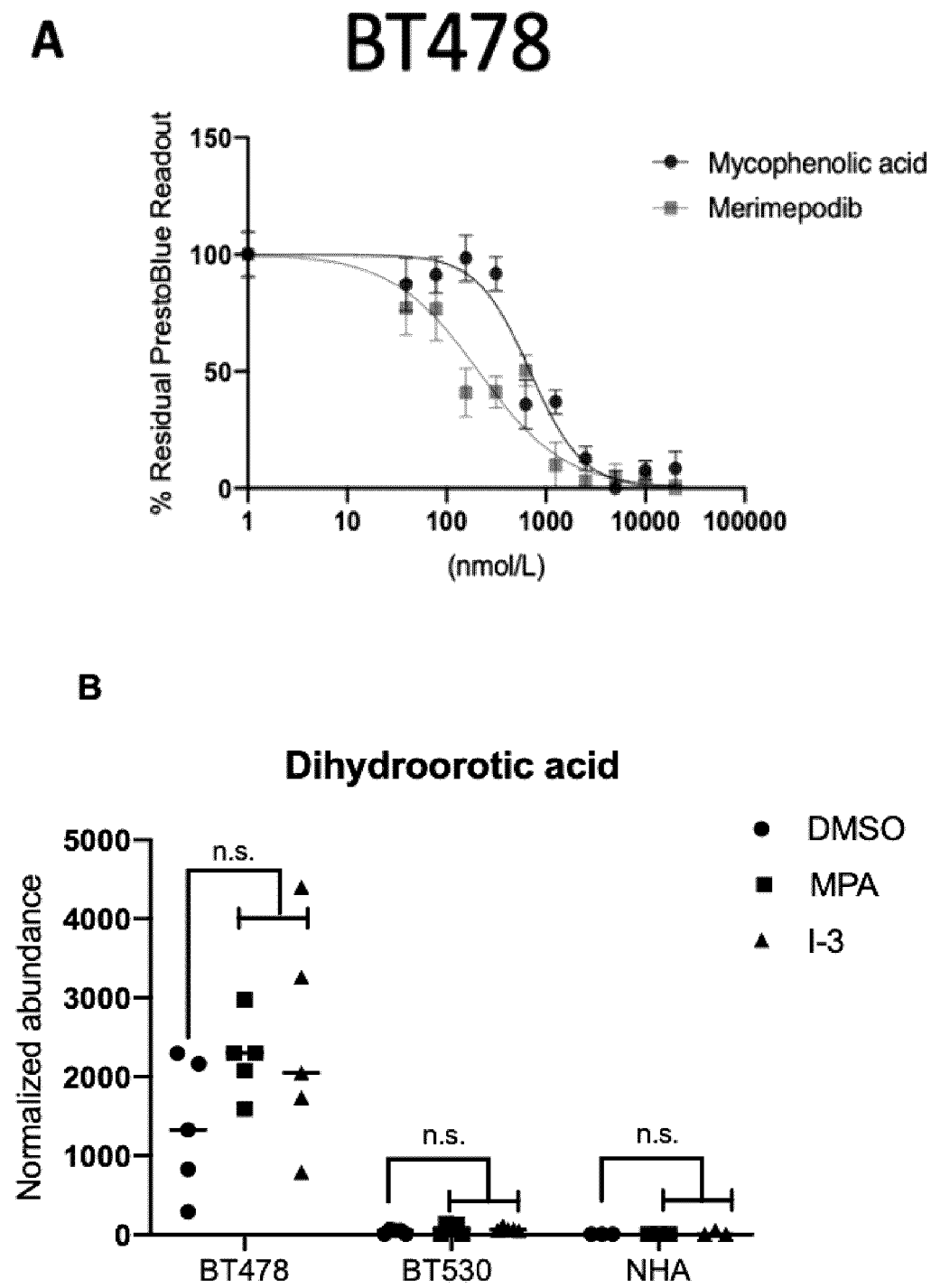


Figure 7



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/050770

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 31/365** (2006.01), **A61K 31/397** (2006.01), **A61K 31/496** (2006.01), **A61K 31/4025** (2006.01),
A61K 31/5377 (2006.01), **A61P 35/04** (2006.01) (more IPCs on the last page)

CPC: **A61K 31/365** (2020.01), **A61K 31/397** (2020.01), **A61K 31/496** (2024.01), **A61K 31/4025** (2020.01),
A61K 31/5377 (2020.01), **A61K 31/7088** (2020.01) (more CPCs on the last page)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **A61K 31/365** (2006.01), **A61K 31/397** (2006.01), **A61K 31/496** (2006.01), **A61K 31/4025** (2006.01), **A61K 31/5377** (2006.01), **A61P 35/04** (2006.01), **C07D 307/88** (2006.01), **C07D 405/12** (2006.01) (continued on the last page)

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

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

Databases: Questel Orbit, STN Registry, STN Caplus, PubMed

Keywords: inosine monophosphate dehydrogenase inhibitor, IMPDH, IMP dehydrogenase, brain metastasis, mycophenolic acid, brain metastasizing initiating cells

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO2023/091964 A2 (LIU, X. et al.) 25 May 2023 (25-05-2023) * abstract, page 2, lines 22-24; page 2, lines 26-31; page 13, lines 1-3; page 13, line 15; page 13, line 27 – page 14, line 7; page 15, line 26 – page 16, line 7; page 16 lines 21-27 *	1-4, 14-20, 30-40, 42, 67, 68 21-26
X	WO2007/109363 A2 (LIU, J. O. et al.) 27 September 2007 (27-09-2007) * abstract, pages 11, 12, 14, 15, 18, 32, 33 *	1-4, 14-20, 30-40, 42, 67, 68
Y	ZHOU, L. et al., "Enhanced expression of IMPDH2 promotes metastasis and advanced tumor progression in patients with prostate cancer" Clin. Transl. Oncol. 2014, Vol. 16, No. 10, pages 906-913 * abstract *	21-26

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

☒ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
30 July 2024 (30-07-2024)

Date of mailing of the international search report
07 August 2024 (07-08-2024)

Name and mailing address of the ISA/CA
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/050770**Box No. I****Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed.
- b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/050770

IPC:

C07D 307/88 (2006.01), **C07D 405/12** (2006.01), *A61K 31/7088* (2006.01), *A61K 38/46* (2006.01),
A61K 39/395 (2006.01)

CPC:

A61K 38/465 (2020.01), *A61K 39/3955* (2020.01), *A61P 35/04* (2020.01), **C07D 307/88** (2020.01),
C07D 405/12 (2020.01)

Continued from Box B

IPC: *A61K 31/7088* (2006.01), *A61K 38/46* (2006.01), *A61K 39/395* (2006.01)

CPC: **A61K 31/365** (2020.01), *A61K 31/397* (2020.01), *A61K 31/496* (2024.01), *A61K 31/4025* (2020.01), *A61K 31/5377*
(2020.01), *A61K 31/7088* (2020.01), *A61K 38/465* (2020.01), *A61K 39/3955* (2020.01), *A61P 35/04* (2020.01), **C07D 307/88**
(2020.01), **C07D 405/12** (2020.01)

International application No.
PCT/CA2024/050770

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2023091964A2	25 May 2023 (25-05-2023)	WO2023091964A3	22 June 2023 (22-06-2023)
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		CN101500579A	05 August 2009 (05-08-2009)
		US2010330148A1	30 December 2010 (30-12-2010)