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(57) Abstract: Provided is a method for inducing innate apolipoprotein B editing catalytic protein (APOBEC) driven C>U RNA deamination of RNA in a cell comprising contacting the cell with an effective amount of atpenin A5 with or without interferon. Specifically, APOBEC3A is a cytidine deaminase enzyme that edits RNA transcripts of hundreds of cellular genes upon stimulation of innate immune cells by low oxygen tension (hypoxia) or antiviral factor interferon. Atpenin A5 induces APOBEC3A-mediated RNA editing in normoxia to levels comparable to those seen in hypoxia. The method can be used to treat viral conditions.



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USE OF ATPENIN TO ACTIVATE INNATE IMMUNITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/145,057, filed on April 9, 2015, the disclosure of which is incorporated herein by reference.

FIELD OF THE DISCLOSURE

[0002] This disclosure relates generally to the field of activation of innate immunity and more particularly to inducing innate immunity by activation of apolipoprotein B editing catalytic polypeptide-like (APOBEC) family by atpenin.

BACKGROUND OF THE INVENTION

[0003] RNA editing is a co- or post-transcriptional process that alters transcript sequences without any change in the encoding DNA sequence. Although various types of RNA editing have been observed in single cell organisms to mammals, base modifications by deamination of adenine to inosine (A>I), or cytidine to uracil (C>U) are the major types of RNA editing in higher eukaryotes. I and U are read as guanosine (G) and thymine (T) respectively by the cellular machinery during mRNA translation and reverse transcription. RNA editing can therefore alter amino acid sequences, thereby modifying and diversifying protein functions.

[0004] The activation-induced deaminase (AID), apolipoprotein B editing catalytic polypeptide-like (APOBEC) family AID causes C>U deamination of DNA. However, multiple studies have failed to identify any RNA editing activity for this protein. Humans have 10 *APOBEC* genes (*APOBEC1*, 2, 3A-D, 3F-H and 4). APOBEC3 proteins can deaminate cytidines in single-stranded (ss) DNA, and although the APOBEC proteins bind RNA, C>U deamination of RNA is known for only APOBEC1, with apolipoprotein B (*APOB*) mRNA as its physiological target⁹. C>U RNA editing alters hundreds of cytidines in chloroplasts and mitochondria of flowering plants, but the underlying deaminating enzymes are unknown.

[0005] APOBEC3A belongs to a family of APOBEC3 enzymes that play an important role in restricting diverse viruses including human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV) and endogenous retrotransposons. Most studies show that cytidine deaminase catalytic activity of APOBEC3s is essential for their antiviral activities.

SUMMARY OF THE DISCLOSURE

[0006] We demonstrate that APOBEC3A is a potent antiviral restriction factor highly expressed in innate immune cells monocytes and macrophages. APOBEC3A is a cytidine deaminase enzyme that edits RNA transcripts of hundreds of cellular genes upon stimulation of innate immune cells by low oxygen tension (hypoxia) or antiviral factor interferon. Thus, biomolecules that activate these enzymes can be used for boosting antiviral immunity. We have identified that hypoxia and interferons activate APOBEC3A-mediated RNA editing in independent and synergistic manners. This disclosure demonstrates that pharmacologic mimics of hypoxia induce APOBEC3A-mediated RNA editing in monocytes and macrophages. We used several cell-permeable compounds. These proteins include atpenin A5 (also referred to herein as atpenin 5) for mitochondrial complex II, DMOG for HIF prolyl hydroxylases and myxothiazol for mitochondrial complex III. We found that atpenin A5 induced APOBEC3A-mediated RNA editing in normoxia to levels comparable to those seen in hypoxia. Similar to hypoxia, atpenin A5 induced RNA editing both independently and synergistically with interferon. In contrast, DMOG and myxothiazol inhibited hypoxia-induced RNA editing. These results suggest that the oxygen sensing process that triggers APOBEC3A-mediated RNA editing in hypoxia occurs in mitochondrial complex II. Based on these results, the present disclosure provides compositions and methods for use as antiviral agents. In one embodiment, the antiviral compositions of the present disclosure are inhibitors of mitochondrial complex II. In one embodiment, the antiviral compositions of the present disclosure boost innate immunity via activation of the APOBEC3A enzyme.

BRIEF DESCRIPTION OF THE FIGURES

[0007] Figure 1. Atpenin A5 (also referred to herein as AtA5), a complex II inhibitor, induces SDHB site-specific RNA editing in MEPs in normoxia, in both independent and additive manners with interferon type 1 (IFN1). Atpenin A5 does not affect RNA editing rates in hypoxia. Results from 4 biological replicates (mean and SEM).

[0008] Figure 2. Effect of atpenin A5 (1-3 micromolar final concentration) in inducing APOBEC3A-mediated RNA editing in normoxia.

[0009] Figure 3. Inhibition of complex II by AtA5 triggers RNA editing in normoxia. (A) Atpenin A5 (AtA5; 1-2 μ M) robustly induces RNA editing in normoxia (N, open bars) in MEPs without interfering with the hypoxic (H, shaded bars, 1% O₂) induction (n=27 biological

replicates). (B) Similar to hypoxia, AtA5 shows additive effect with interferon type 1 (IFN1) in inducing RNA editing (n=4 biological replicates). (C) Inhibition of complex III by myxothiazol (MXT, 1 μ M) does not induce RNA editing in normoxia and inhibits it in hypoxia (n=4 biological replicates). (D) TTFA (1 mM) induces RNA editing in normoxia. Numbers in samples indicate days in culture. Average and SEM are shown. * P<0.05, **P<0.01, ****P<0.0001

[0010] Figure 4. AtA5 treatment in normoxia induces hypoxic gene expression in monocytes. Heat map shows that the most differentially expressed genes (listed on the right), defined as FDR<0.05, log2 fold change >3 and average expression >6 cpm, between normoxic control (c1,c2,c3) and hypoxic (1% O₂) monocytes (h1,h2,h3) also show similar changes in normoxic monocytes exposed to AA5 (a1, a2, a3).

[0011] Figure 5. HIF1 α is stabilized in CD14 negative lymphocytes, but not in CD14 positive monocytes in hypoxia (H, 1% O₂, day 1). Under normoxia (N), DMOG stabilizes HIF1 α in both lymphocytes and monocytes as expected. In contrast, AtA5 does not stabilize HIF1 α in normoxia in lymphocytes or monocytes. CD14 $\pm$ cells are unsorted PBMCs.

[0012] Figure 6. Inhibition of complex II reduces oxygen consumption and antagonizes HIF-1. (Top)(A) Atpenin A5 (AtA5) inhibits HIF1 α stabilization in mild hypoxia (6% O₂), whereas myxothiazol (MXT) inhibits it both in mild and severe hypoxia (1% O₂) in 293T cells (day 1). DMSO indicates the untreated vehicle control. (Bottom) (B) AtA5 (green) reduces oxygen consumption relative to control cells (blue), but does not abolish it as seen with complex III inhibitors MXT (red) and antimycin A (black). The oxygen levels are measured by a fluorescent dye, which is quenched by oxygen (i.e., more fluorescence, less oxygen). Results are averages from 3 donors' monocyte-enriched PBMCs with SEMs shown in dashed lines.

DETAILED DESCRIPTION OF THE INVENTION

[0013] The present methods and compositions are based on our observations relating to the effect of hypoxia and interferon on C>U RNA editing. We found that hypoxia (1% O₂) enhances the C>U editing of RNA. We also found that transcripts of hundreds of genes including those implicated in viral pathogenesis and Alzheimer's disease are targets of editing in monocytes and macrophages. We show that such editing is regulated by oxygen and interferons (IFN). We further found that APOBEC3A, which belongs to the APOBEC3 family of cytidine deaminases, is an RNA editing enzyme. These findings significantly expand our understanding

of C>U RNA editing and open new avenues of inquiry on the role of *APOBEC3* genes in viral and chronic diseases.

[0014] We demonstrate herein that atpenin A5 induces APOBEC3A-mediated RNA editing in normoxia to levels comparable to those seen in hypoxia. Atpenin A5 is an antifungal antibiotic isolated from *Penicillium* sp. It is commercially available (such as from Cayman Chemical, Ann Arbor, Michigan). Similar to hypoxia, atpenin A5 induces RNA editing both independently and synergistically with interferon. We found that DMOG and myxothiazol inhibited hypoxia-induced RNA editing. These results indicate that the oxygen sensing process that triggers APOBEC3A-mediated RNA editing in hypoxia occurs in mitochondrial complex II and identify atpenin A5, a specific and highly potent inhibitor of complex II, as an activator of APOBEC3A's catalytic activity.

[0015] The present disclosure provides compositions and methods for enhancing RNA editing – in particular, C to U deamination in RNA (referred to herein as “C>U” or “C>U change”). While not intending to be bound by any particular theory, it is considered that the present methods boost innate immunity via activation of the APOBEC3A enzyme.

[0016] In one aspect, this disclosure provides a method of inducing C>U deamination in a cell by contacting the cell with a composition comprising one or more interferons. The cells may be isolated, in a tissue, or in an organism. Examples of interferons that are suitable include type 1 interferon (IFN1) and IFN γ .

[0017] In one aspect, this disclosure provides a method of enhancing innate C>U deamination by contacting cells with inducers of hypoxia or hypoxia mimicking agents. The cells may be isolated, in a tissue, or in an organism. Hypoxic conditions may be induced by reducing the amount of oxygen that the cells are exposed to. In one embodiment, hypoxia mimicking agents are used. In one embodiment, hypoxia mimicking agents are inhibitors of mitochondrial complex II, such as, for example, atpenin A5.

[0018] We observed that when monocyte-enriched PBMCs are treated with atpenin A5 in normoxia (normal oxygen tension), a similar additive effect with interferon is also observed in induction of APOBEC3A catalytic activity. Thus atpenin a5 mimics hypoxia and is a potential antiviral compound that can be used to enhance certain interferon effects.

[0019] In one embodiment, the cells are exposed to a combination of hypoxia or hypoxia mimicking agent and interferon.

[0020] In one embodiment, this disclosure provides a method of treating a viral infection in an individual by administering to the individual a composition comprising a therapeutically effective amount of an inhibitor of mitochondrial complex II and/or interferon. A composition is considered to be therapeutically effective if it is considered useful for reducing one or more symptoms of the viral infection. Determination of therapeutically effective amounts is within the purview of those skilled in the art. In one embodiment, the individual is a human. In one embodiment, the individual is a non-human animal, such as for example, domestic or farm animals. The phrase “treating” or “treatment” as used herein means reducing the severity of one or more of the symptoms associated with the indication that the treatment is being used for. Thus treatment includes ameliorating one or more symptoms associated with an indication.

[0021] Hypoxic conditions for cells in vitro may be created by exposing the cells (such as in a controlled environment chamber, e.g., incubator) to a reduced amount of oxygen compared to atmospheric oxygen. For example, less than 15%, 10%, 5% or 1% oxygen may be used. In one embodiment, the amount of oxygen is from 0.1 to 1%, 0.1 to 5%, 0.1 to 10% and 0.1 to 15% and all percentages there between to the tenth decimal point.

[0022] In one embodiment, hypoxic condition or condition that mimic the effects of hypoxia is created by using small molecules that inhibit specific cellular functions. For example, inhibitors of mitochondrial function may be used. In one embodiment, atpenin A5 or other inhibitors of mitochondrial complex II (succinate dehydrogenase) maybe used to mimic hypoxic conditions. Examples of mitochondrial complex II inhibitors also include malonate, diazoxide (DZX), malate and oxaloacetate, 3-nitropropionic acid, nitroxyl, carboxin, TTFA (thenoyltrifluoroacetone). Such agents can therefore, be useful as potential antiviral agents that enhance the C>U cytidine deaminase catalytic activity of APOBEC3A. Thus, these agents may be used in combination with interferons as anti-viral agents via activating APOBEC3A catalytic activity. These agents may be administered in therapeutically effective amounts.

[0023] The term “therapeutically effective amount” of a compound refers to an amount which is effective, upon single or multiple dose administration to an individual, for alleviating the symptoms of, or treating the particular indication – either by itself or with a synergistic agent (such as atpenin A5 with interferon). The exact amount desired or required will vary depending on the particular compound or composition used, its mode of administration and the like. Appropriate effective amount can be determined by one of ordinary skill in the art informed by

the instant disclosure using only routine experimentation. In one embodiment, the dosage of IFN1 or IFN γ is 5 to 2500 units (and all integer amounts therebetween)/ml. This may be administered via a single or multiple doses or one or more of such dosages may be administered. In one embodiment, the dosage of atpenin A5 is such that the cells are exposed to a concentration of 0.5 μ M to 10 μ M and all amounts therebetween to the tenth decimal place. In one embodiment, the cells are exposed to 1 μ M to 3 μ M. The mitochondrial complex II inhibitor and interferon may be administered at the same time or at different times, in the same formulation or different formulations, and by the same route of administration or different routes of administration. The compounds or compositions may be administered as pharmaceutically acceptable salts and may be delivered in pharmaceutically acceptable carriers including liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body.

[0024] Compositions comprising atpenin A5 or atpenin A5 with interferon and a pharmaceutical agent can be provided in combination with any suitable delivery form or vehicle, examples of which include, for example, liquids, caplets, capsules, tablets, inhalants or aerosol, etc. The delivery devices may comprise components that facilitate release of the pharmaceutical agents over certain time periods and/or intervals, and can include compositions that enhance delivery of the pharmaceuticals, such as nanoparticle, microsphere or liposome formulations, a variety of which are known in the art and are commercially available. Further, each composition described herein can comprise one or more pharmaceutical agents. The compositions described can include one or more standard pharmaceutically acceptable carriers. Some examples herein of pharmaceutically acceptable carriers can be found in: *Remington: The Science and Practice of Pharmacy* (2005) 21st Edition, Philadelphia, PA. Lippincott Williams & Wilkins.

[0025] In one embodiment, this disclosure provides compositions comprising atpenin A5, in pharmaceutically acceptable carriers. In one embodiment, interferon is also provided in the composition. The interferon can be IFN1 and/or IFN γ . In one embodiment, kits are provided for use in treatment or amelioration of viral conditions. The kit can comprise separate or combined formulations of atpenin A5 and interferon (such as IFN1 or IFN γ). The kit can also comprise instructions for use of atpenin A5 and/or interferon. The atpenin and/or interferon may be

independently formulated (combined or separately) for delivery via any mode, such as for example, intravenous, intramuscular, intraperitoneal, mucosal, dermal, intradermal or any other route.

[0026] The present method can be used to induce hypoxia induced gene expression in monocytes. The method can be used to suppress oxygen consumption, but not completely inhibit it. Thus, it can be used as a therapeutic agent, but will not act as a toxin. For example, while atpenin 5 was found to suppress oxygen consumption compared to control, myxothiazol and antimycin were observed to completely inhibit oxygen consumption. Thus, myxothiazol and antimycin act as toxins while atpenin A5 can be used as a therapeutic agent. The method can be used to suppress HIF-1.

[0027] Various methods known to those skilled in the art can be used to administer the compositions of the disclosure to an individual. For example, a compound or mixture of compounds, or compositions containing one or more compound, can be administered in any manner including, but not limited to, orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, or combinations thereof. Parenteral administration includes, but is not limited to, intravenous, intraarterial, intracranial, intradermal, subcutaneous, intraperitoneal, subcutaneous, intramuscular, intrathecal, and intraarticular. The compound(s) also can be administered in the form of an implant, which allows a slow release of the compound(s), as well as a slow controlled i.v. infusion.

EXAMPLE 1

[0028] In this example, we use several cell-permeable compounds which are candidates for oxygen sensing. These inhibitors and the inhibited proteins include atpenin A5 for mitochondrial complex II, DMOG for HIF prolyl hydroxylases and myxothiazol for mitochondrial complex III.

METHODS

Isolation and culture of cells

[0029] Peripheral blood mononuclear cells of anonymous platelet donors were isolated from peripheral blood in Trima Accel™ leukoreduction system chambers (Terumo BCT®, Lakewood, CO) after thrombocytapheresis, in accordance with a protocol approved by the institutional review board of Roswell Park Cancer Institute. A density gradient centrifugation

method using polysucrose-containing Lymphocyte Separation Medium (Mediatech®, Manassas, VA) was used for PBMC isolation. MEPs were prepared from PBMCs using the well-established cold aggregation method with slight modification (Mentzer et al 1986). Briefly, PBMCs were subjected to gentle rocking at 4 °C for an hour and aggregated cells that sedimented through fetal bovine serum (FBS; VWR®, Radnor, PA) were collected as MEPs (monocyte-enriched PBMCs) after 0.5-3 hours for high monocyte enrichment (~70% monocytes as assessed by immunofluorocytometry for CD14), or after 8-16 hours for mild enrichment (~20%-40% monocytes). MEPs were cultured at a density of at least 20 million per ml in 1 or 2 ml per well of 6- or 12-well standard tissue culture plates under 5% CO₂ in RPMI-1640 medium (Mediatech®) with 10% FBS, and 100 U per ml penicillin and 100 µg per ml streptomycin (Mediatech®). CD14 positive monocytes were separated in certain experiments from MEPs that were initially cultured for 1 or 2 days using magnetic beads and donut-shaped magnet (EasySep cell isolation kit purchased from STEMCELL Technologies).

Hypoxia and interferon treatments

[0030] For hypoxia, cells were cultured under 1% O₂, 5% CO₂ and 94% N₂ in an Xvivo™ System (Biospherix®, Lacona, NY). 'Universal' type I IFN, a hybrid of N-terminal IFN α -2 and C-terminal IFN α -1, produced in E. coli were obtained from PBL Assay Science® (Piscataway, NJ), and respectively used at 300 U per ml. Unless noted otherwise, hypoxia and/or interferon treatments were for 24 hours. Differential viability of MEPs after 1-day culture in normoxia versus hypoxia was not observed as evaluated by Trypan blue stain.

SDHB RNA editing assay

[0031] **SDHB** c.136C>U site-specific RNA editing, catalyzed by APOBEC3A cytidine deaminase, was quantified by RT-PCR as described (Baysal et al 2013). Total RNA, genomic DNA and plasmid DNA were isolated using material and methods provided with TRIzol™, DNA Wizard Genomic DNA Purification Kit (Promega®), and Plasmid Kit (Qiagen®, Germantown, MD), respectively. RNA/DNA was quantified by spectrophotometry on a Nanodrop™ 2000 instrument (Thermo Fisher®). Proteins were quantified using Bio-Rad® Dc™ assay with bovine serum albumin standards. Statistical tests were two-tailed and were performed using R 3.0, Excel™ 2010 (Microsoft®, Redmond, WA), or Prism™ 6.0 (GraphPad®, San Diego, CA) software.

Cell-permeable inhibitors

[0032] Atpenin A5 is purchased from Cayman Chemical (CAS 119509-24-9), dissolved in DMSO and used in final concentrations of 1-3 μ M in culture. DMOG (dimethylxalylglycine; CAS 89464-63-1) and myxothiazol (CAS 76706-55-3) are purchased from Sigma-Aldrich, dissolved in DMSO and used in final concentrations of 1mM and 1 μ M, respectively.

RESULTS

[0033] We have observed that the innate antiviral restriction factor APOBEC3A modifies the monocyte/macrophage transcriptome by RNA editing in response to pro-inflammatory stimulation by hypoxia and interferons. Because intracellular or viral deamination targets of APOBEC3A could not be previously identified, we considered that APOBEC3A-mediated RNA editing of cellular transcripts is responsible for its antiviral function. Thus, small molecule activators of APOBEC3A-mediated RNA editing may boost antiviral innate immunity.

[0034] Hypoxia is a pervasive feature of inflammatory micro-environments. We have shown that hypoxia and interferons act both independently and in synergy to induce APOBEC3A-mediated RNA editing. This finding suggests that mechanisms by which hypoxia and interferon activates APOBEC3A-mediated RNA editing are distinct and collaborative. Hypoxia sensing is mediated by oxygen sensing complexes. Potential candidates for oxygen sensing include HIF prolyl hydroxylases (also known as Phd enzymes), mitochondrial complex III and mitochondrial complex II (succinate dehydrogenase).

[0035] To identify the proteins involved in hypoxia-regulated APOBEC3A-mediated RNA editing in monocytes, we used cell permeable inhibitors including DMOG, which inhibits Phd enzymes; atpenin A5, which inhibits mitochondrial complex II and myxothiazol, which inhibits mitochondrial complex III. We found that treatment of monocyte-enriched PBMCs by myxothiazol and DMOG inhibited hypoxia-induced RNA editing and did not induce RNA editing in normoxia (data not shown).

[0036] In contrast, atpenin A5 induced APOBEC3A-mediated RNA editing in normoxia without inhibiting the hypoxia-induced RNA editing. We observed normoxic induction of RNA editing by atpenin A5 in 19 of 19 monocyte-enriched PBMCs obtained from anonymous donors (i.e., 19 biological replicates) ($p=0.00013$, two-sided, Wilcoxon pairwise test). The RNA editing levels induced by atpenin A5 in normoxia were comparable to levels observed upon hypoxic stimulation (Fig. 1). We separated CD14 positive and CD14 negative cell populations from MEPs after culture and confirmed that both hypoxia and atpenin A5 induced RNA editing occurs

in CD14+ monocytes. Moreover, atpenin A5 showed a synergistic effect with interferon-induced RNA editing levels. These results indicate that atpenin A5 is a hypoxia mimetic that induces APOBEC3A-mediated RNA editing under normoxia.

EXAMPLE 2

[0037] This example establishes the role of succinate dehydrogenase (mitochondrial complex II) and HIF-1 in regulation of hypoxia-induced gene expression and APOBEC3A-mediated RNA editing in human monocytes.

[0038] We used primary human monocytes and 293T HEK cells to investigate their response to hypoxia and to cell-permeable complex II inhibitors (atpenin A5, TTFA) using cell culture methods, high throughput RNA sequencing, qPCR, immunoblotting and oxygen consumption assays.

[0039] In this example, here we show that inhibition of mitochondrial complex II (succinate dehydrogenase; SDH) by the ubiquinone-analogs atpenin A5 (AtA5) or TTFA induces A3A-mediated RNA editing (Fig.3) and hypoxic gene expression (Fig. 4) in normoxic cultures of monocytes. RNA seq and RNA editing analyses show that normoxic treatment of monocytes with AtA5 closely mimics the hypoxia effects in inducing transcriptome-scale changes (Fig. 4). RT-qPCR validation experiments confirm that normoxic treatment of monocytes with AA5 induces A3A-mediated RNA editing and causes upregulation of many hypoxia-related and pro-angiogenic genes including VEGFA, CXCL8 (IL8) and CXCR4. AtA5 does not inhibit A3A-mediated RNA editing or transcript induction in hypoxic monocytes. In contrast, myxothiazol, a complex III inhibitor, does not induce A3A-mediated RNA editing in normoxia and inhibits it in hypoxia (Fig.3). We find that normoxic treatment with AtA5 does not stabilize HIF-1 α in monocytes (Fig. 5) or in 293T embryonic kidney cells (Fig. 6). On the contrary, AtA5 reduces cellular oxygen consumption in peripheral blood mononuclear cells and inhibits HIF-1 α stabilization in mildly hypoxic conditions in 293T cells (Fig. 6).

[0040] This disclosure identifies mitochondrial complex II inhibitor AtA5 as a hypoxia mimetic that induces the hypoxia-related gene expression and RNA editing activity of the antiviral enzyme A3A in monocytes and suggest that oxygen sensing by SDH controls transcriptional responses to hypoxia through a distinct signaling pathway that does not involve HIF-1 α .

[0041] These data indicate that mitochondrial complex II (succinate dehydrogenase) controls hypoxia-induced transcriptome remodeling in human monocytes through a HIF-independent signaling pathway. Pharmacologic inhibition of complex II by atpenin A5 can provide therapeutic benefit in ischemic conditions by inducing pro-angiogenic gene expression in monocytes.

[0042] While the present disclosure contains description of specific embodiments, it will be recognized by those skilled in the art that routine modifications may be made to the compositions, materials, and methods described herein, and such modifications are intended to be within the scope of this disclosure.

What is claimed is:

1. A method of enhancing apolipoprotein B editing catalytic 3A (APOBEC3A) protein driven C>U RNA deamination of RNA in a cell comprising contacting the cell with an effective amount of Atpenin A5, wherein the APOBEC3A driven C>U deamination of RNA is increased.
2. The method of claim 1, wherein the cell is a monocyte.
3. The method of claim 1 or 2, further comprising contacting the cell with an interferon.
4. The method of claim 3, wherein the interferon is IFN1 or IFN γ .
5. A method of treating a viral infection in an individual by administering to the individual a composition comprising a therapeutically effective amount of Atpenin A5.
6. The method of claim 5, further comprising administering to an individual, a therapeutically effective amount of interferon.
7. The method of claim 6, wherein the interferon is IFN1 or IFN γ .
8. A composition comprising atpenin 5A in a pharmaceutically acceptable carrier.
9. The composition of claim 8, further comprising IFN1 and/or IFN γ .
10. A kit comprising combined or separate formulations of Atpenin A5 and an interferon selected from the group consisting of IFN1 and IFN γ , and instructions for use of the Atpenin A5 and the interferon.

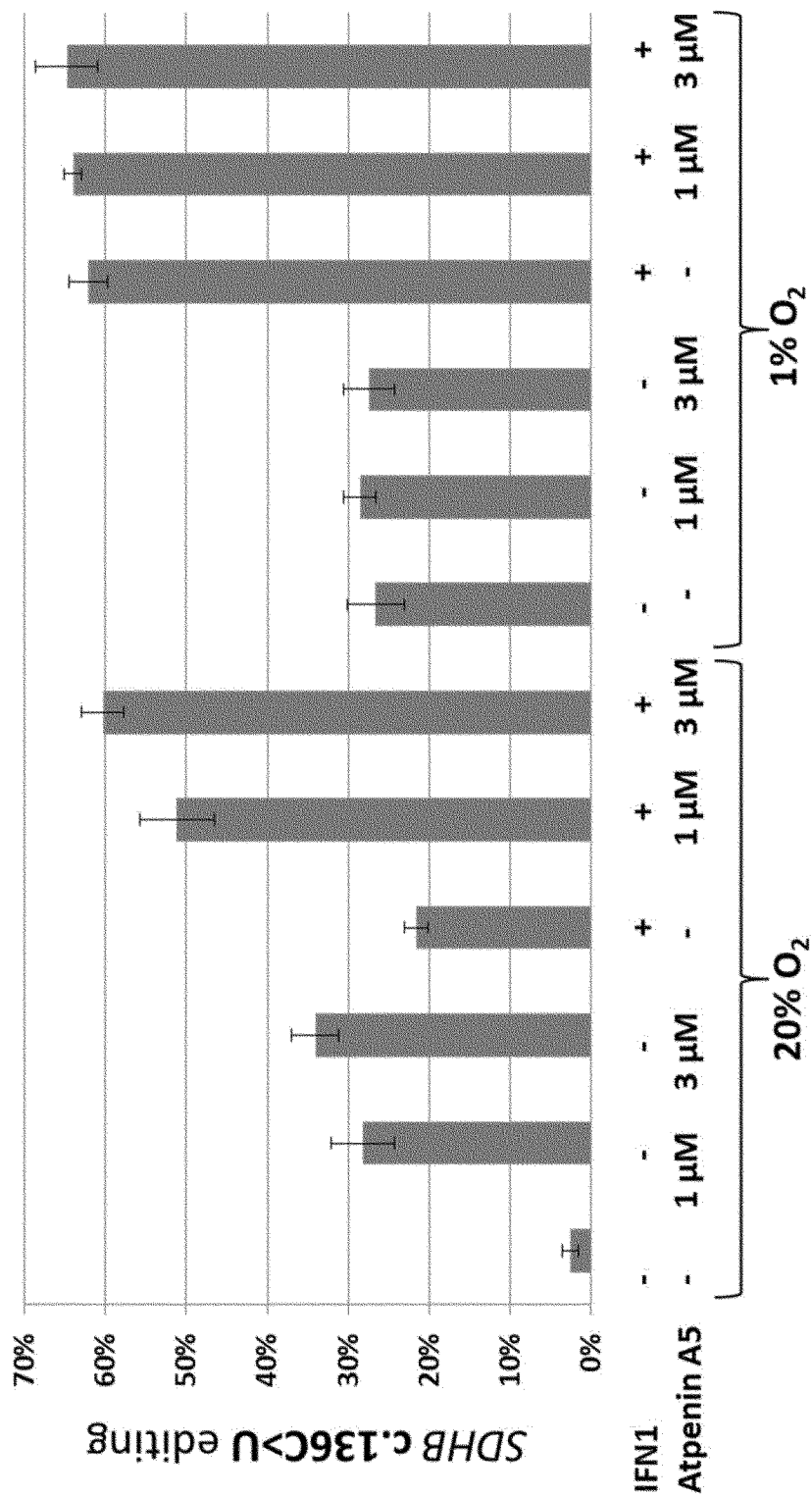


Figure 1

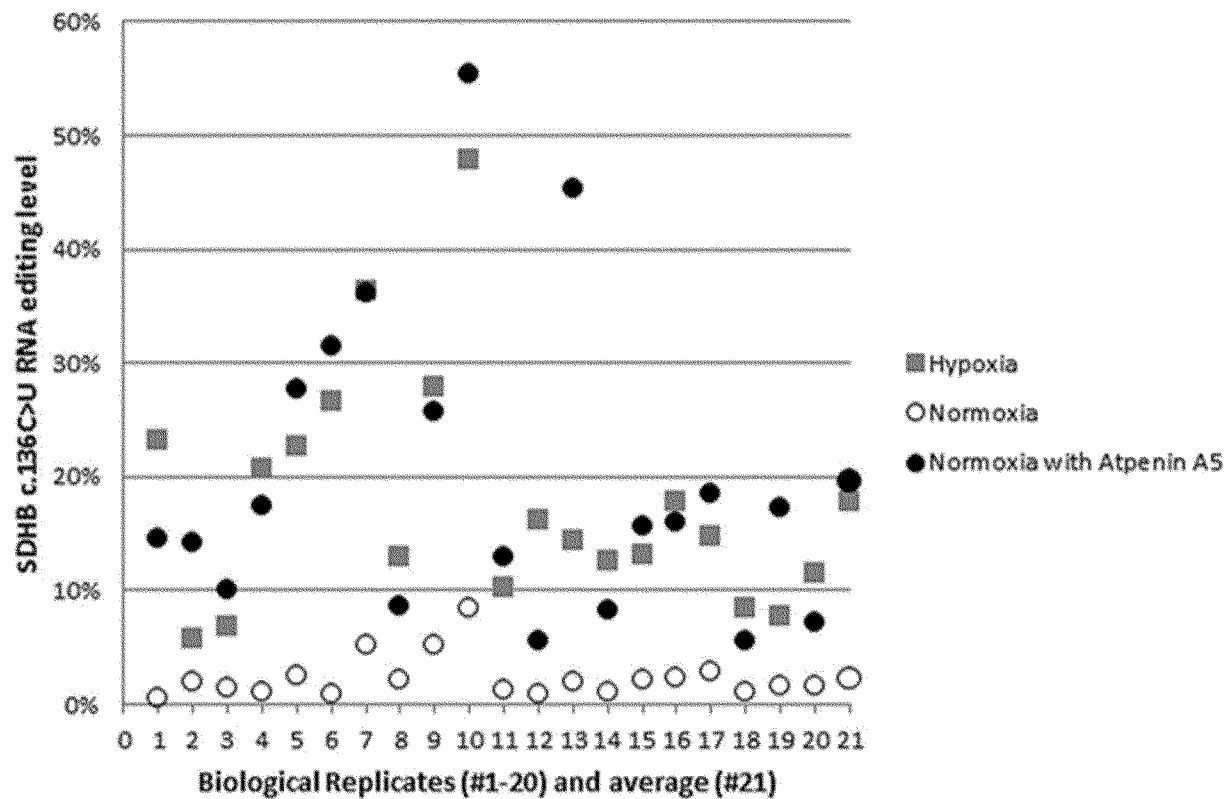
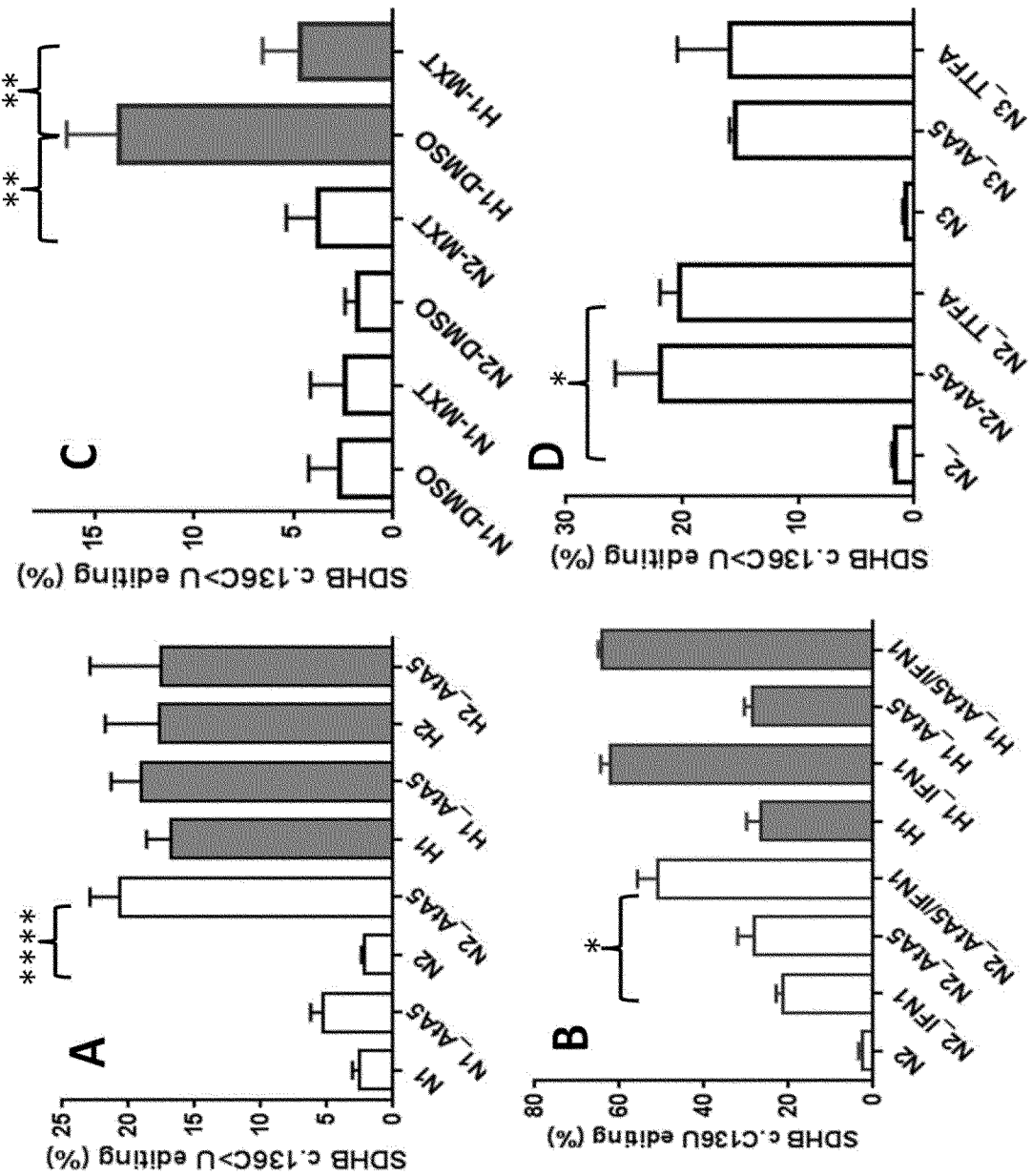


Figure 2

Figure 3



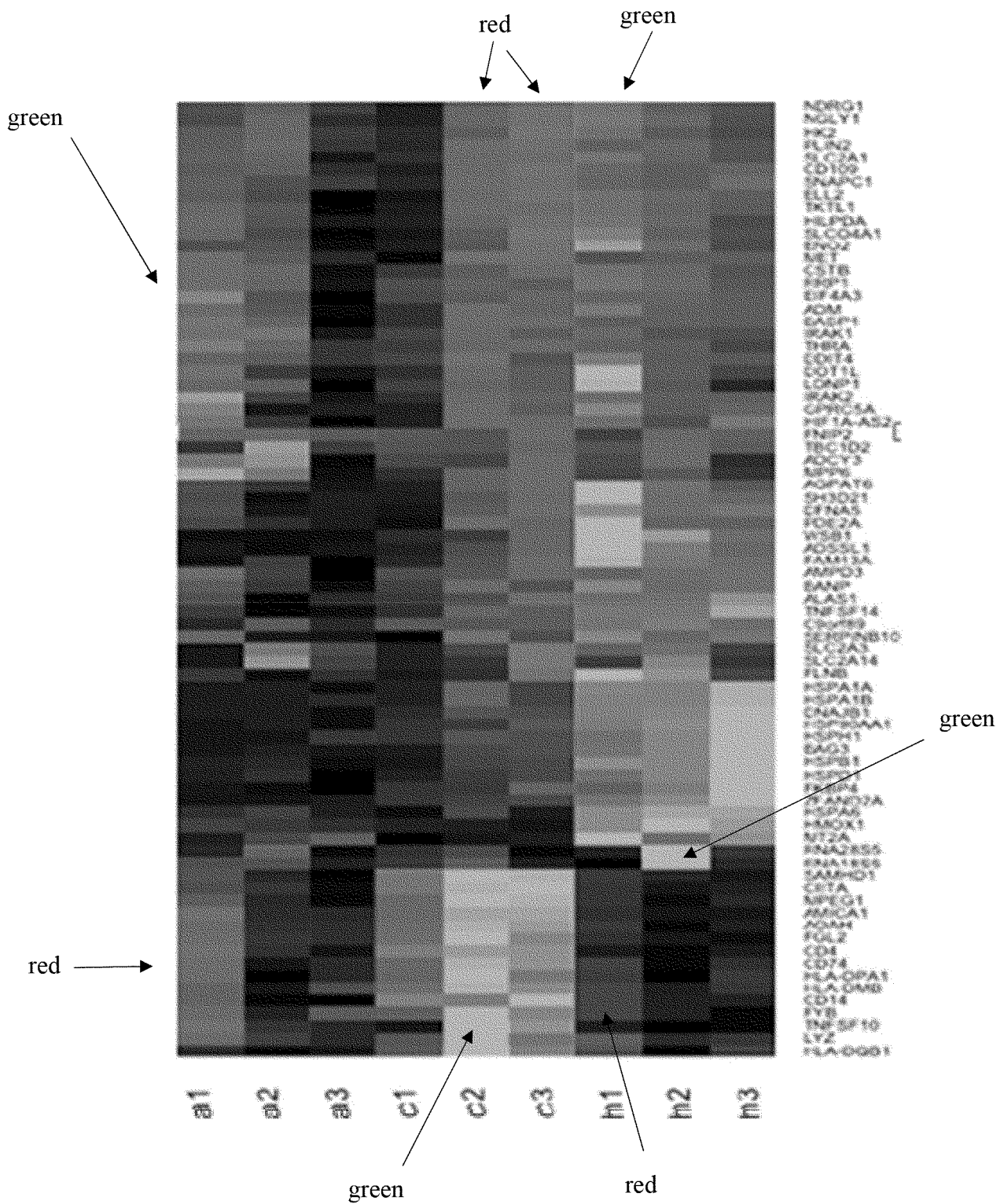


Figure 4

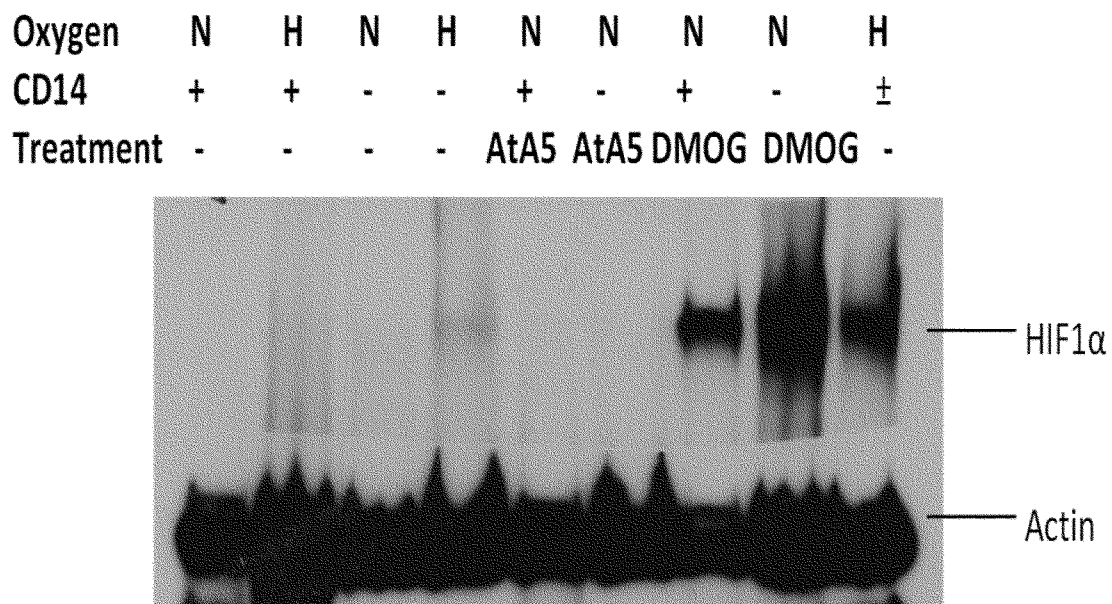


Figure 5

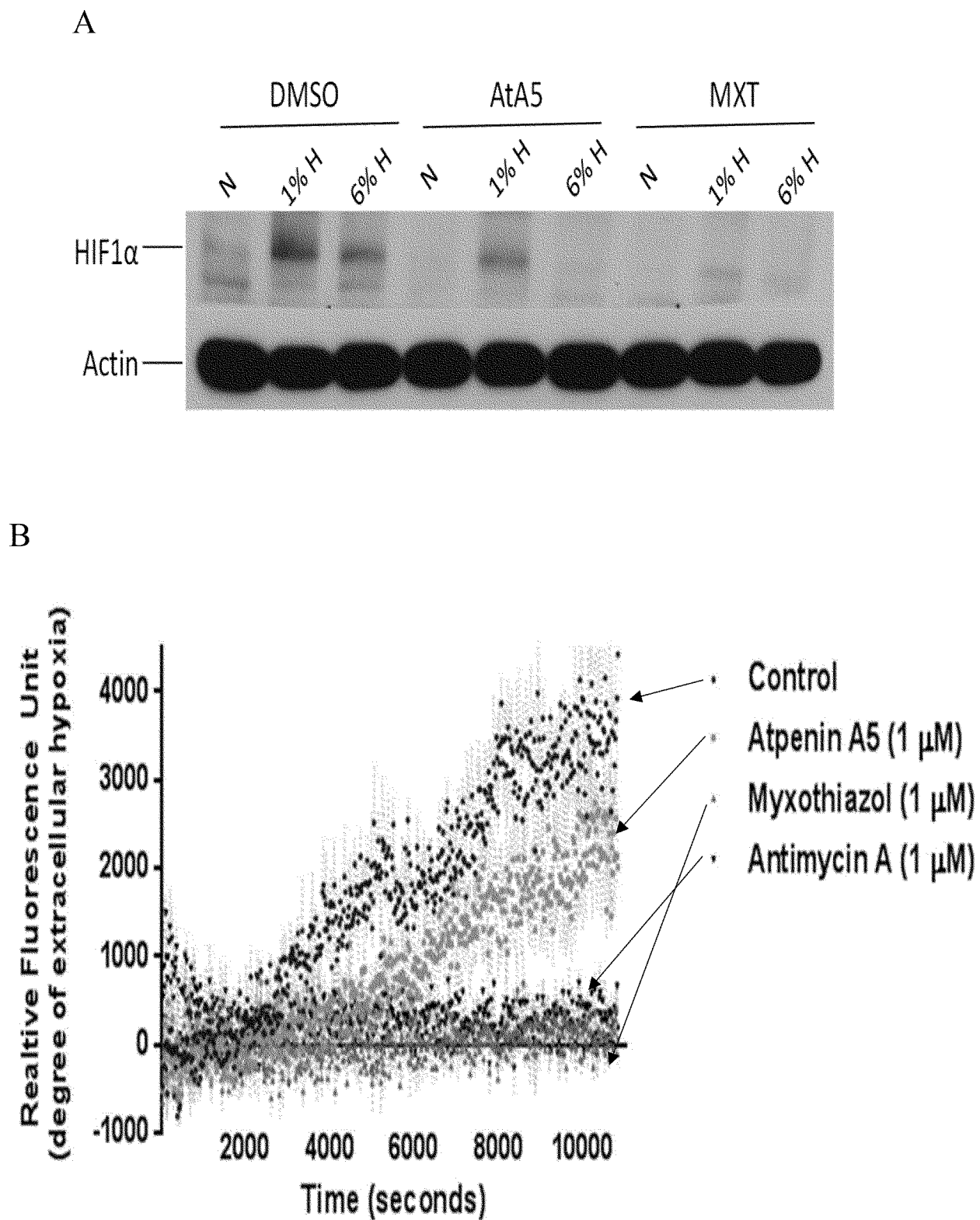


Figure 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/26917

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/00; A61K 31/70; C12N 5/02; A61K 38/00; A61K 31/44 (2016.01)

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

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CPC - C07K 14/47; C07H 19/06; G01N 33/5008; A61K 38/00; A61K 31/44; USPC - 530/350; 514/49; 435/375; 514/3.7; 514/350

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

PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google; Apolipoprotein B editing, catalytic 3A, APOBEC3A, C>U, RNA, deamination, cytosine deaminase, monocyte, interferon, type I, INF.gamma, complex II inhibitor, mitochondria, ROS, hypoxia, reactive oxygen species, cancer, death, contact, screen, administer, enhance, modulate, agent, increase, activ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0059898 A1 (RALPH et al.) 10 March 2011 (10.03.2011), Abstract, para [0025], [0027], [0100]-[0111], and claims 58 and 60	1-4
Y	TAGGART et al., RNA editing of the SDHB transcripts (C to U) in peripheral blood monocytes. ASHG (American Society of Human Genetics) Meeting. Abstract. 2012. [online]. [Retrieved on 2016.06.08]. Retrieved from the Internet:<URL: http://www.ashg.org/2012meeting/abstracts/fulltext/f120120429.htm > Entire Abstract	1-4
Y	KONING et al., Target Cell-Mediated Editing of HIV-1 cDNA by APOBEC3 Proteins in Human Macrophages. J Virol. 2011, Vol. 85(24), p. 13448-52. Abstract; pg 13448, col 2, top para and middle para; pg 13449, Fig 1; and pg 13451, Fig 2	3-4
A	PADDENBERG et al., Essential role of complex II of the respiratory chain in hypoxia-induced ROS generation in the pulmonary vasculature. Am J Physiol Lung Cell Mol Physiol. 2003, Vol. 284(5), p. L710-9. Abstract	1-4
A	CERECER-GIL et al., Mutation of SDHB is a cause of hypoxia-related high-altitude paraganglioma. Clin Cancer Res. 2010, Vol. 16(16), p. 4148-54. Abstract; pg 4148, col 2, lower para; and pg 4149, col 1, para 2	1-4
A	BAYSAL et al., Hypoxia-inducible C-to-U coding RNA editing downregulates SDHB in monocytes. PeerJ. 2013, Vol. 1:e152. PDF File: pg 1-23. Entire documentation, especially Abstract	1-4

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

15 June 2016

Date of mailing of the international search report

31 AUG 2016

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/26917

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

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

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

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

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

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

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

Group I, Claims 1-4, directed to a method of enhancing apolipoprotein B editing catalytic 3A (APOBEC3A) protein driven C>U RNA deamination of RNA in a cell.

Group II, claims 5-7, directed to a method of treating a viral infection in an individual by administering to the individual a composition comprising a therapeutically effective amount of Atpenin A5.

Group III claim 8-10, directed to a composition comprising atpenin 5A in a pharmaceutically acceptable carrier; or a kit comprising combined or separate formulations of Atpenin A5 and an interferon.

*****Continued in the extra sheet*****

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

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/26917

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SUSPENE et al., Somatic hypermutation of human mitochondrial and nuclear DNA by APOBEC3 cytidine deaminases, a pathway for DNA catabolism. Proc Natl Acad Sci U S A. 2011, Vol. 108(12), p. 4858-63. Abstract; pg 4858, col 1, para 1; pg 4859, col 1, lower para; pg 4860, col 1, lower para; pg 4861, col 2, last para; and 4862, col 1, para 2	1-4
A	MIYADERA et al., Atpenins, potent and specific inhibitors of mitochondrial complex II (succinateubiquinone oxidoreductase). Proc Natl Acad Sci U S A. 2003, Vol. 100(2), p. 473-7. Entire documentation, especially Abstract; and pg 474, Fig 1 and Fig 2	1-4
X,P	BAYSAL et al., Oxygen Sensing By Succinate Dehydrogenase Regulates Transcriptional Response to Hypoxia in Monocytes. Abstract No. 998. American Society of Hematology, 57th Annual Meeting & Exposition. 05 December 2015. Section: 201. Granulocytes. : Monocytes and Macrophages: Poster I. [online]. [Retrieved on 2016.05.23]. Retrieved from the Internet: <URL: https://ash.confex.com/ash/2015/webprogram/Paper78859.html> Entire Abstract	1-4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/26917

Continuation of:
Box No III (unity of invention is lacking)

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Feature

Group I includes the special technical feature of enhancing apolipoprotein B editing catalytic 3A (APOBEC3A) protein driven C>U RNA deamination of RNA in a cell, not required by Groups II-III

Groups II-III are partially related as a product (Group III) and a method of using the product (Group II).

Group II includes the special technical feature of treating a viral infection, not required by Groups I and III.

Group III includes the special technical feature of a kit comprising an interferon, not required by Groups I-II

Common Technical Features

The inventions of Groups I-III share the technical feature of Atpenin A5;

Groups I and II further share the technical feature of administering (Group I: contacting a cell; Group II: to an individual) an effective amount of Atpenin A5; and

Groups II and III further share the technical feature of formulation of Atpenin A5 (Group II: a composition comprising a therapeutically effective amount of Atpenin A5).

However, these shared technical features do not represent a contribution over prior art as being anticipated by US 2011/0059898 A1 to RALPH et al. (hereafter 'Ralph') as follows:

Ralph discloses a composition comprising atpenin 5A in a pharmaceutically acceptable carrier (para [0040] - 'a pharmaceutical or veterinary composition comprising the pro-oxidant compound'; para [0048]-[0059] - 'compound may be selected from the group consisting of: ... Atpenin A5'; para [0100]-[0111] - 'the chemical entity is a pro-oxidant vitamin E analogue having a complex II binding affinity ...Atpenin A5'; para [0075] - 'Liquid pharmaceutical compositions generally include a liquid carrier').

Ralph further discloses a method of administering to an individual or contacting a cell a composition comprising an effective amount of Atpenin A5 (para [0027] - 'administering to the subject a therapeutically effective amount of a pro-oxidant compound ... interacting with mitochondrial complex II of a cancerous cell'; para [0100]-[0111] - 'chemical entity is a pro-oxidant vitamin E analogue having a complex II binding affinity ...Atpenin A5'; para [0032] - 'inducing the death of the cancerous cell in a subject ... administering to the subject a therapeutically effective amount of a pro-oxidant compound capable of interacting with mitochondrial complex II of the cancerous cell to generate reactive oxygen species within the cell'; Claim 58 - 'introducing a test compound into mitochondria of a first cancerous cell ... assaying for death of those cancerous cells by the test ...wherein the complex II binding,...the death of the first cancerous cell by the test compound ...indicates that a chemical entity capable of inducing cell death has been identified'; Claim 60 - 'said test compound is a compound selected from the group consisting of...(Atpenin A5)').

Without a shared special technical feature, the inventions lack unity with one another.

Groups I-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.