(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 September 2020 (17.09.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/186211 A1

(51) International Patent Classification:
A61K 35/76 (2015.01) *A61P 31/20* (2006.01)

(21) International Application Number:
PCT/US2020/022751

(22) International Filing Date:
13 March 2020 (13.03.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/817,894 13 March 2019 (13.03.2019) US

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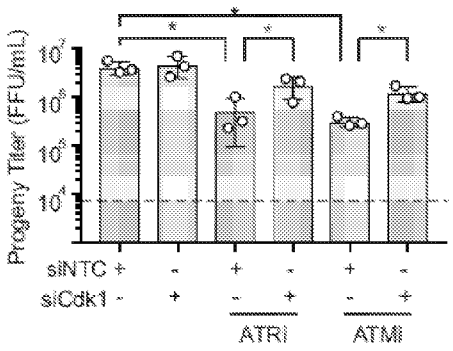
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) Title: METHODS AND COMPOSITIONS FOR TREATING POLYOMA VIRUS INFECTION

FIG. 3A



(57) Abstract: Methods for the treatment of a polyomavirus infection, for decreasing the viral titer of a PyV in a subject having a PyV infection, for treating a adverse effect of a treatment that suppresses the immune system, for inhibiting the DNA damage response pathway, and related methods are provided. Compounds, kits, and composition usefule in the described methods are also provided. Suitable compounds useful in the methods disclosed include inhibitors of ataxia telangiectasia mutated and ATM-Rad 3 related proteins.

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METHODS AND COMPOSITIONS FOR TREATING POLYOMA VIRUS INFECTION

GOVERNMENT LICENSE RIGHTS

This invention was made with government support under Contract 1RO1AI123162 awarded by National Institute for Health. The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

The present disclosure is directed generally to treatment of viral infections and conditions related to a viral infection in a subject and pharmaceutical compositions useful in such treatments. The present disclosure is more specifically dircted to treatment of a polyomavirus infection and conditions related to a polyomavirus infection in a subject and pharmaceutical compositions useful in such treatments.

BACKGROUND

Polyoma virus (PyV) is a family of viruses that contains over 70 species, of which 13 are known to infect humans. Of the thirteen human PyVs that cause human infection, BK polyomavirus (BKPyV), JC polyomavirus (JCPyV), and Merkel cell polyomavirus (MCPyV) cause the majority of PyV related maladies in humans. Most PyV infections appear to cause little or no symptoms and PyVs are probably persistent lifelong among most adults. Diseases caused by human polyomavirus infections are most common among immunocompromised subjects and subjects undergoing certain medical procedures or treatment.

PyV is an emerging pathogen that reactivates in immunosuppressed patients. For example, BKPyV latently infects the genitourinary tract of >90% of the adult population. Immunosuppression increases the risk of viral activation leading to active PyV infection. Immunosuppression can occur as a result of a disease or condition that suppresses the immune system (either as a result of the natural course of the disease or condition or a treatment directed to treating the disease or condition) as well as when a subject is given a treatment that is aimed at suppressing the immune system.

Organ and tissue transplant procedures often require that the subject (the transplant recipient) receive a treatment that suppresses the immune system. Such a treatment is administered in order to decrease the possibility of a host-graft immune response, which can lead to damage to the transplanted organ or tissue (graft dysfunction) or cause failure of the organ or tissue (graft failure). In kidney transplants for example, activation of latent BKPyV is a leading cause of graft dysfunction and graft failure in organ transplant recipients.

In addition, subjects with certain autoimmune conditions are administered drugs to suppress the immune system as a treatment for the autoimmune disease. For example, in patients with multiple sclerosis, treatments may involve the administration of agents that modulates or inhibits the immune system. JCPyV virus has been known to activate or reactivate in multiple sclerosis patients taking immunosuppressive drugs which can lead to progressive multifocal leukoencephalopathy (PML), which can cause dementia, blindness, paralysis and seizures.

A number of agents have been used to treat PyV infections in the art. However, clinical experience has not shown consistent success. There are currently no therapeutics that target PyV generally (including BKPyV). As a result, no effective treatment for PyV activation is available. In subjects experiencing PyV activation associated with an organ transplant, the treatment is to decrease immunosuppression. Such an approach is not optimal as decreasing immunosuppression increases the possibility of graft dysfunction and/or graft failure. The same paradigm is seen in patients treated for autoimmune conditions, wherein the treatment is to decrease immunosuppression which can result in worsening of the autoimmune condition.

The art is lacking agents that can be used to treat PyV infections (including activation of a latent PyV). Such agents would provide a significant benefit to subjects experiencing PyV infections. Such agents would provide a significant benefit to subjects experiencing active PyV infection caused (in whole or in part) by a treatment that suppresses the immune system as such agents would provide for treatment of the PyV infection without requiring that the treatment that suppresses the immune system be decreased.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows BKPyV activation of the DDR induced cell cycle arrest. DNA damage and effects on cell cycle arrest were measured during BKPyV (MOI=0.5) infection in primary renal proximal tubule epithelial (RPTE) cells treated with ATRi, (5μM VE-821), ATMi (10μM KU-55933), or vehicle control (DMSO) at 24 hpi and analyzed at 72 hpi. (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$).

FIG. 1A shows a Western blot for markers of DDR activation (pChk1 and pATM), viral infection (TAg), and β-actin (loading control) Representative of n=3 biological replicates is shown.

FIG. 1B shows DNA damage assessed by nuclear morphology. The left panel shows representative IFA images of nuclear morphologies (DAPI; blue) in BKPyV infected cells

(TAg; red). The right panel shows %TAg positive cells (>100 per n=3) with normal (gray), fragmented (dark gray), mitotic nuclei (green) or abnormal mitosis (blue) phenotypes as % of total.

FIG 1C. shows normal phenotypes from FIG. 1B graphed separately to show differences between groups. Shown is the mean ±SD for n=3 biological replicates.

FIG. 1D shows a fragmented phenotype from FIG. 1B graphed separately to show differences between groups. Shown is the mean ±SD for n=3 biological replicates.

FIG. 1E shows a mitotic phenotype from FIG. 1B graphed separately to show differences between groups. Shown is the mean ±SD for n=3 biological replicates.

FIG. 1F shows an abnormal mitosis phenotype from FIG. 1B graphed separately to show differences between groups. Shown is the mean ±SD for n=3 biological replicates.

FIG. 1G shows the effects of DDR inhibition on cell cycle arrest, mitosis and premature mitosis events as measured by cell cycle analysis (FACS). S phase (EdU pulse labeling) versus M phase ($pH3^{S10}$), and DNA content (FxCycle Violet) displayed by blue to red pseudo-coloring. Representative for n=7 biological repeats.

FIG. 1H shows quantification of mitotic cells ($pH3^{S10}$) from FIG. 1G. The mean ±SD for n=7 biological replicates is shown. Symbols: mock (white) or BKPyV (black) infected, vehicle (square), ATRi (upward triangle), and ATMi (downward triangle).

FIG. 1I shows the % of premature mitotic events (EdU and $pH3^{S10}$ positive) from FIG. 1G as quantified as the % of cells that are positive for mitosis from the total cells in S phase. Error bars represent ±SD for n=7 biological replicates. Symbols are as in FIG. 1H.

FIG. 1J shows a working model of activation of the DDR during a BKPyV. BKPyV infection arrests the cell cycle and prevents entry into mitosis. ATRi, but not ATMi, causes premature mitosis.

FIG. 2 shows blocking mitosis prevented BKPyV-dependent host DNA damage when ATR was inactive. To determine if mitosis is required for DNA damage to occur during BKPyV infection when the DDR is inhibited, RPTE cells were transfected with siCdk1 or control (siNTC) then infected with BKPyV (MOI=0.5). At 24 hpi cells were treated with ATRi (5μM VE-821), ATMi (10μM KU-55933), or vehicle control (DMSO) and harvested at 72 hpi.

FIG. 2A shows a diagram of the G2/M checkpoint. DDR activation phosphorylates Cdk1 (inactive; gray) and arrests the cell cycle. In the absence of DDR activation, Cdk1 (green) is active and it complexes with cyclin B to promote mitosis.

FIG. 2B shows Cdk1 silencing blocked mitosis induced by ATMi and ATRi. Mitotic cells were enumerated by FACS when the DDR was inhibited (ATMi or ATRi) with and without Cdk1 knockdown. Shown is mean ±SD for n=3 biological replicates. (*p<0.05, ****p<0.0001).

FIG. 2C show Cdk1 silencing reduced DNA fragmented nuclei caused by DDRi. Quantification of fragmented nuclei for >100 nuclei per biological replicate (*n*=3) are shown.

FIG. 2D shows quantification of nuclear morphology as in FIG. 1B for >100 nuclei per biological replicate (n=3).

FIG. 2E shows data from FIG. 1B for a direct comparison to siCdk1 nuclear morphology, which were performed in the same manner as the experiments in FIG. 1B.

FIG. 2E shows data from the experiment shown in Fig. 1B are shown again here for a direct comparison to siCdk1 nuclear morphology, which was determined as described for the experiments shown in FIG. 1B. Representative IFA with TAg (red) and DAPI (blue) is also shown. Scale bar, 100 um. Values are the means ± standard deviations for n=3 biological replicates.

FIG. 2F shows DNA damage in BKPyV infected (MOI=1.0) RPTE cells as quantified by alkaline comet assay. Comets from all biological replicates (n=3) were combined. Error bars are the 95% COI. Images are representative comets with varying levels of DNA damage determined by % of comet DNA in tail. Scale bar=50μm.

FIG. 2G shows a working model illustrating that mitotic entry leads to DNA damage during BKPyV infection when ATR is inhibited.

FIG. 3 shows DDR activation enhanced BKPyV titers by arresting the cell cycle._To determine if DDR inhibition reduced BKPyV productive infection due to defective cell cycle arrest, RPTE cells were infected with BKPyV (MOI=0.5). At 24 hpi either ATRi (5μM VE-821), ATMi (10μM KU-55933), or vehicle control (DMSO). Cells were harvested at 72 hpi for analysis. Symbols for are as in FIG. 1. Biological replicates are n=3 for western and viral titers and n=7 for cell cycle analysis.

FIG. 3A shows Cdk1 silencing was used to determine if mitotic entry due to DDRi reduced viral titers. The mean ±SD are shown. Significant differences from the NTC (black asterisks) were determined by One-Way ANOVA with Dunnet's post-test. Significant differences from the NTC within treatment conditions (red asterisks) were determined by Student's t-test (*p<0.05).

FIG. 3B shows representative Western blots of TAg (viral infection) and Cdk1 knockdown.

FIG. 3C shows cell cycle analysis as performed by FACS of mock or BKPyV infected RPTE cells treated with ATRi or ATMi and shown as contour plots (5%) to determine how DDR activation influences the cell cycle profile of a BKPyV infection.

FIG. 3D shows the percent of cells in G1 (gray), S (green) and G2 + M (blue) phases from FIG. 3C as quantified and reported as the % of total.

FIG. 3E shows the average percent of cells in G1 phase (re-graphed from FIG. 3D to show the differences in the populations). Shown are mean±SD.

FIG. 3F shows the average percent of cells in S phase (re-graphed from FIG. 3D to show the differences in the populations). Shown are mean±SD.

FIG. 3G shows the average percent of cells in G2 + M phase (re-graphed from FIG. 3D to show the differences in the populations). Shown are mean±SD.

FIG. 3H shows G2 and M phase population of cells from FIG. 1C as further separated into non-mitotic (gray) and mitotic (orange) cells by $pH3^{S10}$ expression.

FIG. I shows average % of mitotic cells from FIG. 3H quantified as % of total G2 and M phase cells. Shown are mean ±SD.

FIG. 3J shows a working model illustrating that if ATR is inhibited during infection, then mitotic entry is increased, which decreases BKPyV titers and reduces S phase levels.

FIG 4 show the effect of small molecule inhibitors of ATR and ATM prolong BKPyV induced S phase and increase viral titers. To determine if DDR inhibition reduced BKPyV productive infection due to defective cell cycle arrest using different ATR and ATM inhibitors, RPTE cells were infected with BKPyV (MOI=0.5). 24 hpi or 48 hpi either ATRi (5μM, AZD6738) or ATMi (5μM, AZD0156 or 1nM-10μM AZD1390) or DMSO (Vehicle) were added. Cells were harvested at 72 hpi for analysis. **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$; ns, not significant

FIG. 4A shows cell cycle analysis by flow cytometry. S phase was measured by 3 hour EdU pulse labeling to detect nucleotide incorporation; G1 and G2 (or M) phase were separated based on DNA content staining. Contour plots (5%) are representative of n=4 biological replicates.

FIG. 4B shows the percent of cells in G1 (gray), S (green) and G2 or M (blue) phases from FIG. 4A as quantified and reported as the % of the total.

FIG. 4C shows the average percent of cells in G1 phase (re-graphed separately from FIG. 4B to show the differences in the populations). Shown are the mean±SD.

FIG. 4D shows the average percent of cells in S phase (re-graphed separately from FIG. 4B to show the differences in the populations). Shown are the mean±SD.

FIG. 4E shows the average percent of cells in G2 or M phase (re-graphed separately from FIG. 4B to show the differences in the populations). Shown are the mean±SD.

FIG. 4F shows the average percent of cells in M phase determined based on the percent of $pH3^{S10}$ positive cells in the total cell population. Shown are the mean±SD.

FIG. 4G shows the total fraction of S phase (EdU^{+}) cells from FIG. 4A analyzed for whether they were also undergoing mitosis ($pH3^{S10}$) to differentiate premature mitosis (red) from S phase (gray). Cells were plotted based on their DNA content. Shown is representative of n=4 biological replicates.

FIG. 4H shows quantification of the average % of premature mitosis from FIG. 4G. Shown are the mean±SD.

FIG. 4I shows a comparison of the average proportion of cells in S phase caused by chemical inhibition with both sets of inhibitors to ATM and ATR. VE-821 and KU-55933 data are re-graphed from FIG. 3C to visually compare the data. Shown are the mean±SD for n=4-7 biological repeats.

FIG. 4J shows a comparison of the average proportion of cells in premature mitosis caused by chemical inhibition with both sets of inhibitors to ATM and ATR. VE-821 and KU-55933 data are re-graphed from FIG. 3C to visually compare the data. Shown are the mean±SD for n=4-7 biological repeats.

FIG. 4K shows inhibition of ATM reduced BKPyV titers. RPTE cells were infected with BKPyV (MOI=0.5). At 24 hpi ATM inhibitors were added: 10 μM KU-55933 (positive control), 10μM to 1nM AZD1390, or DMSO (vehicle only). Virus was harvested at 72 hpi and titered on RPTE cells by focus forming assays. The mean±SD is shown for n=5 biological replicates.

FIG. 4L shows inhibition of ATM increases mitosis during BKPyV infection. RPTE cells were infected with BKPyV (MOI=0.5). At 24 or 48 hpi ATM inhibitors were added: 10 μM KU-55933 (positive control), 10μM to 1nM AZD1390, or DMSO (vehicle only). At 72 hpi cells were fixed and an IFA (immunofluorescence assay) was performed to determine the percent of cells that were in mitosis ($pH3^{S10}$). n=1

FIG. 5 shows constitutive ATM and ATR activation was required to prolong BKPyV-induced S phase. To determine if DDR activation is required throughout infection by BKPyV to alter the cell cycle, RPTE cells were infected with BKPyV (MOI=0.5) and treated 24 or 48 hpi (as

indicated) with ATRi (5μM VE-821), ATMi (10μM KU-55933) or DMSO (control) and harvested at either 48 or 72 hpi. Full (24-72hpi) treatment data were re-graphed from FIG. 3C for direct comparison. Significant differences were determined by Two-Way ANOVA with Tukey post-hoc test.

FIG. 5A shows a DDRi treatment diagram to indicate the late (48-72 hpi; black) and full (24-72 hpi; dark gray) treatment windows.

FIG. 5B shows cell cycle analysis performed at the late time point (top) or throughout full infection (bottom) by flow cytometry. Phase determination was as follows: S phase (EdU pulse labeling); G1 was separated from G2 (or M) phase by DNA content staining. Contour plots (5%) are representative of n=3 to 7 biological replicates.

FIG. 5C shows the percent of cells in S phase quantified for late or full DDR inhibition times (from FIG. 5B). Box-whisker plots indicate late (black) or full (dark gray) inhibition windows from FIG. 5A. Shown are the mean±SD for n=3 to 7 biological repeats.

FIG. 5D shows the percent of cells in G2+M phase quantified for mid or full DDR inhibition times (from FIG. 5B). Box-whisker plots indicate late (black) or full (dark gray) inhibition windows from FIG. 5A. Shown are the mean±SD for n=3 to 7 biological repeats.

FIG. 5E shows the percent of cells in M phase quantified for mid or full DDR inhibition times (from FIG. 5B). Box-whisker plots indicate late (black) or full (dark gray) inhibition windows from FIG. 5A. Shown are the mean±SD for n=3 to 7 biological repeats.

FIG. 5F shows the percent of cells undergoing premature mitosis (EdU^+ and $pH3^{S10+}$) quantified for mid or full DDR inhibition times (from FIG. 5H) Box-whisker plots indicate late (black) or full (dark gray) inhibition windows from FIG. 5A. Shown are the mean±SD for n=3 to 7 biological repeats.

FIG. 5G shows the S phase cells from FIG. 8B re-plotted to indicate those S-phase cells that were also positive for mitosis ($pH3^{S10}$), which were graphed based on their DNA content to differentiated premature mitosis (red) from S phase (gray). Representative of n=3 to 7 biological replicates are shown.

FIG. 5H shows the cell cycle protein expression profile during BKPyV infection. The top diagram shows the expression levels of cyclins D (gray line), E (blue line), A (green line) and B (orange line) during the phases of a normal cell cycle (x-axis). The bottom diagram is a western blot of cyclin protein levels during BKPyV (MOI=1.0) or mock infection at 1, 2 and 3 dpi. Shown are light (L) and dark (D) exposure times when appropriate to accurately reflect the relative protein abundance. A representative of n=3 biological replicates is shown.

FIG. 5I shows how DDR activation affects the expression of cyclins during infection. Proteins involved in cell cycle control, DDR activation, and viral infection following DDR inhibition late (48-72 hpi) during infection were analyzed by Western blot. RPTE cells were BKPyV infected at an MOI=1.0 to minimize the uninfected population of cells. A representative of n=3 biological replicates is shown.

FIG. 6 shows ATM enhanced S phase entry while ATR induced cell cycle arrest during BKPyV infection. To determine if ATM and ATR are required early during BKPyV infection to alter the cell cycle, RPTE cells were infected with BKPyV (MOI=0.5) and treated 24 hpi with either ATRi (5μM, VE-821), ATMi (10μM, KU-55933), or vehicle control (DMSO). Samples were harvested at either 30 or 48 hpi for cell cycle analysis. Also, data from FIG. 3C (24-72-hour inhibition) is re-presented here for a direct comparison.

FIG. 6A shows a model of the viral life cycle with DDR inhibition scheme. BKPyV trafficks to the nucleus following viral entry. By 24hpi TAg (yellow) is expressed in the nucleus, which induces host cell entry into S phase and promotes viral replication and expression of capsid proteins (triangles). Viral progeny can be detected by 48hpi and peaks by 72hpi. Bars represent the drug treatment window for DDR inhibitors during viral infection: early (24 to 30 hpi), mid (24 to 48 hpi), and full (24 to 72 hpi).

FIG. 6B shows a time course study to reveal the proportion of cells in S phase and their DNA content during a normal BKPyV infection. Cell cycle analyses were performed at 30, 48, and 72 hpi. S phase (EdU^+) (green) cells were superimposed on the total population (grey). The average % of cells with ≥ 4N DNA content were quantified and mean ±SD for n=3 to 7 biological replicates are shown.

FIG. 6C shows how ATRi or ATMi affected the cell cycle in early or mid-infection. Cell cycle analysis was performed by FACS on BKPyV-infected cells. The average percentages of cells in G1 (gray), S (green), and G2 and/or M (blue) phases were quantified and reported as the percentages of total cells for early or mid-DDRi treatments for n=3 biological replicates.

FIG. 6D shows the average fractions of S-phase cells ($pH3^{S10}$) after 30 hpi (white) or 48 hpi (light gray) as quantified by FACS. The mean±SD for n=3 biological replicates are shown. Significant differences were determined by two-way ANOVA with Tukey's post hoc test.

FIG. 6E shows the average number of mitotic cells ($pH3^{S10}$) after 30 hpi (white) or 48 hpi (light gray). The mean±SD for n=3 biological replicates are shown. Significant differences were determined by Two-Way ANOVA with Tukey post-hoc test.

FIG. 6F shows the effect of ATR or ATM inhibition on the incidence of premature mitosis

(red). All S phase cells (grey) were plotted based on DNA content and mitosis ($pH3^{S10}$). FIG. 6G shows the average % of premature mitosis quantified from FIG. 6F. The mean±SD for n=3 biological replicates are shown.

FIG. 6H shows how ATRi or ATMi affected the cell cycle over time during a BKPyV infection. A ternary analysis was utilized to represent the proportional relationship of G1, S, and G2 phases for each of 3-7 replicates from the early (circle), mid (square) and full (triangle) DDRi treatment windows quantified from FIG. 6C and FIG. 3C. 95% COI is represented for each treatment population (line). Symbol color represents vehicle (black), ATMi (green), and ATRi (blue) treatment.

FIG. 6I shows a working model illustrating that ATM is required for efficient S phase entry early during infection while ATR activation prevents mitotic entry of actively replicating cells later during infection. Together, this results in a prolonged S phase during infection.

FIG. 7 shows premature mitosis was the source of DNA damage due to ATR inhibition during BKPyV infection. To determine if ATR activates the Wee1 pathway to block premature mitosis during BKPyV infection (MOI=0.5), Wee1 and/or Cdk1 were silenced with siRNAs to induce or block premature mitosis, respectively. At 72 hpi cells were harvested to assess the amount of premature mitosis and DNA damage during BKPyV infection. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$.

FIG. 7A shows a diagram for how ATM and ATR regulate of the G2/M checkpoint. ATR and ATM activation stimulates Chk1 and Chk2, respectively. Activation of the Wee1 kinase inhibits Cdk1 through phosphorylation leading to G2/M arrest. Simultaneously, DDR activation inhibits Cdc25C, the phosphatase that reactivates Cdk1 to promote mitosis. Cdc25C turnover requires protein PP2A.

FIG. 7B shows a Western blot of G2/M checkpoint control proteins in BKPyV (MOI=1.0) or mock infected cells treated with ATRi, ATMi, or vehicle control (DMSO) from 48-72 hpi. Shown is representative of n=3 biological replicates.

FIG. 7C shows levels of mitosis and premature mitosis measured by FACS as in FIG. 1G. Representative of n=4 is shown.

FIG. 7D shows quantification of cells from FIG. 7C that are mitotic. The mean±SD of n=4 biological replicates is shown. Symbols indicate mock (white) or BKPyV (black) infected, as well as, siNTC (square), siWee1 (upward triangle), siCdk1 (downward triangle), or double knockdown (diamond) knockdowns. Significant differences were determined by Two-Way ANOVA with Tukey post-hoc test.

FIG. 7E shows quantification of cells from FIG. 5C that are undergoing premature mitosis (red bars). The mean ±SD of n=4 biological replicates is shown. Symbols are as in FIG. 7D.

FIG. 7F was used to determine if cells undergoing premature mitosis acquire DNA damage. siWee1 samples stained for FACS (FIG. 7C) were analyzed by IFA for evidence of BKPyV-induced DNA damage. Shown are representative of >20 cells from G1, S, or premature mitosis from FIG. 7C for n=3 biological replicates. EdU incorporation (green), $pH3^{S10}$ (white) and chromatin (blue) are represented. Scale bar=20μm.

FIG. 7G shows the % of DNA damaged nuclei (fragmented) assessed by immunofluorescence microscopy of TAg and DAPI staining quantified from at least 100 nuclei per condition per replicate. Data are mean±SD for n=4 biological replicates. Significant differences were determined by Two-Way ANOVA with Tukey post-hoc test.

FIG. 7H shows Western analysis of markers of viral infection and knockdown efficiency for Wee1 and Cdk1. Representative of n=4 biological replicates is shown.

FIG. 7I shows the impact of Wee1 silencing on BKPyV titers as determined by focus forming assays. The mean ±SD for n=4 biological replicates is shown. The dotted line represents quantifiable limit of detection for the assay. BQL (below quantifiable limit).

FIG. 7J shows the percent of cells in S phase quantified and presented as mean±SD for n=4 biological replicates. Symbols are as in FIG. 7D. Significant differences were determined by Two-Way ANOVA with Tukey post-test.

FIG. 7K shows the percentage of cells in S-phase. RPTE cells were mock or BKPyV infected (multiplicity of infection of 0.5) and then at 24 hpi treated with Wee1i (300 nM MK1775). Cell cycle analysis to identify S phase (EdU) was performed by FACS at 72 hpi. The mean percentage of cells in each phase ± standard deviation is shown for n=3 biological replicates. Symbols are as follows: white, mock infection; black, BKPyV black infection; square, vehicle control; diamond, Wee1i

FIG. 7L shows the percentage of cells with premature mitosis based on $pH3^{S10}$ expression. The experiment was conducted as described in FIG. 7K.

FIG. 7M shows a working model illustrating that ATR prolongs S phase by activating Wee1 to inhibit mitotic entry by inhibiting Cdk1. Thus, ATR activation prevents premature mitosis, which avoids DNA damage and reduced viral titers.

FIG. 8 shows activated Cdk1 is required for S phase exit during BKPyV infection. To determine if the ATR pathway is activated during a BKPyV infection to prevent S phase exit and mitotic entry of actively replicating cells, the contribution of Chk1i (2μM; MK8776),

ATRi (5μM, VE-821), and Cdk1i (10μM, RO-3306) to premature mitosis and cell cycle arrest were measured by FACS of BKPyV infected cells (MOI=0.5) at 72 hpi against a vehicle control (DMSO). *, $P<0.05$;

***, $P<0.001$; ****, $P<0.0001$.

FIG. 8A shows (Top) cell cycle analysis performed on Chk1i treated cells (24hpi-72hpi) by FACS to identify S (EdU), G1 and G2 (based on DNA content staining) , and M ($pH3^{S10}$) phases. Representative contour plots (5%) are shown. (Bottom) The fractions of S phase cells from the top panel were graphed based on their DNA content and mitosis ($pH3^{S10}$) to differentiated premature mitosis (red) from S phase (gray). Data are representative of n=3 biological replicates.

FIG. 8B shows a diagram of Chk1i treatment during BKPyV infection.

FIG. 8C shows the percent of cells in S phase quantified from FIG. 8A. Symbols: mock (white) or BKPyV (black) infected, vehicle (square), Chk1i (diamond).

FIG. 8D shows the percent of cells in premature mitosis quantified from FIG. 8A. Symbols are as described in FIG. 8C.

FIG. 8E shows Cdk1i blocks ATRi-associated mitotic entry. Mock or infected RPTE cells were treated at 48 hpi with ATRi (5μM, VE-821), Cdk1i (10μM, RO-3306) or vehicle control (DMSO). Cells were pulse labeled with EdU at 70-72 hpi to assess DNA synthesis and analyzed by FACS at 72 hpi for cell cycle distribution. Contour plots (5%) are representative of n=3 biological replicates.

FIG. 8F shows the percentage of mitotic ($pH3^{S10}$) cells from FIG. 8E presented as the mean±SD for n=3 biological repeats. Symbols indicate mock (white) or BKPyV (black) infected.

FIG. 8G shows the percentage of S phase (EdU+) cells from FIG. 8E presented as the mean±SD for n=3 biological repeats. Symbols indicate mock (white) or BKPyV (black) infected.

FIG. 8H shows small molecule inhibitor treatment and EdU labeling schema for the ATRi treatment conditions (Control, Cdk1i, and Cdk1i wash). The control, no ATRi treatment schema is not shown. Cdk1i was used to synchronize BKPyV RPTE cells treated with ATRi, then Cdk1 was washed out to determine if cells entered mitosis immediately (premature mitosis) or completed S phase prior to entering mitosis.

FIG. 8I shows cell cycle analysis (FACS) with (bottom) and without (top) ATRi. Shown is a representative of n=3 biological replicates. All events were labeled in gray and mitotic events labeled in orange ($pH3^{S10}$ positive) and plotted as S phase (EdU^{+}) versus DNA content.

FIG. 8J shows the percent of cells in mitosis (orange, positive for $pH3^{S10}$) or undergoing premature mitosis (red, EdU^{+} and $pH3^{S10+}$) from FIG. 8I quantified as mean±SD of n=3 biological replicates. The fraction of premature mitosis is shown as a portion of overall mitosis in each group. Asterisks denote significant differences determined by One-Way ANOVA between the overall mitosis populations (black) and the premature mitosis population (red).

FIG. 8K shows a working model wherein ATR prolongs S phase by activating Wee1 to inhibit mitotic entry by inhibiting Cdk1. ATR activation prevents premature mitosis, which in turn maintains S phase, prevents DNA damage, and enhances viral titers. When ATR is inhibited, Wee1 is no longer activated to inhibit Cdk1. Activated Cdk1 in S phase induces premature mitosis, leading to DNA damage and reducing viral titers.

SUMMARY OF THE DISCLOSURE

The present disclosure provides for methods of treating a PyV infection in a subject as well as methods for treating conditions related to a PyV infection in a subject.

In a first aspect, the present disclosure provides for a method of treating a subject having a PyV infection (including activation of a latent PyV). Such methods comprise administering to the subject an amount of an ATM inhibitor (ATMi), an ATR inhibitor (ATRi), or both an ATMi and an ATRi. In a particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In a second aspect, the present disclosure provides for a method of reducing a viral titer of a PyV in a subject having a PyV infection or a latent PyV. Such methods comprise administering to the subject an amount of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In a third aspect, the present disclosure provides for a method of treating a subject suffering from a disease or condition that suppresses the immune system. Such methods comprise administering to the subject an amount of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In a fourth aspect, the present disclosure provides for a method for treating an adverse effect of a treatment that suppresses the immune system in a subject administered the treatment that suppresses the immune system. Such methods comprise administering to the subject an amount of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In a fifth aspect, the present disclosure provides a method for treating a side effect of immune suppression in a subject who has received or is being prepared to receive an organ or tissue transplant, wherein the subject is administered a treatment that suppresses the immune system and the subject has a PyV infection or a latent PyV. Such methods comprise administering to the subject an amount of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In a sixth aspect, the present disclosure provides for a method for inhibiting the DDR in a subject having a PyV infection. Such methods comprise administering to the subject an amount of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In a further particular

embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In a seventh aspect, the present disclosure provides for a method for treating a disease or condition caused by or resulting from a PyV infection in a subject (including activation of a latent PyV). Such methods comprise administering to the subject an amount of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In an eighth aspect, the present disclosure provides a pharmaceutical composition for use in the methods described herein, including, but not limited to the methods of the first to seventh aspects, wherein the composition comprises an ATMi, an ATRi, and an optional pharmadeutically acceptable carrier or excipient.

In a ninth aspect, the present disclosure provides a pharmaceutical composition for use in the methods described herein, including, but not limited to the methods of the first to seventh aspects, wherein the composition comprises an ATMi and an optional pharmadeutically acceptable carrier or excipient.

In a tenth aspect, the present disclosure provides a pharmaceutical composition for use in the methods described herein, including, but not limited to the methods of the first to seventh aspects, wherein the composition comprises an ATRi and an optional pharmadeutically acceptable carrier or excipient.

In an eleventh aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in a method of treatment or therapy.

In a twelfth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in treating a PyV infection.

In a thirteenth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in reducing a viral titer of a polyomavirus PyV having a PyV infection or a latent PyV.

In a fourteenth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in treating a disease or condition that suppresses the immune system.

In a fifteenth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in treating an adverse effect of a treatment that suppresses the immune system.

In a sixteenth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in treating a side effect of immune suppression in a subject who has receveid or is being prepared to receive an organ or tissue transplant, wherein the subject has a PyV infection or a latent PyV.

In a seventeenth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in decreasing the DDR.

In an eighteenth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in the manufacture of a medicament for treating a disease or condition caused by or resulting from a PyV infection.

In a nineteenth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in the manufacture of a medicament for reducing a viral titer of a polyoma virus PyV having a PyV infection or a latent PyV.

In a twentieth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in the manufacture of a medicament for treating a disease or condition that suppresses the immune system.

In a twenty-first aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in the manufacture of a medicament for decreasing an adverse effect of a treatment that suppresses the immune system.

In a twenty-second aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in the manufacture of a medicament for treating a side effect of immune suppression in a

subject who has received or is being prepared to receive an organ or tissue transplant, wherein the subject has a PyV infection or a latent PyV.

In a twenty-third aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use the manufacture of a medicament for decreasing the DDR.

In a twenty-fourth aspect, the present disclosure provides an ATMi, an ATRi, both an ATMi and an ATRi, or a pharmacetuical composition of the eighth to tenth aspects, for use in the manufacture of a medicament for treating a disease or condition caused by or resulting from a PyV infection.

DETAILED DESCRIPTION

Definitions

As used herein the phrase "a disease or condition that suppresses the immune system" refers to a disease or condition in which the immune system is suppressed, either as a consequence of the pathology of the agent causing the disease or condition or as a result of a treatment of the disease or condition (for example,treating a subject having an autoimmune disease with a compound that modulates or inhibits the immune system). Exemplary diseases or conditions that suppress the immune system include, but are not limited to, acquired immunodeficiency syndrome (AIDS), cancers of the immune system, immune-complex diseases (for example, viral hepatitis and complications of monoclonal antibody treatment), combined immunodeficiency disease, complement deficiencies, DiGeorge syndrome, hypogammaglobulinemia, Job syndrome, leukocyte adhesion defects, panhypogammaglobulinemia, Bruton's disease, congenital agammaglobulinemia, selective deficiency of IgA, Wiskott-Aldrich syndrome, ataxia-telangiectasia, cartilage-hair hypoplasia, PyV nephropathy, nephropathy non-renal solid organ transplants, interstitial nephritis and progressive multifocal leukoencephalopathy (PML), dementia, blindness, paralysis, and seizures associated with PML, Merkel cell cancer, hemorrhagic cystitis, diseases or conditions in which in which the immune system is suppressed during treatment of the disease or condition (such, as, but not limited to autoimmune diseases and diseases or conditions that result in the need for an organ or tissue transplant) and organ or tissue transplants. In a particular embodiment, the condition is an organ or tissue transplant.

As used herein the term "suppressed" in describing the immune system means a change in the function of the immune system that decreases a subject's ability to respond to self and/or non-self-antigens (such as, but not limited to, bacteria, viruses, fungi, and

transplanted organs or tissues). The change may be the result of a treatment that decreases the effectiveness of the immune system or the result of a disease or condition that decreases the effectiveness of the immune system. The normal immune system involves a complex interaction of certain types of cells that can recognize and attack non-self-antigens and in certain cases (i.e., autoimmune diseases) attack self-antigens. The immune system has both innate and adaptive components. Innate immunity is made up of immune protections people are born with. Adaptive immunity develops throughout life. Adaptive immunity is divided into two components: humoral immunity and cellular immunity. The change in the immune system can occur in any component of the immune system or in more than one component of the immune system.

As used herein the phrase "organ or tissue transplant" means a procedure in which an organ or tissue is removed from the body of a donor and placed in the body of a recipient, generally to replace a damaged or dysfunctional organ or tissue, wherein the recipient is administered a treatment in which the immune system is suppressed. Exemplary organs and tissues that may be the subject of an organ or tissue transplant include, but are not limited to, kindey, heart, intestine (small bowel), pancreatic islet cell, liver, lung, thymus, pancrease, bone marrow, bone, tendon (musculoskeletal graft), cornea, skin, heart valve, nerve, and vein. In some embodiments the donor and recipient are the same subject (for example, in the case of a bone marrow transplant). In some embodiments the donor and recipient are not the same subject (for example, in the case of a kidney transplant). In some embodiments the donor is an animal (for example, a heart valve).

As used herein the phrase "polyomavirus" or "PyV" is meant to include any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyV include, but are not limited to, Merkel cell polyomavirus (MCPyV), Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BK polyomavirus (BKPyV), JC polyomavirus (JCPyV), KI, polyomavirus (KIPyV), WU polyomavirus (WUPyV), human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, and Lyon IARC polyomavirus. In a particular embodiment, the polyomavirus is BKPyV. In a particular embodiment, the polyomavirus is JCPyV.

As used herein, the term "phosphatidylinositol 3-kinase-related kinase family" means the proteins ATM, ATR, DNA-dependent protein kinase catalytic subunit (DNA-PKcs),

mammalian target of rapamycin (mTOR), suppressor of morphogenesis in genitalia (SMG1), and transformation/transcription domain-associated protein (TRRAP).

As used herein, the terms “treatment”, “treat” and "treating" refers a course of action (such as administering an ATMi or ATRi of the present disclosure) taken to reduce a symptom, aspect, or characteristics of a disease or condition. Such treating need not be absolute to be useful.

As used herein, the term “in need of treatment” refers to a judgment made by a caregiver that a subject requires or will benefit from treatment. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that includes the knowledge that the subject is ill, or will be ill, as the result of a disease or condition that is treatable by a method or compound of the disclosure.

As used herein, the term “individual”, “subject” or “patient” refers to any animal, including mammals, such as mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and humans. The term may specify male or female or both, or exclude male or female. In a preferred embodiment, a subject is a human.

As used herein, the term “therapeutically effective amount” refers to an amount of a compound (such as an ATMi or ATRi of the present disclosure), either alone or as a part of a pharmaceutical composition, that is capable of having any detectable, positive effect on any symptom, aspect, or characteristics of a disease or condition. Such effect need not be absolute to be beneficial.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of certain embodiments are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Moreover, all ranges disclosed herein are to be understood to encompass any and all subranges subsumed therein. For example, a stated range of “1 to 10” should be considered to include any and all subranges between (and inclusive of) the minimum value of 1 and the maximum value of 10; that is, all subranges beginning with a minimum value of 1 or more, for example 1 to 6.1, and ending with a maximum value of 10 or less, for example, 5.5 to 10.

It is further noted that, as used in this specification, the singular forms “a,” “an,” and ”the” include plural referents unless expressly and unequivocally limited to one referent. The

term "or" is used interchangeably with the term "and/or" unless the context clearly indicates otherwise.

All patent applications, patents, and printed publications cited herein are incorporated herein by reference in the entireties, except for any definitions, subject matter disclaimers or disavowals, and except to the extent that the incorporated material is inconsistent with the express disclosure herein, in which case the language in this disclosure controls.

Introduction

PyVs are the group of DNA tumor viruses with the smallest genome of DNA viruses, encoding only five to eight proteins (1). The BKPyV early genes dramatically alter the host cell cycle and are expressed by alternative splicing of a single transcript to generate large (TAg), small (tAg), and truncated (truncTAg) tumor antigens. The late genes encode for agnoprotein and three structural proteins (VP1, VP2, and VP3). The recently solved structure of the BKPyV virion revealed an outer shell that consists of 72 pentamers of the major capsid protein, VP1, and an inner shell that is not solvent exposed consisting of VP2 and VP3 monomers. The minor capsid proteins, VP2 and VP3 are made from the same transcript, but VP2 has an N-terminal extension. VP1 is required for viral entry through endocytosis and then carried by microtubules to the endoplasmic reticulum at which point the capsid is uncoated and VP2 and/or VP3 are required for nuclear import by the importin pathway where the genome remains episomal. Expression of the early gene TAg drives the cells into S phase by sequestering retinoblastoma protein (pRb) and blocking apoptosis by inactivating the p53 tumor suppressor protein. Viral DNA replication requires only one viral protein, TAg, and several cellular proteins, such as: replication protein A (RPA), DNA polymerase δ (Pol δ), and topoisomerase I. PyV infection has also been shown to require the mismatch repair pathway for cell cycle arrest (*BK polyomavirus infection activates the DNA damage response to prolong S Phase,* Justice, JL, Verhalen, Brandy, and Thompson, SR, J Virol 93:e00130-19).

As a result, PyVs rely on host DNA replication machinery to amplify the viral genome. To hijack the host S phase proteins PyVs drive the host into S phase by expressing TAg and tAg. TAg inactivates the S phase-suppressor, retinoblastoma protein (pRb) (3, 4) while tAg inactivates protein phosphatase 2A (PP2A), a master phosphatase that is essential for cell cycle progression (5, 6). Together these genes commandeer the host cell, promote S phase entry, and induce multiple rounds of viral and host chromatin replication (7). In addition to promoting S phase, all studied PyVs activate the DNA damage response (DDR) during

infection, which is required for efficient amplification of the viral genome, production of virions, and to protect the host from severe DNA damage linked to infection (7-9).

The DDR is a cellular response to genotoxic stress that governs three processes: DNA repair, cell cycle arrest, or cell death (10). The DDRs that impact PyV infection are regulated by ataxia telangiectasia mutated (ATM) and ATM-Rad 3 related (ATR) protein. ATM coordinates homologous recombination to repair double-stranded breaks. RPA association with ssDNA activates the ATR pathway causing replication to slow and mediates recovery from replication fork collapse (11). Both ATR and ATM mediate cell cycle arrest by activating the downstream checkpoint kinases (Chk), Chk1 (ATR) and Chk2 (ATM), respectively. Together, ATM and ATR phosphorylate potentially hundreds of proteins in response to DNA damage (12). Recent proteomic profiling of the nuclear compartment revealed that most of the cellular pathways that are upregulated during BKPyV infection are involved in DNA repair and cell cycle arrest (13). The current paradigm is that ATM and ATR are important for promoting viral chromatin replication by preventing the accumulation of viral replication intermediates (9). However, when the DDR is inactivated only a small fraction of the total viral genomes are affected, which cannot account for the dramatic decrease in viral titers. Furthermore, TAg expression from a mutant virus that was not competent for viral DNA replication still induced DNA damage and failed to activate the DDR despite triggering DNA damage. This suggests that the neither ATM nor ATR are activated by DNA damage during a PyV infection. Instead PyV viral chromatin replication triggers DDR activation early in infection (14), suggesting that activation of the DDR by viral DNA replication may be to prevent DNA damage rather than mediate its repair. These findings suggest that there is an alternative mechanism by which DDR activation enhances viral infection that can be characterized by identifying the origin of host DNA damage that occurs when the DDR is inhibited during infection.

The present disclosure identifies the source of host DNA damage that occurs during PyV infection when the DDR is inhibited. The present disclosure shows that the DDR is required to prevent host DNA damage rather than to repair existing damage caused intrinsically by PyV induced replication stress. Specifically, the present disclosure shows that ATR prevented premature mitosis during PyV infection by blocking activation of cyclin dependent kinase 1 (Cdk1). Consistent with this finding, blocking premature mitosis by Cdk1-depletion prevented DNA damage and rescued viral titers that were attenuated by both ATM and ATR inhibition. The present disclosure shows that the DDR is required late during

infection when the majority of viral replication and assembly occurs to prolong S phase and prevent mitotic entry, thereby extending the window for viral production. The present disclosure also reveals differences between the role of ATM and ATR during a PyV infection. In BKPyV infection, ATM was required for efficient S phase entry as well as prolonging S phase as inhibiting ATM drove cells into regulated mitosis following S phase termination. In contrast, inhibition of ATR during BKPyV infection resulted in a dramatic shift of the population of cells that entered mitosis while actively synthesizing DNA, resulting in severe DNA damage. Taken together, these findings demonstrate that ATR and ATM function synergistically to maintain cells in S phase for sustained PyV replication.

The present disclosure shows PyV activates the DDR in order to keep PyV infected cells in S phase. This extended S phase provides a greater time to replicate the viral DNA. Absent activation of the DDR in PyV infected cells (i.e., when the DDR was inhibited in PyV infected cells), significant DNA damage in PyV infected cells occurs. The DNA damage was primarily due to actively replicating cells with uncondensed chromosomes entering directly into mitosis. The DNA damage decreased PyV viral titer due to death of PyV infected cells.

The present disclosure also provides insights into therapeutic targets that may be used to inhibit PyV replication and to treat PyV infection in a subject. The present disclosure demonstrates that inhibition of ATM and/or ATR represent viable methods of treatment as described herein. Therefore, inhibiting activation of the DDR through the use of the methods and compositions of the present disclosure provides a novel and inventive approach to treating PyV infection and diseases and conditions related to PyV infection.

Methods of Treatment

The present disclosure provides methods for various methods of treatment based on inhibiting ATM, ATR, or both ATM and ATR. As discussed herein, inhibiting ATM results in decreased damage to host cell DNA making inhibition of ATM a particularly preferred option. In certain embodiments, the methods rely on inhibiting the DDR that is induced in PyV infected cells.

The present disclosure provides a method for treating a subject having a PyV infection. Such methods comprise administering to the subject an amount, including a therapeutically effective amount, of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In another particular

embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In one embodiment, the PyV infection result from the activation of a latent PyV in the subject.

In one embodiment, the PyV infection may result from any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyVs include, but are not limited to, MCPyV, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BKPyV, JCPyV, KIPyV, WUPyV, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus, or a combination of the foregoing.

In a particular embodiment, the PyV infection is caused by JCPyV, MCPyV, or BKPyV. In another particular embodiment, the PyV infection is caused by BKPyV.

In a further particular embodiment, the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof.

In a further particular embodiment, the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821.

In a further particular embodiment, the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATMi and an ATRi, such as

but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821.

In certain embodiments of the methods for treating a PyV infection, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits the DDR. In certain embodiments of the methods for treating a PyV infection, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits PyV replication in the subject and/or reduces PyV viral titers in the subject. In certain embodiments, of the methods for treating a PyV infection, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits activation of a latent PyV.

In certain embodiments of the methods for treating a PyV infection, the subject has a disease or condition that suppresses the immune system. In certain embodiments of the methods for treating a PyV infection, the subject is a recipient of an organ or tissue transplant (including, but not limited to, a kidney transplant) and the subject is administered a treatment that suppresses the immune system. In certain embodiments of the methods for treating a PyV infection, the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant (including, but not limited to, a kidney transplant). In certain embodiments of the methods for treating a PyV infection, the subject has an autoimmune disease. In certain embodiments of the methods for treating a PyV infection, the subject has AIDS.

Any ATMi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATMi compounds specifically disclosed herein. Suitable ATMi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATMi is AZD1390, AZD0156, or KU-55933.

Any ATRi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATRi compounds specifically disclosed herein. Suitable ATRi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATRi is AZD6738 or VE-821.

The present disclosure also provides methods for reducing a viral titer of a PyV in a subjectv having a PyV infection or a latent PyV. Such methods comprise administering to the subject an amount, including a therapeutically effective amount, of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically

effective amount of an ATRi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In one embodiment, PyV infection results from the activation of a latent PyV in the subject.

In one embodiment, the viral titer is reduced by over 90%, 75%, 50%, 40%, 30%, or 20% as compared to a subject not administered an ATMi, ATRi, or combination of ATMi and ATRi of the present disclosure.

In one embodiment, the PyV infection may result from any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyVs include, but are not limited to, MCPyV, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BKPyV, JCPyV, KIPyV, WUPyV, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus, or a combination of the foregoing.

In a particular embodiment, the PyV infection is caused by JCPyV, MCPyV, or BKPyV. In another particular embodiment, the PyV infection is caused by BKPyV.

In a further particular embodiment, the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof.

In a further particular embodiment, the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821.

In a further particular embodiment, the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933

and one or more of AZD6738 and VE-821. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821.

In certain embodiments of the methods for reducing a viral titer of a PyV, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits the DDR. In certain embodiments of the methods for reducing a viral titer of a PyV, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits PyV replication in the subject and/or reduces PyV viral titers in the subject. In certain embodiments, of the methods for reducing a viral titer of a PyV, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits activation of a latent PyV.

In certain embodiments of the methods for reducing a viral titer of a PyV, the subject has a disease or condition that suppresses the immune system. In certain embodiments of the methods for reducing a viral titer of a PyV, the subject is a recipient of an organ or tissue transplant (including, but not limited to, a kidney transplant) and the subject is administered a treatment that suppresses the immune system. In certain embodiments of the methods for reducing a viral titer of a PyV, the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant (including, but not limited to, a kidney transplant). In certain embodiments of the methods for reducing a viral titer of a PyV, the subject has an autoimmune disease. In certain embodiments of the methods for reducing a viral titer of a PyV, the subject has AIDS.

Any ATMi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATMi compounds specifically disclosed herein. Suitable ATMi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

Any ATRi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATRi compounds specifically disclosed herein. Suitable ATRi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATRi is AZD6738, VE-821 or a combination of the foregoing.

The present disclosure further provides for methods for treating a subject suffering from a disease or condition that suppresses the immune system. Such methods comprise administering to the subject an amount, including a therapeutically effective amount, of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment, such methods

comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In one embodiment, the disease or condition that suppresses the immune system includes, but is not limited to, AIDS, cancers of the immune system, immune-complex diseases (for example, viral hepatitis and complications of monoclonal antibody treatment), combined immunodeficiency disease, complement deficiencies, DiGeorge syndrome, hypogammaglobulinemia, Job syndrome, leukocyte adhesion defects, panhypogammaglobulinemia, Bruton's disease, congenital agammaglobulinemia, selective deficiency of IgA, Wiskott-Aldrich syndrome, ataxia-telangiectasia, cartilage-hair hypoplasia, PyV nephropathy, nephropathy non-renal solid organ transplants, interstitial nephritis and PML, dementia, blindness, paralysis, and seizures associated with PML, Merkel cell cancer, hemorrhagic cystitis, diseases or conditions in which in which the immune system is suppressed during treatment of the disease or condition (such, as, but not limited to autoimmune diseases and diseases or conditions that result in the need for an organ or tissue transplant) and organ or tissue transplants. In a particular embodiment, the condition is an organ or tissue transplant.

In a particular embodiment, such a disease or condition is an autoimmune disease, AIDS, cancers of the immune system, or cartilage-hair hypoplasia. In a further particular embodiment, the immune system is suppressed in the subject either as a consequence of the pathology of the agent causing the disease or condition or as a result of a treatment of the disease or condition.

In a further embodiment, the subject subject suffering from a disease or condition that suppresses the immune system also has a PyV infection. In a further embodiment, the PyV infection result from the activation of a latent PyV in the subject.

In one embodiment, the PyV infection may result from any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyVs include, but are not limited to, MCPyV, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BKPyV, JCPyV, KIPyV, WUPyV, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus, or a combination of the foregoing.

In a particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV. In another particular embodiment, the PyV infection is caused by BKPyV.

In a further particular embodiment, the disease or condition that suppresses the immune system is a disease or condition listed above and the subject is treated with an ATMi only, such as but not limited to, AZD1390, AZD0156 and/or KU-55933. In a further particular embodiment, the disease or condition that suppresses the immune system is an autoimmune disease, AIDS, or a cancer of the immune system and the subject is treated with an ATMi only, such as but not limited to, AZD1390, AZD0156 and/or KU-55933. In any of the foregoing, the PyV infection may be caused by at least one of JCPyV, MCPyV, or BKPyV. In any of the foregoing, the PyV infection may be caused by BKPyV.

In a further particular embodiment, the disease or condition that suppresses the immune system is a disease or condition listed above and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the disease or condition that suppresses the immune system is an autoimmune disease, AIDS, or a cancer of the immune system, or cartilage-hair hypoplasia and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In any of the foregoing, the PyV infection may be caused by at least one of JCPyV, MCPyV, or BKPyV. In any of the foregoing, the PyV infection may be caused by BKPyV.

In a further particular embodiment, the disease or condition that suppresses the immune system is a disease or condition listed above and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, and KU-55933, and one or more of AZD6738 and VE-821. In a further particular embodiment, the disease or condition that suppresses the immune system is an autoimmune disease, AIDS, or a cancer of the immune system, and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, and KU-55933, and one or more of AZD6738 and VE-821. In any of the foregoing, the PyV infection may be caused by at least one of JCPyV, MCPyV, or BKPyV. In any of the foregoing, the PyV infection may be caused by BKPyV.

In one embodiment of any of the foregoing, the subject is being administered a treatment that suppresses the immune system. In another embodiment of any of the foregoing, the subject is being administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant (including, but not limited to, a kidney transplant).

In certain embodiments of the methods for treating a subject suffering from a disease or condition that suppresses the immune system, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits the DDR. In certain embodiments of the methods for treating a subject suffering from a disease or condition that suppresses the immune system, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits PyV replication in the subject and/or reduces PyV viral titers in the subject. In certain embodiments of the methods for treating a subject suffering from a disease or condition that suppresses the immune system, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits activation of a latent PyV.

Any ATMi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATMi compounds specifically disclosed herein. Suitable ATMi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

Any ATRi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATRi compounds specifically disclosed herein. Suitable ATRi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATRi is AZD6738, VE-821 or a combination of the foregoing.

The present disclosure further provides methods for treating an adverse effect of a treatment that suppresses the immune system in a subject. Such methods comprise administering to the subject an amount, including a therapeutically effective amount, of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In a further embodiment, the subject administered the treatment that suppresses the immune system also has a PyV infection. Such a PyV infection may result from the activation of a latent PyV in the subject.

In one embodiment, the PyV infection may result from any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyVs include, but are not limited to, MCPyV, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BKPyV, JCPyV,

KIPyV, WUPyV, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus, or a combination of the foregoing. In one embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV. In another particular embodiment, the PyV infection is caused by BKPyV.

In a further particular embodiment, the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof.

In a further particular embodiment, the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821.

In a further particular embodiment, the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821.

In certain embodiments of the methods for treating an adverse effect of a treatment that suppresses the immune system, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits the DDR. In certain embodiments of the methods for treating an adverse effect of a treatment that suppresses the immune system, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits PyV replication in the subject and/or reduces PyV viral titers in the subject. In certain embodiments, of the methods for treating an adverse effect of a treatment that

suppresses the immune system, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits activation of a latent PyV.

In certain embodiments of the methods for treating an adverse effect of a treatment that suppresses the immune system, the subject has a disease or condition that suppresses the immune system. In certain embodiments of the methods for treating an adverse effect of a treatment that suppresses the immune system, the subject is a recipient of an organ or tissue transplant (including, but not limited to, a kidney transplant). In certain embodiments of the methods for treating an adverse effect of a treatment that suppresses the immune system, the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant (including, but not limited to, a kidney transplant). In a particular embodiment, the organ or tissue transplant is a heart, intestine (small bowel), pancreatic islet cell, kidney, liver, lung, thymus, pancrease, bone marrow, bone, tendon (musculoskeletal graft), cornea, skin, heart valve, nerve, or vein transplant. In a further particular embodiment, the tissue or organ transplant is a kidney transplant. In certain embodiments of the methods for treating a PyV infection, the subject has an autoimmune disease. In certain embodiments of the methods for treating a PyV infection, the subject has AIDS.

In certain embodiments of the methods for treating an adverse effect of a treatment that suppresses the immune system, the adverse effect an activation of a latent PyV, including, but not limited to, a PyV infection caused by BKPyV, JCPyV, and/or MCPyV. In certain embodiments of the methods for treating an adverse effect of a treatment that suppresses the immune system, the adverse effect is rejection of a transplanted tissue or organ, dysfunction of a transplanted tissue or organ, or failure of a transplanted tissue or organ. In certain embodiments of the methods for treating an adverse effect of a treatment that suppresses the immune system, the adverse effect the occurrence of PML or dementia, blindness, paralysis, and seizures associated with PML.

Any ATMi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATMi compounds specifically disclosed herein. Suitable ATMi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

Any ATRi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATRi compounds specifically disclosed herein. Suitable ATRi

compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATRi is AZD6738, VE-821 or a combination of the foregoing.

The present disclosure further provides a method for treating a side effect of immune suppression in a subject who has received or is being prepared to receive an organ or tissue transplant, wherein the subject is administered a treatment that suppresses the immune system and the subject has a PyV infection or a latent PyV. Such methods comprise administering to the subject an amount, including a therapeutically effective amount, of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In one embodiment, the subject administered the treatment that suppresses the immune system also has a PyV infection. Such a PyV infection may result from the activation of a latent PyV in the subject.

In one embodiment, the PyV infection may result from any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyVs include, but are not limited to, MCPyV, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BKPyV, JCPyV, KIPyV, WUPyV, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus, or a combination of the foregoing.

In one embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV. In another particular embodiment, the PyV infection is caused by BKPyV.

In one embodiment, the side effect of immune suppression is rejection of the tissue or organ, dysfunction of the tissue or organ, and/or failure of the tissue or organ. In another embodiment, the side effect of immune suppression is nephropathy.

In a further particular embodiment, the side effect of immune suppression is a side effect listed above and the subject is treated with an ATMi only, such as but not limited to, AZD1390, AZD0156 and/or KU-55933. In a further particular embodiment, the side effect of immune suppression is rejection of the tissue or organ, dysfunction of the tissue or organ, and/or failure of the tissue or organ and the subject is treated with an ATMi only, such as but not limited to, AZD1390, AZD0156 and/or KU-55933. In a further particular embodiment, the side effect of immune suppression is nephropathy and the subject is treated with an ATMi

only, such as but not limited to, AZD1390, AZD0156 and/or KU-55933. In any of the foregoing, the PyV infection may be caused by at least one of JCPyV, MCPyV, or BKPyV. In any of the foregoing, the PyV infection may be caused by BKPyV.

In a further particular embodiment, the side effect of immune suppression is a side effect listed above and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the side effect of immune suppression is rejection of the tissue or organ, dysfunction of the tissue or organ, and/or failure of the tissue or organ and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the side effect of immune suppression is nephropathy and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In any of the foregoing, the PyV infection may be caused by at least one of JCPyV, MCPyV, or BKPyV. In any of the foregoing, the PyV infection may be caused by BKPyV.

In a further particular embodiment, the side effect of immune suppression is a side effect listed above and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, and KU-55933, and one or more of AZD6738 and VE-821. In a further particular embodiment, the side effect of immune suppression is rejection of the tissue or organ, dysfunction of the tissue or organ, and/or failure of the tissue or organ and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, and KU-55933, and one or more of AZD6738 and VE-821. In a further particular embodiment, the side effect of immune suppression is nephropathy and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, and KU-55933, and one or more of AZD6738 and VE-821. In any of the foregoing, the PyV infection may be caused by at least one of JCPyV, MCPyV, or BKPyV. In any of the foregoing, the PyV infection may be caused by BKPyV.

In a particular embodiment, the organ or tissue transplant is a heart, intestine (small bowel), pancreatic islet cell, kidney, liver, lung, thymus, pancrease, bone marrow, bone, tendon (musculoskeletal graft), cornea, skin, heart valve, nerve, or vein transplant. In a further particular embodiment, the tissue or organ transplant is a kidney transplant. In a further particular embodiment, the organ or tissue transplant is a kidney transplant and the subject has a BKPyV infection, including a BKPyV infection caused by activation of a latent BKPyV virus by the treatment that suppresses the immune system.

In certain embodiments of the methods for treating a side effect of immune suppression in a subject undergoing an organ or tissue transplant, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits the DDR. In certain embodiments of the methods for treating a side effect of immune suppression in a subject undergoing an organ or tissue transplant, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits PyV replication in the subject and/or reduces PyV viral titers in the subject. In certain embodiments, of the methods for treating a side effect of immune suppression in a subject undergoing an organ or tissue transplant, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits activation of a latent PyV.

In certain embodiments of the methods for treating a side effect of immune suppression in a subject undergoing an organ or tissue transplant, the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant (including, but not limited to, a kidney transplant). In certain embodiments of the methods for treating a side effect of immune suppression in a subject undergoing an organ or tissue transplant, the subject has an autoimmune disease. In certain embodiments of the methods for treating a side effect of immune suppression in a subject undergoing an organ or tissue transplant, the subject has AIDS.

Any ATMi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATMi compounds specifically disclosed herein. Suitable ATMi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

Any ATRi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATRi compounds specifically disclosed herein. Suitable ATRi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATRi is AZD6738, VE-821 or a combination of the foregoing.

The present disclosure further provides methods for inhibiting the DDR in a subject having a PyV infection. Such methods comprise administering to the subject an amounttt, including a therapeutically effective amount, of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an

ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In one embodiment, the subject has a PyV infection. In another embodiment, the PyV infection results from the activation of a latent PyV in the subject. Such activation may result from suppression of the immune system (such as by a treatment that suppresses the immune system or a disease or condition that suppresses the immune system).

In one embodiment, the PyV infection may result from any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyVs include, but are not limited to, MCPyV, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BKPyV, JCPyV, KIPyV, WUPyV, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus, or a combination of the foregoing.

In one embodiment, the subject has a PyV infection caused by at least one of JCPyV, MCPyV, or BKPyV. In another particular embodiment, the subject has a PyV infection caused by BKPyV. In one embodiment, the PyV infection is caused by activation of a latent PyV.

In a further particular embodiment, the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the subject has a PyV infection caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the subject has a PyV infection caused by BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof.

In a further particular embodiment, the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the subject has a PyV infection caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the subject has a PyV infection caused by BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821.

In a further particular embodiment, the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the subject has a PyV infection caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with

an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the subject has a PyV infection caused by BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821.

In certain embodiments of the methods for inhibiting a DDR, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits PyV replication in the subject and/or reduces PyV viral titers in the subject. In certain embodiments, of the methods for inhibiting a DDR, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits activation of a latent PyV.

In certain embodiments of the methods for inhibiting a DDR, the subject has a disease or condition that suppresses the immune system. In certain embodiments of the methods for inhibiting a DDR, the subject is a recipient of an organ or tissue transplant (including, but not limited to, a kidney transplant) and the subject is administered a treatment that suppresses the immune system. In certain embodiments of the methods for inhibiting a DDR, the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant (including, but not limited to, a kidney transplant). In certain embodiments of the methods for inhibiting a DDR, the subject has an autoimmune disease. In certain embodiments of the methods for inhibiting a DDR, the subject has AIDS.

Any ATMi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATMi compounds specifically disclosed herein. Suitable ATMi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

Any ATRi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATRi compounds specifically disclosed herein. Suitable ATRi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATRi is AZD6738, VE-821 or a combination of the foregoing.

The present disclosure further provides methods for treating a disease or condition caused by or resulting from a PyV infection in a subject. Such methods comprise administering to the subject an amount, including a therapeutically effective amount, of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject

a therapeutically effective amount of an ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In one embodiment, the subject administered a treatment that suppresses the immune system and the PyV infection that results from the treatment that suppresses the immune system. In another embodiment, the PyV infection results from the activation of a latent PyV in the subject. Such activation may result from suppression of the immune system (such as by a treatment that suppresses the immune system or a disease or condition that suppresses the immune system).

In one embodiment the disease or condition resulting from a PyV infection is nephropathy rejection of a tissue or organ transplant, dysfunction of a tissue or organ transplant, or failure of a tissue or organ transplant, PML (generally associated with JCPyV), and Merkel cell cancer (generally associated with MCPyV).

In one embodiment, the PyV infection may result from any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyVs include, but are not limited to, Merkel cell polyomavirus (MCPyV), Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BK polyomavirus (BKPyV), JC polyomavirus (JCPyV), KI polyomavirus (KIPyV), WU polyomavirus (WUPyV), human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, and Lyon IARC polyomavirus.

In one embodiment, the subject has a PyV infection caused by at least one of JCPyV, MCPyV, or BKPyV. In another particular embodiment, the subject has a PyV infection caused by BKPyV. In one embodiment, the PyV infection is caused by activation of a latent PyV.

In a further particular embodiment, the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the subject has a PyV infection caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the subject has a PyV infection caused by BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof.

In a further particular embodiment, the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the subject

has a PyV infection caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the subject has a PyV infection caused by BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821.

In a further particular embodiment, the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the subject has a PyV infection caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the subject has a PyV infection caused by BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821.

In certain embodiments of the methods for treating a disease or condition caused by or resulting from a PyV infection, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits the DDR. In certain embodiments of the methods for treating a disease or condition caused by or resulting from a PyV infection, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits PyV replication in the subject and/or reduces PyV viral titers in the subject. In certain embodiments, of the methods for treating a disease or condition caused by or resulting from a PyV infection, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits activation of a latent PyV.

In certain embodiments of the methods for treating a disease or condition caused by or resulting from a PyV infection, the subject has a disease or condition that suppresses the immune system. In certain embodiments of the methods for treating a disease or condition caused by or resulting from a PyV infection, the subject is a recipient of an organ or tissue transplant (including, but not limited to, a kidney transplant) and the subject is administered a treatment that suppresses the immune system. In certain embodiments of the methods for treating a disease or condition caused by or resulting from a PyV infection, the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant (including, but not limited to, a kidney transplant). In certain embodiments of the methods for treating a disease or condition caused by or resulting from a PyV infection, the subject has an autoimmune disease. In certain embodiments of the methods for treating a disease or condition caused by or resulting from a PyV infection, the subject has from AIDS.

Any ATMi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATMi compounds specifically disclosed herein. Suitable ATMi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

Any ATRi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATRi compounds specifically disclosed herein. Suitable ATRi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATRi is AZD6738, VE-821 or a combination of the foregoing.

The present disclosure further provides a method for treating a subject at risk for activation of a latent PyV. Such methods comprise administering to the subject an amount, including a therapeutically effective amount, of an ATMi, an ATRi, or both an ATMi and an ATRi. In a particular embodiment of this aspect, such methods comprise administering to the subject a therapeutically effective amount of an ATMi. In another particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATRi. In a further particular embodiment, such methods comprise administering to the subject a therapeutically effective amount of an ATMi and an ATRi.

In one embodiment, the subject is determined to have a latent PyV before such treatment is administered. In another embodiment, the subject is not determined to have a latent PyV but is undergoing a procedure or treatment that suppresses the immune system (which would result in activation of any latent PyV if such were present). In another embodiment, the subject is one who is receiving an organ or tissue transplant. In another embodiment, the subject is one who is donating an organ or tissue for transplantation (including both autologous and allogeneic transplantation). In another embodiment, the subject is undergoing a treatment that suppresses the immune system and a PyV infection results from the activation of the latent PyV in the subject.

In one embodiment, the treatment prevents the activation of a latent PyV in a subject.

In one embodiment, the PyV infection may result from any virus of the family *Polyomaviridae* that infects or is capable of infecting a human subject. Such PyVs include, but are not limited to, MCPyV, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BKPyV, JCPyV, KIPyV, WUPyV, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus, or a combination of the foregoing.

In one embodiment, the latent PyV is at least one of JCPyV, MCPyV, or BKPyV. In another particular embodiment, the latent PyV is BKPyV.

In a further particular embodiment, the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATMi only, such as, but not limited to, AZD1390, AZD0156, KU-55933 or a combination thereof.

In a further particular embodiment, the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATRi only, such as but not limited to, AZD6738 and/or VE-821.

In a further particular embodiment, the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the PyV infection is caused by at least one of JCPyV, MCPyV, or BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821. In a further particular embodiment, the PyV infection is caused by BKPyV and the subject is treated with an ATMi and an ATRi, such as but not limited to, one or more of AZD1390, AZD0156, or KU-55933 and one or more of AZD6738 and VE-821.

In certain embodiments of the methods for treating a subject at risk for activation of a latent PyV, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits the DDR when the latent PyV is activated. In certain embodiments of the methods for treating a subject at risk for activation of a latent PyV, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits PyV replication in the subject and/or reduces PyV viral titers in the subject when the latent PyV is activated. In certain embodiments, of the methods for treating a subject at risk for activation of a latent PyV, treatment with the ATMi, ATRi, or ATMi and ATRi inhibits activation of the latent PyV.

In certain embodiments of the methods for treating a subject at risk for activation of a latent PyV, the subject has a disease or condition that suppresses the immune system. In certain embodiments of the methods for treating a subject at risk for activation of a latent PyV, the subject is a recipient of an organ or tissue transplant (including, but not limited to, a kidney transplant) and the subject is administered a treatment that suppresses the immune system. In certain embodiments of the methods for treating a subject at risk for activation of a latent PyV, the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant (including, but not limited to, a kidney transplant). In certain embodiments of the methods for treating a subject at risk for activation of a latent PyV, the subject has an autoimmune disease. In certain embodiments of the methods for treating a subject at risk for activation of a latent PyV, the subject has AIDS.

Any ATMi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATMi compounds specifically disclosed herein. Suitable ATMi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

Any ATRi known in the art may be used in the methods for treating a subject having a PyV infection, including the ATRi compounds specifically disclosed herein. Suitable ATRi compounds are discussed herein and the discussion is hereby incorporated by reference. In certain embodiments, the ATRi is AZD6738, VE-821 or a combination of the foregoing.

In any of the foregoing methods referring to a disease or condition that suppresses the immune system, such a disease or condition includes, but is not limited to, AIDS, cancers of the immune system, immune-complex diseases (for example, viral hepatitis and complications of monoclonal antibody treatment), combined immunodeficiency disease, complement deficiencies, DiGeorge syndrome, hypogammaglobulinemia, Job syndrome, leukocyte adhesion defects, panhypogammaglobulinemia, Bruton's disease, congenital agammaglobulinemia, selective deficiency of IgA, Wiskott-Aldrich syndrome, ataxia-telangiectasia, cartilage-hair hypoplasia, PyV nephropathy, nephropathy non-renal solid organ transplants, interstitial nephritis and PML, dementia, blindness, paralysis, and seizures associated with PML, Merkel cell cancer, hemorrhagic cystitis, diseases or conditions in which in which the immune system is suppressed during treatment of the disease or condition (such, as, but not limited to autoimmune diseases and diseases or conditions that result in the

need for an organ or tissue transplant) and organ or tissue transplants. In a particular embodiment, the condition is an organ or tissue transplant.

In any of the foregoing methods referring to an autoimmune disease or condition, such a disease or condition includes, but is not limited to,Achalasia, Addison's disease, Adult Still's disease, Agammaglobulinemia, Alopecia areata, Amyloidosis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome, Autoimmune angioedema, Autoimmune dysautonomia, Autoimmune encephalomyelitis, Autoimmune hepatitis, Autoimmune inner ear disease, Autoimmune myocarditis, Autoimmune oophoritis, Autoimmune orchitis, Autoimmune pancreatitis, Autoimmune retinopathy, Autoimmune urticaria, Axonal & neuronal neuropathy, Baló disease, Behcet's disease, Benign mucosal pemphigoid, Bullous pemphigoid, Castleman disease, Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy, Chronic recurrent multifocal osteomyelitis, Churg-Strauss Syndrome or Eosinophilic Granulomatosis, Cicatricial pemphigoid, Cogan's syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST syndrome, Crohn's disease, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic esophagitis, Eosinophilic fasciitis, Erythema nodosum, Essential mixed cryoglobulinemia, Evans syndrome, Fibromyalgia, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Giant cell myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura, Herpes gestationis or pemphigoid gestationis, Hidradenitis Suppurativa (Acne Inversa), Hypogammalglobulinemia, IgA Nephropathy, IgG4-related sclerosing disease, Immune thrombocytopenic purpura, Inclusion body myositis, Interstitial cystitis, Juvenile arthritis, Juvenile diabetes (Type 1 diabetes), Juvenile myositis, Kawasaki disease, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease, Lupus, Lyme disease chronic, Meniere's disease, Microscopic polyangiitis, Mixed connective tissue disease, Mooren's ulcer, Mucha-Habermann disease, Multifocal Motor Neuropathy, Multiple sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neonatal Lupus, Neuromyelitis optica, Neutropenia, Ocular cicatricial pemphigoid, Optic neuritis, Palindromic rheumatism, PANDAS, Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria, Parry Romberg syndrome, Pars planitis (peripheral uveitis), Parsonage-Turner syndrome, Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia,

POEMS syndrome, Polyarteritis nodosa, Polyglandular syndromes type I, II, III, Polymyalgia rheumatica, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Primary biliary cirrhosis, Primary sclerosing cholangitis, Progesterone dermatitis, Psoriasis, Psoriatic arthritis, Pure red cell aplasia, Pyoderma gangrenosum, Raynaud's phenomenon, Reactive Arthritis, Reflex sympathetic dystrophy, Relapsing polychondritis, Restless legs syndrome, Retroperitoneal fibrosis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sjögren's syndrome, Sperm & testicular autoimmunity, Stiff person syndrome, Subacute bacterial endocarditis, Susac's syndrome, Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombocytopenic purpura, Tolosa-Hunt syndrome, Transverse myelitis, Type 1 diabetes, Ulcerative colitis, Undifferentiated connective tissue disease , Uveitis, Vasculitis, Vitiligo, and Vogt-Koyanagi-Harada Disease.

In certain embodiments, the autoimmune disease or condition is Multiple sclerosis, vasculitis, myasthenia gravis, Hashimoto's thyroiditis, Graves' disease, Rheumatoid arthritis, systemic lupus erythematosus (lupus), inflammatory bowel disease, type 1 diabetes mellitus, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, or psoriasis.

Pharmaceutical Compositions

Pharmaceutical compositions are provided that comprise an amount of a compound of the present disclosure (such as an ATMi and/or an ATRi). In one embodiment, such pharmaceutical compositions contain a therapeutically effective amount of a compound of the present disclosure. In a particular embodiment, the compound is an ATMi, an ATRi, or both an ATMi and an ATRi. In addition, other active agents may be included in such pharmaceutical compositions. Additional active agents to be included may be selected based on the disease or condition to be treated.

The pharmaceutical compositions disclosed may comprise one or more compound of the present disclosure, alone or in combination with additional active agents, in combination with a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington: The Science and Practice of Pharmacy (20^{th} Ed., Lippincott, Williams & Wilkins, Daniel Limmer, editor). Such pharmaceutical compositions may be used in the manufacture of a medicament for use in the methods of treatment and prevention described herein. The compounds of the disclosure are useful in both free form and in the form of pharmaceutically acceptable salts.

The pharmaceutically acceptable carriers described herein, including, but not limited to, vehicles, adjuvants, excipients, or diluents, are well-known to those who are skilled in the art. Pharmaceutically acceptable excipients are also well-known to those who are skilled in the art. The choice of excipient will be determined in part by the particular compound(s), as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the present invention. The following methods and excipients are merely exemplary and are in no way limiting. Suitable carriers and excipients include solvents such as water, alcohol, and propylene glycol, solid absorbants and diluents, surface active agents, suspending agent, tableting binders, lubricants, flavors, and coloring agents. The pharmaceutically acceptable carriers can include polymers and polymer matrices. Examples of acceptable pharmaceutical carriers include carboxymethyl cellulose, crystalline cellulose, glycerin, gum arabic, lactose, magnesium stearate, methyl cellulose, powders, saline, sodium alginate, sucrose, starch, talc and water, among others. Typically, the pharmaceutically acceptable carrier is chemically inert to the active agents in the composition and has no detrimental side effects or toxicity under the conditions of use. In some embodiments, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

Surfactants such as, for example, detergents, are also suitable for use in the formulations. Specific examples of surfactants include polyvinylpyrrolidone, polyvinyl alcohols, copolymers of vinyl acetate and of vinylpyrrolidone, polyethylene glycols, benzyl alcohol, mannitol, glycerol, sorbitol or polyoxyethylenated esters of sorbitan; lecithin or sodium carboxymethylcellulose; or acrylic derivatives, such as methacrylates and others, anionic surfactants, such as alkaline stearates, in particular sodium, potassium or ammonium stearate; calcium stearate or triethanolamine stearate; alkyl sulfates, in particular sodium lauryl sufate and sodium cetyl sulfate; sodium dodecylbenzenesulphonate or sodium dioctyl sulphosuccinate; or fatty acids, in particular those derived from coconut oil, cationic surfactants, such as water-soluble quaternary ammonium salts of formula $N^+R'R''R'''R''''Y^-$, in which the R radicals are identical or different optionally hydroxylated hydrocarbon radicals and Y^- is an anion of a strong acid, such as halide, sulfate and sulfonate anions; cetyltrimethylammonium bromide is one of the cationic surfactants which can be used, amine salts of formula $N^+R'R''R'''$, in which the R radicals are identical or different optionally

hydroxylated hydrocarbon radicals; octadecylamine hydrochloride is one of the cationic surfactants which can be used, non-ionic surfactants, such as optionally polyoxyethylenated esters of sorbitan, in particular Polysorbate 80, or polyoxyethylenated alkyl ethers; polyethylene glycol stearate, polyoxyethylenated derivatives of castor oil, polyglycerol esters, polyoxyethylenated fatty alcohols, polyoxyethylenated fatty acids or copolymers of ethylene oxide and of propylene oxide, amphoteric surfactants, such as substituted lauryl compounds of betaine.

In these pharmaceutical compositions, the compound(s) of the present disclosure will ordinarily be present in an amount of about 0.5-95% weight based on the total weight of the composition. Multiple dosage forms may be administered as part of a single treatment.

The active agent can be administered internally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as milk, elixirs, syrups and suspensions. It can also be administered parenterally, in sterile liquid dosage forms. The compound(s) of the present disclosure can also be administered intranasally (nose drops) or by inhalation via the pulmonary system, such as by propellant based metered dose inhalers or dry powders inhalation devices. Other dosage forms include topical administration, such as administration transdermally, via patch mechanism or ointment.

In accordance with the methods of the present disclosure, the compounds of the disclosure are administered to the subject (or are contacted with cells of the subject) in a therapeutically effective amount. This amount is readily determined by the skilled artisan, based upon known procedures, including analysis of titration curves established in vivo and methods and assays disclosed herein. In some embodiments, a therapeutically effective amount decreases the level of PyV in the subject and/or limits or prevents an increase in the level of viral particles in the subject. In some embodiments, a therapeutically effective amount decreases the viral titer of PyV in the subject.

In certain embodiments, the therapeutically effective amount of a compound of the disclosure ranges from about 0.01 mg/kg/day to about 500 mg/kg/day. In certain embodiments, the therapeutically effective amount ranges from about 0.01 mg/kg/day to about 400 mg/kg/day, from about 0.01 mg/kg/day to about 300 mg/kg/day, from about 0.01 mg/kg/day to about 200 mg/kg/day, from about 0.01 mg/kg/day to about 100 mg/kg/day, from about 0.01 mg/kg/day to about 50 mg/kg/day, from about 0.01 mg/kg/day to about 25 mg/kg/day, from about 0.01 mg/kg/day to about 15 mg/kg/day, from about 0.01 mg/kg/day to about 10 mg/kg/day, from about 0.01 mg/kg/day to about 5 mg/kg/day, or from about 0.01

mg/kg/day to about 2.5 mg/kg/day. In some embodiments, the therapeutically effective amount ranges from about 5 mg/kg/day to about 100 mg/kg/day, from about 5 mg/kg/day to about 50 mg/kg/day, from about 2 mg/kg/day to about 30 mg/kg/day, or from about 1 mg/kg/day to about 10 mg/kg/day.

In certain embodiments, the therapeutically effective amount is administered in one or more doses according to a course of treatment (where a dose refers to an amount of a compound administered in a single day). In certain embodiments, the dose is administered q.d. (1 time/administration per day), b.i.d. (2 times/administrations per day; for example, one-half of the therapeutically effective amount in two administrations a day), or t.i.d. (three times/administrations per day; for example, one-third of the therapeutically effective amount in two administrations a day). When a dose is divided into multiple administrations per day, the dose may be divided equally or the dose may be divided unequally at each administration. Any given dose may be delivered in a single dosage form or more than one dosage form (for example, a tablet).

In certain embodiments, only one dose of a compound of the disclosure is administered during a course of treatment and no further doses are administered. Therefore, in the methods described herein the methods may comprise the administration of a single dose of a therapeutically effective amount of a compound of the disclosure during the entire course of treatment. In certain embodiments, the dose is delivered by parenteral administration. In certain embodiments, the dose is delivered by oral administration. The dose may be delivered in a single dosage form or more than one dosage form (for example, a tablet).

In certain embodiments, more than one dose of a compound of the disclosure is administered during a course of treatment. Therefore, the methods may comprise the administration of multiple doses of a therapeutically effective amount of a compound of the disclosure during the course of treatment. In certain embodiments, the course of treatment may range from 2 days to years, from 2 days to months, or from 2 days to 4 weeks. In certain embodiments, the course of treatment for a patient who is receiving an immunosuppressive drug, the course of treatment is for the complete duration of the time he subject is receiving the immunosuppressive drug; the compounds of the disclosure may be administered before the subject receives the immunosuppressive drug and/or extend for a period of time after the subject receives the last dose of the immunosuppressive drug. In certain embodiments, a therapeutically effective amount of a compound of the disclosure may be delivered every day during the course of treatment. The therapeutically effective amount need not be the same for

every dose during a course of treatment. In one embodiment, a course of treatment may comprise administering at least one dose as a loading dose and at least one dose as a maintenance dose, wherein the loading dose contains a greater amount of a compound of the invention as compared to the maintenance dose (such as, but not limited to, 2 to 10 times higher).

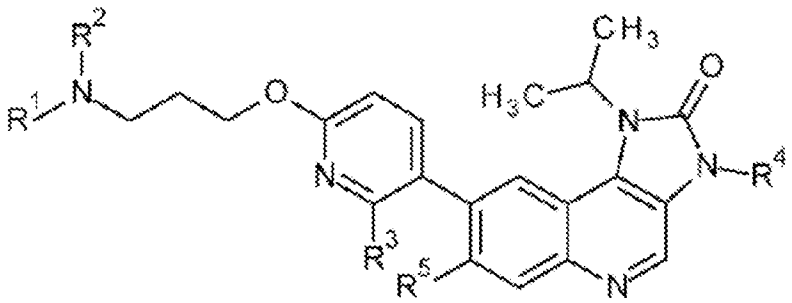
In any of the embodiments herein the dose may comprise a compound of the disclosure alone or a compound of the disclosure in a pharmaceutical composition.

The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the infection or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

Inhibitors

The present disclosure provides for the use of ATMi and ATRi for use in the methods and pharmaceutical compositions described herein. Any known ATMi or ATRi may be used in the methods and pharmaceutical compositions described herein.

In one embodiment, the ATMi is a compound of the formula I:



or a pharmaceutically acceptable salt thereof, wherein:

R^1 is methyl;

R^2 is hydro or methyl; or R^1 and R^2 together with the nitrogen atom to which they are bonded form an azetidinyl, pyrrolidinyl or piperidinyl ring;

R^3 is hydro or fluoro;

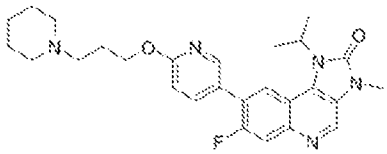
R^4 is hydro or methyl; and

R^5 is hydro or fluoro.

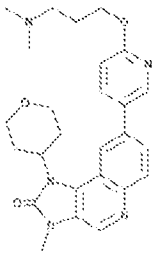
In another embodiment, the ATMi is AZD1390, AZD0156, KU-55933, KU-60014, or KU-59403. In another embodiment, the ATMi is a compound described in one or more of US Patent Nos. 9,428,503, 9822,111, 7,049,313, or 8,802,680, US Patent Publication 2018-134699, Patent Cooperation Treaty application PCT/EP2016/071782, PCT/EP/2016/076416, or PCT/EP2016/076412.

In another embodiment, the ATMi has a selectivity for ATM as compared to other members of the phosphatidylinositol 3-kinase-related kinase family of 50 or more, 100 or more, 250 or more, or 500 or more. In another embodiment, 1 μM of the ATMi does not inhibit another serine/threonine or tyrosine kinase at a level greater than 50% when tested *in vitro* using the Thermo Fisher Scientific Kinase panel. In another embodiment, 1 μM of the ATMi does not inhibit ATR at a level greater than 50% when tested *in vitro* using the Thermo Fisher Scientific Kinase panel.In another embodiment, 1 μM of the ATMi does not inhibit ATM, PI-3K, or mTOR when tested *in vitro* using the Thermo Fisher Scientific Kinase panel.

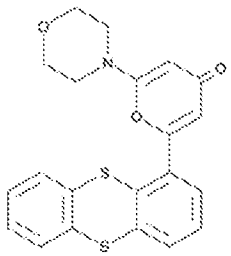
In a particularly preferred embodiment, the ATMi is AZD1390. In another particularly preferred embodiment, the ATMi is AZD0156. In another particularly preferred embodiment, the ATMi is KU-55933.



AZD1390



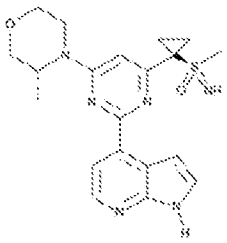
AZD0156



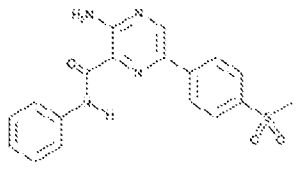
KU-55933

In one embodiment, the ATRi is AZD6738, VE-821, VE-822 (VX-970), MAY1895344, CGK-733, M6620, BAY 1895344, or BEZ-235. In another embodiment, the ATRi is a compound described in one or more of US Patent Nos. 9,334,244, 8,841,308, 9,701,674, 9,365,557, 8,552,004, 8,252,802, 8,999,997, 9,155,742, or 9,421,213, US Patent Publication Nos. 2018-170,922, 2016-311,809, or 2015-164,908, or Patent Cooperation Treaty Publication WO 2016/020320, WO 2010/071837, or WO 2011/154737. In another embodiment, the ATRi has a selectivity for ATR as compared to other members of the phosphatidylinositol 3-kinase-related kinase family of 50 or more, 100 or more, 250 or more, or 500 or more. In another embodiment, 1 μM of the ATMi does not inhibit another serine/threonine or tyrosine kinase at a level greater than 50% when tested *in vitro*. In another embodiment, 1 μM of the ATRi does not inhibit another ATM at a level greater than 50% when tested *in vitro* using the Thermo Fisher Scientific Kinase panel.

In a particularly preferred embodiment, the ATRi is AZD6738. In another particularly preferred embodiment, the ATMi is VE-821.



AZD6738



VE-821

Kits

Kits are provided for use in the methods disclosed herein, comprising any of the active agents disclosed herein. In some embodiments, the active agent is an ATMi only. In some embodiments, the active agent is an ATRi only. In some embodiments, the active agent is an ATMi and an ATRi. Any of the foregoing kits may further comprise compositions useful in the administration of the active agents.

Some embodiments of the kit comprise any of the pharmaceutical compositions disclosed herein.

In some embodiments, instructions for administration may also be provided. In some embodiments, devices for administration of the active agents or pharmaceutical compositions may also be provided.

EXAMPLES

Example 1- BKPyV activation of the DDR induced cell cycle arrest

This example shows that BKPyV activation of the DDR induced cell cycle arrest. DNA damage and effects on cell cycle arrest were measured during BKPyV (MOI=0.5) infection in primary renal proximal tubule epithelial (RPTE) cells treated with ATRi, ATMi, or vehicle at 24 hours post-infection (hpi) and analyzed at 72 hpi. In this example, the ATRi was VE-821 (5μM), the ATMi was KU-55933 (10μM), and the vehicle control was DMSO. RPTE cells were used to study an *in vivo* lytic BKPyV infection to study BKPyV host-pathogen interactions in cells with intact cell cycle control pathways (15) (16), avoiding complications with using immortalized cells that commonly harbor mutations and epigenetic modifications in the DDR and cell cycle control pathways.

As expected, ATM and ATR inhibition (ATMi and ATRi) reduced the BKPyV-induced DDR activation as measured by reduced Chk1 phosphorylation at Ser317 ($pChk1^{S317}$) and ATM autophosphorylation at Ser1981 ($pATM^{S1981}$) (FIG. 1A). The decreased Chk1

phosphorylation observed in the ATMi treatment group, is likely due to cross talk between the ATM and ATR pathways, (19).

Examination of the nuclear morphologies of BKPyV infected cells revealed that ATR and ATM inhibition reduced the percent of normal nuclei and increased the fraction of cells with diffuse TAg staining in the cytoplasm (FIGS. 1B-F, see mitotic cells and abnormal mitosis), as previously reported (7). Closer examination of the cells with diffuse TAg staining revealed alignment of chromatin on the metaphase plate characteristic of mitosis (FIGS. 1B). Nuclear envelope breakdown during mitosis likely explains the presence of cytoplasmic TAg, which is a nuclear protein. Additionally, ATRi caused a decrease in the number of normal nuclei compared to the control and ATMi treatment group (FIG. 1C) and a ~7-fold increase in the number of cells with DNA damage in the form of nuclear fragmentation compared to the control and a ~3-fold increase compared to the ATMi treated group. (FIG. 1D). ATMi inhibition did not result in a statistically significant increase in the number of cells with DNA damage in the form of nuclear fragmentation compared to the control group (FIG. 1D). Under normal conditions PyVs arrest the cell cycle (8), but mitosis was increased by DDR inhibition through the use of the ATMi and ATRi (FIG. 1E). This suggested that DDR activation might be required for cell cycle arrest during infection, such as at the G2/M checkpoint (20). Interestingly, a subset of these mitotic cells possessed chromatin morphologies that are characteristic of severely impaired mitotic progression such as lagging and misaligned chromatin as well as anaphase bridges, which are collectively refer to as abnormal mitosis herein (FIGS. 1F). Cells treated with ATMi showed an increased percentage of mitotic cells and a decreased percentage of cells with abnormal mitosis compared to the control group and ATRi groups. Activation of the ATR pathway prevents metaphase shattering, which is similar to what is observed when an S phase cell goes into mitosis (hereafter referred to as "premature mitosis") (7, 21). Therefore, ATR appears to be important during infection to prevent premature mitotic entry.

To determine if BKPyV requires ATR or ATM activation for cell cycle arrest and to prevent premature mitosis, the cell cycle distribution of BKPyV infected cells was examined when ATM or ATR was inhibited (FIG. 1G). Both ATR and ATM inhibition significantly increased mitotic entry of BKPyV infected cells, but had no effect on uninfected cells (FIG. 1H). ATRi increased premature mitosis 30-fold in BKPyV infected cells (FIG. 1I), while premature mitosis was rarely observed for uninfected cells under any condition (<0.5% of S phase cells). Interestingly, although both ATRi and ATMi induced mitosis during BKPyV

infection, only ATRi significantly induced premature mitosis (FIGS. 1H-1I). Thus, premature mitosis caused by ATRi was not simply a consequence of increased mitotic entry because ATMi did not induce premature mitosis, but did increase overall mitotic entry. Rather inhibition of ATR resulted in abnormal mitotic entry. These data show that the DNA damage observed when the DDR is inhibited during a BKPyV infection may be caused by abnormal mitotic entry during S phase (FIG. 1J).

Example 2- Blocking mitosis prevented BKPyV-dependent host DNA damage when ATR was inactive

This example shows blocking mitosis prevented BKPyV-dependent host DNA damage when ATR was inactive. To determine if mitosis is required for DNA damage to occur during BKPyV infection when the DDR is inhibited, RPTE cells were transfected with siCdk1 or control siRNA (siNTC) then infected with BKPyV (MOI=0.5). At 24 hpi cells were treated with ATRi, ATMi or vehicle control and harvested at 72 hpi. In this example, the ATRi was VE-821 (5μM), the ATMi was KU-55933 (10μM), and the vehicle control was DMSO.

Normally, ATR activation phosphorylates Chk1, which leads to cell cycle at the G2/M checkpoint through inactivation of Cdk1 by phosphorylation (FIG. 2A) (22). To directly test whether the DDR is required to prevent DNA damage by blocking cell cycle progression, mitosis was blocked by knocking down Cdk1 of the mitosis promoting factor (MPF: Cdk1/cyclin B1) to see if DNA damage could be prevented during a BKPyV infection when the DDR is inhibited. Knockdown of Cdk1 prevented mitotic entry when the DDR was inhibited (FIG. 2B). Quantification of fragmented nuclei (FIG. 2C) and nuclear morphology (FIG. 2D) were used to assess DNA damage to determine if Cdk1 silencing reduced DNA damage caused by DDR inhibition. Abnormal mitosis and fragmented nuclei that resulted from ATRi or ATMi during BKPyV infection were dramatically reduced by Cdk1 knockdown, indicating that DNA damage caused by DDRi was dependent upon mitosis (FIGS. 2C-2E). Additionally, cytoplasmic TAg was absent when Cdk1 was depleted indicating that the cells did not enter mitosis.

Since fragmented nuclei are a product of the nuclear envelope forming around severely damaged DNA following mitosis, experiments were conducted to determine if DNA damage occurred independently of mitosis or not (23). The alkaline comet assay, in which DNA damage is visualized with single cell resolution by a comet-like appearance of broken DNA migrating away from the genomic pellet in an electric field (24), was used. As

previously reported, BKPyV infection itself does not cause observable DNA damage, since the comet tails were similar to mock infection (FIG. 2F) (14), However, inhibition of ATR in a BKPyV infected cell increased the amount of DNA within the comet tail, signifying a significant increase in DNA damage (FIG. 2D). This DNA damage was rescued by Cdk1 knockdown. Therefore, BKPyV activation of the ATR pathway is required to prevent Cdk1 activation and subsequent mitotic entry, which is associated with DNA damage (FIG. 2G).

Example 3- Inhibiting DDR activation in BKPyV infected cells decrease BKPyV viral titers

This example shows DDR activation enhanced BKPyV titers by arresting the cell cycle. To determine if DDR inhibition reduced BKPyV productive infection due to defective cell cycle arrest, RPTE cells were infected with BKPyV (MOI=0.5). At 24 hpi cells were treated with ATRi, ATMi or vehicle control and harvested at 72 hpi for analysis. In this example, the ATRi was VE-821 (5μM), the ATMi was KU-55933 (10μM), and the vehicle control was DMSO.

Preventing mitotic entry by knocking down Cdk1 in cells in which DDR was inhibited during a BK infection prevented DNA damage (see Example 2). To determine is a similar effect would be observed on BKPyV titers, Cdk1 was knocked down and the effect on BKPyV viral titers was examined. Blocking mitosis by silencing Cdk1 rescued viral titers that were decreased by both ATRi and ATMi (FIGS. 3A-B). However, since only ATRi was significantly linked to premature mitosis, this suggests that premature mitosis was not responsible for diminished viral titers, but rather overall mitotic entry reduced viral production (FIGS. 1H and 1I).

Cell cycle profiling was performed to determine if ATR and ATM activation reprogramed the cell cycle during BKPyV infection (FIGS. 3C-D). As expected, BKPyV infection significantly increased the fraction of cells in S phase (FIG. 3F) and correspondingly decreased the number of cells that were in G1 phase compared to the uninfected control (FIG. 3E and 3F). A portion of BKPyV-infected, S-phase cells were marked by >4N DNA content reflecting the reduplication of host chromatin (FIG. 3C). Also, since BKPyV infection induces cell cycle arrest, BKPyV infection alone did not significantly increase the portion of cells in G2 or M phase compared to mock infected cells (FIG. 3G). In mock cells, neither ATRi nor ATMi significantly altered the distribution of the cells in G1, S, or G2-M phases (FIGS. 3D-G). In BKPyV infected cells, however, both ATRi and ATMi significantly decreased the percent of cells in S phase while increasing the cells in G1 and G2-M phase (FIG. 3E-G).

Further characterization of the G2-M population revealed that a significant number of the cells had entered mitosis (FIG. 3H-I).

In mock cells, neither ATRi nor ATMi significantly altered the distribution of the cells in G1, S, or G2-M phases (FIGS. 3C-G). In BKPyV infected cells, however, both ATRi and ATMi significantly decreased the percent of cells in S phase while increasing the cells in G1 and G2-M phase (FIG. 3F-G). Treatment with the ATRi, but not the ATMi, induced significant premature mitosis (FIGS. 4G-H).

Overall, this example shows that both ATR and ATM activation are required synergistically to prevent progression from S phase during BKPyV infection (FIG. 3L).

Example 4- The effect of inhibiting DDR activation is observed with multiple ATRis and ATMis

In order to rule out off-target or non-specific effects from using a single set of DDR inhibitors, experiments were conducted using structurally different inhibitors of ATR and ATM (25, 26). RPTE cells were infected with BKPyV (MOI=0.5). At 24 hpi cells were treated with ATRi, ATMi or vehicle control and harvested at 72 hpi for analysis. In these experiments, the additional ATRi was AZD6738 (5μM) as compared to VE-821 (5μM) and the ATMis were AZD0156 (10μM) and AZD1390 (1 nM-10 μM) as compared to KU-55933 (10μM), and the vehicle control was DMSO.

FIG. 4A shows cell cycle analysis after treatment with the ATRi AZD6738. The ATMi AZD0156, and vehicle control in uninfected and BKPyV infected cells. FIGS. 4B-4F show the percentage of cells in S phase, G1 phase, G2 + M phase, and M phase. As expected, BKPyV infection significantly increased the fraction of cells in S phase (FIG. 4D) and correspondingly decreased the number of cells that were in G1 phase compared to the uninfected control (FIG. 4C). A portion of BKPyV-infected, S-phase cells were marked by >4N DNA content reflecting the reduplication of host chromatin (FIG. 4A). Also, since BKPyV infection induces cell cycle arrest, BKPyV infection alone did not significantly increase the portion of cells in G2 or M phase compared to mock infected cells (FIG. 4A and 4E). FIG. 4G shows the total fraction of cells in S phase analyzed to determine if the cells were undergoing premature mitosis with the results quantified as in FIG 4H. As shown in FIG. 4H, treatment with the ATRi AZD6738 induced a higher percentage of cell in premature mitosis than did treatment with the ATMi AZD0156 or vehicle control.

Comparison of the average proportion of cells in S phase and premature mitosis caused by chemical inhibition with structurally different inhibitors of ATM (KU-55933 and

AZD0156) and ATR (VE-821 and AZD0156) is shown in FIGS. 4I-K for percentage of cells in S phase and percentage of cells in premature mitosis, respectively, to show a direct comparison between the various inhibitor compounds.

The same results were obtained with the ATMi AZD1390. AZD1390 is an ATMi that shows less inhibition of other phosphatidylinositol-3-kinase-related kinases, such as, but not limited to, ATR, allowing a more specific inhibition of ATM (Durant, et al., Sci. Adv., 4(6) 2018; DOI: 10.1126/sciadv.aat1719). FIG. 4K shows the ATMi AZD1390 reduced BKPyV viral titers to the same extent as the ATMi KU-55933. FIG. 4L shows the ATMi AZD1390 increased mitosis in BKPyV infected cells to the same extent as the ATMi KU-55933. FIGS 4K-L show that the decrease in viral titers correlates with an increase in mitosis. Without being bound by any particular theory, BKPyV viral titers are decreased when ATM is inhibited as the host replication machinery, which is required for BKPyV replication, is destroyed during the mitotic process. When ATM is inhibited the cells complete S phase and then enter mitosis.

The results in FIGS 4A-L. show the effect of inhibiting the DDR is not specific to a particular ATRi or ATMi.

Example 5- Increased cell populations in BKPyV infected cells is due to increased mitosis

This example shows constitutive ATM and ATR activation is required to prolong BKPyV-induced S phase. To determine if DDR activation is required throughout infection by BKPyV to alter the cell cycle, RPTE cells were infected with BKPyV (MOI=0.5) and treated 24 or 48 hpi (as indicated) with ATRi, ATMi, or vehicle control and harvested at either 48 or 72 hpi. In this example, the ATRi was VE-821 (5μM), the ATMi was KU-55933 (10μM), and the vehicle control was DMSO.

An elevated G1 population may reflect cells that failed to enter S phase or cells that failed to proceed through mitosis due to loss of BKPyV-mediated cell cycle arrest. Since the DDR inhibition treatments were applied after the virus reached the nucleus and TAg was expressed driving the cells into S phase, it is more likely that this cell cycle distribution was due to increased mitosis rather than failure to enter S phase. However, similar results were obtained when the DDR was inhibited at 48 hpi (hours post infection), well after S phase induction (FIGS. 5A-G). These results suggested that DDR is required late in infection to block cell cycle progression.

Characterization of the cell cycle using cyclin expression during a BKPyV infection revealed that BKPyV infection globally upregulated the expression of the S and G2 phase

cyclins E, A and B1 along with their partners Cdk2 and Cdk1 (FIG. 5H). DDR inhibition decreased the expression of cyclin E and Cdk2 supporting the cell cycle analyses that showed that cells were exiting S phase (FIG. 5I).

Taken together, the data in Examples 3-5 show that ATR and ATM prevent cell cycle progression from S phase into mitosis during a BKPyV infection (see model shown in FIG. 3J).

Example 6- ATM enhanced S phase entry while ATR induced cell cycle arrest during BKPyV infection

S phase is a tightly regulated process during which the 2N DNA content of the cell is replicated only once to 4N before entering G2 phase. Following nuclear entry of the viral genome at 24 hpi, TAg expression drives the host cell into S phase and when coupled to cell cycle arrest leads to host polyploidization (>4N DNA content) (7). This results in viral production that peaks at 72 hpi (FIG. 6A) (15, 27). Early during infection (30 hpi) most cells in S phase had <4N DNA content suggesting that host DNA re-replication had not begun for most of the cells (FIG. 6B). By 48 hpi there was a prominent EdU^+ accumulation of cells at 4N and some cells had >4N DNA content. This suggested that BKPyV induced a prolonged S phase by 48 hpi and that re-replication of the host genome had begun, but that a checkpoint is present delaying chromatin accumulation. By 72 hpi the majority of the BKPyV infected cells had >4N DNA content, which is clearly distinguishable from the 4N peak.

Inhibition of either ATM or ATR throughout the infection cycle (24 – 72 hpi) limited the portion of cells in S phase due either to a loss of cell cycle arrest or S phase induction all together (FIG. 3). To better understand how ATR and ATM differentially contributed to S phase maintenance, ATR and ATM were inhibited at different time points of infection and cell cycle analysis was performed. Interestingly, ATMi reduced the amount of S phase cells at early time points (30hpi), but ATRi did not affect S phase levels at this time point (FIGS. 6C-D, compare ATMi to ATRi). Because both forms of DDR induce mitosis when applied throughout infection (FIG. 1H) mitotic entry was examined during early infection to determine if S-phase levels in ATMi treated cells were decreased due to increased S-phase exit and subsequent mitotic entry. Neither ATRi nor ATMi increased mitotic entry during the early treatment window, thus the failure of ATMi treated cells to enter S phase was not due to enhanced mitotic entry (FIG. 6E; white bars). This indicates the reduction in S-phase cells when ATM was inhibited is not due to enhanced mitotic entry. Taken together, these data indicate that ATM activity, but not ATR activity, was required to efficiently enter S phase

during a BKPyV infection. Furthermore, since entry into S-phase was delayed when ATM was inhibited, the data show that ATM must be active prior to the onset of DNA replication in order for BKPyV to efficiently enter the S-phase. By 48 hpi ATMi treated cells eventually entered S phase, indicating that while ATM was required for efficient S-phase entry early in infection, it was not strictly required for S-phase entry overall (FIGS. 6C-D). S-phase levels remained unchanged and were significantly lower from 48 hpi to 72 hpi than those in infected cells with an uninhibited DDR, which continued to increase the proportion of cells in S-phase (data not shown). Failure to accumulate high S phase levels at these later time points (48 and 72hpi) was likely a due to defective cell cycle arrest and exit from S phase since there was a corresponding increase in the number of cells in M phase (FIG. 6E). These data show that ATM activity is required at two different time points during infection: early during infection to induce S phase entry and then later to prevent entry into M phase. However, it is possible that S phase reentry must occur during infection when cells reach 4N DNA content and thus increased mitotic entry due to ATMi may be the result of failure to reenter S phase.

ATR inhibition did not affect S-phase levels at the early time point (30 hpi) (FIGS. 6C-D), but failed to keep pace with the percent of S phase cells at later time points. This suggests that ATR may be required later during infection to prevent mitotic entry but is not required for S-phase entry early during infection. In fact, mitosis levels at 30 hpi were not affected, but by 48 hpi both ATRi and ATMi increased overall mitotic entry (FIG. 6E). Interestingly, at 48 hpi cells that were undergoing premature mitosis had ≥4N DNA content when ATR was inhibited, but ATMi did not induce premature mitosis over the control at any time point (FIGS. 5G and 6F-G). Because the ≥4N DNA content population of cells accumulated during late BKPyV infection when the virus induced rereplication of the cellular DNA (FIG. 6B) and because premature mitosis was only enriched in the ≥4N DNA content cells (FIG. 6F) these data show that ATR was required during late BKPyV infection to prevent premature mitotic entry.

The mitosis and S phase analysis suggested that ATM and ATR were required synergistically but at different time points to maintain S-phase levels during infection (FIGS. 6C-E). To statistically characterize the cell cycle profile of a BKPyV infection treated with DDR inhibitors, a ternary analysis was employed to show significant shifts in the populations (FIG. 6H) (28). Ternary analysis characterizes each cell cycle replicate as a single point with three coordinates (% G1 phase, % S phase and % G2+M phase). From this data a transformation confidence interval can be calculated to determine if populations of points are

statistically dissimilar to each other. The ternary analysis demonstrates that inhibiting ATM early in infection significantly reduced the population of cells in S-phase compared to that in untreated cells (FIG. 6H, left), whereas early ATRi and vehicle control levels were completely overlapping with no statistical difference (FIG. 6H right). This data show that ATM, but not ATR, is required early during infection. Late during BKPyV infection, both ATM and ATR inhibition significantly reduced S-phase and increased mitosis compared to the vehicle control. Taken together with our analysis of premature mitosis and mitotic entry, these data show that ATR and ATM are required synergistically, but for different roles in reprogramming the cell cycle during BKPyV infection. Specifically, ATM is required for efficient S phase entry and blocking mitosis after DNA replication was completed, while ATR is required later in infection for cell cycle arrest to prevent entry into mitosis in actively replicating cells (FIG. 6I).

Example 7- Premature mitosis was the source of DNA damage due to ATR inhibition during BKPyV infection

This example shows premature mitosis was the source of DNA damage due to ATR inhibition during BKPyV infection. To determine if ATR activates the Wee1 pathway to block premature mitosis during BKPyV infection (MOI=0.5), Wee1 and/or Cdk1 were silenced with siRNAs to induce or block premature mitosis, respectively. At 72 hpi cells were harvested to assess the amount of premature mitosis and DNA damage during BKPyV infection.

The DDR activates the G2/M checkpoint and temporarily blocks cell cycle progression, which allows DNA repair to occur in G2 phase (FIG. 7A) (29). ATM and ATR activities were both required to block overall mitotic entry, but only ATR inhibition significantly increased premature mitosis (FIGS. 1H-I). Thus, it was determined if there was a difference in abundance or activation of the G2/M checkpoint proteins during BKPyV infection following ATR versus ATM inhibition. ATR inhibition reduced Chk1 (pChk1) and Wee1 kinase (pWee1) activation, but ATM inhibition did not (FIG. 7B) (30, 31). Total levels of Wee1 were decreased by both ATR and ATM inhibition, which is likely due to the rapid degradation of Wee1 following mitotic entry (32). Cdc25C, which is required for reactivation of Cdk1 (FIG. 7A), was unaffected by either ATR or ATM inhibition (FIG. 7B). Unlike ATM, ATR activity disproportionally contributed to Wee1 activation suggesting that the ATR-Wee1 axis is required to block premature mitotic entry by inactivating Cdk1 during ongoing DNA replication (33, 34). Supporting this, Wee1 knockdown dramatically increased the number of

cells that entered mitosis or premature mitosis in a manner that was reversible by Cdk1 silencing (FIG. 7C-E).

The data from prior studies suggested that the DDR is required to repair DNA damage that accumulates throughout BKPyV infection (7). However, the data in the present application shows that host DNA damage is caused by premature entry into mitosis. To differentiate between these two possibilities, cell cycle arrest was blocked by Wee1 knockdown in cells with active DDR to repair DNA damage. Single cells from either the G1/G2, S, or premature mitosis fractions from FIG. 7C were evaluated for evidence of DNA damage. Cells undergoing premature mitosis had evidence of DNA breaks, with chromatin dispersed throughout the cytoplasm indicating complete misalignment of the chromatin (FIG. 7F). Furthermore, these orphaned fragments occurred adjacent to sites of DNA synthesis (EdU^+) and co-stained for mitosis ($pH3^{S10}$). Quantification of DNA damage by nuclear fragmentation showed that Wee1 silencing induced nuclear fragmentation in 40% of TAg positive nuclei compared to <1% in the siNTC control (FIG. 7G). Silencing Cdk1 along with Wee1 reversed DNA damage and premature mitosis, but not mitotic entry all together (FIGS. 7C-7F). DNA damage caused by Wee1 silencing was specific to BKPyV infection, consistent with what has been shown for ATR depletion (7). This suggests that BKPyV-induced DNA damage caused by DDR inhibition is due to premature Cdk1 activation rather than inefficient DNA repair.

Since Wee1 silencing induced both premature mitosis and DNA damage to a greater extent than had been previously observed by ATR inhibition, the impact of Wee1 silencing on BKPyV productive infection was investigated. The abundance of TAg and capsid proteins were reduced by Wee1 silencing and rescued by co-silencing Cdk1, indicating that viral protein levels were dependent upon cell cycle arrest by Wee1 (FIG. 7H). Similarly, viral titers were reduced to unquantifiable levels in the absence of Wee1, but were partially restored by Cdk1 silencing, suggesting that cell cycle arrest by Wee1 is essential to productive infection (FIG. 7I). To address this possibility, cell cycle profiling of BKPyV infected cells with Wee1 silenced or inhibited revealed that Wee1 was required to prolong S phase during BKPyV (FIGS. 7C and E-J). Because blocking mitosis with Cdk1-cosilencing with Wee1 knockdown partially restored S phase levels, this demonstrates that Wee1 inactivated Cdk1 during infection to prevent mitotic entry (FIGS. 7C. E, and H-M).

To fully delineate the molecular pathway leading to ATR-mediated cell cycle arrest, cell cycle analysis of a BKPyV infection with Chk1 inhibition (Chk1i), a possible

intermediate in the ATR-Wee1 pathway, was performed. The results showed that Chk1i reduced S phase and induced premature mitosis similar to that observed with ATRi and Wee1i (FIGS. 8A-D) The data indicate that productive BKPyV infection is dependent upon cell cycle arrest mediated by the ATR-Chk1-Wee1-Cdk1 axis, which blocks premature mitotic entry. To test this, cell cycle analysis of BKPyV infected cells treated with ATRi in the presence Cdk1 inhibition (Cdk1i) was performed. The results showed that Cdk1i blocked ATRi-associated mitotic entry (FIGS. 7E-F). Reciprocally, S phase levels that were decreased with ATRi were restored to levels approximating the vehicle control (FIG. 7G). Thus, ATR activation during a BKPyV infection prevents mitosis through activation of the Wee1 pathway to block Cdk1 activation. Furthermore, when the Cdk1 inhibitor was washed from ATR inhibited cells, mitosis occurred primarily in cells actively replicating DNA (75% vs 14% in contrast to ATRi alone) (FIGS. 7H-J). Taken together, these findings show that the ATR pathway is activated during BKPyV infection to prevent the activation of Cdk1 during active DNA replication, thereby blocking premature mitotic entry and expanding the window for viral replication by prolonging S phase (FIG. 7K).

Discussion

BKPyV replication activates the DDR (14) and protects the host from DNA damage. The present disclosure shows that the DNA damage resulted not from a lack of DNA repair, but rather from a failure to arrest the cell cycle and allowing cells in S phase to enter mitosis prematurely. The present disclosure shows separate and synergistic roles for ATR and ATM during a BKPyV infection. Inhibiting ATR resulted in cells entering mitosis when S phase DNA synthesis was ongoing. This premature mitosis correlated with severe host DNA damage. In addition, if the block to mitosis was removed in ATR inhibited BKPyV infected cells there was a dramatic and immediate induction of premature mitosis. When ATM was inhibited the cells entered S phase at a reduced rate, but still entered mitosis at a higher rate than cells with an active DDR. In contrast to ATRi, ATMi resulted in cessation of DNA synthesis prior to entry into mitosis, which also correlated with a reduction in host DNA damage. Finally, when cells were induced to enter mitosis by silencing Wee1 (resulting in cells with an active DDR and no reduction in DNA repair capacity), cells acquired DNA damage despite an otherwise fully functional DDR.

Together, these data show that the source of the DNA damage is premature entry into mitosis. In fact, when mitosis was inhibited in cells that did not have an active ATR,

premature mitosis and DNA damage were prevented. This demonstrated that cell cycle arrest, not DNA repair, is critical to prevent host DNA damage during a BKPyV infection.

The present disclosure shows that activation of both ATR and ATM was required non-redundantly to prevent BKPyV infected cells from entering mitosis. This suggests at least two distinct mechanisms by which the DDR promotes cell cycle arrest during infection. Inhibiting the function of ATR and ATM during defined windows of the BKPyV replication cycle revealed Wee1 was required to arrest the cell cycle and prolong S phase. Recently, ATR has been found to mediate the S/G2 phase transition by monitoring DNA replication in an unperturbed cell cycle and blocking Cdk1 activation (34). Thus, DNA replication itself is an essential signal to restrict premature Cdk1 activation (35). This model of ATR activity agrees with the results of the present disclosure showing that ATR inhibition resulted in premature mitosis of S phase cells with a ≥4N DNA content. However, the present disclosure revealed that for cells with <4N DNA content during infection, ATR was not necessary to block premature mitosis through Cdk1 inactivation. This may suggest that in certain primary cells there are additional layers of regulation in S phase that prevent progression into G2 phase in the absence of ATR. However, ATR mutations are lethal *in utero* and the Chk1 response is required to prevent mitotic entry in trophoblast giant cells, therefore it is likely that the ATR pathway regulates genomic reduplication events such as those involved in embryogenesis, cellular differentiation, and tissue repair (36-38). In contrast, ATM activation was required to block mitotic entry, but premature mitosis was not linked to ATM inhibition during BKPyV infection, which is likely due to intact ATR activity in the ATMi cells.

While ATR was not required for S phase initiation, ATM inhibition during the early window of BKPyV infection revealed that expedient entry into S phase was ATM-dependent. As an earlier report has revealed a physical interaction between PCNA and ATM that enhances DNA replication, this finding is not unprecedented, (39). In fact, numerous proteins at the replication fork are ATM targets, including the GINS and MCM complex, and replication itself has been found to modestly activate ATM (19). Another possibility is that ATM is required to form the viral replication compartment, as has been observed with other PyVs, thus absent ATM activation replication is delayed (40). Additional studies will be required to fully understand how ATM facilitates S phase entry during a PyV infection.

The current paradigm for how ATM and ATR contribute to PyV replication is that they are directly required to replicate the circular PyV genome and prevent viral DNA damage. ATM has been suggested to be required to prevent rolling circle replication of the

viral DNA, which must undergo theta replication to produce genomes that are packaged (9, 40). In contrast, ATR has been suggested to prevent replication fork collision of the two forks that fire from the PyV origin of replication on the circular genome (9, 40). However, in those studies ATM or ATR inhibition sharply decreased overall viral DNA replication 60% to 80%, yet only 10-20% of the viral genomes had replication defects (9, 40). The relatively small fraction of viral genomes damaged does not account for the substantial decrease in viral DNA levels. Consistent with the results of the present disclosure, the same study showed that DDR inhibition reduces the fraction of cells in S phase during SV40 infection (Sowd et al., 2014), but did not provide any insight into whether S phase entry or exit was affected. Our findings showed that the DDR was primarily required to prolong S phase by demonstrating efficient viral production was restored by blocking S phase exit in DDR inhibited BKPyV infected cells. The present disclosure shows that DDR is primarily required during BKPyV infection for cell cycle arrest.

A key difference between ATR and ATM activation during BKPyV infection was activation of the Wee1 pathway by ATR but not ATM. Wee1 is one of two inhibitory kinases that are important for governing the G2/M transition by inactivating Cdk1 (42). As such, Wee1 is being explored as a target for chemotherapy to induce mitotic catastrophe (33, 42, 43). Although Cdk1 knockdown rescued DNA damage during BKPyV infection in Wee1 silenced cells, our investigation revealed that viral production and S phase levels were not fully rescued by blocking mitosis, suggesting multiple roles for Wee1 during infection. Since Wee1 also inhibits Cdk2 (Li et al., 2010) and Cdk2 remained inhibited throughout infection, it is possible that Wee1 is required for inhibition of Cdk2 during a BKPyV infection as well.

PyV-associated cancers are not concomitant with a productive infection, but are driven by clonal integration of TAg with mutations in the helicase domain of TAg or in the viral origin of replication (2, 44). In these tumors, episomal viral DNA is depleted and viral replication is limited suggesting that oncogenic transformation does not occur during a normal productive PyV infection (45). In fact, both SV40 and BKPyV cause cancer in a non-permissive host when viral replication is attenuated. (46, 47). Since viral DNA amplification is the trigger for PyV-mediated DDR activation and non-permissive tissue culture models similarly limit DDR activation (14, 48). The results of the present disclosure, in combination with those in the field, provide a framework to understand the cause of oncogenic transformation by PyVs and how DDR activation may be a countermeasure to oncogenesis. The results of the present disclosure support a model by which viral replication activates the

DDR to inhibit cellular proliferation and prevent DNA damage by blocking entry into mitosis. This model underscores the role of the DDR during PyV infection not only to increase viral production, but also as a mechanism to counteract the oncogenic potential of TAg.

The present disclosure therefore provides mechanisms to inhibit PyV replication in a human subject, including inhibition of ATM using an ATMi, inhibition of ATR using an ATRi, and a combination of the foregoing.

MATERIALS AND METHODS

Tissue Culture

RPTE cells were purchased from LONZA (Basel, Switzerland) and maintained according to the supplier's recommendation in renal epithelial growth medium (REGM) (CC-3190) Cells were expanded in T75 flasks twice from the original vial of cells and approximately 2.25 x 10^6 cells were frozen REGM + 10% DMSO and stored in liquid nitrogen (49). All studies utilize these expanded RTPE cell stocks, which can be passaged one additional time.

Viral stocks

BKPyV (Dunlop strain) is maintained on the pBR322 vector (Addgene #25466, a gifr from Peter Howley) ligated into the BamHI site (50). Original stocks of infectious BKPyV were prepared from the pBR322:Dunlop genomic clone which was digested and purified prior to transfection into a T-75 flask of RPTE cells at 60% confluency with Lipofectamine 2000 (Thermo) (7). The cells were scrapped in media after 21 days and subjected to three freeze-thaw cycles and the viral titer was determined by focus forming assay.

Viral Infection

For infection, RPTE cells were chilled at 4°C for 15 min, and then incubated with BKPyV in REGM at 1/4th of the volume of the dish at 4°C for 1h with intermittent shaking before replacing the inoculum with REGM at 37°C (7). Viral infections were at 0.5 FFU (focus forming units)/cell unless otherwise specified. For example, such as when performing comet assay where it is impossible to distinguish an infected cell from an uninfected cell.

siRNA Knockdown

Silencer select siRNA for Wee1 (s21), Cdk1 (s464), and non-targeting siRNA (Silencer Select Negative Control No. 1) were purchased from Ambion (Thermo). Reverse transfected into RPTE cells was performed using 10.8 µl/ml Lipofectamine RNAiMax (Thermo) and 10nM siRNA using a protocol adapted from (7). Briefly, siRNA and lipofectamine were pre-incubated for 20 min in 1/2 of the final volume of the tissue culture plate with REGM in the dish prior to the addition of cells. Subsequently, RPTE cells were suspended at 1.5 x 10^5 cells/mL and added to the lipid:siRNA mixture in 1/2 of the final volume of the well. In the case of double knockdowns, single knockdown and controls conditions were supplemental with additional non-targeting control siRNA such that all conditions were transfected with equal concentrations of siRNA.

Inhibitor Treatment

All inhibitors were added to RPTE cells after nuclear entry of the virus. ATRi and ATMi were by VE-821 (5 μM in DMSO) and KU-55933 (10 μM in DMSO) except for in FIG. 7 in which case AZD6738 (ATRi, 5 μM in DMSO) and AZD0156 (ATMi, 5 μM in DMSO) were utilized. Cdk1 was inhibited by the potent and selective inhibitor RO-3306 (Cdk1i, 10 μM in DMSO) (51). Chk1i and Wee1i were MK8766 (2 μM in DMSO) and MK1775 (0.3 μM in DMSO), respectively. All inhibitors were purchased from Selleck Chemicals (Houston, Tx) and final concentrations of the vehicle (DMSO) were kept constant between conditions.

Immunofluorescent Analysis (IFA)

For IFA RPTE cells grown on coverslips were fixed in 250 μL 4% paraformaldehyde (PFA) for 20 min as in (50). Subsequently, the samples were washed in PBS (NaCl 137mM, KCl 2.7mM, Na_2HPO_4-$7H_2O$ 4.3 mM, KH_2PO_4 1.4mM) prior to permeablization (0.1% Triton X100 in PBS). Blocking was in PBS with 5% goat serum for 1h. TAg expression (pAb416 1:250 in block) was used to mark BKPyV infected cells. Coverslips were mounted onto glass microscopy slides with ProLong Gold anti-fade reagent (Thermo) with 4′,6-diamidino-2-phenylindole (DAPI) and stored overnight prior to quantification. Analysis was performed on a Nikon Eclipse Ti-S inverted microscope using the Nikon 40X/0.60 S Plan Fluor ELWD AMD objective.

Viral titers were determined using a focus forming unit assay (50). Briefly, RPTE cells were infected as above in duplicate with undiluted, 10^{-1}, and 10^{-2} dilutions of viral stocks in REGM. Cells were fixed at 48hpi and stained for TAg as above. The average number of TAg positive nuclei in 7 fields of view (FOV) at 10X magnification per technical replicate were counted for dilutions that had TAg stained between 20 and 200 cells per field of view (FOV). This number was multiplied by the dilution factor and number of FOV per surface area of a 12 well plate (determined by dividing the area of the well by the area of one FOV) then technical duplicates were averaged to determine viral titer. All titers were performed in biological triplicate of technical duplicates.

DNA damage was visualized by IFA for the fragmented nuclei assay to assess nuclear fragmentation of BKPyV infected RPTE cells at 72 hpi (7). At least 100 TAg^+ nuclei per biological replicate were scored on the basis of nuclear morphology. Nuclear classifications were either: normal (rounded), fragmented (multiple smaller nuclei in which DAPI and TAg staining overlap), or diffuse (TAg staining is dispersed throughout the cytoplasm). The diffuse

TAg staining pattern was then subdivided into two categories, normal mitosis and abnormal mitosis, where abnormal mitosis was marked by aberrant DAPI staining such as: misaligned chromatin in reference to the metaphase plate, lagging chromatin in anaphase, and anaphase bridges connecting separating chromatids.

Western Analysis

Protein lysates were collected in E1A lysis buffer (HEPES pH 7, 50mM; NaCl 250mM; NP40 0.1%) with protease inhibitors (50). An equal amount of protein lysates (minimum 20μg) were loaded on either an 8% or 10% polyacrylamide gel for SDS-PAGE. Gel electrophoresis was performed at either 45V overnight or 120V for 6 h then transferred using a wet transfer method at 60V overnight to a PVDF membrane. Membranes were cut on the basis of molecular weight based on the protein marker. Membranes were blocked in either 2% fat free dry milk or 2% FBS in 1X PBS with 0.1% Tiroton X-100 and probed with primary antibodies in blocking buffer (see table S1). Secondary antibody was added depending upon the application. Quantitative western analysis was performed using the Li-Cor Odyssey platform; however, when the Li-Cor system was not amiable to detection of the primary antibody then secondary conjugated to HRP and Immobilon Forte Western HRP substrate was utilized with radiography film. Multiple exposures of each target were collected and presented to best represent the relative abundance of the target protein.

FACS (Fluorescence Activated Cell Sorting) Cell Cycle Analysis

A $10cm^2$ dish of RPTE cells was labeled by adding a thymidine analogue, 10 mM 5-ethynyl-2'-deoxyuridine (EdU) (Click Chemistry Tools; Scottdale, AZ) to the media for 3 h to detect newly synthesized DNA prior to fixation in 4% PFA for 20 min at room temperature (22°C) (52). Samples were washed in wash buffer (1X PBS with 2% FBS) 2x from with centrifugation at 1200 x g for 8 min then permeabilized in 0.3% Triton X-100 in wash buffer for 15 min on ice. Then, cells were incubated with Click-IT staining solution (Alexa Fluor 488 Azide 20μM, $CuSO_4$ 2mM, Na-Ascorbate 10mM) to conjugate EdU to the fluorophore (Alexa Fluor 488, Click Chemistry Tools). To determine which cells were in mitosis cells were stained with anti-$pH3^{S10}$ antibody (53). To stain for DNA content (separating G1 and G2 phase), cells were incubated >30 min with 1 μg/mL FxCycle Violet (Thermo) in wash buffer. FACS analysis was performed on an LSR II flow cytometer using the 405, 488, and 647 laser lines and the FACS DIVA software then analyzed using FlowJo 10. Gating was applied identically to each sample within that experiment. It should be noted that our DNA stain cannot differential between cellular and viral DNA thus viral DNA may contribute to

the DNA signal in cells; however, the viral chromatin only accounts for <5% of total DNA in the cell at the 72hpi time point and thus only minimally affects the analysis of the data (54, 55).

In certain cases, samples that were prepared for flow cytometry as above were fixed onto microscopy slides by addition of Prolong Gold Anti-Fade reagent with DAPI 1:1 (v/v) with the sample and covered with a glass coverslip. After an overnight incubation samples were analyzed using a Nikon Eclipse Ti-S inverted microscope using the Nikon 100X/1.45 oil S Plan APO λ objective. At least 10 images of cells in each of G1/G2 phase, S phase, and premature mitosis were collected per biological replicate. Images were analyzed on the basis of nuclear morphology.

Comet Assay

The comet assay detects DNA damage with single cell resolution by detecting the comet-like appearance of broken DNA migrating away from the unbroken genomic pellet of cells embedded in agarose and pulsed in an electric field. The Trevigen comet assay kit was utilized to prepare samples for analysis as per the manufacturer's instructions for 30 min. Following staining with 1X SyBr Gold nucleic acid stain (Thermo) samples analyzed on a Nikon Eclipse Ti-S inverted microscope using the Nikon 40X/0.60 S Plan Fluor ELWD AMD objective. At least 50 comets in random FOV were captured for each biological repeat (n=3) totaling no fewer than 150 comets for the overall analysis. Comets were scored using the Open Comet plugin for Image J on the basis of % DNA in tail, which is a reflection of the amount of damaged DNA (56). Significance differences were determined using One-Way ANOVA, which is the standard in the field (57)

Statistical analysis

Unless otherwise noted test of significance herein are One-way ANOVA with Dunnet's post-test for multiple comparisons. The p-values are always: *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. In the event of a significant Bartlett's test a Kruskal-Wallace was utilized instead. Significant difference was determined against the BKPyV infected DMSO control unless otherwise noted by brackets on the graph. The GraphPad PRISM analysis suite was utilized for all statistical analysis.

Ternary plots allow visualization of proportional data from three conditions or states allowing to visually and mathematically distinguish distinct populations from the data (28). Ternary analyses were performed using the ggtern package within the R software environment version 3.4.3 using Rstudio version 1.1.447. Briefly, the percent of cells in G1, S, and G2 (or

M) phase under various conditions were determined by flow cytometry and plotted. The 95% confidence interval was determined by the Mahalnobis Distance and log-ratio transformation. Ternary plot visualization was performed using the ggplot2 package version 3.1.0.

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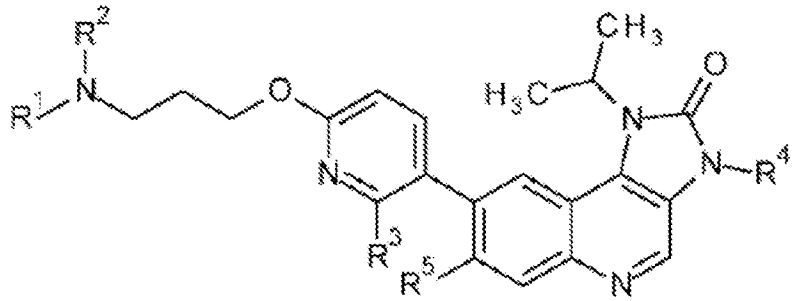
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CLAIMS

What is claimed:

1. A method for treating a subject having a polyoma virus (PyV) infection, the method comprising administering to the subject a therapeutically effective amount of an ATM inhibitor (ATMi,) an ATR inhibitor (ATRi), or both an ATMi and an ATRi.
2. The method of claim 1, wherein the PyV infection result from the activation of a latent PyV in the subject.
3. The method of claim 1, wherein the subject is administered a therapeutically effective amount of an ATMi only.
4. The method of claim 1, wherein the subject is administered a therapeutically effective amount of an ATRi only.
5. The method of claim 1, wherein the subject has a disease or condition that suppresses the immune system.
6. The method of claim 1, wherein the subject is a recipient of an organ or tissue transplant and the subject is administered a treatment that suppresses the immune system.
7. The method of claim 6, wherein the transplant is a kidney transplant.
8. The method of claim 1, wherein the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant.
9. The method of claim 8, wherein the transplant is a kidney transplant.
10. The method of claim 1, wherein the subject has an autoimmune disease.
11. The method of claim 1, wherein the subject has acquired immune deficiency syndrome.
12. The method of claim 1, wherein the PyV infection is caused by Merkel cell polyomavirus, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BK polyomavirus, JC polyomavirus, KI polyomavirus, WU polyomavirus, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus.
13. The method of claim 1, wherein the PyV infection is caused by BK polyomavirus, JC polyomavirus or Merkel cell polyomavirus.
14. The method of claim 1, wherein the ATMi is a compound of the formula I:



or a pharmaceutically acceptable salt thereof, wherein:

R^1 is methyl;

R^2 is hydro or methyl; or R^1 and R^2 together with the nitrogen atom to which they are bonded form an azetidinyl, pyrrolidinyl or piperidinyl ring;

R^3 is hydro or fluoro;

R^4 is hydro or methyl; and

R^5 is hydro or fluoro.

15. The method of claim 1, wherein the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

16. The method of claim 1, wherein the ATRi is AZD6738, VE821, or a combination of the foregoing.

17. A method for reducing a viral titer of a polyomavirus (PyV) in a subject having a PyV infection of a latent PyV, the method comprising administering to the subject a therapeutically effective amount of an ATM inhibitor (ATMi,) an ATR inhibitor (ATRi), or both an ATMi and an ATRi.

18. The method of claim 17, wherein the PyV infection result from the activation of the latent PyV in the subject.

19. The method of claim 17, wherein the subject is administered a therapeutically effective amount of an ATMi only.

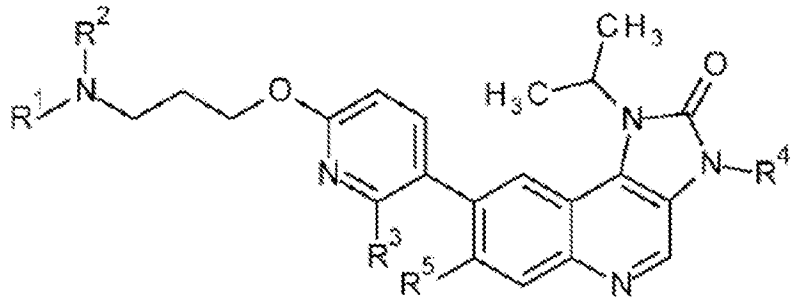
20. The method of claim 17, wherein the subject is administered a therapeutically effective amount of an ATRi only.

21. The method of claim 17, wherein the subject has a disease or condition that suppresses the immune system.

22. The method of claim 17, wherein the subject is a recipient of an organ or tissue transplant and the subject is administered a treatment that suppresses the immune system.

23. The method of claim 22, wherein the transplant is a kidney transplant.

24. The method of claim 17, wherein the subject is administered a treatment that suppresses the immune system in preparation for an organ or tissue transplant.
25. The method of claim 24, wherein the transplant is a kidney transplant.
26. The method of claim 17, wherein the subject has an autoimmune disease.
27. The method of claim 17, wherein the subject has acquired immune deficiency syndrome.
28. The method of claim 17, wherein the PyV infection is caused by Merkel cell polyomavirus, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BK polyomavirus, JC polyomavirus, KI polyomavirus, WU polyomavirus, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus.
29. The method of claim 17, wherein the PyV infection is caused by BK polyomavirus, JC polyomavirus or Merkel cell polyomavirus.
30. The method of claim 17, wherein the ATMi is a compound of the formula I:



or a pharmaceutically acceptable salt thereof, wherein:

R^1 is methyl;

R^2 is hydro or methyl; or R^1 and R^2 together with the nitrogen atom to which they are bonded form an azetidinyl, pyrrolidinyl or piperidinyl ring;

R^3 is hydro or fluoro;

R^4 is hydro or methyl; and

R^5 is hydro or fluoro.

31. The method of claim 17, wherein the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.
32. The method of claim 17, wherein the ATRi is AZD6738, VE821, or a combination of the foregoing.
33. A method for treating a side effect of immune suppression in a subject who has received an organ or tissue transplant or who is being prepared to receive an organ or

tissue transplant, wherein the subject is administered a treatment that suppresses the immune system and the subject has a polyoma (PyV) infection or a latent PyV, the method comprising administering to the subject a therapeutically effective amount of an ATM inhibitor (ATMi,) an ATR inhibitor (ATRi), or both an ATMi and an ATRi.

34. The method of claim 33, wherein the side effect of immune suppression is rejection of the tissue or organ, dysfunction of the tissue or organ, failure of the tissue or organ, or a combination of the foregoing

35. The method of claim 33, wherein the side effect of immune suppression is nephropathy.

36. The method of claim 33, wherein the PyV infection result from the activation of the latent PyV in the subject.

37. The method of claim 33, wherein the PyV infection is a BK PyV infection that result from the activation of a latent BK PyV in the subject.

38. The method of claim 33, wherein the organ or tissue transplant is a heart, intestine, pancreatic islet cell, kidney, liver, lung, thymus, pancrease, bone marrow, bone, tendon, cornea, skin, heart valve, nerve, or vein transplant.

39. The method of claim 33, wherein the transplant is a kidney transplant.

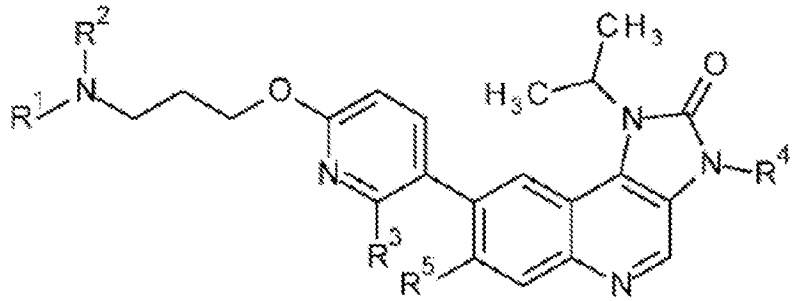
40. The method of claim 33, wherein the subject is administered a therapeutically effective amount of an ATMi only.

41. The method of claim 33, wherein the subject is administered a therapeutically effective amount of an ATRi only.

42. The method of claim 33, wherein the PyV infection is caused by Merkel cell polyomavirus, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BK polyomavirus, JC polyomavirus, KI polyomavirus, WU polyomavirus, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus.

43. The method of claim 33, wherein the PyV infection is caused by BK polyomavirus.

44. The method of claim 33, wherein the ATMi is a compound of the formula I:



or a pharmaceutically acceptable salt thereof, wherein:

R^1 is methyl;

R^2 is hydro or methyl; or R^1 and R^2 together with the nitrogen atom to which they are bonded form an azetidinyl, pyrrolidinyl or piperidinyl ring;

R^3 is hydro or fluoro;

R^4 is hydro or methyl; and

R^5 is hydro or fluoro.

45. The method of claim 33, wherein the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

46. The method of claim 33, wherein the ATRi is AZD6738, VE821, or a combination of the foregoing.

47. A method for treating a subject at risk for activation of a latent polyomavirus (PyV), the method comprising administering to the subject a therapeutically effective amount of an ATM inhibitor (ATMi,) an ATR inhibitor (ATRi), or both an ATMi and an ATRi.

48. The method of claim 47, wherein the subject is determined to have the latent PyV before such treatment is administered.

49. The method of claim 47, wherein the subject is undergoing a procedure or treatment that is likely to suppress the immune system.

50. The method of claim 47, wherein a PyV infection result from the activation of the latent PyV in the subject.

51. The method of claim 47, wherein the latent PyV is a BK PyV.

52. The method of claim 47, wherein the subject is the recipient of a tissue or organ transplant.

53. The method of claim 52, wherein the organ or tissue transplant is a heart, intestine, pancreatic islet cell, kidney, liver, lung, thymus, pancrease, bone marrow, bone, tendon, cornea, skin, heart valve, nerve, or vein transplant.

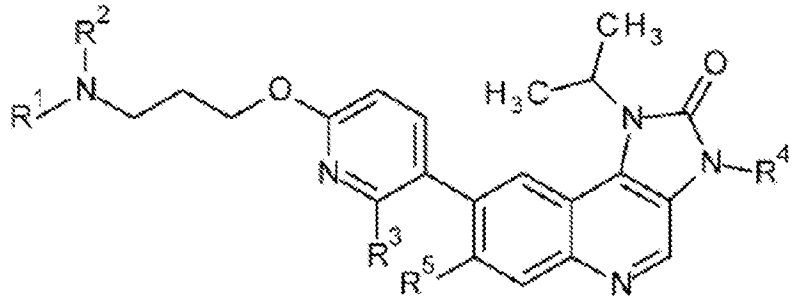
54. The method of claim 52, wherein the transplant is a kidney transplant.

55. The method of claim 47, wherein the subject is administered a therapeutically effective amount of an ATMi only.

56. The method of claim 47, wherein the subject is administered a therapeutically effective amount of an ATRi only.

57. The method of claim 47, wherein the latent PyV infection is Merkel cell polyomavirus, Trichodysplasia spinulosa polyomavirus, human polyomavirus 9, human polyomavirus 12, New Jersey polyomavirus, BK polyomavirus, JC polyomavirus, KI polyomavirus, WU polyomavirus, human polyomavirus 6, human polyomavirus 7, MW polyomavirus, STL polyomavirus, Lyon IARC polyomavirus.

58. The method of claim 47, wherein the ATMi is a compound of the formula I:



or a pharmaceutically acceptable salt thereof, wherein:

R^1 is methyl;

R^2 is hydro or methyl; or R^I and R^2 together with the nitrogen atom to which they are bonded form an azetidinyl, pyrrolidinyl or piperidinyl ring;

R^3 is hydro or fluoro;

R^4 is hydro or methyl; and

R^5 is hydro or fluoro.

59. The method of claim 47, wherein the ATMi is AZD1390, AZD0156, KU-55933, or a combination of the foregoing.

60. The method of claim 47, wherein the ATRi is AZD6738, VE821, or a combination of the foregoing.

FIG. 1A

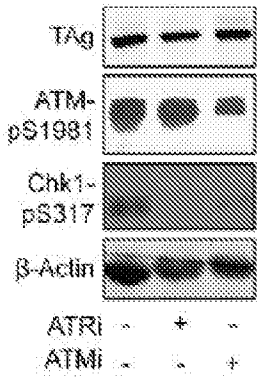


FIG. 1B

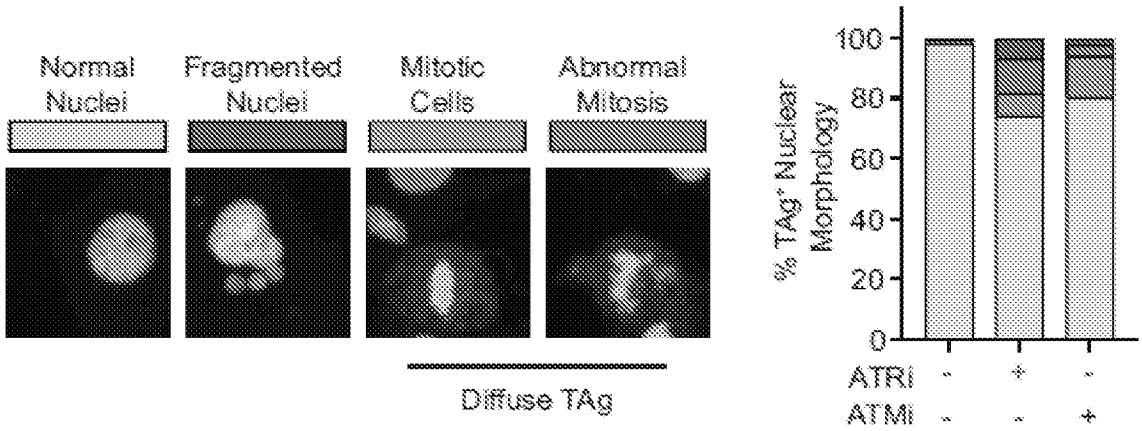


FIG. 1C

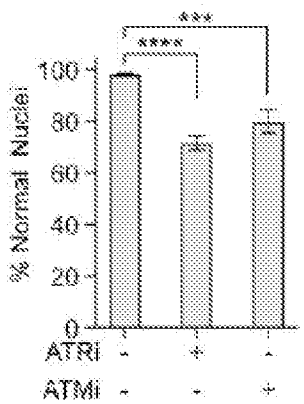


FIG. 1D

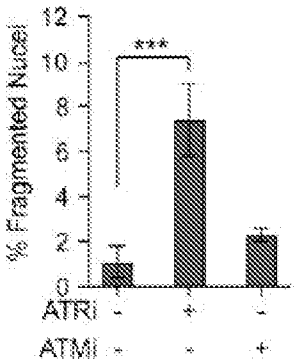


FIG. 1E

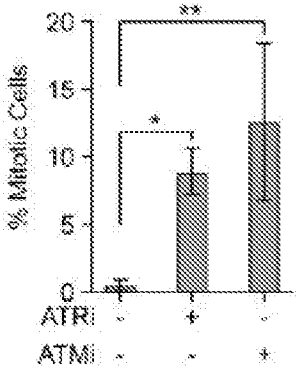


FIG. 1F

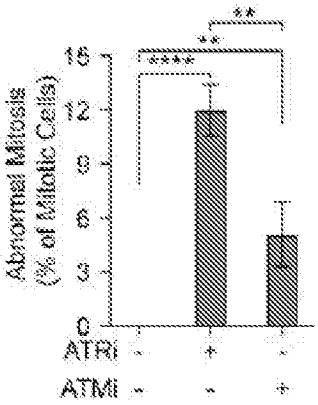


FIG. 1G

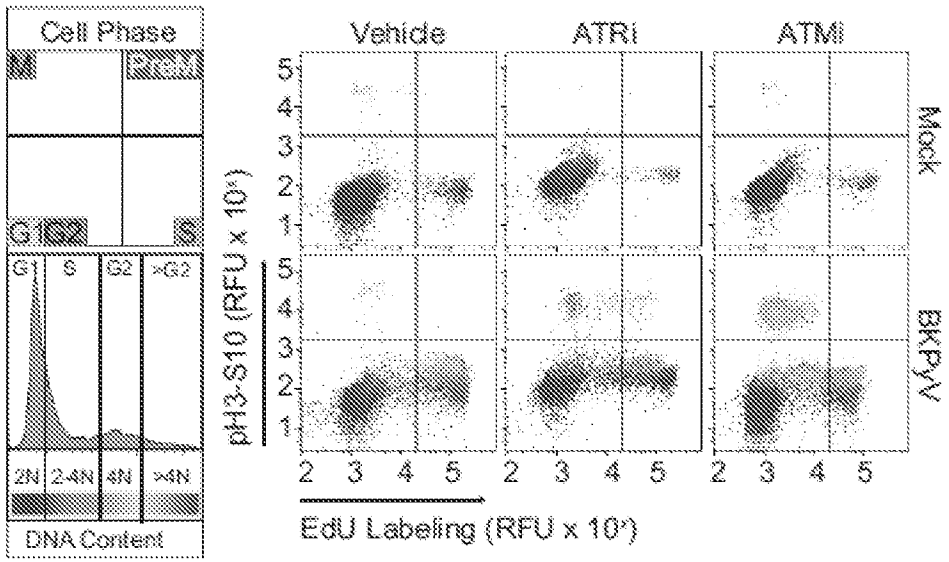


FIG. 1H

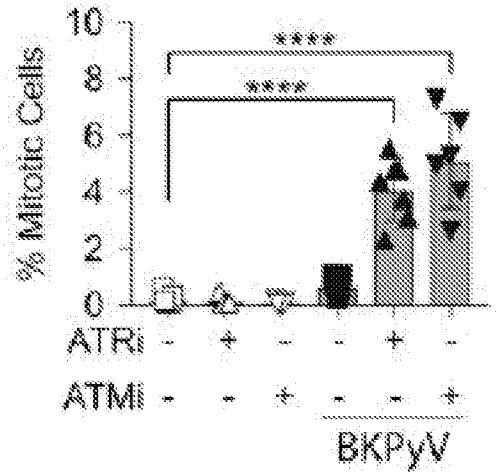

% Mitotic Cells
10
8
6
4
2
0
****
****
ATRi - + - - + -
ATMi - - + - - +
BKPyV

FIG. 1I

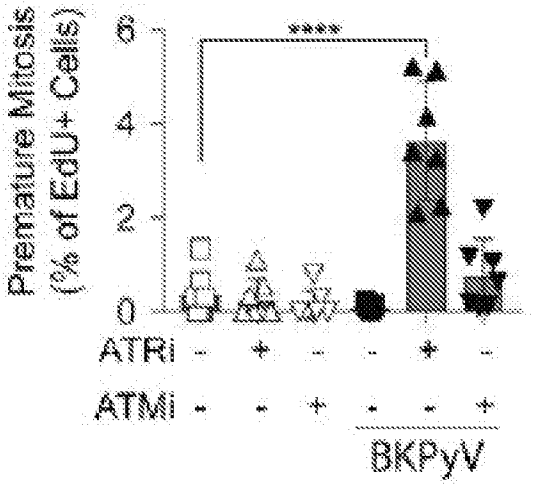

Premature Mitosis
(% of EdU+ Cells)
6
4
2
0
****
ATRi - + - - + -
ATMi - - + - - +
BKPyV

FIG. 1J

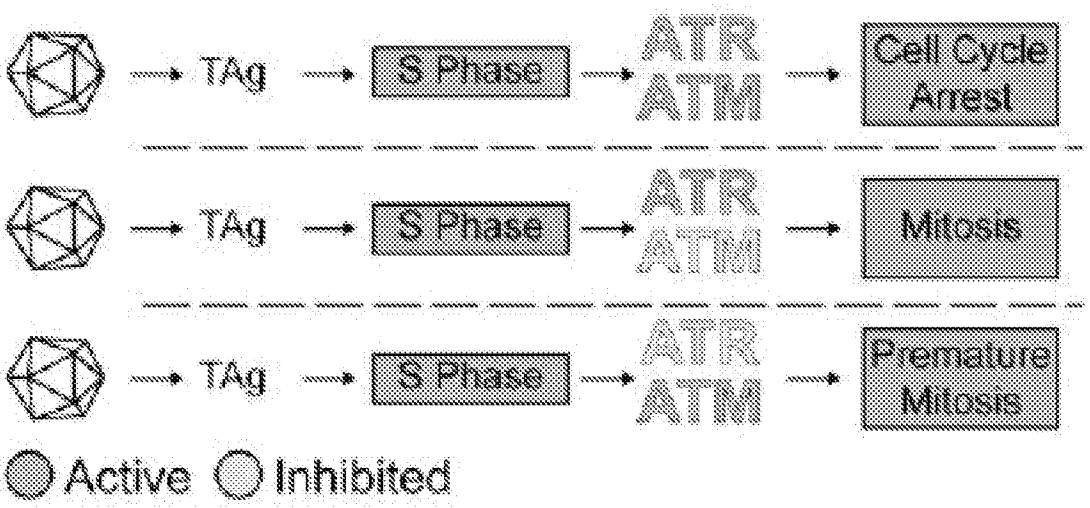


FIG. 2A

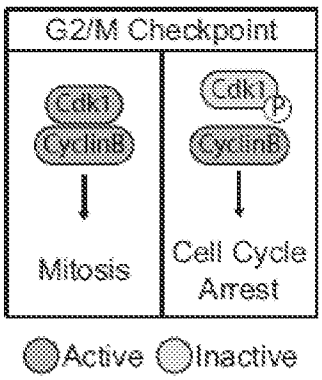


FIG. 2B

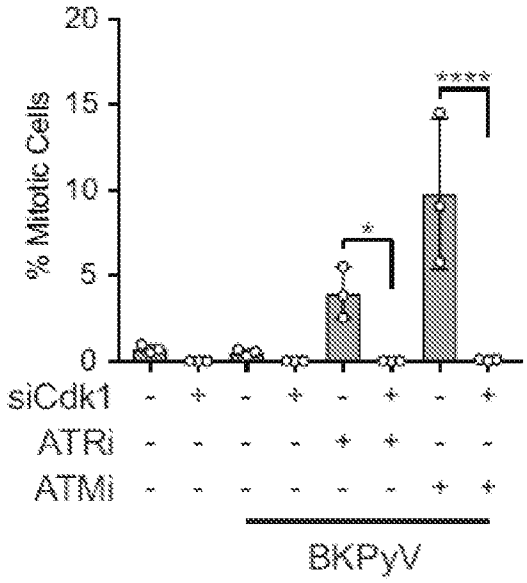


FIG. 2C

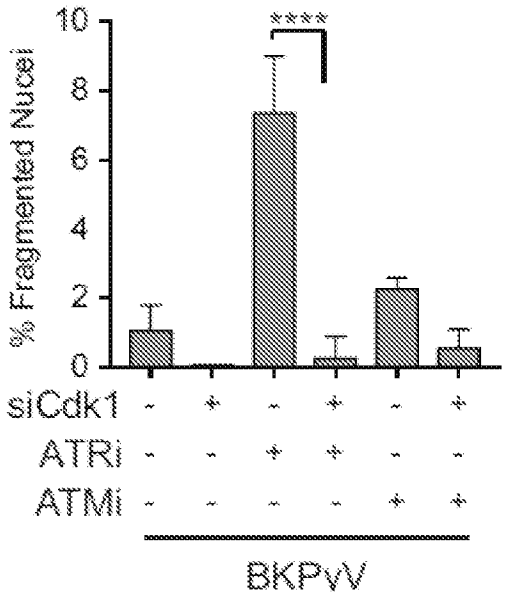

% Fragmented Nuclei
10
8
6
4
2
0
****
siCdk1 - + - + - +
ATRi - - + + - -
ATMi - - - - + +
BKPyV

FIG. 2D

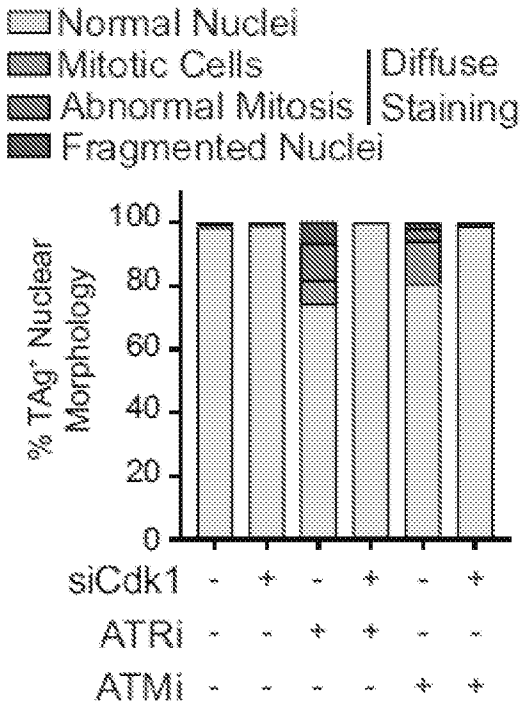

Normal Nuclei
Mitotic Cells
Abnormal Mitosis
Fragmented Nuclei
Diffuse Staining
% TAg+ Nuclear Morphology
100
80
60
40
20
0
siCdk1 - + - + - +
ATRi - - + + - -
ATMi - - - - + +

FIG. 2E

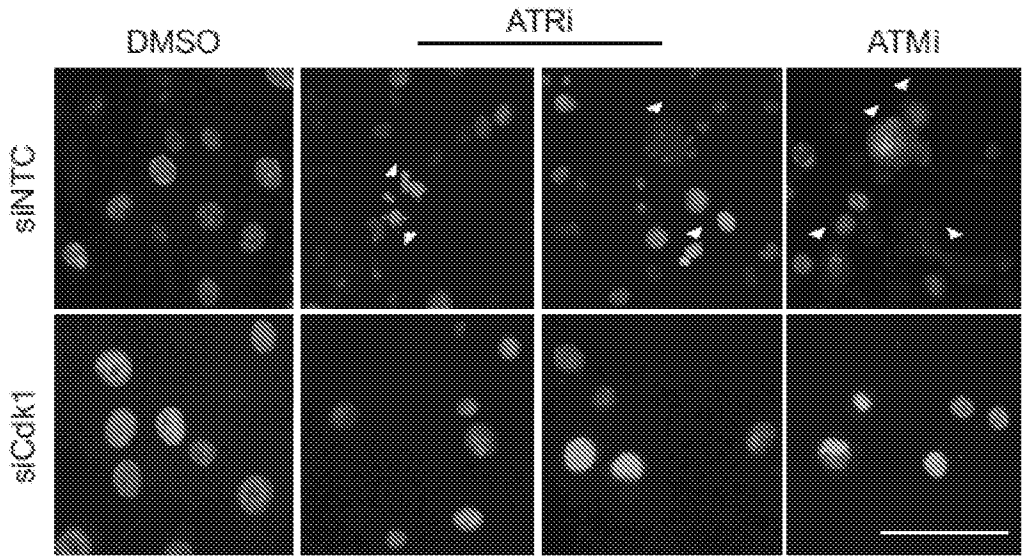

DMSO
ATRi
ATMi
siNTC
siCdk1

FIG. 2F

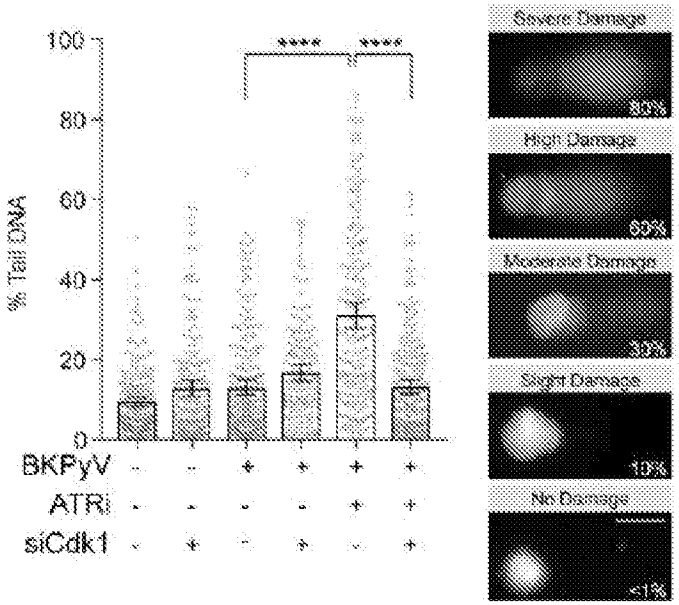

% Tail DNA
100
80
60
40
20
0
****
****
BKPyV
ATRi
siCdk1
Severe Damage
33%
High Damage
60%
Moderate Damage
32%
Slight Damage
10%
No Damage
<1%

FIG. 2G

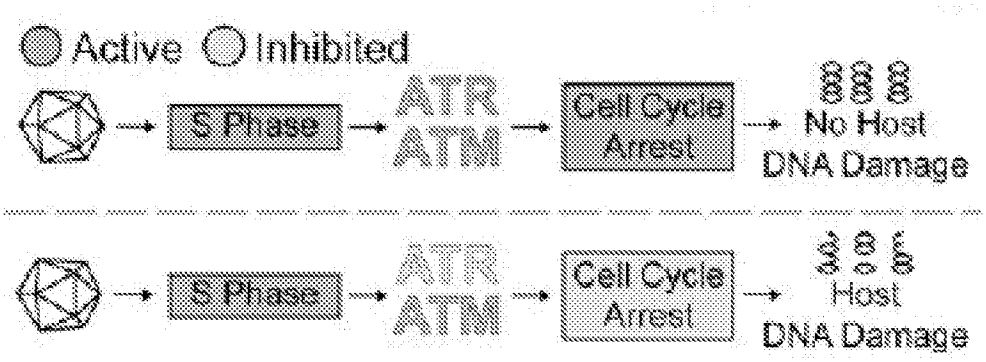


FIG. 3A

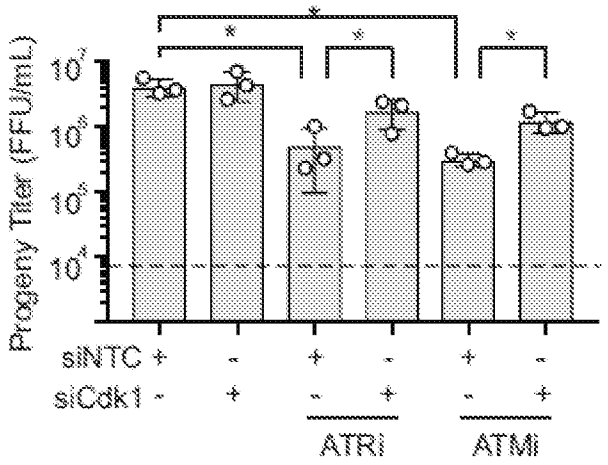


FIG. 3B

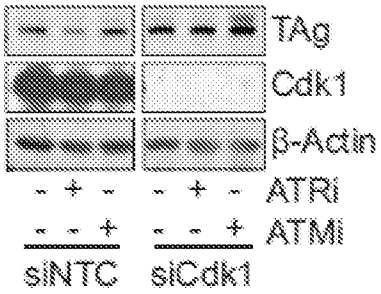


FIG. 3C

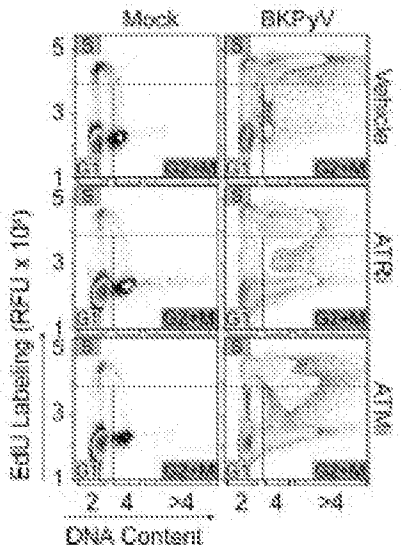


FIG. 3D

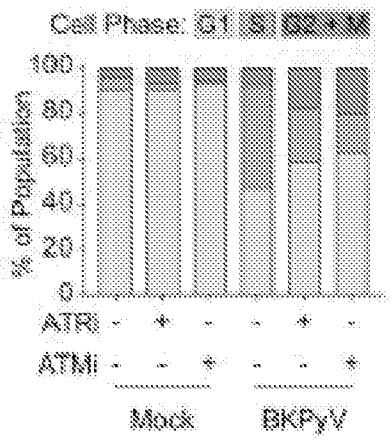


FIG. 3E

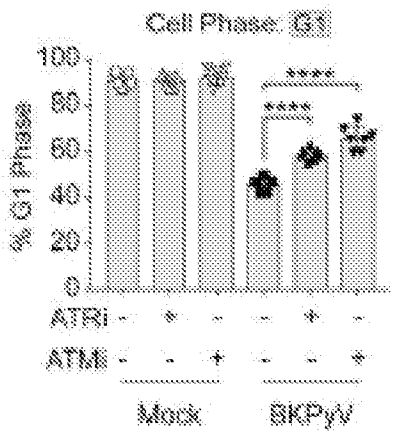


FIG. 3F

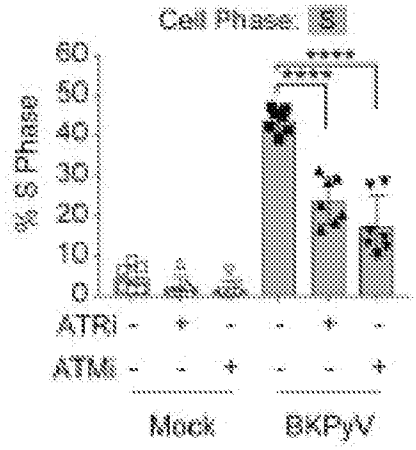


FIG. 3G

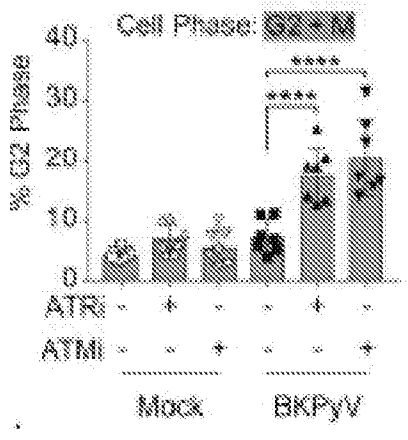


FIG. 3H

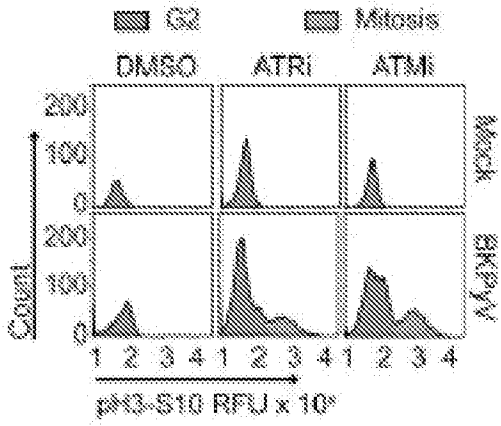


FIG. 3I

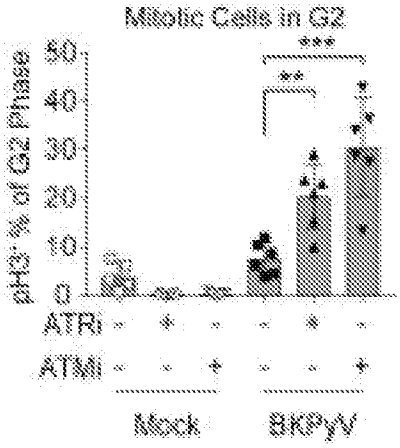


FIG. 3J

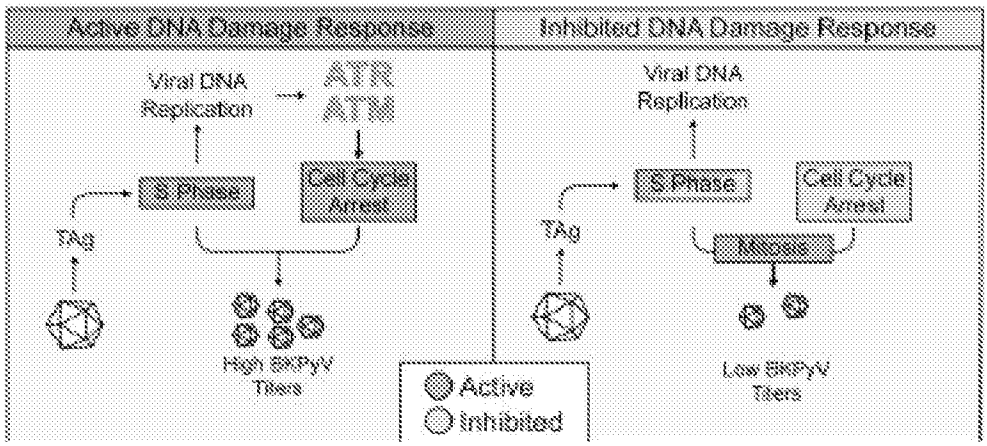

Active DNA Damage Response
Inhibited DNA Damage Response
Viral DNA Replication
ATR
ATM
S Phase
Cell Cycle Arrest
TAg
High BKPyV Titers
Viral DNA Replication
S Phase
Cell Cycle Arrest
TAg
Mitosis
Low BKPyV Titers
Active
Inhibited

FIG. 4A

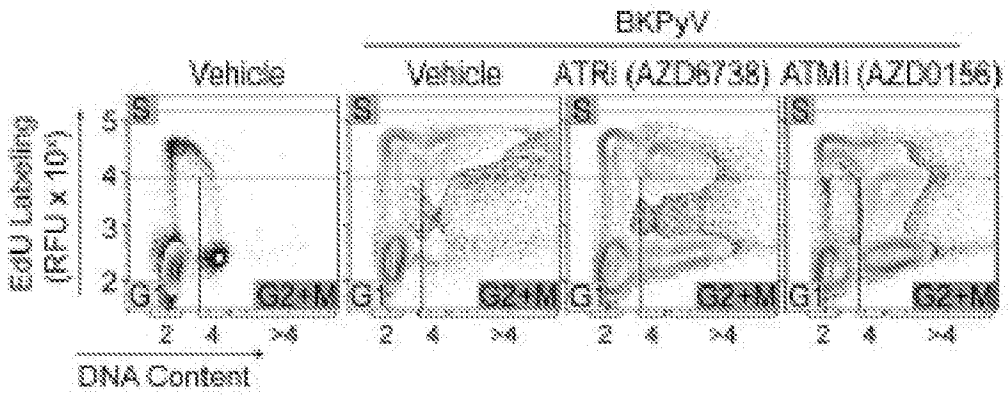

BKPyV
Vehicle
Vehicle
ATRi (AZD6738)
ATMi (AZD0156)
EdU Labeling (RFU x 10⁵)
5
4
3
2
S
G1
G2+M
2
4
>4
DNA Content

FIG. 4B

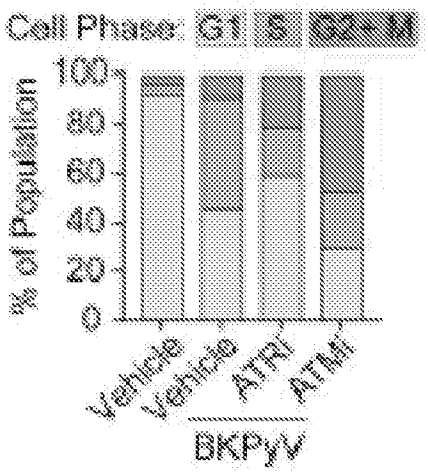


FIG. 4C

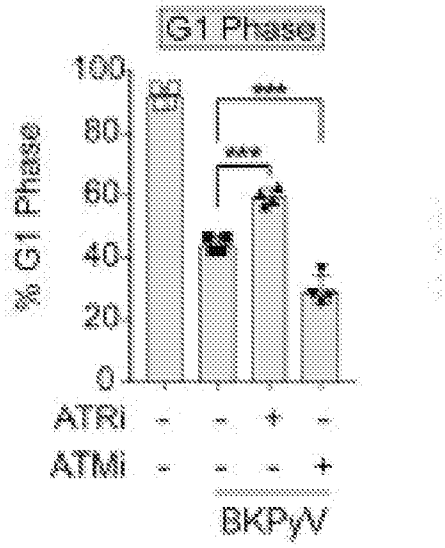


FIG. 4D

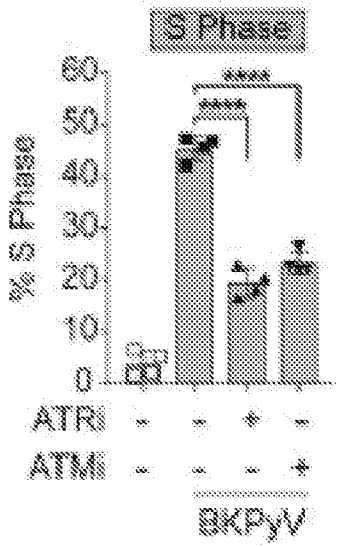


FIG. 4E

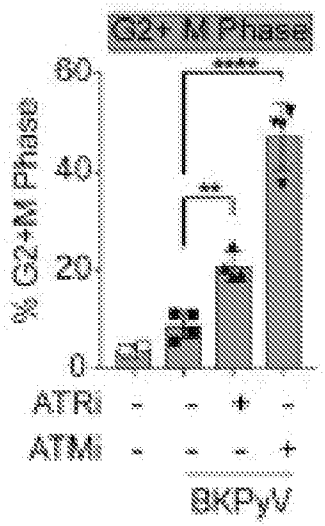


FIG. 4F

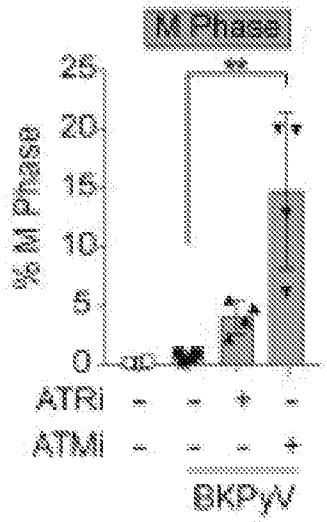


FIG. 4G

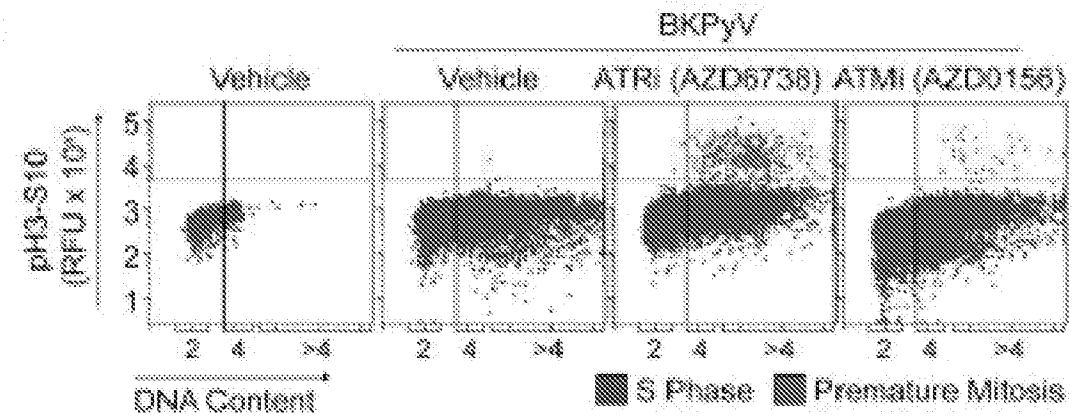


FIG. 4H

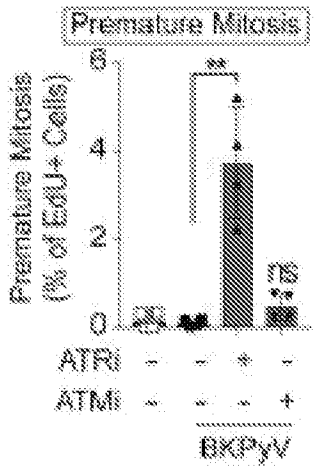


FIG. 4I

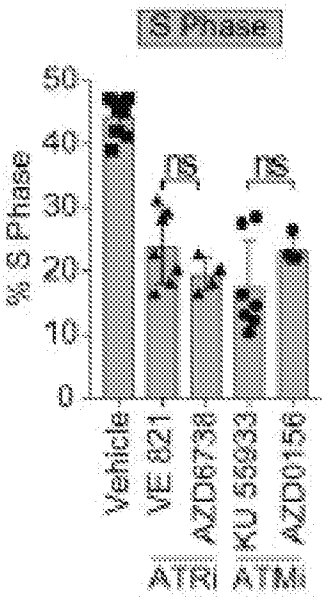


FIG. 4J

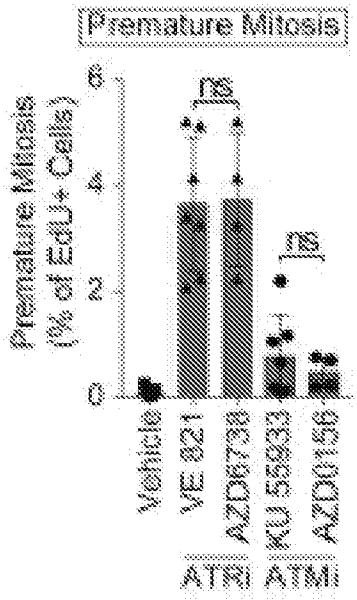


FIG. 4K

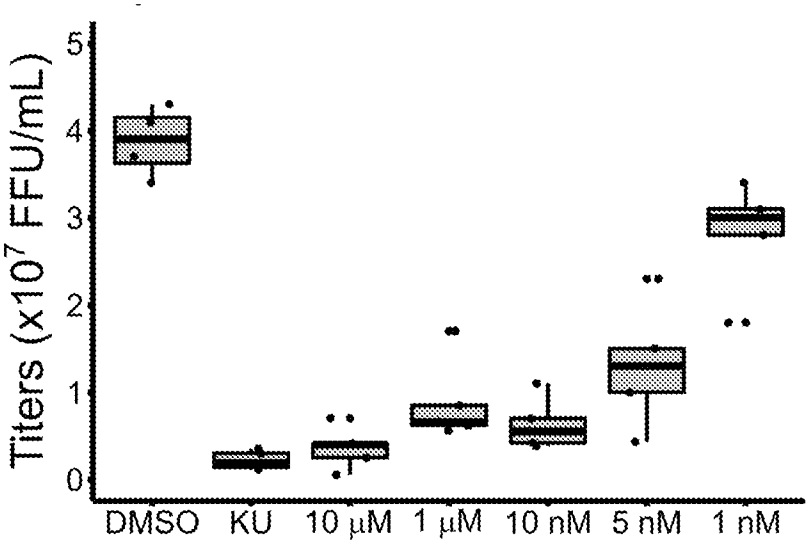

Titers (x10^7 FFU/mL)
0
1
2
3
4
5
DMSO
KU
10 μM
1 μM
10 nM
5 nM
1 nM

FIG. 4L

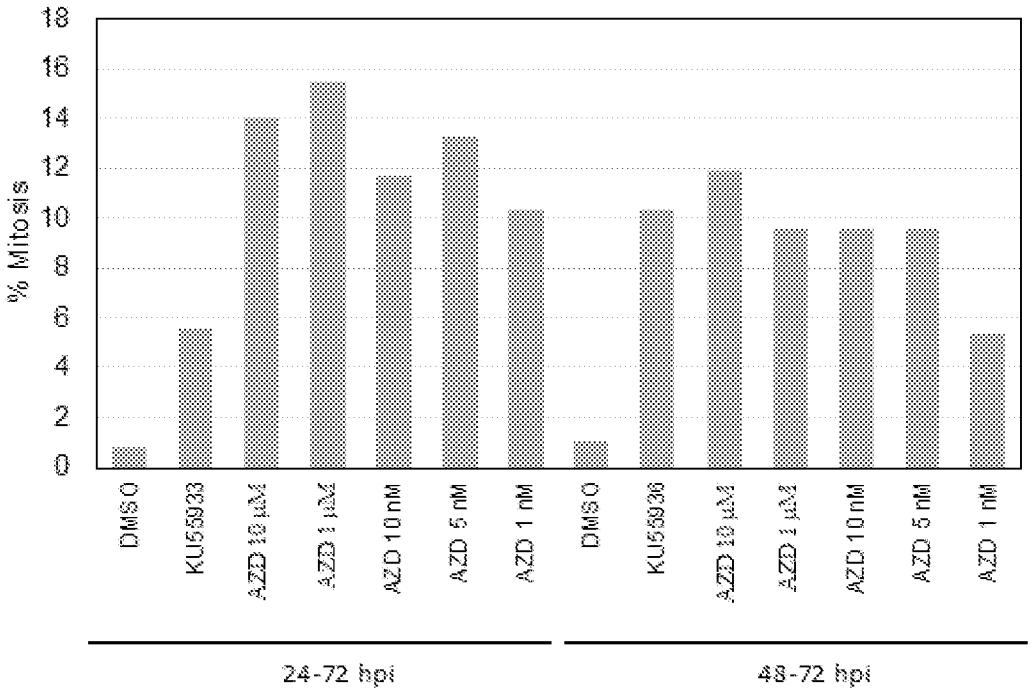

% Mitosis
0
2
4
6
8
10
12
14
16
18
DMSO
KU55933
AZD 10 μM
AZD 1 μM
AZD 10 nM
AZD 5 nM
AZD 1 nM
DMSO
KU55936
AZD 10 μM
AZD 1 μM
AZD 10 nM
AZD 5 nM
AZD 1 nM
24-72 hpi
48-72 hpi

FIG. 5A

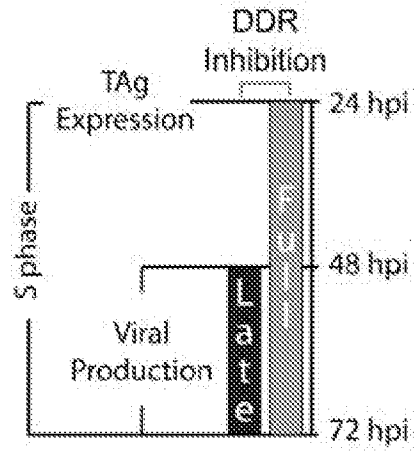

DDR
Inhibition
TAg
Expression
24 hpi
S phase
48 hpi
Late
Viral
Production
72 hpi

FIG. 5B

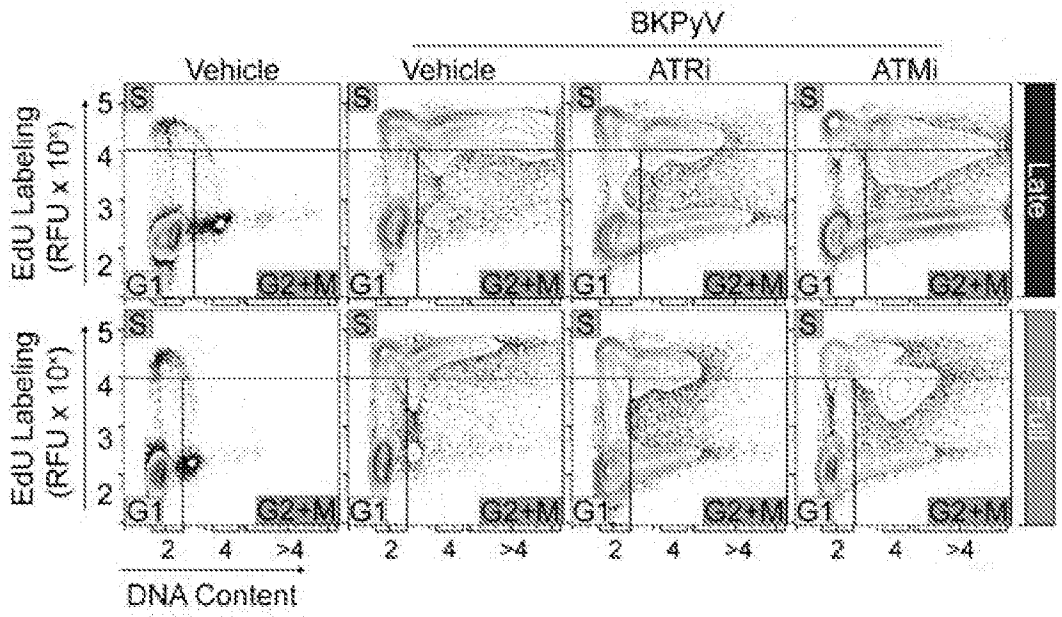

BKPyV
Vehicle
Vehicle
ATRi
ATMi
EdU Labeling (RFU x 10³)
S
G1
G2+M
Late
DNA Content
2
4
>4

FIG. 5C

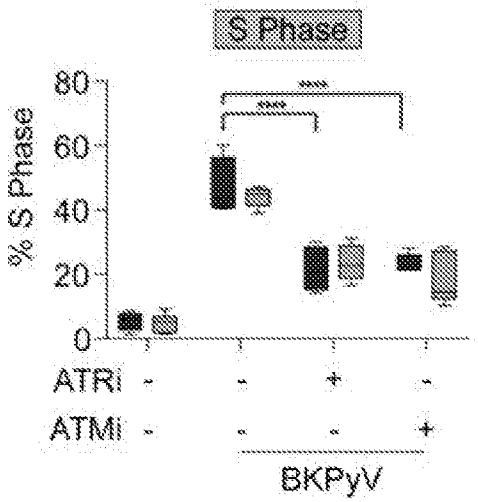


FIG. 5D

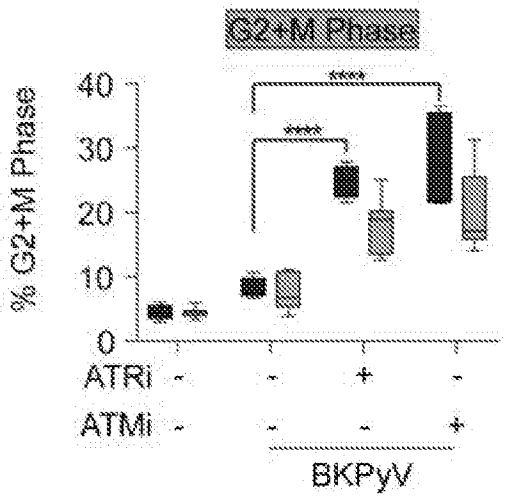


FIG. 5E

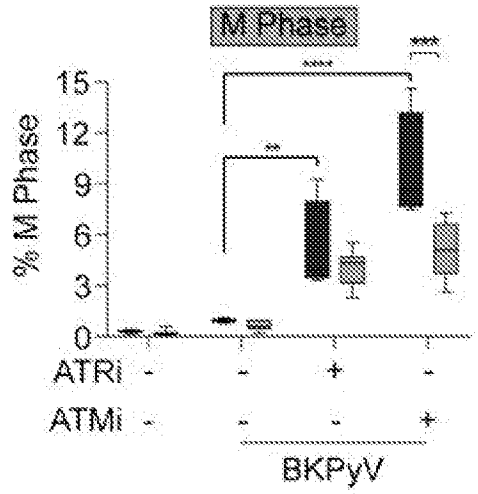


FIG. 5F

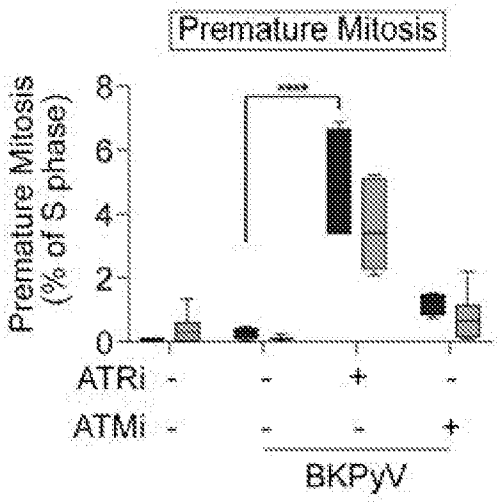


FIG. 5G

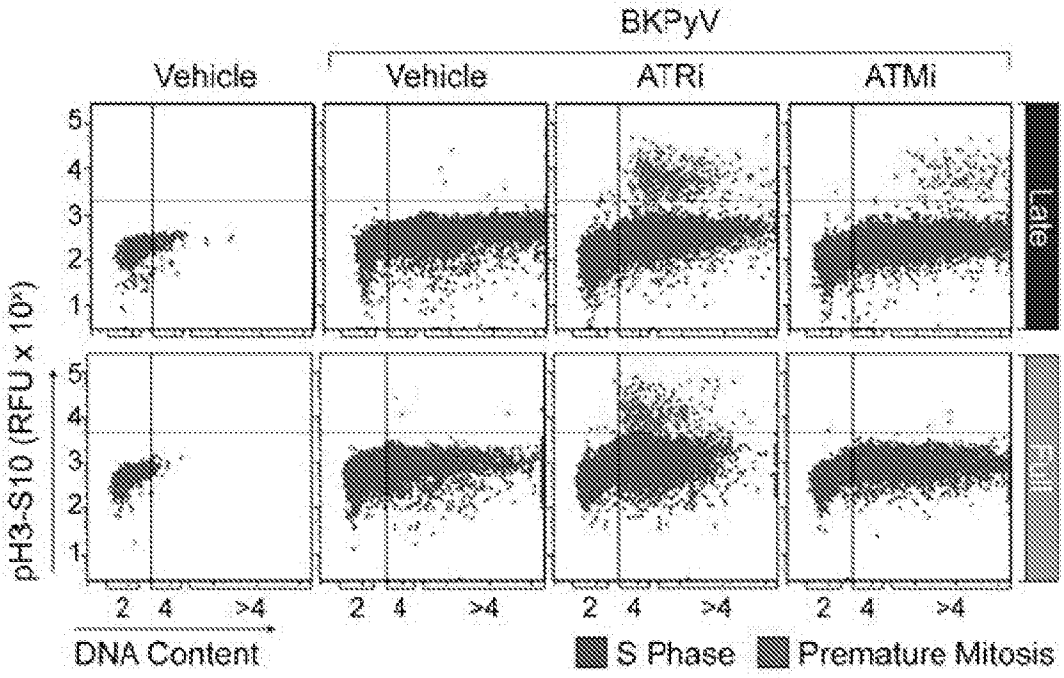

BKPyV
Vehicle
Vehicle
ATRi
ATMi
Late
Early
pH3-S10 (RFU x 10³)
5
4
3
2
1
2 4 >4
DNA Content
S Phase
Premature Mitosis

FIG. 5H

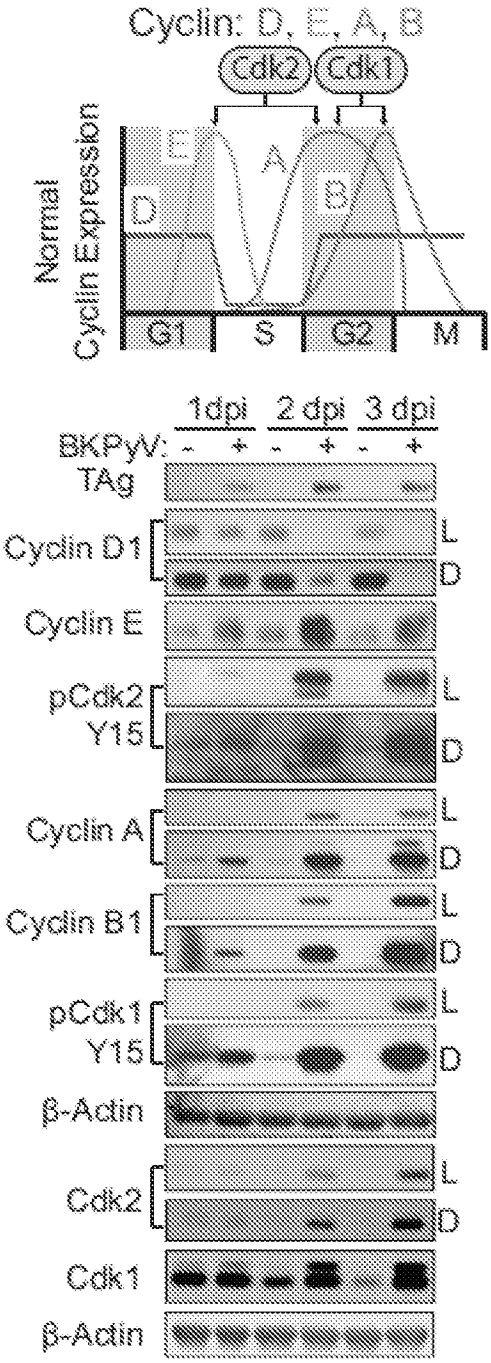

Cyclin: D, E, A, B
Cdk2
Cdk1
Normal Cyclin Expression
E
D
A
B
G1
S
G2
M
1dpi
2 dpi
3 dpi
BKPyV: - + - + - +
TAg
Cyclin D1
L
D
Cyclin E
pCdk2 Y15
Cyclin A
Cyclin B1
pCdk1 Y15
β-Actin
Cdk2
Cdk1
β-Actin

FIG. 5I

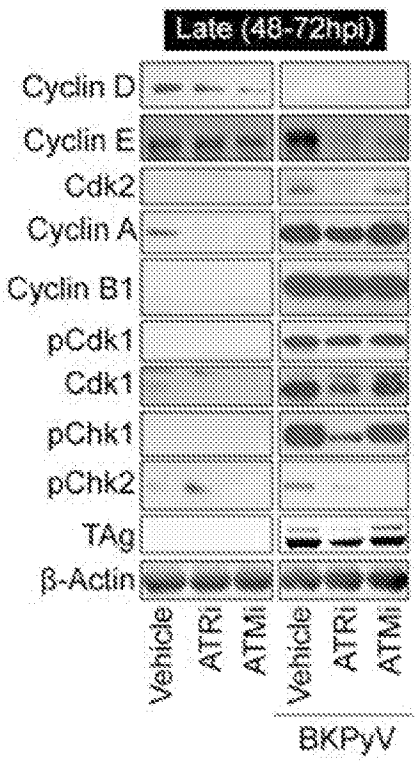

Late (48-72hpi)
Cyclin D
Cyclin E
Cdk2
Cyclin A
Cyclin B1
pCdk1
Cdk1
pChk1
pChk2
TAg
β-Actin
Vehicle
ATRi
ATMi
Vehicle
ATRi
ATMi
BKPyV

FIG. 6A

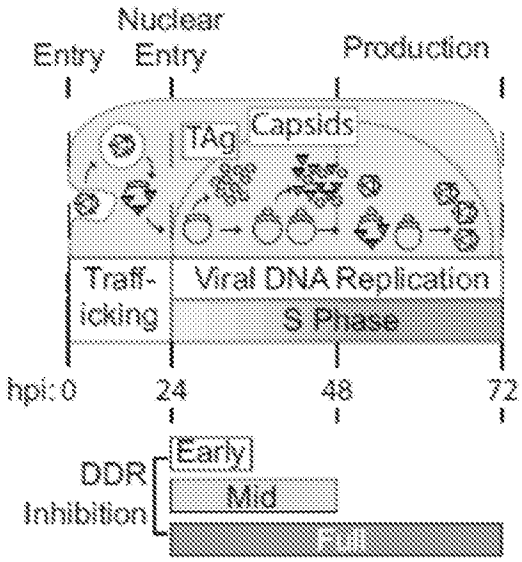

Nuclear
Entry
Entry
Production
TAg
Capsids
Traff-
icking
Viral DNA Replication
S Phase
hpi: 0
24
48
72
DDR
Inhibition
Early
Mid
Full

FIG. 6B

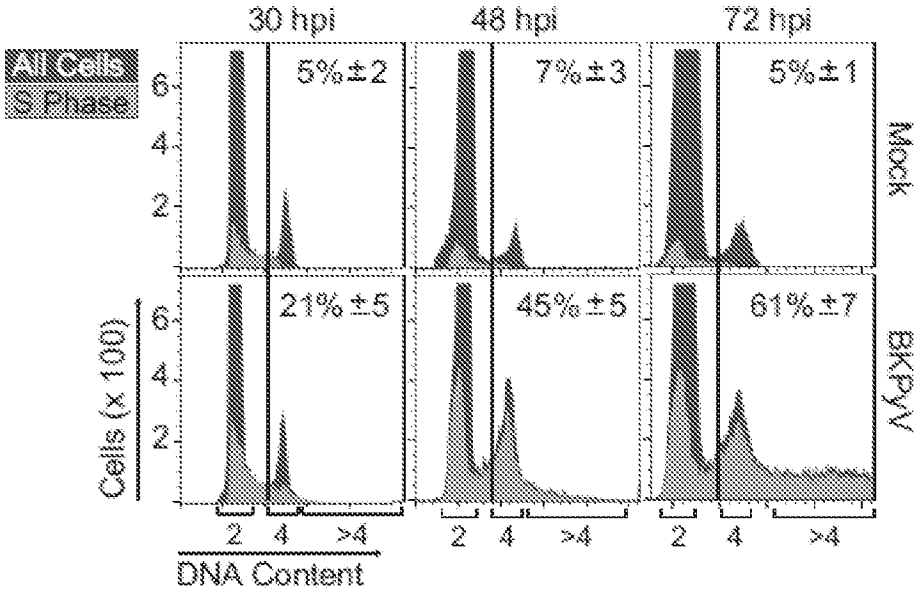

30 hpi
48 hpi
72 hpi
All Cells
S Phase
5%±2
7%±3
5%±1
Mock
21%±5
45%±5
61%±7
BKPyV
Cells (x 100)
6
4
2
2
4
>4
DNA Content

FIG. 6C

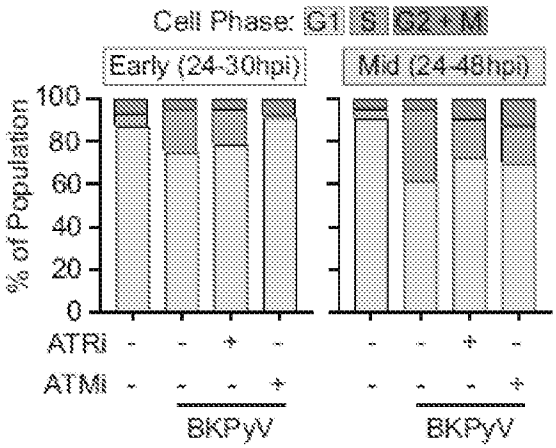


FIG. 6D

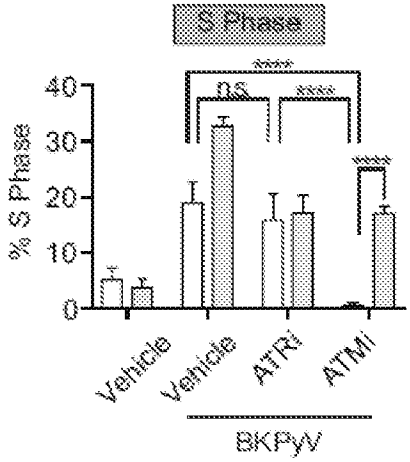


FIG. 6E

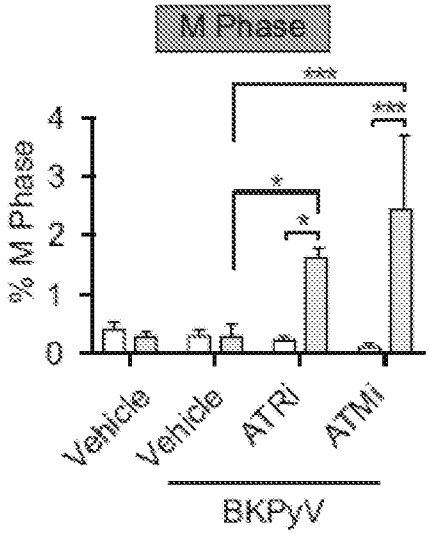


FIG. 6F

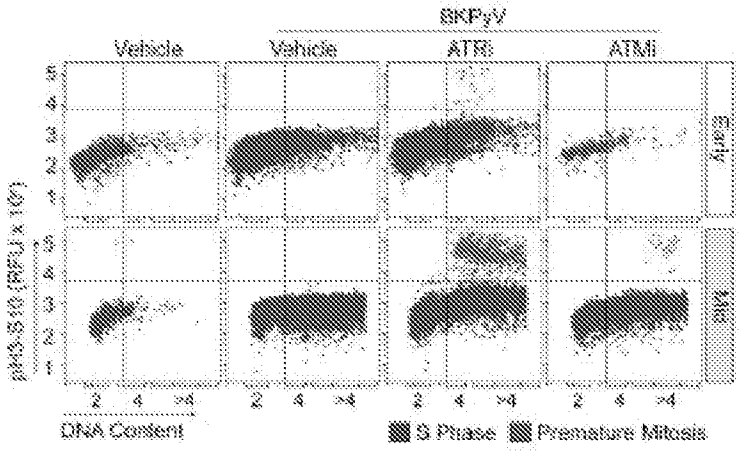


FIG. 6G

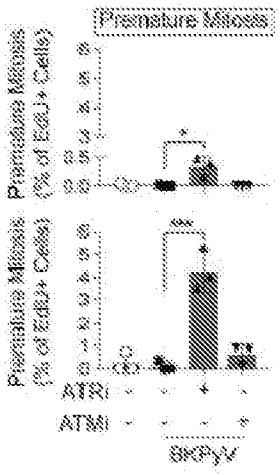


FIG. 6H

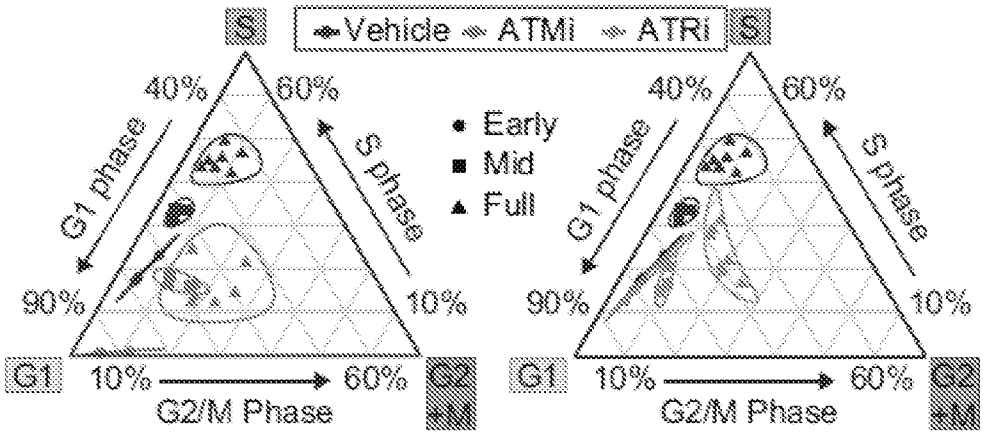


FIG. 6I

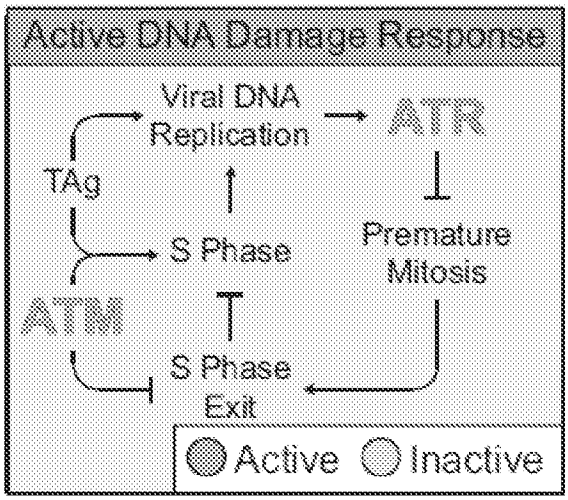


FIG. 7A

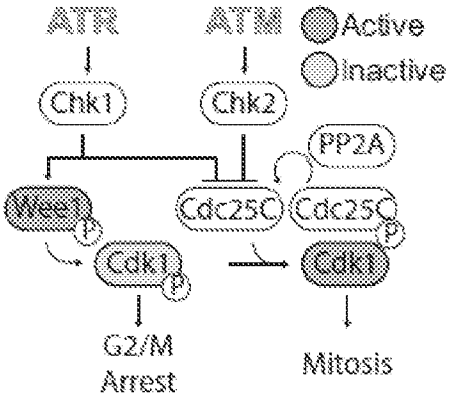


FIG. 7B

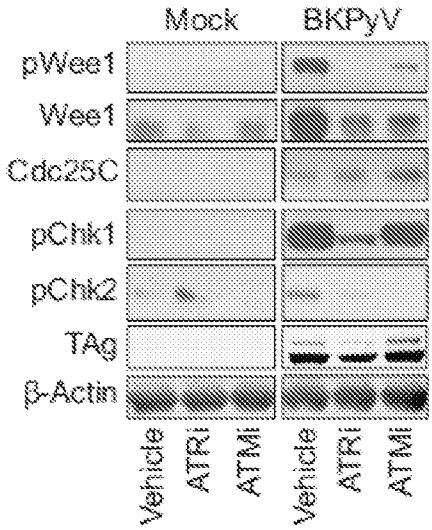


FIG. 7C

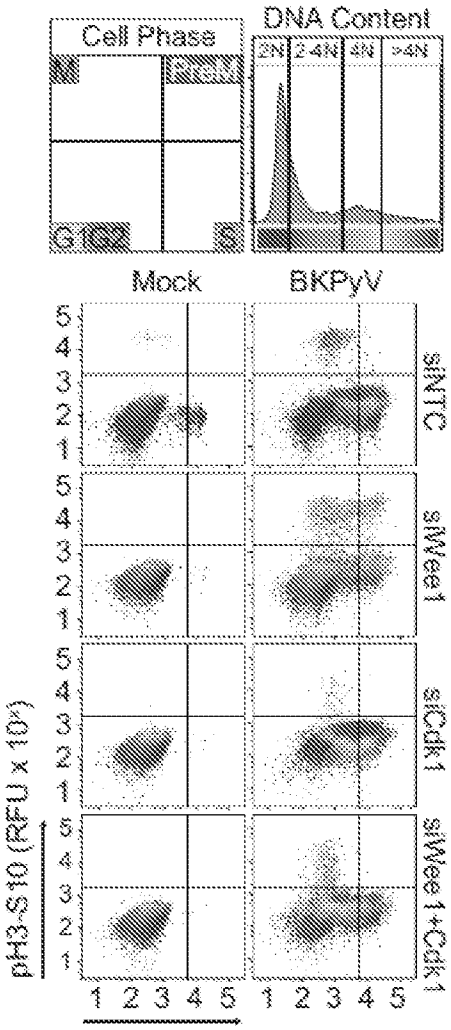

Cell Phase
DNA Content
M
Prophase
G1/G2
S
2N
2-4N
4N
>4N
Mock
BKPyV
siNTC
siWee1
siCdk1
siWee1+Cdk1
pH3-S10 (RFU x 10²)
1 2 3 4 5

FIG. 7D

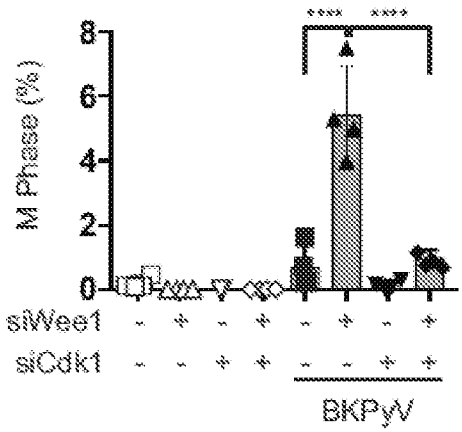

M Phase (%)
0
2
4
6
8
siWee1
siCdk1
BKPyV

FIG. 7E

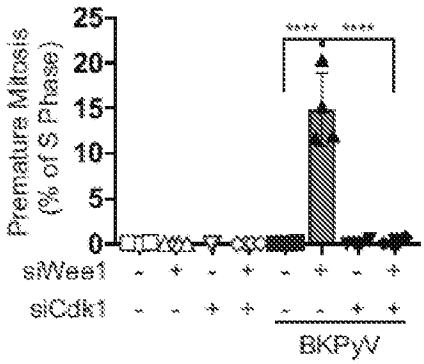


FIG. 7F

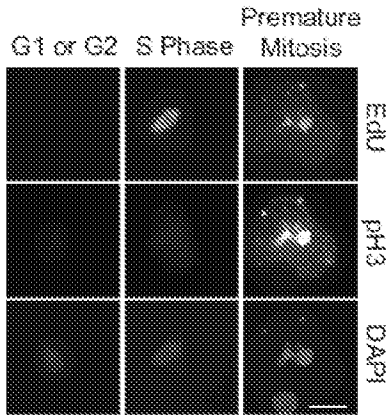


FIG. 7G

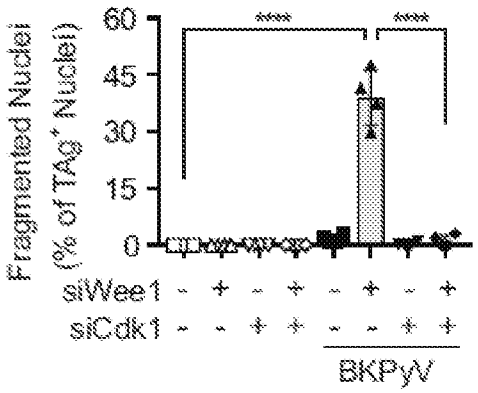


FIG. 7H

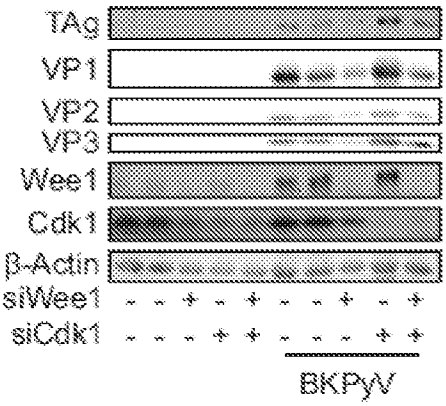


FIG. 7I

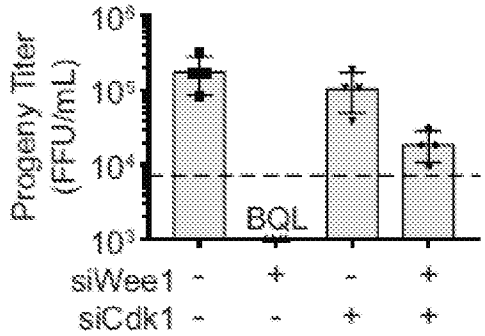


FIG. 7J

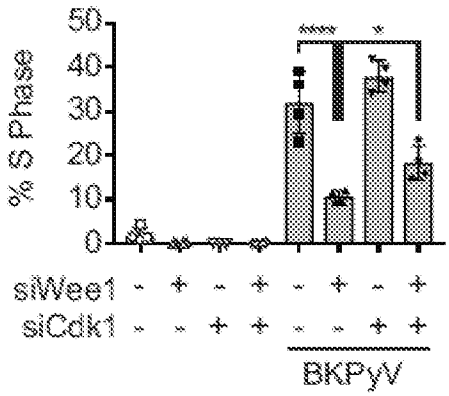


FIG. 7K

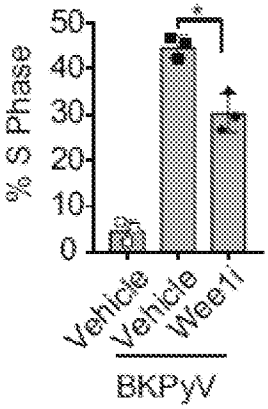


FIG. 7L

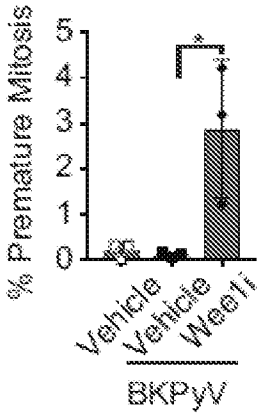


FIG. 7M

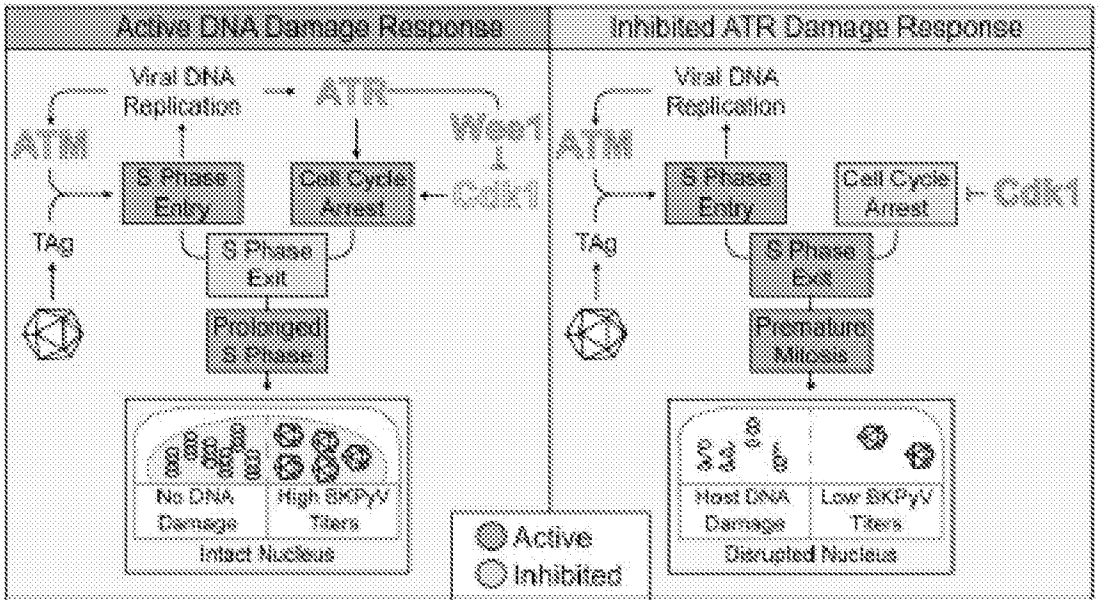

Active DNA Damage Response
Inhibited ATR Damage Response
Viral DNA Replication
ATR
Wee1
ATM
S Phase Entry
Cell Cycle Arrest
Cdk1
TAg
S Phase Exit
Prolonged S Phase
Premature Mitosis
No DNA Damage
High SKPyV Titers
Intact Nucleus
Host DNA Damage
Low SKPyV Titers
Disrupted Nucleus
Active
Inhibited

FIG. 8A

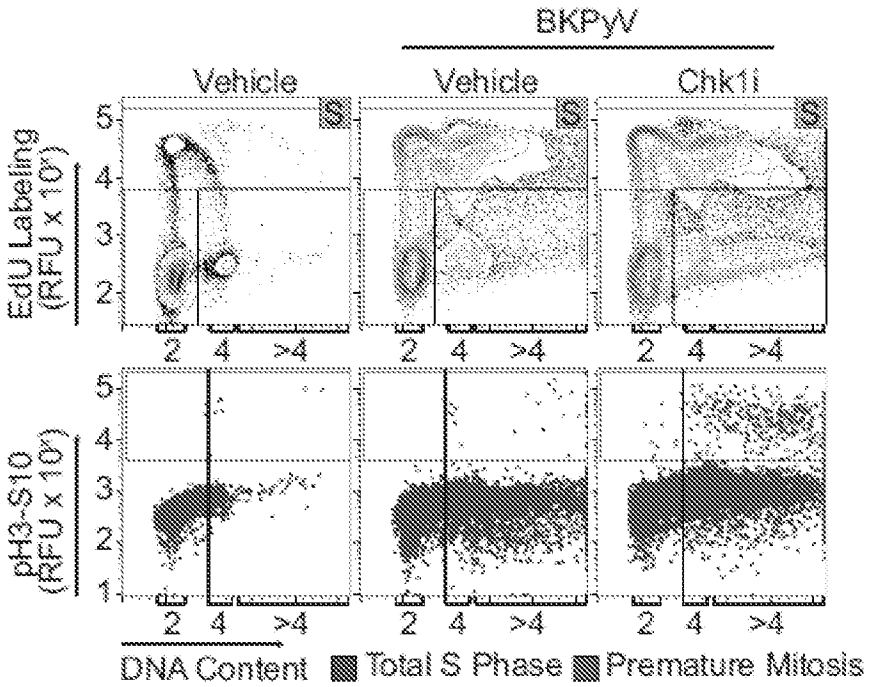


FIG. 8B

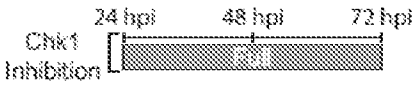


FIG. 8C

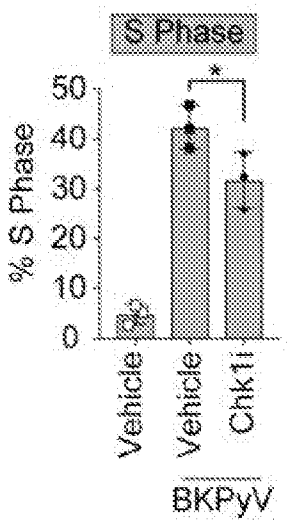


FIG. 8D

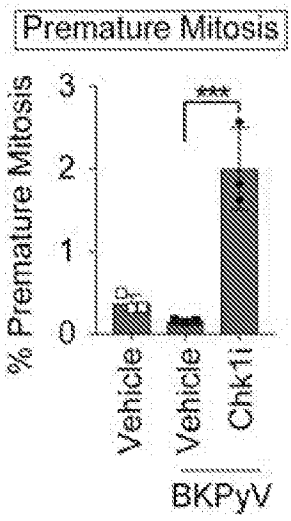


FIG. 8E

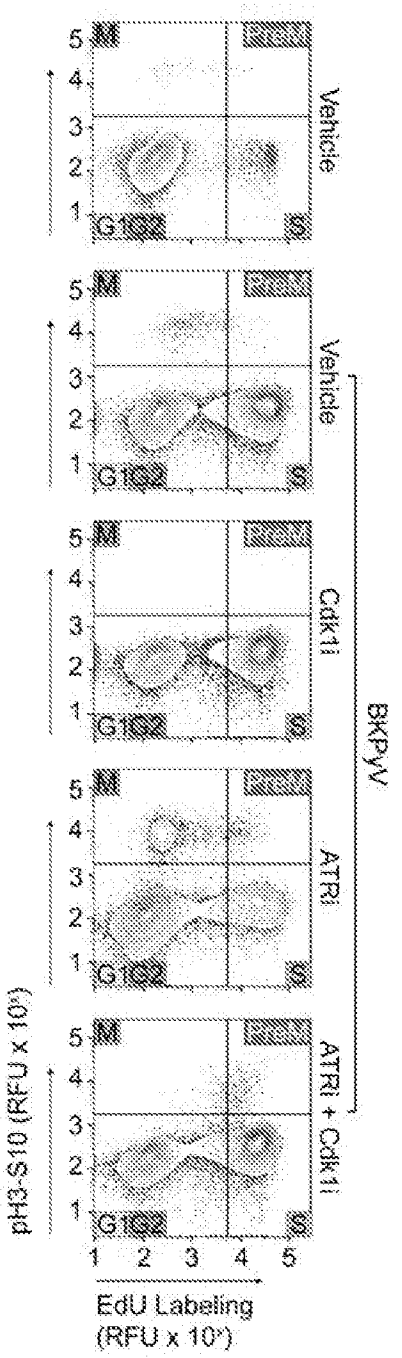

M
G1
G2
S
Vehicle
Vehicle
Cdk1i
ATRi
ATRi + Cdk1i
BKPyV
pH3-S10 (RFU x 10³)
EdU Labeling
(RFU x 10³)
1
2
3
4
5

FIG. 8F

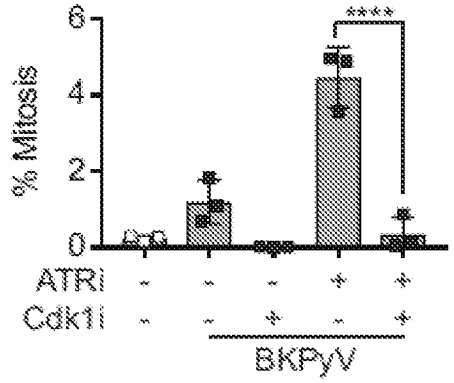


FIG. 8G

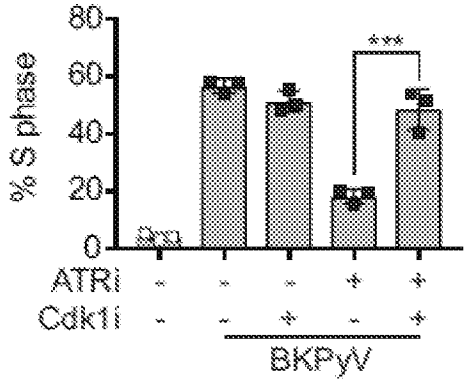


FIG. 8H

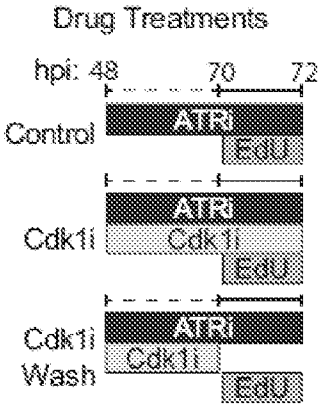


FIG. 8I

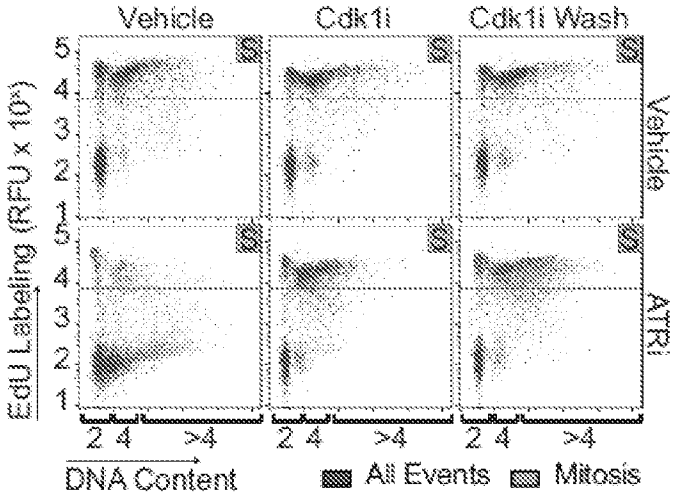


FIG. 8J

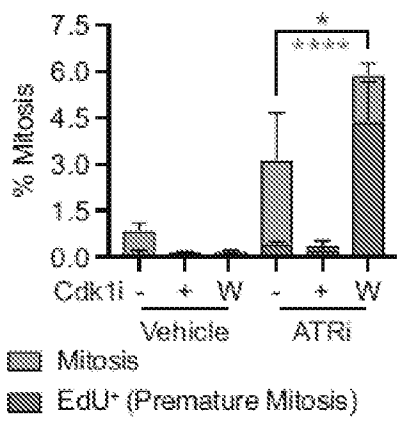
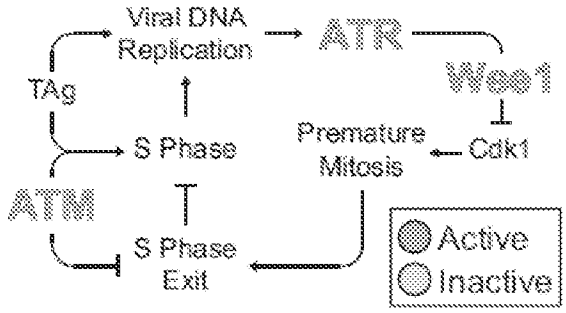

7.5
6.0
4.5
3.0
1.5
0.0
% Mitosis
*
****
Cdk1i - + W - + W
Vehicle
ATRi
Mitosis
EdU+ (Premature Mitosis)

FIG. 8K


Viral DNA Replication
ATR
Wee1
TAg
S Phase
Premature Mitosis
Cdk1
ATM
S Phase Exit
Active
Inactive

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/22751

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 35/76, A61P 31/20 (2020.01)

CPC - A61K 31/5025, A61P 13/12, A61P 31/20, A61P 35/00, Y02A 50/467

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	JIANG et al. "Roles of ATM and ATR-Mediated DNA Damage Responses during Lytic BK Polyomavirus Infection" PloS Pathog, August 2012, Vol 8, No 8, page e1002898. abstract, pg 1, col 1, para 1, pg 3, col 2, para 1, pg 5, col 1, para 2 to col 2, para 1,	1-9, 12-13, 17-25, 28-29, 47-48, 50-57 ----- 10-11, 14-16, 26-27, 30-46, 49, 58-60
Y	US 2016/0250245 A1 (INSERM (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE.) et al.) 01 September 2016 (01.09.2016) Claim 1, Claim 7, para [0017]	10-11, 26-27, 33-46, 49
Y	US 2018/0194782 A1 (ASTRAZENCA AB) 12 July 2018 (12.07.2018) para [0242], [0243]	14-16, 30-32, 44-46, 58-60

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2020	18 JUN 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300