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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 December 2024 (19.12.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/259431 A2

(51) International Patent Classification:
C07K 14/47 (2006.01) *A61K 38/16* (2006.01)

(21) International Application Number:
PCT/US2024/034372

(22) International Filing Date:
17 June 2024 (17.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/508,855 16 June 2023 (16.06.2023) 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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, 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:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: SMALL PEPTIDE (VGN73) DERIVED FROM KSHV LANA PROTEIN INHIBITS LEUKEMIC CELL GROWTH

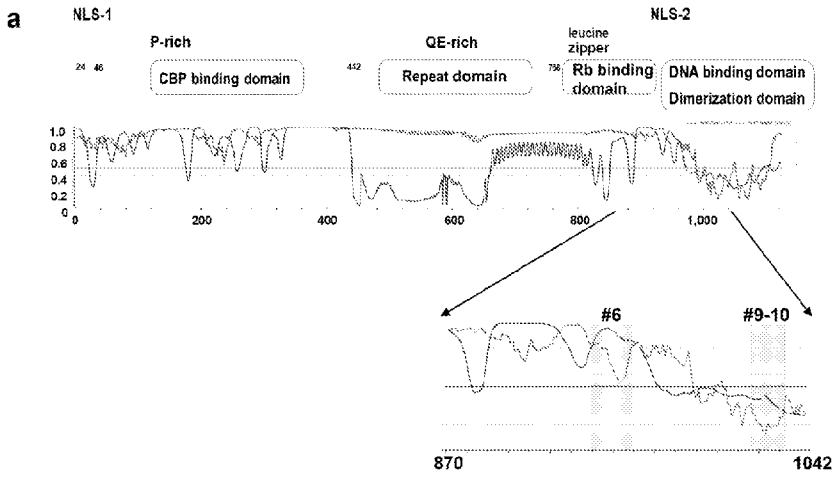

FIG. 1A

(57) Abstract: Disclosed herein are biomimetic cell-penetrating peptides (such as, for example VGN73). It is understood and herein contemplated that the disclosed biomimetic cell-penetrating peptides can be useful in the treatment of a cancer and the induction of apoptosis and differentiation in cancer cells.

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SMALL PEPTIDE (VGN73) DERIVED FROM KSHV LANA PROTEIN INHIBITS LEUKEMIC CELL GROWTH

CROSS-REFERENCE TO RELATED APPLICATIONS

This PCT application claims priority to, and the benefit of, U.S. Provisional Patent Application No. 63/508,855, filed June 16, 2023, which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under R01AI167663 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), is one of eight human herpesviruses. KSHV is a causative agent of Kaposi's Sarcoma (KS), two lymphoproliferative diseases, primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD). KSHV is also responsible for an interleukin-6-related disease called KSHV inflammatory cytokine syndrome (KICS). Like other herpesviruses, KSHV's life cycle consists of a lifelong latent infection phase and a transient lytic replication phase, in which viral progenies are produced. In latency, the expression of most viral genes are silenced and only a few selected genomic segments are actively transcribed. Among the genes expressed in the latent phase, ORF73 encodes the Latency Associated Nuclear Antigen (LANA) protein, and LANA functions to maintain latent episomes in infected cells.

CHD4 is an enzyme that regulates enhancer accessibility and is found in two distinct cellular repressor complexes, the NuRD and ChAHP, and the ChAHP complex plays essential roles in maintaining accurate cell fate decisions during development by regulating enhancer accessibility. Genetic disruption of CHD4 therefore causes spontaneous differentiation concomitant with premature activation of lineage-specific genes. Dysregulation of the gene transcription program by mutations and/or overexpression of transcription-related enzymes prevents cell differentiation and frequently leads to cell transformation. Overexpression of CHD4

is associated with poor progression in hepatocellular carcinoma, colorectal cancer, and ovarian cancer. CHD4 is also identified as essential for breast cancer cell growth, and CHD4 depletion induces a G0/G1 block of the cell cycle with up-regulation of CDKN1A (p21).

Viruses hijack host cell machinery for their replication, because they do not encode the necessary enzymes to replicate outside of infected host cells. They are thus molecular wizards, who control cellular functions, which includes cell apoptosis, cell cycle progression, as well as host immune responses. In viral replicating cells, host cell gene expression is frequently turned off, because the host transcriptional apparatus is diverted to transcribe viral genes.

It is unclear which viral protein is responsible for controlling a specific host cell function and how depletion of CHD4 decreases cancer cell proliferation. There is a need in the art to generate unique peptide drugs based on the viral protein sequence at the surface of the key viral-host protein interaction. Such peptide as well as its variants can be used as a dominant negative to inhibit the protein function.

SUMMARY

Here, we identified a putative CHD4 inhibitor peptide from the LANA protein sequence corresponding to the LANA-CHD4 interaction surface.

In one aspect, disclosed herein are biomimetic cell-penetrating peptides (such as, for example, the peptide as set forth in SEQ ID NO: 1 or a variant thereof including, but not limited to, a biomimetic cell-penetrating peptide comprising a histadine at amino acid residue 3 of SEQ ID NO:1) comprising an amino acid sequence that binds to a Chromodomain Helicase DNA binding protein 4 (CHD4) in host cell; wherein the biomimetic cell-penetrating peptide induces cleavage of CHD4. In some aspects, the amino acid sequence of the biomimetic cell-penetrating peptide is 10-40 amino acids in length. In some aspects, the biomimetic cell-penetrating peptides cleave one or more caspase cleavage sites on CHD4

Also disclosed herein are biomimetic cell-penetrating peptides of any preceding aspect, wherein the amino acid sequence is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV). In some aspects, the amino acid sequence is derived from a binding interface between LANA and CHD4.

In one aspect disclosed herein are biomimetic cell-penetrating peptides of any preceding aspect, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises

between amino acid residues 939 and 1042 of LANA protein. For example, disclosed herein are biomimetic cell-penetrating peptides of any preceding aspect, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73) (such as, for example, SEQ ID NO: 1).

Also disclosed herein are biomimetic cell-penetrating peptides of any preceding aspect, further comprising a cell-penetrating peptide (CPP) sequence (such as for example, a Trans-Activator of Transcription peptide (TAT) including, but not limited to SEQ ID NO: 3) attached to N-terminal of the amino acid sequence.

In one aspect disclosed herein are biomimetic cell-penetrating peptides of any preceding aspect, further comprising a 6 amino acid long peptide at C-terminus (such as, for example, the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21).

Also disclosed herein are compositions comprising the biomimetic cell-penetrating peptide of any preceding aspect and a pharmaceutically acceptable carrier, wherein the composition is formulated for systemic or localized delivery to cancer cells. In some aspects, the composition can further comprise an apoptosis-inducing agent (such as, for example, chemotherapy drugs, radiation therapy sensitizers, or immune checkpoint inhibitors).

In one aspect, disclosed herein are anti-cancer treatments comprising i) a therapeutically effective amount of the biomimetic cell-penetrating peptide of any preceding aspect or the composition of anyone of any preceding aspect and ii) a cancer therapy selected from the group comprising of chemotherapy, radiation therapy, immunotherapy, or targeted therapy.

Also disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis (such as, for example, primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer) in a subject comprising administering to the subject a therapeutically effective amount of the biomimetic cell-penetrating peptide of any preceding aspect or the composition of any preceding aspect or administering the anti-cancer treatment of any preceding aspect. For example, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis (such as, for example, primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer) in a subject comprising administering to the subject a therapeutically effective amount of

a biomimetic cell-penetrating peptide (such as, for example, the peptide as set forth in SEQ ID NO: 1 or a variant thereof including, but not limited to, a biomimetic cell-penetrating peptide comprising a histadine at amino acid residue 3 of SEQ ID NO:1), wherein the biomimetic cell-penetrating peptide comprises an amino acid sequence that binds to a Chromodomain Helicase DNA binding protein 4 (CHD4) in host cell, wherein the biomimetic cell-penetrating peptide induces cleavage of CHD4. In some aspects, the amino acid sequence is 10-40 amino acids in length. In some aspects, the biomimetic cell-penetrating peptides cleave one or more caspase cleavage sites on CHD4.

Also disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis of any preceding aspect, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV). In some aspects, the amino acid sequence is derived from a binding interface between LANA and CHD4.

In one aspect disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis of any preceding aspect, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises between amino acid residues 939 and 1042 of LANA protein. For example, methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis of any preceding aspect, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73) (such as, for example, SEQ ID NO: 1).

Also disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis of any preceding aspect, wherein the biomimetic cell-penetrating peptide further comprises a cell-penetrating peptide (CPP) sequence (such as for example, a Trans-Activator of Transcription peptide (TAT) including, but not limited to SEQ ID NO: 3) attached to N-terminal of the amino acid sequence.

In one aspect disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis of any preceding aspect, wherein the biomimetic cell-penetrating peptide further comprises a 6 amino acid long peptide at C-terminus (such as, for example, the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21).

Also disclosed herein are methods of inducing apoptosis and differentiation in cancer cells (such as, for example, cancer cells derived from a cancer selected from primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer or from a cancer cell line including, but not limited to U937 cells, BC3 cells, or BCBL-1 cells) comprising contacting the cancer cells with the biomimetic cell-penetrating peptide of any preceding aspect, wherein the biomimetic cell-penetrating peptide reduces occupancy of CHD4 on promoters of genes, thus increasing accessibility of transcription factors (including, but not limited to IRF4, KLF6, FOSB, JUN, and/or JUND) and upregulating genes (such as, for example, BRM, SMARCE1, p21, MYADM, RGS2, CCL3, PPP1R15A, Klf6, CD163, CD206, CD38, CD19, TP53 and/or CHD4) whose expression is related to cell differentiation and apoptosis. For example, disclosed herein are methods of inducing apoptosis and differentiation in cancer cells comprising contacting cancer cells (such as, for example, cancer cells derived from a cancer selected from primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer or from a cancer cell line including, but not limited to U937 cells, BC3 cells, or BCBL-1 cells) with a biomimetic cell-penetrating peptide (such as, for example, the peptide as set forth in SEQ ID NO: 1 or a variant thereof including, but not limited to a biomimetic cell-penetrating peptide comprising a histadine at amino acid residue 3 of SEQ ID NO:1), wherein the biomimetic cell-penetrating peptide reduces occupancy of Chromodomain Helicase DNA binding protein 4 (CHD4) on promoters of genes, thus increasing accessibility of transcription factors (including, but not limited to IRF4, KLF6, FOSB, JUN, and/or JUND) and upregulating genes (such as, for example, BRM, SMARCE1, p21, MYADM, RGS2, CCL3, PPP1R15A, Klf6, CD163, CD206, CD38, CD19, TP53 and/or CHD4) whose expression is related to cell differentiation and apoptosis. In some aspects, the amino acid sequence is 10-40 amino acids in length. In some aspects, the biomimetic cell-penetrating peptides cleave one or more caspase cleavage sites on CHD4.

In one aspect, disclosed herein are methods of inducing apoptosis of any preceding aspect, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV). In some aspects, the amino acid sequence of the biomimetic cell-penetrating peptide is derived from a binding interface between LANA and CHD4.

Also disclosed herein are methods of inducing apoptosis of any preceding aspect, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises between amino acid residues 939 and 1042 of LANA protein. For example, disclosed herein are methods of inducing apoptosis of any preceding aspect, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73) (such as, for example, SEQ ID NO: 1).

In one aspect disclosed herein are methods of inducing apoptosis of any preceding aspect, wherein the biomimetic cell-penetrating peptide further comprises a cell-penetrating peptide (CPP) sequence (such as for example, a Trans-Activator of Transcription peptide (TAT) including, but not limited to SEQ ID NO: 3) attached to N-terminal of the amino acid sequence.

Also disclosed herein are methods of inducing apoptosis of any preceding aspect, wherein the biomimetic cell-penetrating peptide further comprises a 6 amino acid long peptide at C-terminus (such as, for example, the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21).

BRIEF DESCRIPTION OF FIGURES

The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

FIGS. 1A – 1E show the identification of the CHD4 binding domain. FIG. 1A shows a schematic diagram of the KSHA LANA protein. The LANA protein domains are depicted, and intrinsically disordered regions were predicted and plotted below the diagram. The CHD4 binding region (amino acids 870-1042) was enlarged. FIG. 1B shows the N-biotinylated peptide sequences that were tiled to cover the entire protein sequence to identify binding regions. Positions of the amino acid sequences are shown. FIG. 1C shows the results of peptide pull-down assays using the peptides in FIG. 1B. 100 nM of purified Flag tagged CHD4 protein was incubated with 1 µg of biotinylated peptides. Peptide bound to Flag-CHD4 was visualized by immunoblotting with anti-Flag antibody. FIG. 1D shows the LANA-CHD4 binding interface and highlights the CHD4 binding domain. Crystal structure of the LANA C-terminal domain was used as a reference to visualize the position of the CHD4 binding region. Each monomer of LANA C-terminal domain was visualized in gray (left-side of dimer) and pink (right-side). The protein backbone and side-chains of the CHD4 binding region on the LANA monomer was highlighted in green. FIG. 1E shows amino acid sequence alignments of gamma herpesvirus

homologs revealing conserved amino acids. Conserved amino acids depicted in bold and similar amino acids were in italics. Underlined amino acid residue was substituted with histidine, which has been found to have better water solubility and cell membrane penetration.

FIGS. 2A-2E show VGN 73 binding to CHD4 and LANA. FIG. 2A depicts peptides and shows the analysis of the VGN73 binding region in CHD4 by ELISA. Increasing concentrations of deletion CHD4 proteins or full-length CHD4 (shown as 1-1912) was incubated in an ELISA plate coated with 1 μM of VGN73 in triplicate. Peptide binding measured as OD450 are shown. FIG. 2B shows the sequences of three peptides used for biolayer interferometry (BLI). Changes in three amino acids were made in the mutant peptide (underlined). FIG. 2C is a profiling of binding kinetics of VGN73 to CHD4 by BLI. BLI sensorgram showing the binding of full-length Flag-CHD4 protein to N-biotinylated VGN73, mutant, or control TAT immobilized on a biosensor tip. Stedy-state analysis of the interaction between VGN73 or mutant peptide and CHD4 protein in solution at various concentrations is shown. FIG. 2D shows subcellular peptide localization by immunofluorescence. Cy5-conjugated VGN73 and mutant peptide were used to track subcellular localization (15 min or 60 min) in BC3 cells. Nuclei were visualized by Hoechst 33342 staining. FIG. 2E shows the peptide-pull down assay with streptavidin beads. BC3 cells were incubated with biotinylated VGN73, biotinylated mutant peptide or biotinylated TAT peptide (10 μM of each) for 4 hours. TAT alone and 2.5% of the input reaction before peptide-pull down were used as control. The authenticity of the CHD4 protein band was confirmed by immunoblotting with specific antibodies. Densitometry values of CHD4 protein were measured with ImageJ and expressed as fold change compared with control values normalized to 100%.

FIGS. 3A-3C show the effect of VGN73 on CHD4 and LANA protein in tissue culture. FIG. 3A shows IFA analyses for CHD4 and LANA. BC3 cells were treated with VGN73 (18 μM) or mutant peptide (18 μM) for 24 hours followed by staining with the indicated antibodies. The scale is indicated in the panel. Cell volume, CHD4 and LANA signal intensity were compared by Wilcoxon signed-rank test (The number of cells measured; ctrl=89, mut=53, and VGN73=55). $^{**}p < 0.01$, $^{***}p < 0.001$ versus the VGN73 group. FIG. 3B shows immunoblotting for CHD4 and LANA. BC3 and BCBL-1 cells were treated with VGN73 at their respective IC50 concentrations either in complete medium or low serum condition for 24 hours. CHD4 and LANA protein levels of total cell lysates were analyzed by immunoblotting with anti-

CHD4 and anti-LANA antibody, respectively. FIG. 3C shows CHD4 cleavage by VGN73. BC3 cells were pretreated with the pan-caspase inhibitor, Z-VAD-FMK (50 μM) or the proteasome inhibitor, bortezomib (25 nM) for 1 hour, followed by treatment with VGN73 (18 μM) for 8 hours. CHD4 protein levels and cleavage were analyzed by immunoblotting with anti-CHD4 antibody. Densitometry values of full-length CHD4 protein were measured with ImageJ and expressed as fold change compared with control values normalized to 100%.

FIGS. 4A-4D shows that VGN73 inhibits cell growth of various cancer cell types and induces apoptosis and autophagy in BC3 cells. FIG. 4A shows the effect of VGN73 on cell viability. MTT assays were performed with the indicated cell lines in triplicate treated with various VGN73 and mutant peptide concentrations for 24 hours. The OD of mock-treated samples were set as 100%, and OD from detergent-treated cells were set as 0%. Mean percentage viability ± SD was calculated for each treatment (n = 3 samples/treatment). FIG. 4B shows that VGN73 induces apoptosis. BC3 cells were incubated with VGN73 (18 μM) for 0, 1, 4 and 24 hours, followed by measurements of percentage apoptosis induction (Annexin V staining) by flow cytometry. Median percentage apoptosis was calculated for each treatment time (n = 3 samples/treatment time). Western blot analysis was performed to detect cleaved caspase 3. BC3 cells were with VGN73 or mutant peptide (18 μM) for 24hours. Cleaved caspase 3 were calculated by normalizing to untreated sample, and untreated sample set as 100%. FIG. 4C shows VGN73 induced autophagy. BC3 cells were incubated with 18 μM VGN73. 50 nM rapamycin and starvation for 24 hours were used as positive controls. Flow cytometry analyses (Autophagy red staining) were performed. Median percentage apoptosis was calculated for each treatment time (n = 3 samples/treatment) (left). Western blot analysis was performed to detect LC3II and LC3I. BC3 cells were pretreated with lysosome inhibitor, chloroquine (7.5 μM) for 1 hour. LC3II/LC3I ratios were calculated by normalizing to the untreated sample, and the untreated sample was set as 100% (right). FIG. 4D shows that pan caspase inhibitor inhibits cell death by VGN73. BC3 and BCBL1 cells were pretreated with Z-VAD-FMK (40 μM) for 1 hour and then incubated with VGN73 (18 or 13 μM, respectively) for 8 hours. Cell viability was determined by MTT assay (n = 5 samples/treatment). b-d *$p < 0.05$, **$p < 0.01$, using by Wilcoxon signed-rank test.

FIGS. 5A-5D U937 cells differentiation enhanced by VGN73. FIG. 5A shows the experimental scheme for total RNA-seq analysis. U937 cells were pretreated with 0, 4, or 8 μM

VGN73 for 24 hours, and then stimulated with PMA at a final concentration of 10 ng/mL for another 24 hours (n = 3 samples/treatment). Red; treated with PMA alone, Green; treated with VGN73 alone, Blue and Purple; pretreated with VGN73 (4 or 8 μM, respectively) and followed by stimulation with PMA. Cells (n=3 samples/treatment group) were harvested after treatment and RNA-seq was performed. PCA performed on the normalized data and the results depicted in the 2D plot. FIG. 5B shows relative levels of top 500 most variable gene expression. Heatmap showed the clustering of samples based on relative levels of gene expression between each treatment determined by RNA-seq. Differential gene expression specifically between PMA alone and VGN73 alone was marked with asterisks. FIG. 5C shows macrophage differentiation and inflammatory markers. Bar graphs showed relative levels of gene expression involved in macrophage differentiation and inflammatory between each treatment for determined by RNA-seq. FIG. 5D shows changes in macrophage or dendritic cell surface marker expression by flow cytometry. Cells were left untreated, treated alone with VGN73 or PMA, or treated with VGN73 followed by PMA. Flow cytometric analysis for the expression of the indicated markers was performed and depicted in the histograms: untreated cells (red), 4 μM VGN73 for 5 days (blue), mock (i.e., no VGN73) pretreatment, stimulation with 10 ng/mL PMA for 3 days (orange). Pretreatment with 4 μM VGN73 for 2 days, then stimulation with 10 ng/mL PMA for 3 days (green).

FIGS. 6A-6E show inhibited BCBL-1 cell growth in xenograft mice by VGN73. BCBL-1 cells (5×10^6 cells) were injected intraperitoneally (i.p.) into 23-30 week-old female NRG mice. After 2 days, mice were randomly assigned to 3 treatment groups: PBS, mutant peptide (10 mg/kg), and VGN73 (10 mg/kg) (n = 6 mice per group). Treatment drugs were administrated i.p. every other day. FIG. 1A shows in vivo bioluminescence imaging. The tumors at day 14 and 19 were measured by in vivo bioluminescence imaging (n = 6 mice per group), and intensities were compared between each group. FIG. 6B shows mouse body weight. Mice were weighed every other day for 18 days and body weight increments were compared among each group at day 14 and 18 (n = 6 mice per group). FIG. 6C shows PEL cell counts in ascites fluid measured at day 19 by flow cytometry. (n = 6 mice per group) FIG. 6D shows the expression of CHD4 and LANA protein in ascites cells after treatment. Cells were stained with the indicated antibodies. The scale is indicated in the panel. FIG. 6E shows gene expression of cell surface markers in PEL cells recovered from ascites after treatment. CD38 and CD19 gene expression was

quantified by RT-qPCR (n = 5 mice per group). 18S ribosomal RNA was used as an internal standard to normalize viral gene expression. a-c and e *p < 0.05, **p <0.01, using by Wilcoxon signed-rank test.

FIGS. 7A-7B show protein expression and VGN73 mediated binding of LANA and CHD4. FIG. 7A shows Flag-tagged CHD4 deletion proteins. These proteins were prepared from recombinant baculovirus infected Sf9 cells. Coommasie blue staining is shown. Purified CHD4 deletion proteins were used in binding studies described in FIG. 2A. FIG. 7B shows the evaluation of VGN73 in LANA interaction with CHD4. (top left) A schematic diagram of peptide-pull down assay. (bottom left) Peptide-pull-down assay. CHD4 (200 nM) and LANA (200 nM) were incubated with VGN73 (1 or 10 μM) or mutant peptide (1 or 10 μM) in 200 μL binding buffer. 5% of the input reaction before peptide-pull down was used as control. The precipitated proteins were confirmed by immunoblotting with anti-CHD4 and anti-LANA antibody. (right) Putative model of VGN73 actions in vitro.

FIGS. 8A-8B shows protein cleavage and changes in gene expression due to VGN73 treatment. FIG. 8A shows selective protein cleavages by VGN73 incubation. Amount of BRM, SMARCE1 and IRF4 protein expression in total cell lysates was evaluated by western blotting. BC3 and BCBL-1 cells were treated with VGN73 at a concentration of IC50 for 24 hours. The indicted protein expression was confirmed by immunoblotting with specific antibody. FIG. 8B shows gene expression after VGN73 treatment in BC3 cells. Human CHD4, LANA and K-Rta gene expression was quantified by RT-qPCR. 18S ribosomal RNA was used as an internal standard to normalize viral gene expression. *; p < 0.05.

FIGS. 9A-9E show the direct targets of VGN73 as determined by SLAM-seq. PBS, VGN73 or mutant peptide at each concentration of IC50 were added to BC3, BCBL-1 or Raji cells 30 mins prior to incubation with 4sU, and RNA was labeled for 1.5 hours in the presence of each peptide. Each treatment was performed in duplicate. FIG. 9A shows the principal component analysis of SLAM-seq from each cell lines treated with PBS, Mutant or VGN73. FIG. 9B shows a venn diagram of up-regulated genes. Venn diagram shows commonly up-regulated gene sets by VGN73 incubation comparing with PBS among BC3, BCBL-1, and Raji cells. FIG. 9C shows cleavage under targets & release using nuclease with the CHD4 antibodies. Peak scores of promoter region were compared between upregulated gene and non-upregulated gene in SLAM-seq data and depicted with violin plots. FIG. 9D shows CUT&RUN with qPCR.

To confirm the occupancies of CHD4 on promoter regions of upregulated gene, CUT&RUN-qPCR was performed with CHD4 antibody in BCBL-1 cells. Each promoter region was compared with negative control genomic locus. FIG. 9E shows VGN73 decreases CHD4 occupancies on promoters. CUT&RUN with qPCR was performed with the selected promoter regions. Ratio between VGN73 treated and non-treated samples was presented. VGN73 incubation decreased the occupancies of CHD4 from the promoter. NC; negative control genomic locus, *; $p < 0.05$ versus the ctrl.

FIG. 10 shows morphological change in macrophage differentiation with or without VGN73 pretreating as evaluated by IFA. U937 cells were pretreated with VGN73 (4 μM) for 24 hours, followed by stimulated with PMA (10 ng/mL) in starved condition for 72hrs. And then, cells were stained with the indicated antibodies.

FIGS. 11A-11B show tolerance for VGN73 treatment in mice. FIG. 11A shows a Kaplan-Meier survival curve. Maximum tolerated dose with NRG mice was determined prior to xenograft studies. The 11-13 weeks old mice (female; n=4, male; n=4) were injected with increasing amount of VGN73 intraperitoneally every other day. FIG. 11B shows the results of toxicology studies. BALB/c mice (8 weeks old, male; n=6, female; n=6/group) were injected with PBS, 10 mg/kg of VGN73 or mutant peptide intraperitoneally every other day for 18 days. (top) Body weight change. Body weight changes were measured every other day. (bottom) Serum biochemistry. At the end of the experiment, fresh serum collected were subjected for serum biochemical analyses.

DETAILED DESCRIPTION

Reference will now be made in detail to the embodiments of the invention, examples of which are illustrated in the drawings and the examples. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein.

Terminology

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. The term "comprising", and variations thereof as used herein is used synonymously with the term "including" and variations thereof and are open, non-limiting terms. Although the

terms “comprising” and “including” have been used herein to describe various embodiments, the terms “consisting essentially of” and “consisting of” can be used in place of “comprising” and “including” to provide for more specific embodiments and are also disclosed. As used in this disclosure and in the appended claims, the singular forms “a”, “an”, “the”, include plural referents unless the context clearly dictates otherwise.

The following definitions are provided for the full understanding of terms used in this specification.

As used herein, the terms "may," "optionally," and "may optionally" are used interchangeably and are meant to include cases in which the condition occurs as well as cases in which the condition does not occur. Thus, for example, the statement that a formulation "may include an excipient" is meant to include cases in which the formulation includes an excipient as well as cases in which the formulation does not include an excipient.

“Composition” refers to any agent that has a beneficial biological effect. Beneficial biological effects include both therapeutic effects, e.g., treatment of a disorder or other undesirable physiological condition, and prophylactic effects, e.g., prevention of a disorder or other undesirable physiological condition. The terms also encompass pharmaceutically acceptable, pharmacologically active derivatives of beneficial agents specifically mentioned herein, including, but not limited to, a vector, polynucleotide, cells, salts, esters, amides, proagents, active metabolites, isomers, fragments, analogs, and the like. When the term “composition” is used, then, or when a particular composition is specifically identified, it is to be understood that the term includes the composition per se as well as pharmaceutically acceptable, pharmacologically active vector, polynucleotide, salts, esters, amides, proagents, conjugates, active metabolites, isomers, fragments, analogs, etc.

The term “comprising”, and variations thereof as used herein is used synonymously with the term “including” and variations thereof and are open, non-limiting terms. Although the terms “comprising” and “including” have been used herein to describe various embodiments, the terms “consisting essentially of” and “consisting of” can be used in place of “comprising” and “including” to provide for more specific embodiments and are also disclosed.

An "increase" can refer to any change that results in a greater amount of a symptom, disease, composition, condition, or activity. An increase can be any individual, median, or average increase in a condition, symptom, activity, composition in a statistically significant

amount. Thus, the increase can be a 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100%, or more increase so long as the increase is statistically significant.

A "decrease" can refer to any change that results in a smaller amount of a symptom, disease, composition, condition, or activity. A substance is also understood to decrease the genetic output of a gene when the genetic output of the gene product with the substance is less relative to the output of the gene product without the substance. Also, for example, a decrease can be a change in the symptoms of a disorder such that the symptoms are less than previously observed. A decrease can be any individual, median, or average decrease in a condition, symptom, activity, composition in a statistically significant amount. Thus, the decrease can be a 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100% decrease so long as the decrease is statistically significant.

"Inhibit," "inhibiting," and "inhibition" mean to decrease an activity, response, condition, disease, or other biological parameter. This can include but is not limited to the complete ablation of the activity, response, condition, or disease. This may also include, for example, a 10% reduction in the activity, response, condition, or disease as compared to the native or control level. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between as compared to native or control levels.

By "reduce" or other forms of the word, such as "reducing" or "reduction," is meant lowering of an event or characteristic. It is understood that this is typically in relation to some standard or expected value, in other words it is relative, but that it is not always necessary for the standard or relative value to be referred to.

By "prevent" or other forms of the word, such as "preventing" or "prevention," is meant to stop a particular event or characteristic, to stabilize or delay the development or progression of a particular event or characteristic, or to minimize the chances that a particular event or characteristic will occur. Prevent does not require comparison to a control as it is typically more absolute than, for example, reduce. As used herein, something could be reduced but not prevented, but something that is reduced could also be prevented. Likewise, something could be prevented but not reduced, but something that is prevented could also be reduced. It is understood that where reduce or prevent are used, unless specifically indicated otherwise, the use of the other word is also expressly disclosed.

The term "subject" refers to any individual who is the target of administration or treatment. The subject can be a vertebrate, for example, a mammal. In one aspect, the subject can be human, non-human primate, bovine, equine, porcine, canine, or feline. The subject can also be a guinea pig, rat, hamster, rabbit, mouse, or mole. Thus, the subject can be a human or veterinary patient.

The term "patient" refers to a subject under the treatment of a clinician, e.g., physician. The term "treatment" refers to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder.

The terms "treat," "treating," "treatment," and grammatical variations thereof as used herein, include partially or completely delaying, alleviating, mitigating, or reducing the intensity of one or more attendant symptoms of a disorder or condition and/or alleviating, mitigating, or impeding one or more causes of a disorder or condition. Treatments according to the disclosure may be applied preventively, prophylactically, palliatively, or remedially. Treatments are administered to a subject prior to onset (e.g., before obvious signs of disease), during early onset (e.g., upon initial signs and symptoms of disease), or after an established development of the disease. Prophylactic administration can occur for several days to years prior to the manifestation of symptoms of an infection. Therapeutic benefit can also mean to effect a cure of one or more diseases, conditions, or symptoms under treatment. Furthermore, therapeutic benefit can also mean to increase survival. For prophylactic benefit, the compositions may be administered to a subject at risk of developing a particular disease, condition, or symptom, or to a subject reporting one or more of the physiological symptoms of a disease, even though the disease, condition, or symptom may not yet be present.

As used herein, the term "administering" includes oral administration, topical contact, administration as a suppository, intravenous, intraperitoneal, intramuscular, intralesional, intratumoral, intrathecal, intranasal, intraosseous, or subcutaneous administration to a subject. Administration is by any route, including parenteral and transmucosal (e.g., buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-arterial, intradermal, subcutaneous, intraperitoneal, intraventricular, intraosseous, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc.

The term "therapeutically effective amount" or "sufficient amount" refers to the amount of a system, recombinant polynucleotide, or composition described herein that is sufficient to effect beneficial or desired results. The therapeutically effective amount may vary depending upon one or more of: the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the immune status of the subject, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The specific amount may vary depending on one or more of: the particular agent chosen, the target cell type, the location of the target cell in the subject, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, and the physical delivery system in which it is carried.

For the purposes herein an effective amount is determined by such considerations as may be known in the art. The amount must be effective to achieve the desired therapeutic effect in a subject suffering from a disease such as an infectious disease or cancer. The desired therapeutic effect may include, for example, amelioration of undesired symptoms associated with the disease, prevention of the manifestation of such symptoms before they occur, slowing down the progression of symptoms associated with the disease, slowing down or limiting any irreversible damage caused by the disease, lessening the severity of or curing the disease, or improving the survival rate or providing more rapid recovery from the disease. Further, in the context of prophylactic treatment the amount may also be effective to prevent the development of the disease.

The term "pharmaceutically acceptable carrier" refers to a substance that aids the administration of an active agent to a cell, an organism, or a subject. "Pharmaceutically acceptable carrier" also refers to a carrier or excipient that can be included in the compositions of

the invention and that causes no significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable carriers include water, sodium chloride (NaCl), normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors and colors, liposomes, dispersion media, microcapsules, cationic lipid carriers, isotonic and absorption delaying agents, and the like. The carrier may also comprise or consist of substances for providing the formulation with stability, sterility and isotonicity (e.g. antimicrobial preservatives, antioxidants, chelating agents and buffers), for preventing the action of microorganisms (e.g. antimicrobial and antifungal agents, such as parabens, chlorobutanol, phenol, sorbic acid and the like) or for providing the formulation with an edible flavor, etc. In some instances, the carrier is an agent that facilitates the delivery of a polypeptide, fusion protein, or polynucleotide to a target cell or tissue. One of skill in the art will recognize that other pharmaceutical carriers are useful in the present invention.

A "nucleotide" is a compound consisting of a nucleoside, which consists of a nitrogenous base and a 5-carbon sugar, linked to a phosphate group forming the basic structural unit of nucleic acids, such as DNA or RNA. The four types of nucleotides are adenine (A), cytosine (C), guanine (G), and thymine (T), each of which are bound together by a phosphodiester bond to form a nucleic acid molecule. As used herein, a "trinucleotide repeat" refers to a repetitive sequence of three base pair motifs in a DNA sequence. For example, the DNA sequence "GAAGAAGAAGAAGAA(n)" contains a repetitive sequence of GAA nucleotides, wherein n = any number. The trinucleotide repeat can be located in a coding or non-coding region of a genome.

A "nucleic acid" is a chemical compound that serves as the primary information-carrying molecules in cells and makes up the cellular genetic material. Nucleic acids are nucleotides, which are the monomers made of a 5-carbon sugar (usually ribose or deoxyribose), a phosphate group, and a nitrogenous base. A nucleic acid can also be a deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA). A chimeric nucleic acid comprises two or more of the same kind of nucleic acid fused together to form one compound comprising genetic material.

The terms "about" and "approximately" as used herein shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Typically, exemplary degrees of error are within 20 percent (%), preferably

within 10%, and more preferably within 5% of a given value or range of values. Any reference to "about X" specifically indicates at least the values X, 0.8X, 0.81X, 0.82X, 0.83X, 0.84X, 0.85X, 0.86X, 0.87X, 0.88X, 0.89X, 0.9X, 0.91X, 0.92X, 0.93X, 0.94X, 0.95X, 0.96X, 0.97X, 0.98X, 0.99X, 1.01X, 1.02X, 1.03X, 1.04X, 1.05X, 1.06X, 1.07X, 1.08X, 1.09X, 1.1X, 1.11X, 1.12X, 1.13X, 1.14X, 1.15X, 1.16X, 1.17X, 1.18X, 1.19X, and 1.2X. Thus, "about X" is intended to teach and provide written description support for a claim limitation of, e.g., "0.98X.

The term "nucleic acid sequence encoding a peptide" refers to a segment of DNA, which in some embodiments may be a gene or a portion thereof, that is involved in producing a peptide chain (e.g., an antigen or fusion protein). A gene will generally include regions preceding and following the coding region (leader and trailer) involved in the transcription/translation of the gene product and the regulation of the transcription/translation. A gene can also include intervening sequences (introns) between individual coding segments (exons). Leaders, trailers, and introns can include regulatory elements that are necessary during the transcription and the translation of a gene (e.g., promoters, terminators, translational regulatory sequences such as ribosome binding sites and internal ribosome entry sites, enhancers, silencers, insulators, boundary elements, replication origins, matrix attachment sites and locus control regions,e.g.). A "gene product" can refer to either the mRNA or protein expressed from a particular gene.

The terms "expression" and "expressed" refer to the production of a transcriptional and/or translational product, e.g., of a nucleic acid sequence encoding a protein (e.g., an antigen or fusion protein). In some embodiments, the term refers to the production of a transcriptional and/or translational product encoded by a gene (e.g., a gene encoding an antigen) or a portion thereof. The level of expression of a DNA molecule in a cell may be assessed on the basis of either the amount of corresponding mRNA that is present within the cell or the amount of protein encoded by that DNA produced by the cell.

The term "recombinant" when used in reference, e.g., to a polynucleotide, protein, vector, or cell, indicates that the polynucleotide, protein, vector, or cell has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. For example, recombinant polynucleotides contain nucleic acid sequences that are not found within the native (non-recombinant) form of the polynucleotide. [0042] As used herein, the terms "polynucleotide" and "nucleic acid" refer to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers

thereof. The term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, and DNA-RNA hybrids, as well as other polymers comprising purine and/or pyrimidine bases or other natural, chemically modified, biochemically modified, non-natural, synthetic, or derivatized nucleotide bases. Unless specifically limited, the term encompasses nucleic acids containing known analogs of natural nucleotides that have similar binding properties as the reference nucleic acid. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), homologs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994).

The terms "vector" and "expression vector" refer to a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid sequence (e.g., encoding an antigen and/or fusion protein of the invention) in a host cell or engineered cell. In some embodiments, a vector includes a polynucleotide to be transcribed, operably linked to a promoter. Other elements that may be present in a vector include those that enhance transcription (e.g., enhancers), those that terminate transcription (e.g., terminators), those that confer certain binding affinity or antigenicity to a protein (e.g., recombinant protein) produced from the vector, and those that enable replication of the vector and its packaging (e.g., into a viral particle). In some embodiments, the vector is a viral vector (i.e., a viral genome or a portion thereof). A vector may contain nucleic acid sequences or mutations, for example, that increase tropism and/or modulate immune function. An "expression cassette" comprises a coding sequence, operably linked to a promoter, and optionally a polyadenylation sequence.

The terms "percent identity" and "% identity," as applied to nucleotide sequences, refer to the percentage of residue matches between at least two nucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences. Percent identity for a

nucleic acid sequence may be determined as understood in the art. (See, e.g., U.S. Pat. No. 7,396,664, which is incorporated herein by reference in its entirety). A suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S. F. et al. (1990) J. Mol. Biol. 215:403 410), which is available from several sources, including the NCBI, Bethesda, Md., at its website. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known nucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at the NCBI website. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed above).

The phrase "specifically binds" refers to a molecule (e.g., an antibody or antibody fragment against a cancer cell antigen) that binds to a target with greater affinity, avidity, more readily, and/or with greater duration to that target in a sample than it binds to a non-target compound. In some embodiments, a molecule that specifically binds a target binds to the target with at least 2-fold greater affinity than non-target compounds, e.g., at least 3 -fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 25-fold, 50-fold or greater affinity.

Compositions

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), is one of eight human herpesviruses. KSHV is a causative agent of Kaposi's Sarcoma (KS), two lymphoproliferative diseases, primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD). KSHV is also responsible for an interleukin-6-related disease called KSHV inflammatory cytokine syndrome (KICS). Like other herpesviruses, KSHV's life cycle consists of a lifelong latent infection phase and a transient lytic replication phase, in which viral progenies are produced. In latency, the expression of most viral genes is silenced and only a few selected genomic segments are actively transcribed. Among the genes expressed in the latent phase, ORF73 encodes the Latency Associated Nuclear Antigen (LANA) protein, and LANA functions to maintain latent episomes in infected cells. LANA tethers KSHV episomes by directly binding to terminal repeat (TR) sequences in the KSHV genomic DNA through its C -terminal DNA binding domain, and docks onto the host chromosome through its N-terminal histone binding domain, which enables the viral genomic DNA to hitch a ride on the host

chromosome during cell division; this is also demonstrated by maintenance of plasmids containing the KSHV latent origin of replication (ori-P) in transfected cells. In addition to histone H2A/B, LANA interacts with many other chromatin-binding proteins; these include bromodomain-containing proteins 2 and 4 (BRD2/4), KDM3A, hSET1complex, MLL1 complex, and CHD4 (Chromodomain Helicase DNA binding protein 4). These interactions regulate histone occupancies and active/suppressive histone modifications for assembly of protein complex at LANA recruited sites. LANA oligomerization, which is likely to be facilitated by DNA binding at TR with increased protein concentration, was found to be essential for many of the protein-protein interaction. The studies showed that the knock-down of CHD4 enhances KSHV reactivation and inhibits latency establishment in de novo infected 293 cells. Consistent with this observation, recent CHD4 studies of host chromatin binding also showed that CHD4 primarily localizes at epigenetically active chromatin sites and suppresses activation of cellular enhancer and promoter activity by preventing the formation of active genomic hubs with enhancers. Enhancers are distal DNA regulatory elements that regulate promoter activity through genomic looping mechanism by which enhancers are brought into proximity to their promoters. This mechanism supports the frequent interaction of enhancer-bounded mediators with transcription factors on promoters in the event of activation at either promoter or enhancer elements. The enhancers often display a high density of transcription factor binding sites to have the flexibility to respond to external stimuli and their regulators such as co-activator enzymes and/or suppressors. CHD4 is an enzyme that regulates enhancer accessibility and is found in two distinct cellular repressor complexes, the NuRD and ChAHP, and the ChAHP complex plays essential roles in maintaining accurate cell fate decisions during development by regulating enhancer accessibility. Genetic disruption of CHD4 therefore causes spontaneous differentiation concomitant with premature activation of lineage-specific genes. Dysregulation of the gene transcription program by mutations and/or overexpression of transcription-related enzymes prevents cell differentiation and frequently leads to cell transformation. Overexpression of CHD4 is associated with poor progression in hepatocellular carcinoma, colorectal cancer, and ovarian cancer. CHD4 is also identified as essential for breast cancer cell growth, and CHD4 depletion induces a G0/G1 block of the cell cycle with up-regulation of CDKN1A (p21). In all cases, depletion of CHD4 decreases cancer cell proliferation, in some cases, through induction of autophagic cell death. Taken together, inhibition of CHD4, which facilitates cell differentiation

by reactivation of enhancers, would be an approach to target the dedifferentiated cancer cells. Viruses have made their way into human bodies, constantly adapting to their host cell environment co-exist. The lineage of viruses dates back to 450 million years, and their uniqueness lies in the fact that they have to utilize host cell enzymes to produce progenies. Viral proteins therefore often possess a higher affinity protein interaction domain to outcompete host cell proteins to transcribe or replicate their genomes within a short time with limited protein coding capability. Identification of such specific protein domains may therefore be used as a competitive inhibitor of interacting host proteins This study has generated a biologically active peptide from the binding interface between LANA and CHD4. The introduction of the small peptide induces caspase dependent cleavage of CHD4, facilitates cell differentiation in the monocyte differentiation model, and prevents cancer cell growth in vitro and in a PEL xenograft mouse model. The comprehensive characterization from identification of peptide sequence to xenograft studies is described in this report.

Disclosed herein is a biomimetic cell-penetrating peptide for treating cancer comprising an amino acid sequence VGN73 that binds to the CHD4 protein in host cell, wherin the peptide induces cleavage of CHD4, which promotes cell differentiation and apoptosis in cancer cells.

In some aspects, the biomimetic cell-penetrating peptide is referred as a “peptide mimic”. A peptide mimic is a small molecule that mimics the structure and function of a peptide. Peptide mimics are designed to replicate the biological activity of natural peptides but often have improved stability, bioavailability, and resistance to enzymatic degradation. Peptide mimics can vary significantly in structure, ranging from small molecules that mimic the essential features of a peptide to larger, more complex structures. They often incorporate non-peptide components to enhance stability and function. The non-peptide components can include synthetic amino acid analogs, D- amino acids, cyclization of peptides, organic or inorganic modifications, and replacing peptide bonds with isosteres like ester or thioester bonds.

In one aspect, disclosed herein are biomimetic cell-penetrating peptides (such as, for example, the peptide as set forth in SEQ ID NO: 1 or a variant thereof including, but not limited to, a biomimetic cell-penetrating peptide comprising a histadine at amino acid residue 3 of SEQ ID NO:1) comprising an amino acid sequence that binds to a Chromodomain Helicase DNA binding protein 4 (CHD4) in host cell; wherein the biomimetic cell-penetrating peptide induces cleavage of CHD4.

In some aspects, the amino acid sequence of the biomimetic cell-penetrating peptide is 10-40 amino acids in length, preferably between 10-30 amino acids more preferably, between 10-20 amino acids in length. For example, the biomimetic cell-penetrating peptide can be 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids in length.

In some aspects, the biomimetic cell-penetrating peptides cleave one or more caspase cleavage sites on CHD4

Also disclosed herein are biomimetic cell-penetrating peptides, wherein the amino acid sequence is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV). In some aspects, the amino acid sequence is derived from a binding interface between LANA and CHD4.

In one aspect disclosed herein are biomimetic cell-penetrating peptides, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises between amino acid residues 939 and 1042 of LANA protein. For example, disclosed herein are biomimetic cell-penetrating peptides, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73) (such as, for example, SEQ ID NO: 1).

Also disclosed herein are biomimetic cell-penetrating peptides, further comprising a cell-penetrating peptide (CPP) sequence (such as for example, a Trans-Activator of Transcription peptide (TAT) including, but not limited to SEQ ID NO: 3) attached to N-terminal of the amino acid sequence.

In one aspect disclosed herein are biomimetic cell-penetrating peptides, further comprising a 6 amino acid long peptide at C-terminus (such as, for example, the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21).

Also disclosed herein are compositions comprising any of the biomimetic cell-penetrating peptides disclosed herein and a pharmaceutically acceptable carrier, wherein the composition is formulated for systemic or localized delivery to cancer cells. In some aspects, the composition can further comprise an apoptosis-inducing agent (such as, for example, chemotherapy drugs, radiation therapy sensitizers, or immune checkpoint inhibitors).

In one aspect, disclosed herein are anti-cancer treatments comprising i) a therapeutically effective amount of any of the biomimetic cell-penetrating peptides disclosed herein or any of

the compositions disclosed herein and ii) a cancer therapy selected from the group comprising of chemotherapy, radiation therapy, immunotherapy, or targeted therapy.

PEPTIDES

Protein variants

The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. All three terms apply to amino acid polymers in which one or more amino acid residues are an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non- naturally occurring amino acid polymers. As used herein, the terms encompass amino acid chains of any length, including full-length proteins, wherein the amino acid residues are linked by covalent peptide bonds.

As discussed herein there are numerous variants of the VGN73 peptide that are known and herein contemplated. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination

thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

TABLE 1: Amino Acid Abbreviations

Amino Acid	**Abbreviations**	
Alanine	Ala	A
allosoleucine	AIle	
Arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
Cysteine	Cys	C
glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isolelucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
phenylalanine	Phe	F
proline	Pro	P
pyroglutamic acid	pGlu	
Serine	Ser	S
Threonine	Thr	T
Tyrosine	Tyr	Y
Tryptophan	Trp	W
Valine	Val	V

TABLE 2: Amino Acid Substitutions

Original Residue	Exemplary Conservative Substitutions, others are known in the art.
Ala	Ser
Arg	Lys; Gln
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn, Lys
Glu	Asp
Gly	Pro
His	Asn;Gln

Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by

glutaminyl or histidyl residues.

Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO: 1 sets forth a particular sequence of VGN73. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90%, 91%, 92%, 93, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. MoL Biol.* 48: 443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989.

It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to

a particular sequence wherein the variants are conservative mutations.

As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein or peptide in the particular VGN73 from which that protein arises is also known and herein disclosed and described.

It is understood that there are numerous amino acid and peptide analogs which can be incorporated into the disclosed compositions. For example, there are numerous D amino acids or amino acids which have a different functional substituent then the amino acids shown in Table 1 and Table 2. The opposite stereo isomers of naturally occurring peptides are disclosed, as well as the stereo isomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site specific way.

Molecules can be produced that resemble peptides, but which are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include CH_2NH--, --CH_2S--, --CH_2--CH_2 --, --CH=CH-- (cis and trans), --$COCH_2$ --, --$CH(OH)CH_2$--, and --CHH_2SO—(These and others can be found in Spatola, A. F. in *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., *Vega Data* (March 1983), Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley, *Trends Pharm Sci* (1980) pp. 463-468; Hudson, D. et al., Int J Pept Prot Res 14:177-185 (1979) (--CH_2NH--, CH_2CH_2--); Spatola et al. *Life Sci* 38:1243-1249 (1986) (--CH H_2--S); Hann *J. Chem. Soc Perkin Trans.* I 307-314 (1982) (--CH--CH--, cis and trans); Almquist et al. *J. Med. Chem.* 23:1392-1398 (1980) (--$COCH_2$--);

Jennings-White et al. *Tetrahedron Lett* 23:2533 (1982) (--$COCH_2$--); Szelke et al. European Appln, EP 45665 CA (1982): 97:39405 (1982) (--$CH(OH)CH_2$--); Holladay et al. *Tetrahedron.* Lett 24:4401-4404 (1983) (--$C(OH)CH_2$--); and Hruby *Life Sci* 31:189-199 (1982) (--CH_2--S--); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is --CH_2NH--. It is understood that peptide analogs can have more than one atom between the bond atoms, such as b-alanine, g-aminobutyric acid, and the like.

Amino acid analogs and analogs and peptide analogs often have enhanced or desirable properties, such as, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others.

D-amino acids can be used to generate more stable peptides, because D amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular conformations.

Pharmaceutical carriers/Delivery of pharmaceutical products

As described above, the compositions can also be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

The compositions may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, including topical intranasal administration or administration by inhalant. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector.

Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., *Bioconjugate Chem.*, 2:447-451, (1991); Bagshawe, K.D., *Br. J. Cancer*, 60:275-281, (1989); Bagshawe, et al., *Br. J. Cancer*, 58:700-703, (1988); Senter, et al., *Bioconjugate Chem.*, 4:3-9, (1993); Battelli, et al., *Cancer Immunol. Immunother.*, 35:421-425, (1992); Pietersz and McKenzie, *Immunolog. Reviews*, 129:57-80, (1992); and Roffler, et al., *Biochem. Pharmacol*, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., *Cancer Research*, 49:6214-6220, (1989); and Litzinger and Huang, *Biochimica et Biophysica Acta*, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either

recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, *DNA and Cell Biology* 10:6, 399-409 (1991)).

Pharmaceutically Acceptable Carriers

The compositions, including antibodies, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable..

Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

Therapeutic Uses

Effective dosages and schedules for administering the compositions may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms of the disorder are effected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, guidance in selecting appropriate doses for antibodies can be found in the literature on therapeutic uses of antibodies, e.g., *Handbook of Monoclonal Antibodies*, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., *Antibodies in Human Diagnosis and Therapy*, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. A typical daily dosage of the antibody used alone might range from about 1 µg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above.

Methods of Treating Diseases or Disorders

It is understood and herein contemplated that the disclosed peptides compositions can be used to treat any disease where uncontrolled cellular proliferation occurs such as cancers. A representative but non-limiting list of cancers that the disclosed compositions can be used to treat is the following: lymphomas such as B cell lymphoma and T cell lymphoma; mycosis fungoides; Hodgkin's Disease; myeloid leukemia (including, but not limited to acute myeloid leukemia (AML) and/or chronic myeloid leukemia (CML)); bladder cancer; brain cancer; nervous system cancer; head and neck cancer; squamous cell carcinoma of head and neck; renal cancer; lung cancers such as small cell lung cancer, non-small cell lung carcinoma (NSCLC), lung squamous cell carcinoma (LUSC), and Lung Adenocarcinomas (LUAD); neuroblastoma/glioblastoma; ovarian cancer; pancreatic cancer; prostate cancer; skin cancer; hepatic cancer; melanoma; squamous cell carcinomas of the mouth, throat, larynx, and lung; cervical cancer; cervical carcinoma; breast cancer including, but not limited to triple negative breast cancer; genitourinary

cancer; pulmonary cancer; esophageal carcinoma; head and neck carcinoma; large bowel cancer; hematopoietic cancers; testicular cancer; and colon and rectal cancers.

Accordingly, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis (such as, for example, primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer) in a subject comprising administering to the subject a therapeutically effective amount any of the biomimetic cell-penetrating peptides disclosed herein or any of the compositions disclosed herein or administering any of the anti-cancer treatments disclosed herein. For example, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis (such as, for example, primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer) in a subject comprising administering to the subject a therapeutically effective amount of a biomimetic cell-penetrating peptide (such as, for example, the peptide as set forth in SEQ ID NO: 1 or a variant thereof including, but not limited to, a biomimetic cell-penetrating peptide comprising a histidine at amino acid residue 3 of SEQ ID NO:1), wherein the biomimetic cell-penetrating peptide comprises an amino acid sequence that binds to a Chromodomain Helicase DNA binding protein 4 (CHD4) in host cell, wherein the biomimetic cell-penetrating peptide induces cleavage of CHD4. In some aspects, the amino acid sequence is 10-40 amino acids in length. In some aspects, the biomimetic cell-penetrating peptides cleave one or more caspase cleavage sites on CHD4.

Also disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV). In some aspects, the amino acid sequence is derived from a binding interface between LANA and CHD4.

In one aspect disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises between amino acid residues 939 and 1042 of LANA protein. For example, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis, wherein the biomimetic

cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73) (such as, for example, SEQ ID NO: 1).

Also disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis, wherein the biomimetic cell-penetrating peptide further comprises a cell-penetrating peptide (CPP) sequence (such as for example, a Trans-Activator of Transcription peptide (TAT) including, but not limited to SEQ ID NO: 3) attached to N-terminal of the amino acid sequence.

In one aspect disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis, wherein the biomimetic cell-penetrating peptide further comprises a 6 amino acid long peptide at C-terminus (such as, for example, the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21).

In one aspect, the treatment of the cancer can include administration of a peptide. For example, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing a cancer and/or metastasis (such as, for example, breast cancer) in a subject comprising administering to the subject VGN37 peptide.

It is understood and herein contemplated that the disclosed treatment regimens can used alone or in combination with any anti-cancer therapy known in the art including, but not limited to Abemaciclib, Abiraterone Acetate, ABITREXATE® (Methotrexate), ABRAXANE® (Paclitaxel Albumin-stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, AC-T, ADCETRIS® (Brentuximab Vedotin), ADE, Ado-Trastuzumab Emtansine, ADRIAMYCIN® (Doxorubicin Hydrochloride), Afatinib Dimaleate, AFINITOR® (Everolimus), AKYNZEO® (Netupitant and Palonosetron Hydrochloride), ALDARA® (Imiquimod), Aldesleukin, ALECENSA® (Alectinib), Alectinib, Alemtuzumab, ALIMTA® (Pemetrexed Disodium), ALIQOPA® (Copanlisib Hydrochloride), ALKERAN™ for Injection (Melphalan Hydrochloride), ALKERAN™ Tablets (Melphalan), ALOXI® (Palonosetron Hydrochloride), ALUNBRIG® (Brigatinib), AMBOCHLORIN® (Chlorambucil), AMBOCLORIN® (Chlorambucil), Amifostine, Aminolevulinic Acid, Anastrozole, Aprepitant, AREDIA® (Pamidronate Disodium), ARIMIDEX® (Anastrozole), AROMASIN® (Exemestane),ARRANON® (Nelarabine), Arsenic Trioxide, ARZERRA® (Ofatumumab), Asparaginase Erwinia chrysanthemi, Atezolizumab, AVASTIN® (Bevacizumab), Avelumab,

Axitinib, Azacitidine, BAVENCIO® (Avelumab), BEACOPP, BECENUM® (Carmustine), BELEODAQ® (Belinostat), Belinostat, Bendamustine Hydrochloride, BEP, BESPONSA® (Inotuzumab Ozogamicin) , Bevacizumab, Bexarotene, BEXXAR® (Tositumomab and Iodine I 131 Tositumomab), Bicalutamide, BICNU® (Carmustine), Bleomycin, Blinatumomab, BLINCYTO® (Blinatumomab), Bortezomib, BOSULIF® (Bosutinib), Bosutinib, Brentuximab Vedotin, Brigatinib, BuMel, Busulfan, BUSULFEX® (Busulfan), Cabazitaxel, CABOMETYX® (Cabozantinib-S-Malate), Cabozantinib-S-Malate, CAF, CAMPATH® (Alemtuzumab), CAMPTOSAR® (Irinotecan Hydrochloride), Capecitabine, CAPOX, CARAC® (Fluorouracil--Topical), Carboplatin, CARBOPLATIN-TAXOL, Carfilzomib, CARMUBRIS® (Carmustine), Carmustine, Carmustine Implant, CASODEX® (Bicalutamide), CEM, Ceritinib, CERUBIDINE® (Daunorubicin Hydrochloride), CERVARIX® (Recombinant HPV Bivalent Vaccine), Cetuximab, CEV, Chlorambucil, CHLORAMBUCIL-PREDNISONE, CHOP, Cisplatin, Cladribine, CLAFEN® (Cyclophosphamide), Clofarabine, CLOFAREX® (Clofarabine), CLOLAR® (Clofarabine), CMF, Cobimetinib, COMETRIQ® (Cabozantinib-S-Malate), Copanlisib Hydrochloride, COPDAC, COPP, COPP-ABV, COSMEGEN® (Dactinomycin), COTELLIC® (Cobimetinib), Crizotinib, CVP, Cyclophosphamide, CYFOS® (Ifosfamide), CYRAMZA® (Ramucirumab), Cytarabine, Cytarabine Liposome, CYTOSAR-U® (Cytarabine), CYTOXAN® (Cyclophosphamide), Dabrafenib, Dacarbazine, DACOGEN® (Decitabine), Dactinomycin, Daratumumab, DARZALEX® (Daratumumab), Dasatinib, Daunorubicin Hydrochloride, Daunorubicin Hydrochloride and Cytarabine Liposome, Decitabine, Defibrotide Sodium, DEFITELIO® (Defibrotide Sodium), Degarelix, Denileukin Diftitox, Denosumab, DEPOCYT® (Cytarabine Liposome), Dexamethasone, Dexrazoxane Hydrochloride, Dinutuximab, Docetaxel, DOXIL® (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, DOX-SL® (Doxorubicin Hydrochloride Liposome), DTIC-DOME® (Dacarbazine), Durvalumab, EFUDEX® (Fluorouracil--Topical), ELITEK® (Rasburicase), ELLENCE® (Epirubicin Hydrochloride), Elotuzumab, ELOXATIN® (Oxaliplatin), Eltrombopag Olamine, EMEND® (Aprepitant), EMPLICITI® (Elotuzumab), Enasidenib Mesylate, Enzalutamide, Epirubicin Hydrochloride , EPOCH, ERBITUX® (Cetuximab), Eribulin Mesylate, ERIVEDGE® (Vismodegib), Erlotinib Hydrochloride, ERWINAZE® (Asparaginase Erwinia chrysanthemi), ETHYOL® (Amifostine), Etopophos ETOPOPHOS® (Etoposide Phosphate), Etoposide, Etoposide Phosphate, EVACET®

(Doxorubicin Hydrochloride Liposome), Everolimus, EVISTA® (Raloxifene Hydrochloride), EVOMELA® (Melphalan Hydrochloride), Exemestane, 5-FU® (Fluorouracil Injection), 5-FU® (Fluorouracil--Topical), FARESTON® (Toremifene), FARYDAK® (Panobinostat), FASLODEX® (Fulvestrant), FEC, FEMARA® (Letrozole), Filgrastim, FLUDARA® (Fludarabine Phosphate), Fludarabine Phosphate, FLUOROPLEX® (Fluorouracil--Topical), Fluorouracil Injection, Fluorouracil--Topical, Flutamide, FOLEX® (Methotrexate), FOLEX PFS® (Methotrexate), FOLFIRI, FOLFIRI-BEVACIZUMAB, FOLFIRI-CETUXIMAB, FOLFIRINOX, FOLFOX, FOLOTYN® (Pralatrexate), FU-LV, Fulvestrant, GARDASIL® (Recombinant HPV Quadrivalent Vaccine), GARDASIL 9® (Recombinant HPV Nonavalent Vaccine), GAZYVA® (Obinutuzumab), Gefitinib, Gemcitabine Hydrochloride, GEMCITABINE-CISPLATIN, GEMCITABINE-OXALIPLATIN, Gemtuzumab Ozogamicin, GEMZAR® (Gemcitabine Hydrochloride), GILOTRIF® (Afatinib Dimaleate), GLEEVEC® (Imatinib Mesylate), GLIADEL® (Carmustine Implant), GLIADEL WAFER® (Carmustine Implant), Glucarpidase, Goserelin Acetate, HALAVEN® (Eribulin Mesylate), HEMANGEOL® (Propranolol Hydrochloride), HERCEPTIN® (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Nonavalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, HYCAMTIN® (Topotecan Hydrochloride), HYDREA® (Hydroxyurea), Hydroxyurea, Hyper-CVAD, IBRANCE® (Palbociclib), Ibritumomab Tiuxetan, Ibrutinib, ICE, ICLUSIG® (Ponatinib Hydrochloride), IDAMYCIN® (Idarubicin Hydrochloride), Idarubicin Hydrochloride, Idelalisib, IDHIFA® (Enasidenib Mesylate), IFEX® (Ifosfamide), Ifosfamide, IFOSFAMIDUM® (Ifosfamide), IL-2 (Aldesleukin), Imatinib Mesylate, IMBRUVICA® (Ibrutinib), IMFINZI® (Durvalumab), Imiquimod, IMLYGIC® (Talimogene Laherparepvec), INLYTA® (Axitinib), Inotuzumab Ozogamicin, Interferon Alfa-2b, Recombinant, Interleukin-2 (Aldesleukin), INTRON A® (Recombinant Interferon Alfa-2b), Iodine I 131 Tositumomab and Tositumomab, Ipilimumab, IRESSA® (Gefitinib), Irinotecan Hydrochloride, Irinotecan Hydrochloride Liposome, ISTODAX® (Romidepsin), Ixabepilone, Ixazomib Citrate, IXEMPRA® (Ixabepilone), JAKAFI® (Ruxolitinib Phosphate), JEB, JEVTANA® (Cabazitaxel), KADCYLA® (Ado-Trastuzumab Emtansine), KEOXIFENE® (Raloxifene Hydrochloride), KEPIVANCE® (Palifermin), KEYTRUDA® (Pembrolizumab), KISQALI® (Ribociclib), KYMRIAH® (Tisagenlecleucel), KYPROLIS® (Carfilzomib), Lanreotide Acetate, Lapatinib Ditosylate, LARTRUVO® (Olaratumab), Lenalidomide, Lenvatinib Mesylate,

LENVIMA® (Lenvatinib Mesylate), Letrozole, Leucovorin Calcium, LEUKERAN® (Chlorambucil), Leuprolide Acetate, LEUSTATIN® (Cladribine), LEVULAN® (Aminolevulinic Acid), LINFOLIZIN® (Chlorambucil), LIPODOX® (Doxorubicin Hydrochloride Liposome), Lomustine, LONSURF® (Trifluridine and Tipiracil Hydrochloride), LUPRON® (Leuprolide Acetate), LUPRON DEPOT® (Leuprolide Acetate), LUPRON DEPOT-PED® (Leuprolide Acetate), LYNPARZA® (Olaparib), MARQIBO® (Vincristine Sulfate Liposome), MATULANE® (Procarbazine Hydrochloride), Mechlorethamine Hydrochloride, Megestrol Acetate, MEKINIST® (Trametinib), Melphalan, Melphalan Hydrochloride, Mercaptopurine, Mesna, MESNEX® (Mesna), METHAZOLASTONE® (Temozolomide), Methotrexate, METHOTREXATE LPF® (Methotrexate), Methylnaltrexone Bromide, MEXATE® (Methotrexate), MEXATE-AQ® (Methotrexate), Midostaurin, Mitomycin C, Mitoxantrone Hydrochloride, MITOZYTREX® (Mitomycin C), MOPP, MOZOBIL® (Plerixafor), MUSTARGEN® (Mechlorethamine Hydrochloride) , MUTAMYCIN® (Mitomycin C), MYLERAN® (Busulfan), MYLOSAR® (Azacitidine), MYLOTARG® (Gemtuzumab Ozogamicin), NANOPARTICLE PACLITAXEL® (Paclitaxel Albumin-stabilized Nanoparticle Formulation), NAVELBINE® (Vinorelbine Tartrate), Necitumumab, Nelarabine, NEOSAR® (Cyclophosphamide), Neratinib Maleate, NERLYNX® (Neratinib Maleate), Netupitant and Palonosetron Hydrochloride, NEULASTA® (Pegfilgrastim), NEUPOGEN® (Filgrastim), NEXAVAR® (Sorafenib Tosylate), NILANDRON® (Nilutamide), Nilotinib, Nilutamide, NINLARO® (Ixazomib Citrate), Niraparib Tosylate Monohydrate, Nivolumab, NOLVADEX® (Tamoxifen Citrate), NPLATE® (Romiplostim), Obinutuzumab, ODOMZO® (Sonidegib), OEPA, Ofatumumab, OFF, Olaparib, Olaratumab, Omacetaxine Mepesuccinate, ONCASPAR® (Pegaspargase), Ondansetron Hydrochloride, ONIVYDE® (Irinotecan Hydrochloride Liposome), ONTAK® (Denileukin Diftitox), OPDIVO® (Nivolumab), OPPA, Osimertinib, Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, PAD, Palbociclib, Palifermin, Palonosetron Hydrochloride, Palonosetron Hydrochloride and Netupitant, Pamidronate Disodium, Panitumumab, Panobinostat, PARAPLAT® (Carboplatin), PARAPLATIN® (Carboplatin), Pazopanib Hydrochloride, PCV, PEB, Pegaspargase, Pegfilgrastim, Peginterferon Alfa-2b, PEG-INTRON® (Peginterferon Alfa-2b), Pembrolizumab, Pemetrexed Disodium, PERJETA® (Pertuzumab), Pertuzumab, PLATINOL® (Cisplatin), PLATINOL-AQ® (Cisplatin), Plerixafor,

Pomalidomide, POMALYST® (Pomalidomide), Ponatinib Hydrochloride, PORTRAZZA® (Necitumumab), Pralatrexate, Prednisone, Procarbazine Hydrochloride, PROLEUKIN® (Aldesleukin), PROLIA® (Denosumab), PROMACTA® (Eltrombopag Olamine), Propranolol Hydrochloride, PROVENGE® (Sipuleucel-T), PURINETHOL® (Mercaptopurine), PURIXAN® (Mercaptopurine), Radium 223 Dichloride, Raloxifene Hydrochloride, Ramucirumab, Rasburicase, R-CHOP, R-CVP, Recombinant Human Papillomavirus (HPV) Bivalent Vaccine, Recombinant Human Papillomavirus (HPV) Nonavalent Vaccine, Recombinant Human Papillomavirus (HPV) Quadrivalent Vaccine, Recombinant Interferon Alfa-2b, Regorafenib, RELISTOR® (Methylnaltrexone Bromide), R-EPOCH, REVLIMID® (Lenalidomide), RHEUMATREX® (Methotrexate), Ribociclib, R-ICE, RITUXAN® (Rituximab), RITUXAN HYCELA® (Rituximab and Hyaluronidase Human), Rituximab, Rituximab and , Hyaluronidase Human, ,Rolapitant Hydrochloride, Romidepsin, Romiplostim, RUBIDOMYCIN® (Daunorubicin Hydrochloride), RUBRACA® (Rucaparib Camsylate), Rucaparib Camsylate, Ruxolitinib Phosphate, RYDAPT® (Midostaurin), Sclerosol Intrapleural Aerosol (Talc), Siltuximab, Sipuleucel-T, SOMATULINE DEPOT® (Lanreotide Acetate), Sonidegib, Sorafenib Tosylate, SPRYCEL® (Dasatinib), STANFORD V, Sterile Talc Powder (Talc), STERITALC® (Talc), STIVARGA® (Regorafenib), Sunitinib Malate, SUTENT® (Sunitinib Malate), SYLATRON® (Peginterferon Alfa-2b), SYLVANT® (Siltuximab), Synribo SYNRIBO® (Omacetaxine Mepesuccinate), TABLOID® (Thioguanine), TAC, TAFINLAR® (Dabrafenib), TAGRISSO® (Osimertinib), Talc, Talimogene Laherparepvec, Tamoxifen Citrate, TARABINE PFS® (Cytarabine), TARCEVA® (Erlotinib Hydrochloride), TARGRETIN® (Bexarotene), TASIGNA® (Nilotinib), TAXOL® (Paclitaxel), TAXOTERE® (Docetaxel), TECENTRIQ® (Atezolizumab), TEMODAR® (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, THALOMID® (Thalidomide), Thioguanine, Thiotepa, Tisagenlecleucel, TOLAK® (Fluorouracil--Topical), Topotecan Hydrochloride, Toremifene, TORISEL® (Temsirolimus), Tositumomab and Iodine I 131 Tositumomab, TOTECT® (Dexrazoxane Hydrochloride), TPF, Trabectedin, Trametinib, Trastuzumab, TREANDA® (Bendamustine Hydrochloride), Trifluridine and Tipiracil Hydrochloride, TRISENOX® (Arsenic Trioxide), TYKERB® (Lapatinib Ditosylate) , UNITUXIN® (Dinutuximab), Uridine Triacetate, VAC, Vandetanib, VAMP, VARUBI® (Rolapitant Hydrochloride), VECTIBIX® (Panitumumab), VeIP, VELBAN® (Vinblastine Sulfate), VELCADE® (Bortezomib), VELSAR® (Vinblastine

Sulfate), Vemurafenib, VENCLEXTA® (Venetoclax), Venetoclax, VERZENIO® (Abemaciclib), VIADUR® (Leuprolide Acetate), VIDAZA® (Azacitidine), Vinblastine Sulfate, VINCASAR PFS® (Vincristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine Tartrate, VIP, Vismodegib, VISTOGARD® (Uridine Triacetate), VORAXAZE® (Glucarpidase), Vorinostat, VOTRIENT® (Pazopanib Hydrochloride), VYXEOS® (Daunorubicin Hydrochloride and Cytarabine Liposome), WELLCOVORIN® (Leucovorin Calcium), XALKORI® (Crizotinib), XELODA® (Capecitabine), XELIRI, XELOX, XGEVA® (Denosumab), XOFIGO® (Radium 223 Dichloride), XTANDI® (Enzalutamide), YERVOY® (Ipilimumab), YONDELIS® (Trabectedin), ZALTRAP® (Ziv-Aflibercept), ZARXIO® (Filgrastim), ZEJULA® (Niraparib Tosylate Monohydrate), ZELBORAF® (Vemurafenib), ZEVALIN® (Ibritumomab Tiuxetan), ZINECARD® (Dexrazoxane Hydrochloride), Ziv-Aflibercept, ZOFRAN® (Ondansetron Hydrochloride), ZOLADEX® (Goserelin Acetate), Zoledronic Acid, ZOLINZA® (Vorinostat), ZOMETA® (Zoledronic Acid), ZYDELIG® (Idelalisib), ZYKADIA® (Ceritinib), and/or ZYTIGA® (Abiraterone Acetate). The treatment methods can include or further include checkpoint inhibitors including, but are not limited to antibodies that block PD-1 (such as, for example, Nivolumab (BMS-936558 or MDX1106), pembrolizumab, cemiplimab , CT-011, MK-3475), PD-L1 (such as, for example, atezolizumab, avelumab, durvalumab, MDX-1105 (BMS-936559), MPDL3280A, or MSB0010718C), PD-L2 (such as, for example, rHIgM12B7), CTLA-4 (such as, for example, Ipilimumab (MDX-010), Tremelimumab (CP-675,206)), IDO, B7-H3 (such as, for example, MGA271, MGD009, omburtamab), B7-H4, B7-H3, T cell immunoreceptor with Ig and ITIM domains (TIGIT)(such as, for example BMS-986207, OMP-313M32, MK-7684, AB-154, ASP-8374, MTIG7192A, or PVSRIPO), CD96, B- and T-lymphocyte attenuator (BTLA), V-domain Ig suppressor of T cell activation (VISTA)(such as, for example, JNJ-61610588, CA-170), TIM3 (such as, for example, TSR-022, MBG453, Sym023, INCAGN2390, LY3321367, BMS-986258, SHR-1702, RO7121661), LAG-3 (such as, for example, BMS-986016, LAG525, MK-4280, REGN3767, TSR-033, BI754111, Sym022, FS118, MGD013, and Immutep).

Following administration of a disclosed composition, such as an antibody, for treating, inhibiting, or preventing a cancer, the efficacy of the therapeutic antibody can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that a composition, such as an antibody, disclosed herein is efficacious in

treating or inhibiting a cancer in a subject by observing that the composition induces immune response to a tumor

The compositions that inhibit CDH4 and LANA interactions disclosed herein may be administered prophylactically to patients or subjects who are at risk for cancers.

The disclosed compositions and methods can also be used for example as tools to isolate and test new drug candidates for a variety of cancer related diseases.

It is understood and herein contemplated that the disclosed biomimetic cell-penetrating peptides can be used in the treatment of inflammation, inflammatory conditions, autoimmune diseases, and/or lympoplroliferative disorders.

The term "inflammation" refers to an organism's (e.g., a mammal's) immune response to irritation, toxic substances, pathogens, or other stimuli. The response can involve innate immune components and/or adaptive immunity. Inflammation is generally characterized as either chronic or acute. Acute inflammation can be characterized by, as non-limiting examples, redness, pain, heat, swelling, and/or loss of function due to infiltration of plasma proteins and leukocytes to the affected area. Chronic inflammation can be characterized by, as non-limiting examples, persistent inflammation, tissue destruction, and/or attempts at repair. Monocytes, macrophages, plasma B cells, and other lymphocytes are commonly recruited to the affected area, and angiogenesis and fibrosis can occur, in some instances leading to scar tissue.

The term "inflammatory condition" or "inflammatory disorder" refers to a condition or disorder that is characterized by or involving an inflammatory response, as described above. A list of exemplary inflammatory conditions includes: systemic lupus erythematosus (SLE), diabetes, chronic renal disease, asthma, autoimmune disease, chronic inflammation, chronic prostatitis, glomerulonephritis, hypersensitivities and allergies, skin disorders such as eczema, inflammatory bowel disease, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, transplant rejection (e.g., graft versus host disease), cytokine storm syndrome, secondary hemophagocytic lymphohistiocytosis, sepsis, macrophage activation syndrome, and vasculitis.

An "autoimmune disease" is a disease in which a patient's immune system recognizes own tissues as foreign and mounts an abnormal immune response to attack the tissue. With the common symptoms of continuous and low grade of inflammation of affected tissue, a large number of autoimmune diseases have been recognized and include (but are 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 (AIED), autoimmune myocarditis, autoimmune oophoritis, autoimmune orchitis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune urticaria, axonal & neuronal neuropathy (AMAN), Balo disease, Behcet's disease, benign mucosal pemphigoid, bullous pemphigoid, Castleman disease (CD), Celiac disease, Chagas disease, chronic inflammatory demyelinating polyneuropathy (CIDP), chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss Syndrome (CSS) or Eosinophilic Granulomatosis (EGPA), 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, Dressier's syndrome, endometriosis, eosinophilic esophagitis (EoE), 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 (HSP), herpes gestationis or pemphigoid gestationis (PG), Hidradenitis Suppurativa (HS) (Acne Inversa), hypogammalglobulinemia, IgA Nephropathy, IgG4-related sclerosing disease, immune thrombocytopenic purpura (ITP), inclusion body myositis (IBM), interstitial cystitis (IC), juvenile arthritis, juvenile diabetes (Type 1 diabetes), juvenile myositis (JM), Kawasaki disease, Lambert-Eaton syndrome, leukocytoclastic vasculitis, lichen planus, lichen sclerosus, ligneous conjunctivitis, linear IgA disease (LAD), lupus, Lyme disease chronic, Meniere's disease, microscopic polyangiitis (MPA), mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multifocal Motor Neuropathy (MMN) or MMNCB, multiple sclerosis, myasthenia gravis, myositis, narcolepsy, neonatal Lupus, neuromyelitis optica, neutropenia, ocular cicatricial pemphigoid, optic neuritis, palindromic rheumatism (PR), PANDAS, paraneoplastic cerebellar degeneration (PCD), paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, pars planitis (peripheral uveitis), Parsonage-Turner syndrome, pemphigus, peripheral neuropathy, perivenous encephalomyelitis, pernicious anemia (PA), 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 (PRCA), pyoderma gangrenosum, Raynaud's phenomenon, Reactive Arthritis, reflex sympathetic dystrophy, relapsing polychondritis, Restless legs syndrome (RLS), retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjogren's syndrome, Sperm & testicular autoimmunity, sperm & testicular autoimmunity, Stiff person syndrome (SPS), subacute bacterial endocarditis (SBE), Susac's syndrome, sympathetic ophthalmia (SO), Takayasu's arteritis, temporal arteritis/giant cell arteritis, thrombocytopenic purpura (TTP), thyroid eye disease (TED), Tolosa-Hunt syndrome (THS), transverse myelitis, type 1 diabetes, ulcerative colitis (UC), undifferentiated connective tissue disease (UC'TD), uveitis, vasculitis, vitiligo, and Vogt-Koyanagi-Harada Disease. [0059] The term "cancer" refers to any of various malignant neoplasms characterized by the proliferation of anaplastic cells that tend to invade surrounding tissue and metastasize to new body sites. Non-limiting examples of different types of cancer suitable for treatment using the compositions and methods of the present invention include colorectal cancer, colon cancer, anal cancer, liver cancer, ovarian cancer, breast cancer, lung cancer, bladder cancer, thyroid cancer, pleural cancer, pancreatic cancer, cervical cancer, prostate cancer, testicular cancer, bile duct cancer, gastrointestinal carcinoid tumors, esophageal cancer, gall bladder cancer, rectal cancer, appendix cancer, small intestine cancer, stomach (gastric) cancer, renal cancer (e.g., renal cell carcinoma), cancer of the central nervous system, skin cancer, oral squamous cell carcinoma, choriocarcinomas, head and neck cancers, bone cancer, osteogenic sarcomas, fibrosarcoma, neuroblastoma, glioma, melanoma, leukemia (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, or hairy cell leukemia), lymphoma (e.g., non-Hodgkin's lymphoma, Hodgkin's lymphoma, B-cell lymphoma, or Burkitt's lymphoma), and multiple myeloma.

The term "lymphoproliferative disorders" refers to any disorders characterized by abnormal proliferation of lymphocytes into a monoclonal lymphocytosis. Non-limiting examples of different types of lymphoproliferative disorders suitable for treatment using the compositions and methods of the present invention besides leukemia as described above include Waldenstrom's macroglobulinemia, Wiskott- Aldrich syndrome, Langerhans cell histiocytosis,

Lymphocyte-variant hypereosinophila, Pityriasis Lichenoides, Post-transplant lymphoproliferative disorder, Autoimmune lymphoproliferative syndrome, Lymphoid interstitial pneumonia, Epstein -Barr virus-associated lymphoproliferative diseases, Castleman disease, and X-linked lymphoproliferative disease.

The term "suppressor cells" refers to any lymphocytes that can suppress productive immune response such as antibody production or T cell proliferation through various mechanisms including cell-cell contact, cytokines and killing. Non-limiting examples of different types of suppressive immune cells suitable for treatment using the compositions and methods of the present invention include T regulatory cells, Tri cells, B regulatory cells, and myeloid-derived suppressor cells.

Accordingly, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing inflammation, inflammatory conditions, autoimmune diseases, and/or lympoplroliferative disorders in a subject comprising administering to the subject a therapeutically effective amount any of the biomimetic cell-penetrating peptides disclosed herein or any of the compositions disclosed herein. For example, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing inflammation, inflammatory conditions, autoimmune diseases, and/or lymphoproliferative disorders in a subject comprising administering to the subject a therapeutically effective amount of a biomimetic cell-penetrating peptide (such as, for example, the peptide as set forth in SEQ ID NO: 1 or a variant thereof including, but not limited to, a biomimetic cell-penetrating peptide comprising a histadine at amino acid residue 3 of SEQ ID NO:1), wherein the biomimetic cell-penetrating peptide comprises an amino acid sequence that binds to a Chromodomain Helicase DNA binding protein 4 (CHD4) in host cell, wherein the biomimetic cell-penetrating peptide induces cleavage of CHD4. In some aspects, the amino acid sequence is 10-40 amino acids in length. In some aspects, the biomimetic cell-penetrating peptides cleave one or more caspase cleavage sites on CHD4.

Also disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing inflammation, inflammatory conditions, autoimmune diseases, and/or lymphoproliferative disorders, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from Latency Associated Nuclear Antigen (LANA) protein of

Kaposi's sarcoma-associated herpesvirus (KSHV). In some aspects, the amino acid sequence is derived from a binding interface between LANA and CHD4.

In one aspect disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing inflammation, inflammatory conditions, autoimmune diseases, and/or lymphoproliferative disorders, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises between amino acid residues 939 and 1042 of LANA protein. For example, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing inflammation, inflammatory conditions, autoimmune diseases, and/or lymphoproliferative disorders, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73) (such as, for example, SEQ ID NO: 1).

Also disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing inflammation, inflammatory conditions, autoimmune diseases, and/or lymphoproliferative disorders, wherein the biomimetic cell-penetrating peptide further comprises a cell-penetrating peptide (CPP) sequence (such as for example, a Trans-Activator of Transcription peptide (TAT) including, but not limited to SEQ ID NO: 3) attached to N-terminal of the amino acid sequence.

In one aspect disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing inflammation, inflammatory conditions, autoimmune diseases, and/or lymphoproliferative disorders, wherein the biomimetic cell-penetrating peptide further comprises a 6 amino acid long peptide at C-terminus (such as, for example, the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21).

In one aspect, the treatment of the inflammation, inflammatory conditions, autoimmune diseases, and/or lymphoproliferative disorders can include administration of a peptide. For example, disclosed herein are methods of treating, inhibiting, reducing, decreasing, ameliorating, and/or preventing inflammation, inflammatory conditions, autoimmune diseases, and/or lymphoproliferative disorders in a subject comprising administering to the subject VGN37 peptide.

Methods of Inducing Apoptosis

It is understood that one mechanism of action for the disclosed biomimetic cell-penetrating peptides is the induction of apoptosis in target cells. Thus, in one aspect, disclosed herein are methods of inducing apoptosis and differentiation in cancer cells (such as, for

example, cancer cells derived from a cancer selected from primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer or from a cancer cell line including, but not limited to U937 cells, BC3 cells, or BCBL-1 cells) comprising contacting the cancer cells with any of the biomimetic cell-penetrating peptides disclosed herein, wherein the biomimetic cell-penetrating peptide reduces occupancy of CHD4 on promoters of genes, thus increasing accessibility of transcription factors (including, but not limited to IRF4, KLF6, FOSB, JUN, and/or JUND) and upregulating genes (such as, for example, BRM, SMARCE1, p21, MYADM, RGS2, CCL3, PPP1R15A, Klf6, CD163, CD206, CD38, CD19, TP53 and/or CHD4) whose expression is related to cell differentiation and apoptosis. For example, disclosed herein are methods of inducing apoptosis and differentiation in cancer cells comprising contacting cancer cells (such as, for example, cancer cells derived from a cancer selected from primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer or from a cancer cell line including, but not limited to U937 cells, BC3 cells, or BCBL-1 cells) with a biomimetic cell-penetrating peptide (such as, for example, the peptide as set forth in SEQ ID NO: 1 or a variant thereof including, but not limited to a biomimetic cell-penetrating peptide comprising a histadine at amino acid residue 3 of SEQ ID NO:1), wherein the biomimetic cell-penetrating peptide reduces occupancy of Chromodomain Helicase DNA binding protein 4 (CHD4) on promoters of genes, thus increasing accessibility of transcription factors (including, but not limited to IRF4, KLF6, FOSB, JUN, and/or JUND) and upregulating genes (such as, for example, BRM, SMARCE1, p21, MYADM, RGS2, CCL3, PPP1R15A, Klf6, CD163, CD206, CD38, CD19, TP53 and/or CHD4) whose expression is related to cell differentiation and apoptosis. In some aspects, the amino acid sequence is 10-40 amino acids in length. In some aspects, the biomimetic cell-penetrating peptides cleave one or more caspase cleavage sites on CHD4.

In one aspect, disclosed herein are methods of inducing apoptosis, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV). In some aspects, the amino acid sequence of the biomimetic cell-penetrating peptide is derived from a binding interface between LANA and CHD4.

Also disclosed herein are methods of inducing apoptosis, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises between amino acid residues 939 and 1042 of LANA protein. For example, disclosed herein are methods of inducing apoptosis, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73) (such as, for example, SEQ ID NO: 1).

In one aspect disclosed herein are methods of inducing apoptosis, wherein the biomimetic cell-penetrating peptide further comprises a cell-penetrating peptide (CPP) sequence (such as for example, a Trans-Activator of Transcription peptide (TAT) including, but not limited to SEQ ID NO: 3) attached to N-terminal of the amino acid sequence.

Also disclosed herein are methods of inducing apoptosis, wherein the biomimetic cell-penetrating peptide further comprises a 6 amino acid long peptide at C-terminus (such as, for example, the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21).

In some embodiments, macrophage (e.g., CD163, CD206) and dendritic cell (CD11c) markers increased on the cell surface of VGN73-pretreated cells (Fig. 5d). VGN73 treatment reduced occupancy of CHD4 on promoters, which presumably increased accessibility of activated transcriptional factors to upregulate gene expression. In other embodiments, the results showed that KLF6, MYADM, FOSB, RGS2, JUN, JUND, CCL3, and PPP1R15A are upregulated genes other than CHD4 in VGN73 treated cancer cells.

It will be apparent to those skilled in the art that various modifications and variations can be made in the present disclosure without departing from the scope or spirit of the invention. Other embodiments of the disclosure will be apparent to those skilled in the art from consideration of the specification and practice of the methods disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

EXAMPLES

The following examples are set forth below to illustrate the compositions, devices, methods, and results according to the disclosed subject matter. These examples are not intended to be inclusive of all aspects of the subject matter disclosed herein, but rather to illustrate

representative methods and results. These examples are not intended to exclude equivalents and variations of the present invention which are apparent to one skilled in the art.

Example 1: Identification of CHD4 interacting peptide from KSHV LANA protein.

The proteomics study identified that CHD4 interacts with LANA and co-occupied both host and viral chromatins. Biochemical mapping studies further showed that the amino acid sequence between 870 and 1042 of LANA was responsible for the interaction (Fig. 1a). The interaction domain was located within relatively well-structured domains, even though LANA contains a very large intrinsically disordered domain at the acidic repeat region (Fig. 1a). To narrow down the interaction surface with CHD4, a series of overlapping N-terminal biotinylated peptides were prepared (Fig. 1b), and peptide pull-down studies were performed. The results showed that the amino acid sequences between 939 and 961 (#6, Fig. 1c) and between 995 and 1042 (#9 and10, Fig 1c) were able to precipitate purified Flag-CHD4 protein. The peptide with LANA amino acids 939-961 (#6, Fig. 1c) was found to have no measurable biological activity following MTT assay (data not shown), and therefore was not studied further.

LANA C-terminal crystal structure, were visualized to find the position of the peptide sequence responsible for the CHD4 interaction. The results showed that the CHD4 interaction surface is located between the DNA binding interface and the LANA homo dimerization domain (Fig. 1d), suggesting that CHD4 may be recruited very close to chromatin. If the interaction with CHD4 is important for the LANA function, this amino acid sequence might be evolutionally conserved in the gamma-herpesviruses. The homologous proteins from other gamma herpesviruses were therefore aligned with KSHV LANA. The alignment indeed showed highly conserved tryptophan, tyrosine, and leucine, which are next to two charged amino acids (arginine or lysine) (Fig. 1e). The C-terminal basic amino acids are predicted to contact DNA. Highly hydrophobic or aromatic residues were also conserved among gamma herpesvirus functional homologs. Based on consensus motifs, we synthesized a small peptide, and attached a cell-penetrating peptide (TAT) to introduce the small peptide into cells. The study designed the peptide with an amino acid change of the LANA protein sequence, which makes it less hydrophobic for water solubility. To increase the half-life of the peptide, D-amino acids at the N-terminus was included, which has been shown to prevent rapid degradation by host cell enzymes. The final design of the cell-penetrating LANA peptide is shown in Fig. 1e. It is found that the

peptide has cancer cell-killing activity, therefore, the peptide is named VGN73 (Virus de Gann wo Naosu ORF73), which means, in Japanese, utilizing virus's wisdom to combat cancers.

Example 2: Characterization of the LANA peptide.

Peptide binding sites on the CHD4 domain and affinity to further confirm the interaction were measured Multiple CHD4 deletion proteins were expressed and purified from recombinant baculovirus-infected Sf9 cells with Flag-agarose beads (Fig. 7a). Biotinylated peptides (1 μM) were coated on an ELISA plate and increasing concentrations of recombinant proteins were incubated. The biotinylated peptide bound to recombinant CHD4 that corresponds 1-512, 363-1245, or the full-length CHD4, while the peptide lacking the PHD domain failed to bind recombinant proteins (Fig. 2a). The results suggested that the peptide may bind to the CHD4 PHD domain, which is known to interact with the acetylated H3 histone tail. Binding affinity to the full-length CHD4 was also determined with Bio-Layer Interferometry (BLI). To demonstrate the specificity of the interaction, we also generated a negative control peptide, which contains three amino acid changes at conserved amino acids (Fig. 2b). The N-biotinylated VGN73 or negative control peptides were first immobilized on a streptavidin-coated chip, and sequentially diluted full-length Flag-CHD4 proteins were applied. The interaction was then detected with interferometry. The results showed that VGN73, but not negative control or TAT alone, binds to CHD4, and the affinity of the interaction was calculated to be approximately 13.5 nM. These results also suggested that the interaction is sequence-specific because conserved amino acid substitution led to lose of the interaction (Fig. 2c).

Since the position of VGN73 is located next to the LANA homo dimerization domain, we next asked if the presence of the VGN73 peptide could influence CHD4-LANA interactions. The mutant peptide or no peptide were used as negative controls. Two purified proteins (Fig. 7b) were first incubated in the presence or absence of peptides, and the protein complex was precipitated with an anti-LANA antibody, and interaction was probed with immunoblotting. The results showed that in the presence of VGN73, CHD4 interacted more with LANA, and a larger amount of LANA was also precipitated in the presence of peptide, suggesting that the peptide facilitates the interactions among LANA and CHD4 in vitro (Fig. 7b). Next this observation was examined in KSHV naturally-infected primary effusion lymphoma (PEL) cells. First, efficacies of cell penetration were confirmed by Cy5 labeled VGN73 or its negative control peptide. Both

peptides effectively penetrated cells within 60 minutes, and there were no noticeable differences in peptide distribution in cells (Fig. 2d). To evaluate if the peptide binds to CHD4 in tissue culture, we incubated cells with biotinylated peptides for 4 hours, and peptide and peptide interacting proteins were precipitated with streptavidin magnetic beads. The amount of peptide-bound CHD4 was then probed with a specific antibody. VGN73 could precipitate CHD4 more when we compare with overall background (Fig. 2e). Taken together, these results suggested that the small peptide isolated from LANA-CHD4 interaction bind CHD4 in vitro and in cells.

Example 3: VGN73 induces caspase-mediated CHD4 cleavage in PELs.

The biological effects of VGN73 on CHD4 and LANA in BC3 and BCBL-1 cells. These cells were incubated with IC50 concentration of the peptides. CHD4 and LANA were then stained with specific antibodies. The results showed that the intensity of the CHD4 signal was substantially lowered in BC3 cells treated with VGN73 (Fig. 3a), which correlated with a decreased amount of full length of CHD4 in total cell lysates (Fig. 3b). LANA also showed different migration patterns in VGN73-treated cells. While full-length CHD4 was decreased, the intensity of smaller forms of CHD4 was increased, suggesting VGN73 might induce CHD4 cleavage in the cells (Fig. 3b). The protein degradation was not seen with other SWI/SNF family proteins, BRM, SMARCE1, or a transcription factor IRF4 in total lysates (Fig. 8a). A pretreatment with Z-VAD-FMK, a pan-caspase inhibitor, but not a proteasome inhibitor, recovered full-length CHD4 (Fig. 3c). The results suggested that CHD4 is cleaved by a caspase in the presence of VGN73 but not mutant peptide. Interestingly, under low serum conditions, CHD4 cleavage was enhanced (Fig. 3b). The results suggested that the induction of cell stress further increased VGN73-mediated CHD4 cleavage, which indicates the involvement of apoptosis and/or autophagy.

Example 4: LANA peptide induces cell apoptosis and autophagy in leukemia cells.

CHD4 is overexpressed in multiple cancer types and, in some cases, CHD4 was suggested to be a driver of tumorigenesis. Cleavage of CHD4 protein mediated by VGN73 prompted to examine general therapeutic effects with cancer cell lines. Because VGN73 is derived from the KSHV protein sequence, VGN73 was first tested to inhibit primary effusion lymphoma (PEL) growth. PEL is a KSHV-associated B-cell lymphoma. Increasing amounts of

VGN73 were incubated, and cell viability was indirectly measured by MTT assays. The results showed that VGN73 inhibited PEL cell growth in a dose-dependent manner (Fig. 4a). Interestingly, other leukemic cancer cell lines are even more sensitive to VGN73, suggesting that the effects of cell killing are independent of KSHV infection. PEL cells that are infected with KSHV only, are less sensitive to the VGN73, compared with cell lines harboring the EBV genome (Raji, BC1). Two monocyte leukemia cells, THP-1 and U937 cells were the most sensitive to among the cell line tested. Interestingly, HaCaT cells, immortalized keratinocytes, are resistant to VGN73, and normal PBMCs are also relatively resistant compared with monocytic leukemia cell lines. Flow cytometry analyses with Annexin V or Autophagy Red showed that VGN73 induces cellular apoptosis and auto-phagosome formation (Fig. 4b, c). VGN73 treatment induced autophagy in BC3 cells similarly to rapamycin treatment, which was used as a positive control (Fig. 4c). Western blotting also confirmed increased levels of apoptosis and autophagy markers. Importantly, pretreatment with Z-VAD-FMK partially rescued BC3 and BCBL-1 from cell death, even though Z-VAD-FMK treatment alone reduced MTT conversion for more than 10% (Fig. 4d). These results demonstrated while the exact molecular mechanism of VGN73-mediated cancer cell killing remained elusive, VGN73 but not the mutant peptide strongly induces cancer cell death by triggering cellular apoptosis and autophagy, and these two pathways are perhaps mutually exclusive.

Example 5: Identification of direct targets of VGN73 with SLAM-seq

Two platforms for transcriptional analyses were employed to better understand the biological activity and molecular actions of VGN73. SLAM-seq analysis with three B-cell lines was first employed to isolate the direct VGN73 target genes. For direct target gene identification, control peptide and vehicle-treated cells were used as comparisons. The common up-regulated genes in the three cell lines were identified. The results showed that KLF6, MYADM, FOSB, RGS2, JUN, JUND, CCL3, and PPP1R15A are commonly upregulated among three cell lines (Fig. 9b). To reveal association with CHD4 occupancies, we next analyzed the CHD4 peak score at promoter regions of the identified direct targets of VGN73 with SLAM-seq. The results showed that promoters of upregulated gene by VGN73 were significantly more enriched with CHD4 than those of genes that transcription rate was not changed (Fig 9c). Furthermore, we also examined the occupancies of CHD4 on each promoter by qPCR with BCBL-1 cells. The BC3

cells were easily undergone to cell apoptosis and were difficult to perform CUT&RUN with VGN73 treating cells, because the cell apoptosis also produces fragmented DNAs. A non-CHD4 recruited region and control IgG were used as negative controls. The results confirmed that the promoter regions of VGN73-upregulated genes showed enrichment of CHD4 (Fig. 9d) and the VGN73 incubation decreased the occupancies of CHD4 from each promoter, except for Klf6 promoter (Fig. 9e). These results suggested that VGN73 treatment decreases CHD4 occupancies that unleashed primarily cellular immediate-early gene expression. The Gene Set Enrichment Analysis (GSEA) of the VGN73 target genes showed that skeletal muscle cell differentiation related genes in Raji and BCBL-1 cells, while BC3 cells did not show statistically significant specific pathway enrichment.

Example 6: VGN73 facilitates transcription reprogramming in U937.

CHD4 is known to regulate cell lineage by restricting cell differentiation Accordingly, we next specifically examined the effects of VGN73 on the regulation of cell differentiation with the U937 monocyte-macrophage model with RNA-sequencing. The U937 cell model was selected because (i) U937 cells were one of the most sensitive cell lines to the VGN73 incubation in MTT assays, and (ii) we frequently observed that U937 displayed the characteristics of macrophage differentiation such as increased cell adhesion, and large cytoplasm volume during experiments (Fig. 10). Figure 5a depicted our experimental scheme. Phorbol-12-myristate-13-acetate (PMA), an analog of diacyl-glycerol, was used as a macrophage differentiation inducer, and it was monitored if VGN73 pretreatment can facilitate the transition. U937 cells were pre-treated with 4 or 8 μM VGN73 for 24 hours, and PMA was added thereafter for another 24 hours. VGN73 or PMA treatment alone was used as a comparison. Treatment with VGN73 or PMA alone displayed similar gene expression patterns, with the exception of highly selective gene sets (Fig. 5b with asterisk), pretreating with VGN73 significantly increased the repertoire of PMA activated genes. A closer look at the VGN73 pre-treated samples showed substantially increased number of overall activated genes that include genes involved in macrophage differentiation and inflammatory cytokines, when compared with PMA treatment alone, without VGN73-pre-treatment (Fig. 5b, c). Flow cytometry independently confirmed increased macrophage (e.g., CD163, CD206) and dendritic cell (CD11c) markers on the cell surface of VGN73-pretreated U937 cells (Fig. 5d). These transcriptomics and genomics studies suggested that VGN73

treatment reduced occupancy of CHD4 on promoters, which presumably increased accessibility of activated transcriptional factors to upregulate gene expression.

Example 7: VGN73 prevents PEL cell growth in the xenograft model.

Induction of cell differentiation in immature cancer cells is a major therapeutic strategy, called differentiation therapy, to reduce tumorigenesis. Terminal differentiation of cancer stem cells or the conversion into non-stem cells increases the sensitivity of tumors to conventional anticancer treatments and also prevents metastasis. Perhaps due to the function of CHD4 in restricting cell differentiation, CHD4 is frequently found overexpressed in many cancer types. Thus examined the utility of VGN73 as a therapeutic drug in a human xenograft mouse model.

The maximum tolerated dose (MTD) in mice by injecting increasing dosages of VGN73 intraperitoneally (IP) was examined. The results found that a VGN73 dose of more than 15 mg/kg showed decreased overall mobility, a sign of discomfort, and one of eight mice died the next day (Fig. 11a). Accordingly, we considered 12.5 mg/kg as the MTD and decided to use 10 mg/kg every other day as a treatment schedule. With this treatment schedule, there was no decreased body weight over three weeks, and blood chemistry analysis showed no sign of organ damage (Fig. 11b).

With a PEL mouse xenograft model, we inoculated 5 x 10^6 luciferase-expressing BCBL-1 cells IP. Two days after the PEL inoculation, IP treatment with VGN73, mutant peptide, or vehicle (PBS) (10 mg/kg/every other day) was started. PEL cell growth was monitored with bioluminescence imaging on days 14 and 19. The results showed that BCBL-1 growth was substantially inhibited in the VGN73-treated group. One of the mutant peptide-treated mice did not show significant luciferase activity (Fig. 6a) and we later found that the BCBL-1 tumor primarily grew subcutaneously. Increased body weight as result of BCBL-1 ascites fluid, which is an indication of BCBL-1 cell growth, was also consistent with the imaging analyses (Fig. 6a and 6b). At Day 19, ascites fluid was collected and the total number of PEL cells in the fluid was counted. Because very little ascites fluid was produced in the VGN73-treated group, we recovered cells by applying PBS and collected residual cells for counting. The results directly confirmed the inhibition of PEL growth in VGN73-treated mice (Fig. 6c). Collected PEL xenograft tumor cells were then stained with CHD4 and LANA. The results showed that VGN73 treatment reduced both CHD4 and LANA signals in BCBL-1 cells in mice, which is consistent

with in vitro studies (Fig. 3a & 6d). Finally, gene expression of cell surface markers were examined for recovered PEL cells from the ascites. The results showed that CD38, an immature cell surface marker and indication of potent proliferative capability, was downregulated in VGN73-treated cells (Fig. 6e). On the other hand, CD19, a selective B cell differentiation marker, was slightly increased, although it showed significant variations within the treated mice and did not meet statistical significance (Fig. 6e). Taken together, VGN73 is a biologically active peptide, which induces CHD4 cleavage in vitro and prevents PEL growth in xenograft mouse model, perhaps by facilitating terminal cell differentiation in immature cancer cells, with no measurable side effects in mice.

Discussion

This study utilizes the fact that viruses depend upon host cell machineries for producing infectious progenies. Accordingly, viral proteins have acquired the ability to control many cellular functions under continuous pressure from host defense mechanisms during millions of years of co-evolution Therefore, it is not surprising to find a functional small peptide derived from a viral protein sequence, which has strong biological activity with high affinity to host cell proteins.

VGN73 identified herein is a 17 amino acids length peptide, and is derived from the KSHV LANA sequence. During screening, we noticed that the C-terminal 6 amino acids (PYGLKK SEQ ID NO: 21) were not essential for leukemic cell-killing activity, but the addition of the segment increases the cell-killing effects approximately 2-fold. Accordingly, in this study, we used the longer version of the VGN73. In addition, while an original KSHV LANA protein sequence has phenylalanine (F) at the third position, changing to a less hydrophobic histidine, which is conserved in other gamma-herpesvirus homologs, increased peptide solubility in water. Additional modifications such as the inclusion of non-natural amino acids at the C-terminus for increasing peptide stability would likely to increase the biological activity of the VGN73.

While we understand that in vitro peptide binding does not prove that VGN73 only targets CHD4 in vivo, VGN73 did indeed bind to purified CHD4 with ~14 nM KD, which is a relatively high affinity when the size of the peptide is considered. Tissue culture studies also showed that VGN73 effectively induced caspase-mediated CHD4 cleavages, while it had little

effect on other cellular proteins such as actin and other nuclear proteins, BRM, SMARCE1 and IRF4. CHD4 indeed contains DXXD and VEXD motifs that can be cleaved by caspases. We also could not rule out a contribution of inducing cell stress responses by VGN73, which would also explain caspase activation; however, mutant peptides with three amino acids substitution, which equally penetrated cultured cells, did not have such effects. Specific and narrowly targeted gene activation with VGN73 as defined with SLAM-seq also demonstrates the specificity of its action. In addition, these highly upregulated target gene promoters were indeed occupied by CHD4, and VGN73 treatment reduced its occupancies. Based on these results, we propose that VGN73's biological function is, at least in part, through inhibition of CHD4. Further large-scale proteomics studies could provide additional details of the molecular actions of VGN73.

Among selected cancer cell types, we found that the monocytic cell lines (U937, THP-1) were the most sensitive to the VGN73, and that PEL cell lines expressing the KSHV LANA protein were relatively resistant. We also noticed that the presence of EBV infection seems to sensitize cells to VGN73 treatment. Non-cancerous cells, such as HaCaT immortalized keratinocytes and PBMCs were not very sensitive to VGN73. We speculate the sensitivity to VGN73 is promoted in cells that maintain an undifferentiated status with CHD4 by restricting promoter activation. Cells that are already differentiated (e.g., keratinocytes, peripheral blood mononuclear cells) might be relatively unaffected by the treatment. This may also explain the minimal side effects in mice. Consistent with this, it has been reported that CD34 positive AML is highly sensitive to CHD4 depletion.

The identification of direct target genes by SLAM-seq suggested that treating with VGN73 preferentially released immediate-early transcription factor expression. These immediate-early genes are poised to be expressed in response to a variety of external stimuli, and VGN73 treatment appears to release promoters from such transcription repression. This is similar to what we have seen in KSHV latent chromosomes with CHD4 knock-down, and VGN73 incubation indeed increased viral immediate-early gene expression (Fig. 8b). Specific to the BC3 cells, we see the induction of TP53 gene expression; this partially explains the activation of a number of apoptosis-related genes and having distinct VGN73-mediated transcription profile (S-Figure 3b). BC3 is known to encode wild-type TP53, while BCBL-1 cells and Raji cells have mutations in TP53.

To our surprise, pre-incubation with VGN73 resulted in marked alteration in the set of PMA-activated genes contained in the top 500 most variable genes. We speculate that such large changes may be attributed to activation of dormant enhancers that are restricted by the CHD4 complex. Further studies with HiChIP (HiC and chromatin immunoprecipitation) should clarify the effects on enhancer-promoter interactions with VGN73.

Analysis of the U937 cell differentiation model by RNA-seq indicated that VGN73 enhanced differentiation from monocytes to macrophages and/or dendric cells. Moreover, in a BCBL-1 xenograft mouse model, an immature marker which relates to cell proliferation was decreased on BCBL-1 cells recovered from ascites of mice treated with VGN73. Cancer cell plasticity is known to drive cancer progression and cancer stemness to reversibly convert their identity associated with drug resistance. The concept of cell differentiation therapy is derived from the fact that terminal cell differentiation irreversibly changes the phenotype and makes the cancer cell sensitive to conventional chemotherapeutic drugs and/or drives the cells into senescence. Differentiation therapy is based on the great success experienced in acute promyelocytic leukemia (APL) APL became highly curable with the combination of retinoic acid and arsenic treatment, which stimulates oncoprotein degradation to induce terminal differentiation of leukemic cells into granulocytes. Similarly, many new therapies for myeloid malignancies entering clinical practice, including epigenetic therapies (e.g., 5-azacitidine), isocitrate dehydrogenase inhibitors, FMS-like tyrosine kinase 3 inhibitors, and lenalidomide for deletion 5q (del5q) myelodysplastic syndrome were found to induce differentiation, which is considered to be a major mechanism by which several of these function as cancer therapeutics. CHD4 is a critical factor to restrict cell differentiation and known to maintain embryonic stem cell identity by controlling differentiation-associated genes. In our study, MYADM (myeloid associated differentiation marker), whose promoter region was enriched for CHD4 occupancy and was the most affected by VGN73 treatment. In addition, considering that cell differentiation renders cancer cells to be sensitive to conventional chemotherapy, VGN73 is likely to synergize with some cancer drugs.

In summary, our study demonstrated that viral proteins are a unique starting material as the basis for designing therapeutics directed at attenuating cellular protein function(s). Future studies will likely increase the utilities of the VGN73 peptide by targeting specific cell types as

antibody-conjugates or may be used as a tool to enhance cell differentiation of inducible pluripotent stem cells in combination with specific stimuli.

Materials and Methods

Cell culture

BC1, BC3, BCBL-1, Raji, THP-1, U937, 293T, and HaCaT cell lines were obtained from ATCC. Leukoreduction system chambers (LRS) from healthy donors were purchased from Vitalant. Peripheral blood mononuclear cells (PBMCs) were prepared by a standard Ficoll gradient method. Cell lines were cultured in RPMI 1640 medium supplemented with 15% FBS, antibiotics, and L-glutamine, or IMDM medium supplemented with 15% FBS, antibiotics, and L-glutamine for BC3, or DMEM medium supplemented with 10% FBS, antibiotics, and L-glutamine for 293T and HaCaT cell lines.

Enzyme linked immunosorbent assays (ELISA)

To evaluate the VGN73 binding region of CHD4, ELISA was carried out. Biotin-conjugated VGN73 was diluted with phosphate buffered saline (PBS) (pH = 7.0) to a final concentration of 1 μM. Each well of a 96-well flat-bottom streptavidin-coated microplate (bioWORLD, Dublin, OH, USA) was coated with 100 μL of biotin-conjugated VGN73 in PBS overnight at 4 °C. Each region of flag conjugated-CHD4 was tested at the following concentrations: 3.1, 6.3. 12.5, 25, 50,100 nM, and vehicle only (0 nM). The wells were washed three times with Tris-buffered saline containing 0.1 % Tween-20 (TBS-T) with 0.1% bovine serum albumin (BSA). Blocking buffer (5% BSA in TBS-T) was added, and the plate was incubated at 37 °C for 1 hour. After washing the wells three times as described above, 200 μL of different concentrations of each region of flag conjugated-CHD4 diluted with 0.1% BSA in TBS-T were applied to each well. The plate was incubated at room temperature for 2 hours and washed 5 times with TBS-T. Bound proteins were probed with 200 μL of streptavidin-horseradish peroxidase (HRP) conjugate (ThermoFisher Scientific, Waltham, MA, USA), which was diluted 1:20,000 in 0.1% BSA in TBS-T and applied to each well. After incubation at room temperature for 1.5 hours and washing 5 times with TBS-T, color development with the TMB Substrate (ThermoFisher Scientific) was performed according to the manufacturer's protocol. Optical densities (ODs) were measured at 450 nm with a Benchmark Plus Microplate

Spectrophotometer (Bio-Rad, Hercules, CA, USA). The assays were performed in triplicate wells. Each absorbance was calculated by subtracting absorbance of the blank from the measured value in each experimental well.

Biolayer interferometry (BLI)

To evaluate of binding kinetics of VGN73 and mutant peptide to CHD4, assays were carried out by biolayer interferometry (BLI) on Octet-Red 384 (ForteBio, CA, USA) at 30 °C with shaking at 1,000 RPM. Streptavidin (SA) tips (Sartorius, Goettingen, Germany) were dipped in 200 µL of biotinylated peptide (VGN73, mutant or TAT) solution (1 µM in 1x kinetic buffer) for the loading step. The tips loaded with peptide were then sampled with CHD4 at various concentrations in 1x kinetic buffer (Sartorius) to obtain the association curve. TAT was used as a reference for background subtraction. After association, the tips were dipped back into 1x kinetic buffer to obtain the dissociation curve. Binding kinetics were evaluated using a 1:1 binding model by ForteBio Data Analysis 8.1 software to obtain the dissociation constant KD.

Peptide-pulldown assay

Flag tagged CHD4 protein and Flag tagged LANA protein were prepared with recombinant baculoviruses. A series of overlapping N-terminal biotinylated LANA peptides were synthesized by GeneScript USA Inc. Recombinant proteins were isolated in the presence of 500 mM NaCl and 2% glycerol with affinity purification. Flag-CHD4 protein (100 nM) was incubated with 1 µg of biotinylated LANA peptides in 200 µL binding buffer (20 mM HEPES [pH 7.9], 150 mM NaCl, 1 mM EDTA, 4 mM MgCl2, 1 mM dithiothreitol, 0.02% NP-40, 10% glycerol supplemented with 1 mg/mL BSA, and protease inhibitor cocktail) and the interacting CHD4 was precipitated with 10 µL of streptavidin-coated magnetic beads. To evaluate VGN73 effects on the interaction between CHD4 and LANA in vitro, Flag-LANA protein (200 nM) and Flag-CHD4 protein (200 nM) were incubated with VGN73 (1 or 10 µM) or mutant peptide (1 or 10 µM) in 200 µL binding buffer. The anti-LANA antibody was incubated at 1:100 dilution for 1 hour at 4°C to form an immunocomplex. The immunocomplex was captured with 10 µL of protein G magnetic beads (Thermo Fisher) (Fig. 7a).

Immunoprecipitation

For examining peptide binding in cells, BC3 cells were incubated with biotinylated VGN73 (10 μM), biotinylated mutant peptide (10 μM) or biotinylated TAT peptide (10 μM) for 4hours. Cells were washed with cold PBS 3 times followed by lysed with RIPA lysis buffer (150 mM NaCl, 5 mM EDTA (pH 8.0), 50 mM Tris (pH 8.0), 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). Lysates were quantified by BCA protein assay kit (Thermo Fisher). The peptides were precipitated with streptavidin-coated magnetic beads (Thermo Fisher). Briefly, 50 μL magnetic beads/sample were pre-washed with Tris-buffered saline containing 0.1 % Tween-20 (TBS-T) 3 times. A total of 1.5 mg of whole cell lysate was incubated with streptavidin beads overnight at 4 °C with rotation. The beads were collected using a magnetic stand and washed 3 times with TBS-T according to the manufacturer's protocol. Protein complex on the beads were eluted in 40μL of SDS-PAGE sample buffer by boiling for 15 minutes. The respective protein band was confirmed by immunoblotting with respective antibodies.

In vitro cell treatment

With VGN73. BC3 cells and BCBL-1 cells were treated with VGN73 at the IC50 concentration for 24 hours. These cells were also treated with VGN73. BC3 cells were pretreated with pan-caspase inhibitor, Z-VAD-FMK (50 μM) or proteasome inhibitor, bortezomib (25 nM) for 1hour, followed by treated with VGN73 (18 μM) for 8 hours. BC3 cells were pretreated with lysosome inhibitor, chloroquine (7.5 μM) for 1 hour, followed by treatment with VGN73 (18 μM) for 24 hours.

U937 cells were pretreated with 0, 4 or 8 μM of VGN73 for 24 hours, followed by stimulation with PMA (10 ng/ml) for another 24 hours. To evaluate molecular action of VGN73, total RNA-sequencing analysis in the U937 monocyte differentiation model was carried out. For flow cytometry or IFA, U937 cells were pretreated with 4 μM VGN73 for 2 days, followed by a stimulation with 10 ng/ml PMA for an additional 3 days for induction of cell differentiation. Cells were treated with VGN73 for 2 days or with PMA for 3 days served as controls.

Immunofluorescence

Staining analysis (IFA) and imaging analysis. Cy5-labeled peptides were used to track subcellular localization. BC3 cells were treated with 10 μM Cy5- labeled VGN73 or mutant peptide for 1 minute and 60 minutes. To evaluate VGN73 effects on CHD4 and LANA in tissue

culture, IFA was carried out. BC3 cells were treated with VGN73 at the IC50 concentration or mutant peptide for 24 hours. The cells were washed with PBS twice after peptide treatment. The cells were fixed with 4% formaldehyde and permeabilized with 0.2% Triton X-100. The cells were stained with anti-CHD4 rabbit monoclonal antibody (Cell Signaling, MA, USA, D4B7; 1:100) and anti-LANA rat monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA, clone LN53; 1:200), followed by Alexa 555-anti rat IgG and Alexa 647-anti rabbit IgG (Thermo Fisher). Nuclei were counterstained with 1 μg/ml of Hoechst 33342 (Thermo Fisher). The labeled cells were observed with a Keyence BZ-X710 fluorescence microscope (Keyence, Osaka, Japan) with standard DAPI, GFP, TRITC, and Cy5 filter sets (Chroma Technologies, Bellows Falls, VT, USA). The exposure settings and image acquisition protocols were fixed in each imaging study to enable quantitative analysis. Mean LANA and CHD4 fluorescence (measured in arbitrary units, or A.U.) per cell was subsequently calculated using onboard software and/or NIH Image.

Western blotting.

Cells were washed twice with PBS and lysed with protein lysis buffer (50 mM Tris-HCl [pH 6.8], 2% SDS, 10% glycerol). The lysates were boiled in SDS-PAGE loading buffer and subjected to SDS-PAGE, and subsequently transferred to a polyvinylidene fluoride membrane (Sigma-Aldrich) using a semidry transfer apparatus (Bio-Rad, Hercules, CA, USA). Final dilutions of the primary antibodies were 1:2,000 for anti-β-actin mouse antibody (Sigma-Aldrich) and 1:1,000 for the anti-LANA rat antibody (Sigma-Aldrich), anti-Flag M2 mouse antibody (Sigma-Aldrich), anti-CHD4 rabbit (Cell Signaling), anti-cleaved caspase 3 rabbit (Cell Signaling), anti-LC3 rabbit (Sigma-Aldrich), anti-BRM rabbit (Cell Signaling), anti-SMARCE1 rabbit (Cell Signaling), and anti-IRF4 rabbit antibodies (Cell Signaling). The streptavidin-HRP conjugate was used at 1:3000 dilution.

RT-qPCR.

BC3 cells treated with VGN73 and BCBL-1 cells from ascites of xenografted mice were used for RT-qPCR. Cells were washed with PBS two times. Total RNA was isolated using the Quick-RNA miniprep kit (Zymo Research, Irvine, CA, USA). First-strand cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher,

Waltham, MA USA). Gene expression was analyzed by real-time qPCR using specific primers. The 18S ribosomal RNA was used as an internal standard to normalize gene expression.

Cell viability assay.

The cytotoxic activity of VGN73 and mutant peptide were measured using the MTT assay. BC1, BC3, BCBL1, Raji, THP-1, U937, 293T, HaCaT cells, and PBMC from a healthy donor were treated with VGN73 or mutant peptide at various concentrations (0, 4, 8, 16, 32, 64 μM) for 24hours. These cells were incubated with MTT (0.5mg/mL) at 37 °C for 4 hours, and lysed with 10% SDS containing lysis buffer for overnight. The optical densities (ODs) were measured at 570 nm with a Benchmark Plus Microplate Spectrophotometer (Bio-Rad). For the caspase inhibition study, BC3 and BCBL-1 cells were pretreated with 40 μM of the Z-VAD-FMK pan caspase inhibitor for 1 hour before incubating with VGN73 for 4 hours.

Flow cytometry.

BC3 cells were treated with 18 μM of VGN73 for 0, 1, 4 and 24 hours. Apoptosis was evaluated after intracellular staining with annexin-V-FITC and 7-AAD (Biolegend) according to the manufacture's protocol. Apoptosis (%) was determined as AnnexinV+ 7AAD- population. Autophagy induction by treating with 18 μM of VGN73 for 24 hours was evaluated with intracellular staining with autophagy probe red (Bio-Rad, Hercules, CA, USA) according to the manufacture's protocol. BC3 cells were also treated with 50 nM rapamycin or cells were cultured under serum starvation as positive controls. U937 cells were treated with 4 μM VGN73 for 5 days, 10 ng/ml PMA for 3 days, or pretreated with 4 μM VGN73 for 2 days, then stimulated with 10 ng/ml PMA for 3 days. Cell differentiation to dendritic cells and macrophages were evaluated after staining with CD11C, CD206 or CD163. Flow cytometry was conducted by using a BD Acuri instrument (BD Biosciences) and the data was analyzed with FlowJo v10.8.0 (Tree Star) software.

RNA-sequencing.

Indexed, stranded mRNA-seq libraries were prepared from total RNA (100 ng) using the KAPA Stranded mRNA-Seq kit (Roche) according to the manufacturer's standard protocol. Libraries were pooled and multiplex sequenced on an Illumina NovaSeq 6000 System (150-bp,

paired-end, >30 x 10^6 reads per sample). RNA-Seq data was analyzed using a Salmon-tximport-DESeq2 pipeline. Raw sequence reads (FASTQ format) were mapped to the reference human genome assembly (GRCh38/hg38, GENCODE release 36) and quantified with Salmon. Gene-level counts were imported with tximport and differential expression analysis was performed with DEseq2. Normalized read counts were utilized for principal component analysis (PCA) and variance analysis, the latter of which was followed by hierarchical clustering and heatmap visualization.

Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

CUT&RUN was performed essentially by following the online protocol developed by Dr. Henikoff's lab with a few modifications to fit our needs. Cells were washed with PBS and wash buffer [20 mM HEPES-KOH pH 7.5, 150 mM NaCl, 0.5 mM Spermidine (Sigma), and proteinase inhibitor (Roche)]. After removing the wash buffer, cells were captured on magnetic concanavalin A (ConA) beads (Polysciences, PA, USA) in the presence of CaCl2. Beads/cells complexes were washed three times with digitonin wash buffer (0.02% digitonin, 20mM HEPES-KOH pH 7.5, 150mM NaCl, 0.5mM Spermidine and 1x proteinase inhibitor), aliquoted, and incubated with anti-CHD4 antibody or human IgG in 250 µL volume. The antibody and concentration used in this study was: rabbit monoclonal anti-CHD4 (Cell Signaling, D4B7; 1:50). After incubation, unbound antibody was removed by washing with digitonin wash buffer three times. Beads were then incubated with recombinant Protein A/G–Micrococcal Nuclease (pAG-MNase) purified from E.coli in 250 µL digitonin wash buffer at 1.0 µg/mL final concentration for 1 hour at 4 °C with rotation. Unbound pAG-MNase was removed by washing with digitonin wash buffer three times. Pre-chilled digitonin wash buffer containing 2mM CaCl2 (200 µL) was added to the beads and incubated on ice for 30 minutes. The pAG-MNase digestion was halted by the addition of 200 µL 2× STOP solution (340mM NaCl, 20mM EDTA, 4mM EGTA, 50 µg/mL RNase A, 50 µg/mL glycogen). The beads were incubated with shaking at 37 °C for 10 minutes in a tube shaker at 500 rpm to release digested DNA fragments from the insoluble nuclear chromatin. The supernatant was collected after centrifugation (16,000×g for 5 minutes at 4 °C) and placed on a magnetic stand. DNA was extracted using the NucleoSpin Gel & PCR kit (Takara Bio, Kusatsu, Shiga, Japan). Sequencing libraries were then prepared from 3 ng of CUT&RUN DNA with the Kapa HyperPrep Kit (Roche) according to the manufacturer's

standard protocol. Libraries were multiplex sequenced (2 × 150bp, paired-end) on an Illumina HiSeq 4000 sequencing system to yield ~15 million mapped reads per sample. The extracted DNA also was used to examine enrichment at selected genomic regions by qPCR (CUT&RUN-qPCR). All PCR data was normalized by using 10% of the input reaction before CUT&RUN. As the negative control, non-CHD4 bound region and control IgG were used. CUT&RUN-qPCR data were analyzed by using the 2-ΔΔ CT method and compared with the negative control.

SLAM-sequencing.

SLAM-seq was performed using the SLAMseq Kinetics Kit (Lexogen GmbH, Vienna, Austria) according to the manufacturer's standard protocol. Briefly, biological replicate cultures of BC3, BCBL-1 or Raji cells were incubated with VGN73 peptide (at each IC50 concentration) for 30 minutes. Subsequently, 4-Thiouridine (s4U; 300 μM) was added to the culture media and the cells incubated for 1.5 hours in order to label newly synthesized RNA. Total RNA was isolated and then the 4-thiol groups in the s4Uracil-labeled transcripts were alkylated with iodoacetamide (IAA). QuantSeq 3' mRNA-Seq (FWD) (Lexogen, Inc.) Illumina-compatible, indexed sequencing libraries were prepared from alkylated RNA samples (100 ng) according to the manufacturer's protocol for oligo(dT)-primed first strand cDNA synthesis, random-primed second strand synthesis, and library amplification. Libraries were multiplex sequenced (1 × 100 bp, single read) on an Illumina HiSeq 4000 sequencing system. SLAM-Seq datasets were analyzed using the T > C conversion-aware SLAMDUNK (Digital Unmasking of Nucleotide conversion-containing k-mers) pipeline utilizing the default parameters. Briefly, nucleotide conversion-aware read mapping of adapter- and poly(A)-trimmed sequences to the human GRCh38/hg38 reference genome assembly was performed with NextGenMap. Alignments were filtered for those with a minimum identity of 95% and minimum of 50% of the read bases mapped. For multi-mappers, ambiguous reads and non-3' UTR alignments were discarded, while one read was randomly selected from multimappers aligned to the same 3' UTR. SNP calling (coverage cut-off of 10X and variant fraction cutoff of 0.8) with VarScan2 was performed in order to mask actual T > C SNPs. Non-SNP T > C conversion events were then counted and the fraction of labeled transcripts determined. All results were exported (i.e., tcount file) and used for downstream analyses, such as principal component analysis and differential expression analysis (DESeq2). For visualization, RefSeq IDs were converted to official gene symbols (refGene). The

resulting data were first filtered by Log2FC >= 1 and sorted by Padj from lowest to highest (<0.01). The resulting differentially-expressed genes in BC3, BCBL-1 and Raji were illustrated in a Venn diagram.

PEL xenografts.

All animal studies were conducted according to a UC Davis Institutional Animal Care and Use Committee (IACUC)-approved protocol. NRG (NOD.Cg-Rag1tm1Mom Il2rgtm1Wjl/SzJ) mouse breeding pairs were purchased from the Jackson Laboratory and the colony was maintained in-house. To evaluate the in vivo antitumor activity of VGN73, twenty-three to thirty-week-old female NRG mice were injected intraperitoneally (i.p.) with 5×10^6 BCBL-1 luciferase cells in 500 µL PBS. On day 2, mice were randomly assigned to PBS control, VGN73 (10 mg/kg), or mutant control peptide (10 mg/kg) groups. Treatments were administered by i.p. injection every other day for 18 days. Mice were monitored for PEL burden based on body weight increment. At day 14 and 19 post-xenograftment, mice were intraperitoneally injected with D-luciferin (2 mg) and imaged with Lago X. Images were analyzed using Aura ver 4.0.7. Mice were sacrificed and ascites fluid was recovered for analysis of ascites cell count, CHD4 and LANA protein expression by IFA and gene expression of CD38 and CD19 by RT-qPCR.

Visualization of KSHV LANA C-terminal domain.

The dimer of LANA C-terminal domain (PDB; 2YPY), and the structure determined by the X-ray crystal structure were visualized and highlighted with the molecular visualization open-source software program PyMOL (Ver 2.5.0).

Statistics and reproducibility

Results are shown as mean ± SD or median [interquartile range (IQR)], from at least three independent experiments. Data were analyzed using Wilcoxon signed-rank test. A value of $p < 0.05$ was defined as statistically significant. Statistical analyses were conducted using JMP version 13.2.1. For animal experiments, sample size is based on our previous study.

All patents, patent applications, and other publications, including GenBank Accession Numbers or similar sequence identification numbers, cited in this application are incorporated by reference in the entirety of their contents for all purposes.

SEQUENCES

1. **SEQ ID NO: 1 – VGN73 (biomimetic cell-penetrating peptide)**
{D-ARG}KKRR{ORN}RRR{BETA-ALA}WKHAVIFWGNDPYGLKK

2. **SEQ ID NO: 2 – Mutant VGN73 with cell-penetrating peptide**
{D-ARG}KKRR{ORN}RRR{BETA-ALA}AKHAVISAGNDPYGLKK

3. **SEQ ID NO: 3 – Cell-penetrating peptide (CPP) / TAT peptide**
{D-ARG}KKRR{ORN}RRR{BETA-ALA}

4. **SEQ ID NO: 4 – LANA Peptide #1; amino acids 872-894 from Kaposi's sarcoma-associated herpesvirus**
EQELEEVEEQEEQELEEVEEQEQ

5. **SEQ ID NO: 5 – LANA Peptide #2; amino acids 876-896 from Kaposi's sarcoma-associated herpesvirus**
EEVEEQEQQGVEQQEQETVEE

6. **SEQ ID NO: 6 – LANA Peptide #3; amino acids 892-914 from Kaposi's sarcoma-associated herpesvirus**
ETVEEPIILHGSSSEDEMEVDYP

7. **SEQ ID NO: 7 – LANA Peptide #4; amino acids 906-928 from Kaposi's sarcoma-associated herpesvirus**
EDEMEVDYPVVSTHEQIASSPPG

8. **SEQ ID NO: 8 – LANA Peptide #5; amino acids 922-945 from Kaposi's sarcoma-associated herpesvirus**
IASSPPGDNTPDDDPQPGPSREYR

9. **SEQ ID NO: 9 – LANA Peptide #6; amino acids 939-961 from Kaposi's sarcoma-associated herpesvirus**
GPSREYRYVLRTSPPHRPGVRMR

10. **SEQ ID NO: 10 – LANA Peptide #7; amino acids 961-981 from Kaposi's sarcoma-associated herpesvirus**
RRVPVTHPKKPHPRYQQPPVP

11. **SEQ ID NO: 11 – LANA Peptide #8; amino acids 977-1000 from Kaposi's sarcoma-associated herpesvirus**
QPPVPYRQIDDCPAKARPQHIFYR

12. **SEQ ID NO: 12 – LANA Peptide #9; amino acids 995-1020 from Kaposi's sarcoma-associated herpesvirus**
QHIFYRRFLGKDGRRDPKCQWKFAVI

13. **SEQ ID NO: 13 – LANA Peptide #10; amino acids 1016-1042 from Kaposi's sarcoma-associated herpesvirus**
KFAVIFWGNDPYGLKKLSQAFQFGGVK

14. **SEQ ID NO: 14 – Kaposi's sarcoma-associated herpesvirus LANA**
WKFAVIFWG---NDPYGLKK

15. **SEQ ID NO: 15 – LANA homolog from Macacine Gammaherpesvirus 5**
WRHGVIFCN---SDPYSLYR

16. **SEQ ID NO: 16 – LANA homolog from Retroperitoneal fibromatosis-associated herpesvirus**

WRHAAIFWT---PRPYPLKK

17. **SEQ ID NO: 17 – LANA homolog from Colobine gammaherpesvirus 1**

WKHAALFWN---PEPYPLKK

18. **SEQ ID NO: 18 – LANA homolog from Macacine Gammaherpesvirus 4**

WPFGVFVYG-PKTSCYNLRR

19. **SEQ ID NO: 19 – LANA homolog from Epstein-Barr Virus**

WVAGVFVYGGSKTSLYNLRR

20. **SEQ ID NO: 20 – LANA homolog from Saimiriine gammaherpesvirus 2**

WRHVVYFWG-PTSALYRLSK

21. **SEQ ID NO: 21 – LANA C-terminal amino acids 1-6**

PYGLKK

22. **SEQ ID NO: 22 – 18S rRNA Foward primer**

TTCGAACGTCTGCCCTATCAA

23. **SEQ ID NO: 23 – 18S rRNA Reverse Primer**

ATGGTAGGCACGGCGACTA

24. **SEQ ID NO: 24 – CHD4 Forward Primer**

GAGCGTATGCTCTTATGCCG

25. **SEQ ID NO: 25 – CHD4 Reverse Primer**

CCTCTTCCTCCTCCTCCACA

26. SEQ ID NO: 26 – LANA Forward Primer

ACTGAACACACGGACAACGG

27. SEQ ID NO: 27 – LANA Reverse Primer

CAGGTTCTCCCATCGACGA

28. SEQ ID NO: 28 – CD19 Forward Primer

ACCTCCTCGCCTCCTCTT

29. SEQ ID NO: 29 – CD19 Reverse Primer

CACAGCGTTATCTCCCTCTTCC

30. SEQ ID NO: 30 – CD38 Forward Primer

GATGCTTTCAAGGGTGCATTT

31. SEQ ID NO: 31 – CD38 Reverse Primer

CTTGTTGCAAGGTACGGTCT

32. SEQ ID NO: 32 – Klf6 coding Forward Primer

GGTGACAGAGGAGCTCAATTT

33. SEQ ID NO: 33 – Klf6 coding Reverse Primer

CTTAGAGACCAACAGCCTGAAC

34. SEQ ID NO: 34 – Klf6 promoter Forward Primer

AACTCTGCTCGTTCTGATTGG

35. SEQ ID NO: 35 – Klf6 promoter Reverse Primer

GCGCCTTATATACCCTGCTAAA

36. SEQ ID NO: 36 – MYADM promoter Forward Primer

GGGTTTCTATGGTAACGGTCTT

37. SEQ ID NO: 37 – MYADM promoter Reverse Primer

CACAGCCCTAGAGGAGTCT

38. SEQ ID NO: 38 – FosB promoter Forward Primer

CCGAGCTCCTTATATGGCTAAT

39. SEQ ID NO: 39 – FosB promoter Reverse Primer

CTAGACTCGATCTGGTCACTTG

40. SEQ ID NO: 40 – RGS2 promoter Forward Primer

CCACACTGAAGACTCTCCATC

41. SEQ ID NO: 41 – RGS2 promoter Reverse Primer

GCACTCGGGCCACATAG

42. SEQ ID NO: 42 – Jun promoter Forward Primer

GCCCGAGCTCAACACTTAT

43. SEQ ID NO: 43 – Jun promoter Reverse Primer

GGGTGACATCATGGGCTATT

44. SEQ ID NO: 44 – JunD promoter Forward Primer

GTCGCTCATTTGCATGGAG

45. SEQ ID NO: 45 – JunD promoter Reverse Primer

ATGACGTCAACCCACAAGG

46. SEQ ID NO: 46 – CCL3 promoter Forward Primer

ACTGGTCTGTGCATGAACTC

47. SEQ ID NO: 47 – CCL3 promter Reverse Primer

GTGGTTATAGCAGCTGAGGAAG

48. SEQ ID NO: 48 – PPP1R15A promoter Forward Primer

ATTCCAGCCTGAGCAACAA

49. SEQ ID NO: 48 – PPP1R15A promoter Reverse Primer

TTCAACAGCGAGCCAGTT

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CLAIMS

What is claimed is:

1. A biomimetic cell-penetrating peptide comprising:

an amino acid sequence that binds to a Chromodomain Helicase DNA binding protein 4 (CHD4) in host cell,

wherein the biomimetic cell-penetrating peptide induces cleavage of CHD4.

2. The biomimetic cell-penetrating peptide of claim 1, wherein the amino acid sequence is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV).

3. The biomimetic cell-penetrating peptide of claim 1 or 2, wherein the amino acid sequence is derived from a binding interface between LANA and CHD4.

4. The biomimetic cell-penetrating peptide of any one of claims 1-3, wherein the amino acid sequence comprises between amino acid residues 939 and 1042 of LANA protein.

5. The biomimetic cell-penetrating peptide of any one of claims 1-4, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73).

6. The biomimetic cell-penetrating peptide of any one of claims 1-5, wherein the amino acid sequence is 10-40 amino acids in length.

7. The biomimetic cell-penetrating peptide of any one of claims 1-6, wherein the amino acid sequence is set forth in SEQ ID NO:1 or variant thereof.

8. The biomimetic cell-penetrating peptide of claim 7, comprising a histadine at amino acid residue 3 of SEQ ID NO:1.

9. The biomimetic cell-penetrating peptide of any one of claims 1-8, further comprising a cell-penetrating peptide (CPP) sequence attached to N-terminal of the amino acid sequence.

10. The biomimetic cell-penetrating peptide of claim 9, wherein the CPP comprises a Trans-Activator of Transcription peptide (TAT).

11. The biomimetic cell-penetrating peptide of claim 10, wherein the TAT peptide comprises the amino acid sequence set forth in SEQ ID NO:3.

12. The biomimetic cell-penetrating peptide of any one of claims 1-11, further comprising a 6 amino acid long peptide at C-terminus.

13. The biomimetic cell-penetrating peptide of claim 12, wherein the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21.

14. The biomimetic cell-penetrating peptide of any of claims 1-13, wherein the biomimetic cell-penetrating peptide cleaves one or more caspase cleavage sites on CHD4.

15. A composition comprising the biomimetic cell-penetrating peptide of any of claims 1-14 and a pharmaceutically acceptable carrier, wherein the composition is formulated for systemic or localized delivery to cancer cells.

16. The composition of claim 15, further comprising an apoptosis-inducing agent.

17. The composition of claim 16, wherein the apoptosis-inducing agent comprises chemotherapy drugs, radiation therapy sensitizers, or immune checkpoint inhibitors.

18. An anti-cancer treatment comprising i) a therapeutically effective amount of the biomimetic cell-penetrating peptide of any of claims 1-14 or the composition of any one of claims 15-17 and ii) a cancer therapy selected from the group comprising of chemotherapy, radiation therapy, immunotherapy, or targeted therapy.

19. A method of treating cancer in a subject comprising administering to the subject a therapeutically effective amount of the biomimetic cell-penetrating peptide of any of claims 1-14 or the composition of any one of claims 15-17 or administering the anti-cancer treatment of claim 18.

20. A method of treating cancer in a subject comprising :

administering to the subject a therapeutically effective amount of a biomimetic cell-penetrating peptide, wherein the biomimetic cell-penetrating peptide comprises an amino acid sequence that binds to a Chromodomain Helicase DNA binding protein 4 (CHD4) in host cell,

wherein the biomimetic cell-penetrating peptide induces cleavage of CHD4.

21. The method of claim 20, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV).

22. The method of claim 20 or 21, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from a binding interface between LANA and CHD4.

23. The method of any one of claims 20-22, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises between amino acid residues 939 and 1042 of LANA protein.

24. The method of any one of claims 20-23, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73).

25. The method of any one of claims 20-24, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is 10-40 amino acids in length.

26. The method of any one of claims 20-25, wherein the biomimetic cell-penetrating peptide comprises the amino acid sequence set forth in SEQ ID NO:1 or a variant thereof.

27. The method of claim 26, wherein biomimetic cell-penetrating peptide comprises comprises a histadine at amino acid residue 3 in SEQ ID NO: 1.

28. The method of any one of claims 20-27, wherein biomimetic cell-penetrating peptide further comprises a cell-penetrating peptide (CPP) sequence attached to N-terminal of the amino acid sequence.

29. The method of claim 28, wherein the CPP comprises Trans-Activator of Transcription peptide (TAT).

30. The method of claim 29, wherein the TAT peptide comprises the amino acid sequence set forth in SEQ ID NO:3.

31. The method of any one of claims 20-30, wherein biomimetic cell-penetrating peptide further comprises a 6 amino acid long peptide at C-terminus.

32. The method of claim 31, wherein the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21.

33. The method of any one of the claims 19 to 32 , wherein the cancer comprises primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer.

34. A method of inducing apoptosis and differentiation in cancer cells comprising contacting the cancer cells with the biomimetic cell-penetrating peptide of any one of claims 1-14, wherein the biomimetic cell-penetrating peptide reduces occupancy of CHD4 on promoters of genes, thus increasing accessibility of transcription factors and upregulating genes expression related to cell differentiation and apoptosis.

35. A method of inducing apoptosis and differentiation in cancer cells comprising contacting cancer cells with a biomimetic cell-penetrating peptide, wherein the biomimetic cell-penetrating peptide reduces occupancy of Chromodomain Helicase DNA binding protein 4 (CHD4) on promoters of genes, thus increasing accessibility of transcription factors and upregulating genes expression related to cell differentiation and apoptosis.

36. The method of claim 35, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from Latency Associated Nuclear Antigen (LANA) protein of Kaposi's sarcoma-associated herpesvirus (KSHV).

37. The method of claim 35 or 36, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is derived from a binding interface between LANA and CHD4.

38. The method of any one of claims 35-37, wherein the amino acid sequence of the biomimetic cell-penetrating peptide comprises between amino acid residues 939 and 1042 of LANA protein.

39. The method of any one of claims 35-38, wherein the biomimetic cell-penetrating peptide comprises Virus de Gann wo Naosu ORF73 (VGN73).

40. The method of any one of claims 35-39, wherein the amino acid sequence of the biomimetic cell-penetrating peptide is 10-40 amino acids in length.

41. The method of any one of claims 35-40, wherein the biomimetic cell-penetrating peptide comprises the amino acid sequence set forth in SEQ ID NO:1 or a variant thereof.

42. The method of claim 41, wherein biomimetic cell-penetrating peptide comprises comprises a histadine at amino acid residue 3 in SEQ ID NO: 1.

43. The method of any one of claims 35-42, wherein biomimetic cell-penetrating peptide further comprises a cell-penetrating peptide (CPP) sequence attached to N-terminal of the amino acid sequence.

44. The method of claim 43, wherein the CPP comprises Trans-Activator of Transcription peptide (TAT).

45. The method of claim 44, wherein the TAT peptide comprises the amino acid sequence set forth in SEQ ID NO:3.

46. The method of any one of claims 35-45, wherein biomimetic cell-penetrating peptide further comprises a 6 amino acid long peptide at C-terminus.

47. The method of claim 46, wherein the C-terminus peptide comprises the amino acid sequence set forth in SEQ ID NO:21.

48. The method of any one of claims 34-47, wherein the transcription factors comprises IRF4, KLF6, FOSB, JUN, or JUND.

49. The method of any one of claims 34-48, wherein the genes comprises BRM, SMARCE1, p21, MYADM, RGS2, CCL3, PPP1R15A, Klf6, CD163, CD206, CD38, CD19, TP53 or CHD4.

50. The method of any one of the claims 34-49, wherein the cancer cells are derived from a cancer selected from primary effusion lymphoma (PEL), Kaposi's Sarcoma (KS), multicentric Castleman's disease (MCD), hepatocellular carcinoma, colorectal cancer, or ovarian cancer.

51. The method of any one of the claims 34-50, wherein the cancer cells comprises U937 cells, BC3 cells, or BCBL-1 cells.

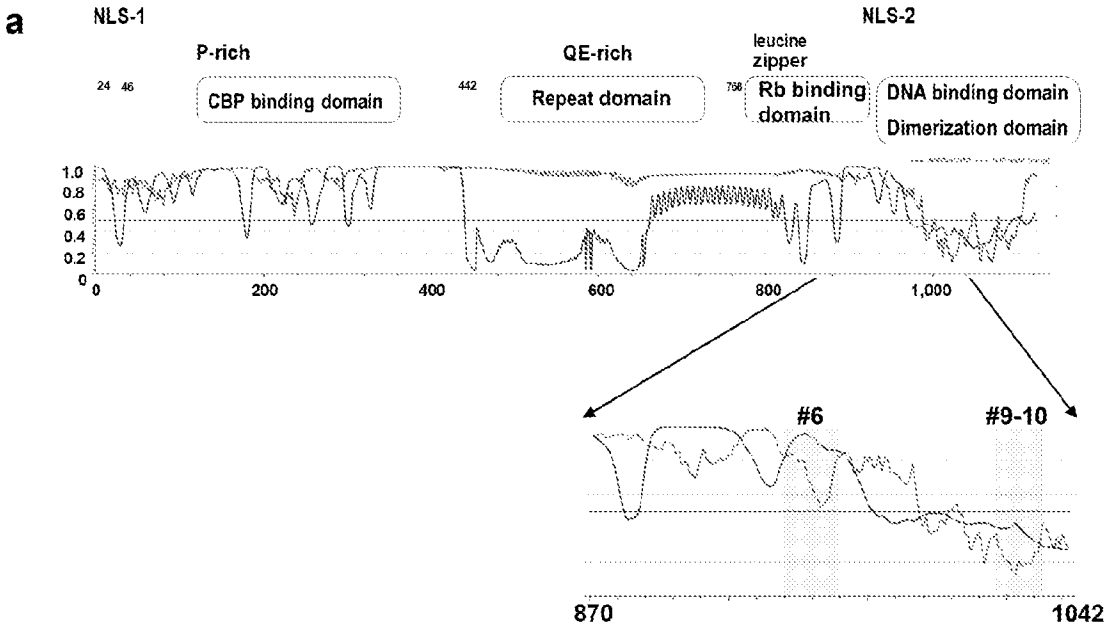


FIG. 1A

b

#1	872	-EQELEEVEEQEEQELEEVEEQEQ-	894 SEQ ID NO: 4
#2	876	-EEVEEQEQQGVEQQEQETVEE-	896 SEQ ID NO: 5
#3	892	-ETVEEPILHGSSSEDEMEVDYP-	914 SEQ ID NO: 6
#4	906	-EDEMEVDYPVVSTHEQIASSPPG-	928 SEQ ID NO: 7
#5	922	-IASSPPGDNTPDDDPQPGPSREYR-	945 SEQ ID NO: 8
#6	939	-GPSREYRYVLRTSPPHRPGVRMR-	961 SEQ ID NO: 9
#7	961	-RRVPVTHPKKPHPRYQQPPVP-	981 SEQ ID NO: 10
#8	977	-QPPVPYRQIDDCPAKARPQHIFYR-	1000 SEQ ID NO: 11
#9	995	-QHIFYRRFLGKDGRRDPKCQWKFAVI-	1020 SEQ ID NO: 12
#10	1016	-KFAVIFWGNDPYGLKKLSQAFQFGGVK-	1042 SEQ ID NO: 13

FIG. 1B

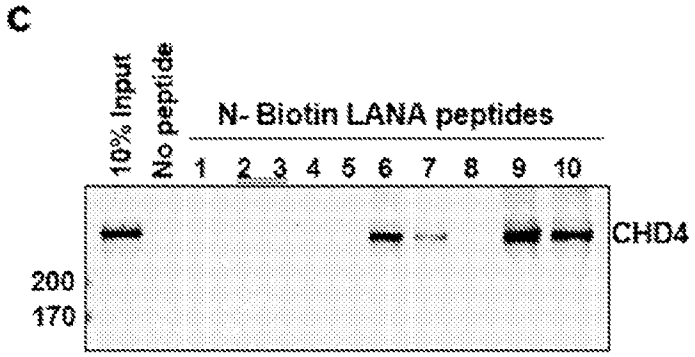


FIG. 1C

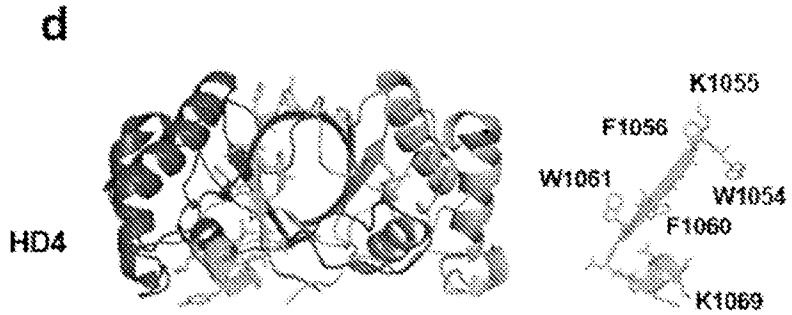


FIG. 1D

e

Kaposi's sarcoma-associated herpesvirus	WKRAVIPWG---NDPYGLKK	SEQ ID NO: 14
Macacine Gammaherpesvirus 5	WKHGVIPGN------SDPYSLGR	SEQ ID NO: 15
Retroperitoneal fibromatosis-associated herpesvirus	WEHAAIPWS------PRPYPLKK	SEQ ID NO: 16
Colobine gammaherpesvirus 1	WKHALLPWR---PEPYPLKK	SEQ ID NO: 17
Macacine Gammaherpesvirus 4	WPFGVPVYG-PKPGCYNLRR	SEQ ID NO: 18
Epstein-Barr Virus	WVAGVPVYGDSKKSLYNLRR	SEQ ID NO: 19
Saimiriine gammaherpesvirus 2	WRSVVIPWG-PTSALYRLSK	SEQ ID NO: 20
Designed peptide (VGH73):	WKHAVIPWG NDPYGLKK	SEQ ID NO: 1

FIG. 1E

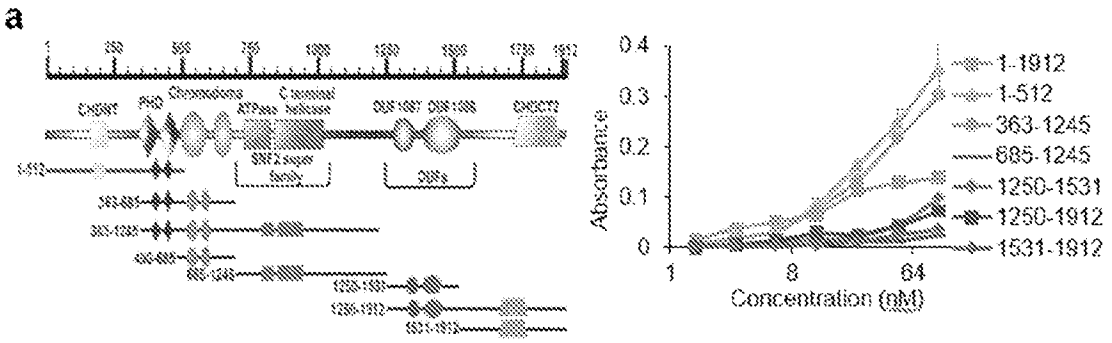


FIG. 2A

b

SEQ ID NO: 1 VGN73: {D-ARG}KKRR{ORN}RRR{BETA-ALA}WKHAVIFWGNDPYGLKK
SEQ ID NO: 2 Mutant: {D-ARG}KKRR{ORN}RRR{BETA-ALA}AKHAVISAGNDPYGLKK
SEQ ID NO: 3 CPP: {D-ARG}KKRR{ORN}RRR{BETA-ALA}

FIG. 2B

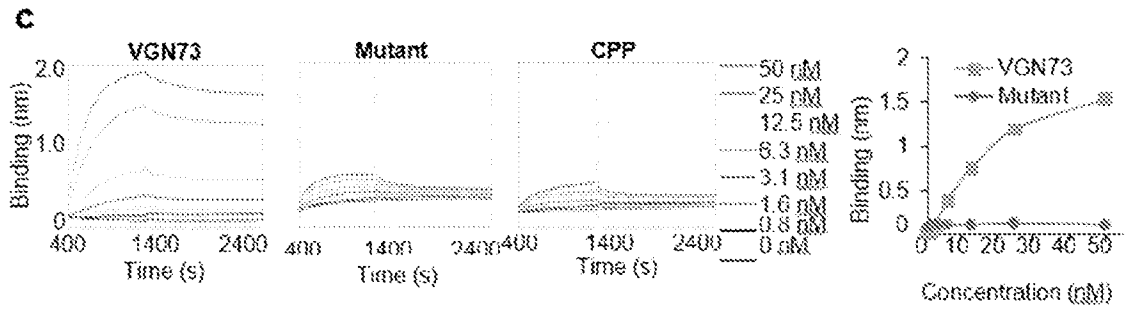


FIG. 2C

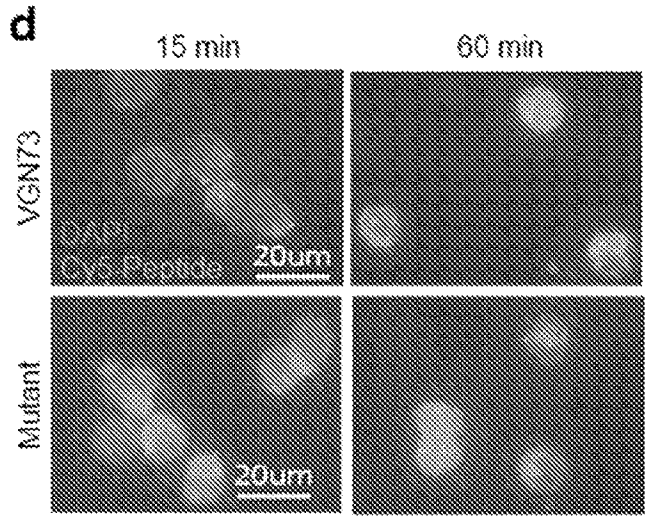

d
15 min
60 min
VGN73
Mutant
20um
20um

FIG. 2D

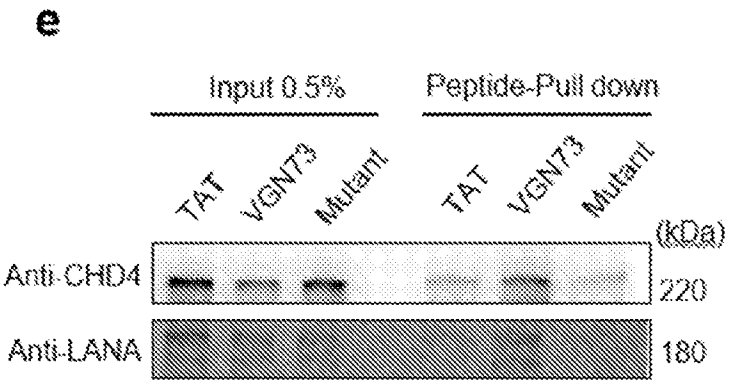

e
Input 0.5%
Peptide-Pull down
TAT
VGN73
Mutant
TAT
VGN73
Mutant
(kDa)
Anti-CHD4
220
Anti-LANA
180

FIG. 2E

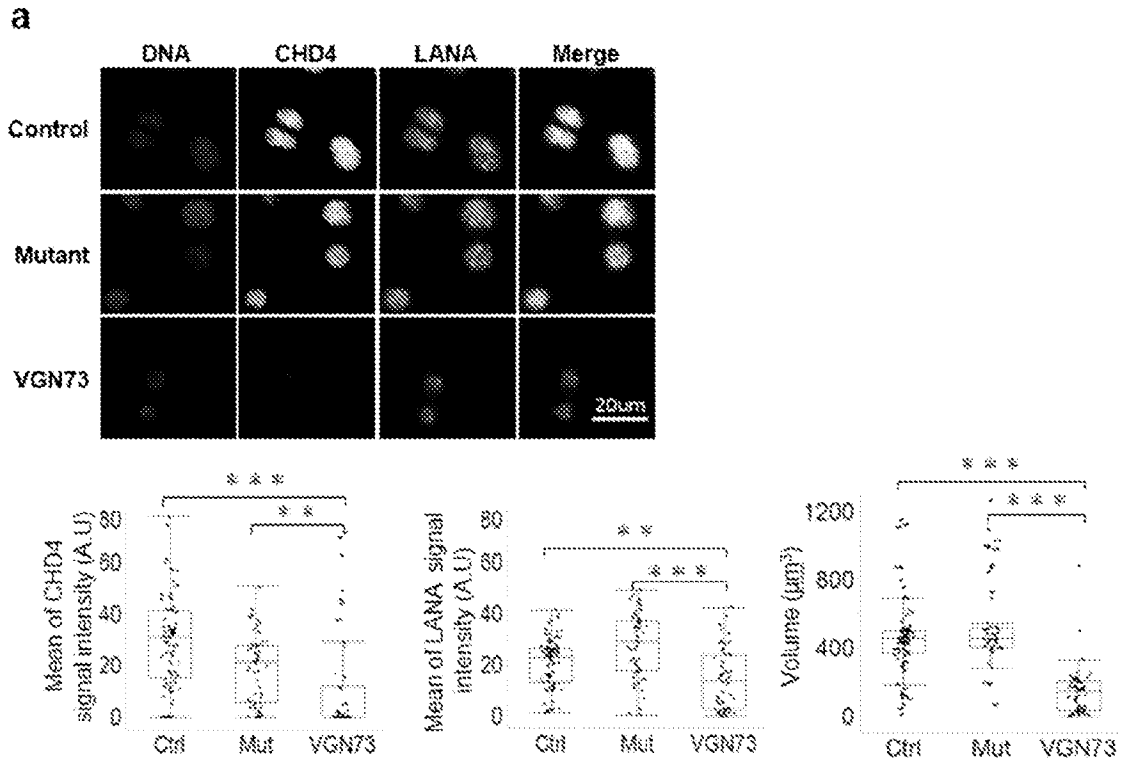

a
DNA
CHD4
LANA
Merge
Control
Mutant
VGN73
20um
Mean of CHD4 signal intensity (A.U)
Mean of LANA signal intensity (A.U)
Volume (μm³)
Ctrl
Mut
VGN73

FIG. 3A

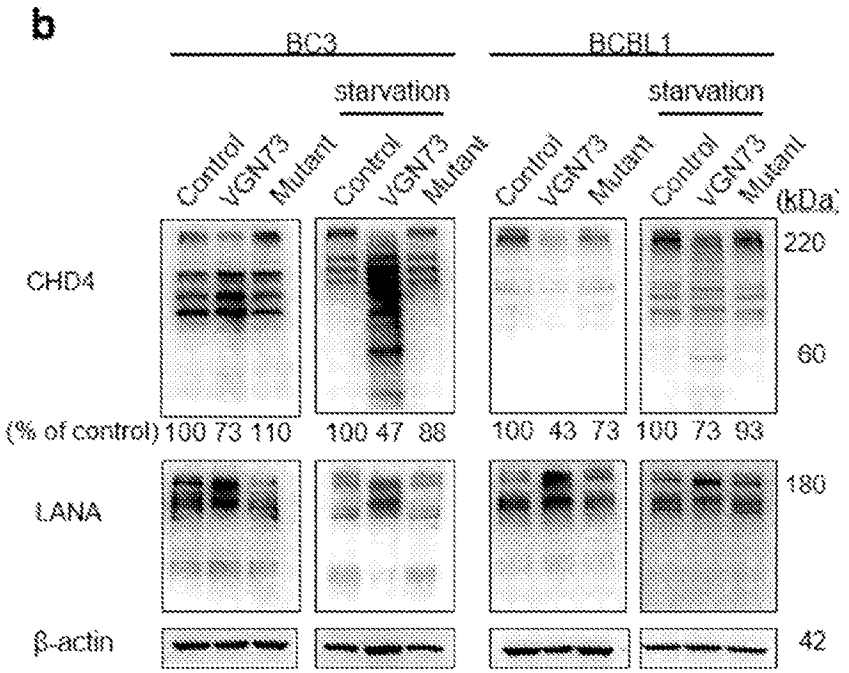

b
BC3
BCBL1
starvation
Control
VGN73
Mutant
(kDa)
CHD4
220
60
(% of control) 100 73 110 100 47 88 100 43 73 100 73 93
LANA
180
β-actin
42

FIG. 3B

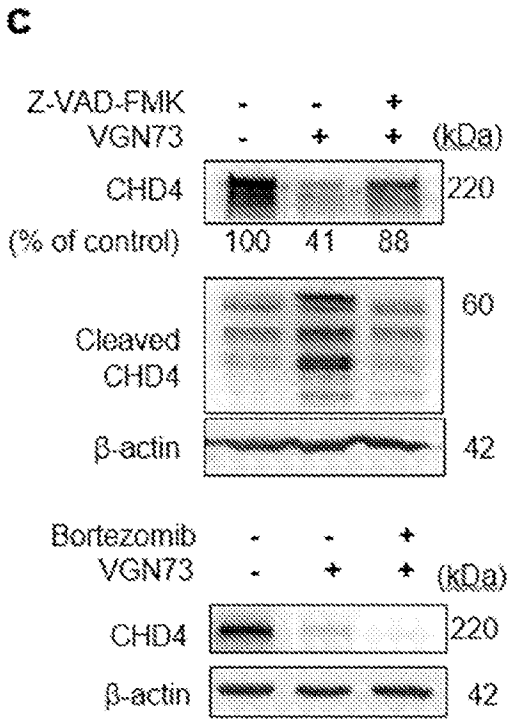

c
Z-VAD-FMK - - +
VGN73 - + + (kDa)
CHD4
220
(% of control) 100 41 88
60
Cleaved CHD4
β-actin
42
Bortezomib - - +
VGN73 - + + (kDa)
CHD4
220
β-actin
42

FIG. 3C

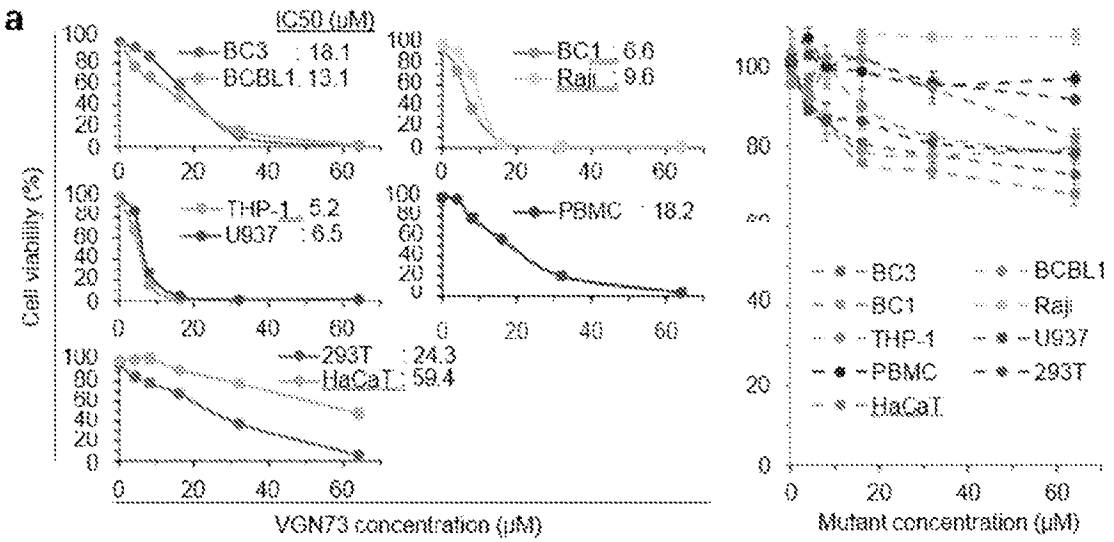

a
IC50 (μM)
BC3 : 18.1
BCBL1 : 13.1
BC1 : 6.6
Raji : 9.6
THP-1 : 5.2
U937 : 6.5
PBMC : 18.2
293T : 24.3
HaCaT : 59.4
Cell viability (%)
VGN73 concentration (μM)
BC3
BCBL1
BC1
Raji
THP-1
U937
PBMC
293T
HaCaT
Mutant concentration (μM)

FIG. 4A

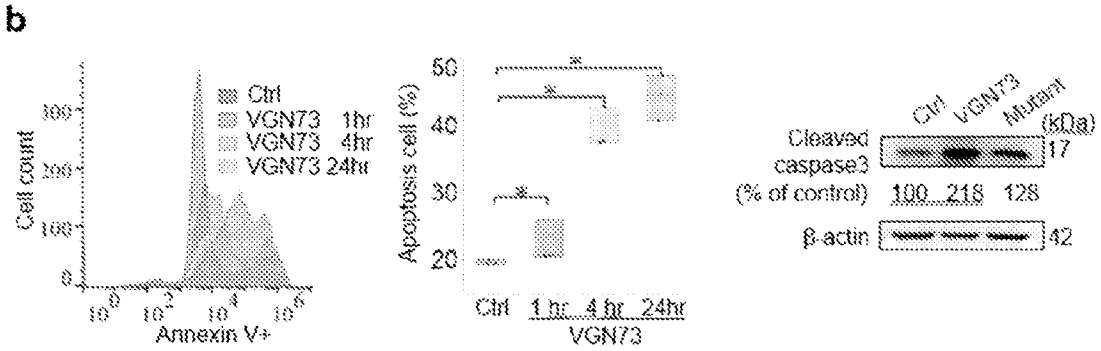


FIG. 4B

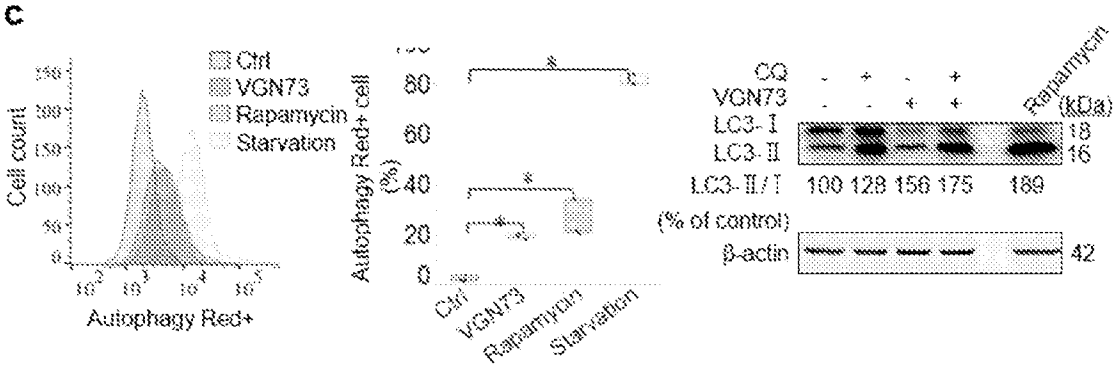


FIG. 4C

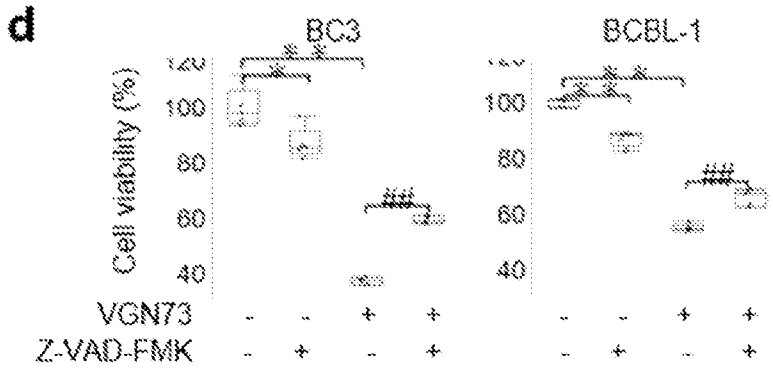


FIG. 4D

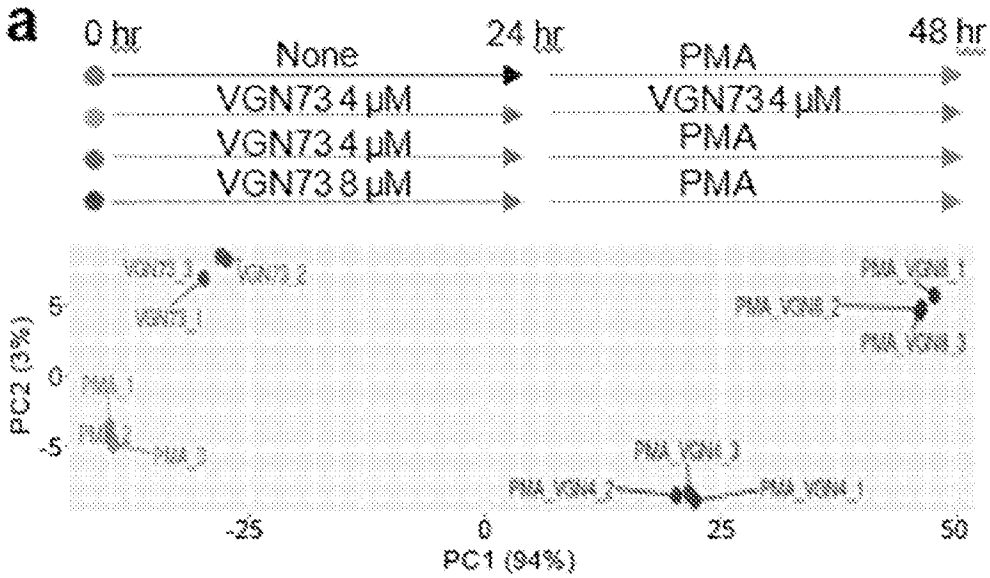

a
0 hr
24 hr
48 hr
None
PMA
VGN734 µM
VGN734 µM
PMA
VGN738 µM
PMA
PC2 (3%)
PC1 (94%)

FIG. 5A

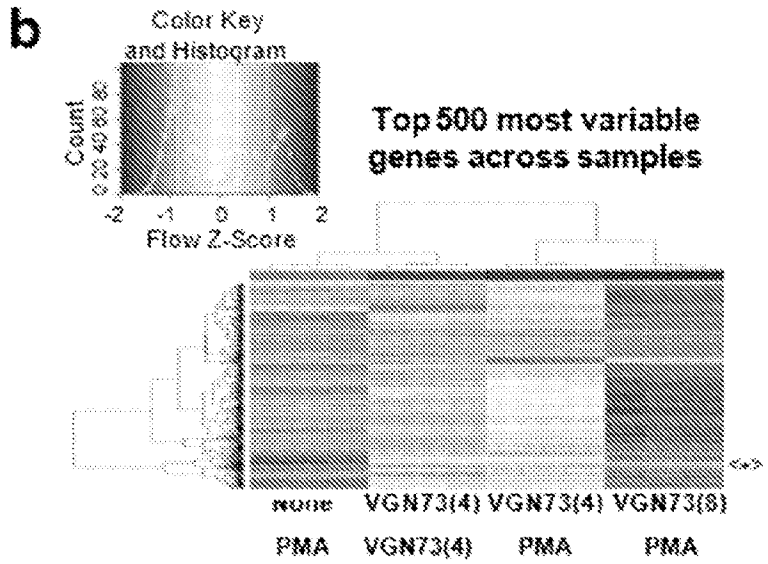

b
Color Key and Histogram
Count
Row Z-Score
Top 500 most variable genes across samples
None
VGN73(4)
VGN73(4)
VGN73(8)
PMA
VGN73(4)
PMA
PMA

FIG. 5B

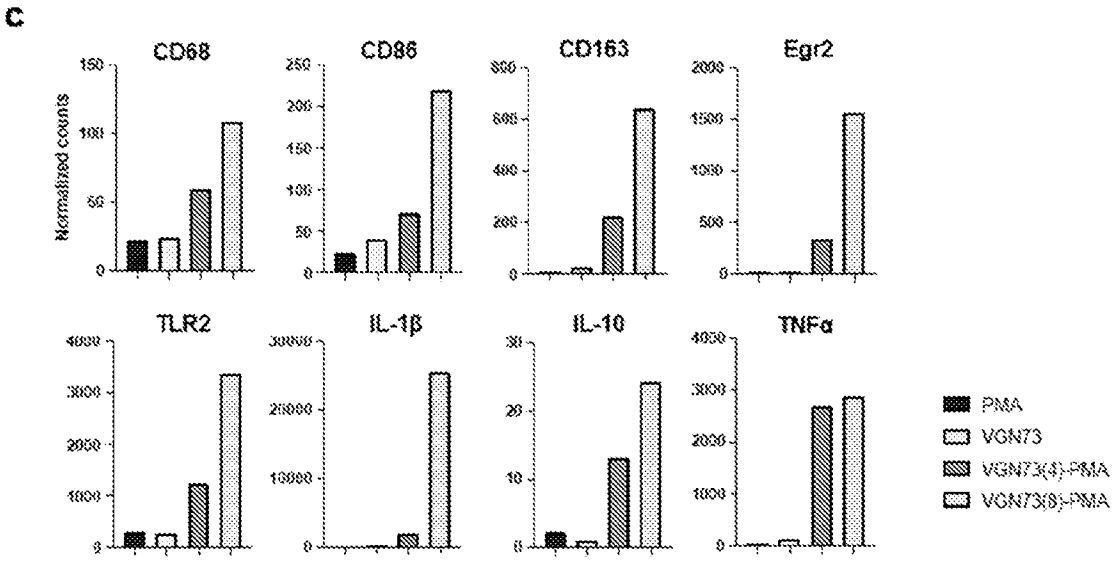

c
CD68
CD86
CD163
Egr2
Normalized counts
TLR2
IL-1β
IL-10
TNFα
PMA
VGN73
VGN73(4)-PMA
VGN73(8)-PMA

FIG. 5C

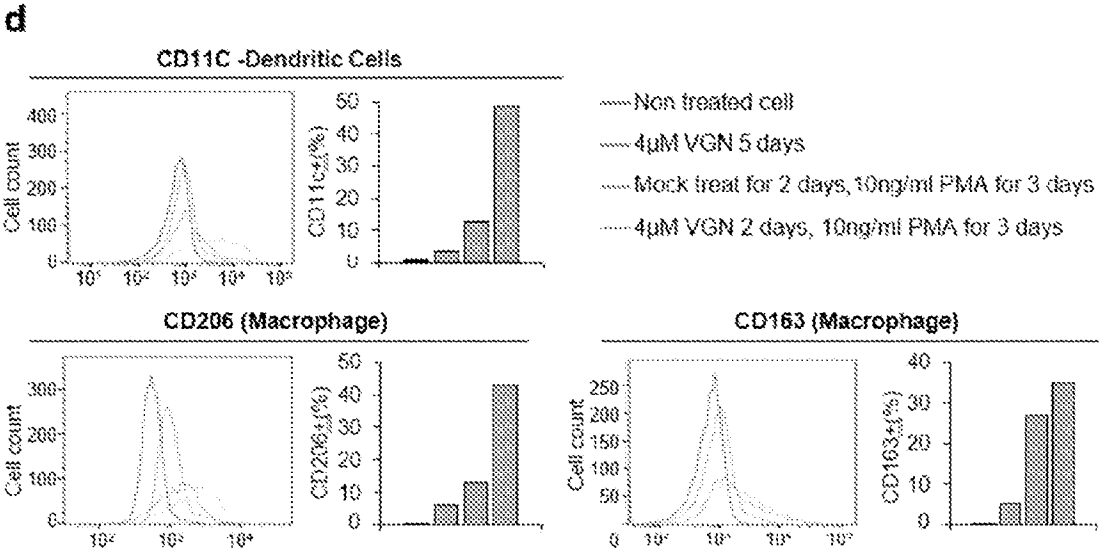

d
CD11C -Dendritic Cells
Cell count
CD11c+(%)
Non treated cell
4μM VGN 5 days
Mock treat for 2 days,10ng/ml PMA for 3 days
4μM VGN 2 days, 10ng/ml PMA for 3 days
CD206 (Macrophage)
CD206+(%)
CD163 (Macrophage)
CD163+(%)

FIG. 5D

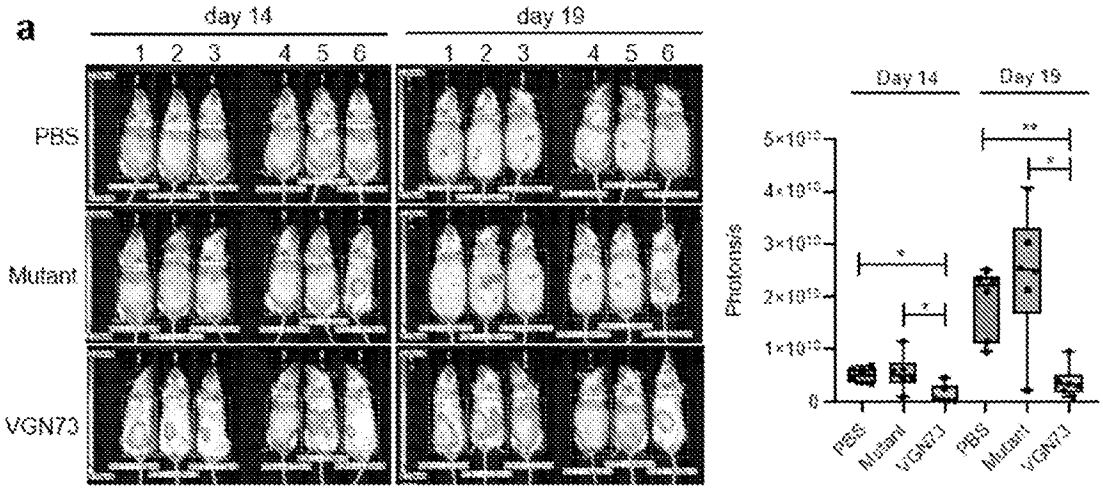

a
day 14
day 19
1 2 3 4 5 6
PBS
Mutant
VGN73
Day 14
Day 19
Photons/s
PBS
Mutant
VGN73

FIG. 6A

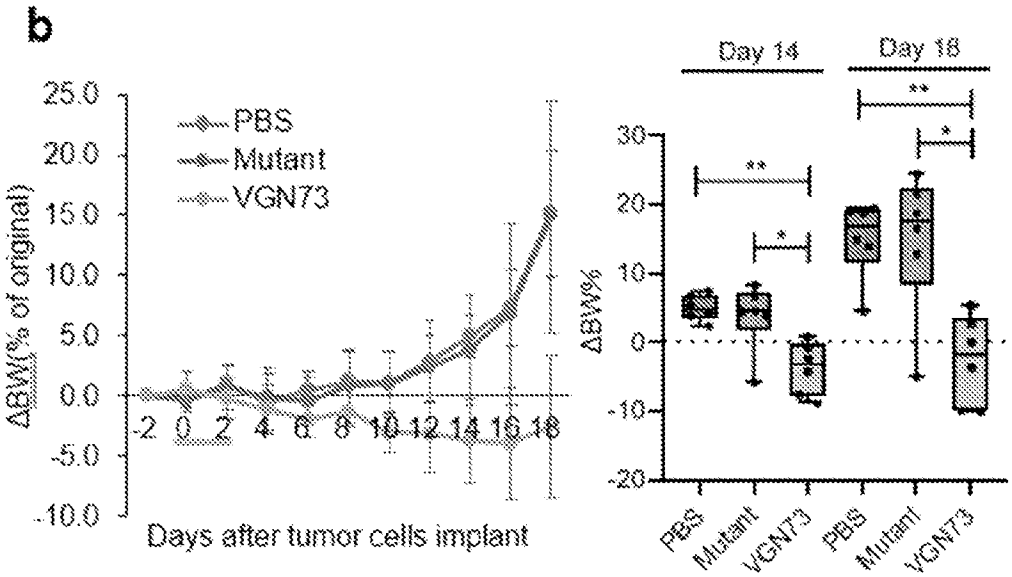

b
PBS
Mutant
VGN73
ΔBW(% of original)
Days after tumor cells implant
Day 14
Day 18
ΔBW%

FIG. 6B

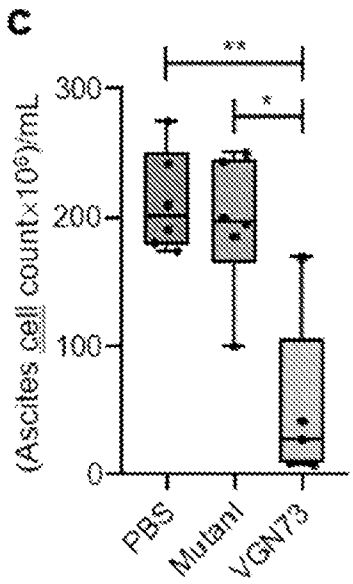

c
**
*
300
200
100
0
(Ascites cell count×10⁶)/mL
PBS
Mutant
VGN73

FIG. 6C

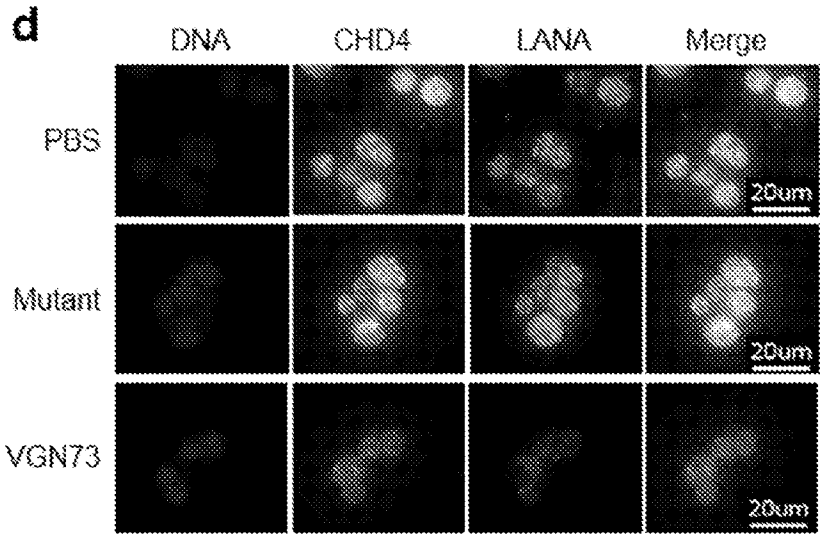

d
DNA
CHD4
LANA
Merge
PBS
Mutant
VGN73
20um
20um
20um

FIG. 6D

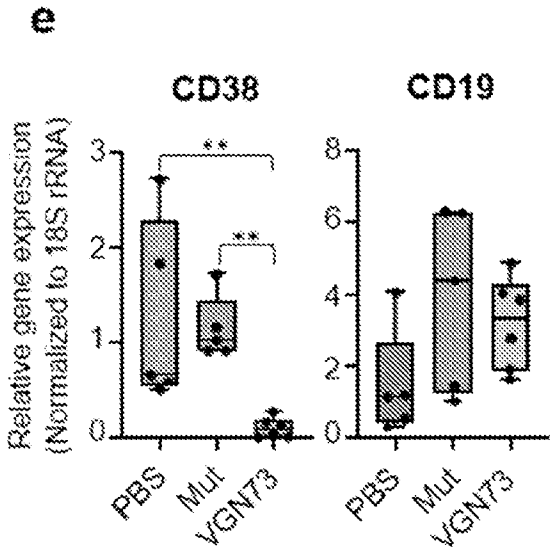

e
CD38
CD19
Relative gene expression
(Normalized to 18S rRNA)
**
**
0
1
2
3
0
2
4
6
8
PBS
Mut
VGN73
PBS
Mut
VGN73

FIG. 6E

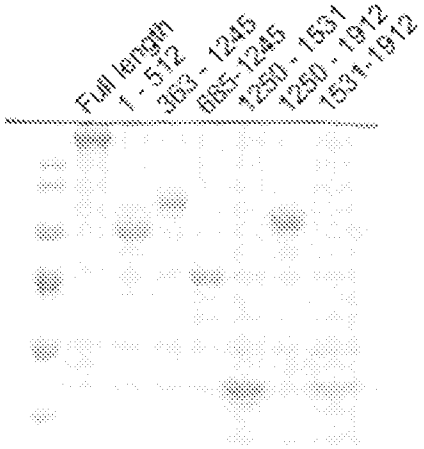

Full length
1 - 512
363 - 1245
865-1245
1250 - 1531
1250 - 1912
1531-1912

FIG. 7A

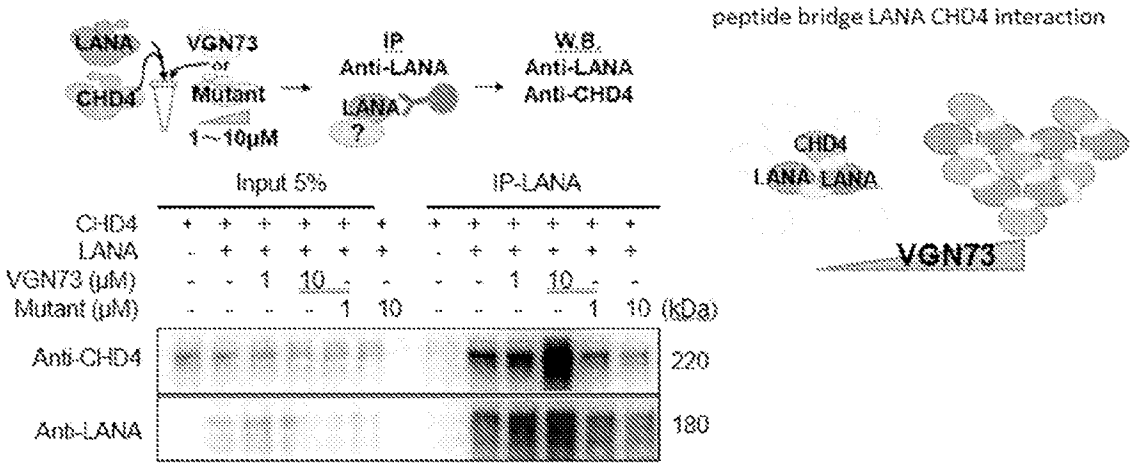

LANA
CHD4
VGN73
or
Mutant
1~10μM
IP
Anti-LANA
LANA
?
W.B.
Anti-LANA
Anti-CHD4
Input 5%
IP-LANA
CHD4
LANA
VGN73 (μM)
Mutant (μM)
(kDa)
Anti-CHD4
Anti-LANA
220
180
peptide bridge LANA CHD4 interaction
CHD4
LANA LANA
VGN73

FIG. 7B

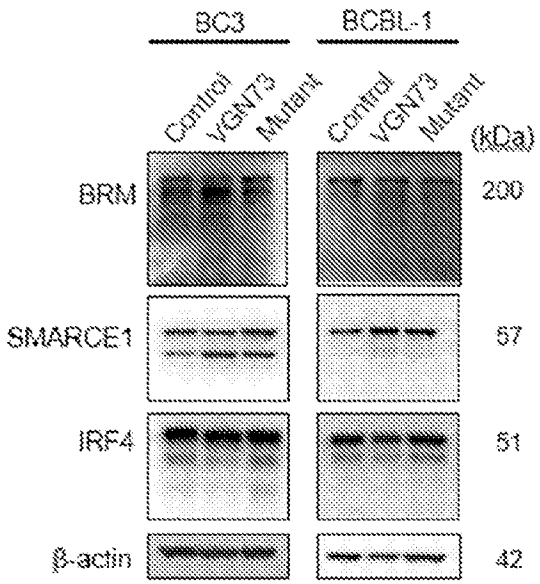

BC3
BCBL-1
Control
VGN73
Mutant
(kDa)
BRM
SMARCE1
IRF4
β-actin
200
57
51
42

FIG. 8A

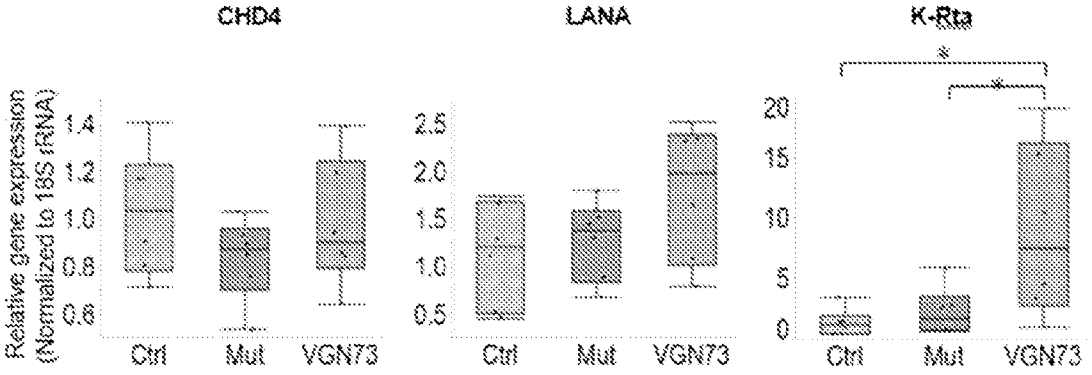


FIG. 8B

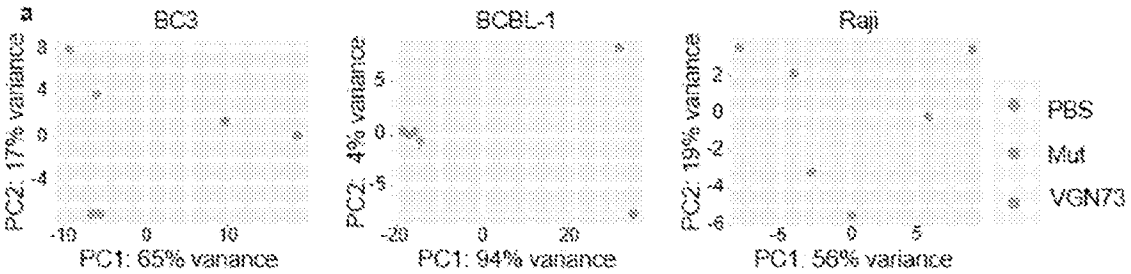


FIG. 9A

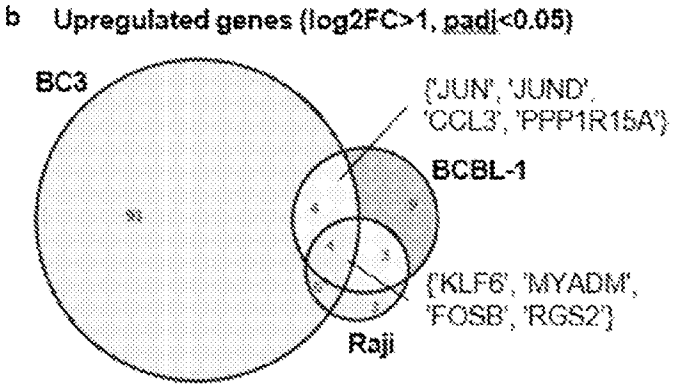


FIG. 9B

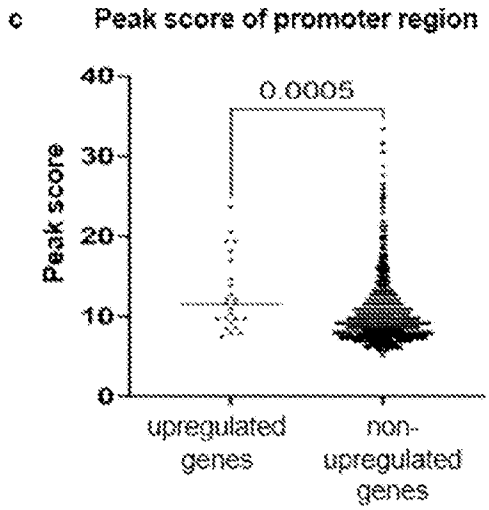


FIG. 9C

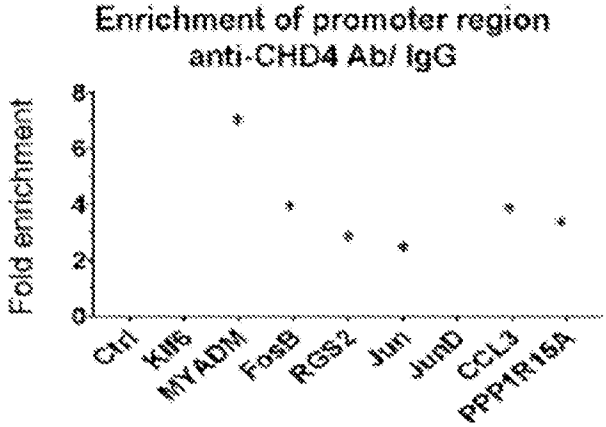


FIG. 9D

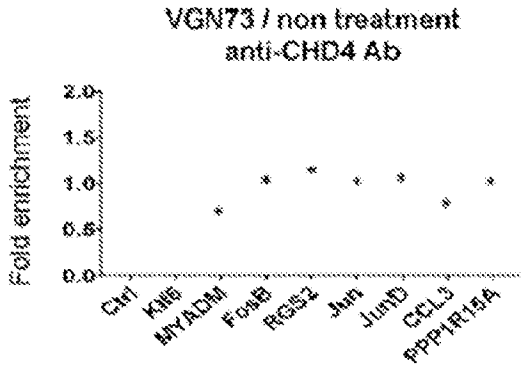


FIG. 9E

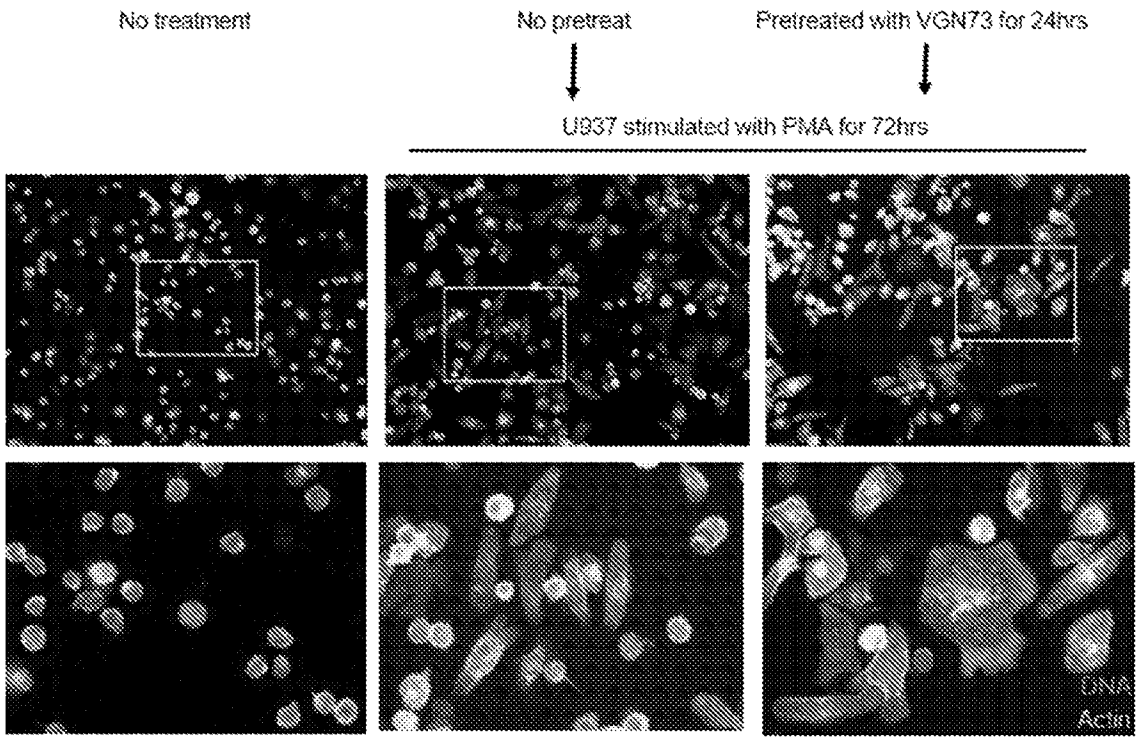


FIG. 10

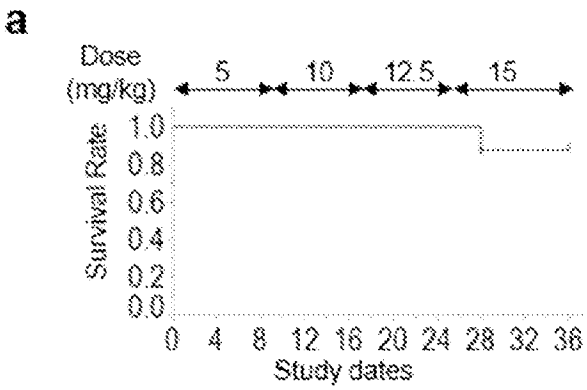
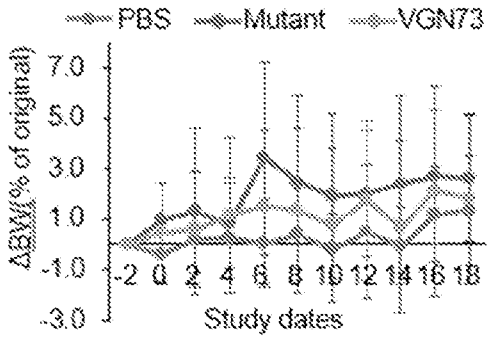


FIG. 11A

b



	PBS		VGN73		Mutant	
	Median	IQR	Median	IQR	Median	IQR
Alanine Transaminase (U/L)	28.7	24.3-32.4	32.0	27.3-42.4	29.6	25.5-33.3
Aspartate Transaminase (U/L)	91.55	75.7-163.6	120.1	90.4-270.5	103.6	68.6-162.4
Albumin (g/dL)	3.48	3.1-3.7	3.3	2.9-3.8	3.3	3.1-3.7
Alkaline Phosphatase (U/L)	127.45	101.4-160.5	99.8	71.0-160.8	121.6	93.9-144.7
Amylase (U/L)	1665.75	1519.8-2072.9	1718.4	1539.9-1928.9	1684.1	1621.5-177
Blood Urea Nitrogen (mg/dL)	21.85	20.5-25.7	20.8	16.6-21.2	22.1	20.2-23.7
Calcium (mg/dL)	9.37	8.1-9.7	9.6	7.5-10.1	9.3	8.9-9.6
Chloride (mmol/L)	110.95	109.3-113.3	110.1	109.5-112.9	111	109.5-112.3
Creatinine (mg/dL)	0.14	0.12-0.15	0.15	0.13-0.17	0.15	0.13-0.22
Glucose (mg/dL)	189.3	136.2-217.4	152.2	124.9-185.5	142.5	118.7-175.9
Potassium (mmol/L)	6.2	5.4-7.1	7.0	5.3-8.1	6.1	5.7-6.9
Sodium (mmol/L)	146.0	143.5-148.8	146.8	144.5-148.0	146.8	143.3-147.0
Phosphorus (mg/dL)	8.0	6.8-9.3	7.2	6.4-8.6	8.7	6.9-7.7
Total Bilirubin (mg/dL)	0.062	0.059-0.084	0.079	0.058-0.080	0.084	0.039-0.088
Total Protein (g/dL)	4.9	4.8-5.2	5.0	4.6-5.5	4.8	4.4-5.5

FIG. 11B