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(54) Title: NON-REPLICATING VIRUS-DERIVED PARTICLES AND USES THEREOF

(57) Abstract: There is described herein a non-replicating Rhabdovirus-derived particle that lacks the ability to spread between cells while having tropism against immortalized cells. The non-replicating Rhabdovirus-derived particle may have cytolytic tropism against immortalized cells. There is also described a non-replicating Rhabdovirus-derived particle that lacks the ability to spread between cells but has innate and/or adaptive immune-stimulating properties.

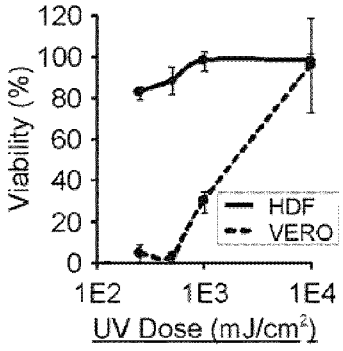


Fig. 1A

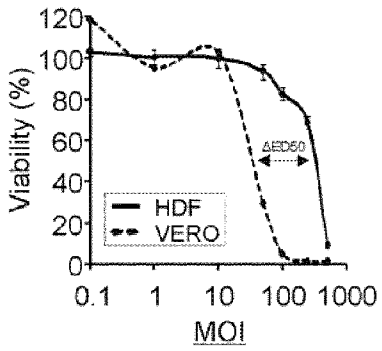


Fig. 1B

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BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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# NON-REPLICATING VIRUS-DERIVED PARTICLES AND USES THEREOF

## CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of priority of U.S. Provisional Patent Application No. 61/740,856 filed December 21, 2012 and of U.S. Provisional Patent Application No. 61/835,310 filed June 14, 2013, which are incorporated herein by reference in their entirety.

## FIELD

**[0002]** The present disclosure relates generally to a non-replicating virus-derived particle and its use as an anti-cancer agent.

## BACKGROUND

**[0003]** The following background discussion does not imply or admit that anything described below is prior art or part of the knowledge of people skilled in the art in any country.

**[0004]** Oncolytic viruses (OVs) have been engineered through attenuating mutations or deletions which allow the virus to replicate exclusively in cells associated with an impaired immune response or enhanced metabolic activity, two key characteristics of tumorigenesis. Examples of current advanced oncolytic therapeutics include the Herpes Simplex Virus OncoVEXGM–CSF and the vaccinia virus (JX594). To date, the main focus of the OV field has been the development of platforms where the live virus has preferential replication/spreading capacity within the local tumor environment.

**[0005]** Rhabdoviruses viruses (RVs), such as vesicular stomatitis virus (VSV) and Maraba, are currently being explored as anti-cancer therapeutics. In tumors, viral propagation is enabled by unrestrained metabolic activities and impaired anti-viral programs. Tumor susceptibility to RV treatment is further enhanced due to pre-disposition towards virus-mediated apoptosis.

**[0006]** In the Rhabdovirus field, oncolytic platforms developed to date utilize a replication competent virus where the virus spreads between tumor cells. In fact, reports describing the use of live replication/expression competent rhabdovirus as a direct virotherapy for cancer typically compare efficacy to non-replicating/non-expressing virus

controls where no measurable efficacy is observed. In these reports, it is concluded that Rhabdovirus genome replication and/or expression is a critical and essential component of tumor cytotoxicity and therapeutic efficacy.

**[0007]** The lack of oncolytic effects in these previous studies is reflected in the methods used to disrupt virus genome replication and/or expression as well as in the number of virus particles used. Indeed, when these previous methods are used to disrupt virus genome replication and/or expression, no bioactivity of the virus is observed. Furthermore, in these studies, non-replicating virus controls are applied at the same dose as their live virus counterparts, and not at higher doses to ensure that each cell encounters a non-replicating particle.

**[0008]** Alternative, and preferably more effective, approaches are desired to treat and cure most forms of cancers. For example, the outcome for the majority of adult patients suffering from acute lymphoblastic or acute myeloid leukemia remains dismal. This is in part due to the significant immunocompromised nature of the disease. For a minority of patients, anti-tumor immune responses are partially restored through allogeneic stem cell transplantation after myeloablative conditioning. This therapy is potentially curative, however is associated with frequent adverse events and significant treatment-related mortality. For many patients with chronic-phase chronic myeloid leukemia (CML), targeted tyrosine kinase inhibitor (TKI) therapy offers excellent disease control. However when progression into acute leukemic blast crisis occurs, very limited therapeutic options exist due to development of multi-drug resistance and the rapid kinetics of this form of recalcitrant leukemia.

**[0009]** Hence there is need for alternative anti-cancer agents, particularly for immunocompromised patients. The anti-cancer agent, by virtue of its design and components, would preferably be able to address current unmet clinical needs and/or overcome at least some of the above-discussed problems.

## SUMMARY

**[0010]** The following summary is intended to introduce the reader to one or more inventions described herein, but not to define any of them. Inventions may reside in any combination of features described anywhere in this document.

**[0011]** While live OV strategies are being pursued to treat a variety of tumor types, their application in hematopoietic malignancies in particular is complicated by several factors.

Limited virion production and reduced spread between leukemia cells requires high-dose viral therapy to overcome these inefficiencies. However, uncontrolled live virus spread and off-target effects in normal tissue compromise the safety of this approach, particularly in immunosuppressed patients.

**[0012]** Issues associated with using live virus include: 1) safety, which relies on the ability of the live Rhabdovirus to spread only in diseased tumor tissue, leaving healthy tissue alone; 2) low doses for administration, since the introduction of live spreadable virus to a patient requires the administration of relatively low doses of these live viral agents to ensure safety; 3) immune diversion from the tumor towards the live virus which effectively decreases the efficiency of anti-tumor immune responses; and 4) engineered live viruses designed with proclivity for tumor often have impaired production capacity compared to wild type virus, and consequently, formulation efficiencies and production costs are sub-optimal from a manufacturing perspective.

**[0013]** It has been previously shown that intra-tumoral injection with VSV engineered to have a deletion of the glycoprotein gene (VSVΔG), which prevents final virion assembly and spread, elicits anti-tumor immune responses. However, treatment with VSVΔG cannot provide a significant reduction of disseminated tumor bulk, partly due to the inability to manufacture and deliver therapeutically effective doses.

**[0014]** The authors are aware of no reports that detail the use of a non-replicating and non-expressing Rhabdovirus-derived platform as an anti-cancer therapeutic. Non-replicating virus-derived particles (NVRP) of the present disclosure, and non-replicating rhabdovirus-derived particles (NRRP) in particular, are wild type virus particles modified so as to lack the ability to spread between cells. Once modified, the non-replicating virus-derived particle (NVRP) cannot sustain virion replication.

**[0015]** NVRPs are unique in that they retain tropism, such as cytolytic tropism, against immortalized cells. This means that NVRPs will induce cell death preferentially in immortalized cells such as tumor or cancer cells and transformed immortalized cells. Specific examples of NVRPs have innate and/or adaptive immune-stimulating properties against immortalized cells.

**[0016]** In one aspect, the present disclosure describes a non-replicating rhabdovirus-derived particle that lacks the ability to spread between cells while having tropism against immortalized cells. The tropism may be a cytolytic tropism. The non-replicating rhabdovirus-

derived particle may have innate or adaptive immune-stimulating properties against immortalized cells.

**[0017]** In yet another aspect, the present disclosure provides a use of a non-replicating rhabdovirus-derived particle to treat a population of hyperproliferative cells or cancer cells. The population of hyperproliferative cells is preferably of hematopoietic nature, and preferably leukemic cells. The population of hyperproliferative cells may be solid tumor cells.

**[0018]** In still another aspect, the present disclosure describes a method of treating a patient having a population of hyperproliferative cells or cancer cells. The method includes administering to the patient non-replicating rhabdovirus-derived particles. The population of hyperproliferative cells may preferably be of hematopoietic nature, preferably leukemic cells. The population of hyperproliferative cells may be solid tumor cells.

**[0019]** Other aspects and features of the present disclosure will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments in conjunction with the accompanying figures.

## BRIEF DESCRIPTION OF DRAWINGS

**[0020]** Embodiments of the present disclosure will now be described, by way of example only, with reference to the attached Figures.

**[0021]** Fig. 1A is a graph showing the impact of UV dosage on NRRP-mediated cytotoxicity on Vero and HDFN cells. No GFP signal was detected following UV-induced NRRP generation. Viability was quantified using the resazurin assay 72h post infection. The MOI of this experiment was set at 100 particles per cell. Error bars represent the standard deviation between triplicate experiments.

**[0022]** Fig. 1B is a graph showing the impact of MOI on the cytotoxicity induced by NRRPs in Vero and HDFN cells as illustrated by the viability as a function MOI. Viability was quantified using the resazurin assay 72h post infection. Error bars represent the standard deviation between triplicate experiments.

**[0023]** Fig. 2A is a set of images show the cytotoxicity of NRRPs in Vero immortalized cells through fluorescent and brightfield microscopy images of Vero cells treated with PBS, Live VSV-GFP or NRRPs taken at 24 and 72 hours post-infection or post-

treatment. The multiplicity of infection (MOI) used in these experiments was set at 100 particles per cell.

**[0024]** Fig. 2B is a graph showing the cytotoxicity of NRRPs through resazurin quantification of cellular viability 72h post treatment. Error bars represent the standard deviation between triplicate experiments.

**[0025]** Fig. 2C is a graph showing viral titers produced. NAN means "not as a number" as no virions were detected.

**[0026]** Fig. 3A is a set of fluorescent microscopy images (4X) of leukemic (L1210) and Vero cells treated with PBS, Live Maraba virus, and Maraba virus-derived NRRPs. Images were taken at 24h post treatment.

**[0027]** Fig. 3B is a graph showing viral titers obtained from tumor cells.

**[0028]** Fig. 3C is a graph showing resazurin quantification of cellular viability of L1210 leukemia cells and HDF normal cells, 72h post infection.

**[0029]** Fig. 4A is a set of images showing fluorescent images of L1210 and Vero cells treated with PBS, Live VSV-GFP, or NRRPs.

**[0030]** Fig. 4B is a graph showing the viral titers generated from L1210 acute leukemia and Vero immortalized cells

**[0031]** Fig. 5 is an image of a Western blot of NRRP genome expression compared to the genome expression of a virus exposed to a UV dose of 20,000 $mJ/cm^2$, where loss of cytotoxicity was observed, and a live virus as a control. Reference to 1x or 2x refers to the amount of protein loaded onto the gel. Proteins were extracted 15h post infection.

**[0032]** Fig 6A is a set of fluorescent and brightfield images of Vero cells treated with chemically- generated, or busulfan-generated, NRRPs.

**[0033]** Fig. 6B is a brightfield microscopy image of Vero cells treated with busulfan alone, at the same dose used to generate NRRPs in Fig. 6A, for 15 hours.

**[0034]** Fig. 6C is a set of fluorescent and brightfield images of Vero cells treated with Live VSV-GFP.

**[0035]** Fig, 7A is a set of brightfield and fluorescent images of Vero cells treated with NRRPs, generated by taking 1E10 frozen wild type VSV and irradiating this preparation with 15 kGy Cobalt-60.

**[0036]** Fig. 7B is a set of brightfield and fluorescent images of Vero cells treated with live wild type VSV-GFP.

**[0037]** Fig. 7C is a set of brightfield and fluorescent images of Vero cells in PBS.

**[0038]** Fig. 8A is a set of brightfield images of L1210 and HDF cells treated with PBS or NRRPs at an MOI of 100.

**[0039]** Fig. 8B is a graph showing resazurin quantification of viability in leukemia and normal cell lines. Murine cell lines are denoted by *.

**[0040]** Fig. 8C is a set of fluorescent microscopy images of PBS, live VSV-GFP, or NRRP treatment in murine human Jurkat T-cell acute leukemia, murine A20 B-cell lymphoblastic leukemia, A301 T-cell lymphoblastic leukemia, and HL60 acute promyelocytic leukemia and GM38 and HDF normal cell lines.

**[0041]** Fig. 9 is a set of graphs showing the flow cytometry analysis of Annexin V-APC and 7-AAD staining in L1210 cells treated with PBS or NRRPs.

**[0042]** Fig. 10 is a graph illustrating cell viability following a resazurin quantification assay for L1210 acute leukemia cell line taken 72 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with bendamustine(300 μM).

**[0043]** Fig. 11 is a graph illustrating cell viability following a resazurin quantification assay for L1210 acute leukemia cell line taken 72 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with dexamethasone (45 μM).

**[0044]** Fig. 12 is a graph illustrating cell viability following a resazurin quantification assay for L1210 acute leukemia cell line taken 72 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with doxorubicin (0.025 μM).

**[0045]** Fig. 13 is a graph illustrating cell viability following a resazurin quantification assay for L1210 acute leukemia cell line taken 72 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with vincristine (0.0125 μM).

**[0046]** Fig. 14 is a graph illustrating cell viability following a resazurin quantification assay for K562 Ph-positive myeloid leukemic cell line taken 15 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with idarubicin (0.05 μM).

**[0047]** Fig. 15A is an illustration of a phenomenological model developed by Le Boeuf et al. to simulate NRRPs cytotoxicity in normal cells and tumors with defects in antiviral signaling pathways. To describe NRRP kinetics, the original model was modified by removing virus replication (X). Hashed lines describe the IFN-defects associated with tumor cells.

**[0048]** Fig. 15B is a graph showing the simulated relationship between defects in the antiviral signaling pathway and viability post-treatment with NRRPs at 72hrs.

**[0049]** Fig. 15C is a graph showing the in vitro relationship between MOI and viability 72h post-infection with NRRPs in normal HDF cells in the presence or absence of IFN.

**[0050]** Fig. 15D is a graph showing the in vitro relationship between MOI and viability 72h post-treatment with NRRPs in leukemic L1210 cells in the presence or absence of IFN.

**[0051]** Fig. 16A is a set of brightfield microscopy images of two Chronic Myeloid Leukemia-blast crisis patient samples treated with PBS or NRRPs.

**[0052]** Fig. 16B is a set of fluorescent microscopy images (4X) of acute leukemia (CML blast-crisis) from human patient peripheral blood samples. Leukemia enriched samples collected from peripheral blood treated with PBS, Live VSV-GFP, or NRRPs encoded for GFP. Images are 24h post infection at MOI=100.

**[0053]** Fig. 16C is a flow cytometry diagram complementing the data presented in Fig. 16A and 16C of Annexin-V and CD33 staining in two CML-blast crisis patient samples treated with PBS or NRRPs (MOI=100) 48h post-treatment. The $CD33^{+}$ blast population was enriched by long term culture of the cells.

**[0054]** Fig. 16D are graphs showing flow cytometry analysis of CD33 staining in the two CML-blast crisis patient samples treated with PBS or NRRPs.

**[0055]** Fig. 17A is a set of brightfield microscopy images of a healthy bone marrow sample treated with PBS or NRRPs for 18 hours.

**[0056]** Fig. 17B is a graph showing the quantification of Annexin-V staining in the healthy bone marrow sample treated with PBS or NRRPs for 65 hours.

**[0057]** Fig. 18A is a graph showing the survival curve in a murine blast crisis treatment model. Following L1210 challenge in mice on day 1, mice received three daily doses NRRPs or PBS.

**[0058]** Fig. 18B is a set of graphs showing Luminex-based quantification of cytokines induced by NRRPs in L1210 bearing mice during acute blast crisis. All identified cytokines are induced over 2 fold by NRRP-treated mice and are statistically significant (non-paired t-test $pV<0.05$). pV has been corrected to account for multiple hypothesis testing (Benjamini & Hochberg Method).

**[0059]** Fig. 19 is a graph showing the survival curve in a murine immunocompetent model of immunogenic apoptosis. Prior to L1210 challenge on day 1, mice received three weekly doses of γ-irradiated L1210 cells incubated or not incubated with NRRPs.

**[0060]** Fig. 20 is a set of brightfield microscopy images of myeloma cell lines MPC-11 and RPMI-8226 taken 15 hours post treatment with PBS or NRRPs. NRRPs were administered at an MOI=250, a dose previously determined to have no impact on normal cell viability.

**[0061]** Fig. 21 is a graph showing cell viability following an resazurin quantification assay for myeloma cell lines MPC-11 and RPMI-8226 taken 15 hours post treatment with NRRPs administered at an MOI = 250. SR4987 is a normal marrow stromal cell line.

**[0062]** Fig. 22 is a graph illustrating cell viability following a resazurin quantification assay for MPC-11 multiple myeloma cell line taken 72 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with melphalan (20μM).

**[0063]** Fig. 23 is a graph illustrating cell viability following a resazurin quantification assay for MPC-11 multiple myeloma cell line taken 72 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with the second mitochondria-derived activator of caspase (SMAC) mimetic, LCL161(15μM).

**[0064]** Fig. 24 is a graph illustrating cell viability following a resazurin quantification assay for RPMI-8226 multiple myeloma cell line taken 72 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with carfilzomib (5nM).

**[0065]** Fig. 25A is a set of brightfield microscopy images of a mouse delayed brain tumor glioblastoma cell line (DBT) taken 24 hrs post treatment with PBS or NRRPs.

**[0066]** Fig. 25B is a set of brightfield microscopy images of an astrocytoma cell line (K1491) taken 24 hrs post treatment with PBS or NRRPs.

**[0067]** Fig. 25C is a set of brightfield microscopy images of a mouse glioma cell line (GL261) taken 24 hrs post treatment with PBS or NRRPs.

**[0068]** Fig. 26 is a graph showing cell viability following a resazurin quantification assay for brain cancer cell lines DBT, K1491, K1492, CT2A, and GL261 relative to normal HDFN control.

**[0069]** Fig. 27 is a graph illustrating cell viability following a resazurin quantification assay for CT2A glioblastoma cell line taken 72 hours post treatment with UV-generated NRRPs and the combinatorial effect of UV-generated NRRPs with the HDAC inhibitor SAHA (10μM).

**[0070]** Fig. 28A is a set of fluorescent microscopy images (4X) of NRRP-mediated tumor cell cytotoxicity in resistant solid tumor cell lines. The set of images show mouse mammary or breast (4T1) and human kidney (786-0) cancer cells treated with PBS, Live VSV, and NRRPs. Images were taken at 24h post infection.

**[0071]** Fig. 28B is a set of brightfield microscopy images taken at 72h post infection of NRRP-mediated tumor cell cytotoxicity in resistant solid tumor cell lines, in breast (4T1) and kidney (786-0) cancer cells treated with PBS, Live VSV, and NRRPs.

**[0072]** Fig. 28C is a graph showing resazurin quantification of cellular viability in resistant solid tumor cell lines, in breast (4T1) and kidney (786-0) cancer cells treated with PBS, Live VSV, and NRRPs, 72h post infection.

**[0073]** Fig. 29 is a graph illustrating survival advantage in sub-cutaneous CT-26 colon cancer treated with 2E9 UV-generated NRRPs on days 16, 18 and 21 post tumor embedment.

## DETAILED DESCRIPTION

**[0074]** Generally, the present disclosure provides a non-replicating virus-derived particle and its use as an anti-cancer agent. A non-replicating virus-derived particle (NRVP) is a virus-derived particle that is able to bind and be internalized by a cell, but has been modified to prevent formation, or substantially reduce formation, of new virus particles when the NRVP is in the cell. One example of a NRVP is a non-replicating rhabdovirus-derived particle (NRRP).

**[0075]** The NRVP includes: an envelope having a sufficient number of functional G proteins on the surface of the envelope to allow the virus-derived particle to bind a surface of a cell and be internalized. It also includes an RNA polynucleotide with a sequence that encodes all the proteins required for new virus particle assembly, and a mixture of proteins that form a structure around the RNA polynucleotide. However, the RNA structure of the NRVP is sufficiently cross-linked, or has been cleaved to form discontinuous segments of RNA, such that the NRVP genome is unable be used to produce the proteins required for

new virus formation. For example, the RNA sequence may not be transcribed into mRNA, translated into protein, or both when the particle is in a cell. The impairment or lack of transcription and/or translation means that insufficient proteins are produced in the cell and new virus particles cannot be assembled.

**[0076]** The functional G protein may have a sequence that includes SEQ ID NO: 1, shown below, which is the sequence of the glycoprotein mature peptide of vesicular stomatitis Indiana virus. This functional G protein has NCBI accession number NP 955548.1.

kftivfphnq kgnwknvpsn yhycpsssdl nwhndligta iqvkmpkshk aiqadgwmch
askwvttcdf rwygpkyitq sirsftpsve qckesieqtk qgtwlnpgfp pqscgyatvt
daeavivqvt phhvlvdeyt gewvdsqfin gkcsnyicpt vhnsttwhsd ykvkglcdsn
lismditffs edgelsslgk egtgfrsnyf ayetggkack mqyckhwgvr lpsgvwfema
dkdlfaaarf pecpegssis apsqtsvdvs liqdverild yslcqetwsk iraglpispv
dlsylapknp gtgpaftiin gtlkyfetry irvdiaapil srmvgmisgt tterelwddw
apyedveigp ngvlrtssgy kfplymighg mldsdlhlss kaqvfehphi qdaasqlpdd
eslffgdtgl sknpielveg wfsswkssia sfffiiglii glflvlrvgi hlciklkhtk
krqiytdiem nrlgk (SEQ ID NO: 1)

**[0077]** Alternatively, the functional G protein may have a sequence that is at least 75% identical to SEQ ID NO: 1 so long as it is capable of binding to a surface of a cell and effecting internalization of the particle. For example, conservative substitutions of amino acids may be made without abrogating the ability of the protein to bind to the surface of a cell and effect internalization of the particle. Examples of conservative substitutions are shown below in Table 1.

| Original Residues | Exemplary Substitutions | Preferred Substitutions |
|---|---|---|
| Ala | Val, Leu, Ile | Val |
| Arg | Lys, Gln, Asn | Lys |
| Asn | Gln | Gln |
| Asp | Glu | Glu |
| Cys | Ser, Ala | Ser |
| Gln | Asn | Asn |
| Glu | Asp | Asp |
| Gly | Pro, Ala | Ala |
| His | Asn, Gln, Lys, Arg | Arg |
| Ile | Leu, Val, Met, Ala, Phe, Norleucine | Leu |
| Leu | Norleucine, Ile, Val, Met, Ala, Phe | Ile |
| Lys | Arg, 1,4 Diamino-butyric Acid, Gln, Asn | Arg |
| Met | Leu, Phe, Ile | Leu |
| Phe | Leu, Val, Ile, Ala, Tyr | Leu |
| Pro | Ala | Gly |
| Ser | Thr, Ala, Cys | Thr |
| Thr | Ser | Ser |
| Trp | Tyr, Phe | Tyr |
| Tyr | Trp, Phe, Thr, Ser | Phe |
| Val | Ile, Met, Leu, Phe, Ala, Norleucine | Leu |

**Table 1 – Conservative Amino Acid Substitutions**

**[0078]** Less conservative substitutions may be made in portions of the G protein that do not take part in the cell surface binding (such as in a trans-membrane domain), while more conservative substitutions might be required in portions of the protein that interact with a G protein receptor. G proteins are known in the art and a skilled person would be able to determine what amino acid substitutions would be possible without abrogating the ability of the protein to bind to the surface of a cell and effect internalization of the particle.

**[0079]** The mixture of proteins that form a structure around the RNA may include at least N, P, M, and L proteins. A NRVP having N, P, M, G and L proteins may include rhabdovirus-derived NRVP. Rhadbovirus-derived NRVPs may be referred to as non-replicating rhabdovirus -derived particles (NRRPs). For the purposes of the present disclosure, the term "Rhabdovirus" (NCBI Taxonomy ID: 11270) may include any one of the following genus of viruses and variants thereof: Cytorhabdovirus (NCBI Taxonomy ID: 11305), Ephemerovirus (NCBI Taxonomy ID: 32613), Vesiculovirus (NCBI Taxonomy ID:

11271), unclassified Dimarhabdovirussupergroup (NCBI Taxonomy ID: 349166), Lyssavirus (NCBI Taxonomy ID: 11286), Novirhabdovirus (NCBI Taxonomy ID: 186778), Nucleorhabdovirus (NCBI Taxonomy ID: 11306 ), unassigned rhabdovirus (NCBI Taxonomy ID: 686606) and unclassified rhabdovirus (NCBI Taxonomy ID: 35303). Species within the Rhabdovirus family include, but are not limited to, Maraba virus, Vesicular stomatitis virus (VSV) and Farmington virus.

**[0080]** The N protein may have a sequence that includes SEQ ID NO: 2, shown below, which is the sequence of the nucleocapsid protein of vesicular stomatitis Indiana virus. This N protein has NCBI accession number NC 041712.1.

msvtvkriid ntvivpklpa nedpveypad yfrkskeipl yinttkslsd lrgyvyqglk
sgnvsiihvn sylygalkdi rgkldkdwss fginigkagd tigifdlvsl kaldgvlpdg
vsdasrtsad dkwlplyllg lyrvgrtqmp eyrkklmdgl tnqckmineq feplvpegrd
ifdvwgndsn ytkivaavdm ffhmfkkhec asfrygtivs rfkdcaalat fghlckitgm
stedvttwil nrevademvq mmlpgqeidk adsympylid fglsskspys svknpafhfw
gqltalllrs trarnarqpd dieytsltta gllyayavgs sadlaqqfcv gdnkytpdds
tgglttnapp qgrdvvewlg wfedqnrkpt pdmmqyakra vmslqglrek tigkyaksef
dk (SEQ ID NO: 2)

**[0081]** Alternatively, the N protein may have a sequence that is at least 80% identical to SEQ ID NO: 2 so long as it is capable of participating in the formation of the protein structure. For example, conservative substitutions of amino acids may be made without abrogating the ability of the protein to participate in the formation of the protein structure. Examples of conservative substitutions are shown in Table 1.

**[0082]** The P protein may have a sequence that includes SEQ ID NO: 3, shown below, which is the sequence of the NS protein of vesicular stomatitis Indiana virus. This P protein has NCBI accession number NC 041713.1.

mdnltkvrey lksysrldqa vgeideieaq raeksnyelf qedgveehtk psyfqaadds
dtesepeied nqglyaqdpe aeqvegfiqg plddyadeev dvvftsdwkp pelesdehgk
tlrltspegl sgeqksqwls tikavvqsak ywnlaectfe asgegvimke rqitpdvykv
tpvmnthpsq seavsdvwsl sktsmtfqpk kaslqpltis ldelfssrge fisvggdgrm
shkeaillgl rykklynqar vkysl (SEQ ID NO: 3)

**[0083]** Alternatively, the P protein may have a sequence that is at least 80% identical to SEQ ID NO: 3 so long as it is capable of participating in the formation of the protein

structure. For example, conservative substitutions of amino acids may be made without abrogating the ability of the protein to participate in the formation of the protein structure. Examples of conservative substitutions are shown in Table 1.

**[0084]** The M protein may have a sequence that includes SEQ ID NO: 4, shown below, which is the sequence of the matrix protein of vesicular stomatitis Indiana virus. This M protein has NCBI accession number NC 041714.1.

msslkkilgl kgkgkkskkl giapppyeed tsmeyapsap idksyfgvde mdtydpnqlr
yekffftvkm tvrsnrpfrt ysdvaaavsh wdhmyigmag krpfykilaf lgssnlkatp
avladqgqpe yhthcegray lphrmgktpp mlnvpehfrr pfniglykgt ieltmtiydd
esleaapmiw dhfnsskfsd frekalmfgl ivekkasgaw vldsishfk (SEQ ID NO: 4)

**[0085]** Alternatively, the M protein may have a sequence that is at least 80% identical to SEQ ID NO: 4 so long as it is capable of participating in the formation of the protein structure. For example, conservative substitutions of amino acids may be made without abrogating the ability of the protein to participate in the formation of the protein structure. Examples of conservative substitutions are shown in Table 1.

**[0086]** The L protein may have a sequence that includes SEQ ID NO: 5, shown below, which is the sequence of the polymerase protein of vesicular stomatitis Indiana virus. This L protein has NCBI accession number NC 041716.1.

mevhdfetde fndfneddya treflnpder mtylnhadyn lnsplisddi dnlirkfnsl
pipsmwdskn wdgvlemlts cqanpistsq mhkwmgswlm sdnhdasqgy sflhevdkea
eitfdvvetf irgwgnkpie yikkerwtds fkilaylcqk fldlhkltli lnavsevell
nlartfkgkv rrsshgtnic rirvpslgpt fisegwayfk kldilmdrnf llmvkdviig
rmqtvlsmvc ridnlfseqd ifsllniyri gdkiverqgn fsydlikmve picnlklmkl
aresrplvpq fphfenhikt svdegakidr girflhdqim svktvdltlv iygsfrhwgh
pfidyytgle klhsqvtmkk didvsyakal asdlarivlf qqfndhkkwf vngdllphdh
pfkshvkent wptaaqvqdf gdkwhelpli kcfeipdlld psiiysdksh smnrsevlkh
vrmnpntpip skkvlqtmld tkatnwkefl keidekgldd ddliiglkgk erelklagrf
fslmswklre yfviteylik thfvpmfkgl tmaddltavi kkmldsssgq glksyeaici
anhidyekwn nhqrklsngp vfrvmgqflg ypslierthe ffeksliyyn grpdlmrvhn
ntlinstsqr vcwqgqeggl eglrqkgwti lnllviqrea kirntavkvl aqgdnqvict
qyktkksrnv velqgalnqm vsnnekimta ikigtgklgl linddetmqs adylnygkip
ifrgvirgle tkrwsrvtcv tndqiptcan imssvstnal tvahfaenpi namiqynyfg

tfarlllmmh dpalrqslye vqdkipglhs stfkyamlyl dpsiggvsgm slsrfliraf
pdpvteslsf wrfihvhars ehlkemsavf gnpeiakfri thidklvedp tslniamgms
panllktevk kcliesrqti rnqvikdati ylyheedrlr sflwsinplf prflsefksg
tflgvadgli slfqnsrtir nsfkkkyhre lddlivrsev ssllhlgklh lrrgsckmwt
csathadtlr ykswgrtvig ttvphpleml gpqhrketpc apcntsgfny vsvhcpdgih
dvfssrgplp aylgsktses tsilqpwere skvplikrat rlrdaiswfv epdsklamti
lsnihsltge ewtkrqhgfk rtgsalhrfs tsrmshggfa sqstaaltrl mattdtmrdl
gdqnfdflfq atllyaqitt tvardgwits ctdhyhiack sclrpieeit ldssmdytpp
dvshvlktwr ngegswgqei kqiyplegnw knlapaeqsy qvgrcigfly gdlayrksth
aedsslfpls iqgrirgrgf lkglldglmr asccqvihrr slahlkrpan avyggliyli
dklsvsppfl sltrsgpird eletiphkip tsyptsnrdm gvivrnyfky qcrliekgky
rshysqlwlf sdvlsidfig pfsisttllq ilykpflsgk dknelrelan lssllrsgeg
wedihvkfft kdillcpeei rhackfgiak dnnkdmsypp wgresrgtit tipvyytttp
ypkmlemppr iqnpllsgir lgqlptgahy kirsilhgmg ihyrdflscg dgsggmtaal
lrenvhsrgi fnsllelsgs vmrgaspepp saletlggdk srcvngetcw eypsdlcdpr
twdyflrlka glglqidliv mdmevrdsst slkietnvrn yvhrildeqg vliyktygty
iceseknavt ilgpmfktvd lvqtefsssq tsevymvckg lkklidepnp dwssineswk
nlyafqsseq efarakkvst yftltgipsq fipdpfvnie tmlqifgvpt gvshaaalks
sdrpadllti slfymaiisy yninhirvgp ippnppsdgi aqnvgiaitg isfwlslmek
diplyqqcla viqqsfpirw eavsvkggyk qkwstrgdgl pkdtrtsdsl apignwirsl
elvrnqvrln pfneilfnql crtvdnhlkw snlrrntgmi ewinrriske drsilmlksd
lheenswrd (SEQ ID NO: 5)

**[0087]** Alternatively, the L protein may have a sequence that is at least 70% identical to SEQ ID NO: 5 so long as it is capable of participating in the formation of the protein structure. For example, conservative substitutions of amino acids may be made without abrogating the ability of the protein to participate in the formation of the protein structure. Examples of conservative substitutions are shown in Table 1.

**[0088]** In some examples, the NRVP may produce functional N, P, M and G proteins after the NRVP binds and is internalized by the cell. However, the NRVP lacks the ability, or has a reduced ability, to produce functional L protein. Without functional L protein, or without the correct amount of functional L protein, new virus particles cannot be assembled.

**[0089]** In other examples, the NRVP may produce functional N, P, and M proteins after the NRVP binds and is internalized by the cell. However, the NRVP lacks the ability, or has a reduced ability, to produce functional G and L proteins. Without functional G and L proteins, or without the correct amounts or ratios of functional G and L proteins, new virus particles cannot be assembled.

**[0090]** In still other examples, the NRVP may produce functional N and P proteins after the NRVP binds and is internalized by the cell. However, the NRVP lacks the ability, or has a reduced ability, to produce functional M, G and L proteins. Without functional M, G and L proteins, or without the correct amounts or ratios of functional M, G and L proteins, new virus particles cannot be assembled.

**[0091]** In still other examples, the NRVP may produce functional N protein after the NRVP binds and is internalized by the cell. However, the NRVP lacks the ability, or has a reduced ability, to produce functional P, M, G and L proteins. Without functional P, M, G and L proteins, or without the correct amounts or ratios of functional P, M, G and L proteins, new virus particles cannot be assembled.

**[0092]** In yet other examples, the NRVP lacks the ability, or has a reduced ability, to produce functional N, P, M, G and L proteins. Without functional N, P, M, G and L proteins, or without the correct amounts or ratios of functional N, P, M, G and L proteins, new virus particles cannot be assembled.

**[0093]** In order for the non-replicating virus-derived particle to be able to bind the surface of a cell and be internalized, the NRVP must have sufficient number of functional G proteins on the envelope of the virus particle. It is expected that a NRVP having at least 5% of the number of G proteins found on the wild-type virus particle would still be able to bind a cell and be internalized. Preferably, the NRVP would have at least 50% of the number of G proteins found on the wild-type virus particle, and more preferably the NRVP would have at least 100% of the number of G proteins found on the wild-type virus particle. In specific examples, the NRVP has at least 60 functional G proteins per particle, at least 600 functional G proteins per particle, or at least 1200 functional G proteins per particle.

**[0094]** As noted above, the NRVP includes RNA having a sequence that encodes all the proteins required for new virus particle assembly. One reason that the RNA sequence may be unable to produce those proteins when the NRVP is in a cell is if the RNA is cross-linked to such an extent that protein production is reduced or stopped. In some examples, at

least 0.05% cross-linked nucleotides may be sufficient to reduce or stop protein production from the RNA sequence. In other examples, the cross-linked RNA may include at least 0.5% cross-linked nucleotides. It may be preferable to have at least 1% of the nucleotides cross-linked, and more preferable to have at least 10% or at least 20% of the nucleotides cross-linked.

**[0095]** Cross-linking the nucleotides may increase the likelihood of rendering G-proteins unable to bind a cell surface. Accordingly, it may be preferable that less than 80% of the nucleotides be cross-linked.

**[0096]** The nucleotides in the RNA structure may be cross-linked to other RNA nucleotides, to amino acids in a protein in the protein structure around the RNA, or both.

**[0097]** In addition to the cross-linked RNA structure, the protein structure around the RNA may include a protein that has an amino acid that is: cross-linked to another protein of the protein structure; cross-linked to another amino acid of the same protein; cross-linked to the RNA structure; or any combination thereof.

**[0098]** Furthermore, the NRVP RNA structure may be unable to replicate by ablating the function of the NRVP RNA polymerase activity encoded by the P and L proteins. This can be effected by sufficient cross-linking of the P and L proteins to the RNA structure, by cross-linking the P and L proteins to other proteins, or by damaging NRVP protein structure such that the function of the P and L proteins are negatively affected.

**[0099]** Another reason that the RNA sequence may be unable to produce those proteins when the NRVP is in a cell is if the RNA structure has been cleaved to form discontinuous segments of RNA. RNA viruses, such as rhabdoviruses, have a single continuous RNA polynucleotide that includes the sequences of all of the genes that encode the proteins required for viral replication. Cleaving the single continuous polynucleotide into two or more discontinuous RNA polynucleotides results defective genome transcription, translation, or both. Proteins that are encoded on a polynucleotide without a transcription initiation site cannot be transcribed. Furthermore, the genome cannot undergo full-length replication and cannot be properly incorporated into a nascent virus particle, thereby preventing virus particle production.

**[00100]** NRVPs may include at least two discontinuous RNA polynucleotides, only one of which comprises a transcription initiation site. However, it may be preferable to cleave the RNA into more than two segments. Accordingly, NRVPs preferably include at least five, more

preferably at least 10, and even more preferably at least 100 discontinuous RNA polynucleotides.

**[00101]** RNA viruses may have an RNA sequence with on the order of 11,000 nucleotides. In RNA viruses having RNA sequences with 11,000 nucleotides or more, it may be desirable to cleave the RNA into segments of no more than 10,000 nucleotides. A NRVP resulting from the cleavage of an RNA virus with 11,000 nucleotides could then have at least one RNA segment of less than 10,000 nucleotides and another RNA segment of less than 1,000 nucleotides. Since only one of the segments includes the transcription initiation site, or since the protein encoding sequence is discontinuous, the other of the segments cannot be transcribed or translated, and any proteins encoded on that segment would not be produced.

**[00102]** It may be preferable to cleave the RNA into smaller portions. For example, the discontinuous RNA polynucleotides may be no more than 7000, no more than 5000, no more than 3000, or no more than 1000 nucleotides.

**[00103]** A non-replicating virus-derived particle is produced from a live virus that includes RNA having a sequence that encodes N, P, M, G and L proteins by: optionally separating the virus-derived particle from a UV absorbing compound; and then subjecting the live virus to an RNA damaging agent to either cross-link the RNA structure, or cleave the RNA structure, thus preventing the RNA from producing sufficient proteins required for new virus particle assembly.

**[00104]** The RNA structure of the live virus is sufficiently cross-linked so that, when the virus-derived particle is in a cell: RNA transcription into mRNA is reduced; mRNA translation into protein is reduced; or both. Similarly, the RNA structure of the live virus is cleaved into sufficiently discontinuous RNA segments so that, when the virus-derived particle is in a cell: RNA transcription into mRNA is reduced; mRNA translation into protein is reduced; or both.

**[00105]** Cross-linking the RNA may be achieved by subjecting the live virus to electromagnetic radiation. The electromagnetic radiation may have a wavelength of less than about 1 mm. The energy associated with electromagnetic radiation increases as the wavelength decreases. Increased energy is associated with damage to DNA, evidenced by increased cancer rates on exposure to UV light, X-rays, and gamma radiation. Accordingly, it is preferable if the electromagnetic radiation has a wavelength of less than about 500 mm, and more preferable if the wavelength is less than about 280 nm. In particular examples, the wavelength is between about 0.1 picometers and 280 nm.

**[00106]** It may be especially desirable to use electromagnetic radiation having a wavelength between about 100 and about 280 nm as such a wavelength preferably induces cross-linking in nucleotides over cross-linking in proteins. When the electromagnetic radiation is in the UV spectrum, i.e. between about 100 nm and about 400 nm, the solution containing the live virus may be subjected to a dose of electromagnetic radiation between about 100 $mJ/cm^2$ and about 8,000 $mJ/cm^2$. Preferably, the dose is between about 150 $mJ/cm^2$ and about 5,000 $mJ/cm^2$. Even more preferably, the dose is between about 150 $mJ/cm^2$ and about 1,000 $mJ/cm^2$. Still even more preferably, the dose is between about 150 $mJ/cm^2$ and about 500 $mJ/cm^2$. Most preferably, the dose is between about 150 $mJ/cm^2$ and about 300 $mJ/cm^2$.

**[00107]** The actual dose may be dependent on the characteristics of the solution. For example, if the solution includes dyes that absorb UV light, then a greater dose is required. Similarly, if the solution is irradiated from a single point and the container is large, there may be live virus that is not exposed to the full intensity of the UV light. In such a situation, a greater dose or stirring the solution may be beneficial. A skilled person would be able to determine the parameters necessary for providing an appropriate dose.

**[00108]** In situations where the media holding the live virus is turbid, includes dye, or otherwise absorbs UV light, it may be desirable to irradiate the live virus with x-rays (i.e. electromagnetic radiation having a wavelength between 0.01 and 10 nm) or gamma rays (i.e. electromagnetic radiation having a wavelength less than 10 picometers). When the electromagnetic radiation is gamma irradiation, the live virus may be subjected to a dose between about 1 kGy and about 50 kGy. More preferably, the dose is between about 5 kGy and about 20 kGy. The gamma radiation may be generated from cobalt-60.

**[00109]** The live virus may be subjected to the electromagnetic radiation at a temperature of 4 °C or lower. For example, the live virus may be subjected to UV radiation at a temperature of about 4 °C. In another example, the live virus may be subjected to gamma radiation at a temperature of about -80 °C. In yet another example, the live virus may be subjected to gamma radiation at a temperature of about -130 °C.

**[00110]** As noted above, the RNA structure may be cross-linked, or cleaved into sufficiently discontinuous RNA segments, to reduce or prevent RNA transcription into mRNA; mRNA translation into protein; or both. In addition to the electromagnetic radiation discussed above, this may be achieved by exposing the live virus to a chemical agent, such as an

alkylating agent capable of crosslinking RNA, or a free radical forming agent capable of cleaving RNA. Examples of such cross-linking agents include busulfan, cyclophosphamide, melphalan, formaldehyde, carbodiimide and bissulfosuccinimidyl suberate . Examples of free radical forming agents include peroxides, hydrogen bromine, ammonium persulfate and hydroxyl radical.

**[00111]** The live virus may be separated from a UV-absorbing compound by fractionating the growth medium used to generate the viral particles. The growth medium maybe fractionated, for example, in a sucrose gradient. Once the NRVP has been prepared, the NRVP may be separated by fractionating or filtering the diluent containing the virus-derived particles. The diluent may be fractionated, for example, in a sucrose gradient or filtered by tangential flow filtration.

**[00112]** The present disclosure also includes a method of stimulating an immune response by administering non-replicating virus-derived particles as described above to a patient. The administration of the NRVPs induces expression and release of cytokines in the patient. Exemplary cytokines which may be released in the patient include: interleukins, interferons, inflammatory cytokines, members of the CXC chemokine family, members of the tumor necrosis factor family, or any combination thereof. These factors can result in the presentation or recognition of tumor antigens.

**[00113]** The disclosure also includes a method of inducing cell death of cancerous cells in a patient. The method includes administering non-replicating virus-derived particles as described above to the patient.

**[00114]** The disclosure further includes a method of preferentially inducing cell death in cancerous cells or non-cancerous cells. The method includes administering non-replicating virus-derived particles as described above to the patient.

**[00115]** The cell death may be through apoptosis, for example caused by the presence of the NRVPs, or constituents of the NRVPs, in the cell. Alternatively, the cell death may be due to recruitment of innate immune effector cells, adaptive immune effector cells, or any combination thereof, for example caused by cytokines released by the cell. The adaptive immune effector cells may be T-cells, B-cells, or both. The innate immune effector cells may include mast cells, phagocytes (such as macrophages, neutrophils, or dendritic cells), basophils, eosinophils, natural killer cells, γδ T cells, or any combination thereof.

**[00116]** The patient is treated with sufficient numbers of NRVPs to stimulate the immune response or induce cell death of cancerous cells. Since the NRVPs do not form live virus particles, it is desirable to administer the NRVPs in an amount that is greater than treatments with replication competent viruses. The patient may be administered with 1E10 to 1E15 non-replicating virus-derived particles, though in preferred examples the patient is administered with 1E11 to 1E13 non-replicating virus-derived particles.

**[00117]** There may be a synergistic benefit when combining treatment of a patient with NRVPs and treatment with a chemotherapeutic agent. The chemotherapeutic may be, for example: bendamustine, dexamethasone, doxorubicin, vincristine, imatinib, disatinib or idarubicin. These agents may improve sensitivity to NRVP-mediated apoptosis, enhance cytokine secretion, improve anti-tumor immune responses, promote vascular shutdown, or any combination thereof.

**[00118]** NRVPs may be used to treat solid tumors or non-solid tumors, such as leukemia. However, since NRVPs do not form live virus particles in a cell, it is especially desirable to expose all cancer cells to the injected NRVPs. This is in contrast to administration of replication competent viruses, where exposure of a portion of the cancer cells to the injected virus results in production of additional virus and subsequent exposure of the remaining cancer cells to the generated virus particles.

**[00119]** In view of the lack of production of virus particles, it is preferable to use NRVPs to treat leukemia since intravenous administration of the NRVPs results in a substantial fraction of the leukemic cells being exposed to the particles. In contrast, with solid tumors, a portion of the cells in the solid tumor may not be exposed to the injected NRVPs. The mode of administration of the non-replicating virus-derived particles may be determined by the cancer to be treated. The NRVPs may be administered to the patient intratumorally, intranasally, intramuscularly, intradermally, intraperitoneally, intra-arterially, intravenously, subcutaneously or intracranially.

**[00120]** Non-replicating virus-derived particles (NRVPs) of the present disclosure may be formed from wild type Rhabdovirus particles modified so as to lack the ability to spread between cells. The non-replicating Rhabdovirus-derived particle may be derived from a replication competent wild type Rhabdovirus particle. Once modified, the NRRP cannot sustain virion replication. NRRPs may retain cytolytic tropism against immortalized cells.

Specific examples of NRRPs have innate and/or adaptive immune-stimulating properties against immortalized cells.

**[00121]** For the purposes of the present disclosure, the expression "immortalized cells" means cells with unchecked cell division, and includes, without limitation, hyperproliferative cells, tumor or cancer cells and transformed immortalized cells. Hyperproliferative cell(s) refer to any neoplasm or any chronically infected cell or tissue. The neoplasm can be, for instance, any benign neoplasm, cystic neoplasm, carcinoma in situ, malignant neoplasm, metastatic neoplasm, or secondary neoplasm. The hyperproliferative cell may be a hematopoietic cancer cell or a cell from a solid tumor.

**[00122]** NRRPs according to the present disclosure may retain cytolytic tropism against immortalized cells. This means that NRRPs will induce cell death preferentially in immortalized cells such as tumor or cancer cells and transformed immortalized cells.

**[00123]** The wild type Rhabdovirus may be modified to generate the NRRP by a means that disrupts its genome replication and/or expression. This means that genome replication and/or expression is decreased over parental baseline expression. Genome expression could also be ablated.

**[00124]** To disrupt genome expression of the wild type Rhabdovirus, electromagnetic (EM) irradiation can be used. Electromagnetic irradiation may include UV irradiation, infrared, X-ray, gamma and other types of irradiation in the EM spectrum such as UVC (200-280 nanometer). Chemical-induced disruption can also be used to disrupt genome expression of the wild type Rhabdovirus. For example, a genome-damaging agent such as busulfan can be used.

**[00125]** The EM dose required to sufficiently disrupt genome expression of the wild type Rhabdovirus will be method dependent, and will vary according to parameters such as virus concentration, turbidity of the virus stock preparation, volume used, the presence of contaminants or purity of the virus stock preparation, the diluent used, and the receptacle in which the virus preparation is stored for the procedure (plastic, glass, etc.). Chemical dosing may also be affected by various parameters.

**[00126]** In one example, 50µl of a 1E10 PFU/ml stock of the wild type Rhabdoviruses purified using the sucrose cushion method was irradiated at 250 $mJ/cm^2$ (for about 40 seconds).

**[00127]** The present disclosure further provides a non-replicating Rhabdovirus-derived particle that has been made from a wild type Rhabdovirus-derived particle. The wild type virus has been modified to lack the ability to spread between cells but to retain innate and/or adaptive immune-stimulating properties.

**[00128]** The present disclosure also provides for a use of a NRVP, and specifically a NRRP, to treat a population of immortalized cells.

**[00129]** For the purposes of the present disclosure, "treat" would be understood to mean applications where the NRVP or NRRP is used alone or in combination with radiation therapies, chemotherapies, immuno-therapies, surgery, oncolytic virus-based therapies or other virus-based therapies.

**[00130]** A person skilled in the art will understand that "chemotherapies" includes, but is not limited to, therapies involving the use of mitotic inhibitors, IMiDS such as lenalidomide or pomalidomide, chromatin modifying agents, HDAC inhibitors such as SAHA, hypomethylating agents, alkylating agents, mTOR inhibitors, tyrosine kinase inhibitors, proteasome inhibitors, antimetabolites, DNA damaging or DNA regulating agents, phosphodiesterase inhibitors, SMAC mimetics such as LCL161, corticosteroids and cytokine/chemokines.

**[00131]** For example, chemotherapy would include therapies that use: alkylating agents, DNA damaging agents or DNA regulating agents, mitotic inhibitors, tyrosine kinase inhibitors, proteasome inhibitors, IMiDS, antimetabolites, mTOR inhibitors, chromatin modifying agents, HDAC inhibitors, hypomethylating agents, phosphodiesterase inhibitors, corticosteroids and cytokines/chemokines. Specific chemotherapies include, but are not limited to; bendamustine, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, lomustine, melphalan, temozolomide, thiotepa, oxaliplatin, procarbazine, pentostatin, cladribine, clofarabine, cytarabine, fludarabine, gemcitabine, hydroxyurea, mercaptopurine, nelarabine, fluorouracil, bleomycin, dactinomycin, daunorubicin, doxorubicin, doxorubicin liposomal, idarubicin, mitoxantrone, capecitabine, topotecan, irinotecan, etoposide, paclitaxel, teniposide, thioguanine, omacetaxin, altretamine, asparaginase, asparaginase, pegaspargase, Isotretinoin, retinoic acid, arsenic, vinblastine, vincristine, vincristine liposomal, bosutinib, dasatinib, imatinib, nilotinib, sunitinib, vemurafenib, regorafenib, bortezomib, carfilzomib, thalidomide, lenalidomide, pomalidomide, methotrexate, pralatrexate, everolimus, Temsirolimus, vorinostat, romidepsin, valproic acid,

decitabine, azacitidine, anagrelide, cortisone, dexamethasone, prednisone and triamcinolone, interferon alfa 2a, interferon alfa 2b, peginterferon alfa 2b, interferon beta 1b, aldesleukin/IL-2, denileukin diftitox, granulocyte colony stimulating factor and granulocyte macrophage colony stimulating factor.

**[00132]** For the purposes of the present disclosure, the term "immunotherapies" shall mean immunotherapies targeting CD20 (such as rituximab, Ibritumomabtiuxetan and tositumomab), CD47, CD33, CD38, CD138, CS1, CD52 (such as alemtuzimab), VEGF (such as bevacizumab), Her2/Neu (such as Trastuzumab), EGFR (such as cetuximab and nimotuzumab), CTLA4 (such as ipilimumab) or IGF-1 (such as ganitumab). Other immunotherapies known to a person skilled in the art may also be included within the scope of the term "immuno-therapies".

**[00133]** The reference to "oncolytic virus-based therapies" includes those known in the art, including Pox virus-based therapies (Vaccinia-based viruses),Herpes Simplex Virus-based therapies (OncoVEXGM–CSF), Rhabdovirus- based therapies (MG1, VSV-IFNb, VSVd51), Reovirus (Reolysin), Adenovirus-based therapies (ONYX 015), Measles virus-based therapies, New Castle Disease virus-based therapies, Alpha virus-based therapies, and Parvovirus species-based therapies.

**[00134]** NRVPs and NRRPs can be administered intratumorally, intranasally, intramuscularly, intradermally, intraperitoneally, intra-arterially, intravenously, subcutaneously or intracranially.

**[00135]** The oncolytic properties of NRRPs in several different in-vitro and in-vivo models using two different Rhabdovirus-derived strains and several different cell types including patient samples were demonstrated, as discussed in greater detail below.

**[00136]** Tumor specific cytotoxicity was characterized in a number of assays including microscopy characterization of cellular phenotype, resazurin cytotoxicity quantification, and flow cytometry of tumor cell killing.

**[00137]** Using an immune-protection model against L1210 indicates that NRRP activation of programmed cell death pathways leads to the generation of innate and adaptive immune response against the tumor. As such, treatment with NRRPs does not require each cell to become infected to maintain efficacy, and therefore may be used as a treatment alone or as an adjuvant in an anticancer therapeutic regimens.

**[00138]** Luminex-based quantification of cytokines induced by NRRPs in L1210 bearing mice during acute blast crisis was also performed. All identified cytokines were induced over 2 fold by NRRP-treated mice and are statistically significant (non-paired t-test pV<0.05). pV has been corrected to account for multiple hypothesis testing (Benjamini & Hochberg Method).

**[00139]** This experiment also shows that NRRPs may be optimally effective when applied at a high NRRP to cell ratio (i.e., > 1). This higher dosing ensures that the majority of cells within a cell population encounter a cytotoxic NRRP. This contrasts live OV therapies, which rely on viral spread to hopefully achieve therapeutic efficacy, and inherently utilize a low OV to cell ratio to promote safe delivery to the recipient.

**Examples**

**[00140]** For all figures except Fig. 1A, NRRPs were generated by UVC-irradiation at a dose of 250 mJ/cm$^2$ of a 50µl sample of 1E10 PFU/ml of live VSV-GFP, purified using a sucrose cushion method where the virus preparation was centrifuged through a 20% (w/v) sucrose cushion in water (5 ml) at 148,000 × g for 120 minutes.

**[00141]** **Example 1: VSV-based NRRPs generated by irradiation with electromagnetic radiation**.

**[00142]** UV photonic damage of rhabdoviruses may be used to generate a non-replicating virus-derived particle that retained bioactivity. Using high-dose UV irradiation ablates the rhabdoviruses's genome, rendering the virus biologically inert. However, it has now been discovered that UV irradiation may be applied at a dose that still allows the virus to bind and be internalized by a cell, but stops, or substantially reduces, the ability of the particle to form new virus particles when the virus particle is in the cell. Accordingly, virus replication is lost, yet biological activities are maintained.

**[00143]** It was determined that irradiation of purified VSV (a Rhabdovirus) expressing green fluorescent protein with a dose between about 100 and about 1000 mJ/cm$^2$ dose of UV fluence generates a NRRP that retains cytolytic tropism against immortalized cells (Figs. 1A and 1B), but that lacks the ability to spread between cells (Fig. 2A).

**[00144]** A 250 mJ/cm$^2$ dose of UV irradiation was applied to the wild type strain of VSV to generate VSV-based NRRPs according to the present disclosure. In Fig. 1A, the UV

dose 1E2 corresponds to 100 mJ/cm$^2$. As such, when irradiated at a dose of 250 mJ/cm$^2$), VSV-eGFP lost its expression capabilities, yet maintained potent cytotoxicity against the immortalized production cell line (Vero) (Fig. 2B). Tittering of the virus following infection confirmed that the resulting particle was unable to replicate in these cells in sharp contrast with live virus infection (Fig. 2C). This effect was equally observed when using other members of the Rhabdovirus family, including Maraba (Fig. 3A, 3B and 3C).

**[00145]** Dose response curves, shown in Fig. 1A, indicate that cytotoxicity is reduced at UV doses above 1000 mJ/cm$^2$ and completely abrogated at a UV dose of 10,000 mJ/cm$^2$. It is believed that cytotoxicity is abrogated at this dose because the G proteins are cross-linked to such an extent that they are unable to allow the treated virus to bind the cell surface and/or be internalized by the cell. By comparing and contrasting with normal neonatal human dermal fibroblats (HDF) (Figs. 1A and 1B), it appears that cytotoxicity is preferential to cancerous cells over non-cancerous cells. Indeed, non-cancerous cells appear to require around 10 times more virus to become sensitive to NRRP-mediated cytotoxicity (Fig. 1B).

**[00146]** To confirm the absence of NRRP replication and spread in acute leukemia cells, GFP synthesis and viral titers were quantified following in-vitro treatment of an aggressive murine acute lymphoblastic leukemia cell line (L1210), alongside the Vero control cell line (normal kidney epithelial cells). In both treated cell lines, no detectable live virus was observed (Figs. 4A and 4B).

**[00147]** Western blot analysis of the viral genome indicates that the NRRPs have a reduced global genome expression (Fig. 5). UV-doses which block virion production and decrease genome expression are associated with distinct oncolytic activity. In these experiments, a high (greater than or equal to 1) multiplicity of infection (MOI), or particle to cell ratio, may be used to ensure that each tumor cell encounters a NRRP and induces extensive cell death across the population (Fig. 1B).

**[00148] Example 2: VSV-based NRRPs generated by exposure to an RNA alkylating agent**

**[00149]** In another example, NRRPs were chemically generated by treating VSV with 6 mg/mL of busulfan at 4 °C for 24 hours and added to Vero cells for 24 hours. Less than 4% of the Vero cells remained viable after treatment (Fig. 6A). This effect was attributable to the NRRPs since treatment with busulfan alone for 24 hours showed that Vero cells remained

around 82% viable (Fig. 6B). Fig. 6C shows cytopathic effect of live VSV-GFP infected Vero cells at 24 hours and that this live virus stock (VSV-GFP), from which the NRRPs were derived, was indeed replication competent – by evidence of GFP expression.

**[00150] Example 3: VSV-based NRRPs generated by exposure to gamma radiation**

**[00151]** In yet another example, NRRPs were generated by irradiating 1E10 frozen VSV with 15kGy Cobalt-60 at -80 °C and 1000 particles per cell were added to Vero cells for 48 hours. Again, the cytopathic effect of NRRPs was clearly evident on these immortalized cells (Fig. 7A). The NRRP-induced morphological effects of cellular apoptosis and death compare to the cytopathic effects of treating the same cells with live VSV-GFP, over the same time period of 48 hours (Fig. 7B). Vero cells treated with PBS alone remained fully viable, without cytopathic effects and showed no fluorescence (Fig. 7C).

**[00152] Example 4: NRRPS are an efficient treatment against leukemia cells in vitro**

**[00153]** Whether acute leukemia cells are susceptible to NRRP-mediated cell death was examined with VSV-based NRRPs generated by the UV method. First, the cytotoxicity induced in the L1210 cell line and that observed in normal Human Dermal Fibroblasts (HDF) was determined. While both cell lines were susceptible to live virus infection, NRRPs exclusively induced death in leukemic L1210 cells (Fig. 8A). The classic apoptotic phenotype, characterized by a reduced cell diameter, a "shriveled" appearance with numerous apoptotic bodies and fragmented nuclear content, was observed in acute leukemia L1210 cells. Cytotoxicity was quantified using a standard resazurin assay in several human and murine cell lines. In these experiments, acute leukemias were highly susceptible to NRRP-mediated cell death while preserving the viability of normal cells (Fig. 8B). Similar results were determined using Maraba-based NRRPs, an alternative Rhabdovirus strain (Fig. 3A and 3B). The absence of genome expression was confirmed by fluorescence microscopy (Fig. 8C).

**[00154]** The level of apoptosis in L1210 cell lines was quantified by flow cytometry. Thirty hours post treatment, NRRPs induced extensive (84% of population) early/late apoptosis (Fig. 9). VSV-induced apoptosis has been shown to directly correlate with the level

of endoplasmic reticulum (ER) stress present (10). Interestingly, when the cell's capacity to mitigate ER stress is breached, immunogenic apoptosis can be induced (16). NRRPs induce this unique form of cellular death as described later.

**[00155]** In other examples, L1210 leukemia cells were treated with NRRPs in combination with either 300 μM bendamustine (Fig. 10); 45 μM dexamethasone (Fig. 11); 0.025 μM doxorubicin (Fig. 12) or 0.0125μM vincristine (Fig.13) for 72 hours. NRRPs are shown to induce cytotoxic effect on their own in the usual manner however in combination with the above drugs additional and/or synergistic cytotoxic effect is observed. This demonstrates that a unique therapeutic potentiation-effect occurs when NRRP-therapy is combined with other chemotherapeutics/pharmacologics.

**[00156]** In yet another example, K562 Ph-positive myeloid leukemic cells were treated with UV-generated NRRPs in combination with 0.05 μM irarubicin (Fig. 14) for 72 hours. In this example as well, the myeloid leukemic cell line was highly susceptible to NRRP-mediated cell death and a potentiation-effect was again observed using this class of chemotherapeutic in combination with NRRPs. These observations indicate that NRRP-therapy may indeed be augmented by the use of additional therapeutics. This represents an alternative strategy to treat cancer, particularly recalcitrant forms of cancer that may require this unique combinatorial approach for increased efficacy.

**[00157]** **Example 5: Modelling depicting NRRPs anti-tumor specificity.**

**[00158]** The model used to describe NRRPs specificity against cells with defects in anti-viral signalling pathways was adapted from our previous work described in LeBoeuf et al 2013 (Fig. 15A). Briefly, this model is represented by a subset of six ordinary differential equations describing the transition between the cell populations (UP, IP, AP and PP) depending on the concentration of NRRPs (N) and interferon (IFN) in the environment. These equations are:

$$\frac{dUP}{dt} = -K_{VI} \times [N] \times [UP] - \left( \frac{-K_{IFN\,on}}{1+\left(\frac{[IFN]}{EC50}\right)^2} + K_{IFN\,on} \right) \times [UP] + K_{IFN\,off} \times [PP],$$

$$\frac{dIP}{dt} = K_{VI} \times [N] \times [UP] - \left( \frac{-K_{IFN\,on}}{1+\frac{[IFN]^2}{EC50}} + K_{IFN\,on} \right) \times [IP] - \gamma_c \times [IP],$$

$$\frac{dAP}{dt} = \left( \frac{-K_{IFN\,on}}{1+\frac{[IFN]^2}{EC50}} + K_{IFN\,on} \right) [IP] - K_{VC} \times [AP] - \gamma_c \times [AP],$$

$$\frac{dPP}{dt} = \left( \frac{-K_{IFN\,on}}{1+\frac{[IFN]^2}{EC50}} + K_{IFN\,on} \right) [UP] + K_{VC} \times [AP] - K_{IFN\,off} \times [PP].$$

**[00159]** The parameters used in the above equations represent the NRRP internalization rate ($K_{NI}$), the rate of IFN-signaling activation ($K_{IFN\,on}$), the rate of IFN-signaling inactivation ($K_{IFN\,off}$), the $EC_{50}$ of IFN ($EC_{50}$), the rate of cell death ($\gamma_C$) and the rate NRRP clearance ($K_{NC}$).

**[00160]** The next subset of equation describes the dynamics of NVRPs (N) and interferon (IFN) whereby:

$$\frac{dN}{dt} = -K_{VI} \times [V] \times [UP] - \gamma_V \times [V],$$

$$\frac{dIFN}{dt} = K_{IFN1} \times [IP] + K_{IFN2.1} \times [AP] + K_{IFN2.2} \times [PP] - \gamma_{IFN} \times IFN.$$

**[00161]** The parameters described in the above equations represent the rate of NRRP internalization ($K_{NI}$), NRRP degradation ($\gamma_N$), IFN production from IP, AP and PP ($K_{IFN1}$, $K_{IFN2.1}$ and $K_{IFN2.2}$, respectively) and IFN degradation ($\gamma_{IFN}$).

**[00162]** The Monte Carlo simulation was performed by randomly varying the above parameters within a 1 log window (Table 2) surrounding physiological parameter derived from literature and experimental evidence (18). Simulations were performed in Matlab using ODE15s imposing a none-negativity constraint. Trends described in Fig. 15B represent the median value over 1000 simulations. The number of cells used in these simulations was

2.5E5, the media volume was set at 1ml, and the PFU to cell ratio was set at 100 particles per cell. In these simulation, defects in IFN-signalling pathways were simulated by decreasing $K_{IFN1}$, $K_{IFN2.1}$, $K_{IFN2.2}$, $K_{vc}$ and $K_{IFN\ on}$ from 100% to 1% of their original value.

**[00163]** To investigate the mechanism by which specificity against the tumor cells is achieved, the authors of the present disclosure simulated the cytotoxicity induced by NRRPs in normal and tumor cells. Recently, the authors of the present disclosure have developed a population-based model describing the relationship between cytotoxicity and live oncolytic virus replication dynamics in normal and tumor cells. According to this model, an infection cycle begins as the uninfected population of cells (UP) encounters virions. This allows the UP population to become infected, and, in the context of live virus, virions and the cytokine known as interferon (IFN) are released into the environment.

**[00164]** As IFN gradually increases, the population of cells activates antiviral signalling (AP) which over time allows this population to clear the viral infection and become protected against further insult (PP). To adapt this model to NRRPs, the authors of the present disclosure removed virus replication dynamics from the model, and simulated the relationship between NRRP-mediated cytotoxicity and the extent of defects in IFN signaling pathways, a process known to occur in ~80% of cancers. These defects were simulated by decreasing the rate of IFN production, the rate of activation of IFN signaling and the rate of NRRP clearance between tumor and normal cells. To ensure that this observation is systematic, a Monte-Carlo simulation platform was utilized. Here, all kinetic parameters were varied within a 1 log window surrounding estimates derived from literature or experimental evidence (Table 2).

**[00165]** Following simulation across 1000 random parameter pairings (Fig. 15B), the authors of the present disclosure determined that as the cancer cells lose their ability to signal or respond to IFN, these cells becomes more sensitive to NRRP-mediated cytotoxicity. To validate this observation, the authors of the present disclosure investigated the impact of IFN on NRRP-mediated cytotoxicity in normal (HDF) and leukemic (L1210) cells. Interestingly, while the IntronA (recombinant IFN) could further increase normal cell protection against NRRP insult (Fig. 15C), IntronA had no detectable impact on leukemic cells (Fig. 15D).

**[00166]** Table 2: List of parameters estimates surrounding the experimental and literature evidence described by Le Boeuf et al (2013)

**Table 2**

| Parameter | Range Utilized |
|---|---|
| $K_{NI}$ | 7.5E-5 to7.5E-4 ($V^{-1}h^{-1}$) |
| $EC50$ | 0.25e-12 to 2.5e-12 (M) |
| $K_{prog}$ | ln(2)/(0.2 to 2.0) ($h^{-1}$) |
| $K_{prog}$ | ln(2)/(5 to 50) ($h^{-1}$) |
| $\gamma_L$ | ln(2)/(2.5 to 25) ($h^{-1}$) |
| $K_{NC}$ | ln(2)/(0.25 to 2.5) ($h^{-1}$) |
| $K_{prog}$ | $K_{prog}$× 10 to 100% (M/h) |
| $K_{prog1}$&$K_{prog2}$ | 8.3e-18 to 8.3e-17 (M/cell/h)<br>(ie 5000-50000 molecules/cell/h) |
| $\gamma_{IFN}$ | ln(2)/(5 to 50) ($h^{-1}$) |
| $\gamma_N$ | ln(2)/(2.5 to 25) ($h^{-1}$) |

**[00167]** **Example 6: NRRP Activity in AML blast crisis**

**[00168]** The translational potential of the NRRP platform was investigated in clinical samples. Peripheral blood mononuclear cells were obtained from two human patients with high-burden acute blast crisis, and susceptibility towards NRRP-mediated cell death was tested. The patients had circulating blasts with a CD33 positive phenotype. Both had previously received extensive treatment for chronic myeloid leukemia (CML) and developed resistance to tyrosine kinase inhibitor (TKI) treatment. Similar to the observation in L1210 blast cells, patient samples developed obvious NRRP-induced apoptosis with the classic morphology (Fig. 16A). Fluorescence microscopy confirmed the absence of NRRP genome expression (Fig.16B). Indeed post NRRP-treatment these CD33+ leukemia cells stained strongly for the apoptotic marker Annexin V (Fig. 16C). Use of the non-cultured patient samples was used to evaluate specificity of this response. Indeed in both patients, the

preponderant leukemic CD33+ population was ablated following NRRP treatment, leaving normal cells to dominate the sample (Fig. 16D).

**[00169]** To ensure that NRRPs do not affect normal white blood cells, bone marrow mononuclear cells isolated from a healthy donor were treated with PBS or NRRPs. At both early (18 hour) and late (65 hour) time points, NRRPs did not induce apoptosis within these samples (Fig. 17A and 17B).

**[00170]** **Example 7: NRRP anti-leukemic activity in-vivo**

**[00171]** A murine model of leukemic blast crisis was used to evaluate the potential of NRRPs as a therapeutic agent. Briefly, on day one, DBA/2 mice were challenged with $1x10^6$ dose of L1210 blast cells. The following day, mice began a regimen of $3x10^9$ NRRPs administered intravenously for three consecutive days, and survival was monitored. In parallel, separate cohorts of mice were treated with live VSV at the MTD of $2x10^6$ virus per injection (19), or PBS under the same treatment schedule. NRRP treated mice achieved 80% survival up to day 40, representing a significant advantage versus those treated with PBS ($P \leq 0.0045$) or live virus ($P \leq 0.044$) (Fig. 18A). NRRPs were well tolerated and administered at the maximal feasible dose for this particular experiment, which represented a 1500x higher dose than the MTD of live virus. Given that acute leukemia frequently disseminates to the central nervous system, and that wild type VSV is highly neurotoxic, intracranial injections of NRRPs and live virus were performed. While mice could tolerate the maximum production dose for intracranial injections of $1x10^8$ particles, all mice rapidly succumbed to a $1x10^4$ dose of live virus.

**[00172]** Prompted by the efficacy and differential MTD afforded by NRRP therapy, it is interesting to know whether the immune system is activated following treatment. Murine blood serum was collected from L1210 tumor bearing mice 20 hours after PBS or NRRPs treatment (Fig. 18B). In this analysis, it is clear that cytokines typically known to recruit and differentiate T-cells are induced following NRRP treatment. Examples of such immune-modulatory cytokines significantly induced by NRRP treatment include the leukemia inhibitory factor LIF, IL-2, IL-4, CCL-2, RANTES and MIP-1$\alpha$ (Fig. 18B).

**[00173]** To confirm immune system stimulation, in particular T-cell activation, the authors of the present disclosure adopted a vaccine strategy described in previous publications. Experimentally, this platform consists of injecting apoptotic cells into immuno-

competent animals and measuring protective adaptive immunity against subsequent tumor challenge. Indeed, L1210 cells treated with NRRPs develop marked apoptosis as can be seen in Fig. 16C by the increase in Annexin-V staining. Therefore, this classical experimental approach was adopted to explore whether NRRPs trigger immunogenic apoptosis.

**[00174]** Two cohorts of DBA/2 mice (syngeneic to L1210) received three weekly intravenous doses of $1x10^6$ γ-irradiated L1210 cells pre-treated with NRRPs. Another cohort received the same number of γ-irradiated L1210 cells. One week following this regimen, a L1210 leukemic challenge ($1x10^6$ cells) was administered via tail vein, and survival recorded. The cohort receiving NRRP-treated L1210 cells had 80% protection after leukemic challenge, which was otherwise uniformly lethal in the untreated L1210-administered cohorts (Fig. 19). Surviving mice were kept for >150 days to ensure long-lasting protection. This is consistent with the notion that NRRP-treated acute leukemia cells undergo immunogenic apoptosis.

**[00175]** Using acute lymphoblastic and myeloid leukemia cell lines, as well as primary leukemia cells from heavily pre-treated CML patients in acute blast crisis, it is demonstrated that NRRPs are at least leukemia-specific cytolytic agents. Through the in-vitro and in-vivo experiments detailed above, it is confirmed that NRRPs offer a multimodal therapeutic platform.

**[00176] Example 8: NRRP activity in multiple myeloma, brain cancer and colon cancer cell lines**

**[00177]** In addition to the experiments detailed above, NRRPs were also shown to be cytopathic in multiple myeloma cell lines MCP-11 and RPMI-8226 (Fig. 20) when the cells lines were treated with PBS or VSV-derived NRRPs for 15 hours. Specifically, Fig. 21 shows cell viability following an Alamar blue cytotoxicity or resazurin assay for myeloma cell lines taken 72 hours post treatment with NRRPs administered at an MOI = 250. In this experiment, SR4987 is a normal marrow stromal cell line. As seen in Fig. 21, SR4987 demonstrates resistance to NRRPs as it is a non-malignant cell. No NRRP or VSV genome replication was found when the NRRPs were generated, since no viral-encoded GFP was produced (data not shown).

**[00178]** In another example, MCP-11 multiple myeloma cell line was treated with 20 μM melphalan (Fig. 22) or 15 μM SMAC mimetic LCL161 (Fig. 23) in combination with NRRPs. Combination therapy augmented the cytopathic effect of NRRPs in both cases.

Synergistic activity between SMAC mimetics and NRRPs represents a promising approach. It is observed that SMAC mimetic anti-tumor activity is significantly augmented or in some cases essentially dependent-upon NRRP co-administration.

**[00179]** In yet another example, RPMI-8226 multiple myeloma cell line was treated with 5 nM carfilzomib with potentiating cytotoxic effect (Fig. 24). It is demonstrated that co-administration of NRRPs with an alkylating agent (such as melphalan), a proteasome inhibitor (such as carfilzomib) or a SMAC mimetic (such as LCL161) represents an alternative treatment strategy for various cancers, particularly promising in hematopoietic-based cancers, such as multiple myeloma.

**[00180]** The usefulness of NRRPs as an anti-cancer therapeutic is further demonstrated by its effect on brain tumor cell lines. NRRPs-mediated cytotoxicity was determined in glioblastoma cell line CT2A, delayed brain tumor glioblastoma cell line (DBT) (Fig. 25A), astrocytoma cell lines K1491 (Fig. 25B) and K1492, and mouse glioma cell line (GL261) (Fig. 25C), compared to HDNF normal cells, when these cells were treated for 24 hrs with PBS or NRRPs (Fig. 26).

**[00181]** Also, in yet another example, glioblastoma cell line CT2A was treated with 10 μM of the HDAC inhibitor SAHA in combination with NRRPs and a potentiation cytopathic effect was observed compared to NRRPs with PBS (Fig. 27). HDAC inhibition has shown a modicum of promise as an anti-cancer agent. However, in combination with NRRPs, significant activity is noted, representing a very promising approach to treat glioblastoma-based malignancies, an unmet clinical need.

**[00182]** Renal (786-0) and breast cancer (4T1) cell lines are equally sensitive to the cytopathic effects of NRRPs (Figs. 28A, 28B, 28C). In this series of experiments, cell lines were treated with NRRPs at an MOI=250 and viability was quantified by resazurin assay over a 72h period. Fluorescence microscopy performed throughout the experiment confirmed the absence of genome expression.

**[00183]** In another example, subcutaneous CT26 colon cancer cells were implanted into mice. The mice were then treated with 2E9 NRRPs on days 16, 18 and 21 post tumor embedment (Fig. 29). Despite large tumor burden prior to NRRP-treatment, prolonged survival and cures were obtained when NRRPs were administered via the intratumoral or intravenous routes. PBS control-treated mice all rapidly reached endpoint. This model

represents additional evidence that solid tumors may also be amenable to NRRP-based regimens.

**[00184]** The Examples above show through in-silico and in-vitro testing that NRRPs, analogous to live virus, are tumor-selective given that they exploit defects in innate immune pathways common to most tumors. However, the safety margin afforded by the NRRP platform was exemplified by the observation that high titer intracranial NRRP administration was well tolerated by murine recipients.

**[00185]** The outcome for the majority of adult patients suffering from acute lymphoblastic or acute myeloid leukemia remains dismal. For a minority of patients, allogeneic stem cell transplantation after myeloablative conditioning is potentially curative, however this procedure is associated with frequent adverse events and significant treatment-related mortality. For many patients with chronic-phase CML, targeted tyrosine kinase inhibitor therapy offers excellent disease control. When progression into acute blast crisis occurs, very limited therapeutic options exist due to development of multi drug resistance and the rapid kinetics of this form of recalcitrant leukemia.

**[00186]** NRRPs exhibit both direct cytolytic and immunogenic properties in multiple acute leukemia murine models. A peculiar form of programmed cell death involves the induction of adaptive immune responses against the dying cell. This process, commonly referred to as immunogenic apoptosis, is essential to the efficacy of several current chemotherapeutics and is required for host defense against viral infection including live RVs. The in-vivo results above indicate that a similar process is induced by NRRPs and is a driving factor to treatment efficacy.

**[00187]** More relevant are the observations that multi-drug resistant primary myeloblasts from patients in CML blast-crisis are forced into apoptosis and finally eradicated by NRRP treatment. In addition, non-leukemic white cells procured from healthy bone marrow were not adversely affected. This observation suggests that despite the potent tumoricidal activity of NRRPs, the leukopenia commonly observed after standard induction and consolidation chemotherapy could be avoided. This may significantly decrease treatment related adverse events. Further, given the preservation of normal white blood cells during leukemic cytoreduction by NRRPs, the simultaneous induction of an effective anti-leukemic immune response may be attainable for the majority of patients who are not candidates for

high-dose radio-chemotherapy followed by allogeneic stem cell transplantation. Following the induction of immunogenic apoptosis by NRRPs, a broad array of immunomodulatory cytokine are released and likely assist in the development of effective adaptive immune activity - a critical component to achieving durable curative responses.

**[00188]** The Examples demonstrate the production of high-titer NRRPs. Through the induction cell lysis mainly via programmed cell death pathways, systemic and intratumoral immune responses, including natural killer cell activation as well as dendritic cell activation, or vasculature shutdown within the tumor - NRRPs harbor several anti-cancer properties. These features may be exploited by using NRRPs alone or as an adjuvant in combination with radiation therapies, chemotherapies, immuno-therapies, surgery, oncolytic-virus derived or other virus-derived therapeutic platforms.

**[00189]** In the preceding description, for purposes of explanation, numerous details are set forth in order to provide a thorough understanding of the examples. However, it will be apparent to one skilled in the art that these specific details are not required.

**[00190]** The above-described examples are intended to be exemplary only. Alterations, modifications and variations can be effected to the particular examples by those of skill in the art without departing from the scope, which is defined solely by the claims appended hereto.

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**WHAT IS CLAIMED IS:**

1. A non-replicating virus-derived particle comprising:

an envelope having sufficient number of functional G proteins on the surface of the envelope to allow for the virus-derived particle to bind a surface of a cell and be internalized;

an RNA polynucleotide having a sequence that encodes all the proteins required for new virus particle assembly; and

a mixture of proteins that form a structure around the RNA;

wherein the RNA:

(a) has a sufficiently cross-linked structure such that the RNA sequence, in the cell, cannot be used to:

transcribe the complete genome;
translate all the proteins; or
both;

in order to produce sufficient macromolecules required for new virus particle assembly;

(b) is sufficiently discontinuous such that the RNA sequence, in the cell, cannot be used to:

transcribe the complete genome;
translate all proteins; or
both;

in order to produce sufficient macromolecules required for new virus particle assembly; or

both (a) and (b).

2. The non-replicating virus-derived particle according to claim 1, wherein the functional G protein:

has a sequence that comprises SEQ ID NO: 1;

has a sequence that is at least 75% identical to SEQ ID NO: 1 and that is capable of binding to a surface of a cell and effecting internalization of the particle.

3. The non-replicating virus-derived particle according to claim 1 or 2, wherein the mixture of proteins that form a structure around the RNA comprises at least N, P, M, and L proteins.

4. The non-replicating virus-derived particle according to claim 3, wherein the N protein:

has a sequence that comprises SEQ ID NO: 2; or

has a sequence that is at least 80% identical to SEQ ID NO: 2 and that is capable of participating in the formation of the protein structure.

5. The non-replicating virus-derived particle according to claim 3 or 4, wherein the P protein:

has a sequence that comprises SEQ ID NO: 3; or

has a sequence that is at least 80% identical to SEQ ID NO: 3 and that is capable of participating in the formation of the protein structure.

6. The non-replicating virus-derived particle according to any one of claims 3-5, wherein the M protein:

has a sequence that comprises SEQ ID NO: 4; or

has a sequence that is at least 80% identical to SEQ ID NO: 4 and that is capable of participating in the formation of the protein structure.

7. The non-replicating virus-derived particle according to any one of claims 3-6, wherein the L protein:

has a sequence that comprises SEQ ID NO: 5; or

has a sequence that is at least 70% identical to SEQ ID NO: 5 and that is capable of participating in the formation of the protein structure.

8. The non-replicating virus-derived particle according to any one of claims 3-7, wherein the RNA sequence produces functional N, P, M and G proteins on binding and internalization of the particle, and lacks the ability to produce functional L protein.

9. The non-replicating virus-derived particle according to any one of claims 3-7, wherein the RNA sequence produces N, P and M proteins on binding and internalization of the particle, and lacks the ability to produce functional G and L proteins.

10. The non-replicating virus-derived particle according to any one of claims 3-7, wherein the RNA sequence produces N and P proteins on binding and internalization of the particle, and lacks the ability to produce functional M, G and L proteins.

11. The non-replicating virus-derived particle according to any one of claims 3-7, wherein the RNA sequence produces N protein on binding and internalization of the particle, and lacks the ability to produce functional P, M, G and L proteins.

12. The non-replicating virus-derived particle according to any one of claims 3-7, wherein the RNA sequence lacks the ability to produce functional N, P, M, G and L proteins.

13. The non-replicating virus-derived particle according to any one of claims 1-12, wherein the surface of the envelope has at least 60 functional G proteins per particle.

14. The non-replicating virus-derived particle according to any one of claims 1-12, wherein the surface of the envelope has at least 600 functional G proteins per particle.

15. The non-replicating virus-derived particle according to any one of claims 1-12, wherein the surface of the envelope has at least 1200 functional G proteins per particle.

16. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the cross-linked RNA includes at least 0.05% cross-linked nucleotides.

17. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the cross-linked RNA includes at least 0.5% cross-linked nucleotides.

18. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the cross-linked RNA includes at least 1% cross-linked nucleotides.

19. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the cross-linked RNA includes at least 10% cross-linked nucleotides.

20. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the cross-linked RNA includes at least 20% cross-linked nucleotides.

21. The non-replicating virus-derived particle according to any one of claims 16-20, wherein the cross-linked RNA includes less than 80% cross-linked nucleotides.

22. The non-replicating virus-derived particle according to any one of claims 1-21, wherein the RNA structure is cross-linked to RNA, is cross-linked to a protein in the protein structure around the RNA, or both.

23. The non-replicating virus-derived particle according to any one of claims 1-22, wherein the protein structure around the RNA comprises a protein that is:

cross-linked to another protein of the protein structure;

internally cross-linked;

cross-linked to the RNA structure; or

any combination thereof.

24. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the RNA comprises at least two discontinuous RNA polynucleotides.

25. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the RNA comprises at least five discontinuous RNA polynucleotides.

26. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the RNA comprises at least 10 discontinuous RNA polynucleotides.

27. The non-replicating virus-derived particle according to any one of claims 1-15, wherein the RNA comprises at least 100 discontinuous RNA polynucleotides.

28. The non-replicating virus-derived particle according to any one of claims 1-15 wherein the RNA comprises discontinuous RNA polynucleotides of no more than 1000 nucleotides.

29. The non-replicating virus-derived particle according to any one of claims 1-15 wherein the RNA comprises discontinuous RNA polynucleotides of no more than 3000 nucleotides.

30. The non-replicating virus-derived particle according to any one of claims 1-15 wherein the RNA comprises discontinuous RNA polynucleotides of no more than 5000 nucleotides.

31. The non-replicating virus-derived particle according to any one of claims 1-15 wherein the RNA comprises discontinuous RNA polynucleotides of no more than 7000 nucleotides.

32. The non-replicating virus-derived particle according to any one of claims 1-15 wherein the RNA comprises discontinuous RNA polynucleotides of no more than 10,000 nucleotides.

33. A method of producing a non-replicating virus-derived particle from a live virus comprising RNA having a sequence that encodes N, P, M, G or L proteins, the method comprising:

optionally separating the live virus from a UV-absorbing compound;

subjecting the live virus to an RNA damaging agent to:

(a) sufficiently cross-link the RNA structure of the live virus so that, when the virus-derived particle is in a cell:

RNA transcription into mRNA is reduced;

mRNA translation into protein is reduced; or

both;

thereby preventing production of sufficient proteins required for new virus particle assembly;

(b) sufficiently cleaving the RNA structure of the live virus into discontinuous RNA segments so that, when the virus-derived particle is in a cell:

RNA transcription into mRNA is reduced;

mRNA translation into protein is reduced; or

both;

thereby preventing production of sufficient proteins required for new virus particle assembly; or

(c) both (a) and (b).

34. The method according to claim 33, wherein the G protein:

has a sequence that comprises SEQ ID NO: 1;

has a sequence that is at least 75% identical to SEQ ID NO: 1 and that is capable of binding to a surface of a cell and effecting internalization of the particle.

35. The method according to claim 33 or 34, wherein the N protein:

has a sequence that comprises SEQ ID NO: 2; or

has a sequence that is at least 80% identical to SEQ ID NO: 2 and that is capable of participating in the formation of the protein structure.

36. The method according to any one of claims 33-35, wherein the P protein:

has a sequence that comprises SEQ ID NO: 3; or

has a sequence that is at least 80% identical to SEQ ID NO: 3 and that is capable of participating in the formation of the protein structure.

37. The method according to any one of claims 33-36, wherein the M protein:

has a sequence that comprises SEQ ID NO: 4; or

has a sequence that is at least 80% identical to SEQ ID NO: 4 and that is capable of participating in the formation of the protein structure.

38. The method according to any one of claims 33-37, wherein the L protein:

has a sequence that comprises SEQ ID NO: 5; or

has a sequence that is at least 70% identical to SEQ ID NO: 5 and that is capable of participating in the formation of the protein structure.

39. The method according to any one of claims 33-38, wherein subjecting the live virus to an RNA damaging agent comprises subjecting the live virus to electromagnetic radiation.

40. The method according to claim 39, wherein the electromagnetic radiation has a wavelength less than about 1 mm.

41. The method according to claim 39, wherein the electromagnetic radiation has a wavelength less than about 500 nm.

42. The method according to claim 39, wherein the electromagnetic radiation has a wavelength less than about 280 nm.

43. The method according to claim 39, wherein the electromagnetic radiation has a wavelength between about 1 picometer and about 300 nm.

44. The method according to claim 39, wherein the electromagnetic radiation has a wavelength between about 100 nm and about 400 nm, and wherein the live virus is subjected to a dose of electromagnetic radiation between about 100 $mJ/cm^2$ and about 8,000 $mJ/cm^2$.

45. The method according to claim 39, wherein the electromagnetic radiation has a wavelength between about 100 nm and about 400 nm, wherein the live virus is subjected to a dose of electromagnetic radiation between about 100 $mJ/cm^2$ and about 1000 $mJ/cm^2$.

46. The method according to claim 39, wherein the electromagnetic radiation has a wavelength between about 100 nm and about 400 nm, wherein the live virus is subjected to a dose of electromagnetic radiation between about 150 $mJ/cm^2$ and about 500 $mJ/cm^2$.

47. The method according to claim 39, wherein the electromagnetic radiation has a wavelength between about 100 nm and about 280 nm, and the live virus is subjected to a dose of electromagnetic radiation between about 150 and about 300 $mJ/cm^2$.

48. The method according to claim 39, wherein the electromagnetic radiation is gamma irradiation, and the live virus is subjected to a dose of electromagnetic radiation between about 1 kGy and about 50 kGy.

49. The method according to claim 39, wherein the electromagnetic radiation is gamma irradiation, and the live virus is subjected to a dose of electromagnetic radiation between about 5 kGy and about 20 kGy.

50. The method according to any one of claims 33-49, wherein the live virus is subjected to the RNA damaging agent at a temperature of 37°C or lower.

51. The method according to any one of claims 44-47, wherein the live virus is subjected to the RNA damaging agent at a temperature of about 25°C.

52. The method according to claim 48 or 49, wherein the live virus is subjected to the RNA damaging agent at a temperature of about -80°C.

53. The method according to any one of claims 33-38, wherein subjecting the live virus to an RNA damaging agent comprises exposing the live virus to an RNA damaging chemical agent.

54. The method according to claim 53, wherein the RNA damaging chemical agent is an alkylating agent capable of crosslinking RNA.

55. The method according to claim 53, wherein the RNA damaging chemical agent is busulfan, cyclophosphamide, melphalan, formaldehyde, carbodiimide or bissulfosuccinimidyl suberate.

56. The method according to claim 53, wherein the RNA damaging chemical agent is a free radical forming agent capable of cleaving RNA.

57. The method according to claim 56 wherein the free radical forming agent is a peroxide, hydrogen bromine, ammonium persulfate, or a hydroxyl radical.

58. The method according to any one of claims 33-57 wherein separating the live virus from a UV-absorbing compound comprises fractionating, in a sucrose gradient, a growth medium containing the live virus.

59. A method comprising:

administering non-replicating virus-derived particles according to any one of claims 1 to 32 to a population of cells in a patient, wherein the population of cells comprises cancerous cells and non-cancerous cells and the administration induces expression and release of cytokines in the patient.

60. The method according to claim 59 wherein the cytokines released in the patient include: interleukins, interferons, inflammatory cytokines, members of the CXC chemokine family, members of the tumor necrosis factor family, or any combination thereof.

61. A method of inducing cell death of cancerous cells in a patient, the method comprising:

administering non-replicating virus-derived particles according to any one of claims 1 to 32 to the patient.

62. A method comprising:

administering non-replicating virus-derived particles according to any one of claims 1 to 32 to a population of cells in a patient, wherein the population of cells comprises cancerous cells and non-cancerous cells and the administration results in preferential cell death of the cancerous cells over the non-cancerous cells.

63. The method according to claim 61 or 62, wherein the cell death is through apoptosis.

64. The method according to claim 61, 62 or 63, wherein the cell death is by innate immune effector cells, adaptive immune effector cells, or both.

65. The method according to claim 64, wherein the adaptive immune effector cells are T-cells, B-cells, or both.

66. The method according to claim 65, wherein the innate immune effector cells are mast cells, phagocytes, basophils, eosinophils, natural killer cells, γδ T cells, or any combination thereof.

67. The method according to claim 66, wherein the phagocytes are macrophages, neutrophils, dendridic cells, or any combination thereof.

68. The method according to any one of claims 59-67, wherein 1E10 to 1E15 non-replicating virus-derived particles are administered to the patient.

69. The method according to any one of claims 59-67, wherein 1E11 to 1E13 non-replicating virus-derived particles are administered to the patient.

70. The method according to any one of claims 59-69 wherein the method further comprises administering a chemotherapeutic to the patient.

71. The method according to claim 70 wherein the chemotherapeutic is bendamustine, dexamethasone, doxorubicin, vincristine, idarubicin, carfilzomib, suberoylanilide hydroxamic acid, melphlan, or the SMAC mimetic LCL161.

72. The method according to claim 72 wherein the chemotherapeutic is bendamustine, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, lomustine, melphalan, temozolomide, thiotepa, oxaliplatin, procarbazine, pentostatin, cladribine, clofarabine, cytarabine, fludarabine, gemcitabine, hydroxyurea, mercaptopurine, nelarabine, fluorouracil, bleomycin, dactinomycin, daunorubicin, doxorubicin, doxorubicin liposomal, idarubicin, mitoxantrone, capecitabine, topotecan, irinotecan, etoposide, paclitaxel, teniposide, thioguanine, omacetaxin, altretamine, asparaginase, asparaginase, pegaspargase, Isotretinoin, retinoic acid, arsenic, vinblastine, vincristine, vincristine

liposomal, bosutinib, dasatinib, imatinib, nilotinib, sunitinib, vemurafenib, regorafenib, bortezomib, carfilzomib, thalidomide, lenalidomide, pomalidomide, methotrexate, pralatrexate, everolimus, Temsirolimus, vorinostat, romidepsin, valproic acid, decitabine, azacitidine, anagrelide, cortisone, dexamethasone, prednisone, triamcinolone, interferon alfa 2a, interferon alfa 2b, peginterferon alfa 2b, interferon beta 1b, aldesleukin/IL-2, denileukin diftitox, granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor, anti-CD20, anti-CD47, anti-CD33, anti-CD38, anti-CD138, anti-CS1, anti-CD52, anti-VEGF, anti-Her2/Neu, anti-EGFR, anti-CTLA4 or anti-IGF-1.

73. The method according to any one of claims 59-72 wherein the cancer is leukemia, lymphoma, multiple myeloma, renal cancer, breast cancer, colon cancer, or glioblastoma.

74. The method according to any one of claims 59-72 wherein the cancer is hepatocellular carcinoma, prostate cancer, lung cancer, mesothelioma, uterine cancer, cervical cancer, bladder cancer, ovarian cancer, pancreatic cancer, melanoma, basal cell carcinoma, melanoma, squamous cell carcinoma, esophageal cancer, gastric cancer, osteosarcoma, chondrosarcoma, a soft tissue cancer, head cancer, neck cancer, thyroid cancer, retinoblastoma, or oral cancer.

75. The method according to any one of claims 59-74, wherein the non-replicating virus-derived particles are administered to the patient intratumorally, intranasally, intramuscularly, intradermally, intraperitoneally, intra-arterially, intravenously, subcutaneously or intracranially.

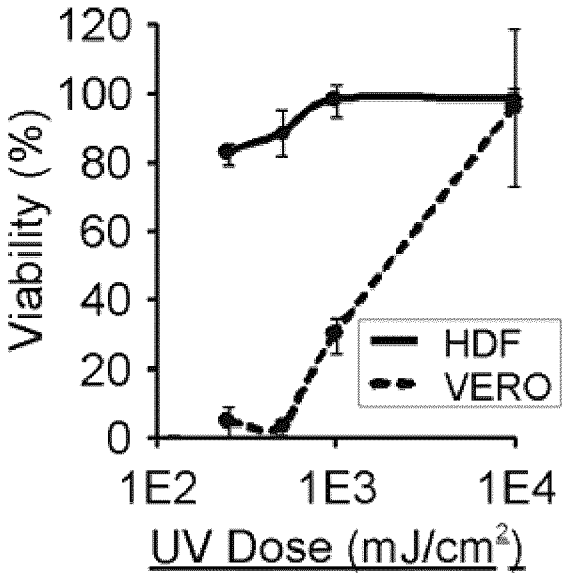

120
100
80
60
40
20
0
Viability (%)
HDF
VERO
1E2
1E3
1E4
UV Dose (mJ/cm2)

Fig. 1A

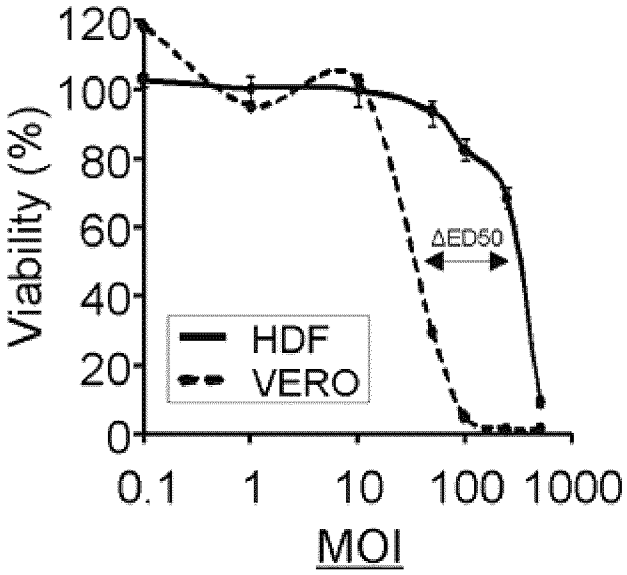

120
100
80
60
40
20
0
Viability (%)
ΔED50
HDF
VERO
0.1
1
10
100
1000
MOI

Fig. 1B

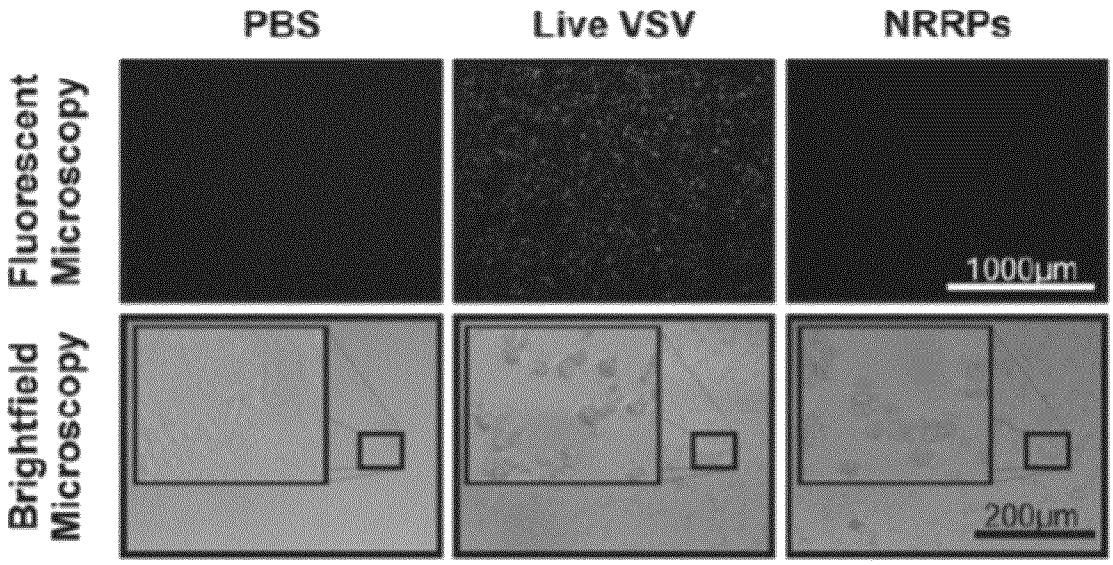



Fig. 2A

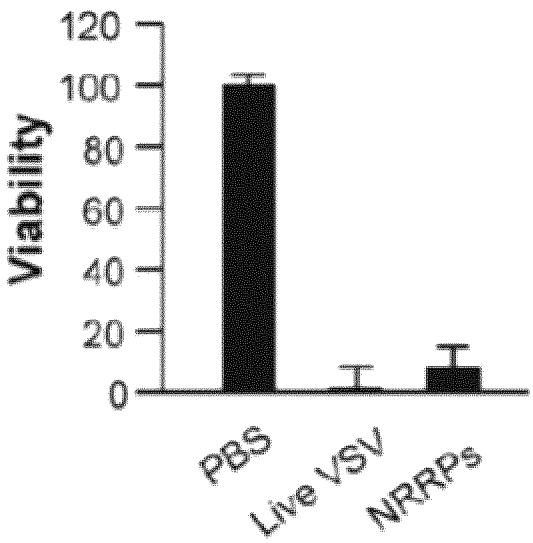



Fig. 2B

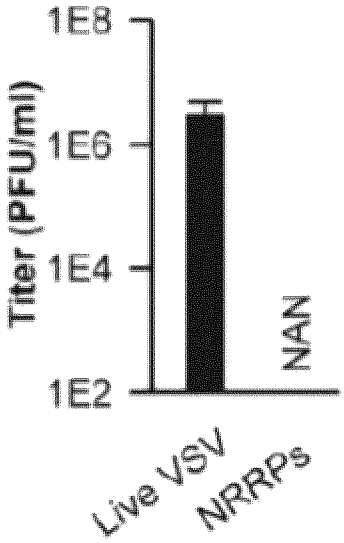



Fig. 2C

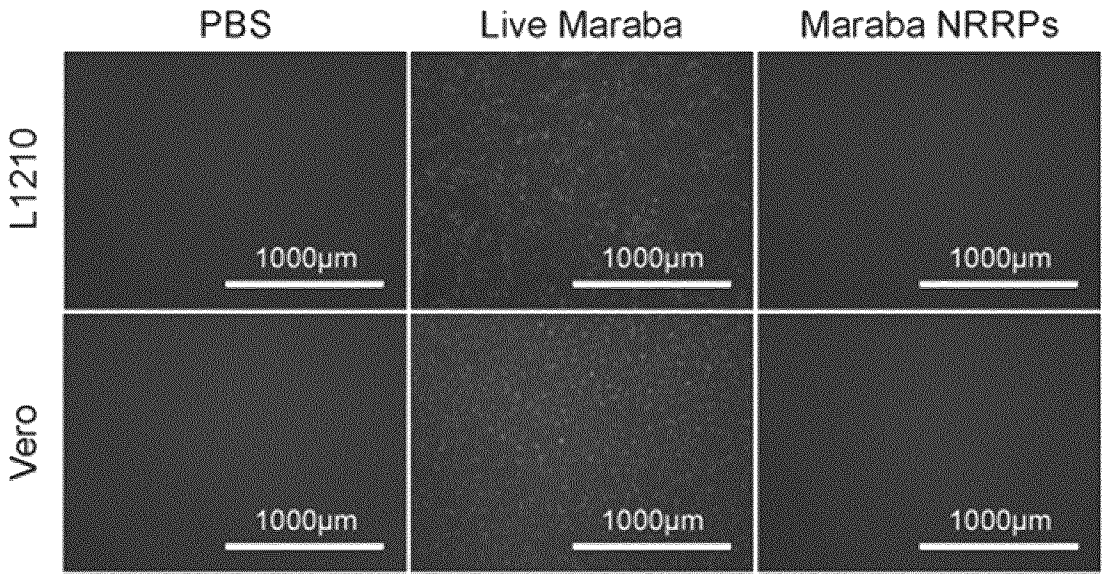

PBS
Live Maraba
Maraba NRRPs
L1210
Vero
1000µm
1000µm
1000µm
1000µm
1000µm
1000µm

Fig. 3A

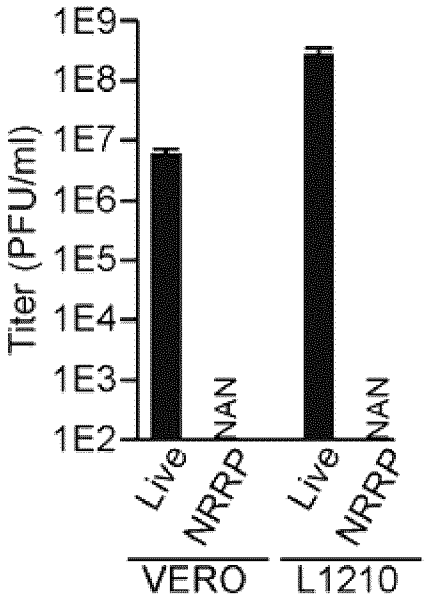

Titer (PFU/ml)
1E9
1E8
1E7
1E6
1E5
1E4
1E3
1E2
NAN
NAN
Live
NRRP
Live
NRRP
VERO
L1210

Fig. 3B

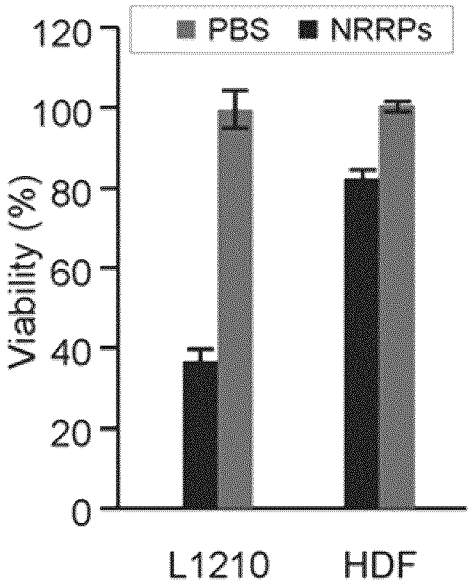

PBS
NRRPs
120
100
80
60
40
20
0
Viability (%)
L1210
HDF

Fig. 3C

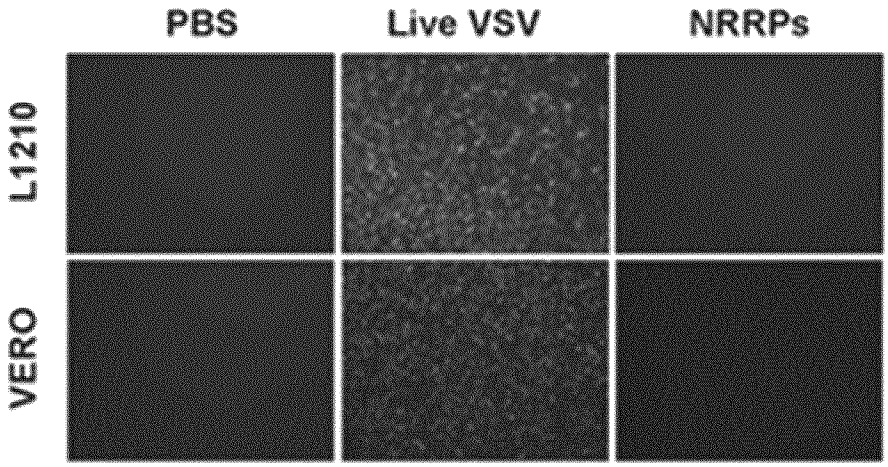

PBS
Live VSV
NRRPs
L1210
VERO

Fig. 4A

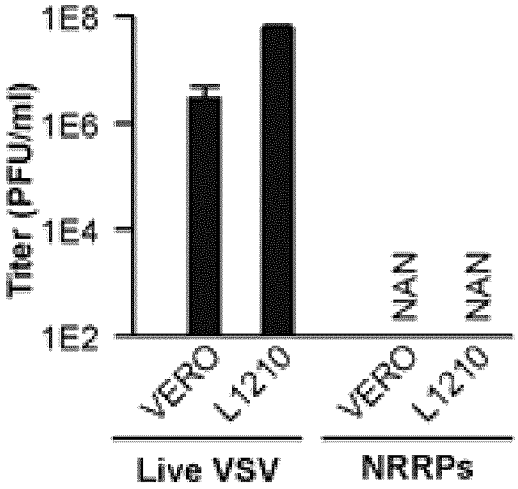

1E8
1E6
1E4
1E2
Titer (PFU/ml)
NAN
NAN
VERO
L1210
VERO
L1210
Live VSV
NRRPs

Fig. 4B

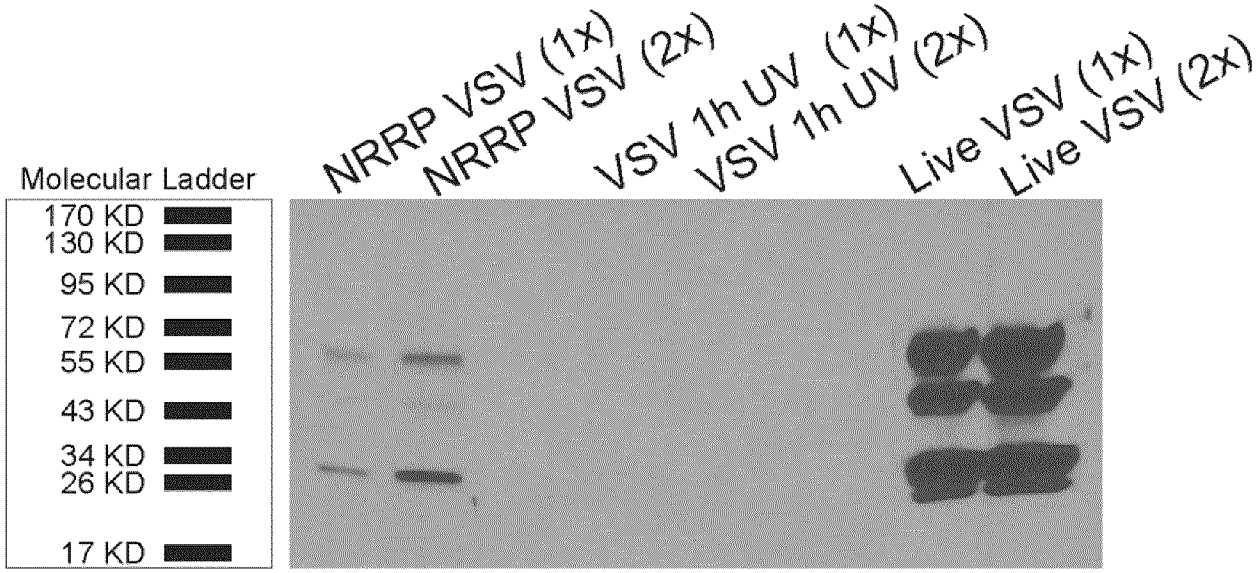

Molecular Ladder
170 KD
130 KD
95 KD
72 KD
55 KD
43 KD
34 KD
26 KD
17 KD
NRRP VSV (1x)
NRRP VSV (2x)
VSV 1h UV (1x)
VSV 1h UV (2x)
Live VSV (1x)
Live VSV (2x)

Fig. 5

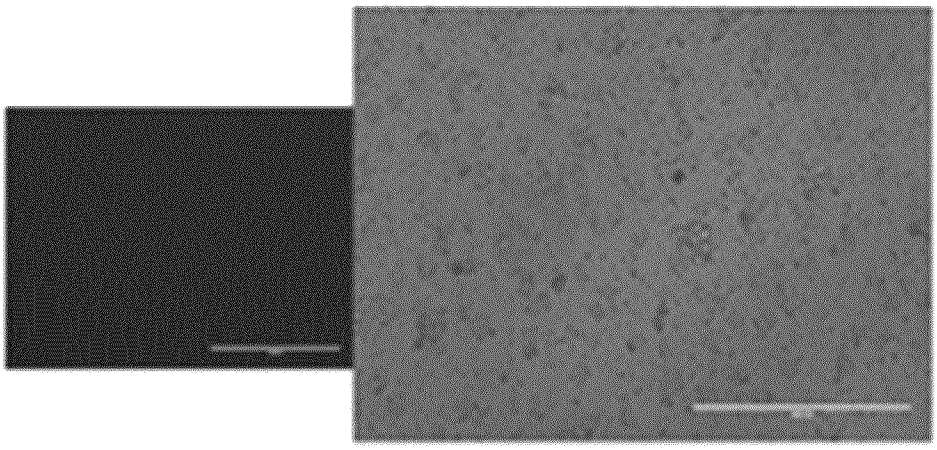


Fig. 6A

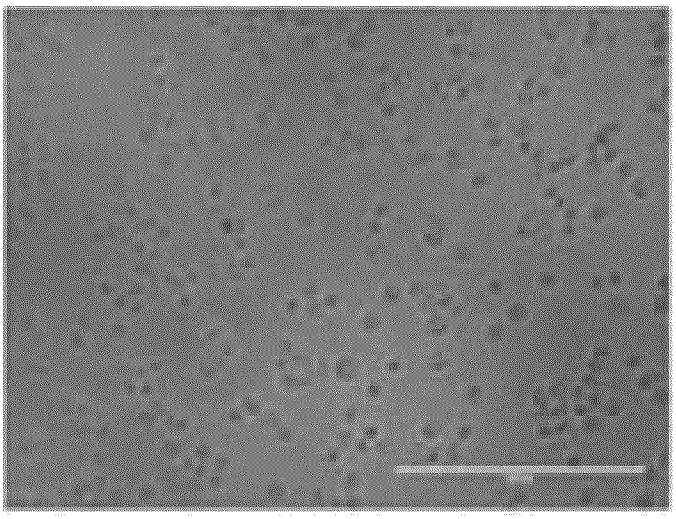


Fig. 6B

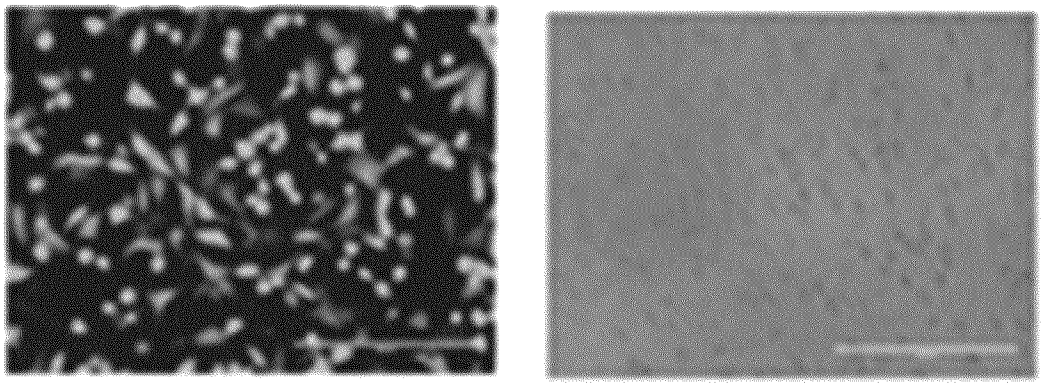


Fig. 6C

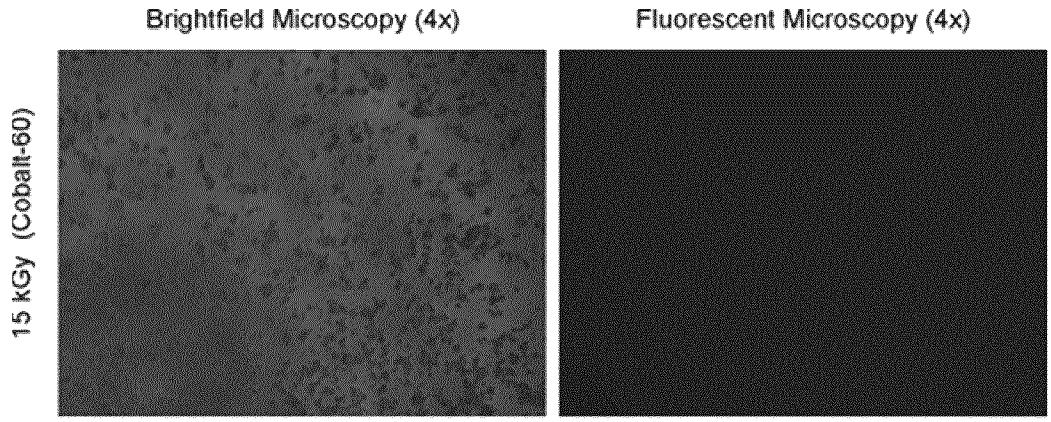



Fig. 7A

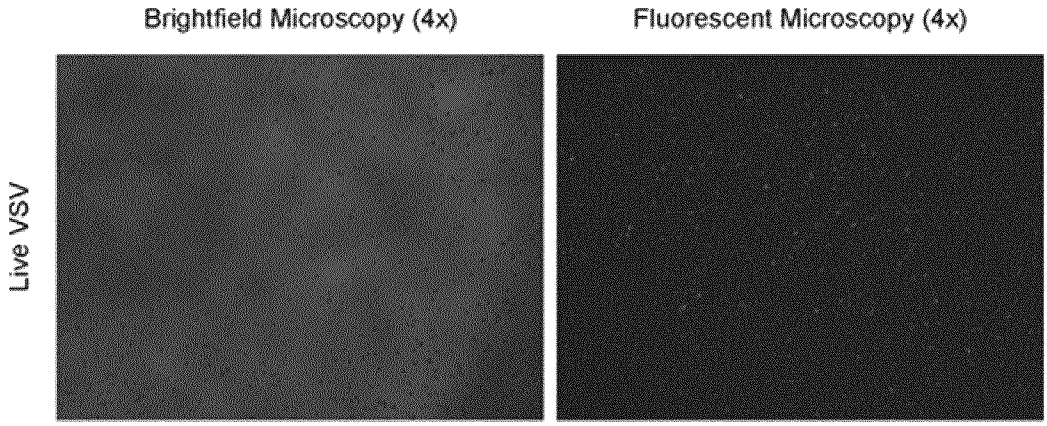



Fig. 7B

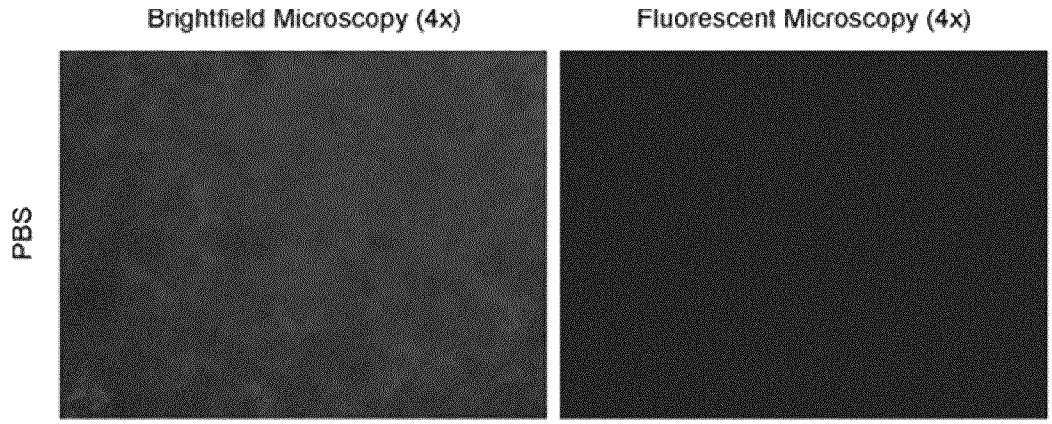



Fig.7C

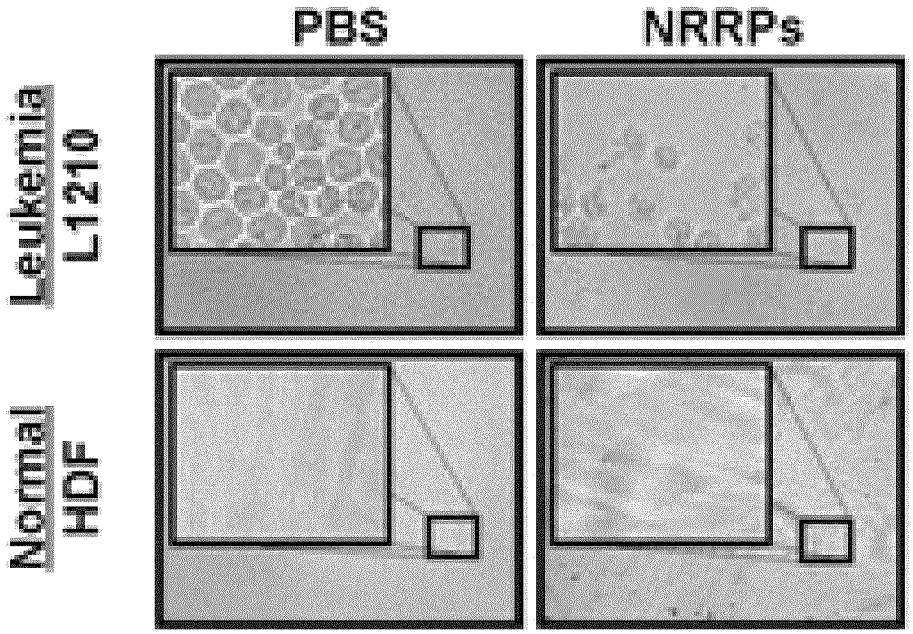

PBS
NRRPs
Leukemia L1210
Normal HDF

Fig. 8A

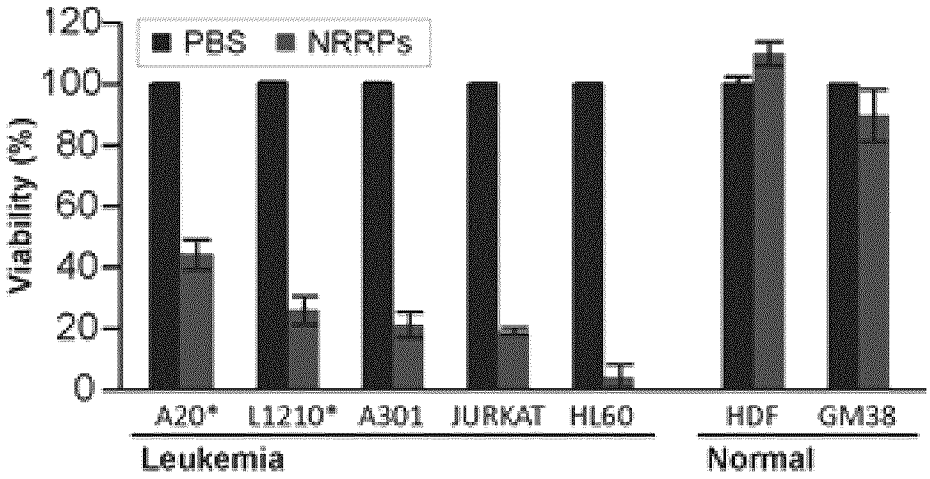

120
100
80
60
40
20
0
Viability (%)
PBS
NRRPs
A20*
L1210*
A301
JURKAT
HL60
HDF
GM38
Leukemia
Normal

Fig. 8B

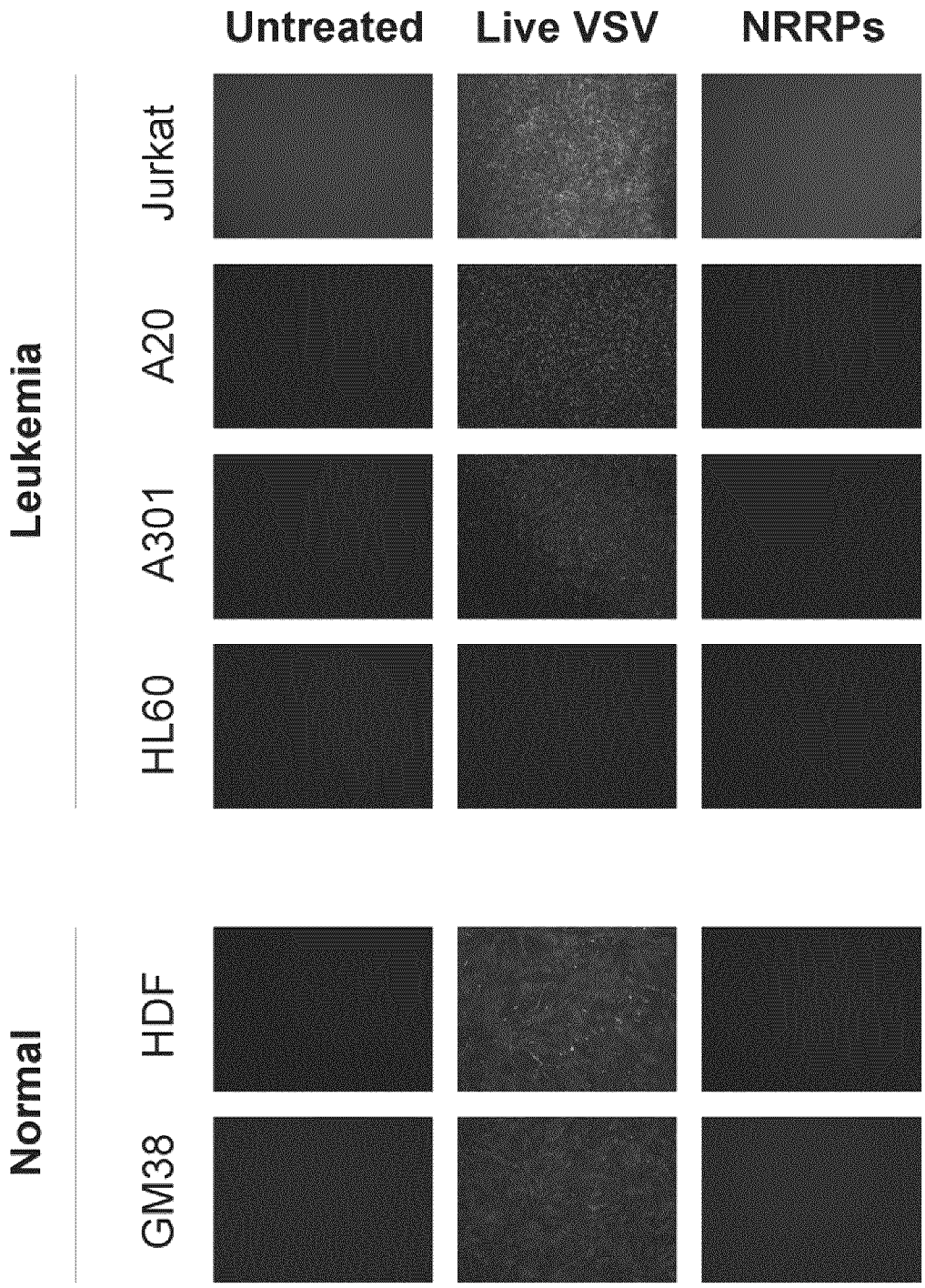

Untreated
Live VSV
NRRPs
Leukemia
Jurkat
A20
A301
HL60
Normal
HDF
GM38

Fig. 6

Fig. 8C

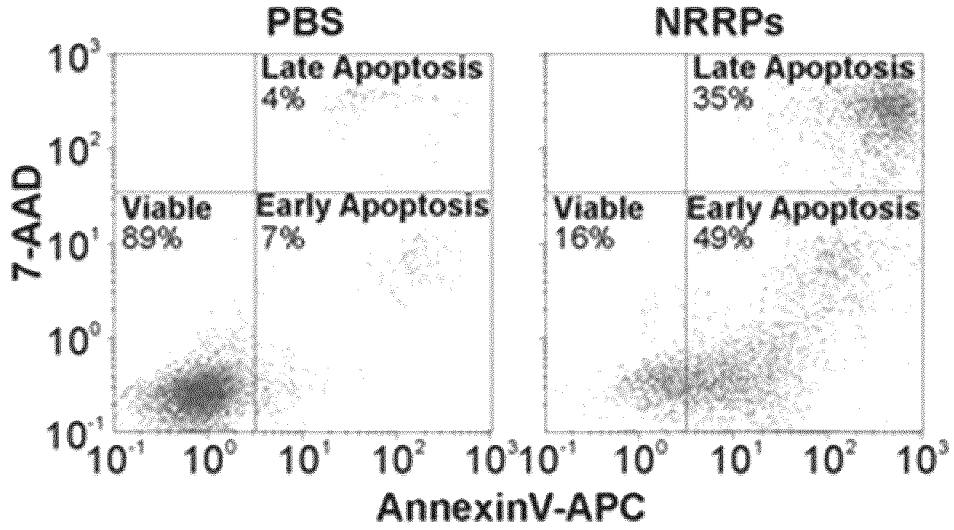

PBS
NRRPs
Late Apoptosis
4%
Late Apoptosis
35%
Viable
89%
Early Apoptosis
7%
Viable
16%
Early Apoptosis
49%
7-AAD
AnnexinV-APC

Fig. 9

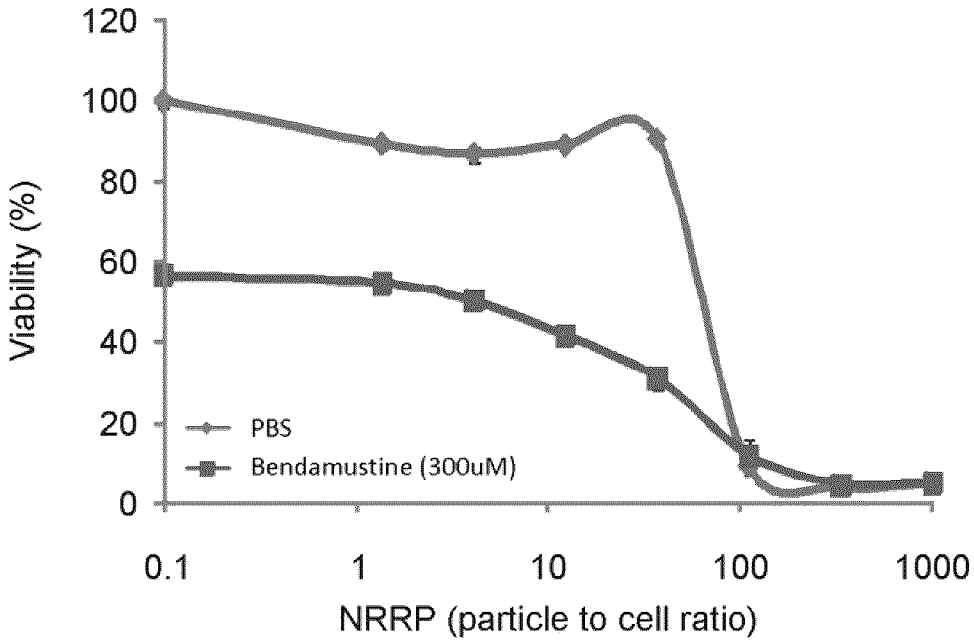

Viability (%)
PBS
Bendamustine (300uM)
NRRP (particle to cell ratio)

Fig. 10

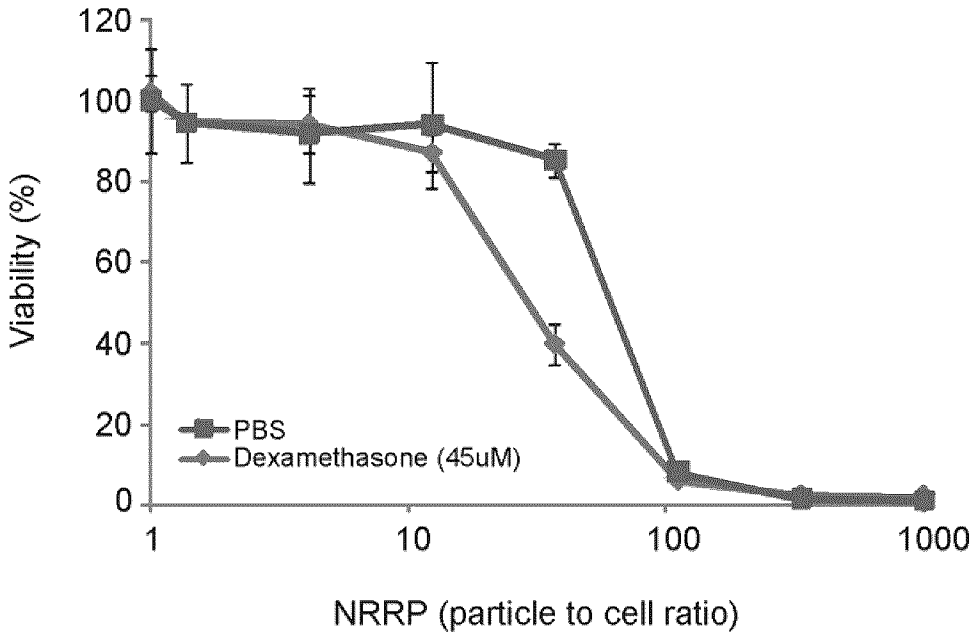

120
100
80
60
40
20
0
Viability (%)
PBS
Dexamethasone (45uM)
1
10
100
1000
NRRP (particle to cell ratio)

Fig. 11

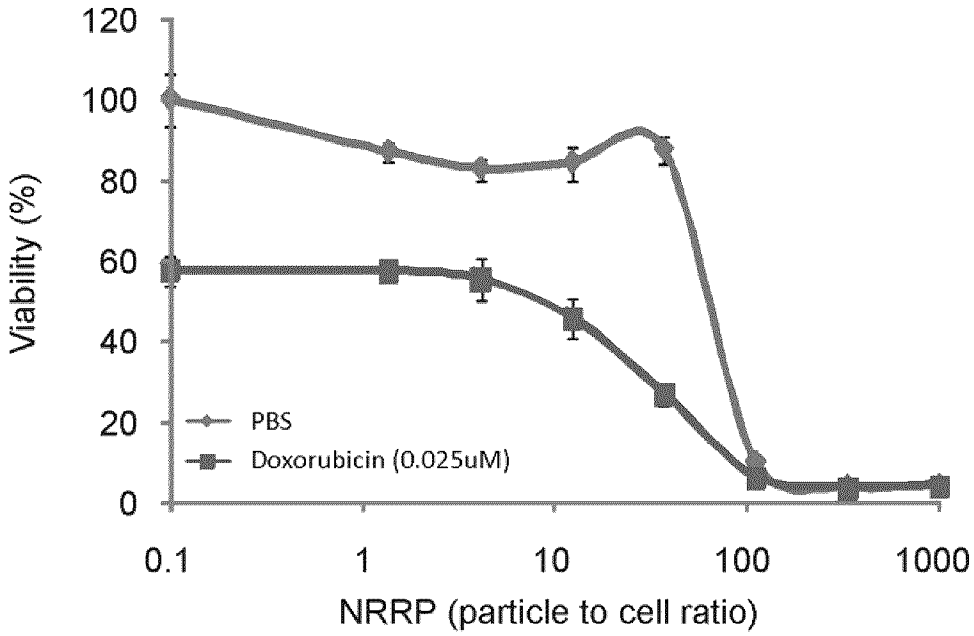

120
100
80
60
40
20
0
Viability (%)
PBS
Doxorubicin (0.025uM)
0.1
1
10
100
1000
NRRP (particle to cell ratio)

Fig. 12

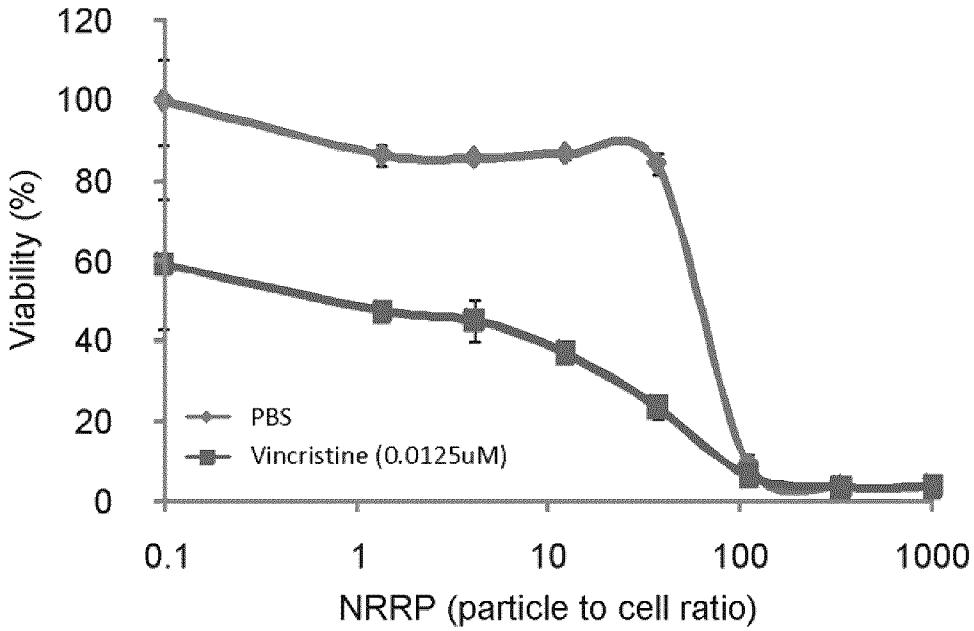

120
100
80
60
40
20
0
Viability (%)
PBS
Vincristine (0.0125uM)
0.1
1
10
100
1000
NRRP (particle to cell ratio)

Fig. 13

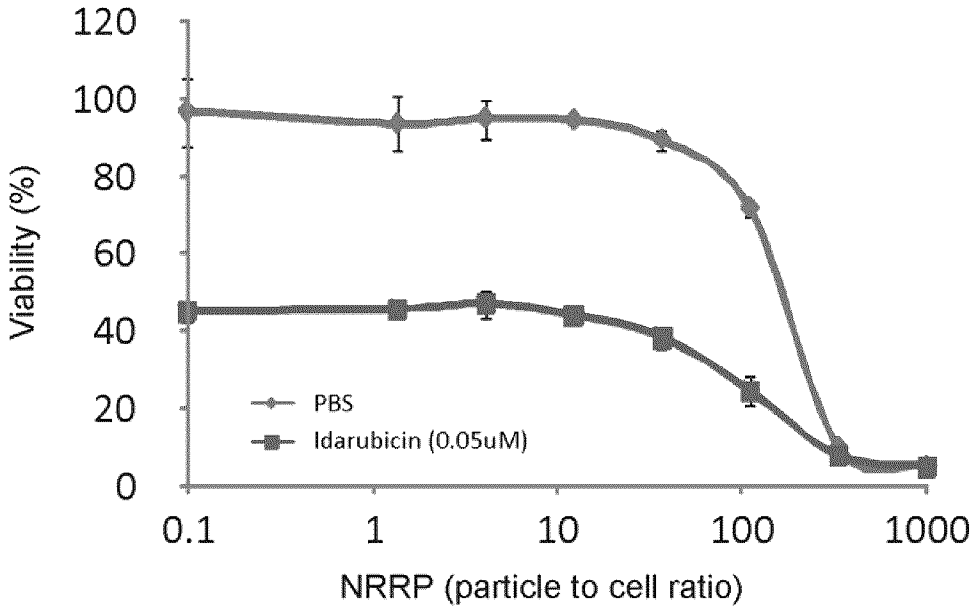

120
100
80
60
40
20
0
Viability (%)
PBS
Idarubicin (0.05uM)
0.1
1
10
100
1000
NRRP (particle to cell ratio)

Fig. 14

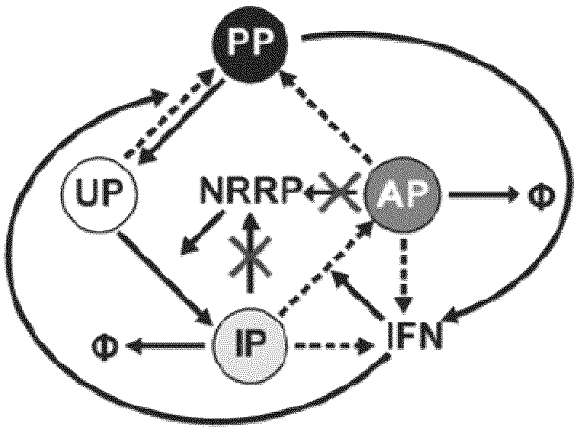



Fig. 15A

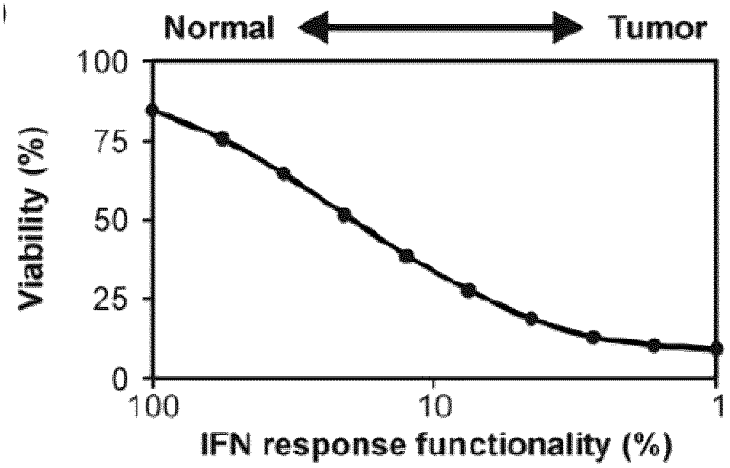



Fig. 15B

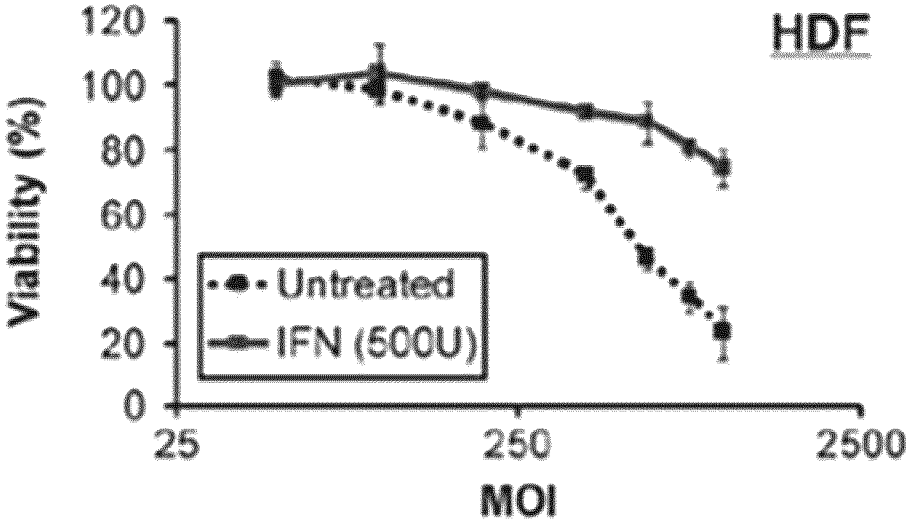



Fig. 15C

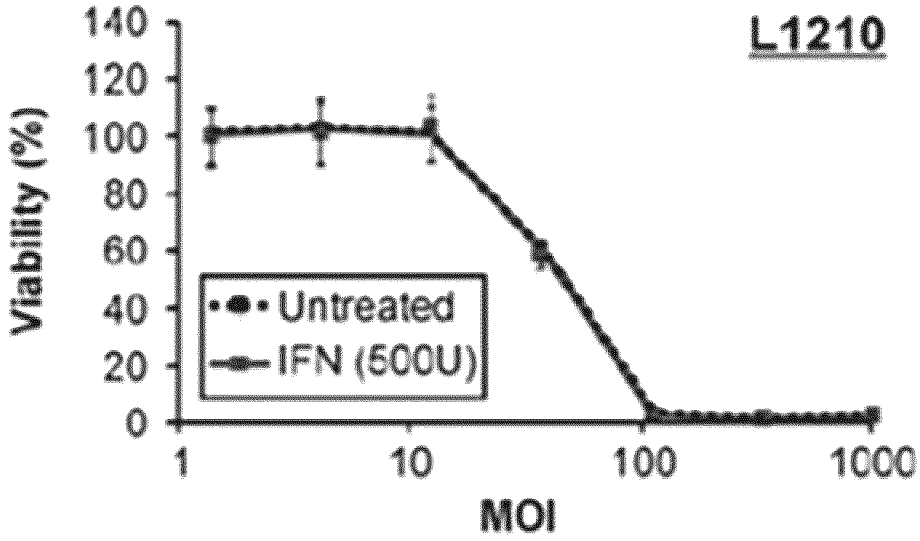



Fig. 15D

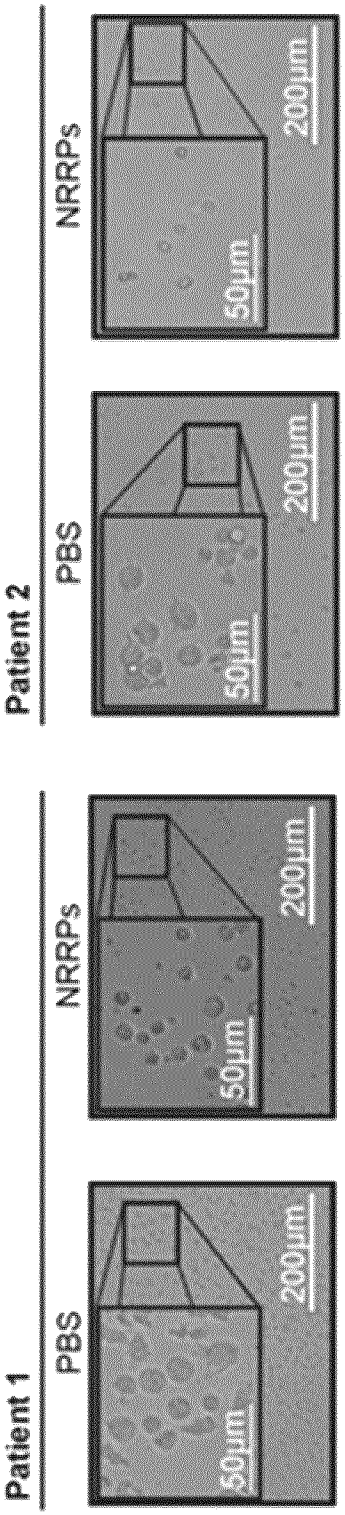

Patient 1
PBS
50µm
200µm
NRRPs
50µm
200µm
Patient 2
PBS
50µm
200µm
NRRPs
50µm
200µm

Fig. 16A

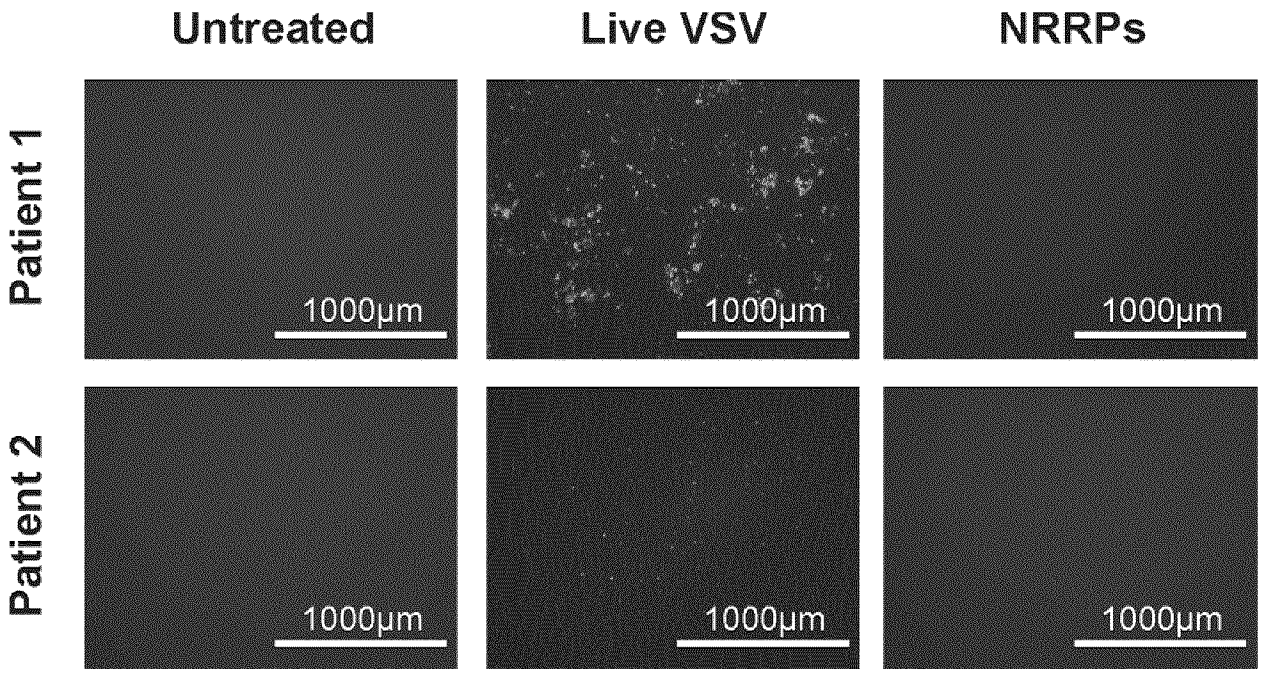

Untreated
Live VSV
NRRPs
Patient 1
Patient 2
1000µm
1000µm
1000µm
1000µm
1000µm
1000µm

Fig. 16B

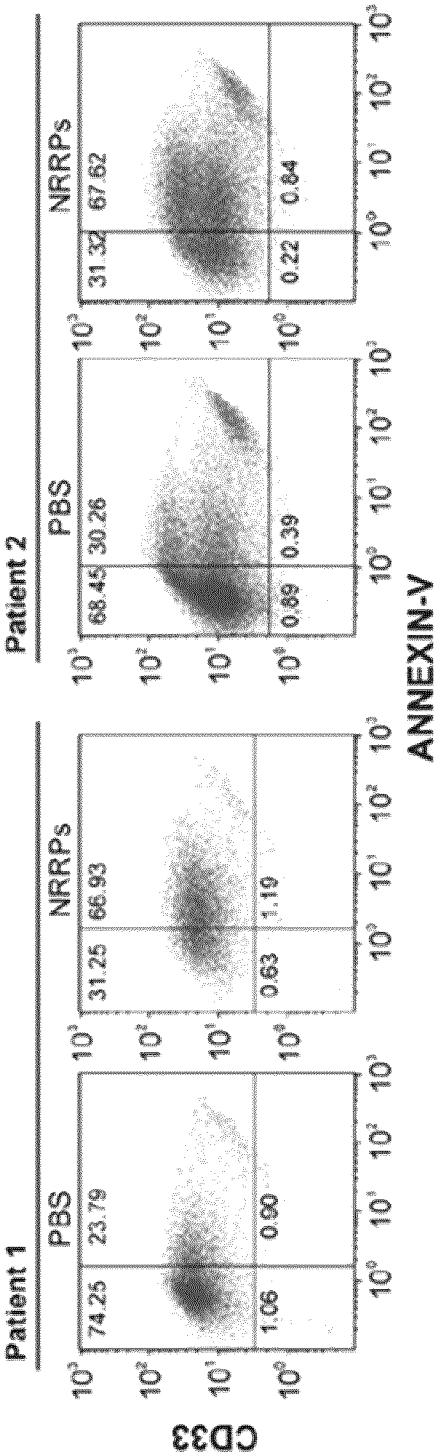



Fig. 16C

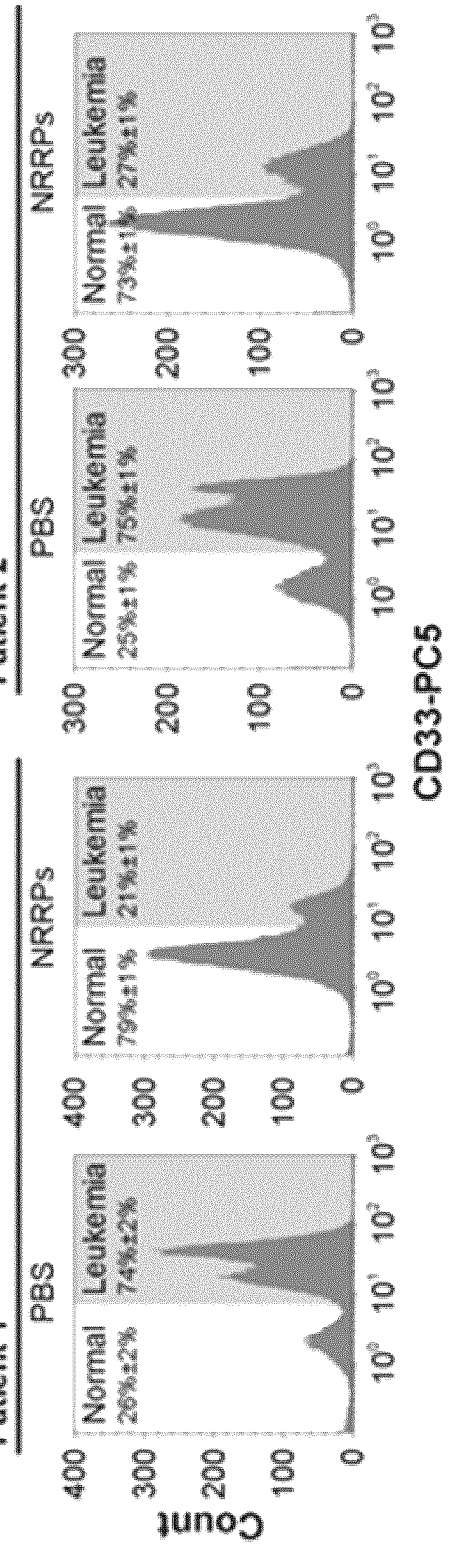



Fig. 16D

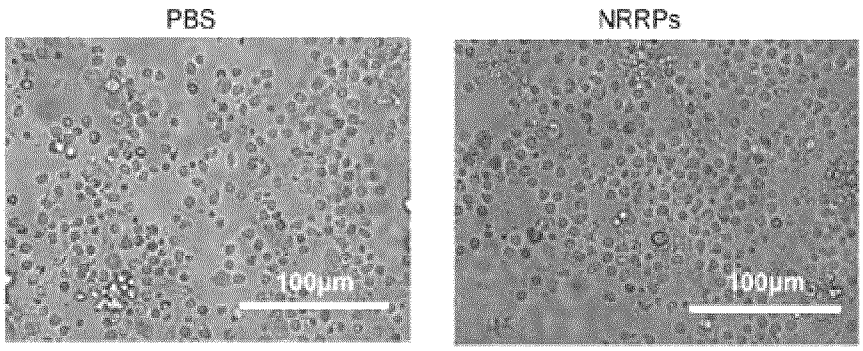



Fig. 17A

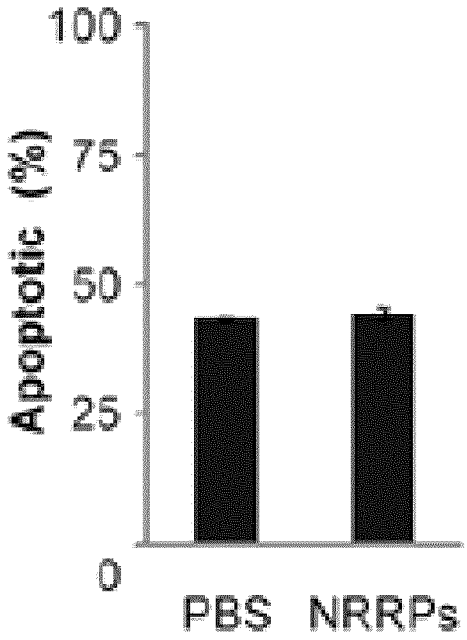



Fig. 17B

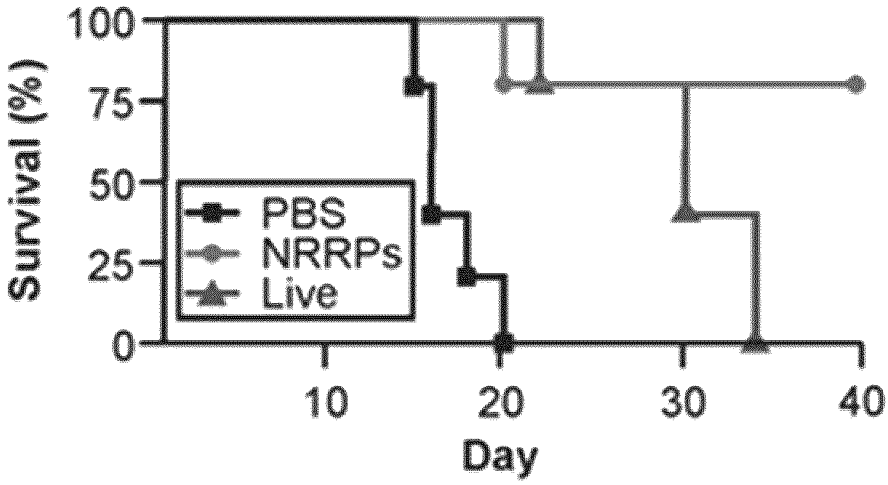



Fig. 18A

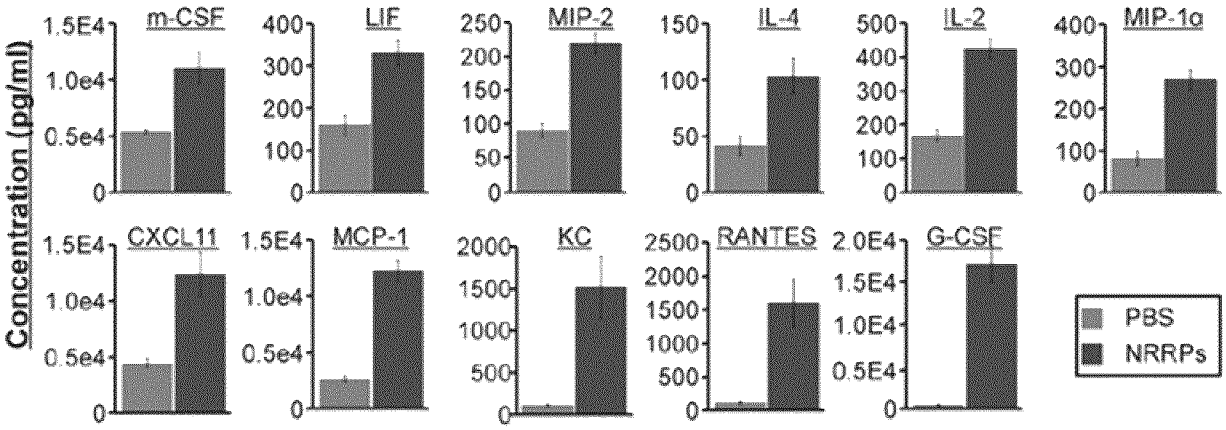



Fig. 18B

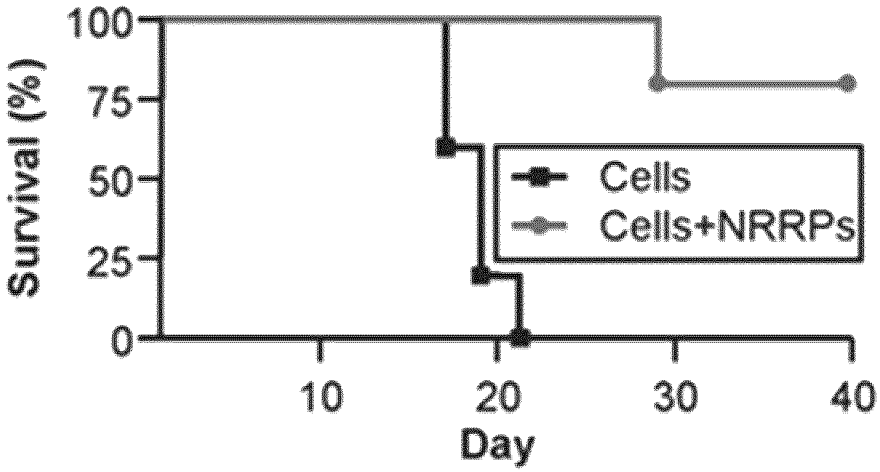

100
75
50
25
0
Survival (%)
10
20
30
40
Day
Cells
Cells+NRRPs

Fig. 19

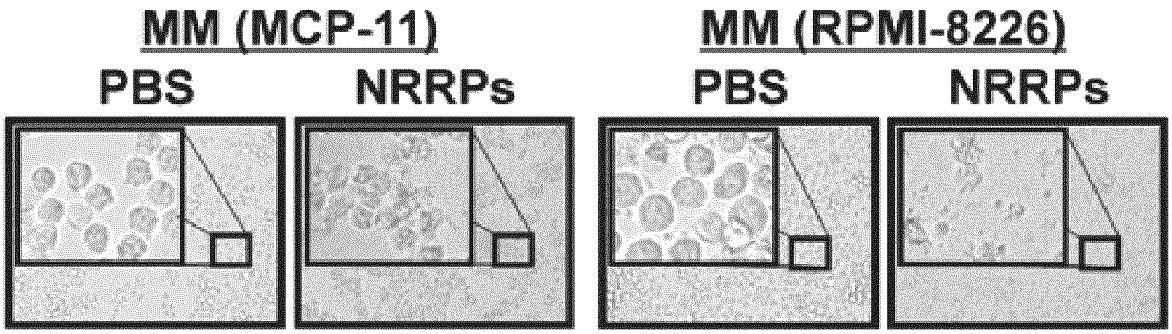

MM (MCP-11)
PBS
NRRPs
MM (RPMI-8226)
PBS
NRRPs

Fig. 20

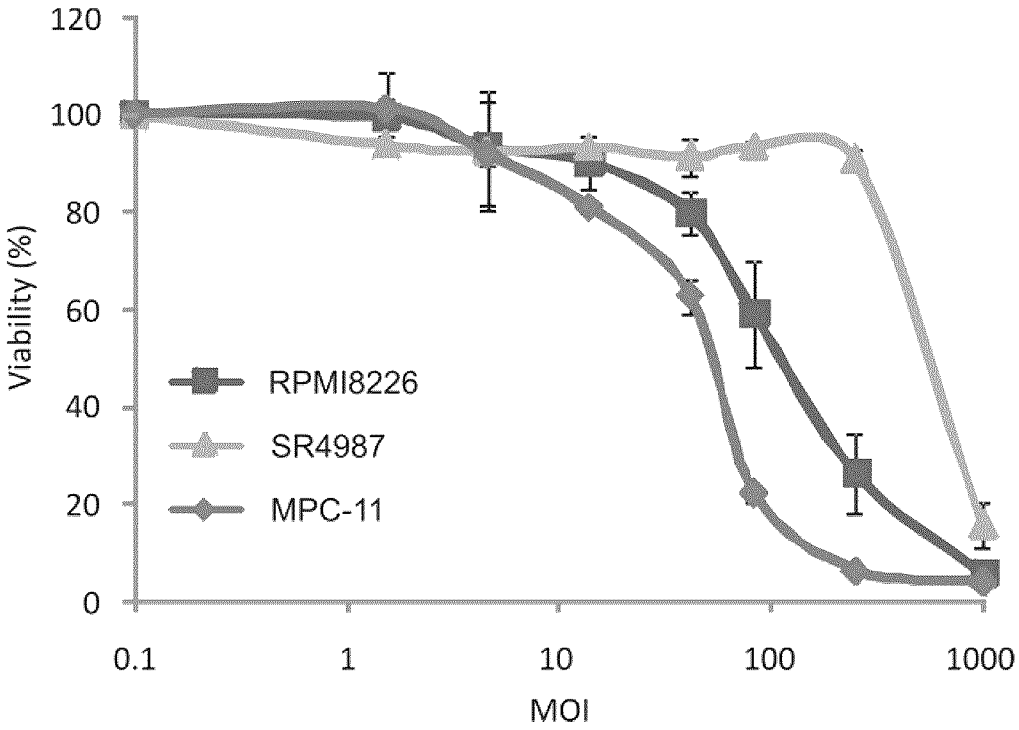

120
100
80
60
40
20
0
Viability (%)
RPMI8226
SR4987
MPC-11
0.1
1
10
100
1000
MOI

Fig. 21

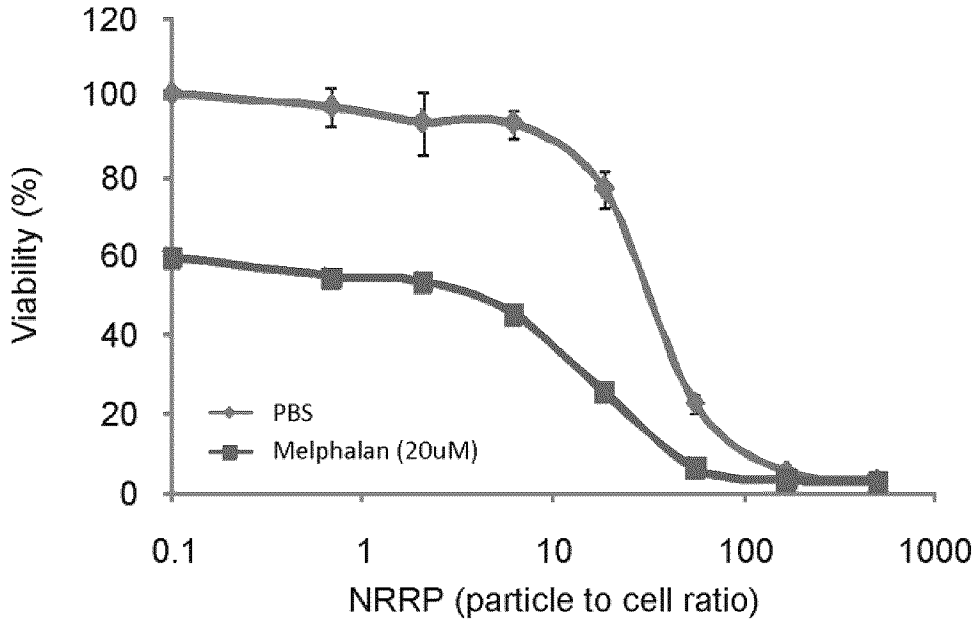

120
100
80
60
40
20
0
Viability (%)
PBS
Melphalan (20uM)
0.1
1
10
100
1000
NRRP (particle to cell ratio)

Fig. 22

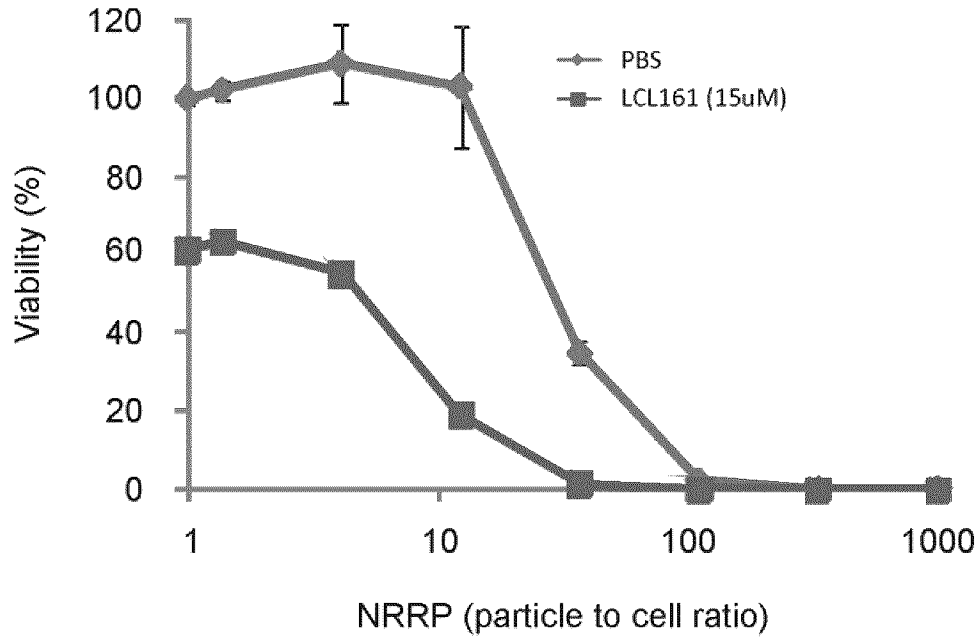

120
100
80
60
40
20
0
Viability (%)
PBS
LCL161 (15uM)
1
10
100
1000
NRRP (particle to cell ratio)

Fig. 23

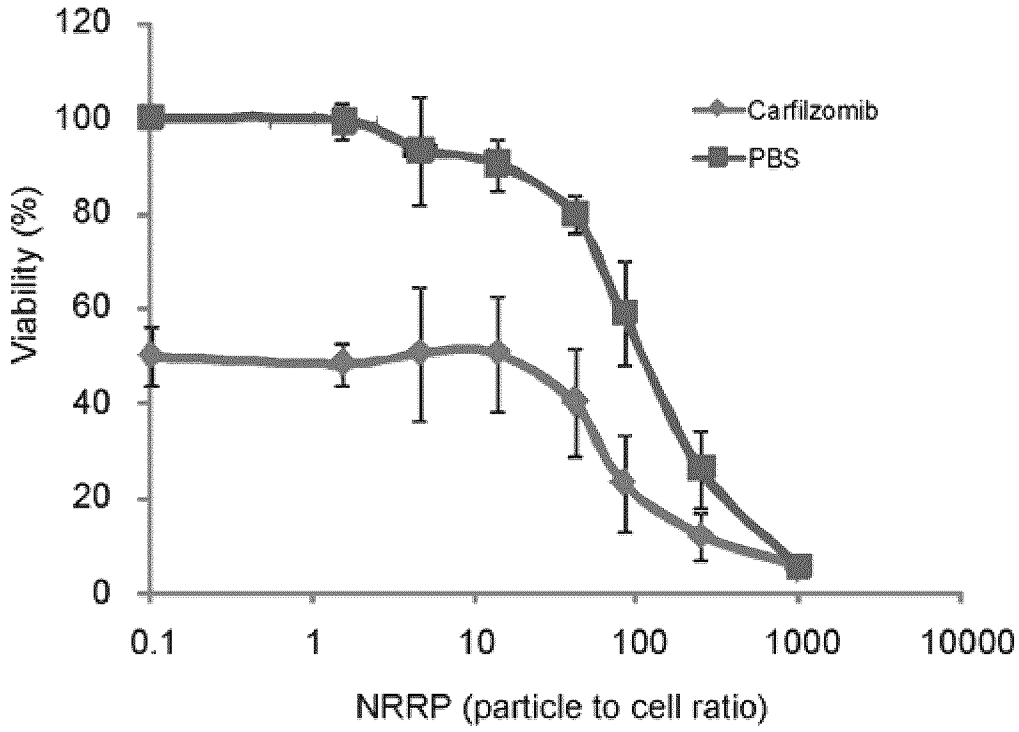
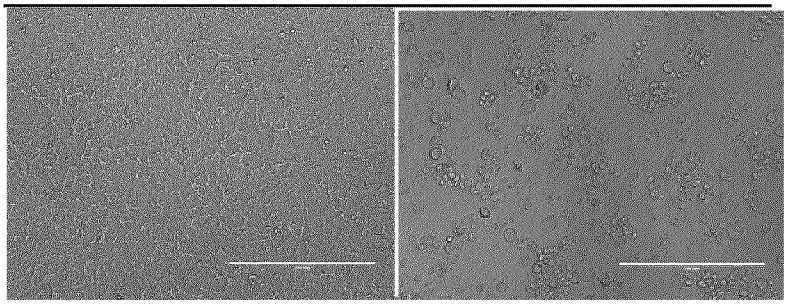

120
100
80
60
40
20
0
Viability (%)
Carfilzomib
PBS
0.1
1
10
100
1000
10000
NRRP (particle to cell ratio)

Fig. 24

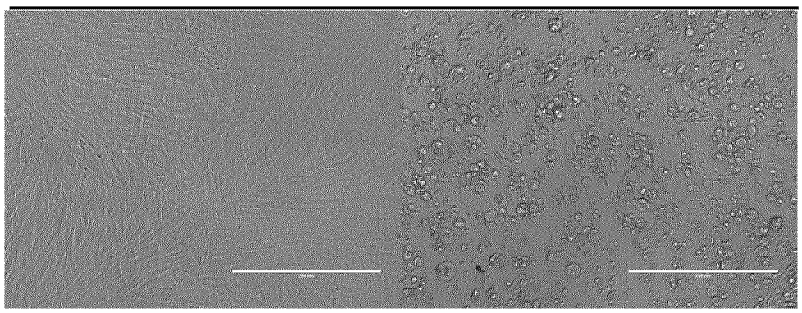
DBT



PBS NRRPs

Fig. 25A

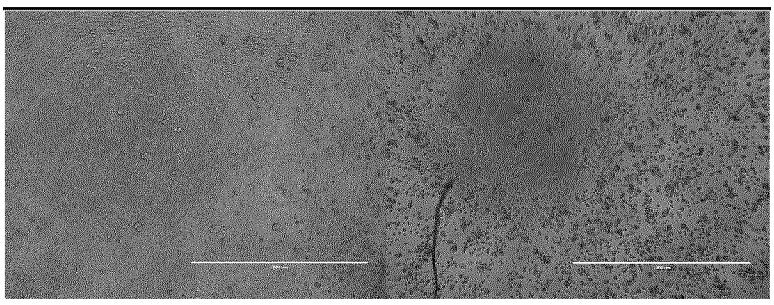
K1491



PBS NRRPs

Fig. 25B

GL261



PBS NRRPs

Fig. 25C

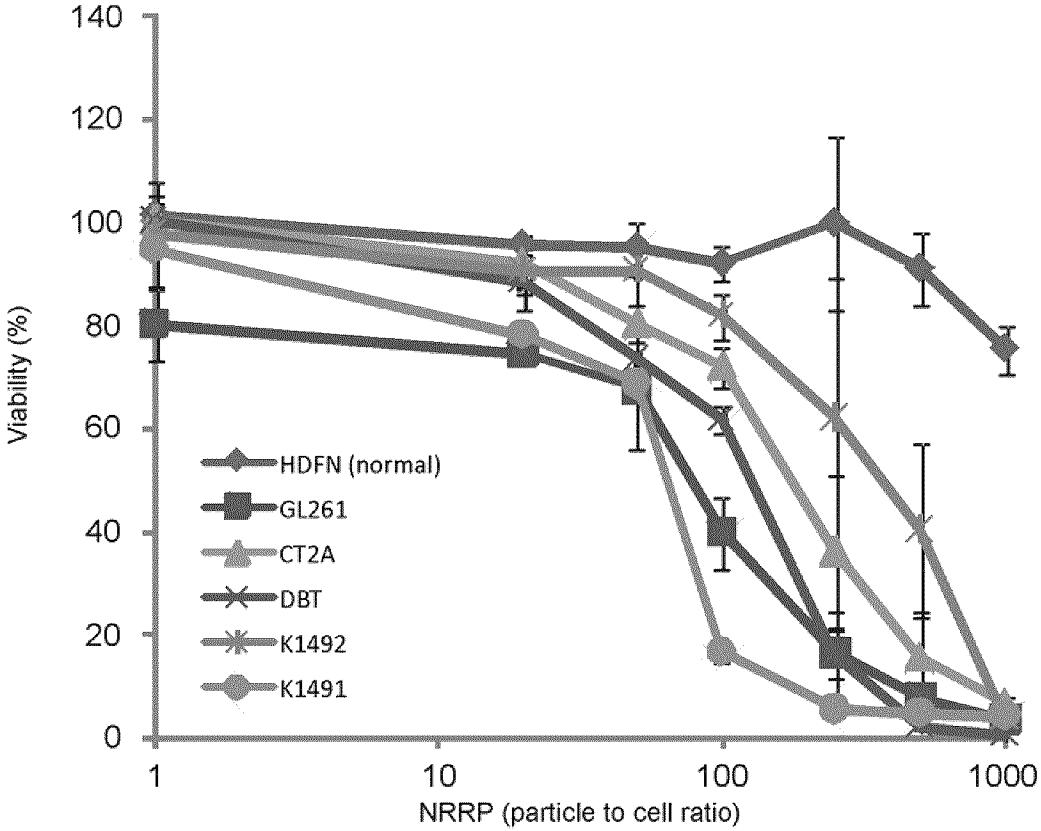

140
120
100
80
60
40
20
0
Viability (%)
HDFN (normal)
GL261
CT2A
DBT
K1492
K1491
1
10
100
1000
NRRP (particle to cell ratio)

Fig. 26

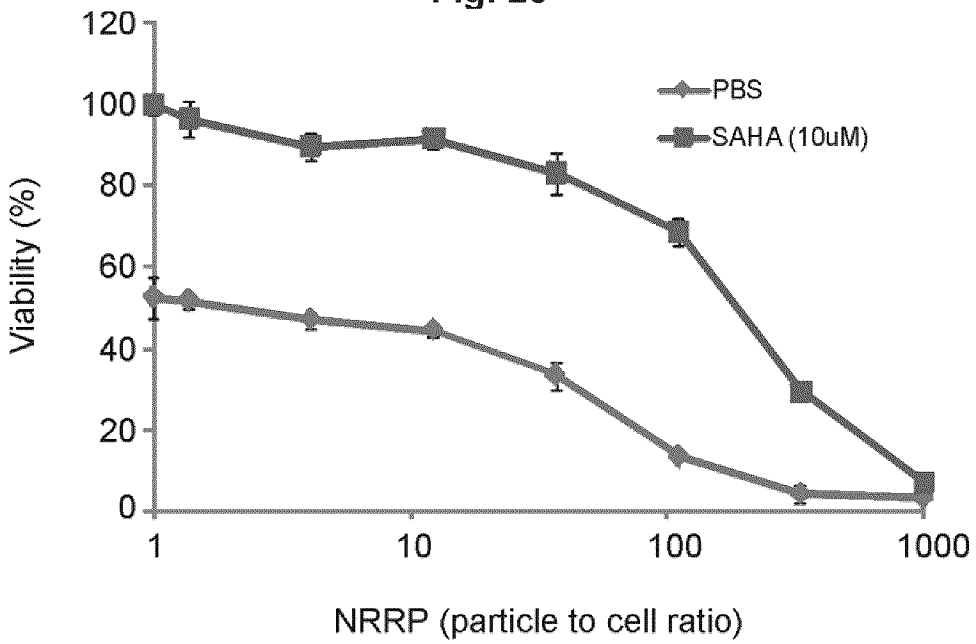

120
100
80
60
40
20
0
Viability (%)
PBS
SAHA (10uM)
1
10
100
1000
NRRP (particle to cell ratio)

Fig. 27

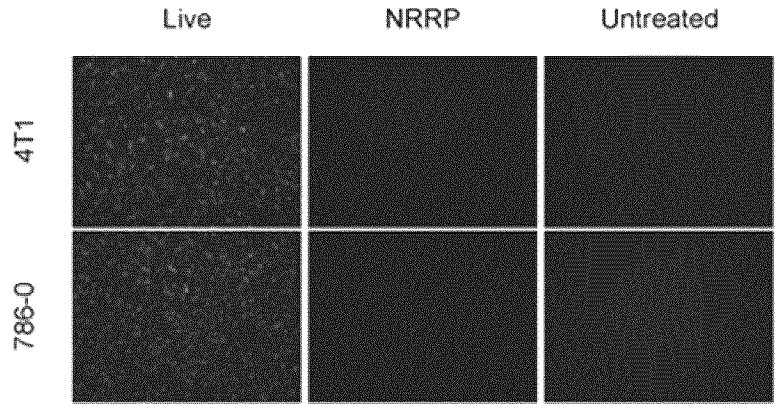



Fig. 28A

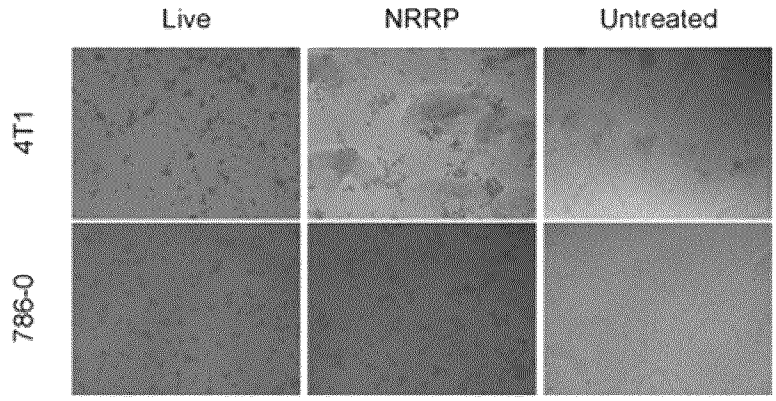



Fig. 28B

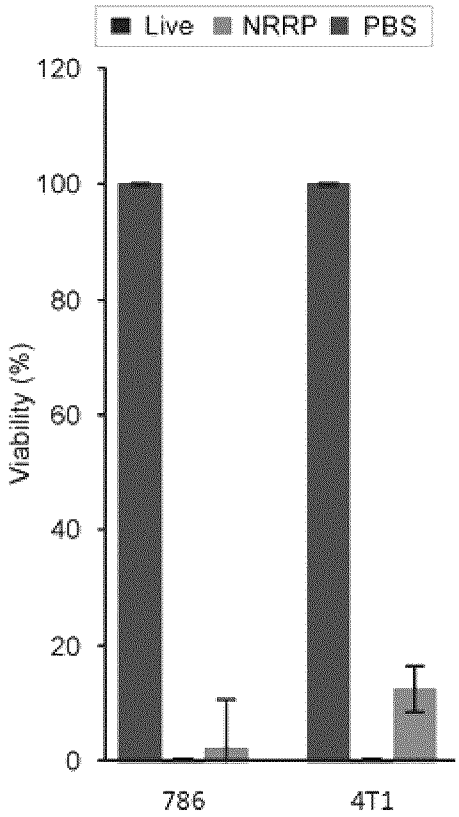



Fig. 28C

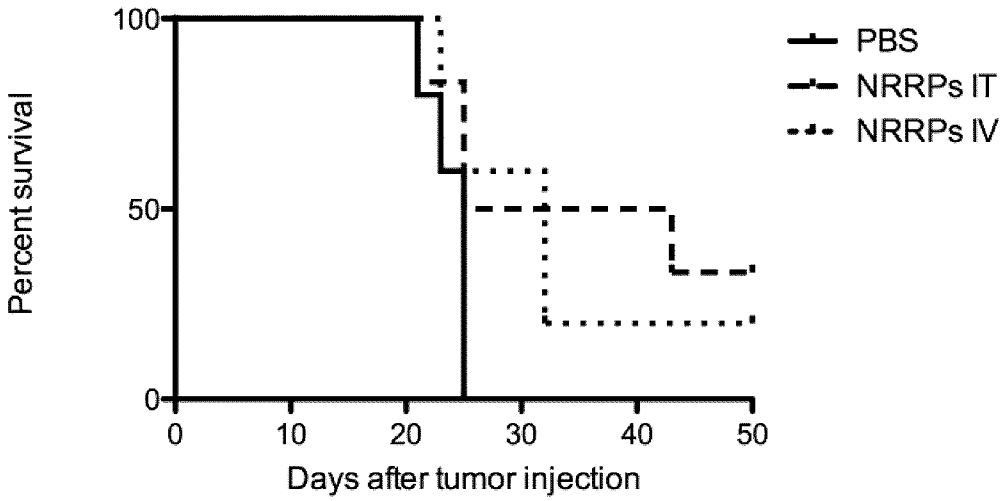



Fig. 29

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/CA2013/051009

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 7/06*** (2006.01) , ***A61K 35/76*** (2006.01) , ***A61P 35/00*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***C12N 7/06*** (2006.01) , ***A61K 35/76*** (2006.01) , ***A61P 35/00*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, TotalPatent, Pubmed, Google Scholar, Google, SCOPUS
VSV, stomatitis, cancer, virus, non-replicating, UV, rhabdovirus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2011/0223148 A1 (KANEDA, Y. ET AL.)<br>15 September 2011<br>see entire document | 1-75 |
| P, X | BATENCHUK, C. ET AL.: "Non-replicating rhabdovirus-derived particles (NRRPs) eradicate acute leukemia by direct cytolysis and induction of antitumor immunity" Blood Cancer Journal, 12 July 2013, vol. 3, e123.<br>ISSN: 2044-5385<br>see entire document | 1-75 |

[ ] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 27 February 2014 (27-02-2014) | 11 March 2014 (11-03-2014) |

| Name and mailing address of the ISA/CA | Authorized officer |
|---|---|
| Canadian Intellectual Property Office<br>Place du Portage I, C114 - 1st Floor, Box PCT<br>50 Victoria Street<br>Gatineau, Quebec K1A 0C9<br>Facsimile No.: 001-819-953-2476 | Stephen Misener (819) 934-4548 |

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/CA2013/051009

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

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

1. [X] Claim Nos. : 59-75

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 59-75 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not obliged to search under Rule 39.1(iv) of the PCT. However, this Authority has carried out a search based on the alleged effect or purpose/use of the composition as defined in claims 1-32.

2. [ ] Claim Nos. :

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

3. [ ] Claim Nos. :

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

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

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**
Information on patent family members

International application No.
PCT/CA2013/051009

| Patent Document Cited in Search Report | Publication Date | Patent Family Member(s) | Publication Date |
|---|---|---|---|
| US2011223148A1 | 15 September 2011 (15-09-2011) | EP2345415A1 | 20 July 2011 (20-07-2011) |
| | | EP2345415A4 | 14 November 2012 (14-11-2012) |
| | | WO2010032764A1 | 25 March 2010 (25-03-2010) |