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(56) Fremdragne publikationer:
WO-A1-2008/018517
WO-A1-2015/052714
WO-A2-2014/172616
US-A1- 2008 254 008
US-A1- 2013 071 414
US-A1- 2014 243 403
AIUTI A. ET AL.: "Lentivirus-based Gene Therapy of Hematopoietic Stem Cells in Wiskott-Aldrich Syndrome", SCIENCE, vol. 341, no. 6148, 1233151, 11 July 2013 (2013-07-11), XP002789810,
SUTLU T. ET AL.: "Inhibition of intracellular antiviral defense mechanisms augments lentiviral transduction of human natural killer cells: Implications for gene therapy.", HUM. GENE THER., vol. 23, October 2012 (2012-10), pages 1090-1100, XP002789811,
FAN Y.ET AL.: "TAK1 inhibitor 5Z-7-oxozeaenol sensitizes neutoblastoma to chemotherapy.", APOPTOSIS, vol. 18, 2013, pages 1224-1234, XP002789812,
NINOMIYA-TSUJI J. ET AL.: "A resorcyclic acid lactone, 5Z-7-oxozeaenol, prevents inflammation by inhibiting

Fortsættes ...

the catalytic activity of TAK1 MAPK kinase kinase.", J. BIOL. CHEM., vol. 278, no. 20, 16 May 2003 (2003-05-16) , pages 18485-18490, XP002789813,
ZHANG, J ET AL.: 'Synergistic Action of 5Z-7-oxozeaenol and Bortezomib in Inducing Apoptosis of Burkitt Lymphoma Cell Line Daudi' TUMOR BIOLOGY vol. 37, no. 1, 01 August 2015, pages 531 - 539, XP036218678
PERTEL T. ET AL.: "TRIM5 is an innate immune sensor for the retrovirus capsid lattice", NATURE, vol. 472, no. 7343, 21 April 2011 (2011-04-21), pages 361-365,

DESCRIPTION

Background

[0001] Natural killer (NK) cells are lymphocytes of the innate immune system. They are cytokine-producing and have cytotoxic ability to kill both viral infected or tumor cells. Thus, adoptive immunotherapy using NK cells is a promising approach for cancer treatment. In the last decade, several NK cell based anti-cancer products have been taken to clinical trial stage with promising clinical outcomes. However, in order to manufacture more efficient NK cell therapy products it is essential to develop novel strategies such as genetic modification of NK cells. Moreover, the use of genetically modified NK cells that have been redirected to tumor targets via the introduction of either activating or chimeric antigen receptors presents an attractive prospect for further clinical applications (Pegram et al., 2009). Introduction of genes expressing various cytokines for enhancement of *in vivo* survival and cytotoxicity of adoptively transferred NK cells are also among various approaches. The problem remains that NK cells are inherently resistant to retroviral infections (Lanier 2008; Alici et al, 2009; Brandstadler and Yang, 2011; Sutlu et al, 2012) While enhanced retroviral and lentiviral gene delivery to NK cells through enhanced proliferation and targeting intracellular viral defense mechanism by small molecule inhibitors has been shown (Sutlu et al., 2012). It is also a problem to deliver genetic materials via viral vectors to macrophages and stem cells. There remains a need in the art for more efficient transfection of cells, in particular NK, hematopoietic stem cells and macrophages.

Brief Description of the Invention

[0002] The present invention concerns the administration of (5Z)-7-Oxozeaenol (iX) in vitro to cells with an RNA viral vector to improve transfection and thereby enhance proliferation of NK, stem cells and macrophages. This method can be applied to the preparation of such cells in view of their use in adoptive cell therapy. While not wanting to be limited by any theory it is believed that the increases in genetic modification observed are due to downregulation of intracellular defense mechanisms mediated by Rig I. Cells treated in this manner can be used to treat cancer, diseases caused by a known genetic mutation and metabolic disorders caused by a known genetic mutation.

[0003] Specifically, the invention is defined in the claims and concerns, in a first aspect, an *in vitro* method of enhancing gene delivery to cells comprising the co-administration of (5Z)-7-Oxozeaenol, along with a retroviral or lentiviral vector to a cell in a nutrient media wherein the cells are selected from natural killer, stem cells and macrophages.

[0004] In one embodiment, the cells are NK92 cells. In a further embodiment, he (5Z)-7-Oxozeaenol is administered in an amount sufficient to achieve a concentration from about 0.04

pM to about 10 pM in the nutrient media.

[0005] In a further aspect, the invention concerns an *ex vivo* method of preparing cells suitable for use in adoptive cell transfer to a patient, said method comprising delivering genes to cells using a retroviral or lentiviral vector with co-administration of 5Z)-7-Oxozeaenol,wherein the cells are selected from natural killer, stem cells and macrophages.

[0006] In one embodiment, the transfected cells are suitable for use in adoptive cell transfer for treating cancer. The cancer may be selcted from: carcinomas, sarcomas, lymphomas, leukemias, and blastomas: acute lymphoblastic leukemia (all), acute myeloid leukemia, adrenocortical carcinoma, aids-related cancers, analcancer, astrocytoma, basal-cell carcinoma, extrahepatic bile duct cancer(cholangiocarcinoma), bladder cancer, bone tumor (osteosarcoma/malignant fibrous histiocytoma), brainstem glioma, brain cancer, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas/carcinoids, burkitt's lymphoma, central nervous system lymphoma, cervical cancer, chondrosarcoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous t-cell lymphoma, desmoplastic small round cell tumor,endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (gist), extracranial, extragonadal, or ovarian germ cell tumor, gestational trophoblastic hodgkin lymphoma, intraocular melanoma, islet cell carcinoma (endocrine pancreas), kaposi sarcoma, kidney cancer (renal cell cancer), acute lymphoblastic leukaemia (also called acute lymphocytic leukemia), acute myeloid leukemia (also called acute myelogenous leukemia), chronic lymphocytic leukemia, chronic myelogenous leukemia (also called chronic myeloid leukemia), hairy cell leukemia, lip and oral cavity cancer, liposarcoma, non small cell lung cancer, small cell lung cancer, macroglobulinemia, waldenstrbm, male breast cancer, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanoma, intraocular (eye)melanoma, Merkel cell cancer, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, chronic myelogenous leukemia, acute myeloid leukemia, myeloid leukemia, multiple myeloma (cancer of the bone-marrow), myeloproliferativedisorders, myxoma, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-small cell lung cancer, oligodendroglioma, oralcancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer (surface epithelial-stromal tumor),ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary adenoma, plasma cell neoplasia/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma (kidney cancer), renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland

cancer, soft tissue sarcoma, uterine sarcoma, Sezary syndrome, melanoma and nonmelanoma skin cancer, merkel cell skin carcinoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer with occult primary, stomach cancer, supratentorial primitive neuroectodermal tumor, t-cell lymphoma (mycosis fungoides and sezarysyndrome), testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, trophoblastic tumor, ureter and renal pelvis transitional cell cancer, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma.

[0007] In another embodiment, the transfected cells are suitable for use in adoptive cell transfer for treating a disease caused by genetic mutation. Such disease may be selected from: 21-hydroxylase deficiency, achondroplasia, acute intermittent porphyria, adenylosuccinate lyase deficiency, adrenoleukodystrophy, Alagille syndrome, Alexander disease, Alström syndrome, amelogenesis imperfecta, biotinidase deficiency, CGD Chronic granulomatous disorder, Di George's syndrome, fanconi anemia, G6PD deficiency, lipoprotein lipase deficiency, muscular dystrophy, Duchenne type, Siderius X-linked mental retardation syndrome caused by mutations in the PHF8 gene, X-linked severe combined immunodeficiency (XSCID), or X-linked sideroblastic anemia (XLSA), x linked severe combined immunodeficiency disease.

[0008] Alternatively, the disease may be a metabolic disorder caused by a known genetic mutation, such as Niemann-Pick disease, Tay-Sachs disease, Gaucher disease, Fabry disease.

Brief Description of the Figures

[0009]

Figure 1 contains two bar graphs showing the inhibition of innate immune signaling with iX enhances lentiviral transduction efficiency in NK cells. NK-92 cells and primary human NK cells isolated from healthy donor PBMCs were subjected to lentiviral transduction in the presence of 6 μM BX795 or iX for 6 hours. GFP expression is acquired 72 hours later. + cell percentages are shown on graph.

Figure 2A is a chart showing that iX has a dose-dependent effect with minimal toxicity to NK cells. Primary human NK cells and NK-92 cells are transduced with LeGO-G2 virus in the presence of varying doses of BX795 or iX for 6 hours. GFP expression is analyzed by flow cytometry 72 hours after transduction. GFP+ cell percentages are shown on graph. Data from three independent experiments, each run in triplicates.

Figure 2B contains two bar graphs comparing apoptotic to dead Annexin -V^+ /PI^-cells.

Figure 3 contains photos of a gel showing iX treatment during lentiviral transduction decreases RIG-I and IRF-3.

Figure 4 contains two bar graphs showing that iX treatment interferes with anti-viral cytokines IFNγ and TNF secretion.

Figure 5 is a graph showing that iX treatment has a unique transcriptome signature compared to BX795.

Figures 6A-d show the purported signaling pathways affected by BXx795.

Figures 6E-H show the purported signaling pathways affected by iX.

Figure 7 is a graph showing that administration of iX increases gene editing.

Detailed Description of the Invention

[0010] Cell therapy is rapidly becoming a viable means for treatment of mammalian diseases. Unfortunately, many therapeutic applications are limited by the low efficiency in which genes can be delivered to target cells. The use of iX together with the methods below achieves unexpected results. Prior to these experiments it was unknown that a small molecule could target RIG I and down regulate intracellular defense mechanisms. Using the present invention desired genes can be more readily introduced to target cells. It is believed that analogs, derivatives or mimetics of iX would also be effective.

Example 1

Materials and Methods:

Cell lines:

[0011] 293FT cells were purchased from Invitrogen (Life Technologies, Grand Island, NY, USA) and maintained in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO, Life Technologies, Grand Island, NY, USA) supplemented with 10% Fetal Bovine Serum (FBS) (GIBCO), 0.1 mM non-essential amino acids (Sigma-Aldrich, St. Louis, MO, USA), 6 mM L-glutamine (Sigma-Aldrich), 1 mM sodium pyruvate (Sigma-Aldrich) and 20 mM HEPES (Sigma-Aldrich). NK92 cells were maintained in CellGro SCGM (Cellgenix) medium supplemented with 20% FBS and 1000 U/ml rhIL-2 (Proleukin, Chiron Corporation).

Production of lentiviral vectors

[0012] For production of VSV-G pseudotyped lentiviral vectors, $14x10^6$ 293FT cells were plated into a poly-D-lysine coated 150 mm dish (BD Biosciences, San Jose, CA, USA). Next day cells were transfected with 30 µg of LeGO-G2 plasmid (courtesy of Prof. Boris Fehse, University Medical Center Hamburg-Eppendorf, Hamburg, Germany), 15 µg of pMDLg/pRRE (Addgene, Cambridge, MA, USA), 10 µg of pRSV-REV (Addgene) and 5 µg of phCMV-VSV-G (Addgene) using calcium phosphate transfection kit (Sigma-Aldrich) in the presence of 25 µM Chloroquine (Sigma-Aldrich). 10 hours after transfection, the medium was changed and thereafter virus containing supernatant was collected every 24 hours for 2-3 days and stored in - 80°C until further use. A small aliquot from each production was used to determine viral titers by transduction of 293FT cells with serially diluted amounts of virus supernatant.

Primary natural killer cell isolation and culture

[0013] Buffy coats were obtained from healthy donors via the blood bank at the Karolinska University Hospital, Huddinge. The experimental protocol was approved by the local research ethics committee.

[0014] The peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation, using Lymphoprep (Nyegaard, Oslo, Norway) and washed twice with phosphate-buffered saline (PBS) (GIBCO, Grand Island, NY, USA). Cell count and viability were assessed by Turk and Trypan Blue dye exclusion. NK cells were obtained by using NK cell isolation kit (Miltenyi Biotec, Cologne, Germany) and the AutoMACS machine (Miltenyi Biotec) according to manufacturer's instructions. After isolation, NK cells were put into culture at a concentration of $1x10^6$ cells/ml in CellGro SCGM (Cellgenix) supplemented with 10% Human AB Serum (Lonza, Basel, Switzerland) and 1000 U/ml rhIL-2 (Proleukin, Chiron Corporation). For initial testing of cytokine stimulations prior to transduction (Figure 1), IL-12 (Peprotech, Rocky Hill, NJ, USA), IL-15 (Peprotech) and IL-21 (Peprotech) were used at a concentration of 20 ng/ml. For the rest of the experiments, only 1000 U/ml rhIL-2 and 20 ng/ml IL-21 were used.

Lentiviral Transduction of Natural Killer Cells

[0015] For each lentiviral transduction, $0.25x10^6$ NK cells per well were seeded in a 24-well plate (BD Biosciences) and mixed with an appropriate amount of virus supernatant in the presence of 8 µg/ml of protamine sulfate (Sigma-Aldrich) or polybrene (Sigma-Aldrich) in a final volume of no more than 1 ml. The cytokines were replenished and plates were centrifuged at 1000xg for 1 hour at room temperature. After centrifugation, without removing viral supernatants, the plates were incubated at 37°C, 5% CO_2 for 4-6 hours. At the end of the incubation, a second centrifugation at 1000xg for 1 hour at room temperature was carried out,

after which the supernatants were removed from the wells and 1 ml of fresh NK cell growth medium (CellGro SCGM supplemented with 10% Human AB Serum) per well was added. The cells were maintained in this medium with daily addition cytokines (IL-2: 1000 U/ml, and IL-21: 20 ng/ml. Combinations of cytokines were used as indicated in Figure 1. Only IL-2 and IL-21 were used in the rest of the experiments) for at least 3 days before acquisition of eGFP expression was carried out. In indicated experiments, the following inhibitors were present during the transduction: BX795 (Invivogen) and (5Z)-7-Oxozeaenol (iX) (Tocris).

Lentiviral Transduction of NK92 Cell Line

[0016] For the transduction of cell lines, $2x10^5$ cells per well were seeded into 24-well plates. Treatment with 6μM BX795 and (5Z)-7-Oxozeaenol (iX) was initiated simultaneously with the transduction process. Appropriate amount of viral supernatants were added into the wells along with protamine sulfate (NK92, cells) and the total volume was adjusted to 500 μl. The plates were centrifuged at 1000Xg for 1 hour followed by incubation at 37°C, 5% CO_2 for 4-6 hours, after which the virus containing supernatant was removed and fresh growth media was added. The cells were grown for at least 3 days before acquisition of eGFP expression was carried out.

Gene editing

[0017] The recent emergence of the clustered, regularly interspaced, palindromic repeats (CRISPR) system for gene editing has the potential to overcome these limitations. The CRISPR technology utilizes a fixed nuclease, often the CRISPR-associated protein 9 (Cas9) from Streptococcus pyogenes, in combination with a short guide RNA (gRNA) to target the nuclease to a specific DNA sequence. We confirmed that gRNA recognition and thus editing efficacy could be increased with introduction of iX inhibitor. Human primary macrophages, CD34+ HSCs and NK92 cell line were transfected with Cas9-2A-GFP and gRNA encoding plasmids using respective Amaxa kits and cell-specific program using Nucleofector II device (VAPA-1003 for CD34+ HSCs, VAPA-1008 for Macrophages cells, and VVPA-1005 for NK92 cells) according to manufacturer's instructions except in half of the groups, we have added 1uM iX into the manufacturer's transfection reagent.

[0018] The following commercially available guide RNAs from Origene Technologies were tested against Beta 2 Microglobulin: GATGTCTCGCTCCGTGGCCT (Seq. ID No. 1); CTCGCGCTACTCTCTCTTTC (Seq. ID No. 2); GACTCACGCTGGATAGCCTC (Seq. ID No. 3); CCAGAAAGAGAGAGTAGCGC (Seq. ID No. 4) CACAGCTAAGGCCACGGAGC (Seq. ID No. 5); GGCCGAGATGTCTCGCTCCG (Seq. ID No. 6); TTGCGGGAGCGCATGCCTTT (Seq. ID No. 7); CCACCTCTTGATGGGGCTAG (Seq. ID No. 8); ATACCTTGGGTTGATCCACT (Seq. ID No. 9); CGTGAGTAAACCTGAATCTT (Seq. ID No. 10); AAGTCAACTTCAATGTCGGA (Seq. ID No. 11); CATAGATCGAGACATGTAAG (Seq. ID No. 12); GCTACTCTCTCTTTCTGGCC (Seq. ID

No. 13);ACCCAAACCAAGCCTTTCTA (Seq. ID No. 14); and TATAAGTGGAGGCGTCGCGC (Seq. ID No. 15). 48 hours post-transfection, cells were stained with anti-B2M antibody (clone:2M2, Biolegend) to compare loss of β_2m expression. Cells were then analysed using flowcytometry as described below.

Flow cytometry

[0019] All antibody stainings for flow cytometry were done according to the following protocol: For surface stainings, the cells were washed once with PBS and incubated with appropriate amounts of antibody at 4°C for 30 min. The labeled cells were then washed with PBS and fixed in 1% PFA prior to data acquisition. Data acquisition was done on a FACSCalibur (BD Biosciences), CyFlow ML (Partec GmbH, Munster, Germany) and LSRII-Fortessa (BD Biosciences) with standard filters. Data were analysed with the FlowJo software (TreeStar Inc.). The antibodies used for NK cells were, CD56 (NCAM16.2), CD56 (B159), CD3 (SK7), CD3 (SP34-2) from BD Biosciences.

Results and Discussion:

Inhibition of intracellular innate immune sensor pathways enhances lentiviral gene delivery efficiency in NK cells

[0020] We have shown that innate immune sensor mediated detection of viral vector components activate an anti-viral response in NK cells, negatively effecting the efficiency of lentiviral transduction. We successfully used small molecule inhibitors of innate immune signaling during lentiviral transduction to deactivate or reduce the anti-viral response.

[0021] As we have previously reported (Sutlu et al., 2012), the use of BX795 dramatically increases transduction efficiency in NK cells. BX795 is an inhibitor of TBK1/IKKε complex that acts as a common mediator in the signaling pathways of RIG-I, MDA-5 and TLR3 (Clark, et al. 2009). Therefore, it might be possible to state that the lentiviral RNA is recognized by one or more of these receptors and an anti-viral response is triggered, which can be inhibited by the use of BX795.

[0022] Here we show that the use of the TAK1 inhibitor iX enhances lentiviral gene delivery to NK cells to a further extent compared to BX795. When used at a 6 μM concentration during lentiviral transduction, iX provides a statistically significant improvement in gene delivery to both primary human NK cells and the human NK cell line NK-92 (Figure 1).

[0023] Taken together, these results support the hypothesis that during transduction, intracellular anti-viral defense mechanisms are activated and contribute significantly to the

resistance of NK cells to lentiviral genetic modification. The inhibition of this response using small molecule inhibitors significantly enhances the gene delivery efficiency.

[0024] We have shown that iX increases gene editing efficacy in hematopoietic stem cells, macrophages and NK cells. Testing iX at 1μM level for a short period of time has increased β_2M editing significantly in all three cell types. The results are summarized in Figure 7 (n=4).

iX shows a dose dependent effect with minimal toxicity to NK cells

[0025] Testing different concentrations of iX has shown that the inhibitor, as was shown previously for BX795, has a dose-dependent effect on increasing genetic modification efficiency in NK cells (Figure 2A). For iX, although a significant effect is seen already at 0.5μM concentration, this effect increases even more up to 1.5μM after which it seems to stabilize. Compared to BX795, iX performs better at lower doses and enhances lentiviral gene delivery efficiency to a significantly higher extent.

[0026] Remarkably, the use of iX at the optimum concentration presents no immediate toxic effects on NK cells as determined by Annexin-V/PI staining after BX795 treatment (Figure 2b). At the optimum dose of 1.5μM, iX shows no significant toxicity on NK cells when compared to DMSO controls. At 1.5μM, BX795 similarly shows no toxicity while at the optimum dose of 6μM, a quite small but statistically significant increase is observed in apoptotic and dead cells in culture. Conversely, no signs of immediate toxicity to the NK cells were observed for iX even at the 6μM concentration.

[0027] Taken together, these results indicate that iX is not only more efficient than BX795 in enhancing lentiviral gene delivery efficiency but also significantly less toxic to the cells.

iX treatment during lentiviral gene delivery downregulates RIG-I and IRF3

[0028] In order to better characterize the effects of the inhibitors on innate immune signaling during lentiviral transduction, we have analyzed the expression of the viral RNA sensor molecule RIG-I and the transcription factor IRF3 that acts downstream of RIG-I. While the changes expression of these genes were not detectable at the protein level after the 6 hour transduction period, a significant decrease in both RIG-I and IRF3 expression attributable to the use of BX795 and iX was observed at 24 hours (Figure 3).

[0029] Moreover, we have observed that the extent of downregulation in RIG-I and IRF3 was significantly more when iX was used, compared to the use of BX795. This might be a possible explanation to why iX performs better than BX795 in enhancing lentiviral gene delivery.

iX treatment during Lentiviral gene delivery suppresses IFNγ and TNF responses.

[0030] In order to further characterize mechanisms behind superior lentiviral gene delivery triggered by iX, we tested cytokine mediated responses upon exposure to lentiviral particles in the presence of BX795 and iX. NK92 cells treated with iX during lentiviral gene delivery, significantly decreased the secretion of anti-viral cytokine, IFNy, already at six hours while BX795 could only significantly trigger similar effect 24 hours after the exposure to the lentiviral particles. (Figure 4). Moreover, similar trend was observed in the same setup for an other antiviral cytokine TNF. (Figure 4). Overall, using iX during lentiviral gene delivery is superior than BX795 and this could be due to inactivation of RIG-I pathway and downstream signaling molecule IRF-3 which leads to decreased secretion of danger signals, anti-viral cytokines. (Figure 4).

iX and BX795 have differential gene expression profile.

[0031] Gene expression profiling by RNAseq for identification of pathways triggered by lentivirus entry in the absence and presence of small molecule inhibitors are assessed. NK-92 cells are transduced as previously described. At the end of the 6 hour transduction protocol, RNA from the cells is extracted and sequenced on a HiSeq 2500 instrument. After quality control, the reads are mapped to the human genome using the STAR aligner and gene abundance is estimated with HT-Seq. Differential expression is analyzed using DeSeq software. Briefly, pretreatment of iX and BX795 resulted in different mRNA profiles. (Figure 5). Moreover, in line with previous in vitro experiments, treatment of iX during lentiviral gene delivery affected pathways associated with anti-viral responses such as TNF, IFN and pattern recognition pathways (Figures 6E-H) whereas BX795 failed to fully suppress similar anti-viral responses at mRNA level (Figures 6A-D).

Prophetic Example 2: In Vivo Administration

[0032] Although not claimed as part of the invention and recommended due to iX's inherent suppressive effect on intracellular RNA recognition pathways and the increased susceptibility for uncontrollable viral infection that would result, it is conceivable that iX might be used for in vivo gene delivery with serum concentrations varying between 0.2 and 6mM. Ix can be administered via injection, oral, nasal or mucosal delivery using technologies known in the art. Potential therapeutic applications of iX include cancer, liver gene therapy, single gene disorders, storage disorders as well as tumor retargeting genes.

[0033] In this context, cancers treatable by the present invention include carcinomas, sarcomas, lymphomas, leukemias, and blastomas: acute lymphoblastic leukemia (all), acute myeloid leukemia, adrenocortical carcinoma, aids-related cancers, anal cancer, astrocytoma, basal-cell carcinoma, extrahepatic bile duct cancer (cholangiocarcinoma), bladder cancer, bone tumor (osteosarcoma/malignant fibrous histiocytoma), brainstem glioma, brain cancer,

cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas/carcinoids, burkitt's lymphoma, central nervous system lymphoma, cervical cancer, chondrosarcoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous t-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (gist), extracranial, extragonadal, or ovarian germ cell tumor, gestational trophoblastic tumor, glioma of the brain stem, childhood cerebral astrocytoma glioma, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, hodgkin lymphoma, intraocular melanoma, islet cell carcinoma (endocrine pancreas), kaposi sarcoma, kidney cancer (renal cell cancer), acute lymphoblastic leukaemia (also called acute lymphocytic leukemia), acute myeloid leukemia (also called acute myelogenous leukemia), chronic lymphocytic leukemia, chronic myelogenous leukemia (also called chronic myeloid leukemia), hairy cell leukemia, lip and oral cavity cancer, liposarcoma, non small cell lung cancer, small cell lung cancer, macroglobulinemia, waldenstrom, male breast cancer, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanoma, intraocular (eye)melanoma, Merkel cell cancer, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, chronic myelogenous leukemia, acute myeloid leukemia, myeloid leukemia, multiple myeloma (cancer of the bone-marrow), myeloproliferative disorders, myxoma, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-small cell lung cancer, oligodendroglioma, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer (surface epithelial-stromal tumor), ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary adenoma, plasma cell neoplasia/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma (kidney cancer), renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, soft tissue sarcoma, uterine sarcoma, Sezary syndrome, melanoma and non-melanoma skin cancer, merkel cell skin carcinoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer with occult primary, stomach cancer, supratentorial primitive neuroectodermal tumor, t-cell lymphoma (mycosis fungoides and sezary syndrome), testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, trophoblastic tumor, ureter and renal pelvis transitional cell cancer, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor (kidney cancer).

[0034] The present invention is also useful in treating genetic disorders such as: 21-

hydroxylase deficiency, achondroplasia, acute intermittent porphyria, adenylosuccinate lyase deficiency, Adrenoleukodystrophy, Alagille syndrome, Alexander disease, Alstrom syndrome, Amelogenesis imperfecta, biotinidase deficiency, CGD Chronic granulomatous disorder, Di George's syndrome, fanconi anemia, G6PD deficiency, lipoprotein lipase deficiency, Muscular dystrophy, Duchenne type, Siderius X-linked mental retardation syndrome *caused by mutations in the PHF8 gene,* X-linked severe combined immunodeficiency (X-SCID), X-linked sideroblastic anemia (XLSA).

[0035] Metabolic disoders treatable with the present invention include: Niemann-Pick disease, Tay-Sachs disease, Gaucher disease, Fabry disease.

Example 3: Adoptive Cell Transfer

[0036] In a most preferred use, iX is used to treat cells prior to adoptive cell transfer to a patient. Using the protocols described above iX can be used at any time during ex vivo manipulation. Preferred concentration of iX in culture ranges are from about 0.4mM to about 10mM. Most preferred is 0.5mM to 6mM These cells can thereby be immediately infused or frozen for a later infusion time point using protocols well known in the art. iX administration can be done both in vivo and ex vivo as a single dose or repetitively using the dose window where serum concentration of the inhibitor can be between 0.4-6 uM, preferentially the lower dose. The cells so treated can be used in adoptive cell transfer to treat the conditions described under Example 2 above

Prophetic Example 4 Treatment of Viral Inflammation

[0037] Although this aspect is not claimed as part of the invention, it may also be possible to treat patients suffering from inflammation caused by a viral infection such as myositis, myocarditis, viral arthritis, viral encephalitis and meningitis. In such diseases iX can be co-administered with anti viral therapy to reduce or stop the recognition of the virus by the immune system thereby reducing, preventing or eliminating inflammation. This could only be used with antiviral therapies that do not rely on or fully utilize immune responses. Such antivirals include: adamantane antivirals such as amandatind and rimantidine; antiviral boosters such as ritonavir and cobicistat; chemokine receptor antagonists such as maraviroc; integrase strand transfer inhibitors such as maraviroc, dolutegravir and elvitegravir; miscellaneous antivirals such as sofosbuvir, enfuvirtide, foscarnet and fomivirsen; neuraminidase inhibitors such as peramivir,oseltamivir and zanamivir; non nucleoside reverse transcriptase inhibitors (NNRTIs) such as favirenz, nevirapine, delavirdine, etravirine andrilpivirine; NS5a inhibitors such as daclatasvir; nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine, didanosine,,stavudine, lamivudine, □abacavir"emtricitabine and entecavir; protease inhibitors such as saquinavir, ritonavir,i ndinavir,nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir and darunavir; and purine nucleosides such as

ribavirin valacyclovir, famciclovir, acyclovir, ganciclovir, valganciclovir and cidofovir. Dosing instructions for these drugs alone and in combination are well known in the art.

Prophetic Example 5: Increasing in Vivo Efficacy of RNA Viral Therapies

[0038] Although this aspect is not claimed as part of the invention, it may be conceivable to use iX to increase in vivo efficacy of RNA virus based oncolytic virus therapies such as vesicular stomatitis virus, poliovirus, reovirus, senecavirus, ECHO viruses such as Rigvir for indications such as bladder carcinoma, brain tumors, gynecological tumors, Hepatocellular carcinoma, melanoma, multiple myeloma, prostate carcinoma, soft tissue sarcoma and Solid tumors. The iX would help inhibit intracellular antiviral defense mechanisms thus increase the efficacy of distribution of the oncolytic virus within the tumor. Target serum levels for in vivo administration of iX are between 0.2 and 6mM.

References

[0039]

Alici, E., Sutlu, T., and Sirac Dilber, M. (2009). Retroviral gene transfer into primary human natural killer cells. Methods Mol Biol 506, 127-137.

Brandstadter, J.D., and Yang, Y. (2011). Natural killer cell responses to viral infection. J Innate Immun 3, 274-279.

Clark, K., Plater, L., Peggie, M., and Cohen, P. (2009). Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB kinase epsilon: a distinct upstream kinase mediates Ser-172 phosphorylation and activation. J Biol Chem 284, 14136-14146.

Lanier, L.L. (2008). Evolutionary struggles between NK cells and viruses. Nat Rev Immunol 8, 259-268.

Pegram, H.J., Kershaw, M.H., and Darcy, P.K. (2009). Genetic modification of natural killer cells for adoptive cellular immunotherapy. Immunotherapy 1, 623-630.

Sutlu, T., Nystrom, S., Gilljam, M., Stellan, B., Applequist, S.E., and Alici, E. (2012). Inhibition of intracellular antiviral defense mechanisms augments lentiviral transduction of human natural killer cells: implications for gene therapy. Hum Gene Ther 23, 1090-1100.

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REFERENCES CITED IN THE DESCRIPTION

Cited references

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liability in this regard.

Patent documents cited in the description

- WO62235427A [0040]

Non-patent literature cited in the description

- **ALICI, E.SUTLU, T.SIRAC DILBER, M.**Retroviral gene transfer into primary human natural killer cellsMethods Mol Biol, 2009, vol. 506, 127-137 [0039]
- **BRANDSTADTER, J.D.YANG, Y.**Natural killer cell responses to viral infectionJ Innate Immun, 2011, vol. 3, 274-279 [0039]
- **CLARK, K.PLATER, L.PEGGIE, M.COHEN, P.**Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB kinase epsilon: a distinct upstream kinase mediates Ser-172 phosphorylation and activationJ Biol Chem, 2009, vol. 284, 14136-14146 [0039]
- **LANIER, L.L.**Evolutionary struggles between NK cells and virusesNat Rev Immunol, 2008, vol. 8, 259-268 [0039]
- **PEGRAM, H.J.KERSHAW, M.H.DARCY, P.K.**Genetic modification of natural killer cells for adoptive cellular immunotherapyImmunotherapy, 2009, vol. 1, 623-630 [0039]
- **SUTLU, T.NYSTROM, S.GILLJAM, M.STELLAN, B.APPLEQUIST, S.E.ALICI, E.**Inhibition of intracellular antiviral defense mechanisms augments lentiviral transduction of human natural killer cells: implications for gene therapyHum Gene Ther, 2012, vol. 23, 1090-1100 [0039]

Patentkrav

1. *In vitro* fremgangsmåde til at forøge genlevering til celler omfattende co-administration af (5Z)-7-oxozeaenol, sammen med en retrovirus- eller lentivirus-vektor til en celle i et næringsmedie, hvor cellerne er valgt fra naturlige dræberceller, stamceller og makrofager.

2. Fremgangsmåden ifølge krav 1, hvor cellerne er NK92-celler.

3. Fremgangsmåden ifølge krav 1 eller 2, hvor (5Z)-7-oxozeaenol indgives i en mængde, som er tilstrækkelig for at opnå en koncentration fra ca. 0,04 µM til ca. 10 µM i næringsmediet.

4. Ex vivo fremgangsmåde til fremstilling af celler, som er egnede til anvendelse i adoptiv celleoverførsel til en patient, hvor nævnte fremgangsmåde omfatter levering af gener til celler under anvendelse af en retrovirus- eller lentivirusvektor med co-administration af 5Z)-7-oxozeaenol, hvor cellerne er valgt fra naturlige dræberceller, stamceller og makrofager.

5. Ex vivo fremgangsmåden ifølge krav 4, hvor de transficerede celler er egnede til anvendelse i adoptiv celleoverførsel til behandling af kræft.

6. Ex vivo fremgangsmåden ifølge krav 5, hvor kræftformen er valgt fra: karcinomer, sarkomer, lymfomer, leukæmiformer og blastomer: akut lymfatisk leukæmi (alle), akut myeloid leukæmi, adrenokortikalt karcinom, aids-relaterede kræftformer, endetarmskræft, astrocytom, basalcellekarcinom, ekstrahepatisk galdegangskræft (kolangiokarcinom), blærekræft, knogletumor (osteosarkom/malignt fibrøst histiocytom), hjernestammegliom, hjernekræft, cerebral astrocytom/malignt gliom, ependymom, medulloblastom, supratentoriel primitiv neuroektodermal tumor, gliom i den visuelle vej og hypothalamus, brystkræft, bronkieadenomer/karcinoider, Burkitts lymfom, lymfom i centralnervesystemet, livmoderhalskræft, kondrosarkom, kronisk lymfatisk leukæmi, kronisk myelogen leukæmi, kroniske myeloproliferative lidelser, tyktarmskræft, kutant t-celle-lymfom, desmoplastic lille rundcelletumor, endometriekræft, ependymom, spiserørskræft, Ewings sarkom, intraokulært

melanom, retinoblastom, galdeblærekræft, gastrisk (mave-) kræft, gastrointestinal karcinoidtumor, gastrointestinal stromal tumor (gist), ekstrakranial, ekstragonadal eller ovarial kimcelletumor, gestationel trofoblastisk tumor, hjernestammegliom, cerebral astrocytomgliom hos børn, hårcelleleukæmi, hoved- og halskræft, hjertekræft, hepatocellulær (lever-) kræft, Hodgkins lymfom, intraokulært melanom, øcelle-karcinom (endokrin pancreas), kaposis sarkom, nyrekræft (nyrecellekræft), akut lymfoblast leukæmi (også kaldet akut lymfocytisk leukæmi), akut myeloid leukæmi (også kaldet akut myelogen leukæmi), kronisk lymfocytisk leukæmi, kronisk myelogen leukæmi (også kaldet kronisk myeloid leukæmi), hårcelleleukæmi, læbe- og mundhulekræft, liposarkom, ikke-småcellet lungekræft, småcellet lungekræft, makroglobulinæmi, Waldenstrøm, mandebrystkræft, malignt fibrøst histiocytom i knogle-/osteosarkom, medulloblastom, melanom, intraokulært (øjen-)melanom, Merkelcellekræft, mesotheliom, metastatisk pladecellehalskræft med ukendt primær, mundkræft, multipelt endokrint neoplasisyndrom, multipelt myelom/plasmacelleneoplasma, mycosis fungoides, myelodysplastiske syndromer, myelodysplastiske/myeloproliferative sygdomme, kronisk myelogen leukæmi, akut myeloid leukæmi, myeloid leukæmi, multipelt myelom (knoglemarvskræft), myeloproliferative lidelser, myxom, næsehule- og paranasal sinuskræft, næsesvælgkarcinom, neuroblastom, ikke-småcellet lungekræft, oligodendrogliom, oralkræft, orofaryngeal kræft, osteosarkom/malignt fibrøst knoglehistiocytom, æggestokkræft, epitelovariekræft (overfladeepitel-stromal tumor), ovarial kimcelletumor, ovarial tumor med lavt malignt potentiale, bugspytkirtelkræft, bugspytkirtelkræft, paranasal sinus- og næsehulekræft, biskjoldbruskkræft, peniskræft, svælgkræft, fæokromocytom, pineal astrocytom, pineal germinom, pineoblastom og supratentorial primitiv neuroektodermal tumor, hypofyseadenom, plasmacelleneoplasi/multipelt myelom, pleuropulmonært blastom, primært lymfom i centralnervesystemet, prostatakræft, endetarmskræft, nyrecellekarcinom (nyrekræft), nyrebækken- og transitional urinledercellekræft, retinoblastom, rhabdomyosarkoma, spytkirtelkræft, blødvævssarkom, livmodersarkom, Sezarys syndrom, melanom og non-melanom hudkræft, Merkelcelle hudkarcinom, småcellet lungekræft, tyndtarmskræft, blødvævssarkom, pladecellekarcinom, pladecellehalskræft med ukendt primær, mavekræft, supratentorial primitiv neuroektodermal tumor, t-celle-lymfom (mycosis fungoides og Sezarys syndrom), testikelkræft, strubekræft, thymom og

thymos karcinom, skjoldbruskkirtelkræft, transitional cellekræft nyrebækken og urinleder, trofoblastisk tumor, urinleder og nyrebækken transitional cellekræft, uretral kræft, livmoderkræft, livmodersarkom, vaginal kræft, gliom i den visuelle vej og hypothalamus, vulvakræft, Waldenstrøms makroglobulinæmi, Wilms tumor (nyrekræft), leverkræft, multipelt myelom eller et sarkom.

7. Ex vivo fremgangsmåden ifølge krav 4, hvor de transficerede celler er egnede til anvendelse i adoptiv celleoverførsel til behandling af en sygdom forårsaget af genetisk mutation.

8. Ex vivo fremgangsmåden ifølge krav 7, hvor sygdommen er valgt fra: 21-hydroxylasemangel, akondroplasi, akut intermitterende porfyri, adenylosuccinat-lyasemangel, adrenoleukodystrofi, Alagilles syndrom, Alexanders sygdom, Alströms syndrom, amelogenesis imperfecta, biotinidasemangel, CGD kronisk granulomatøs lidelse, DiGeorge syndrom, Fanconi anæmi, G6PD-mangel, lipoprotein-lipasemangel, Duchennes muskeldystrofi, Siderius X-bunden mental retardationssyndrom *forårsaget af mutationer i PHF8-genet,* X-bunden alvorlig kombineret immundefekft (X-SCID), eller X-bunden sideroblastisk anæmi (XLSA), x-bunden alvorlig kombineret immundefektsygdom.

9. Ex vivo fremgangsmåden ifølge krav 4, hvor sygdommen er en stofskiftesygdom forårsaget af en kendt genetisk mutation.

10. Ex vivo fremgangsmåden ifølge krav 9, hvor stofskiftesygdommen er valgt fra Niemann-Picks sygdom, Tay-Sachs' sygdom, Gauchers sygdom, Fabrys sygdom.

DRAWINGS

Transduction efficiency is increased significantly in primary NK cells and NK92 cell line with BX or iX

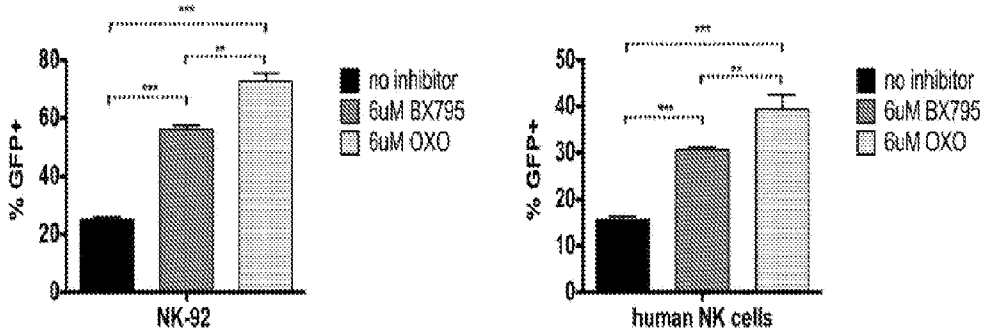
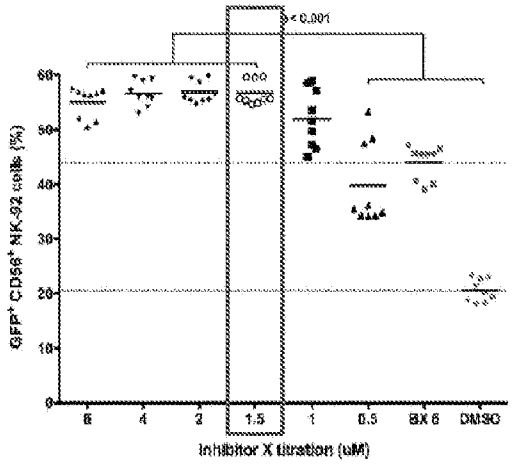


Figure 1

BX795 and iX dose titration in NK92 cells



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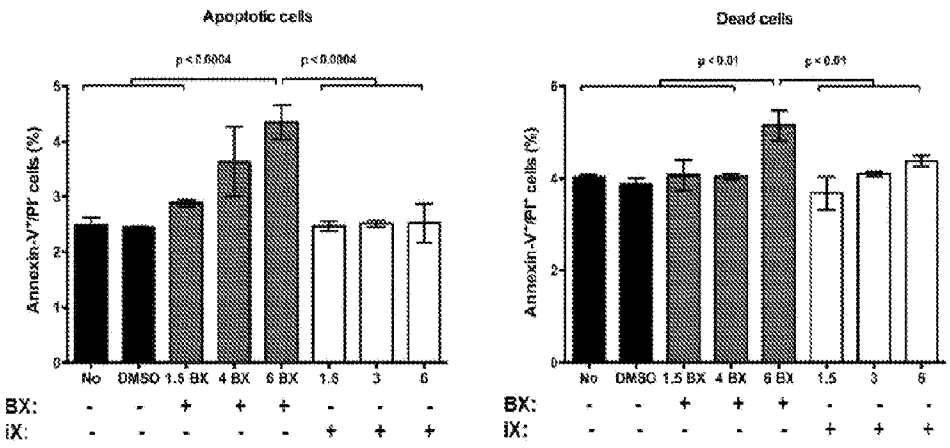

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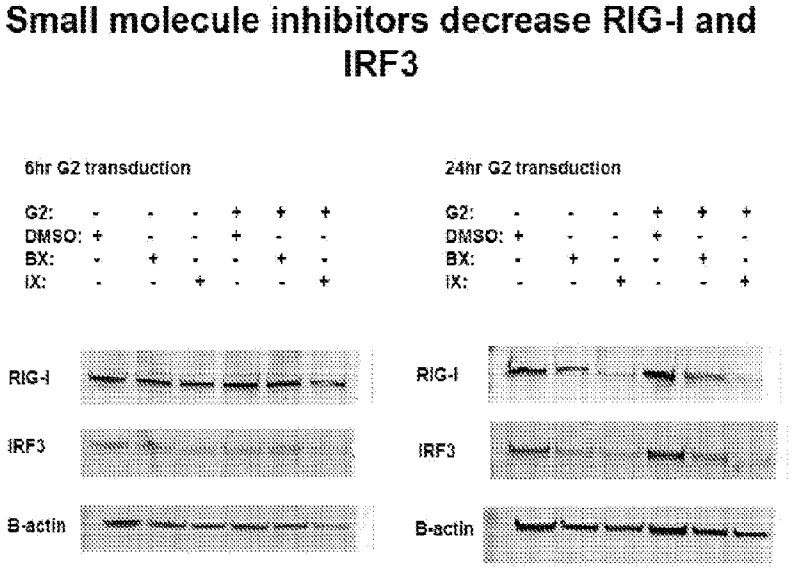

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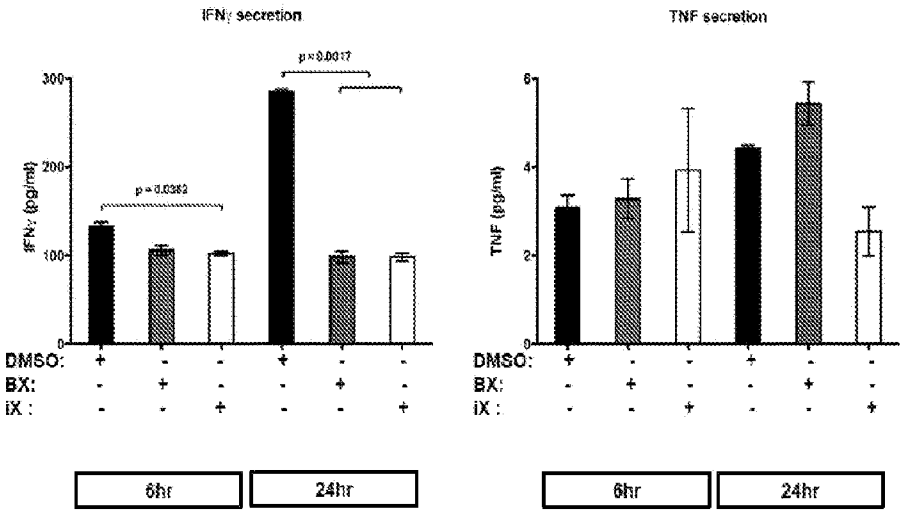
Small molecule inhibitor treatment interferes with IFNγ responses
IFNγ secretion
TNF secretion
IFNγ (pg/ml)
TNF (pg/ml)
p = 0.0017
p = 0.0382
DMSO:
BX:
iX :
6hr
24hr

Figure 4


No inh
iX
Bx795
z-score
3
2
1
0
-1
-2
-3
Treatment
mockiup_BX795
mockiup_DMSO
viniup_BX795
viniup_DMSO
PS_OXO
PS_OXO_Vina
vinisup_BX795
vinisup_DMSO
Timepoint
HR24
HR48
HR6
ETS1:ETS1
LSP1:LSP1
ACTN4:ACTN4
TMSB4X:TMSB4X
CTSW:CTSW
ARPC2:ARPC2
CORO1A:CORO1A
COTL1:COTL1
YWHAZ:YWHAZ
GSTP1:GSTP1
LCP1:LCP1
ARHGDIB:ARHGDIB
PRKDC:PRKDC
SRRM2:SRRM2
HLA-E:HLA-E
HLA-A:HLA-A
HLA-B:HLA-B
HLA-C:HLA-C
IQGAP1:IQGAP1
WDR1:WDR1
MCM7:MCM7
MYH9:MYH9
TLN1:TLN1
WIPF1:WIPF1
PTPRC:PTPRC
TOP2A:TOP2A
SON:SON
GBP5:GBP5
IL2RB:IL2RB
ANXA6:ANXA6
HUWE1:HUWE1
CCL5:CCL5
ITGAL:ITGAL
RNF213:RNF213
HSPA8:HSPA8
HSP90AA1:HSP90AA1
MKI67:MKI67
PRF1:PRF1
GNAS:GNAS
B2M:B2M
HMGB2:HMGB2
CD3E:CD3E
RPS3:RPS3
TPT1:TPT1
RPS6:RPS6
EEF1A1:EEF1A1
NKG7:NKG7
GZMA:GZMA
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Figure 5

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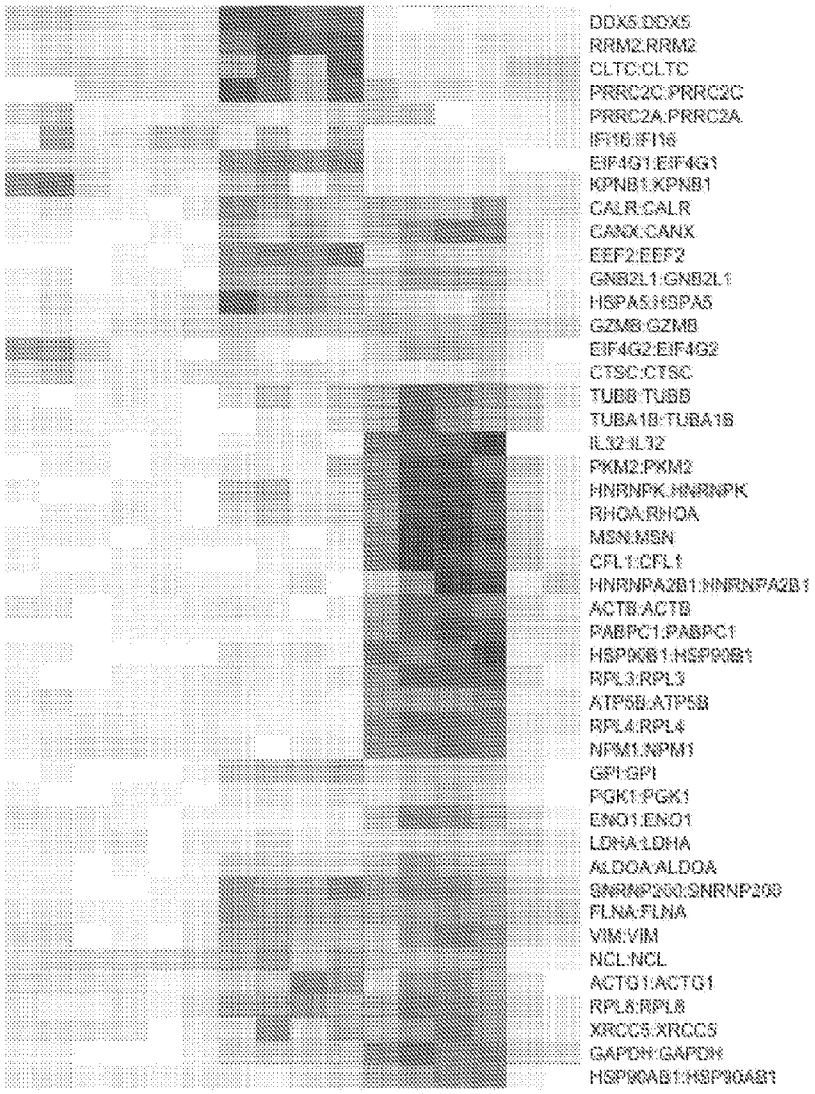

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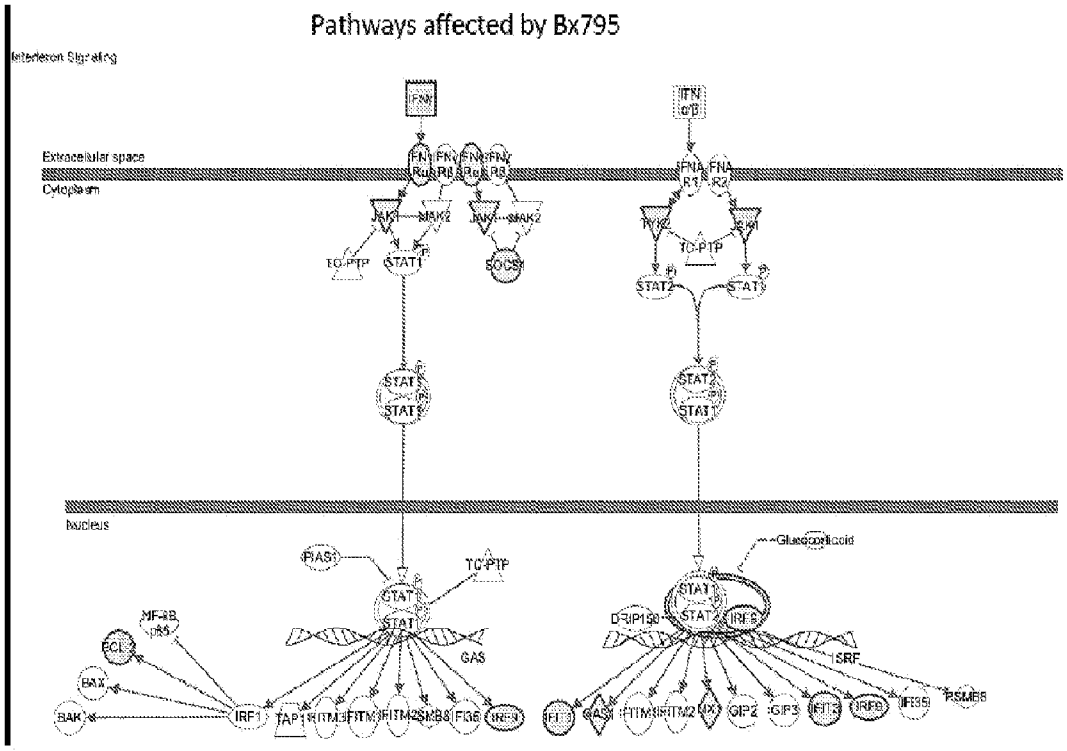
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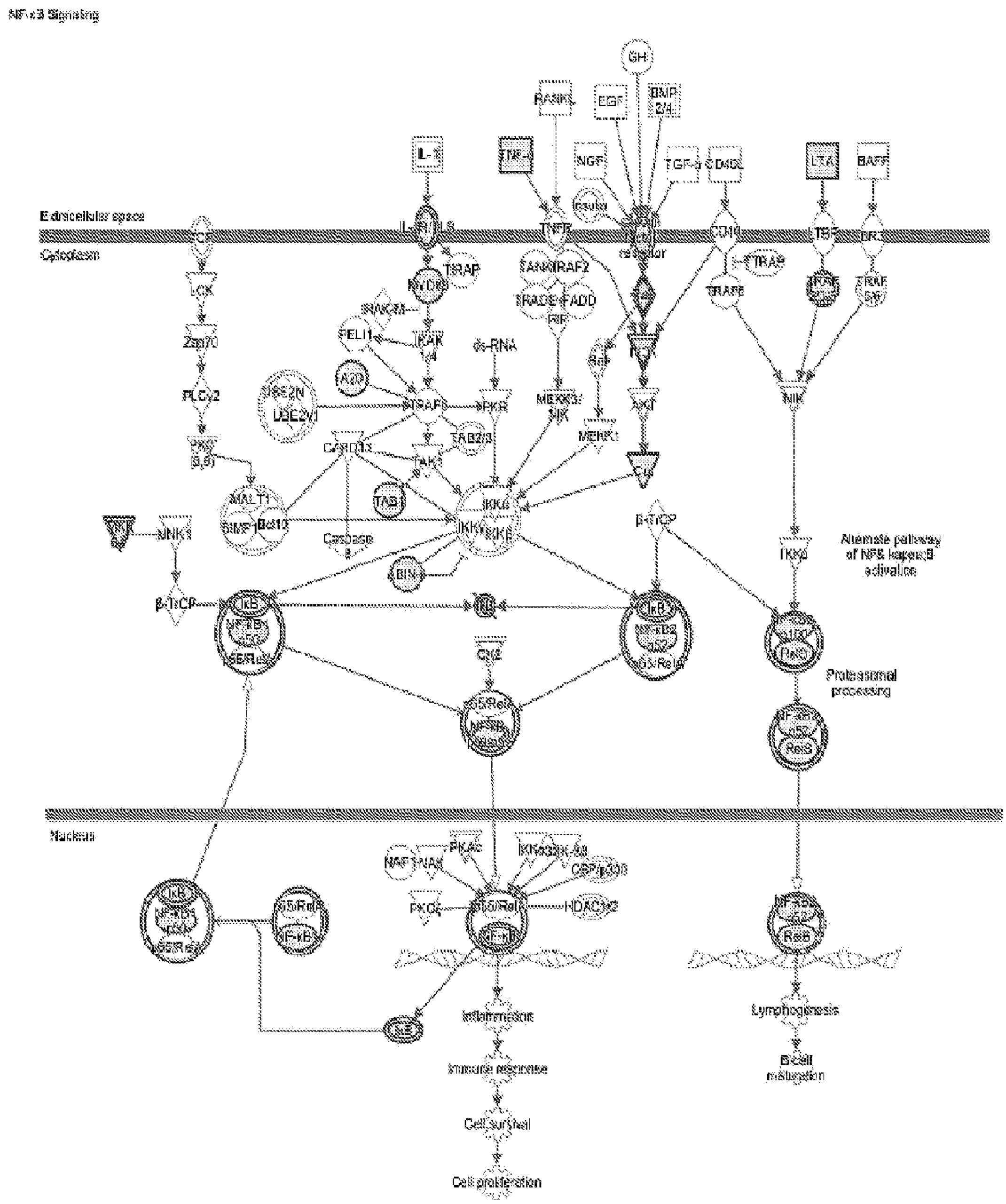
IFN α/β
Extracellular space
Cytoplasm
IFNAR1
IFNAR2
JAK1
JAK2
TYK2
TC-PTP
STAT1
SOCS1
STAT2
P
Nucleus
PIAS1
Glucocorticoid
MCL1
BAX
BAK
IRF1
TAP1
IFITM3
IFITM2
MX1
GIP2
GIP3
IFIT3
IRF9
IFI35
PSMB8
GAS
ISRE
SRF

Figure 6A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Cytoplasm
Nucleus
GH
RANKL
EGF
BMP 2/4
IL-1
NGF
TGF-α
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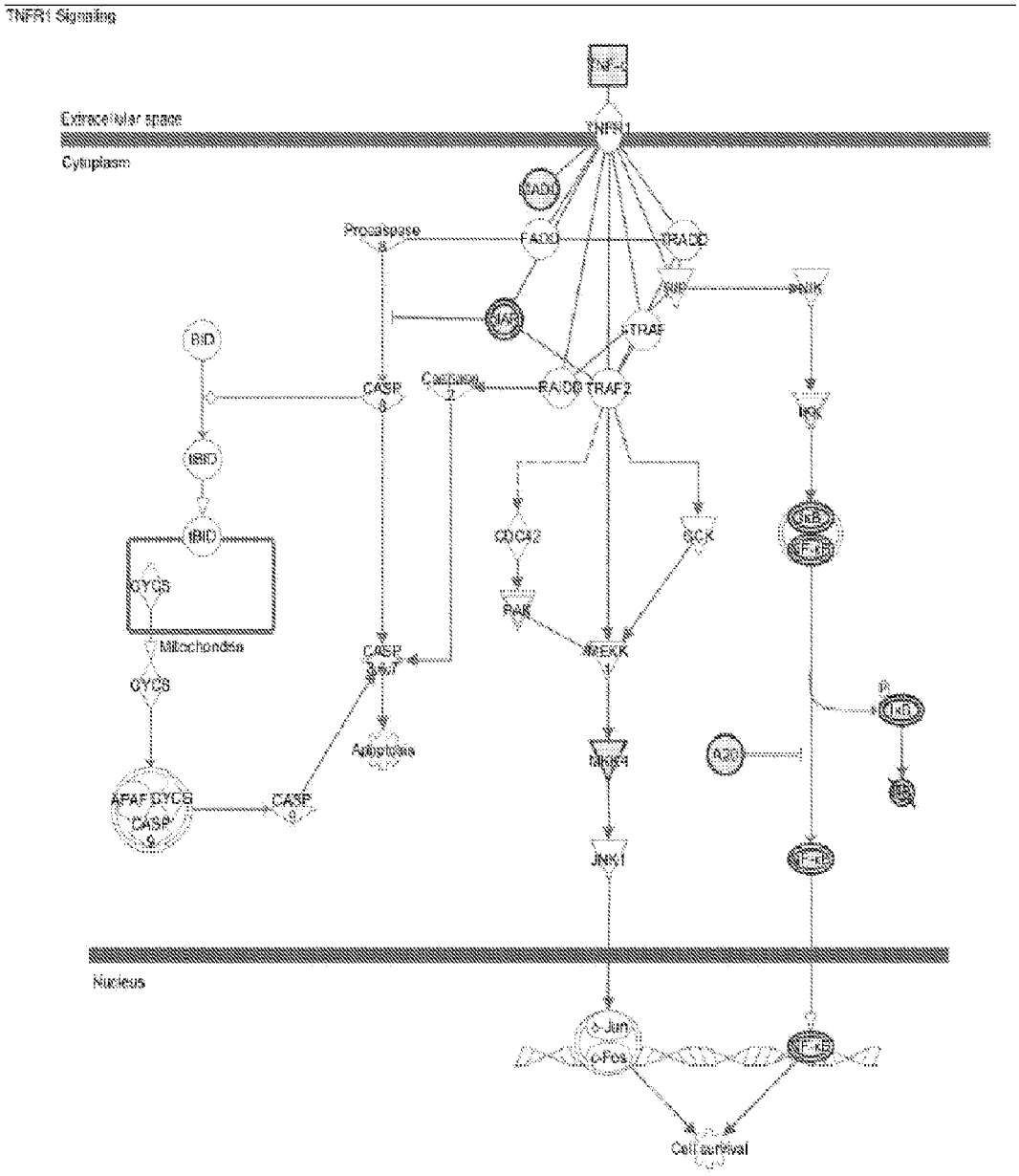
BAFF
TNFR
TIRAP
TANK
TRAF2
TRADD
FADD
RIP
LCK
Zap70
PLCγ2
PELI1
ds-RNA
A20
UBE2N
UBE2V1
PKR
MEKK1
TAB2/3
CARD11
AKT
NIK
TRAF6
MALT1
Bcl10
Caspase 8
ABIN
NNK1
β-TrCP
IKKα
Proteasomal processing
NAF1
PKAc
CBP/p300
HDAC1/2
Inflammation
Immune response
Cell survival
Cell proliferation
Lymphogenesis
B-cell maturation

Figure 6B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Cytoplasm
TNFR1
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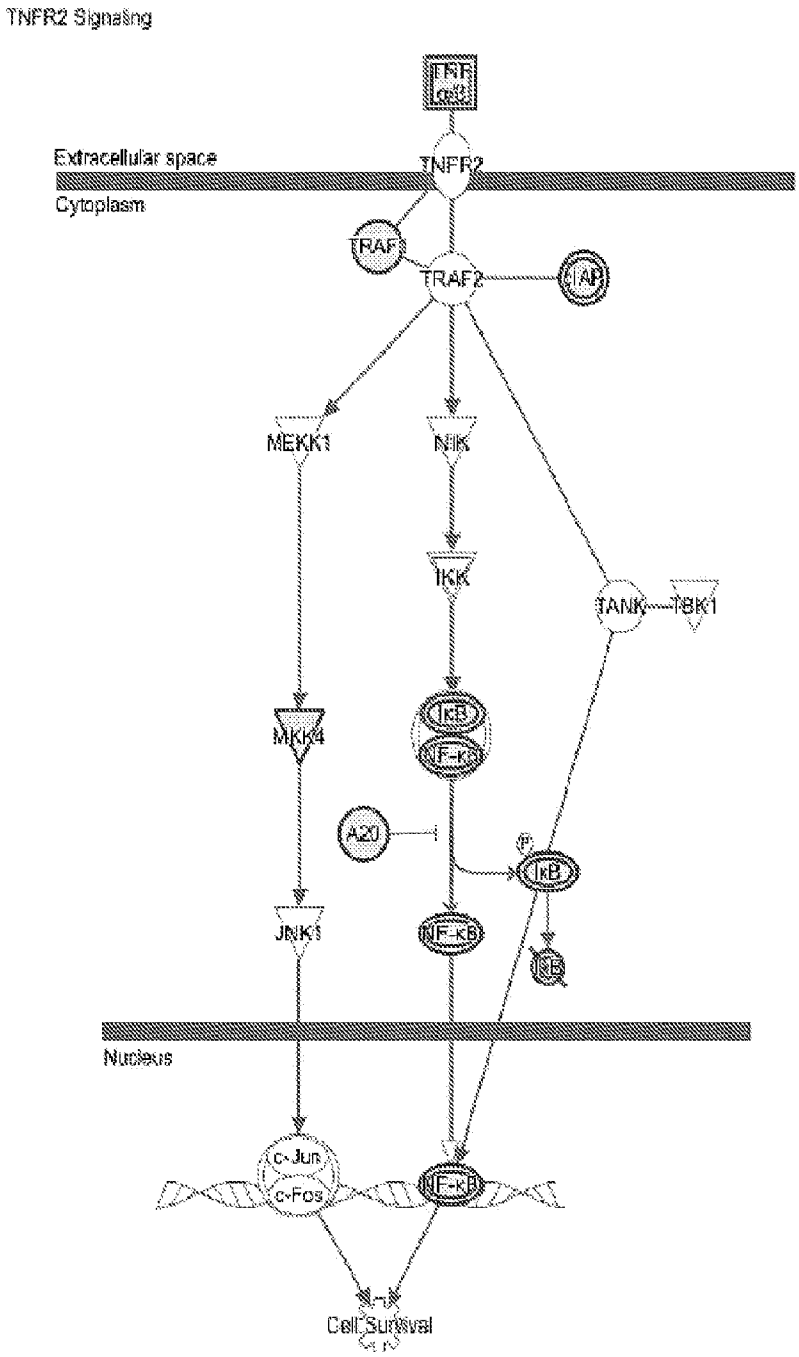
Procaspase 8
FADD
TRADD
RIP
MAP3K
TRAF
BID
CASP 8
Caspase 2
RADD TRAF2
IKK
tBID
CDC42
RICK
CYCS
PAK
Mitochondria
CASP 3&7
MEKK
CYCS
IKB
Apoptosis
NIK/4
APAF CYCS CASP 9
CASP 8
JNK1
Nucleus
c-Jun
c-Fos
Cell survival

Figure 6C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TNF
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Cytoplasm
TRAF1
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NIK
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TBK1
IκB
NF-κB
MKK4
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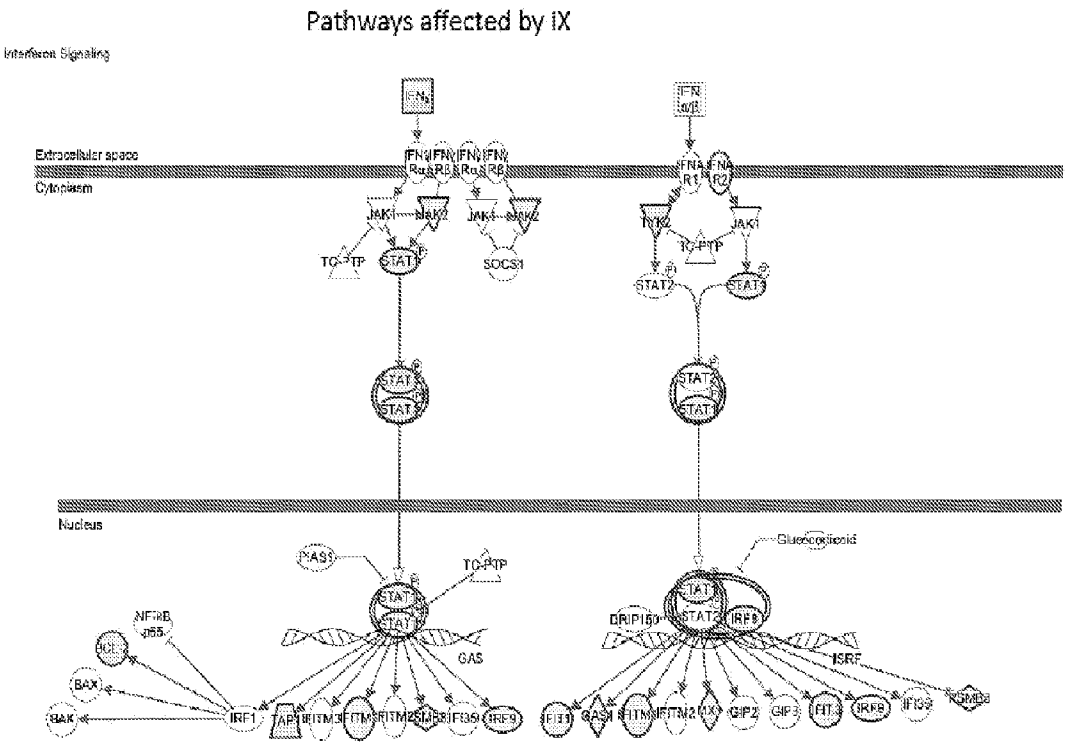
NF-κB
IκB
Nucleus
c-Jun
c-Fos
NF-κB
Cell Survival

Figure 6D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Interferon Signaling
Extracellular space
Cytoplasm
Nucleus
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JAK2
TYK2
TC-PTP
STAT1
STAT2
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PIAS1
Glucocorticoid
GRIP1
IRF9
GAS
ISRE
NFκB
p65
BAX
BAK
IRF1
TAP1
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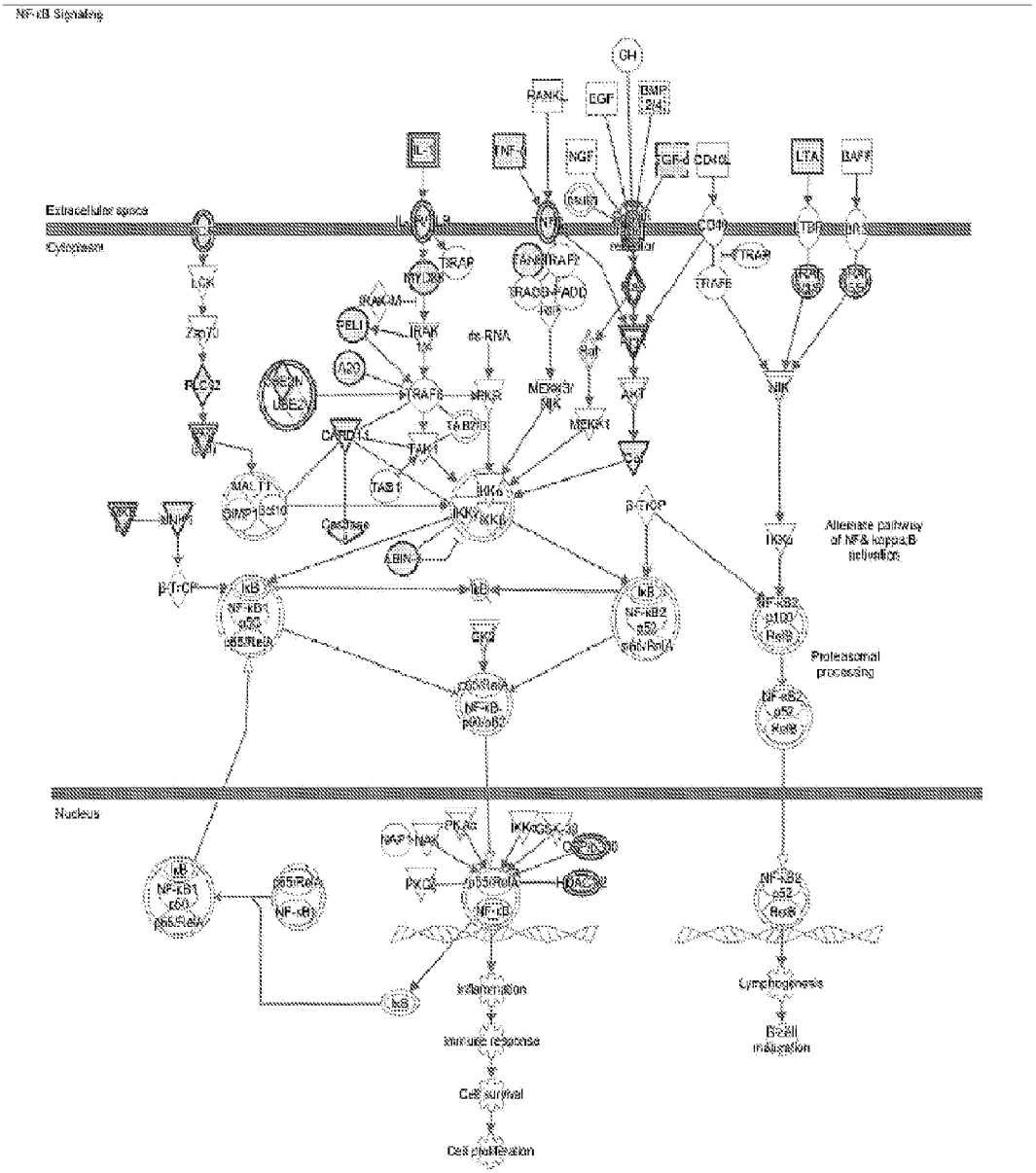
IFITM2
MX1
GIP2
GIP3
FIT1
IRF8
PSMB8

Figure 6E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Extracellular space
Cytoplasm
Nucleus
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TNF
NGF
EGF
BMP 2/4
RANKL
CD40L
LTA
BAFF
TRAF
TRADD
FADD
RIP
MEKK3/ NIK
MEKK1
AKT
NIK
IKKα
IKKγ (NEMO)
IKKε
TAK1
TAB2/3
MALT1
BCL10
CARD11
PKCβ
PKCζ
β-TrCP
IκB
NF-κB1 p50 p65/RelA
NF-κB2 p52 RelB
p65/RelA
NF-κB
Alternate pathway of NF-κappaB activation
Proteasomal processing
Inflammation
Immune response
Cell survival
Cell proliferation
Lymphogenesis
B-cell maturation

Figure 6F

Pathways affected by iX

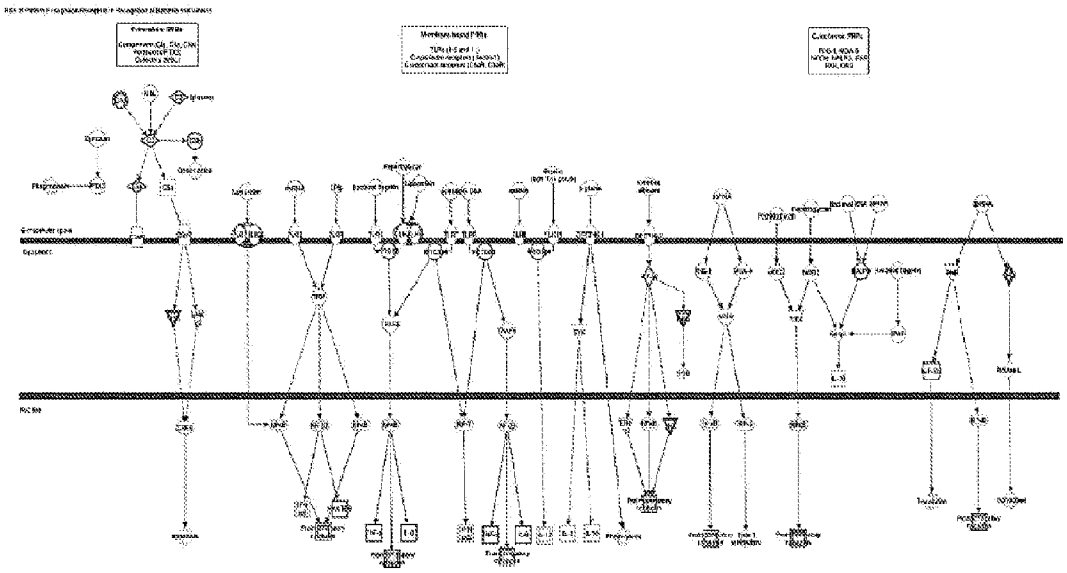

Figure 6G

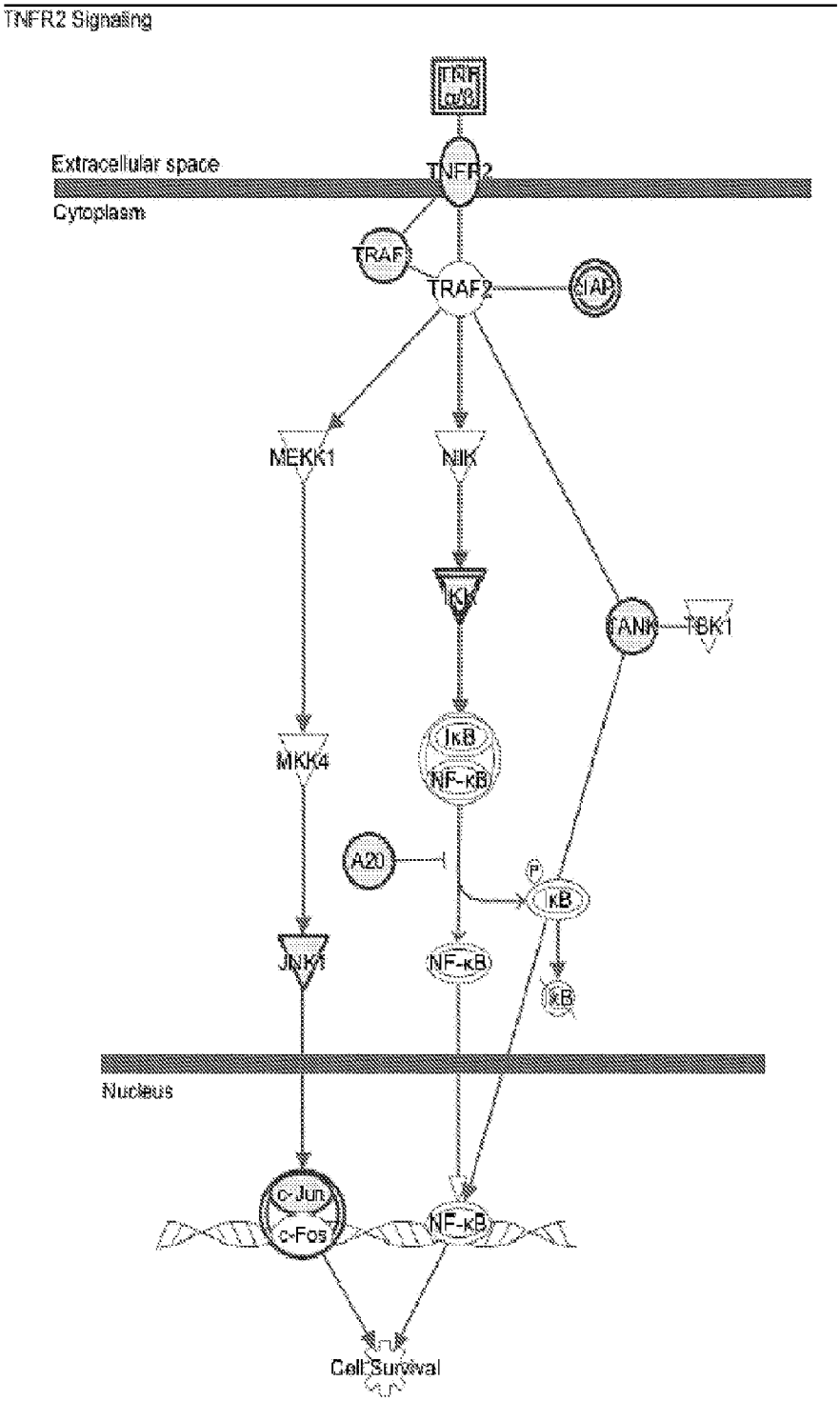
TNFR2 Signaling
TNFR
(trimer)
Extracellular space
TNFR2
Cytoplasm
TRAF
TRAF2
cIAP
MEKK1
NIK
IKK
TANK
TBK1
MKK4
IκB
NF-κB
A20
P
κB
JNK1
NF-κB
κB
Nucleus
c-Jun
c-Fos
NF-κB
Cell Survival

Figure 6H

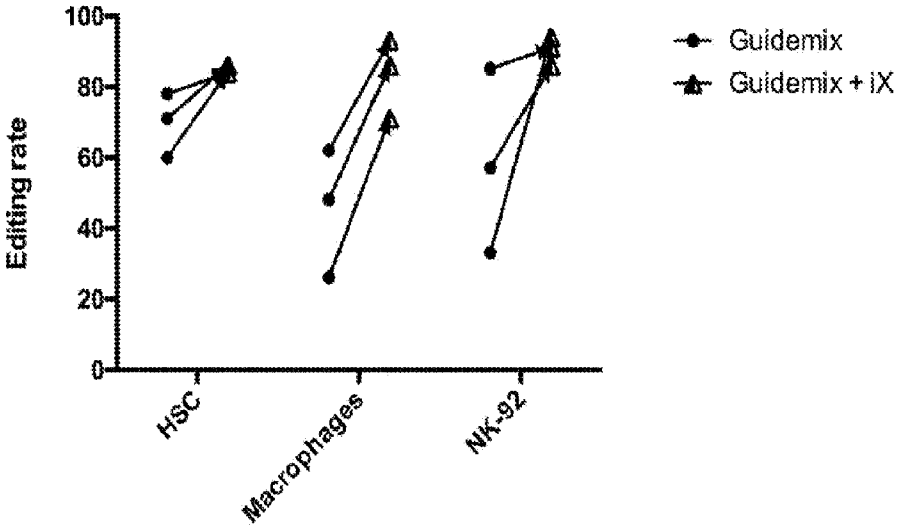
Editing rate
100
80
60
40
20
0
HSC
Macrophages
NK-92
Guidemix
Guidemix + iX

Figure 7