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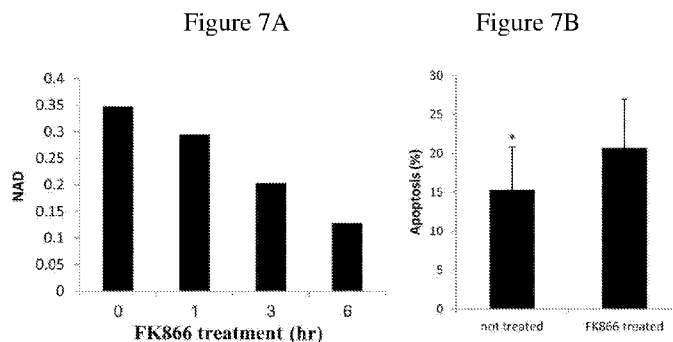
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(54) **Title:** COMPOSITIONS FOR TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA AND METHODS OF USE THEREOF



(57) **Abstract:** Provided herein are compositions for treatment of Acute Lymphoblastic Leukemia (ALL) and methods of their use, including inhibiting ALL relapse. Further provided herein are systems of treatment that are directed by a health care provider, and which combine prognostic methods for determining ALL relapse and the described treatments.

COMPOSITIONS FOR TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

5 Benefit is claimed to U.S. Provisional Patent Application No. 62/030,629, filed July 30, 2014, the contents of which are incorporated by reference in their entirety.

FIELD

10 Provided herein are compositions for treatment of Acute Lymphoblastic Leukemia (ALL) and methods of their use, including inhibiting ALL relapse. Further provided herein are systems of treatment that are directed by a health care provider, and which combine prognostic methods for determining ALL relapse and the described treatments.

BACKGROUND

15 Leukemia is a cancer of the blood or bone marrow characterized by an abnormal increase of blood cells, usually leukocytes. Leukemia is clinically and pathologically subdivided into a variety of large groups, including it acute and chronic forms. Acute leukemia is characterized by the rapid increase of immature blood cells. This crowding makes the bone marrow unable to produce healthy blood cells. Immediate treatment is required in acute
20 leukemia due to the rapid progression and accumulation of the malignant cells, which then spill over into the bloodstream and spread to other organs of the body. Acute forms of leukemia are the most common forms of leukemia in children, of which, acute lymphoblastic leukemia (ALL) is the most prevalent.

 Current treatments for ALL are guided by patient assessment and classification into a
25 particular risk group. Examples of such classifications include the Berlin-Frankfurt-Münster (BFM), the Children Oncology Group (COG) (Schrappe, *Ann Hematol.* (2004); 83: S121-3; Vrooman LM *et al.*, *Curr Opin Pediatr.* (2009); 21(1):1-8), UKALL, from the United Kingdom, the Chinese Children's Leukemia Group (CCLG), and the Dana-Farber Cancer Institute ALL Consortium (DFCI). In the classifications, patients are classified *inter alia* on white blood cell
30 count, chromosomal rearrangement, and responsiveness to prednisone treatment at day 8 following treatment initiation. Classification into a particular group will determine how aggressively a patient is treated in order to provide effective treatment and to reduce the possibility of disease relapse.

While current methods of diagnosis and treatment have improved the cure rate up to 80-90%, certain children are still over- or under- treated (*Schrappe M et al.*, Leukemia. (2010); 24: 253-254; *Pui CH and Evans WE*, N. Engl. J. Med. (2006); 354: 166-178; *Bhojwani D et al.*, Clin. Lymphoma. Myeloma. (2009); 3:S222-230]. Thus, a continuing need exists for improved ALL treatments.

SUMMARY

Described herein are compositions for use in treatment of acute lymphoblastic leukemia (ALL). In particular embodiments, the compositions include. an inhibitor of nicotinamide phosphoribosyltransferase (NAMPT), an inhibitor of miR-1290, a ribonucleic acid sequence at least 90% identical to a miR-451 ribonucleic acid sequence set forth as SEQ ID NO: 2, and/or an inhibitor of janus kinase 2 (JAK2), for use in treatment of acute lymphoblastic leukemia (ALL).

The described compositions can all be used in methods of treatment of acute lymphoblastic leukemia (ALL) in a subject, including reducing the risk of relapse in a subject or even preventing relapse in the subject, wherein the described compositions are administered to the subject, thereby treating the ALL.

Also described herein are systems of ALL treatment that include first determining the expression level of miR-1290 and at least one of miR-151-5p and miR-451; and comparing the determined expression of miR-1290, and miR-151-5p and/or miR-451 with control expression of miR-1290, and miR-151-5p and/or miR-451, wherein a significant increase in miR-1290 expression in the subject in comparison to the control miR-1290 expression, combined with a significant decrease in expression of the at least one of miR-151-5p and miR-451 in comparison to the control expression of miR-151-5p and/or miR-451, indicates that the subject has an increased risk of ALL relapse, and requires treatment appropriate for a subject with an increased risk of ALL relapse; and then administering to the patient a therapeutically effective amount of a composition comprising an inhibitor of nicotinamide phosphoribosyltransferase (NAMPT), or any of the other compounds or compositions described herein for use in treating ALL.

The foregoing and other objects, features, and advantages will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a Kaplan Meier estimation of Relapse Free Survival (RFS) in a cohort of 125 ALL patients. In the plot, the line representing high or low expression of miR-1290 is accordingly indicated.

Figure 2 is a Kaplan Meier plot of relapse free survival only for B-lineage ALL cohort (n=105). In the plot, the line representing high or low expression of miR-1290 is accordingly indicated.

Figure 3 is a Kaplan Meier analysis for relapse-free survival by expression levels of the combined miRs: both downregulated miRs together with the upregulated miR-1290 in precursor B-cell ALL patients. The lower line represents a combination of down-regulated miR-451 and miR-151-5p, and up-regulated miR-1290. The upper line represents all other expression combinations for miR-451, miR-151-5p, and miR-1290.

Figures 4A-4C show that *Hsa-miR-451* transfection decreases ALL cell growth *in vivo*. **Figure 4A:** expression analysis of *hsa-miR-451* measured by quantitative reverse transcription-PCR (RT-PCR). Expression of *hsa-miR-451* in Nalm-6 cells transfected with miR-451 24hr after transfection, 5 day and 10 days after transfection and scrambled control. **Figure 4B:** comparison of tumor size in female NOD/SCID mice transplanted with Nalm-6 cells transfected with miR-451, Nalm-6 transfected with scrambled cells for 31 days after s.c. injection of cells. **Figure 4C:** mean tumor weight in NOD/SCID mice transplanted with Nalm-6 cells transfected with miR-451, Nalm-6 transfected with scrambled cells at the end of the experiment. bars, SE. *, $P < 0.05$.

Figures 5A-5B show the effect of miR-451 on NAMPT expression. **Figure 5A:** expression analysis of NAMPT measured by FACS in NALM-6 cell line expressing miR-451 mimic, inhibitor and scrambled control. **Figure 5B:** Luciferase reporter assay validating the direct interaction of miR-451 with the 3'UTR of NAMPT. bars, SE. *, $P < 0.05$.

Figures 6A-6B show the effect of NAMPT expression on NAD levels. **Figure 6A:** expression analysis of *NAMPT* measured by quantitative reverse transcription-PCR (RT-PCR) in NALM-6 cell line treated with 50ng/ml TPA for 24 hours. **Figure 6B:** NAD⁺ assay in cells treated with 50nM TPA for 24 hours. bars, SE.

Figures 7A-7B show that NAMPT inhibitor FK866 induced apoptosis and reduction in NAD levels in NALM-6 cells. **Figure 7A:** NAD levels were measured using NAD assay in cells treated with FK866 for 1, 3 and 6 hours. **Figure 7B:** Apoptosis was measured with apoptosis kit in cells treated with the NAMPT inhibitor, FK866. bars, SE. *, $P < 0.05$.

Figures 8A-8B show sensitivity of ALL cell line to NAMPT inhibitor, FK866. NAD levels were measured using NAD assay in cells treated for 3 hours with FK866. Assayed cells were transfected with miR-451 mimic (Fig. 8A), inhibitor (Fig. 8B), or scrambled negative control (representative figure of one experiment).

Figure 9 shows SOCS4 protein expression levels following the over-expression of miR-1290 (mimic) in comparison to control (scramble). Protein expression was determined and quantified by Western blotting.

Figures 10A-10B show SOCS4 protein levels in ALL BM samples with high and low miR-1290 levels. Representative Western blot and (**Figure 10A**) and quantitation (**Figure 10B**) are shown.

Figure 11 shows a quantitation of phosphorylated-STAT5 protein levels following overexpression of miR-1290.

BRIEF DESCRIPTION OF THE DESCRIBED SEQUENCES

The nucleic acid sequences provided herewith are shown using standard letter abbreviations for nucleotide bases as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file named 3044_3_SEQLIST_TREAT, created July 30, 2015, about 1.03 KB, which is incorporated by reference herein. In the accompanying sequence listing:

SEQ ID NO: 1 is the nucleotide sequence of miR-151-5p.

SEQ ID NO: 2 is the nucleotide sequence of miR-451.

SEQ ID NO: 3 is the nucleotide sequence of miR-1290.

SEQ ID NO: 4 is the nucleotide sequence of a miR-1290 mimic.

SEQ ID NO: 5 is the nucleotide sequence of a miR-1290 inhibitor.

DETAILED DESCRIPTION

I. Abbreviations

ALL Acute lymphoblastic leukemia

MRD Minimal residual disease

II. Terms

Unless otherwise noted, technical terms are used according to conventional usage, which for example can be found in Benjamin Lewin, *Genes V*, published by Oxford University

Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The term “comprises” means “includes.” The abbreviation, “*e.g.*” is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation “*e.g.*” is synonymous with the term “for example.” In case of conflict, the present specification, including explanations of terms, will control. In addition, all the materials, methods, and examples are illustrative and not intended to be limiting.

Abnormal: Deviation from normal characteristics. Normal characteristics can be found in a control, a standard for a population, etc. For instance, where the abnormal condition is a disease condition, such as ALL, a few appropriate sources of normal characteristics might include an individual who is not suffering from the disease, or a population who did not experience a particular prognosis outcome of the disease, such as ALL relapse. Similarly, abnormal may refer to a condition that is associated with a disease or disease relapse. The term “associated with” includes an increased risk of developing the disease or a relapse thereof. For instance, a certain abnormality (such as an abnormality in expression of a miRNA) can be described as being associated with the biological condition of ALL relapse. Controls or standards appropriate for comparison to a sample, for the determination of abnormality, such as in the determination of an expression cutoff value, include samples believed to be normal as well as laboratory-determined values, even though such values are possibly arbitrarily set, and keeping in mind that such values may vary from laboratory to laboratory. Laboratory standards and values may be set based on a known or determined population value and may be supplied in the format of a graph or table that permits easy comparison of measured, experimentally determined values.

Administration: The introduction of a composition into a subject by a chosen route. Administration of an active compound or composition can be by any route known to one of skill in the art. Administration can be local or systemic. Examples of local administration include, but are not limited to, topical administration, subcutaneous administration, intramuscular administration, or administration to the nasal mucosa or lungs by inhalational

administration. In addition, local administration includes routes of administration typically used for systemic administration, for example by directing intravascular administration to the arterial supply for a particular organ. Thus, in particular embodiments, local administration includes intra-arterial administration and intravenous administration when such administration is targeted to the vasculature supplying a particular organ. Systemic administration includes any route of administration designed to distribute an active compound or composition widely throughout the body via the circulatory system. Thus, systemic administration includes, but is not limited to intra-arterial and intravenous administration. Systemic administration also includes, but is not limited to, topical administration, subcutaneous administration, intramuscular administration, or administration by inhalation, when such administration is directed at absorption and distribution throughout the body by the circulatory system.

Altered expression: Expression of a biological molecule (for example, a miRNA) in a subject or biological sample from a subject that deviates from expression of the same biological molecule in a subject or biological sample from a subject having normal or unaltered characteristics for the biological condition associated with the molecule. Normal expression can be found in a control, a standard for a population, etc. Altered expression of a biological molecule may be associated with a disease or condition thereof, such as ALL relapse.

Amplification: When used in reference to a nucleic acid, any technique that increases the number of copies of a nucleic acid molecule in a sample or specimen. An example of amplification is the polymerase chain reaction (in all of its forms), in which a biological sample collected from a subject is contacted with a pair of oligonucleotide primers, under conditions that allow for the hybridization of the primers to nucleic acid template in the sample. The primers are extended under suitable conditions, dissociated from the template, and then re-annealed, extended, and dissociated to amplify the number of copies of the nucleic acid. The product of in vitro amplification can be characterized by electrophoresis, restriction endonuclease cleavage patterns, oligonucleotide hybridization or ligation, and/or nucleic acid sequencing, using standard techniques. Other examples of in vitro amplification techniques include strand displacement amplification (see U.S. Patent No. 5,744,311); transcription-free isothermal amplification (see U.S. Patent No. 6,033,881); repair chain reaction amplification (see WO 90/01069); ligase chain reaction amplification (see EP-A-320 308); gap filling ligase chain reaction amplification (see U.S. Patent No. 5,427,930); coupled ligase detection and PCR (see U.S. Patent No. 6,027,889); and NASBA™ RNA transcription-free amplification (see U.S. Patent No. 6,025,134).

Analog, derivative or mimetic: An analog is a molecule that differs in chemical structure from a parent compound, for example a homolog (differing by an increment in the chemical structure, such as a difference in the length of an alkyl chain), a molecular fragment, a structure that differs by one or more functional groups, a change in ionization. Structural
5 analogs are often found using quantitative structure activity relationships (QSAR), with techniques such as those disclosed in *Remington (The Science and Practice of Pharmacology, 19th Edition (1995), chapter 28)*. A derivative is a biologically active molecule derived from the base structure. A mimetic is a molecule that mimics the activity of another molecule, such as a biologically active molecule. Biologically active molecules can include chemical
10 structures that mimic the biological activities of a compound. It is acknowledged that these terms may overlap in some circumstances.

Antagonist: A molecule or compound that tends to nullify the action of another, or in some instances that blocks the ability of a given chemical to bind to its receptor or other interacting molecule, preventing a biological response. Antagonists are not limited to a
15 specific type of compound, and may include in various embodiments peptides, antibodies and fragments thereof, and other organic or inorganic compounds (for example, peptidomimetics and small molecules).

Antibody: A polypeptide ligand comprising at least a light chain or heavy chain immunoglobulin variable region, which specifically recognizes and binds an epitope of an
20 antigen, such as the NAMPT or JAK2 protein or a fragment thereof. Antibodies are composed of a heavy and a light chain, each of which has a variable region, termed the variable heavy (VH) region and the variable light (VL) region. Together, the VH region and the VL region are responsible for binding the antigen recognized by the antibody. This includes intact immunoglobulins and the variants and portions of them well known in the art, such as Fab'
25 fragments, F(ab)'₂ fragments, single chain Fv proteins ("scFv"), and disulfide stabilized Fv proteins ("dsFv"). The term also includes recombinant forms such as chimeric antibodies (for example, humanized murine antibodies), heteroconjugate antibodies (such as, bispecific antibodies). See also, *Pierce Catalog and Handbook, 1994-1995* (Pierce Chemical Co., Rockford, IL); *Kuby, Immunology, 3rd Ed., W.H. Freeman & Co., New York, 1997*. A
30 "monoclonal antibody" is an antibody produced by a single clone of B-lymphocytes or by a cell into which the light and heavy chain genes of a single antibody have been transfected. Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen

cells. These fused cells and their progeny are termed “hybridomas.” Monoclonal antibodies include humanized monoclonal antibodies.

Antisense inhibitor: Refers to an oligomeric compound that is at least partially complementary to the region of a target nucleic acid molecule to which it hybridizes. As used herein, an antisense inhibitor (also referred to as an “antisense compound”) that is “**specific for**” a target nucleic acid molecule is one which specifically hybridizes with and modulates expression of the target nucleic acid molecule. As used herein, a “**target**” nucleic acid is a nucleic acid molecule to which an antisense compound is designed to specifically hybridize and modulation expression. Non-limiting examples of antisense compounds include primers, probes, antisense oligonucleotides, siRNAs, miRNAs, shRNAs and ribozymes. As such, these compounds can be introduced as single-stranded, double-stranded, circular, branched or hairpin compounds and can contain structural elements such as internal or terminal bulges or loops. Double-stranded antisense compounds can be two strands hybridized to form double-stranded compounds or a single strand with sufficient self complementarity to allow for hybridization and formation of a fully or partially double-stranded compound.

Biological Sample: Any sample that may be obtained directly or indirectly from an organism, including whole blood, plasma, serum, tears, mucus, saliva, urine, pleural fluid, spinal fluid, gastric fluid, sweat, semen, vaginal secretion, sputum, fluid from ulcers and/or other surface eruptions, blisters, abscesses, tissues, cells (such as, fibroblasts, peripheral blood mononuclear cells, or muscle cells), organs, and/or extracts of tissues, cells (such as, fibroblasts, peripheral blood mononuclear cells, or muscle cells), or organs. A sample is collected or obtained using methods well known to those skilled in the art.

cDNA (complementary DNA): A piece of DNA lacking internal, non-coding segments (introns) and transcriptional regulatory sequences. cDNA can also contain untranslated regions (UTRs), such as those that are responsible for translational control in the corresponding RNA molecule. cDNA is synthesized in the laboratory by reverse transcription from RNA extracted from cells.

Chemotherapeutic agent: An agent with therapeutic usefulness in the treatment of diseases characterized by abnormal cell growth or hyperplasia. Such diseases include cancer, autoimmune disease as well as diseases characterized by hyperplastic growth such as psoriasis. One of skill in the art can readily identify a chemotherapeutic agent (for instance, see Slapak and Kufe, *Principles of Cancer Therapy*, Chapter 86 in *Harrison's Principles of Internal Medicine*, 14th edition; Perry *et al.*, *Chemotherapy*, Ch. 17 in Abeloff, *Clinical Oncology* 2nd ed., © 2000 Churchill Livingstone, Inc; Baltzer L, Berkery R (eds): *Oncology Pocket Guide to*

Chemotherapy, 2nd ed. St. Louis, Mosby-Year Book, 1995; Fischer DS, Knobf MF, Durivage HJ (eds): *The Cancer Chemotherapy Handbook*, 4th ed. St. Louis, Mosby-Year Book, 1993). Examples of chemotherapeutic agents include ICL-inducing agents, such as melphalan (AlkeranTM), cyclophosphamide (CytosanTM), cisplatin (PlatinolTM) and busulfan (BusilvexTM, MyleranTM).

Contacting: Placement in direct physical association. Includes both in solid and liquid form. Contacting can occur *in vitro* with isolated cells or *in vivo* by administering to a subject.

Control: A reference standard. A control can be a known value indicative of basal expression of a diagnostic molecule such as miR-1290. In particular examples a control sample is taken from a subject that is known not to have a disease or condition, including ALL patients who did or did not experience disease relapse. In other examples a control is taken from the subject being diagnosed, but at an earlier time point, either before disease onset or prior to or at an earlier time point in disease treatment. A difference between a test sample and a control can be an increase or conversely a decrease. The difference can be a qualitative difference or a quantitative difference, for example a statistically significant difference. In some examples, a difference is an increase or decrease, relative to a control, of at least about 10%, such as at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, at least about 500%, or greater than 500%.

Detect: To determine if an agent (such as a signal or particular nucleic acid probe) is present or absent. In some examples, this can further include quantification.

Determining expression of a gene product: Detection of a level of expression (for example a nucleic acid) in either a qualitative or a quantitative manner. In one example, it is the detection of a miRNA, as described herein.

Diagnosis: The process of identifying a disease or a predisposition to developing a disease or condition, for example ALL or its relapse, by its signs, symptoms, and results of various tests and methods, for example the methods disclosed herein. The conclusion reached through that process is also called “a diagnosis.” The term “predisposition” refers to an effect of a factor or factors that render a subject susceptible to or at risk for a condition, disease, or disorder, such as ALL or its relapse. In the disclosed methods, specific miRNA expression determination to identify a subject predisposed to (or at an increased risk for) ALL relapse.

Effective amount of a compound: A quantity of compound sufficient to achieve a desired effect in a subject being treated. An effective amount of a compound can be

administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the effective amount of the compound will be dependent on the compound applied, the subject being treated, the severity and type of the affliction, and the manner of administration of the compound.

5 **Expression Control Sequences:** Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked, for example the expression of miR-451. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can
10 include appropriate promoters, enhancers, transcription terminators, a start codon (ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. A polynucleotide can be inserted into an expression vector that contains a promoter sequence, which facilitates the efficient transcription of the inserted genetic sequence of the host.

15 **Increased risk:** As used herein “increased risk” of ALL relapse refers to an increase in the statistical probability of an ALL patient relapsing relative to the general population, following standard disease treatment. As described herein, the risk of a subject determined to have an increased risk of ALL relapse may have a high risk or intermediate risk, both of which are an increased risk in comparison to “standard risk”.

20 **Inhibiting protein activity:** To decrease, limit, or block an action, function or expression of a protein. The phrase inhibit protein activity is not intended to be an absolute term. Instead, the phrase is intended to convey a wide-range of inhibitory effects that various agents may have on the normal (for example, uninhibited or control) protein activity. Inhibition of protein activity may, but need not, result in an increase in the level or activity of
25 an indicator of the protein’s activity. By way of example, this can happen when the protein of interest is acting as an inhibitor or suppressor of a downstream indicator. Thus, protein activity may be inhibited when the level or activity of any direct or indirect indicator of the protein’s activity is changed (for example, increased or decreased) by at least 10%, at least 20%, at least 30%, at least 50%, at least 80%, at least 100% or at least 250% or more as compared to control
30 measurements of the same indicator.

Isolated: A biological component (such as a nucleic acid molecule, protein or organelle) that has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, *i.e.*, other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles. Nucleic acids

and proteins that have been **isolated** include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

Label: A detectable compound or composition that is conjugated directly or indirectly to another molecule to facilitate detection of that molecule. Specific, non-limiting examples of labels include radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens, and enzymes.

Mammal: This term includes both human and non-human mammals. Similarly, the term **subject** includes both human and veterinary subjects.

MicroRNA (miRNA): Short, single-stranded RNA molecule of 18-24 nucleotides long. Endogenously produced in cells from longer precursor molecules of transcribed non-coding DNA, miRNAs can inhibit translation, or can direct cleavage of target mRNAs through complementary or near-complementary hybridization to a target nucleic acid (Boyd, *Lab Invest.*, 88:569-578, 2008). As used herein, a “microRNA sequence” includes both mature miRNA sequences and precursor sequences such as pri-miRNA, pre-miRNA, and the like.

Oligonucleotide: A plurality of joined nucleotides, between about 6 and about 300 nucleotides in length. An oligonucleotide analog refers to a subclass of oligonucleotides that contain moieties that function similarly to oligonucleotides but have non-naturally occurring portions. For example, oligonucleotide analogs can contain non-naturally occurring portions, such as altered sugar moieties or inter-sugar linkages, such as a phosphorothioate oligodeoxynucleotide. Functional analogs of naturally occurring polynucleotides can bind to RNA or DNA, and include peptide nucleic acid (PNA) molecules. Particular oligonucleotides and oligonucleotide analogs can include linear sequences up to about 200 nucleotides in length, for example a sequence (such as DNA or RNA) that is at least 6 bases, for example at least 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100 or even 200 bases long, or from about 6 to about 50 bases, for example about 10-25 bases, such as 12, 15 or 20 bases.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers useful in this disclosure are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the compounds herein disclosed. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid

compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Pharmaceutical agent: A chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject or a cell. Incubating includes exposing a target to an agent for a sufficient period of time for the agent to interact with a cell. Contacting includes incubating an agent in solid or in liquid form with a cell.

Preventing or treating a disease: Preventing a disease refers to inhibiting the full development of a disease, for example inhibiting the development of myocardial infarction in a person who has coronary artery disease or inhibiting the progression or metastasis of a tumor in a subject with a neoplasm. Treatment refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. In particular embodiments, a treatment will decrease the probability that a condition will develop, such as ALL relapse.

Probes and primers: Nucleic acid probes and primers can be readily prepared based on the nucleic acid molecules provided in this invention. A probe comprises an isolated nucleic acid attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens, and enzymes. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed, for example, in Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989 and Ausubel *et al.* *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999.

Primers are short nucleic acid molecules, preferably DNA oligonucleotides 10 nucleotides or more in length. More preferably, longer DNA oligonucleotides can be about 15, 17, 20, or 23 nucleotides or more in length. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then the primer extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, *e.g.*, by the PCR or other nucleic-acid amplification methods known in the art.

PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose. One of ordinary skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, in order to obtain greater specificity, probes and primers can be selected that comprise at least 17, 20, 23, 25, 30, 35, 40, 45, 50 or more consecutive nucleotides of the target sequence being amplified.

Quantitative real time PCR: A method for detecting and measuring products generated during each cycle of a PCR, which products are proportionate to the amount of template nucleic acid present prior to the start of PCR. The information obtained, such as an amplification curve, can be used to quantitate the initial amounts of template nucleic acid sequence.

Small interfering RNAs: Synthetic or naturally-produced small double stranded RNAs (dsRNAs) that can induce gene-specific inhibition of expression in invertebrate and vertebrate species are provided. These RNAs are suitable for interference or inhibition of expression of a target gene and comprise double stranded RNAs of about 15 to about 40 nucleotides containing a 3' and/or 5' overhang on each strand having a length of 0- to about 5-nucleotides, wherein the sequence of the double stranded RNAs is essentially identical to a portion of a coding region of the target gene for which interference or inhibition of expression is desired. The double stranded RNAs can be formed from complementary ssRNAs or from a single stranded RNA that forms a hairpin or from expression from a DNA vector.

Small molecule inhibitor: A molecule, typically with a molecular weight less than 1000, or in some embodiments, less than about 500 Daltons, wherein the molecule is capable of inhibiting, to some measurable extent, an activity of some target molecule.

System of Treatment: A multi-step health care method directed by a single actor, such as a public health care provider (e.g. a national health service or provider thereof), health maintenance organization (HMO), or hospital organization. While individual steps with a system of treatment can be carried out by multiple actors, the total method is organized by a single actor from whom the health care is provided.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transfected host cell. Recombinant DNA vectors are vectors having recombinant DNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Viral vectors are recombinant DNA vectors having at least some nucleic acid sequences derived from one or more viruses.

III. Overview of Several Embodiments

Described herein is a composition including an inhibitor of nicotinamide phosphoribosyltransferase (NAMPT), for use in treatment of acute lymphoblastic leukemia (ALL), such as in preventing relapse of ALL after remission of the disease.

5 In particular embodiments, the inhibitor of NAMPT is selected from the group consisting of a small molecule inhibitor, antibody, antisense nucleic acid, and RNA interference agent. In a particular embodiment, the inhibitor is FK866 or a functional variant thereof.

Also described herein are compositions that include an inhibitor of miR-1290, for use in
10 treatment of acute lymphoblastic leukemia (ALL) in a subject. In particular embodiments, the inhibitor of miR-1290 includes a nucleic acid that is at least 90% identical to the reverse complement of the miR-1290 sequence as set forth in SEQ ID NO: 3. In other embodiments, the inhibitor of mir-1290 includes a nucleic acid expressing a nucleic acid that is at least 90% identical to the reverse complement of the miR-1290 sequence as set forth in SEQ ID NO: 3.

15 In particular embodiments, the inhibitor of miR-1290 is selected from the group consisting of a DNA inhibitor or an RNA interference (RNAi) agent.

Further described herein are compositions including a ribonucleic acid sequence at least 90% identical to a miR-451 ribonucleic acid sequence set forth as SEQ ID NO: 2, or a nucleic acid expressing a ribonucleic acid sequence at least 90% identical to a miR-451 ribonucleic
20 acid sequence set forth as SEQ ID NO: 2, for use in treatment of acute lymphoblastic leukemia (ALL) in a subject. In a particular embodiment, the nucleic acid expressing miR-451 is operably linked to a recombinant expression plasmid. In other embodiments, the composition further includes an inhibitor of mir-1290 comprising a nucleic acid expressing a nucleic acid that is at least 90% identical to the reverse complement of the miR-1290 sequence as set forth
25 in SEQ ID NO: 3

Further described herein are compositions comprising an inhibitor of janus kinase 2 (JAK2), for use in treatment of acute lymphoblastic leukemia (ALL). Particular examples of the JAK2 inhibitor include a small molecule inhibitor, antibody, antisense nucleic acid, and RNA interference agent.

30 The described compositions can all be used in methods of treatment of acute lymphoblastic leukemia (ALL) in a subject, including reducing the risk of relapse in a subject or even preventing relapse in the subject, wherein the described compositions are administered to the subject, thereby treating the ALL.

Also described herein are systems of ALL treatment that include first determining the expression level of miR-1290 and at least one of miR-151-5p and miR-451; and comparing the determined expression of miR-1290, and miR-151-5p and/or miR-451 with control expression of miR-1290, and miR-151-5p and/or miR-451, wherein a significant increase in miR-1290 expression in the subject in comparison to the control miR-1290 expression, combined with a significant decrease in expression of the at least one of miR-151-5p and miR-451 in comparison to the control expression of miR-151-5p and/or miR-451, indicates that the subject has an increased risk of ALL relapse, and requires treatment appropriate for a subject with an increased risk of ALL relapse; and then administering to the patient a therapeutically effective amount of a composition comprising an inhibitor of nicotinamide phosphoribosyltransferase (NAMPT), or any of the other compounds or compositions described herein for use in treating ALL.

IV. ALL Prognosis by detection of miR-1290, miR-151-5p, and miR-451

Prediction of relapse has proved to be the key for successful treatment of pediatric ALL. Described herein is the observation that even on the day of ALL diagnosis, differences in miRNA expression are predictive of disease relapse, and indicative of the appropriate form of treatment to provide a patient. In particular, described herein is the observation that overexpression of miR-1290 correlates with ALL relapse, and the predictive power of combination determinations of miR-151 and miR-451 expression (underexpressed, compared with a standard), and miR-1290 expression (overexpressed, compared with a standard) is greater than any subcombination thereof.

Current practice for ALL treatment includes determining the risk of disease relapse following standard treatment. The determined risk prognosis is determinative of the treatments given to the patient. Standard prognosis determining methods include the COG, BFM, MRD, UKALL, CCLG, DFCI systems, from which a patient is determined to be high risk (HR), intermediate risk (IR), and standard risk (SR). Accordingly, under current practice, ALL treatment is provided as a risk-based treatment, i.e., high risk patients receive a more intensive treatment while the standard risk patients receive treatment reduction.

According to the BFM system (Vrooman L M et al., Curr Opin Pediatr. (2009); 21:1-8), standard risk includes (1) no adverse cytogenetics, (2) age between 1 and 6 years, (3) good response to prednisone treatment on day 8. High risk includes at least one of (1) cytogenetic abnormalities (e.g. t(9;22) and t(4;11)), (2) under 1 year of age or above 6 years, (3) poor response to prednisone treatment on day 8 and (4) hypodiploidy. Intermediate risk includes

those whose age is between 1 to 6, show no adverse cytogenetics, no hypodiploidy and a good response to prednisone on day 8 of treatment, as well as those whose condition does not meet the criteria for either standard risk or high risk.

An alternative definition of relapse risk is MRD diagnosis, which is based on an indication of the amount of remaining leukemic blasts in a patient's bone marrow (BM) during and/or after treatment, which can be measured by means of flow cytometry. (FACS) and polymerase-chain reaction (PCR) (van Dongen J J M et al., *Lancet*. (1998); 352:1731-1738). MRD risk stratification is performed after MRD analysis on days 33 and 78 from the beginning of treatment. MRD standard risk is defined as a negative MRD finding on day 33. MRD high risk is defined as a finding of 10^{-3} leukemic cells (1 leukemic cell in 1000 normal cells) on day 78. All other findings are defined as intermediate risk. In the present invention, the MRD test was performed by PCR amplification of immunoglobulin and T-cell rearrangement sites (PCR-MRD), and interpreted according to the guidelines of the European Study Group for MRD detection in ALL (ESG-MRD-ALL).

Prognostic grouping by BFM-2000 clinical risk grouping does not normally dictate a different treatment regime for the diagnosed patient. However, once MRD risk classification becomes available after day 78 of treatment, it replaces the previous classification and provides a basis for planning treatment for the patient. Until such time that the MRD risk group prognosis replaces the previous risk classification, a standard treatment is provided to all patients.

According to the COG system (Smith et al., *J Clin Oncol*. (1996); 14:18-24; Hunger, *Am Soc Clin Oncol Educ Book* (2012); 611-615), NCI standard risk includes (1) WBC count less than 50,000/ μ L and (2) age 1 to younger than 10 years. NCI high risk includes (1) WBC count 50,000/ μ L or greater and/or (2) age 10 years or older.

Induction drugs are given at first four weeks of treatment. NCI standard risk without CNS3 or overt testicular disease induction drugs includes (1) dexamethasone, (2) vincristine and (3) asparaginase. NCI high risk drugs or with CNS3 or overt testicular disease includes (1) dexamethasone, (2) vincristine, (3) asparaginase and (4) an anthracycline such as daunorubicin (Borowitz et al., *Blood* (2008); 111:5477-5485).

The described methods therefore not only allow for improved determination of ALL prognosis and relapse risk, but also improved overall systems of treatment for ALL, which include providing the most appropriate treatment protocol as determined by the determined relapse risk at a significantly earlier time point than currently achievable with MRD testing.

Accordingly, provided herein are methods for the prognosis of ALL in a subject, by determining the level of expression of miR-1290, alone or in combination with the expression of miR-151-5p and/or miR-451, and comparing the determined expression to a control or standard, such as a predetermined cutoff value. In a particular embodiment, the expression of miR-1290 is detected. In another embodiment, the expression of miR-1290 and miR-151-5p is detected. In yet another embodiment, the expression of miR-1290 and miR-451.

In the described methods, the expression in the subject sample of miR-1290, alone or in combination with the expression of miR-151-5p and/or miR-451 is compared to the expression of the specific miRNAs in a control sample, wherein a comparative significant increase in miR-1290 expression alone or in combination with a significant decrease in at least one of miR-151-5p and miR-451 indicates an increased risk of relapse. As understood herein, a control is a standard defined by the amount of specific miRNA expression in samples from one of more subjects who are either ALL free, or alternatively who had ALL but did not relapse. Such standards can change over time as additional patient data is accumulated.

In some embodiments, the predetermined control value to which a subject sample is compared, is described as a cutoff value, wherein a departure from the cutoff indicates a significant difference from the control value, and an increased risk of ALL relapse. In such embodiments, the expression of the miRNAs in relation to the cutoff value determines how the patient should be grouped with those pre-established ALL patient populations associated with specific relapse rates. For example, determination that a patient is expressing miR-1290 at levels greater than a cutoff, combined with determination that at least one of miR-151-5p and miR-451 are expressed lower than a cutoff indicates that the patient has higher risk for relapse than a patient that does not exhibit such miRNA expression levels. As used herein, such expression (a detected downregulation of miR-151-5p and miR-451, and a detected upregulation of miR-1290) can be termed a "positive expression value"

As described herein, a "cutoff value", sometimes referred to as a "cutoff", is a value that meets the requirements for both high diagnostic sensitivity (true positive rate) and high diagnostic specificity (true negative rate). Determined cutoff values are the result of a statistical analysis of miRNA expression value differences in pre-established populations which either relapsed or remained disease-free.

It should be emphasized that the accumulation of further patient data may improve the accuracy of the presently provided cutoff values, which are based on an ROC (Receiver Operating Characteristic) curve generated according to said patient data using, for example, a commercially available analytical software program. The miR-151-5p and/or miR-451

expression values are selected along the ROC curve for optimal combination of prognostic sensitivity and prognostic specificity which are as close to 100% as possible, and the resulting values are used as the cutoff values that distinguish between patients who will relapse at a certain rate, and those who will not (with said given sensitivity and specificity). The ROC curve may evolve as more and more patient-relapse data and related miR-151-5p, miR-451, and miR-1290 expression values are recorded and taken into consideration, modifying the optimal cutoff values and improving sensitivity and specificity. Thus, the provided cutoff values for miR-151-5p and miR-451 should be viewed as not limiting, but merely illustrative of the statistical analysis.

In a particular embodiment, the cutoff values for miR-151-5p and miR-451 respectively are 0.00015 and 0.001 (units relative to expression of an internal standard; the determination of which is described in International Patent Application No. PCT/IL2011/000754). Accordingly, respective miR-151-5p and miR-451 expression levels that are lower than 0.00015 and 0.001 indicates that a subject is expressing these miRNAs at lower levels than a control. With regard to miR-1290, if a subject is determined to be expressing miR-1290 above a determined cut-off value, the subject is identified as expressing miR-1290 at significantly higher levels than a control.

In particular embodiments, the determination of the miR-1290 expression combined with determination at least one of miR-151-5p and miR-451 is correlated with particular risks of relapse, depending on the determined expression levels. In other embodiments, the determined miRNA expression is combined with other clinical features, including white blood cell (WBC) count, age, minimal residual disease (MRD) risk index, cytogenetic aberrations, response to prednisone treatment on day 8, and ploidy to determine disease prognosis and relapse risk.

In particular embodiments, the ALL patient population group that may be examined by the described methods is optionally further defined by sub-grouping of the patient according to at least one clinical criterion, and each patient sub-group belongs to a specific pre-established ALL patient population associated with a specific relapse rate. According to certain embodiments, the clinical criteria comprise subgroupings according to: B-ALL and/or T-ALL diagnosis; minimal residual disease (MRD) high and low risk definitions; response to prednisone on day 8 of treatment; BFM high and low risk definitions; white blood count (WBC) being over or below 20,000 cells/ml; patient age being over one and under six years old or otherwise; CCG high and low risk definitions; and gender.

Typically, a good response to prednisone on day 8 of treatment is defined as a finding of less than 1000 leukemic blast cells/ml of blood, whereas a poor response is defined as a finding of more than 1000 leukemic blast cells/ml of blood.

5 In particular embodiments, the method of the invention is specifically applicable for predicting B-ALL relapse.

The miRNAs described herein can be detected by any methods known to the art, including use of standard oligonucleotides primers and probes, each of which can specifically hybridize to a nucleic acid sequence of at least one of miR-151-5p (SEQ ID NO: 1), miR-451 (SEQ ID NO: 2), and miR-1290 (SEQ ID NO: 3), and of at least one control reference miRNA.
10 Such sequences include sequences that are 100% identical to the reverse complement of SEQ ID NOs 1-3. It is understood that such primers and probes can also be less than identical to the reverse complement of SEQ ID NOs 1-3, such as 98%, 95%, 90%, 85% or even less, and that the design of such primers is well known in the art.

Non-limiting examples of standard nucleic acid detection methods include PCR (in all
15 of its forms, including qPCR), nucleic acid microarrays, Northern blot analysis, and various forms of primer extension.

Primers and probes for use in detecting the described miRNAs can be RNA or DNA, or analogs thereof. Examples of DNA/RNA analogs include, but are not limited to, 2-'O-alkyl sugar modifications, methylphosphonate, phosphorothiate, phosphorodithioate, formacetal, 3 -
20 thioformacetal, sulfone, sulfamate, and nitroxide backbone modifications, and analogs, for example, LNA analogs, wherein the base moieties have been modified. In addition, analogs of oligomers may be polymers in which the sugar moiety has been modified or replaced by another suitable moiety, resulting in polymers which include, but are not limited to, morpholino analogs and peptide nucleic acid (PNA) analogs. Probes may also be mixtures of
25 any of the oligonucleotide analog types together or in combination with native DNA or RNA. In particular embodiments, the oligonucleotides and analogs can be used alone; in other embodiments, they can be used in combination with one or more additional oligonucleotides or analogs.

In a particular embodiment, the described oligonucleotides are any one of a pair of
30 primers or nucleotide probe, for use in detecting the level of expression of miR-1290 and at least one of miR-151-5p and miR-451, using a nucleic acid amplification assay including but not limited to Real-Time PCR, micro arrays, PCR, in situ Hybridization and Comparative Genomic Hybridization. Methods and hybridization assays using self-quenching fluorescence probes with and/or without internal controls for detection of nucleic acid application products

are known in the art, for example, U.S. Pat. Nos. 6,258,569; 6,030,787; 5,952,202; 5,876,930; 5,866,336; 5,736,333; 5,723,591; 5,691,146; and 5,538,848.

In particular embodiments, in addition to detection of the miR of interest (miR-1290, etc.), the particular detection methods also utilizes primers and/or probes to detect the

expression of a nucleic acid to be used as an internal normalizing control. According to this embodiment, the detecting nucleic acid molecules used by the described methods include isolated oligonucleotides that specifically hybridize to a nucleic acid sequence of miR-1290 and at least one of miR-151-5p and miR-451; and isolated oligonucleotides that specifically hybridize to a nucleic acid sequence of at least one reference RNA. Non-limiting examples of such reference RNAs include a reference miRNA (whose expression is known to be the same, regardless of ALL condition), the 5S ribosomal RNA (rRNA), the U6 small nuclear RNA, or the miRXPlore Universal Reference (UR) (Miltényi biotech), which represents a pool of 979 synthetic miRNA for comparison of multiple samples.

The described methods relate to prognosis of ALL based on examining the expression of certain miRNA's, specifically, miR-151-5p, miR-451, and miR-1290 in a test sample, specifically, a biological sample obtained from a subject, methods of processing such samples to isolate nucleic acids for use in the described methods are known to the art. In particular embodiments, the sample is derived from the bone marrow of the subject.

V. Compositions for ALL Treatment and Methods of use thereof

Described herein are the observations that miR-451 and mir-151-5p expression are significantly decreased in ALL subjects with a greater risk of ALL relapse. Conversely, it was also observed that miR-1290 expression is significantly increased in ALL subjects with a greater risk of ALL relapse. Additionally described herein are specific targets of miR-451 and mir-1290 translational regulation. From these observations compositions, and methods of their use, for ALL treatment (including inhibiting ALL relapse) are accordingly indicated as detailed below.

miR-451 and mir-151-5p expression

Described herein are compositions for use in methods of treating ALL, including by decreasing the risk for ALL relapse, by increasing the levels of mir-151-5p and/or miR-451 in a subject. The described methods include administering to a subject in need thereof a therapeutically effective amount of at least one of miR-151-5p and miR-451, or any nucleic acid sequence encoding at least one of miR-151-5p and miR-451, or the pri-miRNA or miRNA

thereof. Administration of the miR-151-5p and/or miR-451 increases the intracellular level of the miRNA in a treated subject by 10%, 15%, 20%, 25%, 30%, 40%, 50% or even more than in a non-treated subject, thereby treating the ALL and/or inhibiting its relapse. In particular embodiments, the miRNA is administered to the subject. In other particular embodiments, the administered nucleic acid sequence includes an expression vector or plasmid encoding the at least one of miR-151-5p and miR-451.

The miR-151-5p and/or miR-451 sequences to be administered (whether directly or in a miRNA-encoding plasmid) are set forth as SEQ ID NOs 1 and 2, respectively. Functional variants of these sequences can also be used in the described methods, as long as the specific translation regulation function of miR-151-5p and miR-451 is retained. Such sequence variants can be 98%, 95%, 90%, 85%, or even less identical to the wild type miRNA sequences set forth as SEQ ID NOs 1 and 2.

Inhibition of miR-1290 expression

Additionally described herein are methods of treating ALL, including inhibiting ALL relapse, by administering to a subject an inhibitor of miR-1290 expression. Administration of the miR-1290 inhibitor (or nucleic acid encoding such inhibitors) decreases the intracellular level of the miRNA in a treated subject by 10%, 15%, 20%, 25%, 30%, 40%, 50% or even more than in a non-treated subject, thereby treating the ALL and/or inhibiting its relapse. In particular embodiments, the inhibitor is a nucleic acid molecule capable of specifically hybridizing to miR-1290, such as a nucleic acid comprising the reverse complement of the miR-1290 sequence set forth herein as SEQ ID NO: 3. Such nucleic acids include DNA and RNA antisense inhibitors as known in the art and as described herein. Particular non-limiting examples of miR-1290 inhibitors include DNA antisense oligonucleotides, morpholino oligonucleotides, and RNA interference agents such as siRNA which target the miR-1290 sequence. It will be appreciated that the antisense inhibitor or targeting agent of miR-1290 need not contain a reverse complementary sequence that is 100% complementary to the miR-1290 sequence; antisense sequence variants can be used in the described methods. Such sequence variants can be 98%, 95%, 90%, 85%, or even less identical to the reverse complementary sequence set forth as SEQ ID NO 3.

A wide variety of methods for delivering a nucleic acid to a subject are known in the art. Such methods are contemplated equally for administration of nucleic acids whether for use in increasing expression of miR-151-5p and miR-451 or use in inhibiting expression of miR-1290.

In particular embodiments, a miRNA-encoding nucleic acid or nucleic acid encoding a miRNA inhibitor is operably linked to a recombinant plasmid that is operable in a mammalian cell. In other embodiments, the miRNA-encoding nucleic acid or miRNA inhibitor (or nucleic acid encoding the inhibitor) is incorporated into a viral vector.

5 In still other embodiments, the provided miRNA or miRNA inhibitor is not provided in a vector or plasmid, but is provided in a form for immediate use (*e.g.* the miRNA or inhibitor is not encoded by another nucleic acid that needs to be transcribed to carry out its function as a miRNA or miRNA antisense inhibitor). In such embodiments, the miRNA or miRNA inhibitor is provided in any composition that provides stability to nucleic acid and/or facilitates the
10 uptake of the nucleic acid to a cell. Such delivery compositions are well known in the art, and include but are not limited to, liposomes, micelles (and inverted micelles), micro- and nano-particles of nucleic acid complexed with a degradable polymer, degradable nucleic acid-polymeric implants, and the like.

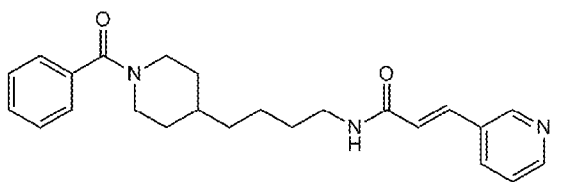
15 ***Inhibitors of NAMPT or JAK2***

Described herein is the observation that NAMPT (nicotinamide phosphoribosyl transferase), which regulates NAD⁺ synthesis, and by extension apoptosis, is negatively regulated by miR-451. Similarly, it was discovered that SOCS4, an inhibitor of cancer-promoting gene JAK2 (Janus Kinase 2) is negatively regulated by miR-1290. Accordingly,
20 compositions and methods of their use are described herein for treating ALL, including inhibiting ALL relapse. The methods include administering to a subject in need thereof a therapeutically effective amount of an inhibitor or antagonist of NAMPT or and/or an inhibitor or antagonist of JAK2.

Non-limiting examples of the antagonists and inhibitors NAMPT for use in the
25 described uses, compositions, and methods include: anti-NAMPT antibodies or fragments thereof which are able to bind NAMPT ; small molecule agents (such as but not limited to FK866, also known as WK175 or APO866 (Sigma), CHS828 also known as GMX1778 or GMX1777 or EB1627 or teglarinad (Galli et al. 2013, Journal of Medical Chemistry 56:6279-96, which interact with NAMPT and interfere with its biological function; NAMPT
30 competing derivatives (peptide and non-peptide based); antisense oligonucleotides; a nucleic acid which is capable of hybridizing with at least part of a gene encoding NAMPT, and inhibit its expression, such as siRNA and miRNA; ribozymes; molecules that target NAMPT promoter transcription factors; or that bind to the NAMPT promoter, thereby blocking access to such transcription factors and preventing its expression.

Likewise, non-limiting examples of antagonists and inhibitors of JAK2 for use in the described uses, compositions, and methods include: anti-JAK2 antibodies or fragments thereof which are able to bind JAK2; small molecule agents, which interact with JAK2 and interfere with its biological function; JAK2 competing derivatives (peptide and non-peptide based); antisense oligonucleotides; a nucleic acid which is capable of hybridizing with at least part of a gene encoding JAK2, and inhibit its expression, such as siRNA and miRNA; ribozymes; molecules that target JAK2 promoter transcription factors; or that bind to the JAK2 promoter, thereby blocking access to such transcription factors and preventing its expression.

In particular embodiments, the NAMPT antagonist is a small molecule antagonist that interacts with NAMPT and inhibits NAD⁺ synthesis. A non-limiting example of a small molecule for use in the current disclosure is FK866, which has a structure:



Functionally equivalent variants and derivatives, as described herein, of FK866 are also described.

In other embodiments, the antagonist for use in the described methods is an anti-NAMPT or anti-JAK2 antibody or fragments thereof which are able to recognize and bind NAMPT or JAK2. Antibodies that specifically recognize NAMPT or JAK2 would recognize and bind the particular protein (and peptides derived therefrom) and would not substantially recognize or bind to other proteins or peptides found in a biological sample. The determination that an antibody specifically detects its target protein is made by any one of a number of standard immunoassay methods; for instance, the Western blotting technique (Sambrook *et al.*, In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1989).

Also contemplated are humanized antibodies, for instance humanized equivalents of a murine monoclonal antibodies. A “humanized” immunoglobulin is an immunoglobulin including a human framework region and one or more CDRs from a non-human (for example a mouse, rat, or synthetic) immunoglobulin. The non-human immunoglobulin providing the CDRs is termed a “donor,” and the human immunoglobulin providing the framework is termed an “acceptor.” In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, such as at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human

immunoglobulin sequences. A “humanized antibody” is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. A humanized antibody binds to the same antigen as the donor antibody that provides the CDRs. The acceptor framework of a humanized immunoglobulin or antibody may have a limited number of substitutions by amino acids taken from the donor framework. Humanized or other monoclonal antibodies can have additional conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. Humanized immunoglobulins can be constructed by means of genetic engineering (see for example, U.S. Patent No. 5,585,089).

In still further embodiments, the NAMPT or JAK2 inhibitor is an inhibitor of NAMPT or JAK2 gene expression. NAMPT or JAK2 expression can be inhibited or eliminated at the level of transcription or at the level of translation. In particular examples, NAMPT or JAK2 expression is inhibited by use of antisense oligonucleotides; antisense morpholinos oligonucleotides, or any other nucleic acid which is capable of hybridizing with at least part of a gene encoding NAMPT or JAK2 (or the RNA product thereof), and inhibiting its expression. Such nucleic acids include as siRNA, shRNA, and miRNA.

Suppression of endogenous NAMPT or JAK2 expression can also be achieved using ribozymes. Ribozymes are synthetic molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 and U.S. Patent No. 5,543,508. The inclusion of ribozyme sequences within antisense RNAs may be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that bind to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression. Suppression can also be achieved using RNA interference, using known and previously disclosed methods as described herein.

It will be appreciated that when administered to a subject, the compositions for use in the described treatment methods, including NAMPT or JAK2 inhibitory and antagonist compounds, and the miR-451, miR-151-5p, and miR-1290-inhibitory nucleic acids described herein are formulated in standard formulations known to the art for pharmaceutical compositions. Such compositions can further include any standard pharmaceutically acceptable salts, carriers, and excipients known in the art which are appropriate for any particular mode of administration.

Combination Therapies

In particular embodiments, the compositions for use in treating ALL can be administered in varying combinations and with additional therapeutic agents, such as one or more immunomodulatory agents or known cancer therapeutic, such as a chemotherapeutic agent of the anthracycline family used when a subject is determined to have an increased risk of relapse. For example, in particular embodiments, miR-451 can be co-administered with an inhibitor of miR-1290. Similarly, in particular embodiments, a NAMPT antagonist, such as FK866 is co-administered with an inhibitor of miR-1290, such as an antisense DNA oligonucleotide containing the reverse complement of SEQ ID NO: 3.

When administered as part of a combination treatment, each composition can be administered separately in an additional separate step having an optional different mode of administration, or together in a single pharmaceutical combination.

VI. Systems of ALL treatment

Additionally described herein are systems of treating an ALL patient. The described systems involve first determining the risk of ALL relapse, through the described methods of detecting the expression of miR-1290, and at least one of miR-151-5p and miR-451. Once ALL relapse risk is determined (*e.g.* once a subject is grouped as a standard risk, intermediate risk, or high risk), an appropriate treatment is given, tailored to the determined relapse risk, and tailored to the molecule determined to be deficient or overexpressed.

Prior to the described systems, a determining appropriate ALL treatment not only relied on entirely different clinical parameters (*e.g.* WBC count, prednisone response), but such determinations were made days or even weeks after the initial diagnosis. In contrast, the current systems can determine appropriate treatment at the time of initial ALL diagnosis, following a test for expression of miR-1290, and at least one of miR-151-5p and miR-451. For example, if miR-451 is determined to be significantly underexpressed, the described systems include administering miR-451 to the subject or similarly, administering an antagonist of NAMPT, such as FK866.

The current systems are based on the understanding that modern healthcare services are provided by large entities within which multiple healthcare services are given to a patient. Particular non-limiting examples of such entities include physicians groups, hospital consortiums or networks, and public or private health maintenance organizations. Within these entities, a patient's health care may be managed by a single actor, such as a physician, nurse practitioner, and the like, but specialized services are provided to the patient by multiple actors

within the system, such as diagnosticians and specialists. It is recognized that in particular embodiments, certain services may be outsourced to a provider outside of the main service provider. However, in all embodiments, it is the main service provider, or representative or employee thereof, who is directing the described systems of treatment.

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

EXAMPLES

Example 1: General Methods

Patient material collection: Bone marrow (BM) biopsies at the time of diagnosis were obtained from 95 pediatric ALL patients and the percentage of leukemic blasts in those samples was at least 80%. 53 males and 42 females, median age 6.6 years (range 0.3-18), 28 patients had T-ALL, 46 patients had a WBC >20000, 18 patients were poor prednisone responders, 32 patients were clinically classified as BFM high-risk, 40 intermediate-risk and 23 standard-risk, 31 patients relapsed. The median follow-up of patients was 69 months (range 6-296). All patients were treated at Schneider children's medical center of Israel. 4, 16, 25 and 50 patients were treated according to the INS-84, INS-89, INS-98 and INS-2003 protocols respectively [Stark B et al., Leukemia. (2010); 24: 419-424; INS-2003].

RNA isolation: RNA was isolated out of 10^7 cells from BM biopsies according to the miRNeasy mini kit (Qiagen). RNA concentration was determined by measuring the absorbance at 260 nm with a A_{260}/A_{280} ratio of 1.8.

miRNA expression profile: Microarray analysis was performed on 48 ALL samples using the Miltenyi biotech microarray platform (Miltenyi biotech, Germany). RNA quality was assessed by Agilent 2100 Bioanalyzer platform (Agilent technologies) and visualized by means of agarose gel electrophoresis. Sample labeling was performed according to the miRXplore™ microarray platform user manual (Miltenyi biotech, Germany). For those samples which revealed a sufficient RNA yield, 2 µg total RNA were used for the labeling, for all other samples the available amount of total RNA was used. Subsequently, the fluorescently labeled samples were hybridized overnight to miRXplore™ microarrays using the a-Hyb™ hybridization station (Miltenyi biotech, Germany). Control samples were labeled with Hy3 and

experimental samples were labeled with Hy5. The miRXplore Universal Reference (UR) was used as control samples and it represents a pool of 979 synthetic miRNA for comparison of multiple samples. Fluorescent signals of the hybridized miRXplore™ microarray were detected using a laser scanner from Agilent (Agilent technologies). Normalized Hy5/Hy3 ratios were calculated for each quadruplicate by PIQORTM analyzer (Miltényi biotech, Germany). Only miRNAs that had a signal that was equal or higher than the 50% percentile of the background signal intensities were used for the Hy5/Hy3 ratio calculation. Data was transformed to Log2 ratios for data clustering (2D-clustering using Pearson correlation and average linkage).

qRT-PCR: miRNA-microarray results were verified by qRT-PCR on 95 samples. cDNA was made from 100 ng according to the manufacturer instructions (Exiqon, Denmark). qRT-PCR was performed with LNA™ primers (Exiqon) for the selected miRNAs. The 5S rRNA was used as a reference. qRT-PCR was performed in duplicate with the LightCycler 480.

Statistical analyses: miRNA expression data were analyzed with PASW Statistics 18 (SPSS Inc. Chicago, Illinois). For correlation with age, gender, WBC, d8, type and risk group the Fisher's exact test was used. In order to determine the optimal cutoff value, ROC analysis was performed for each miRNA. Kaplan-Meier analyses were performed to evaluate whether the selected miRNA correlate with relapse and COX-regression was used to determine whether those miRNA can be regarded as independent risk factors. A *p*-value of < 0.05 was considered as significant for the survival analyses.

Example 2: miR-151-5p, miR-451 and miR-1290 expression correlates with various ALL clinical parameters

This example shows that ALL prognosis can be accurately predicted by decreased expression of the miR-151-5p and miR-451, accompanied by an increased expression of miR-1290.

Microarray analysis was used to determine ALL-specific miRNA expression. From a panel of 979 synthetic miRNA, only 116 were significantly higher and 116 were significantly lower, relative to the universal reference (UR). Clustering with age, type, WBC, d8, risk group and relapse revealed 10, 33, 20, 14, 19 and 33, respectively, miRNA that were significantly lower expressed in ALL, while 9, 36, 16, 12, 14 and 28 (respectively) miRNA were significantly higher expressed in ALL. Analysis of the lower-expressed miRNAs was described

in International Patent Application No. PCT/IL2011/000754, the entirety of which is incorporated by reference herein. Therein, it was described that combined decrease in miR-151-5p and miR-451 expression is predictive of increased risk of ALL relapse and worse disease prognosis.

In a further analysis of the miR-ALL microarray, 4 miRs were chosen that were upregulated and associated with at least 3 adverse prognostic markers: miR-196b, miR-424, miR-1248, and miR-1290. To confirm the correlation between expression and ALL, the expression levels of these 4 miRs were further analyzed by real-time quantitative PCR in a cohort of 125 pediatric ALL patients (B-lineage and T-cell). Of the 4 miRs analyzed, only miR-1290 significantly correlated with ALL outcome.

Using quartile 3 as a cut-off, patients expressing high levels of miR-1290 had a 48% relapse free survival (RFS) versus 77% RFS in those expressing low levels of the miR ($p=0.005$, Figure 1). When the median expression level was used as the cut-off, the significant correlation with outcome was maintained. RFS was 59% for those expressing high levels versus 81% for those expressing low levels of the miR ($p=0.017$, data not shown). A significant correlation with outcome was also observed when analyzing only the B-lineage ALL patients ($n=105$). Patients expressing high levels of miR-1290 had a 52% RFS versus 80% for those expressing low levels ($p=0.010$; Figure 2).

When applying multivariate cox regression analysis with the variants: miR-1290, age, WBC, and prednisone response in the B-lineage cohort, both miR-1290 and WBC were identified as significant independent prognostic markers. From this analysis, it was determined that a patient expressing high levels of miR-1290 has a 3 fold increased risk of relapse (Table I).

Table A: Multivariate cox regression analysis for relapse in the B-lineage cohort ($n=105$)

Variant	Univariate P	Multivariate p	HR	95% CI
miR-1290 high vs low expression	0.010	0.006	3.03	1.4-6.6
Age 1 to 6 vs <1 or >6 years	NS			
WBC below versus above 20 X10 ⁹ /L	0.001	0.001	3.8	1.7-8.4
Prednisone response poor versus good	NS			

Currently, the risk of ALL relapse is based on the detection of minimal residual disease following treatment on days 33 and 78 from diagnosis. The amount of residual leukemic cells determines the risk groups and treatment is adjusted accordingly. The aim is to increase treatment in the high risk group, and reduce in the favorable group. Multivariate cox regression analysis was applied again including the MRD data, which was available for 61 B-lineage ALL patients. All patients excluding 2, were MRD non-high risk patients. A patient expressing high levels of miR-1290 had an increased risk fold of 4.8 to relapse ($p=0.027$; Table II).

Table B: Multivariate cox regression analysis for relapse in the B-lineage MRD non-high risk cohort (n=61)

Variant	Univariate p	Multivariate p	HR	95% CI
miR-1290 high vs low expression	0.014	0.027	4.8	1.2-19.5
Age 1 to 6 vs <1 or >6 years	NS			
WBC below versus above 20 X10 ⁹ /L	0.037	0.055	4.1	0.1-17.2
Prednisone response poor versus good	NS			
MRD	NS			

When data related to the downregulated and upregulated miRs was combined, it was revealed that the patients expressing low levels of both miRs together (miR-151 and miR-451) with high levels of miR-1290 had a very poor outcome; 33% versus 79% RFS for all other combinations ($p=0.008$; Figure 3).

When Multivariate Cox regression analysis was applied to the risk of relapse in the combined results of the down and up-regulated miRs, it was shown that a patient expressing low levels of both miR-151 and miR-451) with high levels of miR-1290, had an increased risk of 16.7 to relapse ($p=0.006$; Table III).

Table C: Multivariate Cox regression analysis for relapse in the PCR-MRD non-high risk cohort (n=54)

Variant	Univariate p	Multivariate p	HR	95% CI
Combination of all 3 miRs	0.02	0.006	16.7	2.3-122
Upregulated miR-1290	0.021			
Both miR-151 and miR-451 downregulated	0.06			
Age 1 to 6 vs <1 or >6 years	NS			
WBC below versus above 20 X10 ⁹ /L	0.037			
Prednisone response poor versus good	NS			
MRD	0.075	0.017	6.9	1.4-33

Based on these analysis it can be concluded that combining detection of miR-151, miR-451, and miR-1290 together, very high risk patients can be accurately detected, within a cohort of non-high risk patients, so that those patients at high risk of relapse could benefit from a more intensive therapy, already at the time of diagnosis.

Example 3: Up-regulation of *miR-451* decreases ALL cell growth

As described herein, decreased expression of miR-451 in comparison to a control level can serve as a prognostic factor for ALL relapse risk, as low expression of miR-451 at diagnosis predict worse outcome. To demonstrate the effect of miR-451 in ALL, miR-451 was up regulated in ALL derived Nalm-6 cell line using miR-451 mimic (SEQ ID NO: 2) transfection (Nalm-6/miR-451) by electroporation (Amaxa Nucleofector technology; kit T; program c-005). RQ-PCR was used to confirm miR-451 expression in the transfected cells. The RQ-PCR results showed a significant increase in the expression of miR-451 in Nalm-6/miR-451 versus the negative control cells (Nalm-6/miR-NC) (Fig. 4A).

To further study the putative tumor-suppressive function of *hsa-miR-451 in vivo*, 10⁷ viable Nalm-6 untransfected cells, transfected *in vitro* with miR-451, or transfected with scrambled control nucleic acid, were injected s.c. into the right flanks of 6-week-old female NOD/SCID mice. Whereas animals transplanted with scrambled miR control cells developed large tumors after 20 days, animals receiving Nalm-6/miR-451 cells showed significantly decreased tumor growth (Fig. 4B). On day 26, the median tumor volume in the scrambled control mice and the miR-451 mice were 204.69 mm³ (SE=63.96) and 23.32 mm³ (SE=13.12)

respectively ($P = 0.019$). At the end of the experiment mice were sacrificed and the tumors were weighted. The median tumor weight in the scrambled control mice and the miR-451 mice were 0.0966gr (SE=0.040) and 0.0159gr (SE=0.0009) respectively ($P = 0.046$) (Fig. 4C). These results indicate that up regulation of *hsa-miR-451* mediates cell growth in ALL and supports the role of *hsa-miR-451* as a tumor suppressor gene.

Example 4: miR-451 Inhibits NAMPT expression by targeting NAMPT 3'-UTR in ALL cell lines

This example demonstrates that the NAMPT mRNA is a specific target of miR-451 translation inhibition,

Using open access software programs (TargetScan and miRanda), nicotinamide phosphoribosyltransferase (NAMPT) was identified as a predicted target of miR-451.

To determine the effects of miR-451 on NAMPT expression, Nalm-6 cells were transfected with miR-451 mimic (SEQ ID NO: 2) and miR-451 inhibitor (GeneCopoeia miArrest miR-451an inhibitor expression clone) and NAMPT expression was measured by FACS analysis using a specific NAMPT antibody. Following the over-expression of miR-451 in cells, NAMPT protein expression was decreased by 46% of NAMPT while miR-451 inhibitor caused a 60% increase in NAMPT expression in Nalm-6/miR-451 cells (Fig. 5a , $P < 0.05$).

To confirm that NAMPT is a direct target of miR-451, luciferase reporter vectors were generated that contained the NAMPT 3'-UTR (LightSwitch NAMPT 3'UTR Reporter GoClone). Luciferase reporter assays (LightSwitch Luciferase Assay; SwitchGear) were then performed in the presence and absence of miR-451mimic and inhibitor to determine whether NAMPT was a direct downstream target of miR-451. The relative luciferase activity of the reporter that contained NAMPT 3'-UTR was decreased in 80% when miR-451 mimics were transfected. In contrast, miR-451 inhibitor showed a significant 17% increase in the relative luciferase activity of the reporter (Fig. 5B , $P < 0.05$). These results confirm that that miR-451 directly binds the 3'-UTR of NAMPT transcript, and negatively regulating its protein levels.

Studies by several investigators have shown that 12–0-tetradecanoylphorbol-13-acetate (TPA) (Sigma) is an extraordinarily potent tumor promoter and stimulates protein kinase C (PKC). Since NAMPT is over-expressed in several tumors, it was believed that it might be possible to achieve NAMPT stimulation by TPA treatment. To test this hypothesis. Peripheral blood cells were treated with 50ng/ml TPA for 24 hours and NAMPT expression was measured

by FACS using a specific NAMPT antibody. Cells treated with TPA showed an increase of more than 4 folds in NAMPT expression levels (Fig 6A).

NAMPT is the rate-limiting enzyme in the NAD⁺ biosynthetic pathway. Thus, NAD⁺ levels in the stimulated cells were measured using a standard NAD⁺ assay (Biovision NADH/NAD Quantification Kit). It was found that the cellular NAD⁺ levels in the TPA stimulated cells were 2 fold higher (Fig. 6B).

Example 5: Increased Expression of NAMPT increases sensitivity of ALL cells to the NAMPT inhibitor, FK866

Example 4 shows that miR-451 regulates NAMPT expression, and by extension, cellular NAD⁺ levels. This example demonstrates that ALL cells in which miR-451 expression is decreased have increased sensitivity to the NAMPT inhibitor FK866.

FK866 is a potent NAMPT inhibitor that is known to cause the depletion of intracellular NAD⁺ levels in the cells and ultimately induces apoptosis. The effect of FK866 treatment in Nalm-6 cell line on apoptosis and NAD⁺ levels was thus characterized.

Nalm-6 cells were treated for 1, 3, and 6 hours with 1nM FK866 (Sigma) and NAD⁺ formation was measured using NAD assay as described. The results show a gradual decrease in NAD⁺ detection following FK866 treatment (Fig. 7A). Hence, FK866 is a specific inhibitor of NAD⁺ formation in Nalm-6 cell line. To measure the effect of NAD⁺ depletion following FK866 treatment on Nalm-6 cells, apoptosis and viability were measured. Nalm-6 cells were treated for 48 hours with 1nM FK866 and apoptosis was measured using FACS. As shown in Figure 7, FK866 both potently inhibited NAD⁺ formation (Fig. 7A) and induced apoptosis of Nalm-6 cells (Fig. 7B).

The sensitivity of NALM-6 cells to the NAMPT inhibitor FK866 was measured in cells following transfection with miR-451 mimic or miR-451 inhibitor (Fig. 5). The sensitivity was measured by levels of NAD⁺. Nalm-6/miR-451 cells showed less change in NAD⁺ production after FK866 treatment compared to Nalm-6/miR-NC (Fig. 8A). However, Nalm-6/miR-inhibitor cells showed more than 5 fold change in NAD⁺ production after FK866 treatment compared to Nalm-6/miR-NC (Fig. 8B; p=0.003). These results suggest that ALL cells expressing low levels of miR-451 are more sensitive to NAMPT inhibitors. Thus, miR-451 expression can distinguish between patients that could benefit from treatment with NAMPT inhibitors, such as FK866.

Example 6: miR-1290 targets expression of SOCS4

This example describes the determination of SOCS4 as a target of miR-1290, and which will be affected by the miR-1290 overexpression observed in ALL subjects with a higher rate of relapse.

Using target prediction softwares (miRDB, miRANDA), SOCS4 was chosen as a potential target of miR-1290. The Socs4 gene encodes a member of the STAT-induced STAT inhibitor (SSI), also known as suppressor of cytokine signaling (SOCS), family. SSI family members are cytokine-inducible negative regulators of cytokine signaling. SOCS4 negatively regulates the STAT family. The expression of this gene is induced by various cytokines, including IL6, IL10, and interferon (IFN)-gamma. The protein encoded by this gene can bind to JAK kinase, and inhibit the activity of JAK kinase. The JAK kinase is known to be activated in leukemia.

Following transfection of miR-1290 mimic (SEQ ID NO: 4, over-expression), miR-1290 inhibitor (SEQ ID NO: 5, silencing), and miR-scramble (control), into the Nalm6 cell line, the protein levels of SOCS4 were measured using Western blotting ($p=0.029$; Figure 9). The values shown in the figure are mean $\pm$ S.D from 3 experiments.

Additionally, SOCS4 protein levels were measured in 31 BM samples of ALL patients and compared to the levels of miR-1290. SOCS4 protein levels were significantly reduced in the samples harboring high miR-1290 expression levels ($p<0.0001$; Figure 10).

Phosphorylated STAT5 (phospho-STAT5, activated by JAK) levels were measured by FACS analysis following transfection of miR-1290 mimic (overexpression) into NALM-6 cell line. An increase of 50% in the levels of pSTAT5 protein is evident in the cells expressing high levels of miR-1290 (Figure 11).

JAK2 is an essential gene in the leukemic process. We have shown that the over-expression of miR-1290 results in the down-regulation of SOCS4. SOCS4 normally inhibits the activity of JAK2, thus its down-regulation results in the increase activity of JAK2, with no need in external signals (as cytokines).

This result suggests that the expression levels of miR-1290 may predict the presence or absence of an activated JAK/STAT pathway, and predict who may benefit from JAK2 inhibitors.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated embodiments are only preferred examples of the invention and should not be taken as limiting the scope of the

invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A composition comprising an inhibitor of nicotinamide phosphoribosyltransferase (NAMPT), for use in treatment of acute lymphoblastic leukemia (ALL).

5 2. The composition of claim 1, wherein the inhibitor of NAMPT is selected from the group consisting of a small molecule inhibitor, antibody, antisense nucleic acid, and RNA interference agent.

10 3. The composition of claims 1 or 2, wherein the inhibitor is FK866 or a functional variant thereof.

4. The composition of any one of claims 1-3, wherein the treatment of ALL includes reducing risk of relapse in a subject.

15 5. A composition comprising an inhibitor of miR-1290, for use in treatment of acute lymphoblastic leukemia (ALL) in a subject.

20 6. The composition of claim 5, wherein the inhibitor of miR-1290 comprises a nucleic acid that is at least 90% identical to the reverse complement of the miR-1290 sequence as set forth in SEQ ID NO: 3.

25 7. The composition of claim 5, wherein the inhibitor of mir-1290 comprises a nucleic acid expressing a nucleic acid that is at least 90% identical to the reverse complement of the miR-1290 sequence as set forth in SEQ ID NO: 3.

8. The composition of claim 5 or claim 6, wherein the inhibitor is selected from the group consisting of a DNA inhibitor or an RNA interference (RNAi) agent.

30 9. A composition comprising a ribonucleic acid sequence at least 90% identical to a miR-451 ribonucleic acid sequence set forth as SEQ ID NO: 2, or a nucleic acid expressing a ribonucleic acid sequence at least 90% identical to a miR-451 ribonucleic acid sequence set forth as SEQ ID NO: 2, for use in treatment of acute lymphoblastic leukemia (ALL) in a subject.

10. The composition of claim 9, wherein the nucleic acid expressing miR-451 is operably linked to a recombinant expression plasmid.

11. The composition of claim 9 or claim 10, further comprising an inhibitor of mir-1290 comprising a nucleic acid expressing a nucleic acid that is at least 90% identical to the reverse complement of the miR-1290 sequence as set forth in SEQ ID NO: 3

12. A composition comprising an inhibitor of janus kinase 2 (JAK2), for use in treatment of acute lymphoblastic leukemia (ALL).

13. The composition of claim 12, wherein the inhibitor of JAK2 is selected from the group consisting of a small molecule inhibitor, antibody, antisense nucleic acid, and RNA interference agent.

14. A method for treatment of acute lymphoblastic leukemia (ALL) in a subject, comprising:

administering to the subject a therapeutically effective amount of an inhibitor of nicotinamide phosphoribosyltransferase (NAMPT), thereby treating the patient.

15. The method of claim 14, wherein the inhibitor of NAMPT is selected from the group consisting of a small molecule inhibitor, antibody, antisense nucleic acid, and RNA interference agent.

16. The method of claim 14 or claim 15, wherein the inhibitor is FK866 or a functional variant thereof.

17. The method of any one of claims 14-16, wherein the treatment of ALL comprises reducing risk of relapse in a subject.

18. The method of any one of claims 14-17, wherein the subject has been diagnosed as having a non-standard risk of ALL relapse.

19. A method for treatment of acute lymphoblastic leukemia (ALL) in a subject, comprising:

administering to the subject a therapeutically effective amount of an inhibitor of miR-1290, thereby treating the patient.

20. A method for treatment of acute lymphoblastic leukemia (ALL) in a subject,
5 comprising:

administering to the subject a therapeutically effective amount of a ribonucleic acid sequence at least 90% identical to a miR-451 ribonucleic acid sequence set forth as SEQ ID NO: 2, or a nucleic acid expressing a ribonucleic acid sequence at least 90% identical to a miR-451 ribonucleic acid sequence set forth as SEQ ID NO: 2, thereby treating the patient.

10 21. A system of ALL treatment comprising:

determining the expression level of miR-1290 and at least one of miR-151-5p and miR-451; and

comparing the determined expression of miR-1290, and miR-151-5p and/or miR-451
15 with control expression of miR-1290, and miR-151-5p and/or miR-451,

wherein a significant increase in miR-1290 expression in the subject in comparison to the control miR-1290 expression, combined with a significant decrease in expression of the at least one of miR-151-5p and miR-451 in comparison to the control expression of miR-151-5p and/or miR-451,
20 indicates that the subject has an increased risk of ALL relapse, and requires treatment appropriate for a subject with an increased risk of ALL relapse; and

administering to the patient a therapeutically effective amount of a composition comprising an inhibitor of nicotinamide phosphoribosyltransferase (NAMPT).

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Figure 1

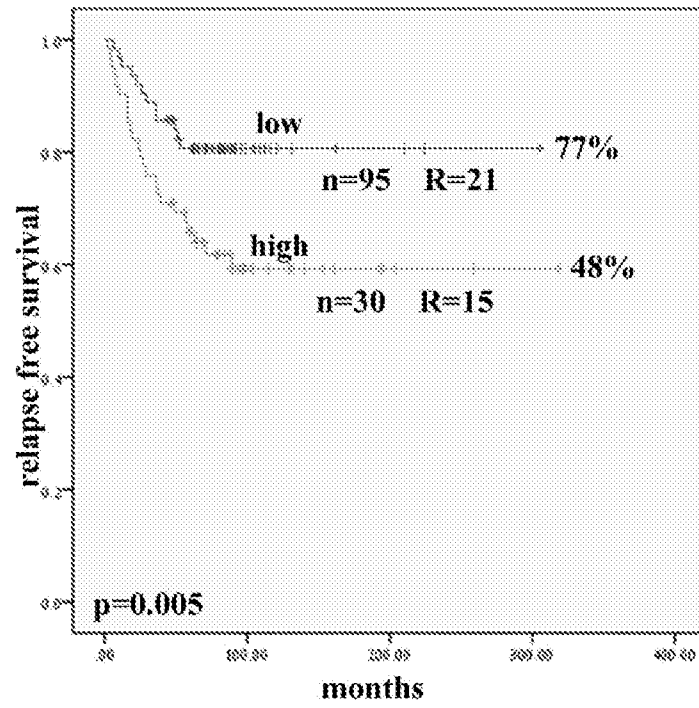
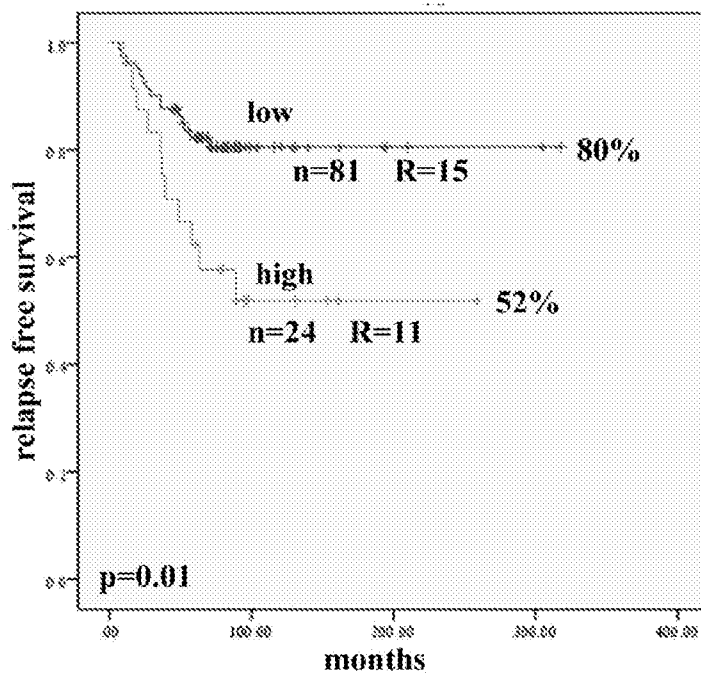


Figure 2



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Figure 3

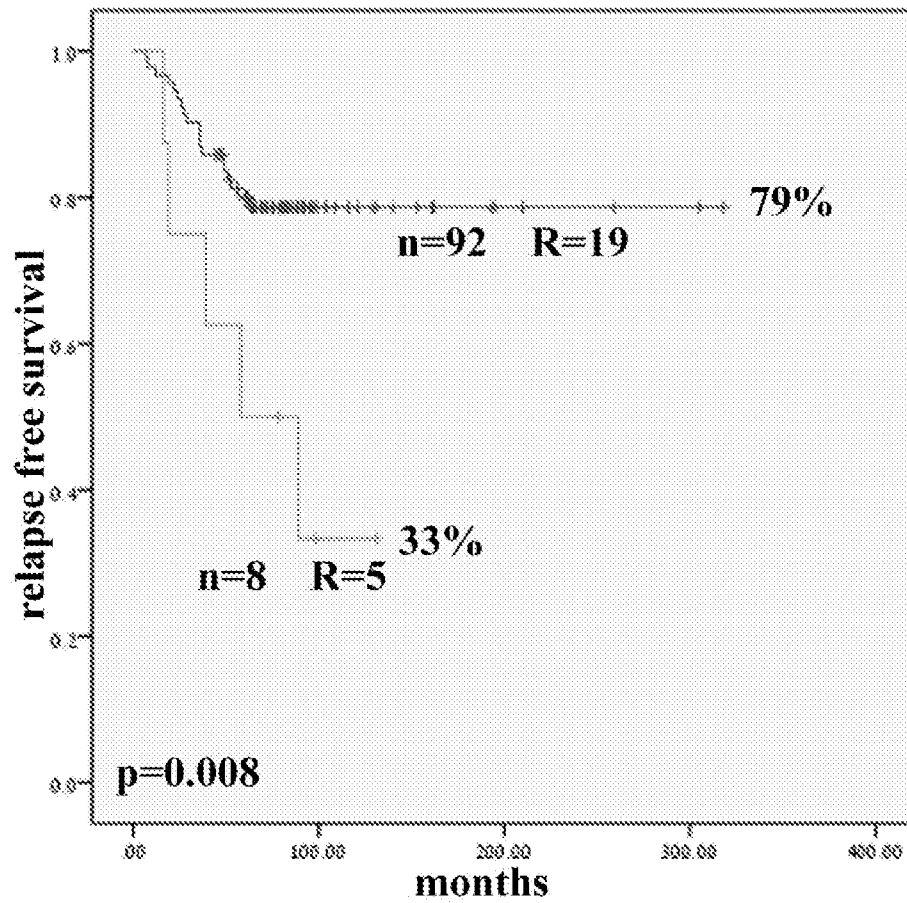


Figure 4A

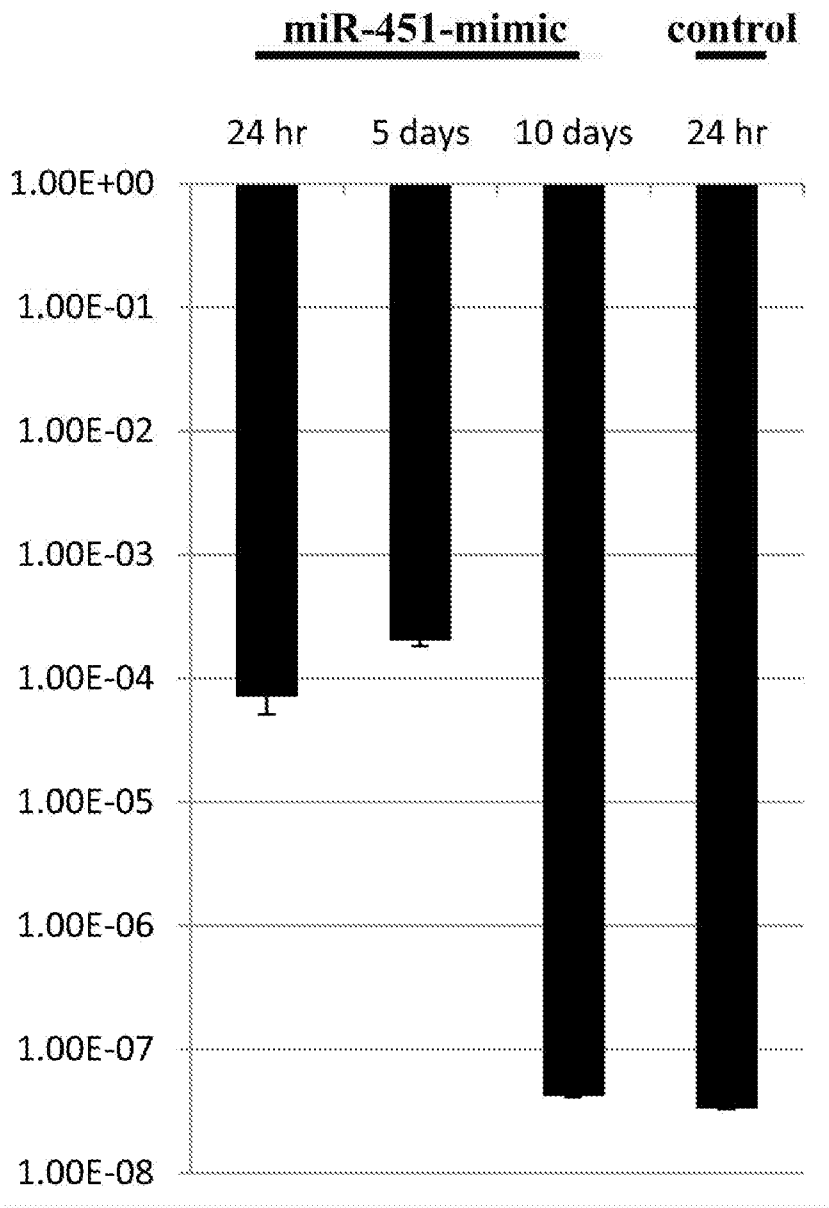


Figure 4B

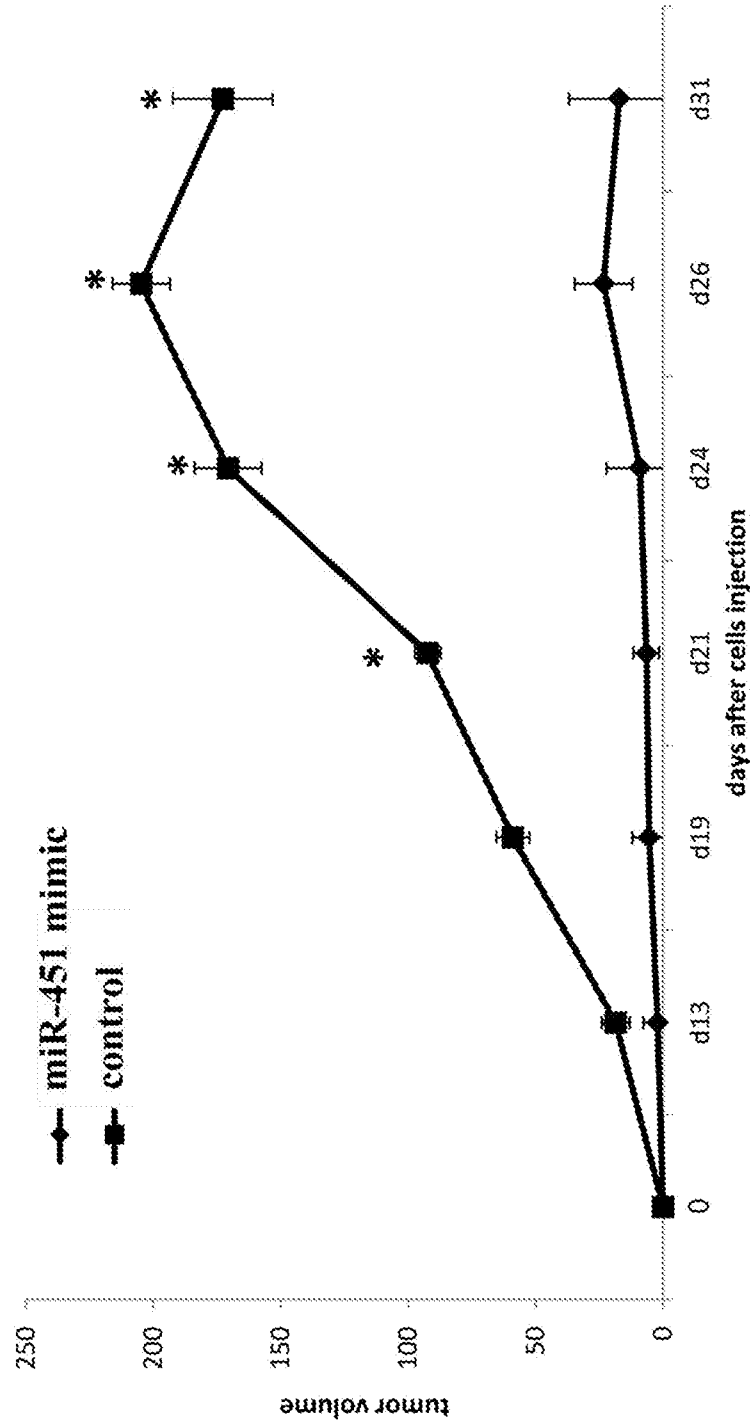


Figure 4C

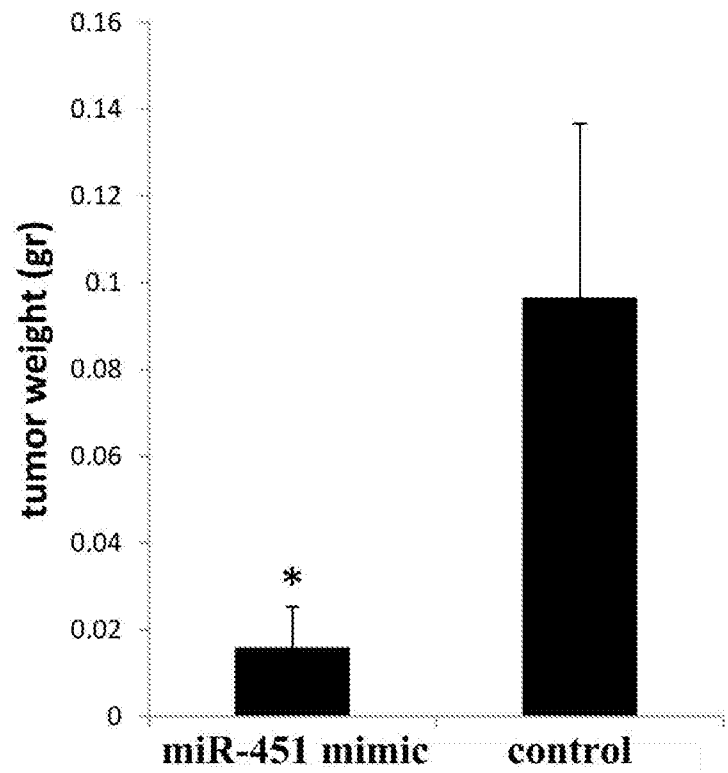
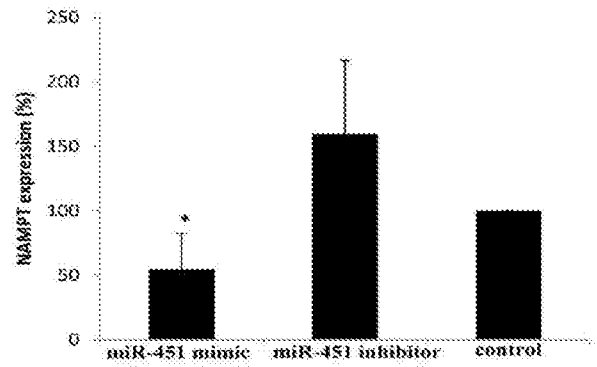


Figure 5A



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Figure 5B

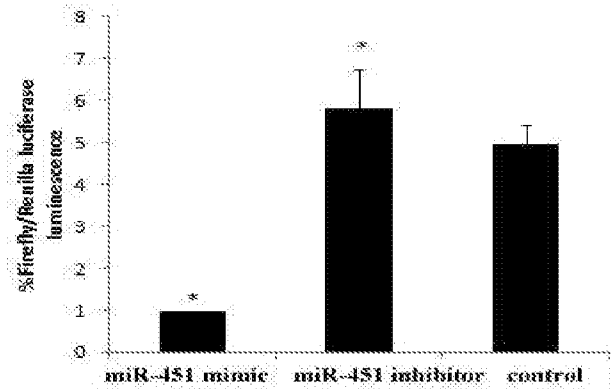


Figure 6A

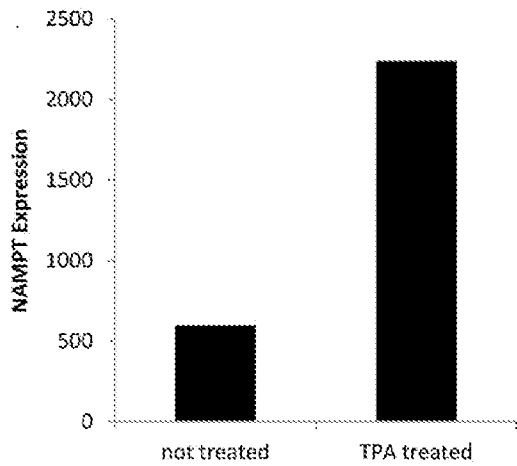


Figure 6B

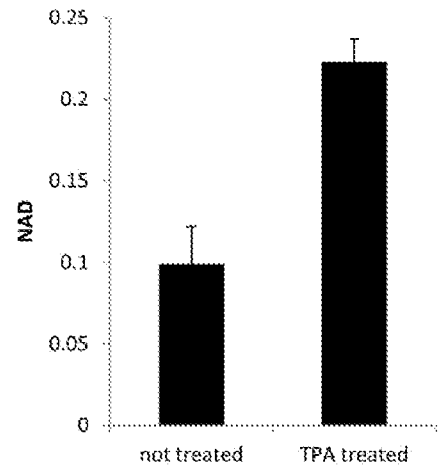


Figure 7A

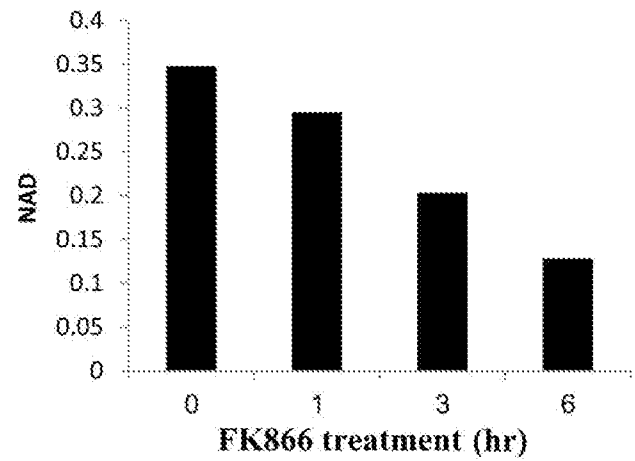


Figure 7B

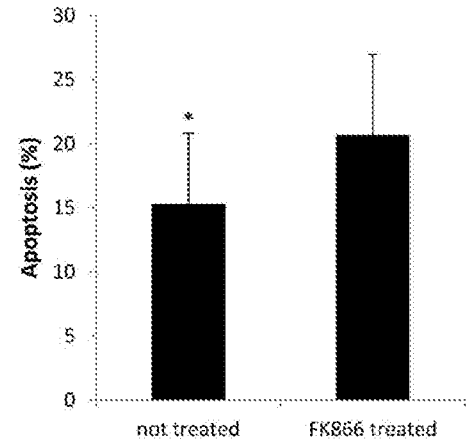


Figure 8A

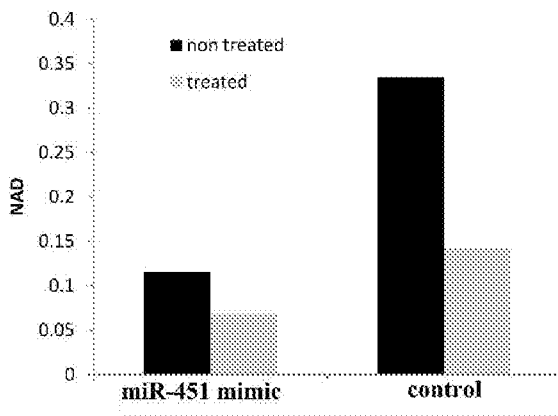


Figure 8B

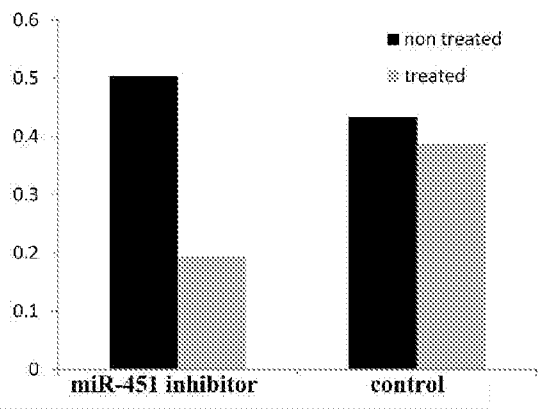


Figure 9

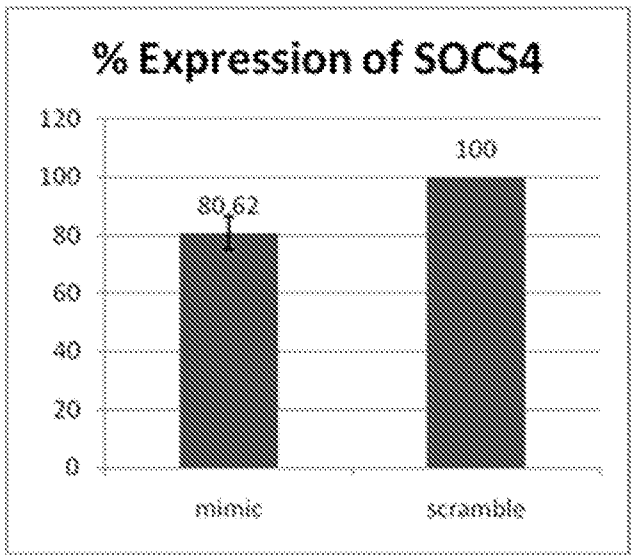


Figure 10A

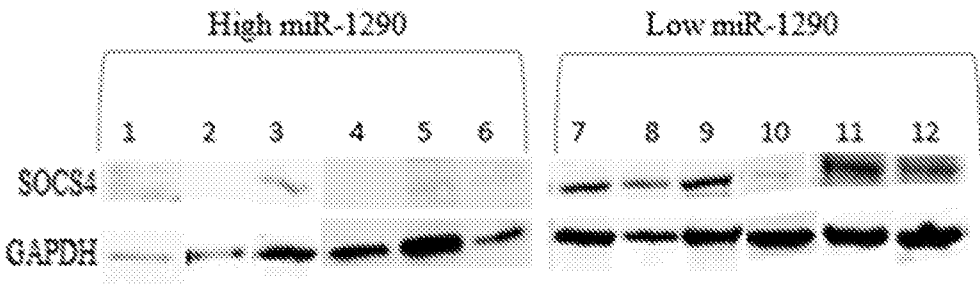


Figure 10B

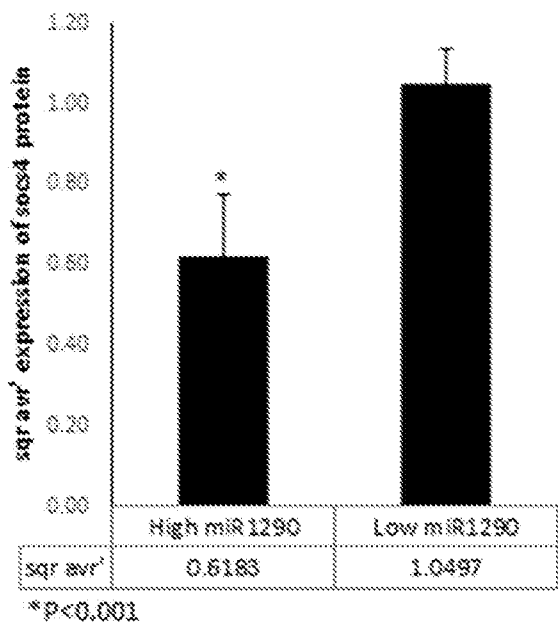
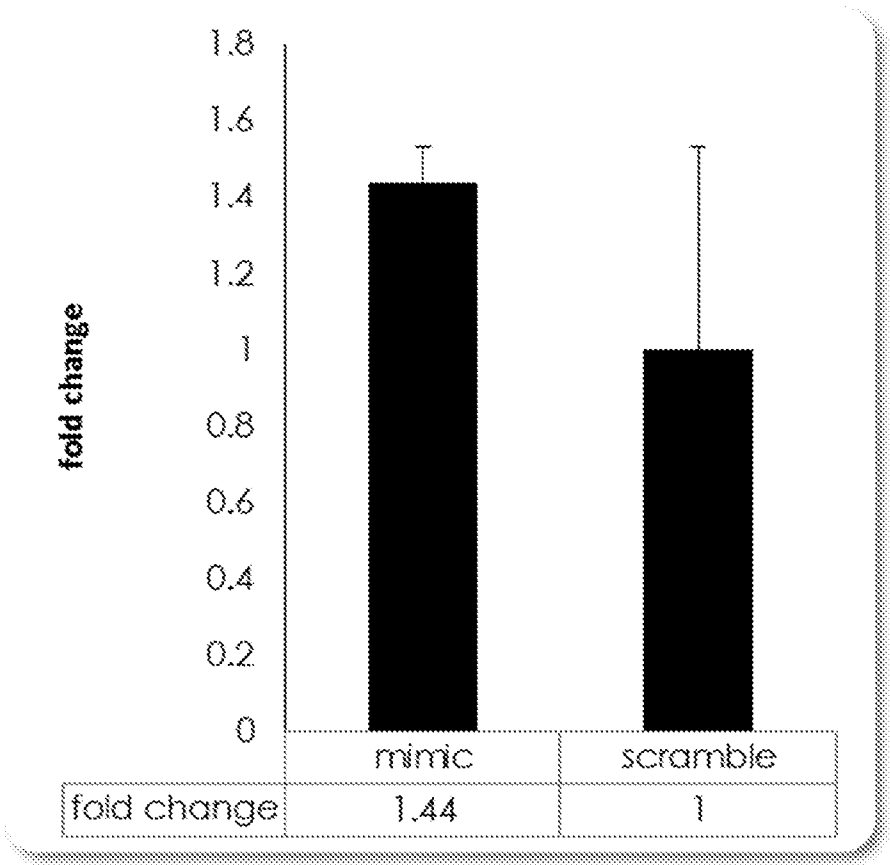


Figure 11



INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2015/050791

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2015.01) G01N 33/574, C12Q 1/68

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 (2015.01) G01N, C12Q

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

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

Databases consulted: BLAST, PATENTSCOPE, THOMSON INNOVATION, Esp@cenet, Google Patents, CAPLUS, BIOSIS, EMBASE, REGISTRY, PubMed, Google Scholar, PatBase

Search terms used: acute lymphoblastic leukemia, relapse, NAMPT, FK866, miR-1290 inhibitor, miR-451, JAK2, miR-151-5p

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Matheny, Christina J., et al. "Next-generation NAMPT inhibitors identified by sequential high-throughput phenotypic chemical and functional genomic screens." Chemistry & biology 20.11 (31.10.2013): 1352-1363. Retrieved from the internet on: 15.11.15; URL: http://www.sciencedirect.com/science/article/pii/S1074552113003554 31 Oct 2013 (2013/10/31) summary, page 3 1st, 3rd – 4th para., page 7 5th para., Figure 5, Figure 1D, Table S1	1-4,9-11,14-18,20, 21
X	WO 2012042516 A3 MOR RESEARCH APPLIC LTD?[IL]; AVIGAD SMADAR?[IL]; YANIV ISAAC?[IL] 09 Aug 2012 (2012/08/09) whole document	1-4,9-11,14-18,20, 21
Y	Chen, Xiaomei, et al. "Analysis of Microvesicle Microrna Expression Profiles and Their Functional Roles in ALL Subtypes." 53rd ASH Annual Meeting and Exposition, 10.12.2011. retrieved from the internet on: 16.11.2015; URL: https://ash.confex.com/ash/2011/webprogram/Paper37549.html 10 Dec 2011 (2011/12/10) whole document	5-8,11,19

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

* Special categories of cited documents:

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

"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

17 Nov 2015

Date of mailing of the international search report

26 Nov 2015

Name and mailing address of the ISA:

Israel Patent Office

Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel

Facsimile No. 972-2-5651616

Authorized officer

HERMAN Karin

Telephone No. 972-2-5651749

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2015/050791

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Li, Wenying, et al. "Oncomirnas and Tumor Suppressors In Microvesicles From Four Types Of Cancer." Blood 122.21 (15.11.2013): 4900-4900. retrieved from the internet on: 16.11.2015; URL: retrieved from the internet on: 16.11.2015; URL: http://www.bloodjournal.org/content/122/21/4900?sso-checked=true 15 Nov 2013 (2013/11/15) para. 3, 4, 6	5-8,11,19
X	Meydan, Naftaly, et al. "Inhibition of acute lymphoblastic leukaemia by a Jak-2 inhibitor." Nature 379, 645 - 648 (15.02.1996);. Retrieved from the internet on: 16.11.2015; URL: http://www.nature.com/nature/journal/v379/n6566/abs/379645a0.html 15 Feb 1996 (1996/02/15) whole document	12,13

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2015/050791

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:

See extra sheet.

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

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/IL2015/050791

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2012042516 A3	09 Aug 2012	WO 2012042516 A2	05 Apr 2012
		WO 2012042516 A3	09 Aug 2012
		CA 2812512 A1	05 Apr 2012
		EP 2622097 A2	07 Aug 2013
		US 2013196335 A1	01 Aug 2013

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2015/050791

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:

Invention/s 1	Compositions and methods for treating Acute Lymphoblastic Leukemia (ALL) comprising the administration of Nicotinamide phosphoribosyltransferase (NAMPT) inhibitor or miR-451 as set forth in SEQ. ID. 2.	Claim/s 1-4,9-11,14-18,20,21
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Invention/s 2	Compositions and methods for treating Acute Lymphoblastic Leukemia (ALL) comprising the administration of miR-1290 inhibitor or inhibitor of Janus Kinase 2 (JAK2).	Claim/s 5-8,11-13,19
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