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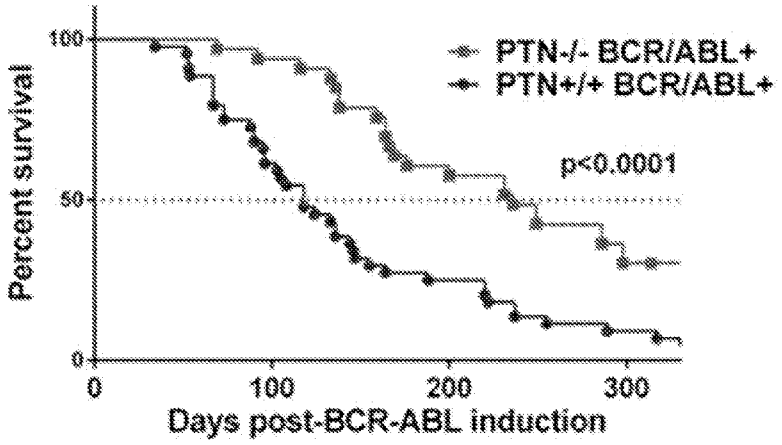
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(54) Title: METHODS FOR THE TREATMENT OF HEMATOLOGIC CANCER

FIGURE 4:



(57) Abstract: Provided herein are methods of treating hematologic cancer, such as chronic myelogenous leukemia, or splenomegaly by inhibiting PTN expression or activity. Additionally, provided herein are methods of reducing white blood cell count in a subject with elevated white blood cell count (e.g., as a result of a pathological condition), as well as methods of reducing the number of leukemia stem cells in bone marrow by inhibiting PTN expression or activity.

METHODS FOR THE TREATMENT OF HEMATOLOGIC CANCER

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 62/299,822, filed February 25, 2016, which is incorporated by reference herein in its entirety.

BACKGROUND

Hematologic cancers, or blood cancers, affect the production, development, and function of blood cells. Leukemia, a hematological cancer that affects the bone marrow, impairs the normal function of bone marrow and is correlated with increased levels of white blood cells. Chronic myelogenous leukemia accounts for approximately 60% of leukemia diagnoses, as well as one of the leading causes of splenomegaly (enlarged spleen). New therapies are needed to treat hematologic cancer and its pathological progression.

SUMMARY

In certain aspects, provided herein is a method of treating and/or preventing a hematologic cancer (*e.g.*, chronic myelogenous leukemia) in a subject comprising administering to the subject an agent that inhibits the activity or the expression of pleiotrophin (PTN). In some embodiments, the agent is a small molecule, a polypeptide (*e.g.,* a PTN protein or a fragment thereof), or a polynucleotide (*e.g.,* encoding a PTN protein or an inhibitory nucleic acid). In some embodiments, the agent is administered with a second agent. In some embodiments, the second agent is a chemotherapeutic agent (*e.g.,* cladribine fludarabine, topotecan, etoposide, 6-thioguanine, hydroxyurea, methotrexate 6-mercaptopurine, azacitidine, decitabine, doxycycline, corticosteroids, or, preferably, tetracycline). In some embodiments, the second agent is an immune checkpoint inhibitor.

Provided herein are methods of decreasing the count of white blood cells (WBC) in a subject with an elevated white blood cell (WBC) count (*e.g.,* as a result of a disease or disorder), comprising administering to the subject an agent that inhibits the expression or activity of PTN. In some embodiments, the white blood cells are neutrophils. In some embodiments, the disease or disorder is an autoimmune disorder. In some embodiments, the agent is a small molecule, a polypeptide (*e.g.,* a PTN protein or a fragment thereof), or a polynucleotide (*e.g.,* encoding a PTN protein or an inhibitory nucleic acid).

In some aspects, provided herein are methods related to decreasing the number of leukemia stem cells in bone marrow (BM), comprising contacting bone marrow with an agent that inhibits the expression or activity of PTN. In some embodiments, the bone marrow is implanted into a subject with chronic myelogenous leukemia. In some embodiments, the bone marrow is bone marrow from an individual with chronic myelogenous leukemia. In some embodiments, the agent is a small molecule, a polypeptide (*e.g.*, a PTN protein or a fragment thereof), or a polynucleotide (*e.g.*, encoding a PTN protein or an inhibitory nucleic acid).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 includes two sections, A and B. A shows the WBC count in BCR-ABL; $PTN^{+/+}$ versus BCR-ABL;PTN-/- mice. Section B shows neutrophil counts in BCR-ABL;$PTN^{+/+}$ versus BCR-ABL;PTN-/- mice.

Figure 2 includes three sections, A, B, and C. Section A shows femur sections from BCR-ABL;PTN+/+ mice and BCR-ABL;PTN-/- mice. **Section B shows** representative spleens from BCR-ABL;PTN+/+ mice and BCR-ABL;PTN-/- mice. Section C shows histologic analysis of enlarged spleens in BCR-ABL;PTN+/+ mice.

Figure 3 includes two sections, A and B. A shows a flow cytometric analysis of BCR-ABL;PTN+/+ mice and BCR-ABL;PTN-/- mice, displaying increased KSL cells in BCR-ABL;PTN+/+ mice. Section B shows scatter plots comparing mean percentage KSL cells in each group and controls.

Figure 4 shows Log Rank analysis of survival of BCR-ABL mice is shown in presence or absence of PTN deletion.

Figure 5 includes two sections, A and B. A shows PTN protein levels in the spleens of CML mice. Section B shows PTN mRNA in spleens of CML mice compared to healthy spleens.

Figure 6 shows colony growth of healthy bone marrow CD34+ CML cells undergoing anti-PTN treatment at different concentrations.

DETAILED DESCRIPTION

As described herein, PTN is a signaling molecule that plays a role in hematologic cancer pathogenesis. In some aspects, provided herein are methods of preventing or treating an hematologic cancer, such as chronic myelogenous leukemia, or splenomegaly in a subject comprising administering to the subject an agent that inhibits the activity or expression of PTN. In some aspects, provided herein are methods of decreasing the count of white blood cells

(WBC) in a subject with an elevated white blood cell count (*e.g.*, as a result of a disease or disorder, such as an autoimmune disorder), comprising administering to the subject an agent that inhibits the expression or activity of PTN. In some embodiments, the white blood cells are neutrophils.

In some aspects, the provided herein are methods related to decreasing the number of leukemia stem cells in bone marrow, comprising contacting bone marrow with an agent that inhibits the expression or activity of PTN.

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The terms "antagonist" and "inhibitor" are used herein to denote a chemical compound (such as an organic or inorganic compound, a mixture of chemical compounds), a biological macromolecule (such as a nucleic acid, an antibody, including parts thereof as well as humanized, chimeric and human antibodies and monoclonal antibodies, a protein or portion thereof, *e.g.*, a peptide, a lipid, a carbohydrate), or an extract made from biological materials such as bacteria, plants, fungi, or animal cells or tissues. They include, for example, agents whose structure is known, and those whose structure is not known. The terms "antagonist" and "inhibitor" are used interchangeably herein. An inhibitor may, for example, reduce the activity of PTN. The inhibitor may inhibit a target such as PTN by reducing the amount of translation of a PTN mRNA, e.g., the inhibitor may be an interfering nucleic acid.

The term "agent" is used to refer to an "agonist", "antagonist", or "inhibitor", and the term includes small molecules, interfering nucleic acids, and viral vectors. The term "agent" is used interchangeably with the term "compound" herein.

The terms "patient," "subject," or "individual" are used interchangeably and refer to either a human or a non-human animal. These terms include mammals, such as humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (*e.g.*, canines, felines, etc.) and rodents (*e.g.*, mice and rats).

"Treating" a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or

more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "preventing" is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition to an asymptomatic subject which reduces the frequency or severity of, or delays the onset of, symptoms of a medical condition in the subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

"Administering" or "administration of" a substance, a compound or an agent to a subject can be carried out using one of a variety of methods known to those skilled in the art. For example, a compound or an agent can be administered, intravenously, arterially, intradermally, intramuscularly, intraperitoneally, subcutaneously, ocularly, sublingually, orally (by ingestion), intranasally (by inhalation), intraspinally, intracerebrally, and transdermally (by absorption, *e.g.*, through a skin duct). A compound or agent can also appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, *e.g.*, patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound or agent. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

Appropriate methods of administering a substance, a compound or an agent to a subject will also depend, for example, on the age and/or the physical condition of the subject and the chemical and biological properties of the compound or agent (*e.g.*, solubility, digestibility, bioavailability, stability and toxicity). In some embodiments, a compound or an agent is administered orally, *e.g.*, to a subject by ingestion. In some embodiments, the orally administered compound or agent is in an extended release or slow release formulation, or administered using a device for such slow or extended release.

As used herein, the phrase "conjoint administration" refers to any form of administration of two or more different therapies such that the second therapy is administered while the previously administered therapy is still effective in the body (e.g., the two compounds are simultaneously effective in the patient, which may include synergistic effects of the two compounds). For example, the different therapies can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially. In certain embodiments, the different therapies can be administered within one hour, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, or a week of one another. Thus, an individual who receives such treatment can benefit from a combined effect of different therapies.

A “therapeutically effective amount” (“effective amount”) or a “therapeutically effective dose” of a therapy or agent, such as an agonist, antagonist, or inhibitor, is an amount of a drug or therapy that, when administered to a subject will have the intended therapeutic effect. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations. The precise effective amount needed for a subject will depend upon, for example, the subject’s size, health and age, and the nature and extent of the condition being treated. The skilled worker can readily determine the effective amount for a given situation by routine experimentation.

The phrase “pharmaceutically acceptable” is art-recognized. In certain embodiments, the term includes compositions, excipients, adjuvants, polymers and other materials and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

"Pharmaceutically acceptable salt" or "salt" is used herein to refer to an acid addition salt or a basic addition salt which is suitable for or compatible with the treatment of patients.

The term "pharmaceutically acceptable acid addition salt" as used herein means any non-toxic organic or inorganic salt. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono- or di-acid salts can be formed, and such salts may exist in either a hydrated, solvated or substantially anhydrous form. The selection of the appropriate salt will be known to one skilled in the art.

The term "pharmaceutically acceptable basic addition salt" as used herein means any non-toxic organic or inorganic base addition salt of any acid compounds. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium, or barium hydroxide. Illustrative organic bases which form suitable salts include aliphatic, alicyclic, or aromatic organic amines such as methylamine, trimethylamine and picoline or ammonia. The selection of the appropriate salt will be known to a person skilled in the art.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filter, diluent, excipient, solvent or encapsulating material useful for formulating a drug for medicinal or therapeutic use.

The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity, e.g., inhibition of a PTN activity.

The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th Ed., Daniel P. Stites, Abba I.

Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha ("α"), delta ("δ"), epsilon ("ε"), gamma ("γ") and mu ("μ"), respectively. The γ and α classes are further divided into subclasses (isotypes) on the basis of relatively minor differences in the CH sequence and function, e.g., humans express the following subclasses: IgGl, IgG2, IgG3, IgG4, IgAl, and IgA2. The subunit structures and three dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al., Cellular and Molecular Immunology, 4th ed. (W.B. Saunders Co., 2000).

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_R) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

An "isolated" antibody is one that has been identified, separated and/or recovered from a component of its production environment (e.g., naturally or recombinantly). Preferably, the isolated polypeptide is free of association with all other contaminant components from its production environment.

Contaminant components from its production environment, such as those resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be

purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant T-cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as "V_H" and "V_L", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies, such as the antibodies described herein. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat et al., Sequences of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent- cellular toxicity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-

translation modifications (e.g., isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler and Milstein, Nature, 256:495-97 (1975); Hongo et al., Hybridoma, 14 (3):253-260 (1995), Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2d ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage-display technologies (see, e.g., Clackson et al., Nature, 352:624- 628 (1991); Marks et al., J. Mol. Biol. 222:581-597 (1992); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Nat'l Acad. Sci. USA 101(34): 12467-472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al., Proc. Nat'l Acad. Sci. USA 90:2551 (1993); Jakobovits et al., Nature 362:255- 258 (1993); Bruggemann et al., Year in Immunol. 7:33 (1993); U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks et al., Bio/Technology 10:779-783 (1992); Lonberg et al., Nature 368:856-859 (1994); Morrison, Nature 368:812-813 (1994); Fishwild et al., Nature Biotechnol. 14:845-851 (1996); Neuberger, Nature Biotechnol. 14:826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13:65-93 (1995).

The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an

Fc region. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

An "antibody fragment" comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments; diabodies; linear antibodies (see U.S. Patent 5,641,870, Example 2; Zapata et al., Protein Eng. 8(10): 1057-1062 (1995)); single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_RI). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_HI domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

"Fv" is the minimum antibody fragment which contains a complete antigen- recognition and -binding site. This fragment consists of a dimer of one heavy- and one light- chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an

antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pliickthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

"Functional fragments" of antibodies comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the F region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10) residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, i.e., a fragment having two antigen-binding sites.

Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger et al., Proc. Nat'l Acad. Set USA 90:6444-48 (1993).

As used herein, a "chimeric antibody" refers to an antibody (immunoglobulin) of the present disclosure, in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Nat'l Acad. Sci. USA, 81:6851-55 (1984)). Chimeric antibodies of interest herein include PREVIATIZED® antibodies wherein the antigen-binding region of the antibody is

derived from an antibody produced by, e.g., immunizing macaque monkeys with an antigen of interest. As used herein, "humanized antibody" is a subset of "chimeric antibodies."

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, and the like. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, e.g., Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also, for example, Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1: 105- 115 (1998); Harris, Biochem. Soc. Transactions 23: 1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.

A "human antibody" is one that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, Mol. Biol., 227:381 (1991); Marks et al., Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods

described in Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(l):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e.g., immunized xenomice {see, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li et al., Proc. Nat'l Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

Antibodies that selectively or broadly target PTN are useful in the compositions and methods described herein, e.g., as inhibitors of PTN activity. For example, in certain embodiments, the compositions and methods described herein employ an antibody that inhibits or blocks PTN activity. Antibodies include antibodies of different isotypes, such as IgM, IgG, IgA, IgD, and IgE antibodies. The antibody may be a polyclonal antibody, a monoclonal antibody, a chimerized or chimeric antibody, a humanized antibody, a primatized antibody, a deimmunized antibody, or a fully human antibody. The antibody can be made in or of any variety of species, *e.g.*, mammals such as humans, non-human primates (*e.g.*, orangutan, baboons, or chimpanzees), horses, cattle, pigs, sheep, goats, dogs, cats, rabbits, guinea pigs, gerbils, hamsters, rats, and mice. The antibody can be a purified and/or a recombinant antibody. The antibody may be a bispecific or multispecific antibody, or antigen-binding fragment thereof, for PTN. The antibody, or antigen-binding fragment thereof, may be murine, chimeric, humanized, composite, or human. The antibody may be detectably labeled.

The terms "*polynucleotide*" and "*nucleic acid*" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before

or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified, such as by conjugation with a labeling component. The term "recombinant" polynucleotide means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in a non-natural arrangement.

The term "*small molecule*" is a term of the art and includes molecules that are less than about 2000 amu, less than about 1000 amu, or even less than about 500 amu. In some embodiments, small molecules do not exclusively comprise peptide bonds. In some embodiments, small molecules are not oligomeric. Small molecule compounds which can be screened for activity include, but are not limited to, peptides, peptidomimetics, nucleic acids, carbohydrates, small organic molecules (*e.g.*, polyketides) (Cane *et al.* (1998) *Science* 282:63), and natural product extract libraries. In some embodiments, the compounds are organic non-peptidic compounds. In some embodiments, a small molecule is not biosynthetic.

Modulators of PTN Activity

In certain embodiments, the method relates to the use of a small molecule to inhibit PTN (e.g., to inhibit the activity or expression of PTN). The small molecule may be a small molecule identified from a library of test molecules.

Certain embodiments of the present disclosure relate to methods of inhibiting PTN activity. These methods include administering an agent that decreases the activity and/or expression of PTN. Agents which may be used to modulate the activity of PTN include antibodies, pheromones, proteins, peptides, small molecules and inhibitory RNA molecules, *e.g.*, siRNA molecules, shRNA, ribozymes, and antisense oligonucleotides specific for PTN.

In some embodiments, the agent is an antibody (*e.g.*, monoclonal antibodies, polyclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, multispecific antibodies, single-chain antibodies and antigen-binding antibody fragments).

In some embodiments, any agent that inhibits the activity and/or expression of PTN can be used to practice the methods provided herein. Such agents can be those described herein, those known in the art, or those identified through routine screening assays.

Polypeptides

In certain embodiments, provided herein are isolated polypeptides capable of inhibiting the activity of PTN. The isolated polypeptides may be a binding partner of PTN, or a fragment thereof. Such polypeptides can be useful, for example, for inhibiting the activity of PTN and for identifying and/or generating agents that specifically bind to PTN and modulate its activity.

In some embodiments, the polypeptide described herein is able to bind to PTN and inhibit its activity. In some embodiments, the binding of the polypeptide to PTN alters the pathogenesis of hematologic cancers, such as chronic myelogenous leukemia. In some embodiments, the binding of the polypeptide to PTN alters the pathogenesis of splenomegaly. In some embodiments, the binding of the polypeptide to PTN decreases the WBCs in an individual with an elevated WBCs as a result of pathology. In some embodiments, the polypeptides can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In other embodiments, polypeptides are produced by recombinant DNA techniques. Alternatively, polypeptides can be chemically synthesized using standard peptide synthesis techniques.

In some embodiments, the test agent is a chimeric or fusion polypeptide. A fusion or chimeric polypeptide can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety.

The polypeptides described herein can be produced in prokaryotic or eukaryotic host cells by expression of polynucleotides encoding a polypeptide(s). Alternatively, such peptides can be synthesized by chemical methods. Methods for expression of heterologous polypeptides in

recombinant hosts, chemical synthesis of polypeptides, and *in vitro* translation are well known in the art and are described further in Maniatis et al., Molecular Cloning: A Laboratory Manual (1989), 2nd Ed., Cold Spring Harbor, N. Y.; Berger and Kimmel, Methods in Enzymology, Volume 152, Guide to Molecular Cloning Techniques (1987), Academic Press, Inc., San Diego, Calif.; Merrifield, J. (1969) J. Am. Chem. Soc. 91:501; Chaiken I. M. (1981) CRC Crit. Rev. Biochem. 11:255; Kaiser et al. (1989) Science 243:187; Merrifield, B. (1986) Science 232:342; Kent, S. B. H. (1988) Annu. Rev. Biochem. 57:957; and Offord, R. E. (1980) Semisynthetic Proteins, Wiley Publishing, which are incorporated herein by reference.

Inhibitory RNA Molecules

In some embodiments, provided herein are inhibitory RNA molecules for inhibiting PTN expression. In some embodiments, the inhibitory RNA molecules may be contacted with a cell or administered to an organism. Alternatively, constructs encoding these may be contacted with or introduced into a cell or organism. Antisense constructs, antisense oligonucleotides, RNA interference constructs or siRNA duplex RNA molecules can be used to interfere with expression or activity of a molecule of interest *e.g.,* a PTN molecule. Typically at least 15, 17, 19, or 21 nucleotides of the complement of PTN mRNA sequence are sufficient for an antisense molecule. Typically at least 19, 21, 22, or 23 nucleotides of a target sequence are sufficient for an RNA interference molecule. The RNA interference molecule may have a 2 nucleotide 3' overhang. If the RNA interference molecule is expressed in a cell from a construct, for example from a hairpin molecule or from an inverted repeat of the desired PTN sequence, then the endogenous cellular machinery will create the overhangs. Inhibitory RNA molecules can be prepared by chemical synthesis, in vitro transcription, or digestion of long dsRNA by Rnase III or Dicer. These can be introduced into cells by transfection, electroporation, or other methods known in the art. See Hannon, GJ, 2002, RNA Interference, Nature 418: 244-251; Bernstein E et al., 2002, The rest is silence. RNA 7: 1509-1521; Hutvagner G et al., RNAi: Nature abhors a double-strand. Curr. Opin. Genetics & Development 12: 225-232; Brummelkamp, 2002, A system for stable expression of short interfering RNAs in mammalian cells. Science 296: 550-553; Lee NS, Dohjima T, Bauer G, Li H, Li M-J, Ehsani A, Salvaterra P, and Rossi J. (2002). Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nature Biotechnol. 20:500-505; Miyagishi M, and Taira K. (2002). U6-promoter-driven siRNAs with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells.

Nature Biotechnol. 20:497-500; Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, and Conklin DS. (2002). Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. Genes & Dev. 16:948-958; Paul CP, Good PD, Winer I, and Engelke DR. (2002). Effective expression of small interfering RNA in human cells. Nature Biotechnol. 20:505-508; Sui G, Soohoo C, Affar E-B, Gay F, Shi Y, Forrester WC, and Shi Y. (2002). A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. Proc. Natl. Acad. Sci. USA 99(6):5515-5520; Yu J-Y, DeRuiter SL, and Turner DL. (2002). RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. Proc. Natl. Acad. Sci. USA 99(9):6047-6052.

Antisense or RNA interference molecules can be delivered in vitro to cells or in vivo, *e.g.*, injected into tissues of a mammal. Typical delivery means known in the art can be used. For example, an interfering RNA can be delivered systemically using, for example, the methods and compositions described in PCT Application No: PCT/US09/036223, PCT/US09/061381 PCT/US09/063927, PCT/US09/063931 and PCT/US09/063933, each of which is hereby incorporated by reference in its entirety. In certain embodiments the siRNA is delivered locally. For example, when the siRNA described herein is used to treat splenomegaly, delivery to the spleen can be accomplished by injections. When the interfering RNA described herein is used to treat chronic myelogenous leukemia, the interfering RNA can be delivered intravenously or parenterally.

Interfering nucleic acid molecules provided herein can contain RNA bases, non-RNA bases or a mixture of RNA bases and non-RNA bases. For example, interfering nucleic acid molecules provided herein can be primarily composed of RNA bases but also contain DNA bases or non-naturally occurring nucleotides.

The interfering nucleic acids can employ a variety of oligonucleotide chemistries. Examples of oligonucleotide chemistries include, without limitation, peptide nucleic acid (PNA), linked nucleic acid (LNA), phosphorothioate, 2'O-Me-modified oligonucleotides, and morpholino chemistries, including combinations of any of the foregoing. In general, PNA and LNA chemistries can utilize shorter targeting sequences because of their relatively high target binding strength relative to 2'O-Me oligonucleotides. Phosphorothioate and 2'O-Me-modified chemistries are often combined to generate 2'O-Me-modified oligonucleotides having a

phosphorothioate backbone (*See, e.g.*, PCT Publication Nos. WO/2013/112053; U.S Patent No. 8,609,065, incorporated by reference).

Peptide nucleic acids (PNAs) are analogs of DNA in which the backbone is structurally homomorphous with a deoxyribose backbone, consisting of N-(2-aminoethyl) glycine units to which pyrimidine or purine bases are attached. PNAs containing natural pyrimidine and purine bases hybridize to complementary oligonucleotides obeying Watson-Crick base-pairing rules, and mimic DNA in terms of base pair recognition (Egholm, Buchardt et al., Nature, 365:566-68 (1993)). The backbone of PNAs is formed by peptide bonds rather than phosphodiester bonds, making them well-suited for antisense applications (see structure below). The backbone is uncharged, resulting in PNA/DNA or PNA/RNA duplexes that exhibit greater than normal thermal stability. PNAs are not recognized by nucleases or proteases. One type of PNA is an antisense oligonucleotide (ASO), in which 15 to 20 chemically modified deoxynucleotides or ribonucleotides form a polymer that has sequence complementarity to an mRNA sequence of interest.

Despite a radical structural change to the natural structure, PNAs are capable of sequence-specific binding in a helix form to DNA or RNA. Characteristics of PNAs include a high binding affinity to complementary DNA or RNA, a destabilizing effect caused by single-base mismatch, resistance to nucleases and proteases, hybridization with DNA or RNA independent of salt concentration and triplex formation with homopurine DNA. Panagene has developed proprietary benzothiazole-2-sulfonyl-PNA monomers (Bts PNA) and proprietary oligomerization processes. The PNA oligomerization using Bts PNA monomers is composed of repetitive cycles of deprotection, coupling and capping. PNAs can be produced synthetically using any technique known in the art (*See, e.g.*, U.S. Pat. Nos. 6,969,766, 7,211,668, 7,022,851, 7,125,994, 7,145,006 and 7,179,896, which are incorporated by reference. See also U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262 for the preparation of PNAs, which are incorporated by reference). Further teaching of PNA compounds can be found in Nielsen et al., Science, 254:1497-1500 (1991).

Interfering nucleic acids may also contain "locked nucleic acid" subunits (LNAs). "LNAs" are a member of a class of modifications called bridged nucleic acid (BNA). BNA is characterized by a covalent linkage that locks the conformation of the ribose ring in a C30-endo

(northern) sugar pucker. For LNA, the bridge is composed of a methylene between the 2'-O and the 4'-C positions. LNA enhances backbone preorganization and base stacking to increase hybridization and thermal stability.

The structures of LNAs can be found, for example, in Chemical Communications, 455-56 (1998); Tetrahedron, 54:3607 (1998); Accounts Chemical Research, 32:301(1999); Tetrahedron Letters, 38:8735-38 (1997); Tetrahedron Letters, 39:5401-04 (1998); and Bioorganic Medicinal Chemistry, 16:9230-37 (2008).

Compounds provided herein may incorporate one or more LNAs; in some cases, the compounds may be entirely composed of LNAs. Methods for the synthesis of individual LNA nucleoside subunits and their incorporation into oligonucleotides are described, for example, in U.S. Pat. Nos. 7,572,582, 7,569,575, 7,084,125, 7,060,809, 7,053,207, 7,034,133, 6,794,499, and 6,670,461, each of which is incorporated by reference. Typical intersubunit linkers include phosphodiester and phosphorothioate moieties; alternatively, non-phosphorous containing linkers may be employed. One embodiment is an LNA containing compound where each LNA subunit is separated by a DNA subunit. Certain compounds are composed of alternating LNA and DNA subunits where the intersubunit linker is phosphorothioate.

"Phosphorothioates" (or S-oligos) are a variant of normal DNA in which one of the nonbridging oxygens is replaced by a sulfur. The sulfurization of the internucleotide bond reduces the action of endo-and exonucleases including 5' to 3' and 3' to 5' DNA POL 1 exonuclease, nucleases S1 and P1, RNases, serum nucleases and snake venom phosphodiesterase. Phosphorothioates are made by two principal routes: by the action of a solution of elemental sulfur in carbon disulfide on a hydrogen phosphonate, or by the method of sulfurizing phosphite triesters with either tetraethylthiuram disulfide (TETD) or 3H-1,2-bensodithiol-3-one 1,1-dioxide (BDTD) (*See, e.g.*, Iyer et al., J. Organic Chemistry 55:4693-4699 (1990)). The latter methods avoid the problem of elemental sulfur's insolubility in most organic solvents and the toxicity of carbon disulfide. The TETD and BDTD methods also yield higher purity phosphorothioates.

"2'O-Me oligonucleotides" molecules carry a methyl group at the 2'-OH residue of the ribose molecule. 2'-O-Me-RNAs show the same (or similar) behavior as DNA, but are protected against nuclease degradation. 2'-O-Me-RNAs can also be combined with phosphorothioate oligonucleotides (PTOs) for further stabilization. 2'O-Me oligonucleotides (phosphodiester or

phosphorothioate) can be synthesized according to routine techniques in the art (*See, e.g.*, Yoo et al., Nucleic Acids Research 32:2008-16 (2004)).

The interfering nucleic acids described herein may be contacted with a cell or administered to an organism (*e.g.*, a human). Alternatively, constructs and/or vectors encoding the interfering RNA molecules may be contacted with or introduced into a cell or organism. In certain embodiments, a viral vector is used. The viral vector may be an adenovirus vector; an adeno-associated virus vector; a pox virus vector, such as a fowlpox virus vector; an alpha virus vector; a bacloviral vector; a herpes virus vector; a retrovirus vector, such as a lentivirus vector; a Modified Vaccinia virus Ankara vector; a Ross River virus vector; a Sindbis virus vector; a Semliki Forest virus vector; and a Venezuelan Equine Encephalitis virus vector. In some embodiments, the vector has a tropism for hematopoietic cells. In some embodiments the vector is a lentiviral vector.

Typically at least 17, 18, 19, 20, 21, 22 or 23 nucleotides of the complement of the target mRNA sequence are sufficient to mediate inhibition of a target transcript. Perfect complementarity is not necessary. In some embodiments, the interfering nucleic acids contain a 1, 2 or 3 nucleotide mismatch with the target sequence. The interfering nucleic acid molecule may have a 2 nucleotide 3' overhang. If the interfering nucleic acid molecule is expressed in a cell from a construct, for example from a hairpin molecule or from an inverted repeat of the desired sequence, then the endogenous cellular machinery will create the overhangs. shRNA molecules can contain hairpins derived from microRNA molecules. For example, an RNAi vector can be constructed by cloning the interfering RNA sequence into a pCAG-miR30 construct containing the hairpin from the miR30 miRNA. RNA interference molecules may include DNA residues, as well as RNA residues.

In some embodiments, the interfering nucleic acid molecule is a siRNA molecule. Such siRNA molecules should include a region of sufficient homology to the target region, and be of sufficient length in terms of nucleotides, such that the siRNA molecule down-regulate target RNA. The term "ribonucleotide" or "nucleotide" can, in the case of a modified RNA or nucleotide surrogate, also refer to a modified nucleotide, or surrogate replacement moiety at one or more positions. It is not necessary that there be perfect complementarity between the siRNA molecule and the target, but the correspondence must be sufficient to enable the siRNA molecule to direct sequence-specific silencing, such as by RNAi cleavage of the target RNA. In some

embodiments, the sense strand need only be sufficiently complementary with the antisense strand to maintain the overall double-strand character of the molecule.

In addition, an siRNA molecule may be modified or include nucleoside surrogates. Single stranded regions of an siRNA molecule may be modified or include nucleoside surrogates, *e.g.*, the unpaired region or regions of a hairpin structure, *e.g.*, a region which links two complementary regions, can have modifications or nucleoside surrogates. Modification to stabilize one or more 3′- or 5′-terminus of an siRNA molecule, *e.g.*, against exonucleases, or to favor the antisense siRNA agent to enter into RISC are also useful. Modifications can include C3 (or C6, C7, C12) amino linkers, thiol linkers, carboxyl linkers, non-nucleotidic spacers (C3, C6, C9, C12, abasic, triethylene glycol, hexaethylene glycol), special biotin or fluorescein reagents that come as phosphoramidites and that have another DMT-protected hydroxyl group, allowing multiple couplings during RNA synthesis.

Each strand of an siRNA molecule can be equal to or less than 35, 30, 25, 24, 23, 22, 21, or 20 nucleotides in length. In some embodiments, the strand is at least 19 nucleotides in length. For example, each strand can be between 21 and 25 nucleotides in length. In some embodiments, siRNA agents have a duplex region of 17, 18, 19, 29, 21, 22, 23, 24, or 25 nucleotide pairs, and one or more overhangs, such as one or two 3′ overhangs, of 2-3 nucleotides.

A "small hairpin RNA" or "short hairpin RNA" or "shRNA" includes a short RNA sequence that makes a tight hairpin turn that can be used to silence gene expression via RNA interference. The shRNAs provided herein may be chemically synthesized or transcribed from a transcriptional cassette in a DNA plasmid. The shRNA hairpin structure is cleaved by the cellular machinery into siRNA, which is then bound to the RNA-induced silencing complex (RISC).

In some embodiments, shRNAs are about 15-60, 15-50, or 15-40 (duplex) nucleotides in length, about 15-30, 15-25, or 19-25 (duplex) nucleotides in length, or are about 20-24, 21-22, or 21-23 (duplex) nucleotides in length (*e.g.*, each complementary sequence of the double-stranded shRNA is 15-60, 15-50, 15-40, 15-30, 15-25, or 19-25 nucleotides in length, or about 20-24, 21-22, or 21-23 nucleotides in length, and the double-stranded shRNA is about 15-60, 15-50, 15-40, 15-30, 15-25, or 19-25 base pairs in length, or about 18-22, 19-20, or 19-21 base pairs in length). shRNA duplexes may comprise 3' overhangs of about 1 to about 4 nucleotides or about 2 to about 3 nucleotides on the antisense strand and/or 5'-phosphate termini on the sense strand. In

some embodiments, the shRNA comprises a sense strand and/or antisense strand sequence of from about 15 to about 60 nucleotides in length (*e.g.*, about 15-60, 15-55, 15-50, 15-45, 15-40, 15-35, 15-30, or 15-25 nucleotides in length),or from about 19 to about 40 nucleotides in length (*e.g.*, about 19-40, 19-35, 19-30, or 19-25 nucleotides in length), or from about 19 to about 23 nucleotides in length (*e.g.*, 19, 20, 21, 22, or 23 nucleotides in length).

Non-limiting examples of shRNA include a double-stranded polynucleotide molecule assembled from a single-stranded molecule, where the sense and antisense regions are linked by a nucleic acid-based or non-nucleic acid-based linker; and a double-stranded polynucleotide molecule with a hairpin secondary structure having self-complementary sense and antisense regions. In some embodiments, the sense and antisense strands of the shRNA are linked by a loop structure comprising from about 1 to about 25 nucleotides, from about 2 to about 20 nucleotides, from about 4 to about 15 nucleotides, from about 5 to about 12 nucleotides, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more nucleotides.

Additional embodiments related to the shRNAs, as well as methods of designing and synthesizing such shRNAs, are described in U.S. patent application publication number 2011/0071208, which is herein incorporated by reference.

Suitable methods for making shRNAs are described in the examples, and those with ordinary skill in the art will recognize that many other nucleotide sequences may be designed to inhibit the PTN pathway.

In some embodiments, provided herein are micro RNAs (miRNAs). miRNAs represent a large group of small RNAs produced naturally in organisms, some of which regulate the expression of target genes. miRNAs are formed from an approximately 70 nucleotide single-stranded hairpin precursor transcript by Dicer. miRNAs are not translated into proteins, but instead bind to specific messenger RNAs, thereby blocking translation. In some instances, miRNAs base-pair imprecisely with their targets to inhibit translation.

In some embodiments, antisense oligonucleotide compounds are provided herein. In certain embodiments, the degree of complementarity between the target sequence and antisense targeting sequence is sufficient to form a stable duplex. The region of complementarity of the antisense oligonucleotides with the target RNA sequence may be as short as 8-11 bases, but can be 12-15 bases or more, *e.g.*, 10-40 bases, 12-30 bases, 12-25 bases, 15-25 bases, 12-20 bases, or

15-20 bases, including all integers in between these ranges. An antisense oligonucleotide of about 14-15 bases is generally long enough to have a unique complementary sequence.

In certain embodiments, antisense oligonucleotides may be 100% complementary to the target sequence, or may include mismatches, *e.g.*, to improve selective targeting of allele containing the disease-associated mutation, as long as a heteroduplex formed between the oligonucleotide and target sequence is sufficiently stable to withstand the action of cellular nucleases and other modes of degradation which may occur *in vivo*. Hence, certain oligonucleotides may have about or at least about 70% sequence complementarity, *e.g.*, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence complementarity, between the oligonucleotide and the target sequence. Oligonucleotide backbones that are less susceptible to cleavage by nucleases are discussed herein. Mismatches, if present, are typically less destabilizing toward the end regions of the hybrid duplex than in the middle. The number of mismatches allowed will depend on the length of the oligonucleotide, the percentage of G:C base pairs in the duplex, and the position of the mismatch(es) in the duplex, according to well understood principles of duplex stability.

Interfering nucleic acid molecules can be prepared, for example, by chemical synthesis, in vitro transcription, or digestion of long dsRNA by Rnase III or Dicer. These can be introduced into cells by transfection, electroporation, or other methods known in the art (See Hannon, Nature, 418:244-51 (2002); Bernstein et al., RNA, 7:1509-21 (2002); Hutvagner et al., Current Opinion Genetics & Development, 12:225-32 (2002); Brummelkamp, Science, 296:550-53 (2002); Lee et al., Nature Biotechnology, 20:500-05 (2002); Miyagishi & Taira, Nature Biotechnology, 20:497-00 (2002); Paddison et al., Genes & Development, 16:948-58 (2002); Paul et al., Nature Biotechnology, 20:505-08 (2002); Sui et al., Proceedings Nat'l Academy Sci. USA, 99:5515-20 (2002); Yu et al., Proceedings Nat'l Academy Sci. USA, 99:6047-52 (2002)).

In the present methods, an interfering nucleic acid molecule or an interfering nucleic acid encoding polynucleotide can be administered to the subject, for example, as naked nucleic acid, in combination with a delivery reagent, and/or as a nucleic acid comprising sequences that express an interfering nucleic acid molecule. In some embodiments the nucleic acid comprising sequences that express the interfering nucleic acid molecules are delivered within vectors, *e.g.*, plasmid, viral and bacterial vectors. Any nucleic acid delivery method known in the art can be

used in the methods described herein. Suitable delivery reagents include, but are not limited to, *e.g.,* the Mirus Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; polycations (*e.g.,* polylysine), atelocollagen, nanoplexes and liposomes. The use of atelocollagen as a delivery vehicle for nucleic acid molecules is described in Minakuchi *et al.* Nucleic Acids Research, 32:e109 (2004); Hanai *et al.* Annals N.Y. Acad. Sci., 1082:9-17 (2006); Kawata *et al.* Molecular Cancer Therapeutics, 7:2904-12 (2008). Exemplary interfering nucleic acid delivery systems are provided in U.S. Patent Nos. 8,283,461, 8,313,772, 8,501,930. 8,426,554, 8,268,798 and 8,324,366, each of which is hereby incorporated by reference.

Polynucleotide/ Nucleic Acid Molecules

Also provided herein are nucleic acid or polynucleotide molecules that encode PTN, antibodies, antigen binding fragments thereof and/or polypeptides described herein. For example, the polynucleotide may encode a PTN protein or fragment thereof, or the polynucleotide may be an inhibitory polynucleotide specific for PTN. The nucleic acids may be present, for example, in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

Nucleic acids described herein can be obtained using standard molecular biology techniques. For example, nucleic acid molecules described herein can be cloned using standard PCR techniques or chemically synthesized. For antibodies obtained from an immunoglobulin gene library (*e.g.,* using phage or yeast display techniques), nucleic acids encoding the antibody can be recovered from the library.

In certain embodiments, provided herein are vectors that contain the isolated nucleic acid molecules described herein (e.g., PTN). As used herein, the term "vector," refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.,* bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.,* non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby be replicated along with the host genome. Moreover,

certain vectors are capable of directing the expression of genes. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

In certain embodiments, the provided herein are cells that contain a nucleic acid described herein (*e.g.,* a nucleic acid encoding an antibody, antigen binding fragment thereof or polypeptide described herein). The cell can be, for example, prokaryotic, eukaryotic, mammalian, avian, murine and/or human. In certain embodiments, the nucleic acid is operably linked to a transcription control element such as a promoter. In some embodiments the cell transcribes the nucleic acid and thereby expresses an antibody, antigen binding fragment thereof or polypeptide described herein. The nucleic acid molecule can be integrated into the genome of the cell or it can be extrachromosomal.

Pharmaceutical Compositions

In some embodiments (such as the uses described above), the agents of the disclosure are formulated into pharmaceutical compositions for administration to subjects (such as human subjects) in a biologically compatible form suitable for administration *in vivo*. Accordingly, in another aspect, aspects disclosed herein provide a pharmaceutical composition comprising an agent of the disclosure in admixture with a suitable diluent or carrier. Such a composition is useful for treating the conditions described herein.

The compositions containing the agents of the disclosure can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The agents disclosed herein may be used in treating the conditions described herein, in the form of the free base, salts (preferably pharmaceutically acceptable salts), solvates, hydrates, prodrugs, isomers, or mixtures thereof. All forms are within the scope of the disclosure. Acid addition salts may be formed and provide a more convenient form for use; in practice, use of the

salt form inherently amounts to use of the base form. The acids which can be used to prepare the acid addition salts include preferably those which produce, when combined with the free base, pharmaceutically acceptable salts, that is, salts whose anions are non-toxic to the subject organism in pharmaceutical doses of the salts, so that the beneficial properties inherent in the free base are not vitiated by side effects ascribable to the anions.

Pharmaceutically acceptable salts within the scope of the disclosure include those derived from the following acids; mineral acids such as hydrochloric acid, sulfuric acid, phosphoric acid and sulfamic acid; and organic acids such as acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, quinic acid, and the like.

Pharmaceutically acceptable carriers that may be used in compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene polyoxypropylene block polymers, polyethylene glycol, and wool fat.

In accordance with the methods of the disclosure, the described agents may be administered to a patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compositions of the disclosure may be administered orally or parenterally.

Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time.

In certain embodiments, pharmaceutical compositions suitable for parenteral administration may comprise the agents of the present disclosure in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats,

solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

A composition comprising an agent of the present disclosure may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

In certain embodiments of the disclosure, compositions comprising an agent of the present disclosure can be administered orally, *e.g.*, in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and the like, each containing a predetermined amount of the agent of the present disclosure as an active ingredient.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), one or more compositions comprising the agent of the present disclosure may be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution

retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the agents of the present disclosure, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol (ethanol), isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

Suspensions, in addition to the active agents, salts and/or prodrugs thereof, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol, and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

A person skilled in the art would know how to prepare suitable formulations. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (1990 - 18th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions.

The agents of the disclosure may be administered to a subject in need thereof alone or in combination with pharmaceutically acceptable carriers, as noted above, the proportion of which is determined by the solubility and chemical nature of the agent, chosen route of administration and standard pharmaceutical practice.

The dosage of the agents and/or compositions of the disclosure can vary depending on many factors such as the pharmacodynamic properties of the agent, the mode of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the frequency of the treatment and the type of concurrent treatment, if any, and the clearance rate of the agent in the subject to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. The agents of the disclosure may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response. To calculate the human equivalent dose (HED) from a dosage used in the treatment of age-dependent cognitive impairment in rats, the formula HED (mg/kg) = rat dose (mg/kg) x 0.16 may be employed (*see* Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, December 2002, Center for Biologics Evaluation and Research). For example, using that formula, a dosage of 10 mg/kg in rats is equivalent to 1.6 mg/kg in humans. This conversion is based on a more general formula HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$. Similarly, to calculate the HED from a dosage used in the treatment in mouse, the formula HED (mg/kg) = mouse dose (mg/kg) x 0.08 may be employed (*see* Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, December 2002, Center for Biologics Evaluation and Research).

Therapeutic Methods

Disclosed herein are novel therapeutic methods of treatment or prevention of hematologic cancers, such as chronic myelogenous leukemia, and/or splenomegaly.

In some embodiments, provided herein are therapeutic methods of treating hematologic cancer, comprising administering to a subject, (*e.g.,* a subject in need thereof), an effective amount of an agent that inhibits PTN expression or activity.

The agent and/or pharmaceutical compositions may be delivered by any suitable route of administration, including orally, intravenously, parenterally, or through intraosseous infusion. In certain embodiments the pharmaceutical compositions are delivered generally (*e.g.,* via oral or

parenteral administration). In certain other embodiments the agent and/or pharmaceutical compositions are delivered locally through injection.

The therapeutic described herein may be administered through conjunctive therapy. Conjunctive therapy includes sequential, simultaneous and separate, and/or co-administration of the active compounds in such a way that the therapeutic effects of the first agent administered have not entirely disappeared when the subsequent agent is administered. In certain embodiments, the second agent may be co-formulated with the first agent or be formulated in a separate pharmaceutical composition. In some embodiments, the second agent is a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is cladribine fludarabine, topotecan, etoposide, 6-thioguanine, hydroxyurea, methotrexate 6-mercaptopurine, azacitidine, decitabine, doxycycline, tetracycline, and corticosteroids.

In some embodiments, the second agent is an immune checkpoint inhibitor. Immune Checkpoint inhibition broadly refers to inhibiting the checkpoints that cancer cells can produce to prevent or downregulate an immune response. Examples of immune checkpoint proteins include, but are not limited to, CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA. Immune checkpoint inhibitors can be antibodies or antigen binding fragments thereof that bind to and inhibit an immune checkpoint protein. Examples of immune checkpoint inhibitors include, but are not limited to, nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

In certain embodiments, provided herein are therapeutic methods of treating chronic myelogenous leukemia, splenomegaly, or an elevated white blood cell count (*e.g.,* as a result of a pathological condition) that include administering to a subject (*e.g.,* a subject in need thereof), an effective amount of an agent described herein. In certain embodiments, provided herein are therapeutic methods of hematologic cancer (*e.g.,* chronic myelogenous leukemia), splenomegaly, or an elevated white blood cell count (*e.g.,* as a result of a pathological condition) that include administering to a subject (*e.g.,* a subject in need thereof), an effective amount of an agent described herein. A subject in need thereof may include, for example, a subject who has been diagnosed with chronic myelogenous leukemia, splenomegaly, or an elevated white blood cell count, a subject predisposed to chronic myelogenous leukemia, splenomegaly, or an elevated white blood cell count or a subject who has been treated for chronic myelogenous leukemia,

splenomegaly, or an elevated white blood cell count, including subjects that have been refractory to the previous treatment.

In certain aspects, provided herein are methods of decreasing the number of leukemia stem cells in bone marrow, comprising contacting bone marrow with an agent that inhibits the expression or activity of PTN. In some embodiments, the bone marrow is implanted into a subject with individual with chronic myelogenous leukemia. In some embodiments, the bone marrow is bone marrow from an individual with chronic myelogenous leukemia.

In certain embodiments, provided herein are therapeutic methods of treating chronic myelogenous leukemia, splenomegaly, or an elevated white blood cell count comprising administering to a subject, (*e.g.,* a subject in need thereof), an effective amount of an agent described herein.

Actual dosage levels of the active ingredients or agents in the pharmaceutical compositions may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular agent employed, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could prescribe and/or administer doses of the compounds employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

This disclosure will be better understood from the Exemplification which follows. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the disclosure as described more fully in the embodiments which follow thereafter.

EXEMPLIFICATION

Example 1: PTN deletion inhibits CML progression in vivo.

Pleiotrophin (PTN), a 17 kD heparin binding growth factor, is secreted by bone marrow endothelial cells (BM ECs) and regulates the growth of normal hematopoietic stem cells (HSCs) in vivo. In order to test whether PTN regulates the growth of hematopoietic malignancies, such as chronic myeloid leukemia (CML), an SCL-tTA/BCR-ABL inducible transgenic mouse model of CML and crossed these mice with $PTN^{-/-}$ mice (PTN-deficient) and $PTN^{+/+}$ mice, white blood cell count, and peripheral blood neutrophils concentrations were measured. BCR-ABL;$PTN^{+/+}$ mice developed worsening leukocytosis and progressive myeloid predominance in the peripheral blood after withdrawal of doxycycline **(Figure 1, Sections A and B)**. In contrast, BCR-ABL;PTN-/- mice maintained normal range WBCs and neutrophil counts, comparable to normal control mice **(Figure 1, Sections A and B)**. Mice that are deficient in PTN would have delayed or decreased progression of CML in the BCR-ABL transgenic model. In this tetracycline-off (TET-OFF) inducible model, the BCR-ABL fusion protein is expressed under the control of the 3′ enhancer of the murine stem cell leukemia (SCL) gene. The SCL gene is hematopoietic specific; therefore, expression of BCR-ABL is contained to the hematopoietic compartment. Myeloid leukemia is spontaneously induced in these mice by cessation of tetracycline or doxycycline treatment.

Example 2: PTN deletion inhibits CML pathogenesis in vivo.

BCR-ABL;PTN+/+ mice also displayed progressive fibrosis in the BM and marked splenomegaly at 6 months of age, whereas BCR-ABL;PTN-/- mice displayed normal BM cellularity and maintenance of normal spleen sizes at the same time point. Representative femur sections from BCR-ABL;PTN+/+ mice and BCR-ABL;PTN-/- mice are shown in **Figure 2, Section A.** Representative spleens are shown in **Figure 2, Section B.** Histologic analysis of enlarged spleens in BCR-ABL;PTN+/+ mice revealed effacement with pathologic $Mac1^{+}Gr1^{+}$ myeloid cells **(Figure 2, Section C).** Taken together, these results suggested that PTN was necessary for progression of CML in this model and that PTN deficiency markedly inhibited CML pathogenesis.

Example 3: PTN deletion in murine CML model reduces LSC content

CML in mice is characterized by the expansion of pathologic ckit+sca-1+lin- (KSL) leukemia stem cells (LSC) in the spleen, which are capable of perpetuating disease upon transplantation into secondary recipient mice. In BCR-ABL;PTN+/+ mice, we observed a large increase in splenic LSCs compared to BCR-ABL;PTN-/- mice **(Figure 3).** While in vivo transplantation studies will be necessary to confirm the effect of PTN deletion on functional LSC content, these data suggest PTN deletion inhibits the accumulation of LSCs in CML.

Example 4: PTN deletion significantly increases survival in CML mice

To determine if deletion of PTN impacted survival in the presence of CML, the survival of BCR-ABL;PTN+/+ and BCR-ABL;PTN-/- mice were compared. BCR-ABL;PTN-/- mice displayed markedly improved survival compared to BCR-ABL;PTN+/+ mice **(Figure 4)**. Survival was followed in a group of 44 PTN+/+BCR-ABL+ mice over one year. After a year, only two of the PTN+/+ BCR-ABL+ mice remained alive (4.5%), with a median survival of 118 days. Conversely, 8/33 (24%) mice in the PTN-/-BCR-ABL+ group survived at least 1 year past induction of BCR-ABL expression. Median survival in the PTN-/-BCR-ABL+ group was 236 days. Log-rank analysis demonstrated a highly significant difference in the survival of the 2 groups ($p<0.0001$). These data suggest that PTN is necessary for the lethal pathogenesis of CML.

Example 5: PTN expression is increased in CML spleen cells.

In order to test whether PTN mediated growth of CML cells is BM niche-dependent or supports CML growth in a cell-autonomous manner, PTN concentration was measured in the spleens of diseased mice **(Figure 5, Section A)**. This data confirmed that PTN protein levels are unregulated in CML disease. Isolated KSL cells from spleens in CML mice showed PTN expression was significantly increased in CML cells, exclusive of any contribution from the microenvironment **(Figure 5, Section B).** PTN mRNA was 100 fold greater in the KSL fraction of splenic cells from BCR-ABL+ mice compared to the whole spleen. This marked increase in PTN expression in CML cells suggests that CML cells contribute autonomously to PTN-dependent CML growth in vivo.

Example 6: Anti-PTN treatment reduces human CML colony growth

In order to test whether PTN regulates human CML growth, colony forming cell assays of human CML cells were treated with and without a neutralizing anti-PTN antibody (50 μg). Treatment with anti-PTN significantly inhibited human CML colony formation in vitro **(Figure 6)**. Anti-PTN antibody treatment had little inhibitory effect on normal human BM $CD34^+$ cells. These results suggest that PTN is secreted by CML cells and promotes CML growth in an autocrine manner.

Incorporation by Reference

All publications, patents, and patent applications mentioned herein are hereby incorporated by reference in their entirety as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Equivalents

It will be understood by one of skill in the art that the compositions and methods described herein may be adapted and modified as is appropriate for the application being addressed and that the compositions and methods described herein may be employed in other suitable applications, and that such other additions and modifications will not depart from the scope hereof. The disclosure contemplates all uses of the agents and compositions of the disclosure, including their use in therapeutic methods, in diagnostic assays, and their use as research tools.

What is claimed is:

1. A method of treating or preventing hematologic cancer in a subject, comprising administering to the subject an agent that inhibits the expression or activity of pleiotrophin (PTN).

2. The method of claim 1, wherein the hematopoietic cancer is leukemia.

3. The method of claim 2, wherein the hematopoietic cancer is chronic myelogenous leukemia.

4. The method of any one of claims 1 to 3, wherein the agent is a small molecule.

5. The method of claim 4, wherein the small molecule decreases the activity of PTN.

6. The method of any one of claims 1 to 3, wherein the agent is a polypeptide.

7. The method of claim 6, wherein the polypeptide is a PTN protein or a fragment thereof.

8. The method of claims 6 or 7, wherein the polypeptide inhibits the activity of PTN.

9. The method of any one of claims 1 to 3, wherein the agent is a polynucleotide.

10. The method of claim 9, wherein the polynucleotide is an inhibitory polynucleotide specific for PTN.

11. The method of claim 10, wherein the inhibitory polynucleotide is selected from siRNA, shRNA, and an antisense RNA molecule, or is a polynucleotide that encodes a molecule selected from siRNA, shRNA, and/or an antisense RNA molecule.

12. The method of any one of claims 9 to 11, wherein the polynucleotide inhibits the expression of PTN.

13. The method of any one of claims 1 to 3, wherein the agent is an antibody.

14. The method of any one of claims 1 to 13, wherein the method further comprises conjoint therapy with a second agent for the treatment of prevention of hematopoietic cancer.

15. The method of claim 14, wherein the second agent is a chemotherapeutic agent.

16. The method of claim 15, wherein the second agent is selected from cladribine fludarabine, topotecan, etoposide, 6-thioguanine, hydroxyurea, methotrexate 6-mercaptopurine, azacitidine, decitabine, doxycycline, tetracycline, and corticosteroids.

17. The method of claim 16, wherein the second agent is a tetracycline.

18. The method of claim 14, wherein the second agent is an immune checkpoint inhibitor.

19. A method of decreasing the count of white blood cells (WBC) in a subject with an elevated white blood cell count, comprising administering to the subject an agent that inhibits the expression or activity of PTN.

20. The method of claim 19, wherein the white blood cells are neutrophils.

21. The method of claims 19 or 20, wherein the agent is a small molecule.

22. The method of claim 21, wherein the small molecule decreases the activity of PTN.

23. The method of claims 19 or 208, wherein the agent is a polypeptide.

24. The method of claim 23, wherein the polypeptide is a PTN protein or a fragment thereof.

25. The method of claims 23 or 24, wherein the polypeptide inhibits the activity of PTN.

26. The method of claims 19 or 20, wherein the agent is a polynucleotide.

27. The method of claim 26, wherein the polynucleotide is an inhibitory polynucleotide specific for PTN.

28. The method of claim 27, wherein the inhibitory polynucleotide is selected from siRNA, shRNA, and an antisense RNA molecule, or a polynucleotide that encodes a molecule selected from the group consisting of siRNA, shRNA, and/or an antisense RNA molecule.

29. The method of any one of claims 26 to 28, wherein the polynucleotide inhibits the expression of PTN.

30. The method of claims 19 or 20, wherein the agent is an antibody.

31. A method of treating or preventing splenomegaly in a subject, comprising administering to the subject an agent that inhibits the expression or activity of PTN.

32. The method of claim 31, wherein the subject has leukemia.

33. The method of claim 32, wherein the subject has chronic myelogenous leukemia.

34. The method of any one of claims 31 to 33, wherein the agent is a small molecule.

35. The method of claim 34, wherein the small molecule decreases the activity of PTN.

36. The method of any one of claims 31 to 33, wherein the agent is a polypeptide.

37. The method of claim 36, wherein the polypeptide is a PTN protein or a fragment thereof.

38. The method of claims 36 to 37, wherein the polypeptide inhibits the activity of PTN.

39. The method of any one of claims 31 to 33, wherein the agent is a polynucleotide.

40. The method of claim 39, wherein the polynucleotide is an inhibitory polynucleotide specific for PTN.

41. The method of claim 40, wherein the inhibitory polynucleotide is selected from siRNA, shRNA, and an antisense RNA molecule, or a polynucleotide that encodes a molecule selected from siRNA, shRNA, and/or an antisense RNA molecule.

42. The method of any one of claims 39 to 41, wherein the polynucleotide inhibits the expression of PTN.

43. The method of any one of claims 31 to 33, wherein the agent is an antibody.

44. A method of decreasing the number of leukemia stem cells in bone marrow, comprising contacting the bone marrow with an agent that inhibits the expression or activity of PTN.

45. The method of claim 44, wherein the bone marrow is implanted into a subject with chronic myelogenous leukemia.

46. The method of claim 44 or claim45, wherein the bone marrow is bone marrow from a subject with chronic myelogenous leukemia.

47. The method of any one of claims 44 to 46, wherein the agent is a small molecule.

48. The method of claim 47, wherein the small molecule decreases the activity of PTN.

49. The method of any one of claims 44 to 46, wherein the agent is a polypeptide.

50. The method of claim 49, wherein the polypeptide is a PTN protein or a fragment thereof.

51. The method of claim 49 or claim 50, wherein the polypeptide inhibits the activity of PTN.

52. The method of any one of claims 44 to 46, , wherein the agent is a polynucleotide.

53. The method of claim 52, wherein the polynucleotide is an inhibitory polynucleotide specific for PTN.

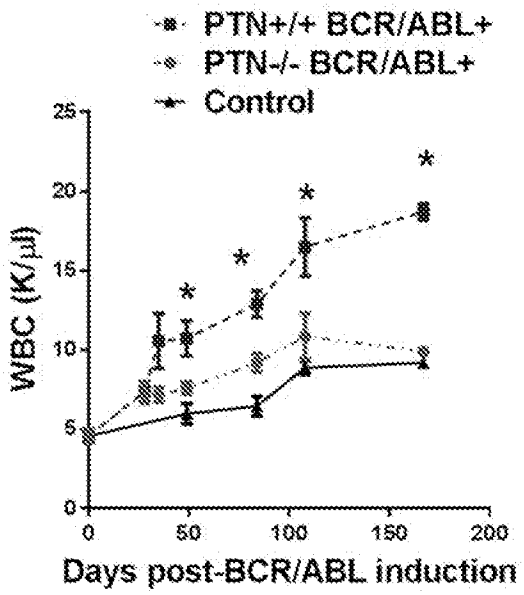
54. The method of claim 53, wherein the inhibitory polynucleotide is selected from siRNA, shRNA, and an antisense RNA molecule, or a polynucleotide that encodes a molecule selected from siRNA, shRNA, and/or an antisense RNA molecule.

55. The method of any one of claims 52 to 54, wherein the polynucleotide inhibits the expression of PTN.

56. The method of any one of claims 44 to 46, wherein the agent is an antibody.

FIGURE 1:

A


PTN+/+ BCR/ABL+
PTN-/- BCR/ABL+
Control
WBC (K/μl)
25
20
15
10
5
0
0
50
100
150
200
Days post-BCR/ABL induction
*
*
*
*

B

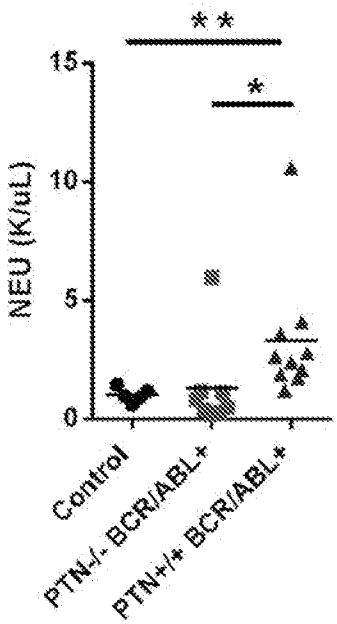

**
*
NEU (K/uL)
15
10
5
0
Control
PTN-/- BCR/ABL+
PTN+/+ BCR/ABL+

FIGURE 2:

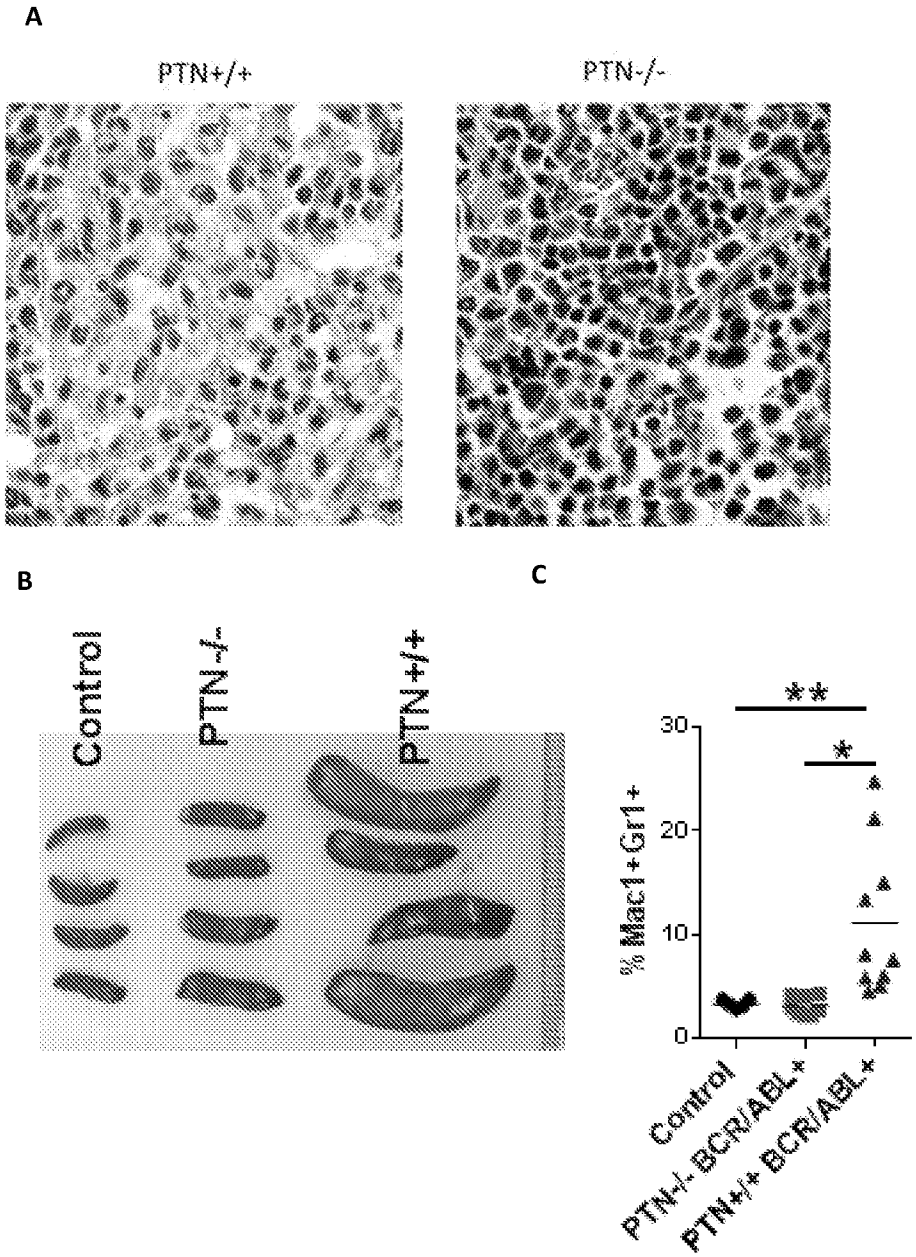

A
PTN+/+
PTN-/-
B
Control
PTN-/-
PTN+/+
C
% Mac1+Gr1+
30
20
10
0
**
*
Control
PTN-/- BCR/ABL+
PTN+/+ BCR/ABL+

FIGURE 3:

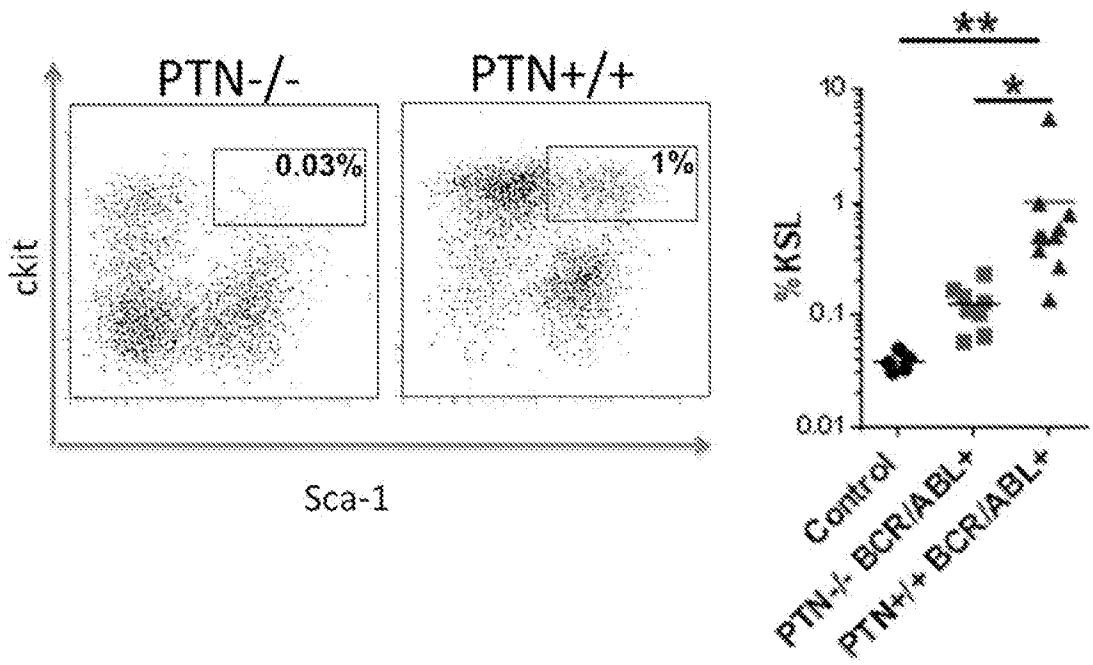

PTN-/-
PTN+/+
0.03%
1%
ckit
Sca-1
**
*
10
1
0.1
0.01
%KSL
Control
PTN-/- BCR/ABL+
PTN+/+ BCR/ABL+

FIGURE 4:

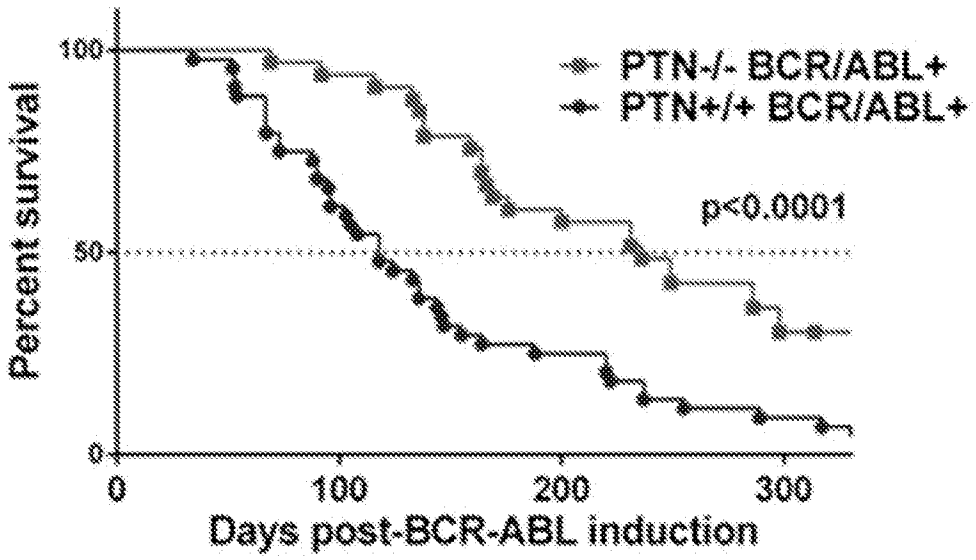
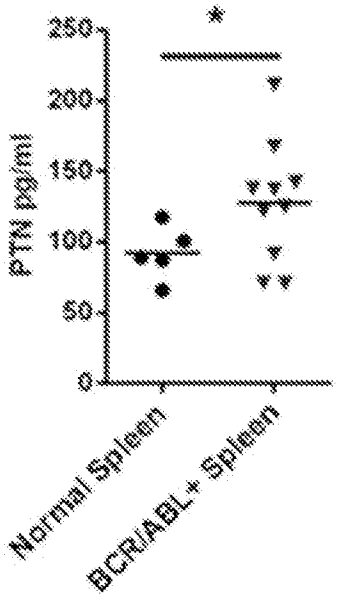

PTN-/- BCR/ABL+
PTN+/+ BCR/ABL+
p<0.0001
Percent survival
100
50
0
0
100
200
300
Days post-BCR-ABL induction

FIGURE 5:

A


PTN pg/ml
250
200
150
100
50
0
*
Normal Spleen
BCR/ABL+ Spleen

B

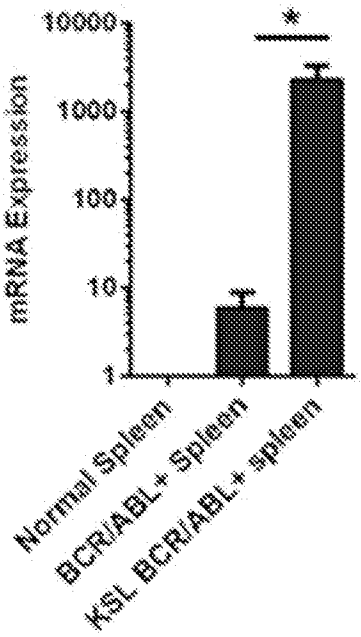
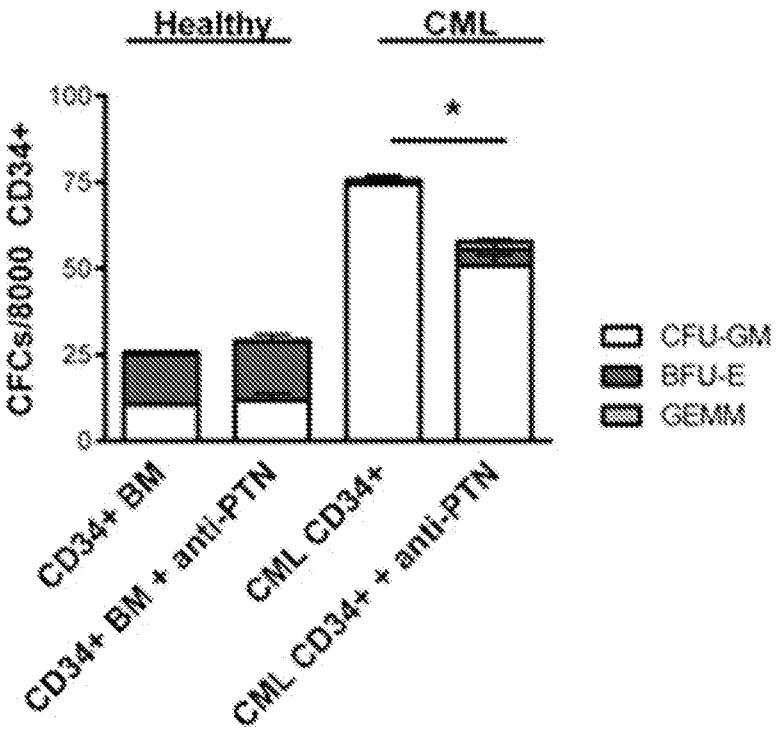

mRNA Expression
10000
1000
100
10
1
*
Normal Spleen
BCR/ABL+ Spleen
KSL BCR/ABL+ spleen

FIGURE 6:


Healthy
CML
*
CFCs/8000 CD34+
100
75
50
25
0
CFU-GM
BFU-E
GEMM
CD34+ BM
CD34+ BM + anti-PTN
CML CD34+
CML CD34+ + anti-PTN

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/019334

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2017.01) C07K 16/22, A61K 39/395, A61K 47/68, A61P 35/00, A61K 31/708800, A61K 38/02

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 (2017.01) C07K, A61K, A61P

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)

See extra sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014294845 A1 UNIV GEORGETOWN 02 Oct 2014 (2014/10/02) whole document, para. 0036, para.0056, para. 0133, Examples 1&2	1-18
X	Himburg, Heather A., Liman Zhao, and John P. Chute. "Deletion of Pleiotrophin (PTN), a Vascular Niche Paracrine Factor, Abrogates Chronic Myeloid Leukemia Pathogenesis In Vivo." Blood 126.23 (31.12.2015): 4268-4268. Retrieved from the Internet on: 23.5.17 URL: http://www.bloodjournal.org/content/126/23/4268?sso-checked=true 31 Dec 2015 (2015/12/31) whole document	1-3,13,19,20,30-33, 43
X	whole document	4-12,21-29,34-42, 44-56

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 May 2017	24 May 2017
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/US2017/019334

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007124610 A1 ESBATECH AG [CH]; AUF DER MAUR ADRIAN [CH]; LICHTLEN PETER [CH]; BARBERIS ALCIDE [CH] 08 Nov 2007 (2007/11/08) whole document, page 33 last para., Example 5	1,13
P,X	Himburg, Heather A., et al. "Deletion of Pleiotrophin (PTN) Abrogates Chronic Myeloid Leukemia Pathogenesis In Vivo." Biology of Blood and Marrow Transplantation 22.3 (March 2016): S203-S204. Retrieved from the Internet on: 23.5.17 URL: http://www.bbmt.org/article/S1083-8791(15)01353-1/pdf 31 Mar 2016 (2016/03/31) whole document	1-56

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2017/019334

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
US 2014294845 A1	02 Oct 2014	US 2014294845 A1	02 Oct 2014
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2017/019334

Patent document cited search report	Publication date	Patent family member(s)		Publication Date
		US 7902340	B2	08 Mar 2011
		US 2011159008	A1	30 Jun 2011
		US 8945563	B2	03 Feb 2015
		US 2015203829	A1	23 Jul 2015
		US 9512411	B2	06 Dec 2016

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/019334

B. FIELDS SEARCHED:

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

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

Search terms used: Pleiotrophin, PTN, hematologic cancer, leukemia, chronic myelogenous leukemia, chemotherap*, ofwhite blood cells, WBC, neutrophils, splenomegaly, ofleukemia stem cells, bone marrow