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(54) Title: METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF BETA-THALASSEMIAS

(57) Abstract: The present invention relates to methods and pharmaceutical compositions for the treatment of beta-thalassemias. In particular, the present invention relates to an XPO1 inhibitor for use in a method for treating beta-thalassemia in a subject in need thereof.



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METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF BETA-THALASSEMIAS

FIELD OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of beta-thalassemias.

BACKGROUND OF THE INVENTION:

Adult mammalian Hg is a multimeric protein that includes two α and two β globin chains which together form the $(\alpha/\beta)_2$ tetrameric hemoglobin (Hb) molecule. Beta-thalassemias are a group of inherited blood disorders caused by a quantitative defect in the synthesis of the β chains of hemoglobin. In individuals with this disorder, the synthesis of β -globin chains is reduced or absent. Three main forms of the disease have been described: β -thalassemia major (β -TM or β^0 -TM) in which no β chain is produced, and β -thalassemia intermedia and β -thalassemia minor, in which β chain is produced but in lower than normal amounts. These conditions cause variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. Individuals with β -TM usually present within the first two years of life with severe anemia, poor growth, and skeletal abnormalities during infancy. Affected children will require regular lifelong blood transfusions. β -thalassemia intermedia is less severe than β -thalassemia major and may require episodic blood transfusions. Transfusion-dependent patients will develop iron overload and require chelation therapy to remove the excess iron.

Despite extensive knowledge of the molecular defects causing β -thalassemia major (TM), less is known about the mechanisms responsible of the ineffective erythropoiesis. This latter is characterized by accelerated erythroid differentiation, apoptosis and maturation arrest at the polychromatophilic stage. It explains, at least in part, the profound anemia observed in this disease. Although it has been proposed that both the precipitation of unmatched globin chains as well as the accumulation of unbound iron could lead to oxidative stress and subsequent hemolysis, the mechanism by which apoptosis and maturation arrest are induced remains unclear.

The present inventors have elucidated the consequences of α chain cytoplasmic accumulation and the cascade of failed reactions that result therefrom which ultimately cause β -TM symptoms such as anemia. The discovery is based on the further clarification of the

roles of the chaperone protein HSP70 and the erythrocyte maturation protein GATA-1 (<https://ash.confex.com/ash/2012/webprogram/Paper48181.html>). The inventors have discovered that HSP70 has important functions in both the cytoplasm and the nucleus of erythroblasts. A primary function of HSP70 in the nucleus is binding to the GATA-1 protein and preventing its cleavage and proteolytic degradation (by the protease caspase-3). HSP70 thus prevents inactivation of GATA-1 and preserves its function as a key factor in erythrocyte maturation. A secondary function of HSP70 is binding to α globin in the cytoplasm and ensuring that the protein chains are properly folded and can form tetrameric $(\alpha/\beta)_2$ Hb. Ordinarily, there is sufficient HSP70 available in the cell to carry out both of these functions. However, in β -TM cells, the HSP70 is monopolized by the excess of free α chains which accumulate in the cytoplasm. Thus, a disproportionate amount of the HSP70 is sequestered in the cytoplasm, and there is not sufficient HSP70 available for binding and protecting GATA-1 in the nucleus. Unprotected GATA-1 is proteolytically cleaved and inactivated, and proper erythrocyte maturation does not occur. Rather, the absence of active GATA-1 results in maturation arrest and apoptosis of immature erythrocytes at the polychromatophilic stage. This sequence of events is thus initially triggered by a lack of hemoglobin β chains and ultimately results in low (or no) erythrocyte production, causing anemia.

SUMMARY OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of beta-thalassemias. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

The present invention provides methods and pharmaceutical compositions designed to intervene in this defective process and to promote or restore erythrocyte maturation in individuals suffering from β -TM. It is noted that because β globin is not formed in β -TM erythrocytes, the type of erythrocytes that are produced in individuals treated with the methods and compositions described herein contain $(\alpha/\gamma)_2$ Hb, and the invention provides methods and compositions for increasing the production of $(\alpha/\gamma)_2$ Hb erythrocytes in β -TM cells and β -TM individuals. The methods involve maintaining the activity of GATA-1 by preventing nuclear export of HSP70 in the cytoplasm. Accordingly, it is an object of this invention to provide methods of restoring or increasing erythrocyte maturation in a subject suffering from β -thalassemia major (β -TM) by preventing proteolytic inactivation of GATA-

1. In some embodiments, preventing is achieved by administering to the subject a compound that inhibits the XPO1 nuclear transporter.

5 The present invention relates to an XPO1 inhibitor for use in a method for treating beta-thalassemia in a subject in need thereof.

The present invention relates to an XPO1 inhibitor for use in a method for promoting or restoring erythrocyte maturation in a subject suffering from beta-thalassemia.

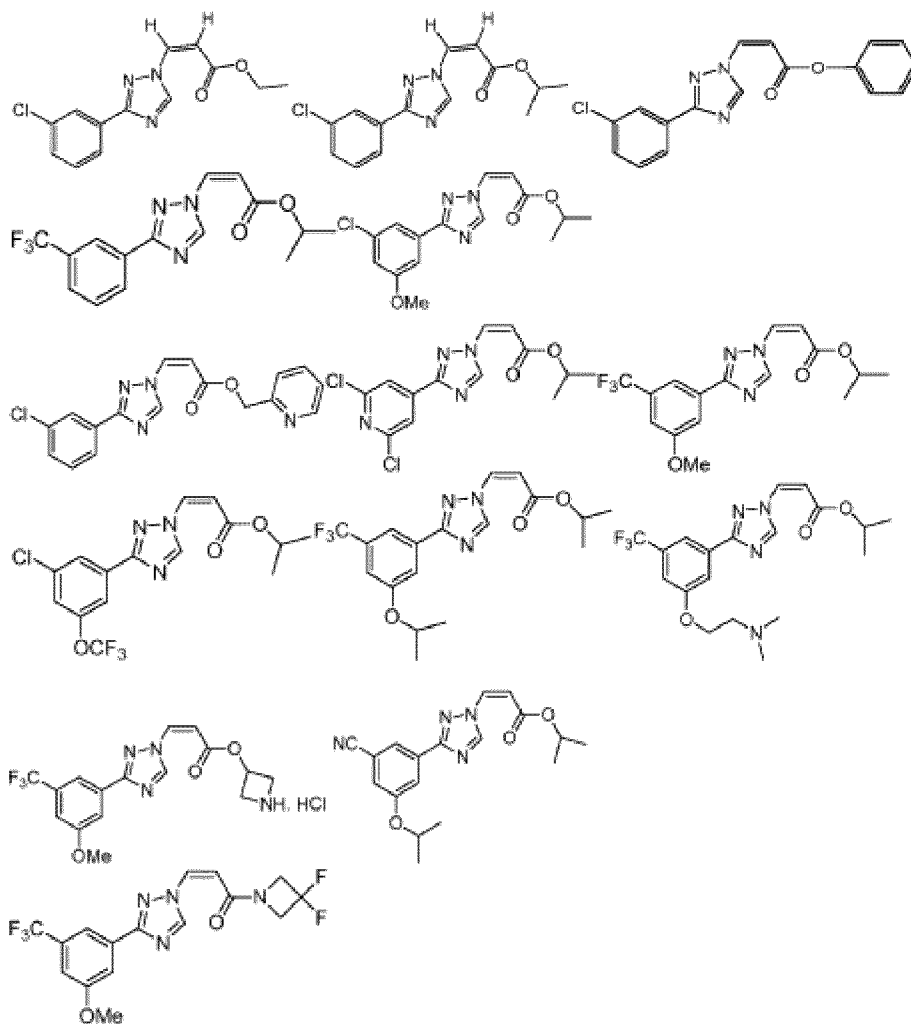
10 The present invention relates to an XPO1 inhibitor for use in a method for increasing the production of $(\alpha/\gamma)_2$ Hb erythrocytes in a subject suffering from beta-thalassemia.

15 As used herein the term "XPO1" has its general meaning in the art and refers to the exportin 1 protein. The protein mediates leucine-rich nuclear export signal (NES)-dependent protein transport. The protein is also called Chromosomal Region Maintenance 1 (Crm1).

20 As used herein the term "XPO1 inhibitor" designates any compound or treatment that reduces or blocks the activity of XPO1. The term also includes inhibitors of XPO1 expression.

XPO1 inhibitors are well known in the art. In certain embodiments of the invention the XPO1 inhibitor is selected from small molecule compounds that have been disclosed in the following publications WO2011109799, WO2012099807, WO2013020024, WO 2013019548, and WO2013019561.

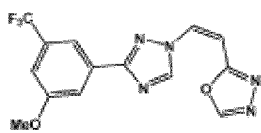
25 In some embodiments, the XPO1 inhibitor is selected from the group consisting of the following compounds:



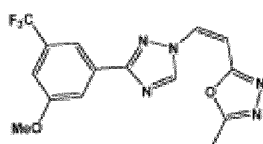
and pharmaceutically acceptable salts thereof.

- 5 In some embodiments, the XPO1 inhibitor is selected from the group consisting of (Z)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid ethyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid ethyl ester; (Z)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid isopropyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid isopropyl ester; (Z)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid tert-butyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid tert-butyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-N-phenyl-acryl amide; (E)-N-(2-Chloro-phenyl)-3-[3-(3-chloro-phenyl)-[1,2,4]-triazol-1-yl]-acryl amide; (4-{(E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acryloylamino}-phenyl)-carbamic acid tert-butyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-N-(4-methoxy-phenyl)-acryl amide; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-N-methyl-N-phenyl-acryl amide; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-N-methyl-N-phenyl-acryl amide; (E)-N-(4-Amino-phenyl)-3-[3-(3-chloro-phenyl)-[1,2,4]-triazol-1-yl]-acryl amide and/or a pharmaceutically-acceptable salt thereof.
- 10
- 15

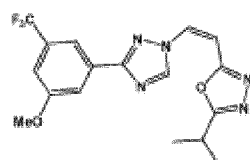
In some embodiments, the XPO1 inhibitor is selected from the group consisting of the following compounds:



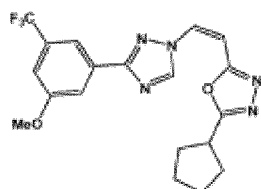
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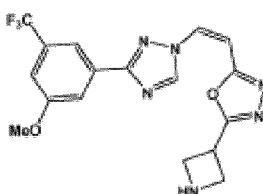
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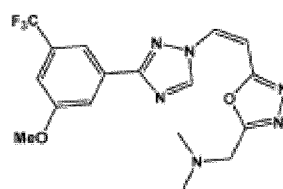
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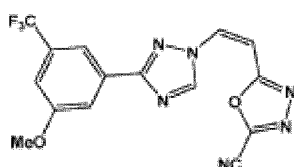
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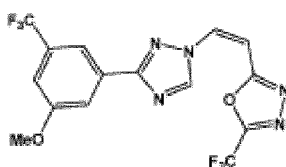
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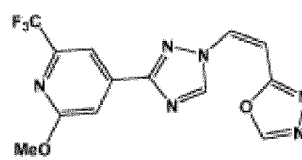
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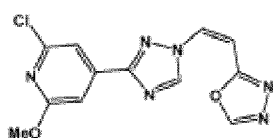
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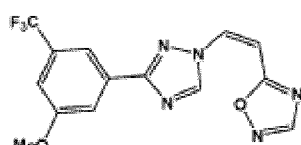
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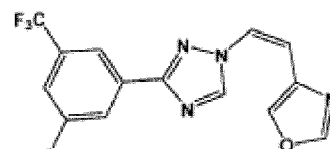
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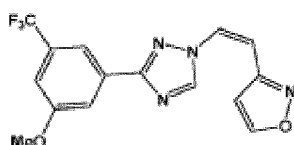
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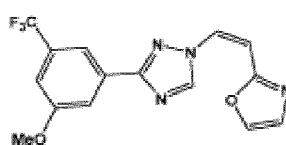
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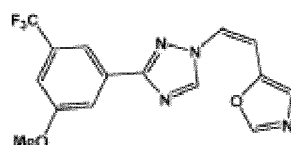
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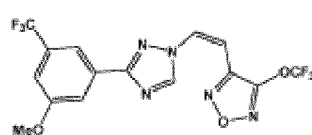
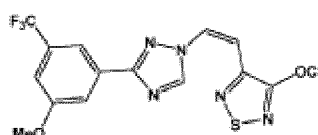
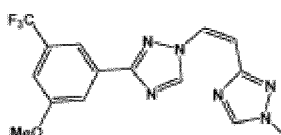
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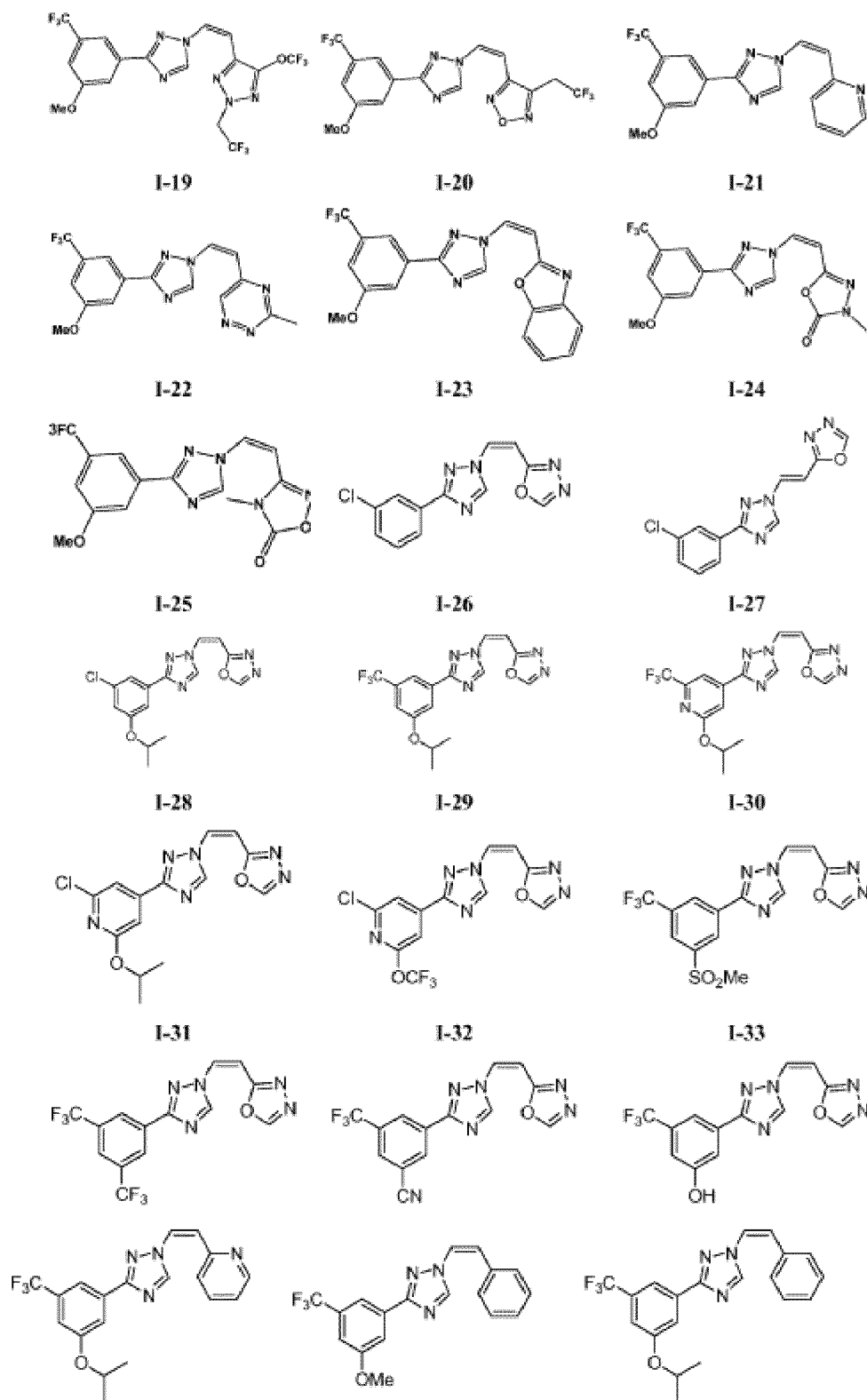


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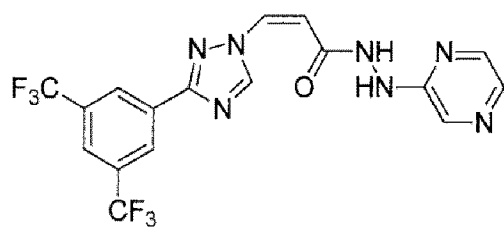


or a pharmaceutically acceptable salt thereof.

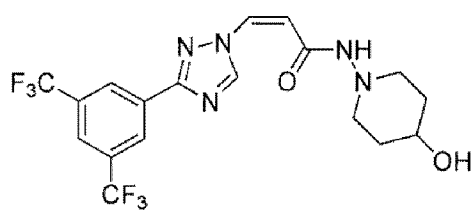
5 In some embodiments, the XPO1 inhibitor is selected from the group consisting of a compound selected from the group consisting of 1-(4-methoxyphenyl)-1H-pyrazole-2,5-dione; 1-(4-bromo-2,5-difluorophenyl)-1H-pyrazole-2,5-dione; 3-methyl-1-(1-methyl-1H-pyrazol-3-yl)-

1H-pyrrole-2,5-dione; 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide; 1-(3-benzoyl-4-methylthiophen-2-yl)-1H-pyrrole-2,5-dione; 1-(4-(3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)-1H-pyrrole-2,5-dione; 1-(4-(4-chlorophenyl)thiazol-2-yl)-3-methyl-1H-pyrrole-2,5-dione; 1-(benzo[b]thiophen-3-ylmethyl)-1H-pyrrole-2,5-dione; 1-(3,4-dimethoxyphenethyl)-1H-pyrrole-2,5-dione; 1-(naphthalen-1-yl)-1H-pyrrole-2,5-dione; 1-(4-cyclohexylphenyl)-1H-pyrrole-2,5-dione; 1-(2-benzoylphenyl)-1H-pyrrole-2,5-dione; 1-(4-morpholinophenyl)-1H-pyrrole-2,5-dione; 1-(4-chlorophenethyl)-1H-pyrrole-2,5-dione; 1-(2-(thiophen-2-yl)ethyl)-1H-pyrrole-2,5-dione; 1-([3,4]methylenedioxybenzyl)-1H-pyrrole-2,5-dione amide and/or a pharmaceutically-acceptable salt thereof.

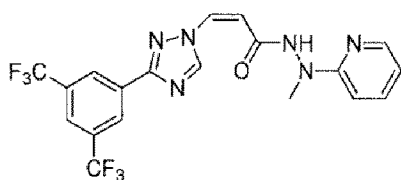
In some embodiments, the XPO1 inhibitor is selected from the group consisting of the following compounds:



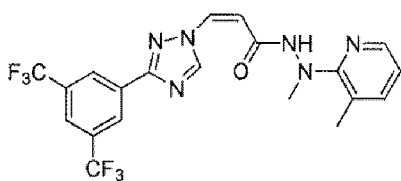
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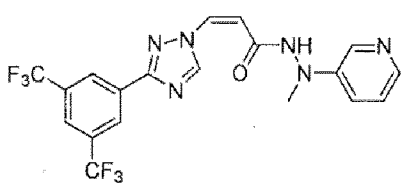
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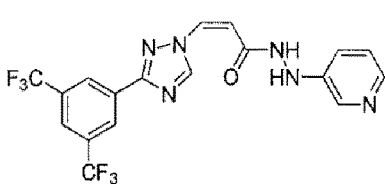
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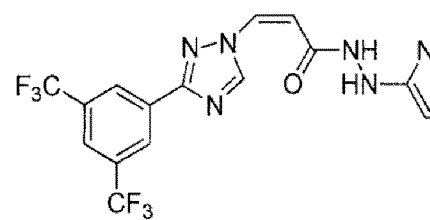
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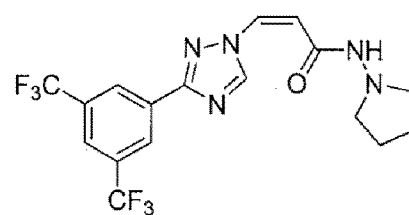
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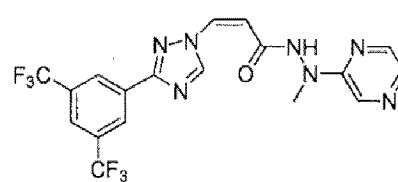
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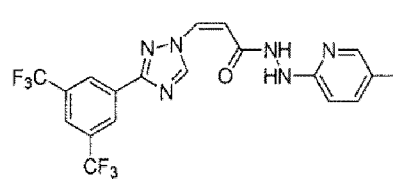
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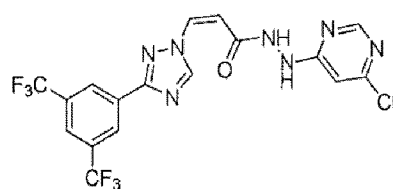
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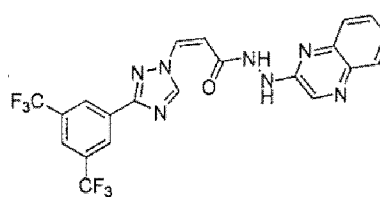
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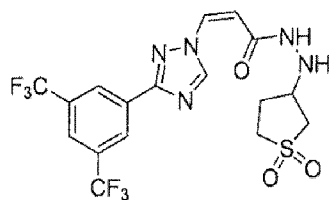
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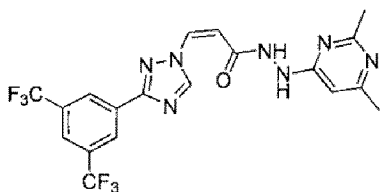
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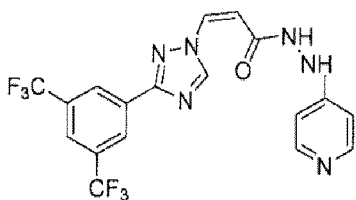
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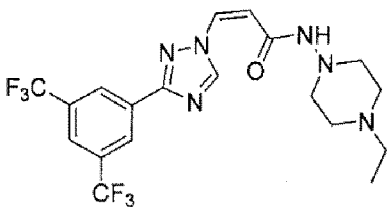
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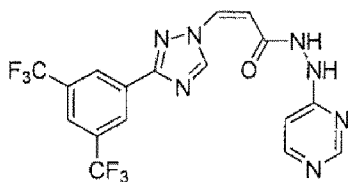
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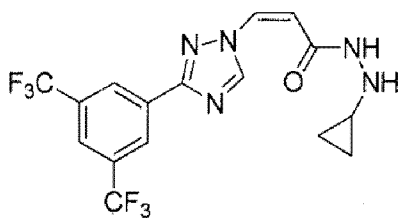
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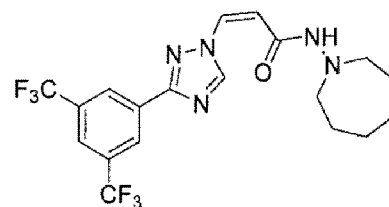


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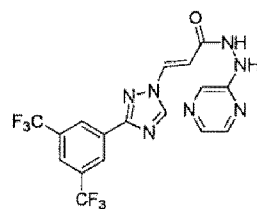


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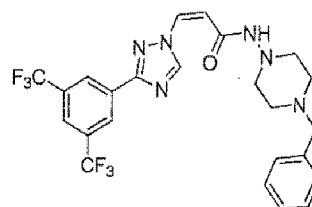
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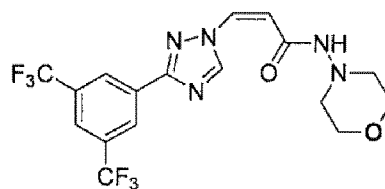
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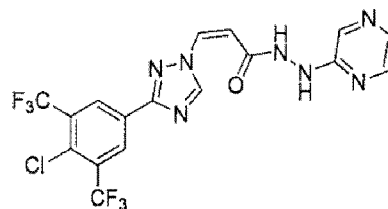
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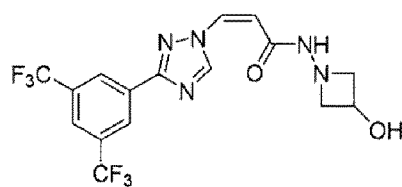
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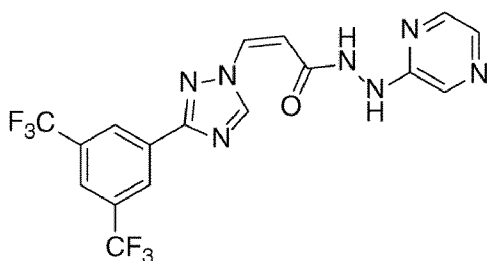


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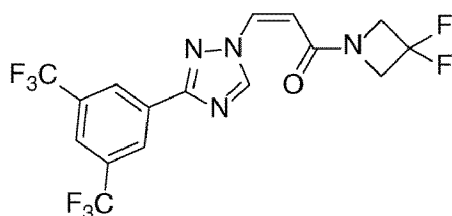


I-26.

In some embodiments, the XPO1 inhibitor is KPT-330 (Etchin J, Sanda T, Mansour MR, Kentsis A, Montero J, Le BT, Christie AL, McCauley D, Rodig SJ, Kauffman M, Shacham S, Stone R, Letai A, Kung AL, Thomas Look A. KPT-330 inhibitor of CRM1 (XPO1)-mediated nuclear export has selective anti-leukaemic activity in preclinical models of
5 T-cell acute lymphoblastic leukaemia and acute myeloid leukaemia. Br J Haematol. 2013 Apr;161(1):117-27. doi: 10.1111/bjh.12231. Epub 2013 Feb 4).

**KPT-330**

In some embodiments, the XPO1 inhibitor is KPT-276 (Schmidt J, Braggio E, Kortuem KM, Egan JB, Zhu YX, Xin CS, Tiedemann RE, Palmer SE, Garbitt VM, McCauley D, Kauffman M, Shacham S, Chesi M, Bergsagel PL, Stewart AK. Genome-wide studies in multiple myeloma identify XPO1/CRM1 as a critical target validated using the selective nuclear export inhibitor KPT-276. Leukemia. 2013 Jun 11. doi: 10.1038/leu.2013.172.).
10

**KPT-276**

15 An "inhibitor of expression" refers to a natural or synthetic compound that has a biological effect to inhibit the expression of a gene.

In a preferred embodiment of the invention, said inhibitor of gene expression is a
20 siRNA, an antisense oligonucleotide or a ribozyme.

Inhibitors of gene expression for use in the present invention may be based on antisense oligonucleotide constructs. Anti-sense oligonucleotides, including anti-sense RNA

molecules and anti-sense DNA molecules, would act to directly block the translation of the targeted mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of the targeted protein (i.e. XPO1), and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding the target protein can be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically inhibiting gene expression of genes whose sequence is known are well known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

Small inhibitory RNAs (siRNAs) can also function as inhibitors of gene expression for use in the present invention. Gene expression can be reduced by contacting the tumor, subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that gene expression is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see Tuschl, T. et al. (1999); Elbashir, S. M. et al. (2001); Hannon, GJ. (2002); McManus, MT. et al. (2002); Brummelkamp, TR. et al. (2002); U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

Ribozymes can also function as inhibitors of gene expression for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of the targeted mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated

by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

Both antisense oligonucleotides and ribozymes useful as inhibitors of gene expression can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramidite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

Antisense oligonucleotides siRNAs and ribozymes of the invention may be delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the antisense oligonucleotide siRNA or ribozyme nucleic acid to the cells. Preferably, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the the antisense oligonucleotide siRNA or ribozyme nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rouse sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic

viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in KRIEGLER (A Laboratory Manual," W.H. Freeman C.O., New York, 1990) and in MURRY ("Methods in Molecular Biology," vol.7, Humana Press, Inc., Clifton, N.J., 1991).

Preferred viruses for certain applications are the adeno-viruses and adeno-associated viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy. The adeno-associated virus can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as, heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hematopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g., SANBROOK et al., "Molecular Cloning: A Laboratory Manual," Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are particularly advantageous for this because they do not have the same safety concerns as with many of the viral vectors. These plasmids, however, having a promoter compatible with the host cell, can

express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUC19, pRC/CMV, SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be injected by intramuscular, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation.

Typically the XPO1 inhibitor is administered to the subject in a therapeutically effective amount.

By a "therapeutically effective amount" of the XPO1 inhibitor of the invention as above described is meant a sufficient amount of the compound. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Preferably, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily

supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

5 The XPO1 inhibitor of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

10 "Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

15 In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and 20 buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms. Galenic adaptations may be done for specific delivery in the small intestine or colon.

25 Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized 30 water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol ; and sterile powders for the extemporaneous preparation of sterile injectable solutions or

dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

5

Solutions comprising XPO1 inhibitors of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

10

The XPO1 inhibitor of the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

20

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifusoluble agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

30

Sterile injectable solutions are prepared by incorporating the active polypeptides in the required amount in the appropriate solvent with various of the other ingredients enumerated

above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The XPO1 inhibitor of the invention may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses can also be administered.

In addition to the XPO1 inhibitors of the invention formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; liposomal formulations ; time release capsules ; and any other form currently used.

A further object of the invention relates to a method for screening a drug suitable for the treatment of beta-thalassemia comprising the steps consisting of i) providing a candidate compound, ii) testing whether the candidate compound for its ability to inhibit XPO1 activity or expression and iii) selecting the candidate compound that is able to inhibit XPO1 activity or expression.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: Expression of XPO1 gene in CD36+ erythroid precursors (immunoblot)
N= Nucleus, C= Cytoplasm. From day 3 to day 6 of CD36+ culture.

Figure 2: Expression of XPO1 gene (and HSP70) in CD36+ erythroid precursors (fluorescence microscopy).

Figure 3A. KPT is not toxic for CD36+ erythroid progenitors from cord blood at day 6 (D6) of culture, when diluted at concentrations 33nM, 100nM, 333nM and 1000nM in CD36+ culture media, with normal concentrations of erythropoietin (EPO), until 20h of treatment.

Figure 3B. EPO starvation during 20h results in 80% of cell death. Treatment of CD36+ D6 with KPT at 100nM, 333nM during EPO starvation decreases cell death, of 49% and 39% respectively, compared to the absence of KPT treatment.

Figure 4: KPT induces HSP70 nuclear retention in CD36+ D6 from cord blood, in conditions of EPO starvation, after 20h of treatment.

Figure 5: In beta-thalassemia CD36+ D8 cells, KPT induces HSP70 nuclear localization after 72h of treatment at 100nM compared to non treated cells.

Figure 6. CD36+ cells, derived from circulating CD34+ cells from Beta thalassemia major patient, were treated or not with KPT molecule (KPT 0= DMSO, KPT 100 = 100nM, KPT 1000 = 1000nM), and analysed at 24h, 48h, 72h and 96h of treatment. Day 0 of treatment corresponds to day 5 of CD36+ culture. a) Proliferation of CD36+ cells between 24h and 96h, for the different conditions of KPT treatment. b) Cell death of CD36+ cells between 48h and 96h, for the different conditions of KPT treatment. c) Flow cytometry analysis of Mean fluorescence intensity (MFI) for Band 3 protein, in CD36+ cells, in the different conditions of KPT treatment. d) MGG count of immature, polychromatophiles and acidophiles+reticulocytes at 96h of treatment, for the different conditions of KPT treatment and e) corresponding maturation indexes.

Figure 7: CD36+ cells, derived from circulating CD34+ cells from Beta thalassemia major patient, were treated or not with KPT molecule (KPT 0= DMSO, KPT 100 = 100nM, KPT 1000 = 1000nM), and analysed at 24h, 48h, and 72h of treatment. Day 0 of treatment corresponds to day 6 of CD36+ culture. a) Proliferation of CD36+ cells between 24h and 72h, for the different conditions of KPT treatment. b) Cell death of CD36+ cells between 24h and 72h, for the different conditions of KPT treatment. c) Flow cytometry analysis of MFI for Band 3 protein, in CD36+ cells, in the different conditions of KPT treatment. d) Flow cytometry analysis of the number of small, alive, CD36+ cells positive for foetal haemoglobin in the different conditions of KPT treatment. e) MGG count of immature, polychromatophiles and acidophiles+reticulocytes at 48h of treatment, for the different conditions KPT 0 and KPT 100nM and e) corresponding maturation indexes.

Figure 8. CD36+ cells from the same experiment as Figure 8, at 24h of treatment. Percentages of cells containing HSP70 located in the nucleus (black) or in the cytoplasm (grey), gated on the total population of cells analyzed, for the different treatment conditions: KPT 0 (a), KPT 100nM (b) and KPT 1000nM (c). d) HSP70 cytoplasm/nuclear ratio for the three treatment conditions e) Mean intensity and standard deviation for GATA1 transcription factor fluorescence.

EXAMPLE 1:

Background

It has been published by our lab (Ribeil et al., Nature 2007) that Leptomycin B (LMB), a chemical inhibitor of XPO1, induces HSP70 nuclear retention in erythroid precursor cells. This observation suggests that XPO1 might be involved in the nuclear export of HSP70 protein in erythroid precursor.

During erythroid precursor cell differentiation, the nuclear accumulation of HSP70 protein is essential. In fact, in the nucleus, HSP70 protects GATA1 transcription factor from Caspase-3 degradation.

In the case of two pathologies with dyserythropoiesis: myelodysplastic syndrome (MDS) and beta-thalassemia, a defect in nuclear localization of HSP70 in erythroid progenitor cells has been assessed (Frisan et al. Blood 2012, Arlet et al unpublished data). Thus, we suggest that it would be of interest to repress XPO1-induced HSP70 nuclear export to restore HSP70 protein nuclear localization in erythroid precursors cells, and therefore improve erythroid precursors differentiation.

KPT-330 (Selinexor*) is the first selective inhibitor of XPO1 and produced by Karyopharm. In vitro analysis and safety studies in human patients have shown a good tolerability of the molecule.

We suggest assessing the effect of KPT molecule (KPT-251 suitable for in vitro tests) both in beta-thalassemia and MDS erythroid precursor cells.

Material and methods:

Material

Umbilical cord blood units from normal full-term deliveries were obtained, after informed mother's consent, from the Obstetrics Unit of Hôpital Necker-Enfants Malades. CD36+ erythroid progenitors, generated from 7 days IL-6 + IL3 +SCF- cultured CD34 progenitors isolated from cord blood (Miltenyi CD34 Progenitor Cell Isolation Kit), were cultured in the presence of IL3+SCF+Epo in IMDM (Gibco cell culture) supplemented with 15% BIT 9500 (Stem Cell Technologies).

Immunoblot analyses

Separate cytoplasmic and nuclear protein fractions were extracted from erythroid progenitors using NE-PER Nuclear and cytoplasmic extraction reagents (Thermo Scientific), following manufacturer's protocol.

40µg of proteins of nuclear or cytoplasmic extracts were resolved on 14% acrylamide gels and analysed by immunoblotting. Antigens were visualized by chemiluminescence using SuperSignal West Dura (Thermo Scientific).

5 *Reagents*

Antibodies used are anti HDAC mouse clone 3F3 #05-814 (Millipore), anti HSP70 rabbit ADI SPA 812 (Enzo lifesciences), and anti CRM1 (XPO1) rabbit #ST1100 (Calbiochem).

10 *Cell permeabilisation and labelling for Fluorescence microscopy*

5.10⁴ cells were spin on slides, acetone fixated, hydrated with cold 1X PBS 1% BSA for 30 minutes, treated with formaldehyde for 15 min (Sigma), then with methanol (Prolabo) for 10 minutes at room temperature. Cells were then permeabilized with 1X PBS 0,2% triton X100 (Sigma) for 10min at 4°C, washed and incubated in 10% BSA for 30minutes. They
15 were then sequentially incubated with antibodies diluted in 1X PBS 1% BSA 0,1% tween (Sigma). Nuclei were stained with DAPI and slides were examined with a confocal laser microscope (LSM 700 Carl Zeiss).

20 **Results:**

20

We investigated the expression of XPO1 protein in CD36+ erythroid progenitors from cord blood by immunoblot (Figure1) and fluorescence microscopy (Figure 2). In Figure 1, XPO1 protein is present majoritary in the cytoplasm (C) and to a lesser extend in the nucleus (N) compartment of CD36+ erythroid progenitors, from day 3 (D3) to day 6 (D6) of culture.
25 In figure 2, the expression of XPO1 protein observed by immunoblotting is confirmed by confocal microscopy, on permeabilised CD36+ erythroid progenitors, at day 6 of culture in Epo. Here we report for the first time the expression of XPO1, a nuclear export protein of the beta importin family, in CD36+ erythroid progenitor cells.

30 **EXAMPLE 2:**

Data from Figures 6 and Figure 7 are from two different experiments on CD36+ cells from a beta-thalassemia patient. Data from Figure 8 have been performed with cells from Figure 7 experiment. Beta-thalassemia erythroblasts were generated in vitro from peripheral

blood circulating CD34+ cells from an adult patient with beta-thalassemia major (β^0 -TM). This study was performed according to the Helsinki Declaration with approval from the ethics committee of our institution.

5 Treatment with KPT at 1000nM shows decreased proliferation compared to KPT 0 and KPT 100nM, only in the experiment where patient cells have a high proliferation rate in control condition (Figure 6a and Figure 7a). Treatment with KPT at 1000nM shows little cytotoxicity compared to KPT 0 and KPT 100nM (Figure 6b, Figure 7b).

10 In vitro, maturation is arrested at the polychromatophilic stage in beta-thalassemia erythroid progenitors. To quantify the maturation arrest in beta-thalassemia cells, we use an index of terminal maturation as the number (acidophilic cells + reticulocytes per slide)*100 divided by the number of polychromatophilic cells per slide (Arlet Jb et al. Nature oct 2014). We show that the maturation index of beta-thalassemia cells increases with KPT treatment at
15 100nM and 1000nM compared to control condition KPT 0 (Figure 6d, 6e, Figure 7e and 7f).

Flow cytometry analyses show that the presence of KPT 100nM or 1000nM in culture media of CD36+ beta-thalassemia cells increases the mean fluorescence intensity (MFI) of Band 3 protein labelling, a marker of terminal erythroid differentiation (Hu J et al. Blood apr
20 2013), at 96h of treatment (Figure 6c), and at 72h of treatment (Figure 7c).

In beta-thalassemia patients, the lack of beta chain synthesis is compensated by an increased proportion of cells producing fetal haemoglobin (HbF: composed of two alpha and to gamma subunits) and the only surviving mature erythroblasts are HbF cells. Here we
25 observe that after 72 hours of treatment with 1000nM of KPT, the proportion of HbF positive, among the population of small alive cells, is higher compared to KPT 0 and KPT 100nM conditions.

Amnis Technology enables to analyse fluorescence localization in a large number of
30 cells beforehand labelled. Here we show the percentage of cells positive for HSP70 labelling in the nucleus, or in the cytoplasm, for conditions KPT 0, 100nM and 1000nM (Figure 8). We can observe that the fraction of cells positive for HSP70 in the nucleus increases in KPT 100nM and 1000nM conditions (Figure 8b and 8c), compared to KPT 0 (Figure 8a).

This observation is also evidenced by the decrease in HSP70 cyto/nuclear fraction in conditions KPT 100nM and 1000nM compared to KPT 0 (Figure 8d).

During terminal erythroid differentiation, HSP70 nuclear translocation is required to protect GATA1 transcription factor from the cleavage by activated caspase 3. In beta-thalassemia erythroid progenitors, HSP70 is sequestered in the cytoplasm by the excess of alpha globin chains (Arlet Jb et al. Nature oct 2014). Thus, GATA1 is cleaved and this results in maturation arrest and apoptosis of erythroid progenitors. Here we show that the increasing fraction of cells with nuclear HSP70 coincides with an increase in the mean of fluorescence intensity of GATA1 labelling.

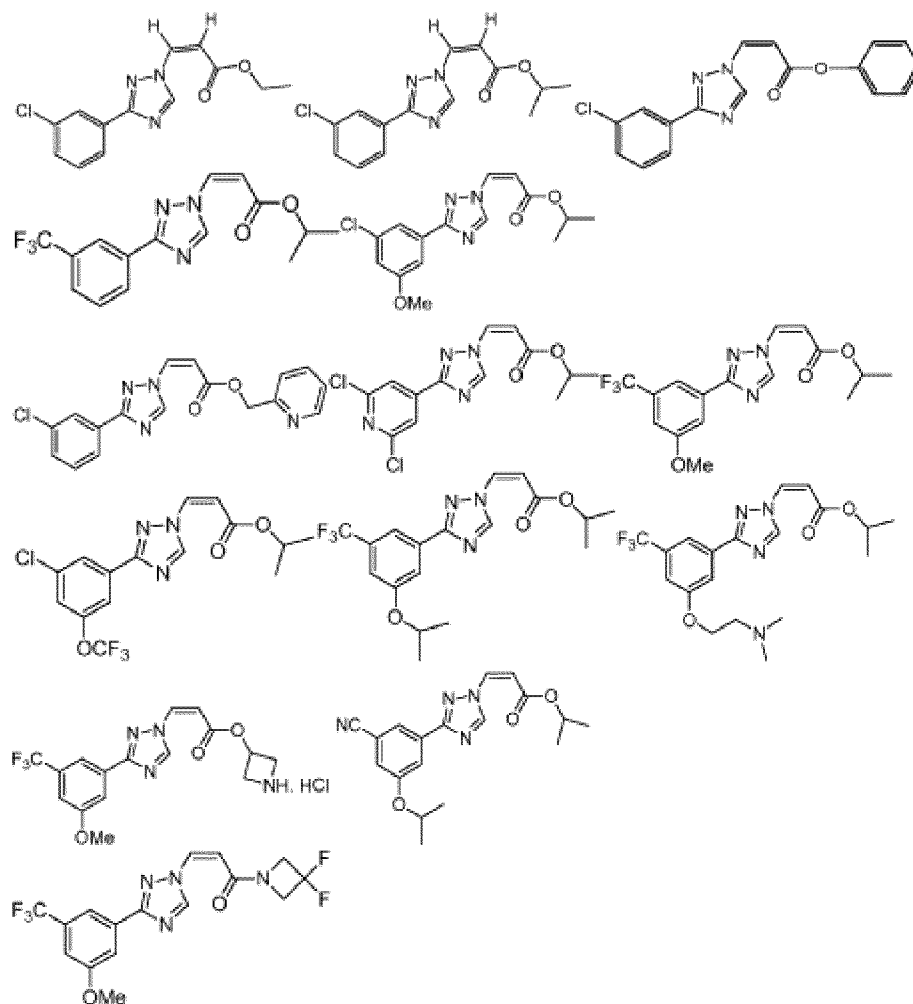
Taken together, these data show that treatment of CD36+ cells from beta-thalassemia patients in vitro with KPT, a specific inhibitor of XPO1 exportin, shows few cytotoxicity, and can improve terminal erythroid differentiation by repressing HSP70 export from the nucleus of erythroid progenitors. This nuclear translocation of HSP70 is associated with the protection of GATA1 erythroid transcription factor from cleavage.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

CLAIMS:

1. A method of restoring or increasing erythrocyte maturation in a subject suffering from β -thalassemia major (β -TM) by preventing proteolytic inactivation of GATA-1
5 wherein preventing is achieved by administering to the subject a compound that inhibits the XPO1 nuclear transporter.
2. The method of claim 1 wherein the compound that inhibits the XPO1 nuclear transporter is an XPO1 inhibitor.
3. A method of treating beta-thalassemia in a subject in need thereof comprising
10 administering the subject with a therapeutically effective amount of an XPO1 inhibitor.
4. A method for promoting or restoring erythrocyte maturation in a subject suffering from beta-thalassemia comprising administering the subject with a therapeutically effective amount of an XPO1 inhibitor.
- 15 5. A method for increasing the production of $(\alpha/\gamma)_2$ Hb erythrocytes in a subject suffering from beta-thalassemia comprising administering the subject with a therapeutically effective amount of an XPO1 inhibitor.
6. The method of claim 1, 3, 4 or 5 wherein the XPO1 inhibitor is selected from the group consisting of the following compounds:

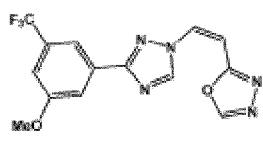


and pharmaceutically acceptable salts thereof.

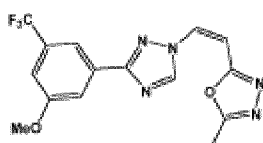
7. The method of claim 1, 3, 4 or 5 wherein the XPO1 inhibitor is selected from the group consisting of (Z)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid ethyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid ethyl ester; (Z)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid isopropyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid isopropyl ester; (Z)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid tert-butyl ester; (Z)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acrylic acid tert-butyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-N-phenyl-acryl amide; (E)-N-(2-Chloro-phenyl)-3-[3-(3-chloro-phenyl)-[1,2,4]-triazol-1-yl]-acryl amide; (4-{(E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-acryloylamino}-phenyl)-carbamic acid tert-butyl ester; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-N-(4-methoxy-phenyl)-acryl amide; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-N-methyl-N-phenyl-acryl amide; (E)-3-[3-(3-Chloro-phenyl)-[1,2,4]-triazol-1-yl]-N-methyl-N-phenyl-acryl amide; (E)-N-(4-Amino-

phenyl)-3-[3-(3-chloro-phenyl)-[1,2,4]-triazol-1-yl]-acryl amide and a pharmaceutically-acceptable salts thereof.

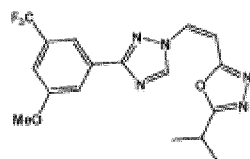
8. The method of claim 1, 3, 4 or 5 wherein the XPO1 inhibitor is selected from the group consisting of the following compounds:



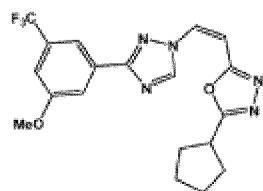
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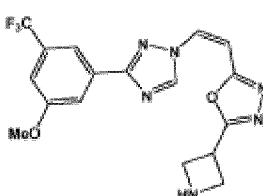
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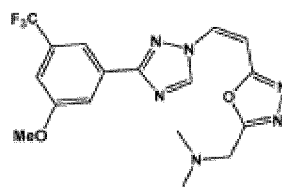
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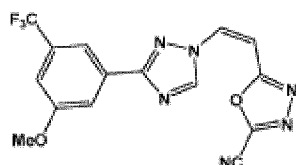
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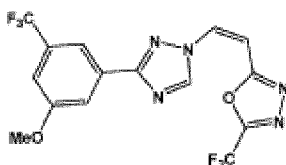
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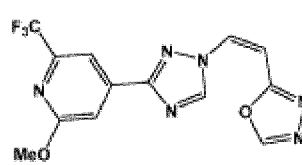
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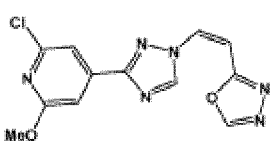
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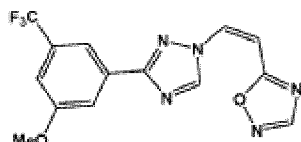
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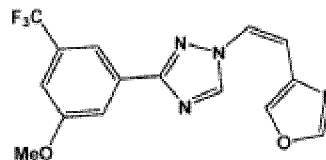
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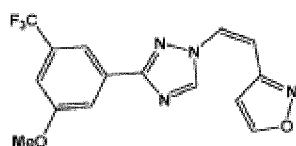
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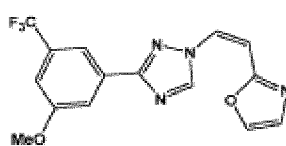
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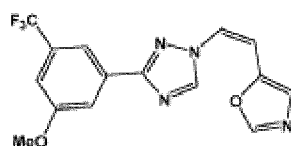
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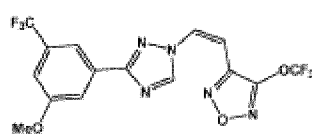
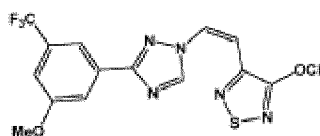
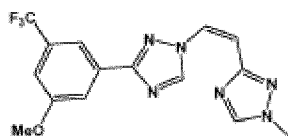
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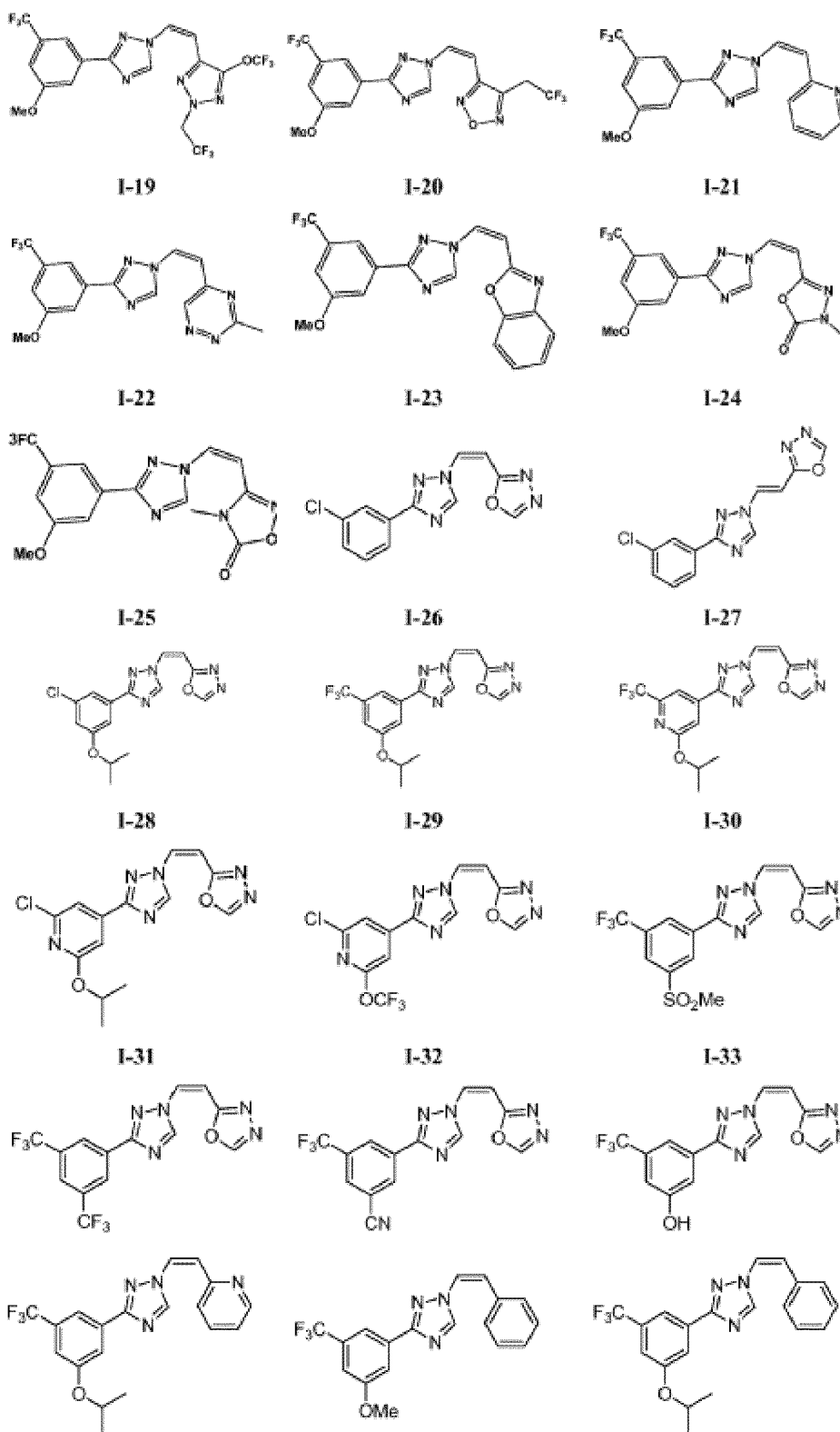


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I-15



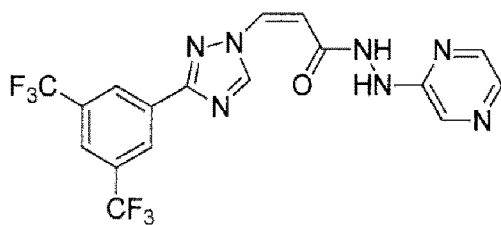


and pharmaceutically acceptable salts thereof.

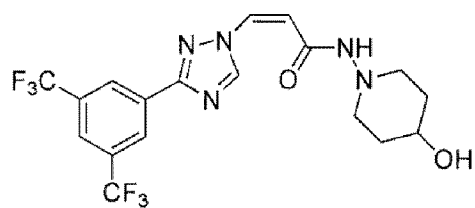
9. The method of claim 1, 3, 4 or 5 wherein the XPO1 inhibitor is selected from the group consisting of a compound selected from the group consisting of 1-(4-methoxyphenyl)-1H-pyrrole-2,5-dione; 1-(4-bromo-2,5-difluorophenyl)-1H-pyrrole-

2,5-dione; 3-methyl-1-(1-methyl-1H-pyrazol-3-yl)-1H-pyrrole-2,5-dione; 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide; 1-(3-benzoyl-4-methylthiophen-2-yl)-1H-pyrrole-2,5-dione; 1-(4-(3-(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)-1H-pyrrole-2,5-dione; 1-(4-(4-chlorophenyl)thiazol-2-yl)-3-methyl-1H-pyrrole-2,5-dione; 1-(benzo[b]thiophen-3-ylmethyl)-1H-pyrrole-2,5-dione; 1-(3,4-dimethoxyphenethyl)-1H-pyrrole-2,5-dione; 1-(naphthalen-1-yl)-1H-pyrrole-2,5-dione; 1-(4-cyclohexylphenyl)-1H-pyrrole-2,5-dione; 1-(2-benzoylphenyl)-1H-pyrrole-2,5-dione; 1-(4-morpholinophenyl)-1H-pyrrole-2,5-dione; 1-(4-chlorophenethyl)-1H-pyrrole-2,5-dione; 1-(2-(thiophen-2-yl)ethyl)-1H-pyrrole-2,5-dione; 1-([3,4]methylenedioxybenzyl)-1H-pyrrole-2,5-dione amide and pharmaceutically-acceptable salts thereof.

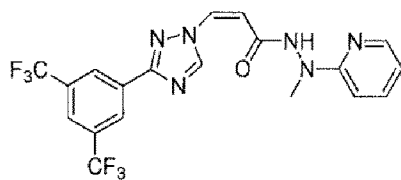
10. The method of claim 1, 3, 4 or 5 wherein the XPO1 is selected from the group consisting of the following compounds:



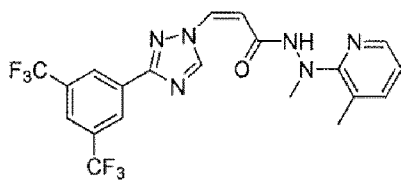
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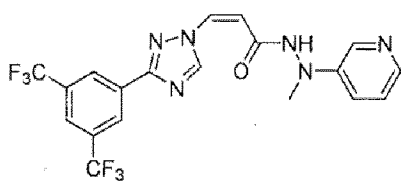
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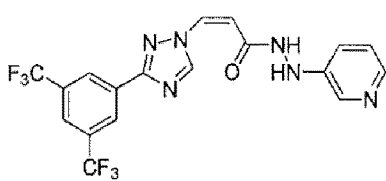
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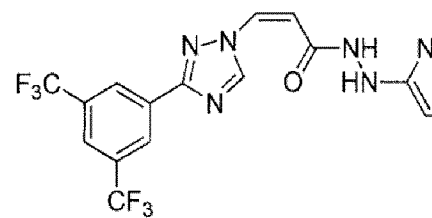
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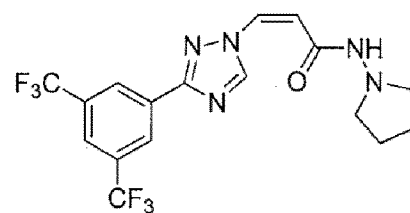
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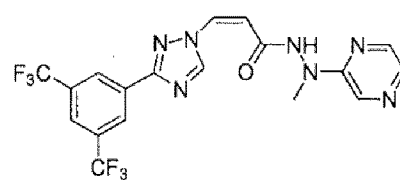
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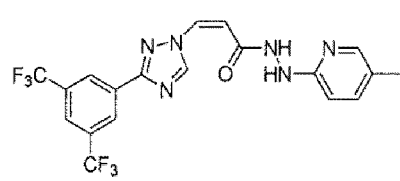
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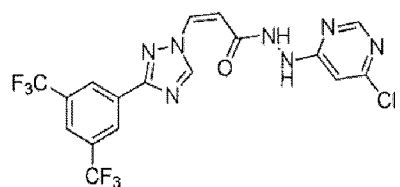
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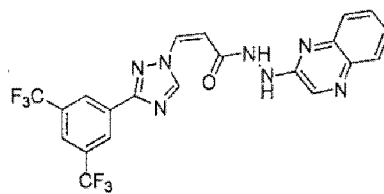
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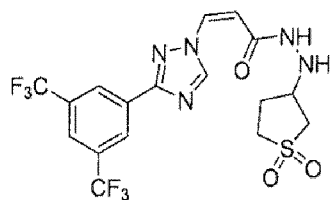
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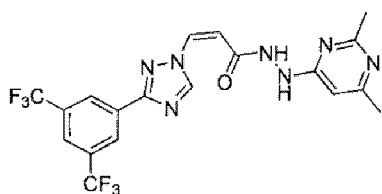
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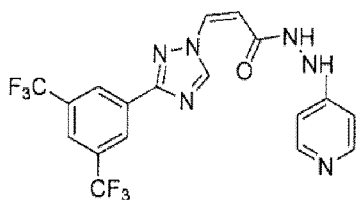
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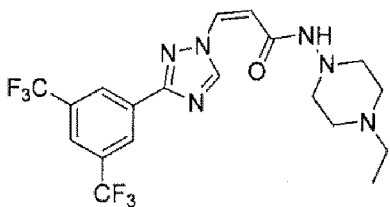
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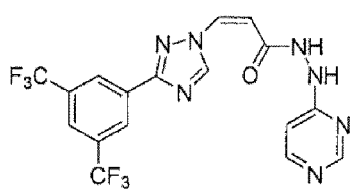
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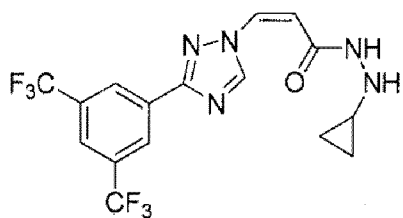
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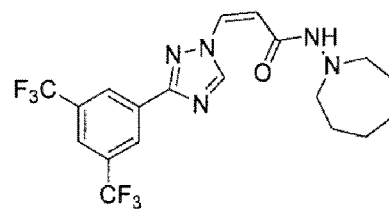


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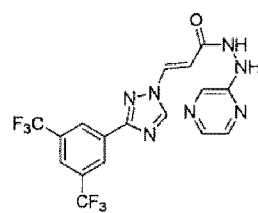


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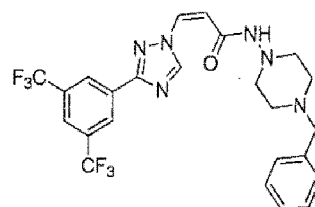
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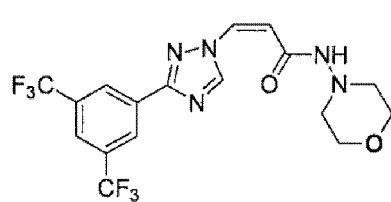
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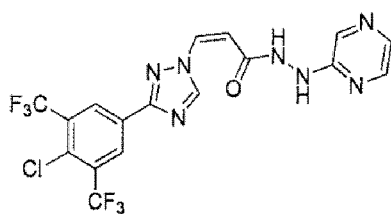
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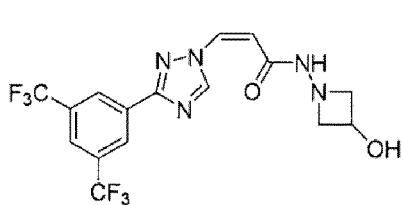
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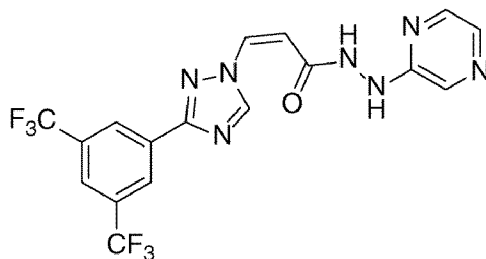


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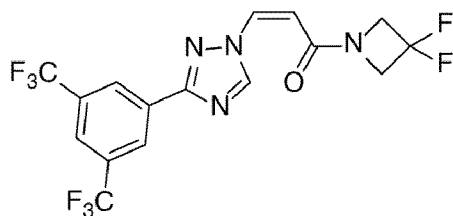


I-26.

11. The method of claim 1, 3, 4 or 5 wherein the XPO1 inhibitor is KPT-330 which has formula of:



12. The method of claim 1, 3, 4 or 5 wherein the XPO1 inhibitor is KPT-276 which has formula of



13. The method of claim 1, 3, 4 or 5 wherein the XPO1 inhibitor is an inhibitor of XPO1 expression

14. The method of claim 13 wherein the inhibitor of gene expression is a siRNA, an antisense oligonucleotide or a ribozyme.

15. A method for screening a drug suitable for the treatment of beta-thalassemia comprising the steps consisting of i) providing a candidate compound, ii) testing whether the candidate compound for its ability to inhibit XPO1 activity or expression and iii) selecting the candidate compound that is able to inhibit XPO1 activity or expression.

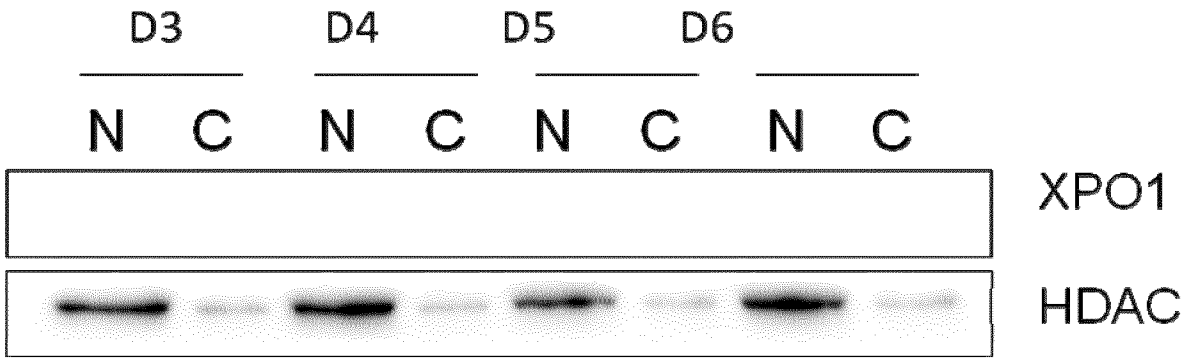


Figure 1

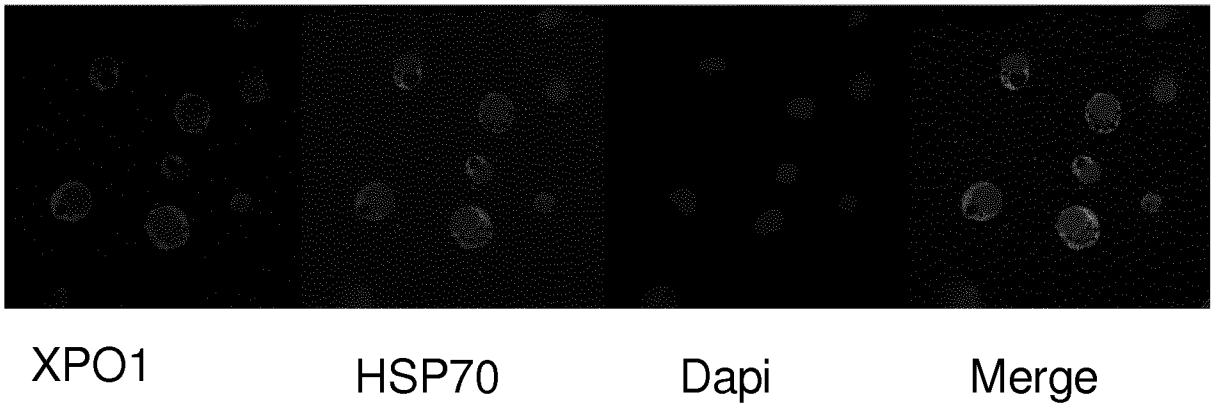
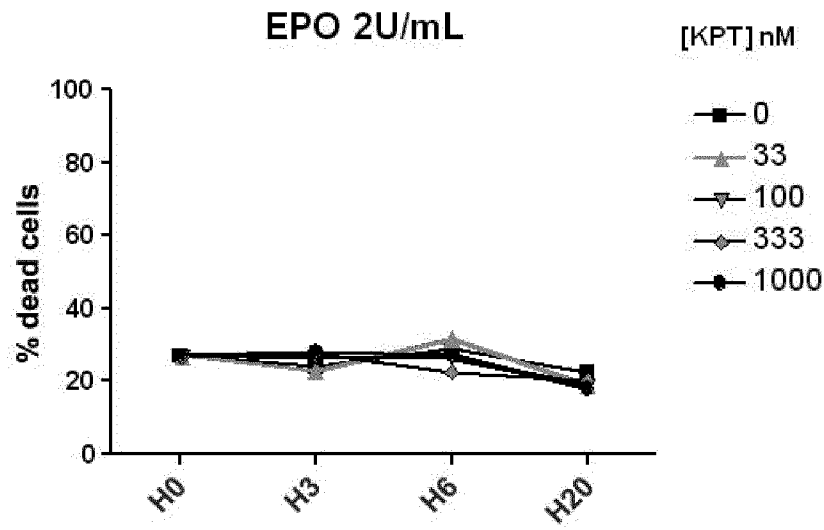


Figure 2

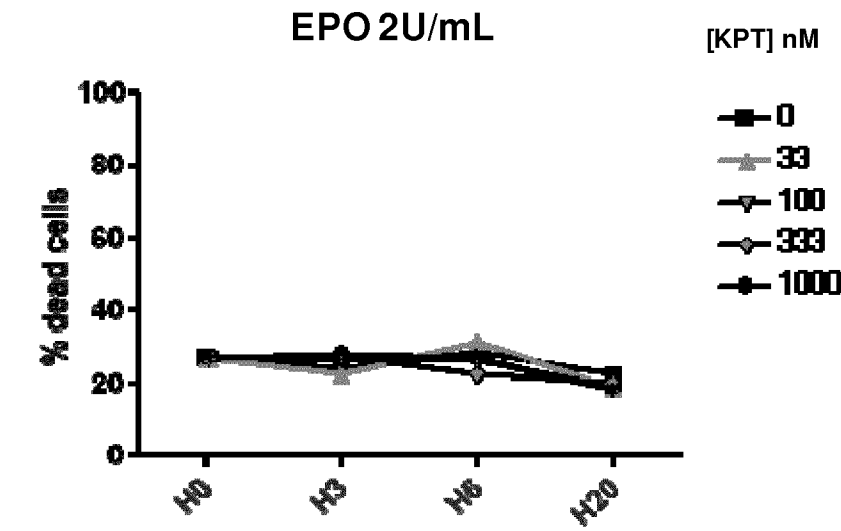


Figure 3A

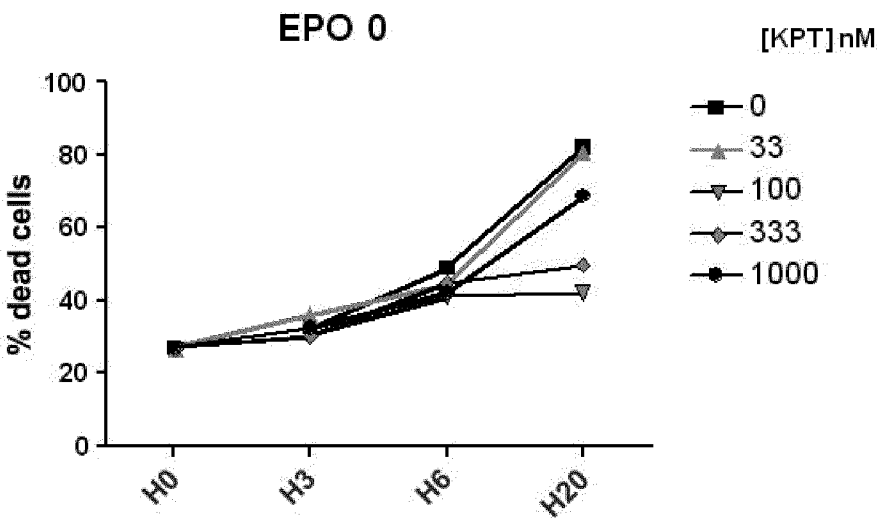


Figure 3B

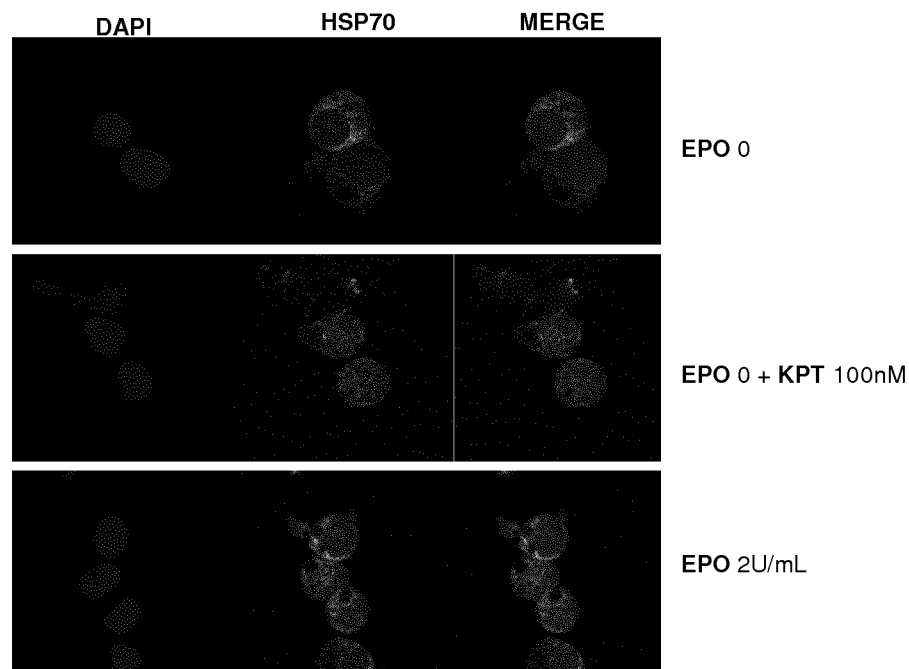


Figure 4

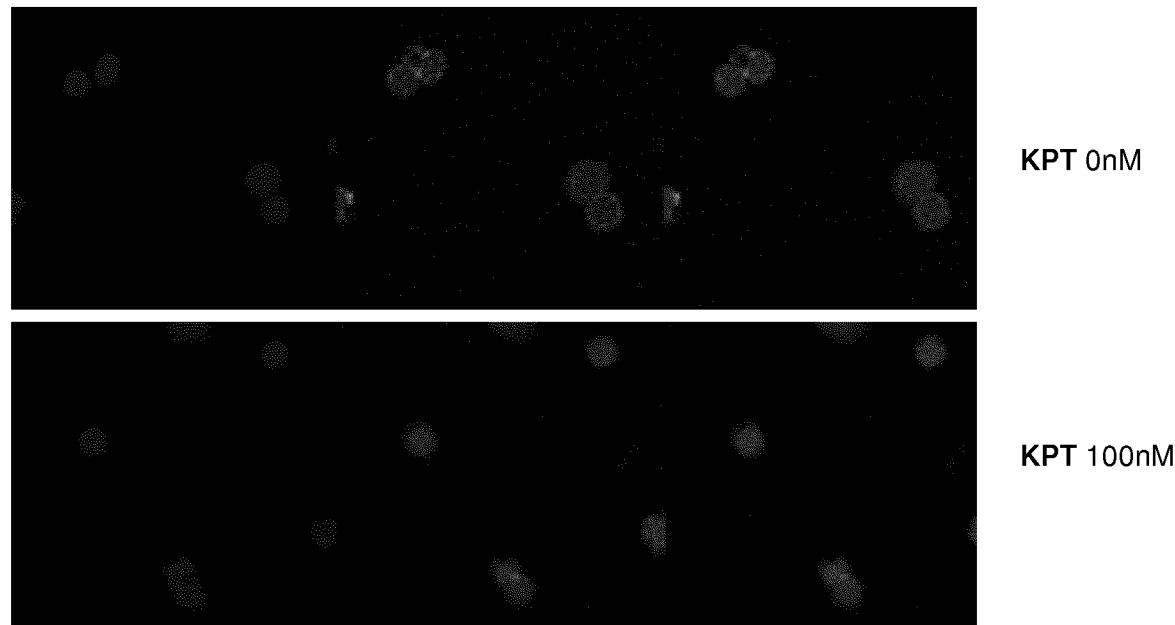


Figure 5

**Proliferation study of CD36+ cells from beta thalassemia maj patient,
treated or not with KPT**

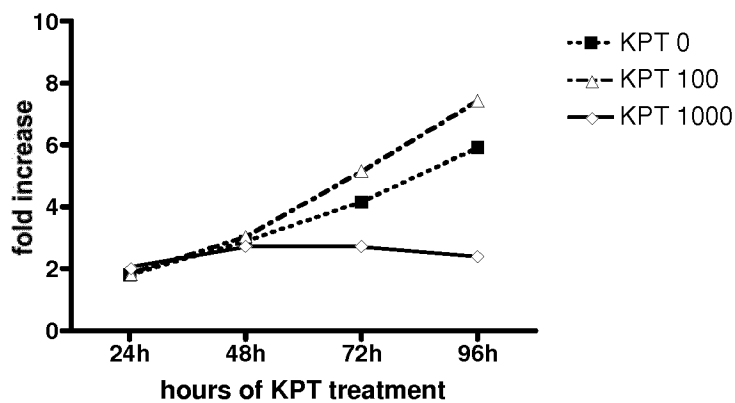


Figure 6A

**Cell death of CD36+ cells from beta thalassemia maj patient
treated or not with KPT**

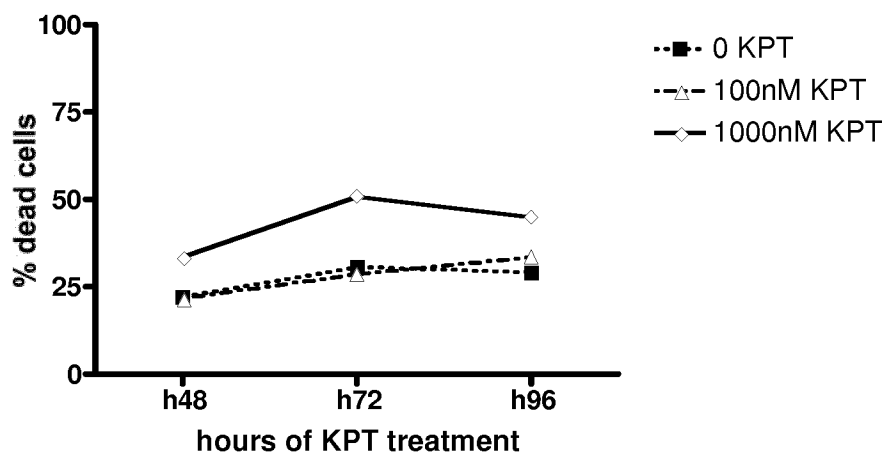


Figure 6B

**MFI of Band 3 protein in CD36+ cells from beta thalassemia maj
patient treated or not with KPT**

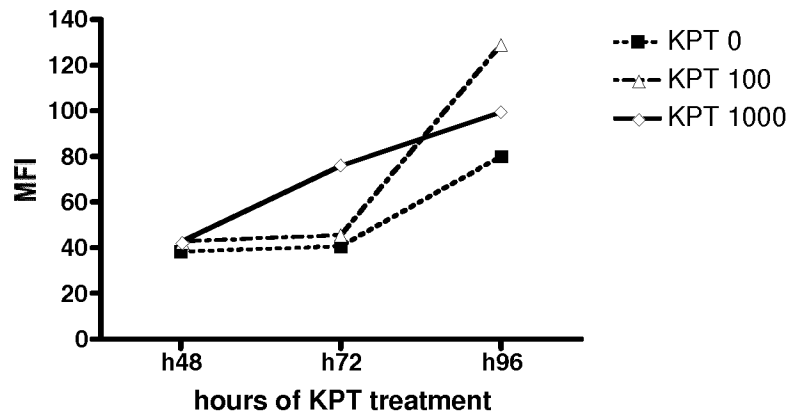


Figure 6C

**MGG count of Beta thalassemia CD36+ at day 9 of culture
(96h treatment KPT)**

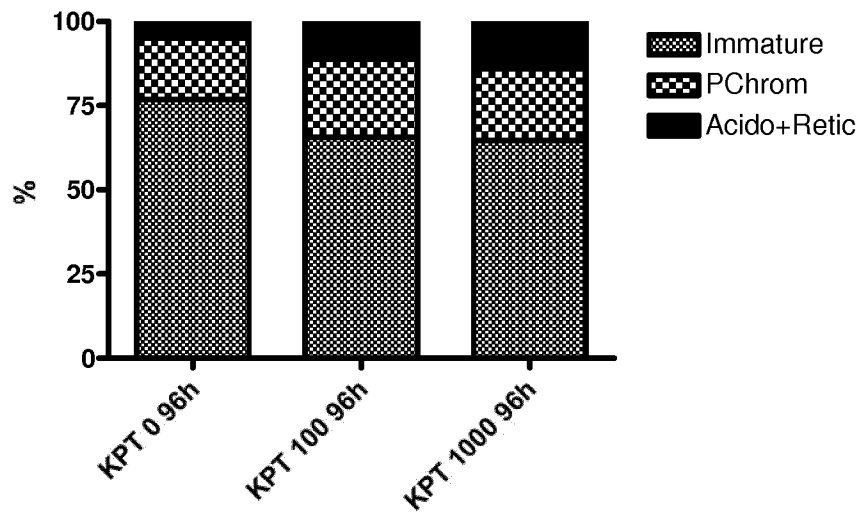


Figure 6D

**Maturation index of Beta thalassemia CD36+ D9
(96h treatment)**

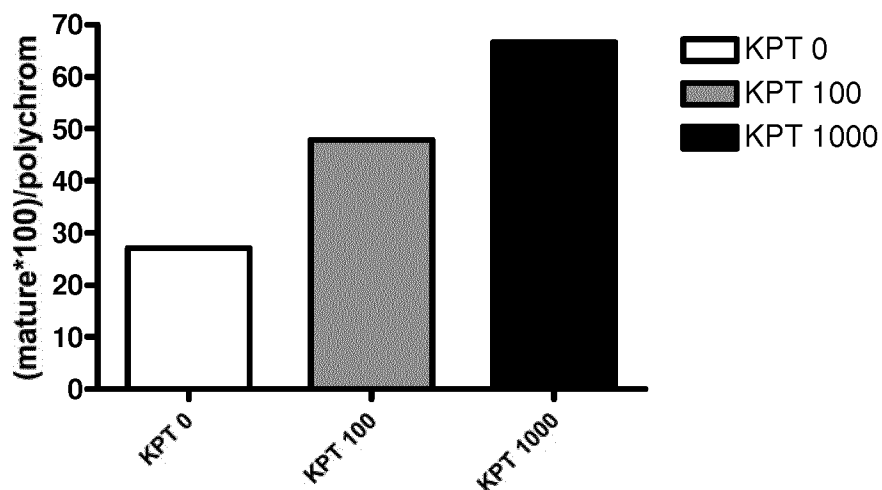


Figure 6E

**Proliferation study of CD36+ cells from beta thalassemia maj patient,
treated or not with KPT**

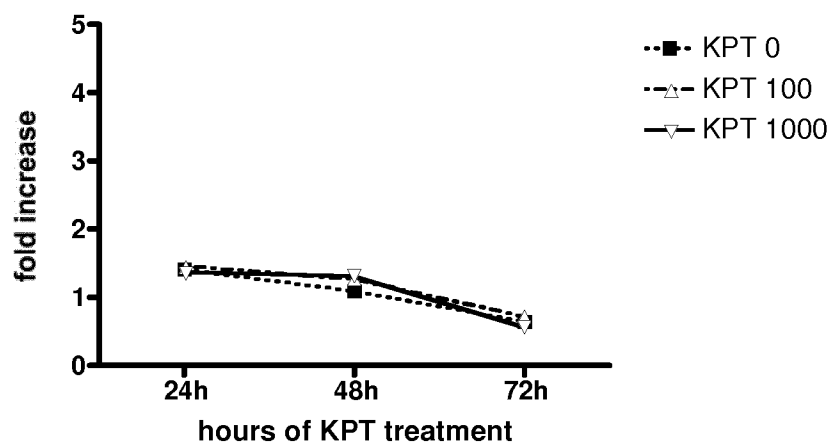


Figure 7A

**Cell death of CD36+ cells from beta thalassemia maj patient
treated or not with KPT**

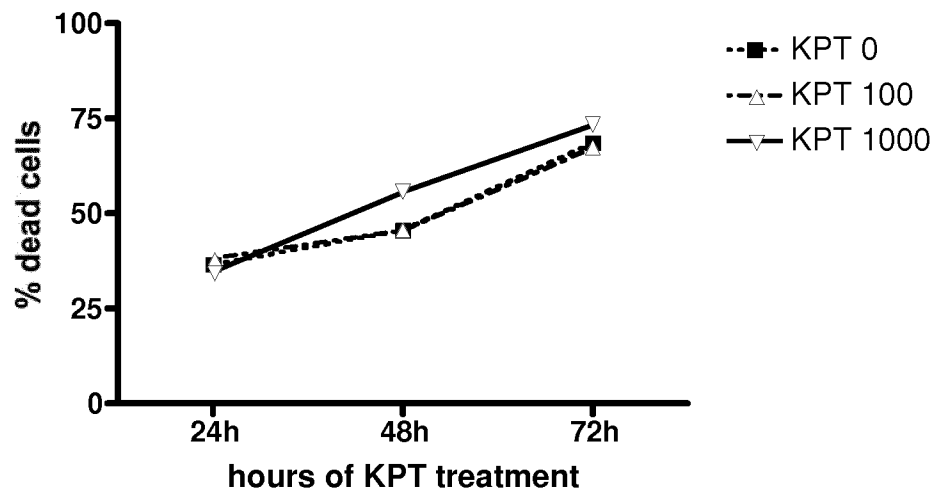


Figure 7B

**MFI of Band 3 protein in CD36+ cells from beta thalassemia maj
patient treated or not with KPT**

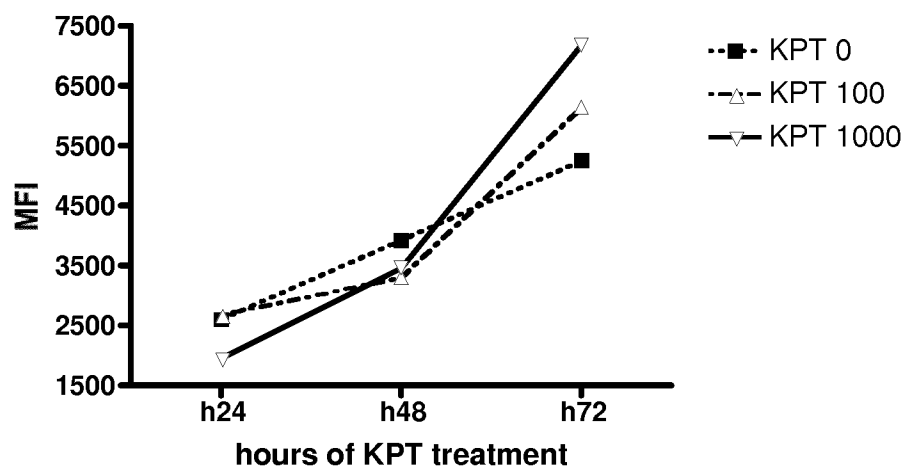


Figure 7C

**Percentage of small alive beta thalassemia cells HbF+
in the different conditions of KPT treatment**

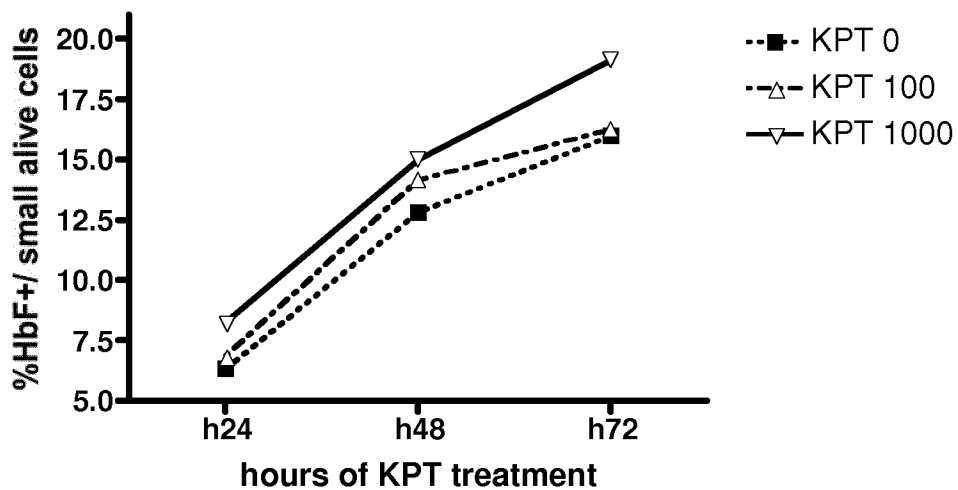


Figure 7D

**MGG count of Beta thalassemia CD36+ at day 8 of culture
(48h treatment KPT)**

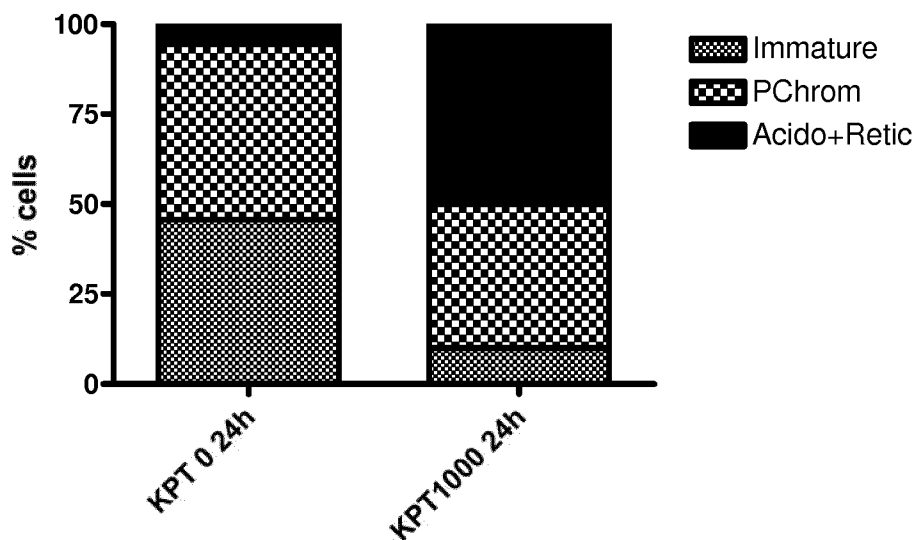


Figure 7E

**Maturation index of Beta thalassemia CD36+ D8
(48h treatment)**

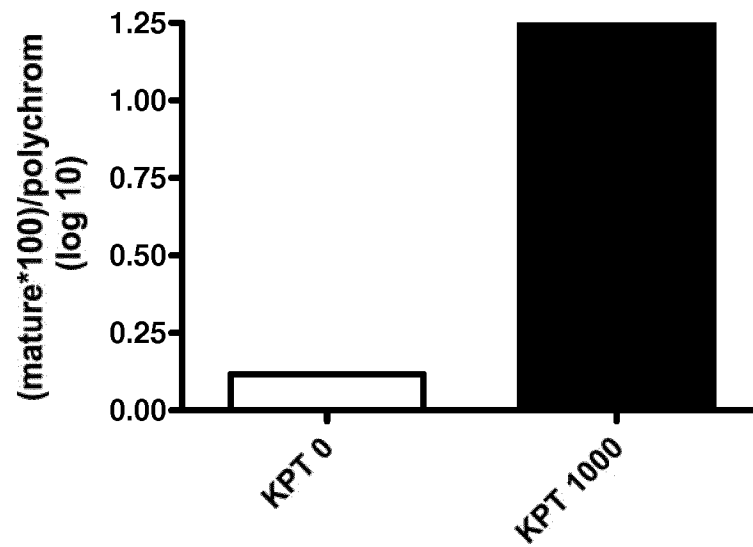


Figure 7F

KPT 0

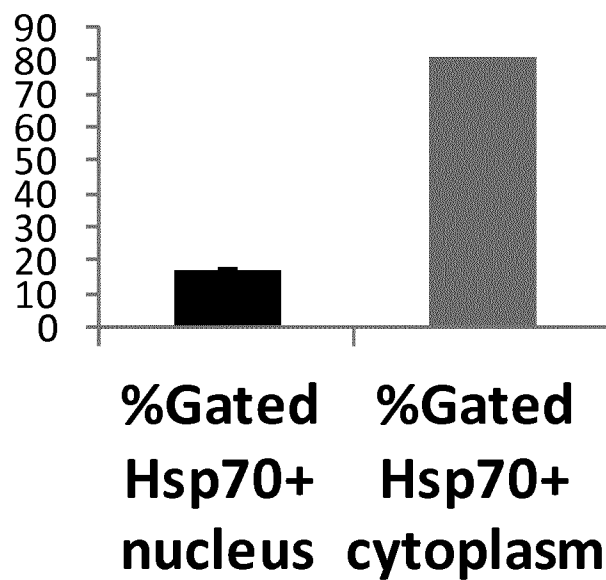


Figure 8A

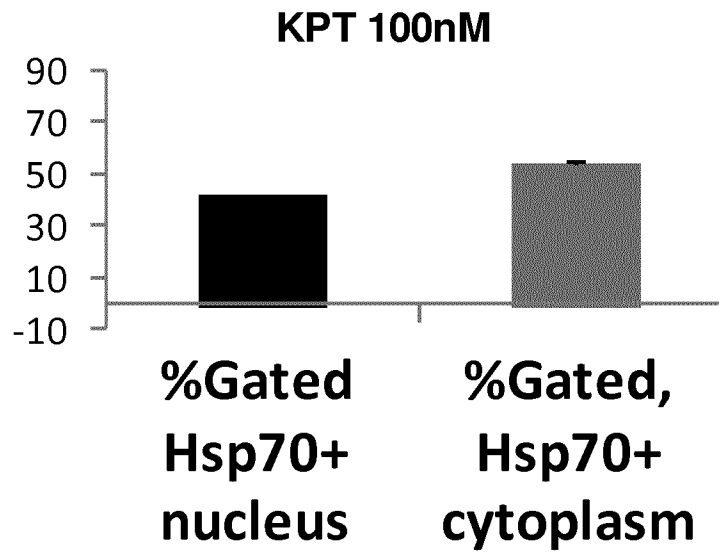


Figure 8B

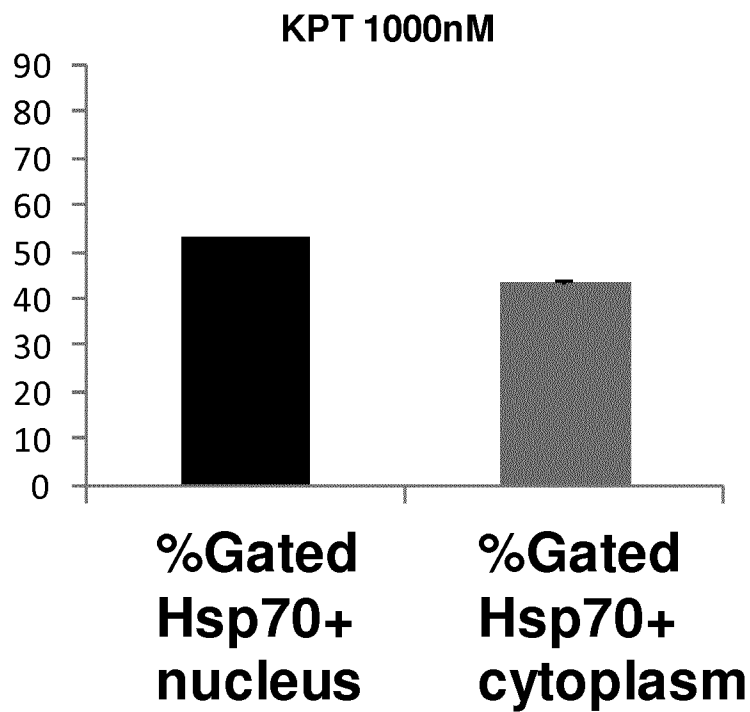


Figure 8C

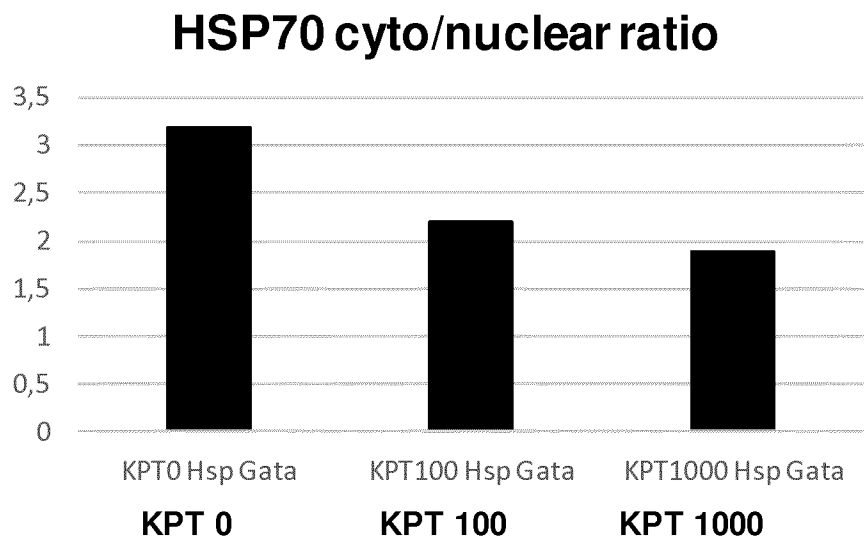


Figure 8D

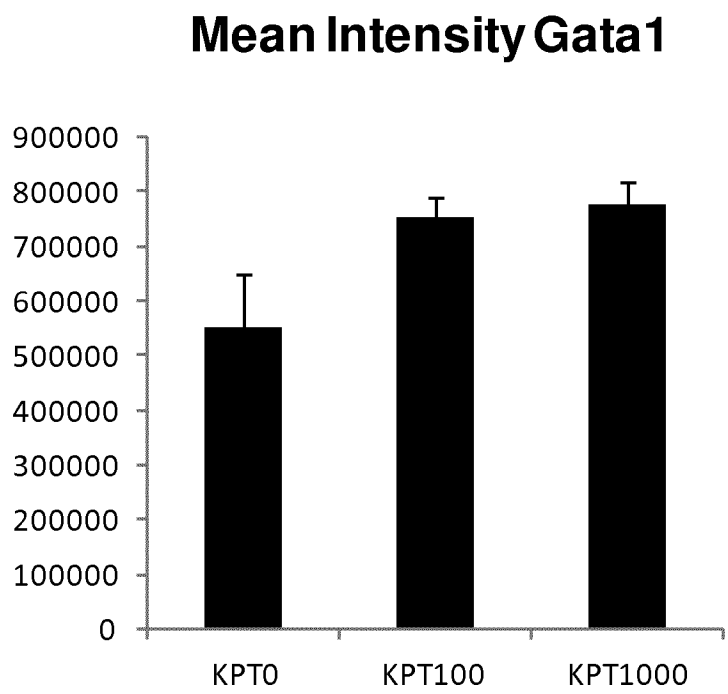


Figure 8E

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/075980

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/396 A61P7/00
ADD.

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)
A61K

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)

EPO-Internal, BIOSIS, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WALKER ET AL: "Preclinical and clinical efficacy of XP01/CRM1 inhibition by the karyopheerin inhibitor KPT-333 in Ph1 leukemias.", BLOOD, vol. 122, no. 17, 22 August 2013 (2013-08-22), pages 3034-3044, XP002718690, Results.	1-3
A	WO 2012/099807 A1 (KARYOPHARM THERAPEUTICS INC [US]; SANDANAYAKA VINCENT P [US]; SHACHAM) 26 July 2012 (2012-07-26) claim 19	1-3



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

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Date of the actual completion of the international search

29 January 2015

Date of mailing of the international search report

10/02/2015

Name and mailing address of the ISA/

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Fax: (+31-70) 340-3016

Authorized officer

Cattell, James

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2014/075980

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012099807 A1	26-07-2012	AU 2012207565 A1	22-08-2013
		CA 2824808 A1	26-07-2012
		CN 103402359 A	20-11-2013
		EP 2665362 A1	27-11-2013
		JP 2014502641 A	03-02-2014
		US 2012258986 A1	11-10-2012
		WO 2012099807 A1	26-07-2012
