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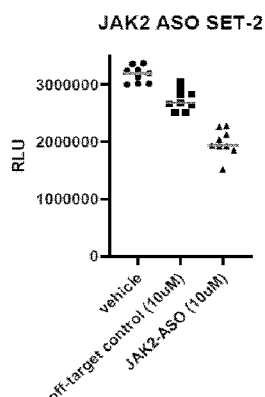
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(54) Title: ANTISENSE OLIGONUCLEOTIDE (ASO) GENE INHIBITION AND TREATMENT

FIG. 1



(57) Abstract: Embodiments of the invention related generally to antisense oligonucleotides (ASOs) and, more particularly, to compositions and methods for regulating protein synthesis using ASOs. In one embodiment, the invention provides a method of treating a patient diagnosed with myelodysplastic syn-
drome (MDS) comprising: administering to the patient an amount of an anti-
sense oligonucleotide (ASO) targeted to a nucleic acid molecule encoding JAK2
(ASO-T-JAK2) compound effective to treat such disease.

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Antisense Oligonucleotide (ASO) Gene Inhibition and Treatment

Cross-Reference to Related Applications

- This application claims priority to co-pending US Provisional Patent Application
5 Serial No. 63/222,336, filed 15 July 2021, and US Provisional Patent Application
Serial No. 63/224,362, filed 21 July 2021, each of which is hereby incorporated
herein as though fully set forth.

Sequence Listing

- 10 The sequence listing contained in the electronic file titled “\txt_VAND-0224-
PCT.xml,” created 19 June 2022 and comprising 63 kb, is hereby incorporated
herein.

Background

- 15 The present invention relates to certain novel antisense oligonucleotides (ASOs) and,
more particularly, to pharmaceutical compositions thereof and to methods for treating
certain diseases or conditions in which regulating protein synthesis using ASOs can
have therapeutic value. More particularly aspects of the present invention relate to the
treatment of known disorders such as polycythemia vera (PV) and myelodysplastic
20 syndrome (MDS), as well as Charcot-Marie-Tooth type 2 (CMT2) disease.

PV is a myeloproliferative disorder characterized by erythroid hyperplasia, myeloid
leukocytosis, thrombocytosis, and splenomegaly. Most individuals diagnosed with
PV carry a valine-to-phenylalanine mutation at position 617 (V617F) of the JAK2
gene.

- 25 MDS is a group of clonal hematologic stem cell disorders characterized by
ineffective hematopoiesis, often resulting in anemia. Some cases of MDS have been
associated with the same V617F mutation of the JAK2 gene and specifically with an
increased platelet count.

- The JAK2 gene codes for a non-receptor tyrosine kinase involved in cell growth,
30 development, differentiation, and histone modifications, as well as cytokine and
growth factor signaling.

Charcot-Marie-Tooth disease (CMT), also known as hereditary motor and sensory
neuropathy, causes damage to the peripheral nerves and nerves involved in muscle

control. Symptoms are progressive, often beginning in the feet and lower legs and then the fingers, hands, and arms.

Nearly all cases of CMT are inherited. More than 40 genes have been associated with CMT. Some genes are associated with more than one form of CMT and some forms of CMT are associated with more than one gene. As such, it is possible for an individual to suffer from more than one form of CMT as a result of more than one inherited mutation. More than half of all CMT cases are caused by a duplication of the PMP22 gene on chromosome 17. Other forms of CMT are caused by X-linked mutations.

- 5
- 10 CMT type 2 (CMT2) is a less common subtype of CMT affecting nerve axons. Most forms of CMT2 are inherited in an autosomal dominant pattern, though some forms, including CMT type 2S (CMT2S), are inherited in an autosomal recessive pattern. As such, symptoms associated with CMT2S are typically less severe than in autosomal dominant forms of CMT2. The mutation associated with CMT2S is a mutation of the
- 15 IGHMBP2 (immunoglobulin mu DNA binding protein 2) gene on chromosome 11, leading to abnormal RNA processing and axonal neuropathy.

Summary

- 20 An “antisense oligonucleotide” or “ASO” refers to a synthetic, multi-nucleotide RNA compound that hybridizes to a target RNA sequence in a manner such that gene expression can be inhibited, including by inactivating an mRNA molecule.

- The invention provides a method of treating a patient diagnosed with MDS which comprises administering to the patient an amount of an ASO-T-JAK2 compound effective to treat MDS. In accordance with the present invention an ASO-T-JAK2
- 25 compound refers to any antisense oligonucleotide (ASO) targeted to a nucleic acid molecule encoding JAK2. In addition, an amount of the ASO-T-JAK2 compound effective to treat MDS requires administration of the compound in a quantity effective to inhibit JAK2 protein expression on the patient.

- 30 As noted above, the term “ASO-T-JAK2” refers to an ASO targeted to a nucleic acid molecule encoding the gene JAK2. The term “nucleic acid molecule” is a reference to a polynucleotide compound, *i.e.*, RNA. An “antisense oligonucleotide” or “ASO” references a synthetic, multi-nucleotide DNA compound that hybridizes to a target RNA in a manner such that gene expression can be inhibited, including by inactivating an mRNA compound. Illustrative ASO-T-JAK2 compounds are
- 35 disclosed herein (SEQ. ID. 4 and 5 targeting SEQ. ID. 2 and 3, respectively). Additional ASO-T-JAK2 compounds can be identified and prepared by methods

known in the art for developing antisense oligonucleotides specific to a target RNA sequence.

The term “ASO-T-IGHMBP2” refers to an ASO targeted to a nucleic acid molecule encoding the IGHMBP2 gene. The term “nucleic acid molecule” is a reference to a polynucleotide compound, *i.e.*, RNA. Illustrative ASO-T-IGHMBP2 compounds are disclosed and described in detail below. Additional ASO-T-IGHMBP2 compounds can be identified and prepared by methods known in the art for developing ASOs specific to a target RNA sequence.

A “patient” as referenced above references an individual diagnosed with, suffering from, or at risk of developing, being diagnosed with, or suffering from a disease or disorder, specifically PV or MDS or CMT2S.

In another embodiment, the invention provides a method of treating a patient diagnosed with PV which comprises administering to the patient an amount of an ASO-T-JAK2 compound effective to treat PV. An amount effective of the ASO-T-JAK2 compound effective to treat PV is an amount effective to inhibit JAK2 protein expression in the patient.

In yet another embodiment, the invention provides a method of inhibiting expression of the JAK2 gene in an individual which comprises administering to the individual an amount of an ASO-T-JAK2 compound effective to accomplish such inhibition.

In still another embodiment, the invention provides novel ASO-T-JAK2 compounds. As noted above, such compounds included, but are not limited to the oligonucleotides of SEQ ID 4 and SEQ ID 5. Numerous methods exist in the art for preparing ASOs once the nucleotide base sequence thereof have been determined. The determination of the appropriate sequence of bases for an ASO-T-JAK2 compound is likewise accomplished by strategies known in the art for identifying gene sequences for which an ASO would inhibit protein expression by the gene upon RNA binding.

In the treatment of an individual patient by administration of an ASO-T-JAK2, the dose and dosage regiment can be readily determined. Numerous examples of approved dosing for ASOs are known in the art, including nusinersen (marketed as Spinraza), mipomersen (marketed as Kynamro), and fomivirsen (marketed as Vitravene). In certain circumstances, it may be advantageous to administer the ASO-T-JAK2 in a derivatized or modified form in order to optimize bioavailability, duration of action, or therapeutic effect otherwise. Methods are known in the art for such modification/derivatization. *See, generally*, Drug Delivery Trends in Clinical Trails and Translational Medicine: challenges and opportunities in the delivery of nucleic acid-based therapeutics, J Pharm Sci. (2011 Jan); 100(1): 38–52. For the

purposes of the present invention the ASOs are administered intravenously, though other routes of administration are possible, including oral administration. Such alternative routes of administration are applicable to the ASOs described herein.

Accordingly, a further aspect of the present invention are pharmaceutical compositions that are used for the administration of the ASO-T-JAK2 compound to the patient being treated. These include sterile parenteral dosage forms of the type conventionally employed for the intravenous administration of a medicine. Such pharmaceutical compositions contain excipients that may be required. These can include one or more diluents, carriers, adjuvants, or combinations thereof as are known in the art for the production of a finished dosage form.

In one embodiment, the invention provides a method of treating a patient diagnosed with Charcot-Marie-Tooth type 2 (CMT2) which comprises: administering to said patient an amount of an ASO-T-IGHMBP2 compound effective to treat such disease.

In another embodiment, the invention provides a method of inhibiting expression of a mutated IGHMBP2 gene in an individual carrying a C31401A mutation of the IGHMBP2 gene, the method comprising: administering to the individual an amount of an ASO-T-IGHMBP2 compound effective to inhibit expression of the mutant IGHMBP2 gene in the individual.

In still another embodiment, the invention provides an antisense oligonucleotide (ASO) targeted to a nucleic acid molecule encoding a mutated IGHMBP2 gene. Such an ASO may be included in a pharmaceutical composition in combination with a pharmaceutically acceptable diluent, carrier, adjuvant, or combination thereof.

Numerous methods exist in the art for preparing ASOs once the nucleotide base sequence thereof have been determined. The determination of the appropriate sequence of bases for an ASO-T-IGHMBP2 compound is likewise accomplished by strategies known in the art for identifying gene sequences for which an ASO would inhibit protein expression by the gene upon RNA binding.

In the treatment of an individual patient by administration of an ASO-T-IGHMBP2, the dose and dosage regiment can be readily determined. Numerous examples of approved dosing for ASOs are known in the art, including nusinersen (marketed as Spinraza), mipomersen (marketed as Kynamro), and fomivirsen (marketed as Vitravene). In certain circumstances, it may be advantageous to administer the ASO-T-IGHMBP2 in a derivatized or modified form in order to optimize bioavailability, duration of action, or therapeutic effect otherwise. Methods are known in the art for such modification/derivatization. *See, generally*, Drug Delivery Trends in Clinical Trails and Translational Medicine: challenges and opportunities in the delivery of

nucleic acid-based therapeutics, J Pharm Sci. (2011 Jan); 100(1): 38–52. For the purposes of the present invention the ASOs are administered intravenously, though other routes of administration are possible, including oral administration. Such alternative routes of administration are applicable to the ASOs described herein.

- 5 Accordingly, a further aspect of the present invention are pharmaceutical compositions that are used for the administration of the ASO-T- IGHMBP2 compound to the patient being treated. These include sterile parenteral dosage forms of the type conventionally employed for the intravenous administration of a medicine. Such pharmaceutical compositions contain excipients that may be required.
- 10 These can include one or more diluents, carriers, adjuvants, or combinations thereof as are known in the art for the production of a finished dosage form.

Brief Description of the Drawings

- These and other features of this invention will be more readily understood from the following detailed description of the various aspects of the invention taken in
- 15 conjunction with the accompanying drawings that depict various embodiments of the invention, in which:

FIG. 1 shows the results of a bioluminescence assay in which ASO treatment inhibits JAK2 expression and decreases cell viability;

- 20 FIGS. 2A and 2B show, respectively, fluorescent and brightfield images of SET-2 cells treated with ASOs according to embodiments of the invention;

FIGS. 3A and 3B show, respectively, fluorescent and brightfield images of HEL cells treated with ASOs according to embodiments of the invention;

- 25 FIGS. 4A and 4B show, respectively, fluorescent and brightfield images of SET-2 cells treated with ASOs according to embodiments of the invention; and

FIGS. 5A and 5B show, respectively, fluorescent and brightfield images of HEL cells treated with ASOs according to embodiments of the invention.

- It is noted that the drawings of the invention are not to scale. The drawings are intended to depict only typical aspects of the invention, and therefore should not be
- 30 considered as limiting the scope of the invention. In the drawings, like numbering represents like elements among the drawings.

Detailed Description

The present invention provides target sequences in the JAK2 pre-mRNA that are amenable to binding by a synthetic ASO. When so bound, expression of the JAK2 gene is inhibited. In patients diagnosed with MDS and PV, such inhibition of JAK2 expression provides an effective method of treatment of the disorder.

As noted above, MDS and PV are both associated with the V617F mutation of the JAK2 protein. The wildtype JAK2 protein sequence is shown in SEQ ID NO 1 in which the amino acid at position 617 is valine.

In one aspect of the present invention, a target region within the JAK2 pre-mRNA molecule is identified that, when bound with an ASO, is capable of inhibiting synthesis of the JAK2 protein. This region includes a 19 bp sequence:

TTTCCTTAGTCCTTTCTTTG (SEQ ID NO 2)

and the shorter 16 bp sequence:

CCTTAGTCCTTTCTTTG (SEQ ID NO 3).

Complementary ASO sequences are, respectively:

5'-CAAAGAAAGACUAAGGAAA-3' (SEQ ID NO 4)

and:

5'-CAAAGAAAGACUAAGG-3' (SEQ ID NO 5).

Each of these sequences bridges an intron and exon of the JAK2 pre-mRNA. When ASOs containing these sequences are bound to JAK2 pre-mRNA, processing of the pre-mRNA into mature mRNA is prevented, as is synthesis of the JAK2 protein.

To test the efficacy of ASOs having SEQ ID NO 4 and/or SEQ ID NO 5 to bind to the JAK2 pre-mRNA and inhibit JAK2 protein synthesis, three human cell lines (SET-2, HEL, CMK) are incubated with each of these ASOs and treated with cycloheximide. After a 48-hour incubation, the treated cell lines are collected and subjected to a CellTiter-Glo® (CTG) assay and RNA extraction. Data related to the cell lines and culture media are shown in Table 1 below.

Table 1

Cell Line	Growth Period	Culture Medium
SET-2	60–80 hours	80% RPMI 1640 + 20% heat-inactivated FBS
HEL	36 hours	90% RPMI 1640 + 10% heat-inactivated FBS
CMK	40–50 hours	80% RPMI 1640 + 20% heat-inactivated FBS

Cells are harvested during the growth period and counted using a Cellometer K2. One million cells are incubated in the listed medium in each well of a six-well plate and incubated in a humidified incubator at 37 °C with 5% CO₂.

After 24 hours, the medium is changed, the ASO is added, and incubation is continued under the same conditions for another 24 hours. Cycloheximide is then added to a final concentration of 0.1 mg/mL. Twenty-four hours after addition of the cycloheximide, the medium and cells are combined in a 15 mL conical tube and 50 μ L aliquots of cells are added to a 96-well assay plate for CTG assaying. Luminescence is recorded using a BioTek Synergy neo2 Multi-mode Reader. The remaining cells are spun at 4 °C to collect a cell pellet for RNA extraction by Tryzol reagent.

FIG. 1 shows results of the CTG assay described above for the SET-2 cell line. In the first column is the result for the assay vehicle, which contains no ASOs. Average relative luminescence units (RLUs) are in excess of 3,000,000. RLU is proportional to the number of viable cells in the assay well.

The second column shows the results of the assay when off-target control ASOs (*i.e.*, ASOs not targeting JAK2 pre-mRNA or mRNA) are used. Average RLUs are reduced as compared to vehicle, but are still in excess of 2,500,000.

The third column of FIG. 1 shows the results of the assay when ASOs containing SEQ ID NO 4 or SEQ ID NO 5 are added. Here, average RLUs are reduced to less than 2,000,000, with some samples exhibiting a reduction to approximately 1,500,000. This constitutes a significant reduction in RLUs, and consequently cell viability, as compared to vehicle.

These results are confirmed with RNA sequencing using next-generation sequencing (NGS) to quantify expression of the JAK2 gene. FIGS. 2A and 2B show, respectively, fluorescent (GFP) and brightfield images of assay wells for SET-2 cells treated with ASOs containing SEQ ID NO 4 or SEQ ID NO 5. FIGS. 3A and 3B show, respectively, fluorescent and brightfield images for HEL cells treated with the

same ASOs. In each case, fluorescence is lower than in untreated cells, demonstrating decreased mature JAK2 mRNA in samples incubated with ASOs containing SEQ ID NO 4 or SEQ ID NO 5.

Thus, embodiments of the invention include the administration of an ASO containing
5 SEQ ID NO 4, SEQ ID NO 5, or both, to an individual in order to inhibit expression of JAK2; such administration as a treatment for MDS, PV, or both; ASOs targeting SEQ ID NO 2, SEQ ID NO 3, or both; ASOs having nucleotide sequences that include SEQ ID NO 4 or SEQ ID NO 5; and compositions containing such ASOs in combination with pharmaceutically acceptable diluents, carriers, adjuvants, or
10 combinations thereof.

In other aspects, the present invention provides target sequences in the mutant IGHMBP2 pre-mRNA that are amenable to binding by a synthetic ASO. When so bound, expression of the mutant IGHMBP2 gene is inhibited. In patients diagnosed with CMT2S who are heterozygous for the mutation, such inhibition may result in the
15 “rescue” or return to normal function of the IGHMBP2 protein.

The full sequence of the wildtype IGHMBP2 gene is shown in the attached sequence listing as SEQ ID NO 6. The nucleotide at position 31401 is cytosine. In the mutation associated with CMT2S, this nucleotide is adenine.

In one aspect of the present invention, a target region within the resulting mutant
20 IGHMBP2 pre-mRNA molecule is identified that, when bound with an ASO, is capable of inhibiting synthesis of the mutant IGHMBP2 protein. That region within the mutant IGHMBP2 gene comprises a 19-bp sequence that includes the C31401A mutation:

CACTTCCACAGGGGGAAGA (SEQ ID NO 7).

25 The complementary ASO sequence is:

UCUUCUUUUUUGUGGAAGUG (SEQ ID NO 8).

An ASO comprising SEQ ID NO 3 bridges an intron and exon of the mutant IGHMBP2 pre-mRNA and, when bound to the pre-mRNA, prevents synthesis of the mutant IGHMBP2 protein.

30 To test the efficacy of an ASO comprising SEQ ID NO 8 in binding to the mutant IGHMBP2 pre-mRNA and inhibiting mutant IGHMBP2 protein synthesis, a fibroblast cell line of a patient with CMT2S is incubated with an ASO including SEQ ID NO 8 in RPMI 1640 medium supplemented with 10% FBS and treated with cycloheximide. After a 48-hour incubation, the treated cells are collected and for

RNA extraction and Western Blot analysis to determine IGHMBP2 protein expression.

- More specifically, one million cells are incubated in 2 mL in the RPMI 1640 medium described above in a humidified incubator at 37 °C with 5% carbon dioxide. After 24 hours, the medium is changed, the ASO is added, and incubation is continued for another 24 hours. Cycloheximide is then added to a final concentration of 0.1 mg/mL. Twenty-four hours after addition of the cycloheximide, the medium and cells are combined in a 15 mL conical tube and spun to collect a cell pellet for RNA extraction by Tryzol reagent. RNA extraction is carried out using, for example, Qiagen's RNeasy Mini Kit. Western Blot analysis is then carried out to determine the concentration of wild type and mutant proteins in the samples.

FIGS. 4A and 4B show fluorescent and brightfield photomicrographs, respectively, of SET-2 cells following treatment with ASOs including SEQ ID NO 8, demonstrating the ability of these ASOs to penetrate the cell membrane.

- FIGS. 5A and 5B show similar fluorescent and brightfield photomicrographs, respectively, of HEL cells following treatment with ASOs including SEQ ID NO 8. Again, these results demonstrate the ability of such ASOs to penetrate the cell membrane.

- ASOs other than or in addition to those including SEQ ID NO 8 may be useful in practicing the invention. Applicant has identified a "core" 11 nucleotide sequence within SEQ ID NO 8 that would provide a basis for sequences having sufficient specificity to enable discriminate binding of an ASO to the mutant IGHMBP2 pre-mRNA. As one skilled in the art will understand, efficient and effective ASO therapies such as those described herein require the use of ASOs having a length sufficient to ensure discriminate binding of the ASO to the target sequence. ASOs including sequences of between 13 nucleotides and 35 nucleotides in length and including the core sequence of SEQ ID NO 9 are of sufficient length for use in practicing embodiments of the invention.

5'-CCCCCUGUGGA-3' (SEQ ID NO 9)

- Thus, an ASO having a nucleotide sequence that includes SEQ ID NO 4 and between two and 24 adjacent nucleotides are useful in practicing embodiments of the invention. Such nucleotide sequences include, for example, sequences as short as the 13-nucleotide sequences of SEQ ID NOS, 10, 11, and 12:

5'-CCCCCUGUGGAAG-3' (SEQ ID NO 10)

- 5'-UCCCCCUGUGGAA-3' (SEQ ID NO 11)

5'-UCCCCCUGUGGA-3' (SEQ ID NO 12)

Such nucleotide sequences also include, for example, sequences as long as the 35-nucleotide sequences of SEQ ID NOS 13 and 14.

5'-GAAGAAAUCAAUCUCCCCCUGUGGAAGUGAGGGC-3'

5 (SEQ ID NO 13)

5'-GAAAUCAAUCUCCCCCUGUGGAAGUGAGGGCCAG-3'

(SEQ ID NO 14)

More generally, ASOs having a nucleotide sequence between 13 nucleotides and 35 nucleotides in length comprising the core sequence of SEQ ID NO 9 and as many as 10 15 upstream nucleotides and/or 12 downstream nucleotides. The ASO "sequence window" is shown in SEQ ID NO 15.

5'-GAAGAAAUCAAUCUCCCCCUGUGGAAGUGAGGGCCAG-3'

(SEQ ID NO 15)

As used herein, the singular forms "a," "an," and "the" are intended to include the 15 plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" and/or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups 20 thereof.

This written description uses examples to disclose the invention, including the best mode, and also to enable any person skilled in the art to practice the invention, including making and using any devices or systems and performing any related or incorporated methods. The patentable scope of the invention is defined by the claims, 25 and may include other examples that occur to those skilled in the art. Such other examples are intended to be within the scope of the claims if they have structural elements that do not differ from the literal language of the claims, or if they include equivalent structural elements with insubstantial differences from the literal language of the claims.

CLAIMS

1. A method of treating a patient diagnosed with myelodysplastic syndrome (MDS) which comprises:
5 administering to said patient an amount of an ASO-T-JAK2 compound effective to treat such disease.
2. The method of claim 1, wherein the ASO-T-JAK2 compound is targeted to SEQ ID NO 2, SEQ ID NO 3, or both.
10
3. The method of claim 2, wherein the ASO-T-JAK2 compound comprises a nucleotide sequence including SEQ ID NO 4 or SEQ ID NO 5.
4. The method of claim 1, wherein the amount of said ASO-T-JAK2 compound
15 is an amount sufficient to inhibit expression of JAK2 in the patient.
5. A method of treating a patient diagnosed with polycythemia vera (PV) which comprises:
administering to the patient an amount of an ASO-T-JAK2 compound
20 effective to treat such disease.
6. The method of claim 5, wherein the ASO-T-JAK2 compound is targeted to SEQ ID NO 2, SEQ ID NO 3, or both.
- 25 7. The method of claim 6, wherein the ASO-T-JAK2 compound comprises a nucleotide sequence including SEQ ID NO 4 or SEQ ID NO 5.
8. The method of claim 5, wherein the amount of the ASO-T-JAK2 compound is an amount sufficient to inhibit expression of JAK2 in the patient.
30
9. A method of inhibiting expression of the JAK2 gene in an individual which comprises:
administering to the individual an amount of an ASO-T-JAK2 compound
effective to inhibit JAK2 expression in the individual.
35
10. The method of claim 9, wherein the ASO-T-JAK2 compound is targeted to SEQ ID NO 2, SEQ ID NO 3, or both.

11. The method of claim 10, wherein the ASO-T-JAK2 compound comprises a nucleotide sequence including SEQ ID NO 4 or SEQ ID NO 5.
12. An antisense oligonucleotide (ASO) targeted to a nucleic acid molecule
5 encoding JAK2.
13. The ASO of claim 12, wherein the ASO is targeted to SEQ ID NO 2, SEQ ID NO 3, or both.
- 10 14. The ASO of claim 13, wherein the ASO comprises a nucleotide sequence selected from a group consisting of: SEQ ID No 4 and SEQ ID NO 5.
- 15 15. A pharmaceutical composition which comprises:
the ASO of claim 12; and
15 a pharmaceutically acceptable diluent, carrier, adjuvant, or combination thereof.
16. The composition of claim 15 which further comprises a sterile parenteral dosage form.
20
17. A method of treating a patient diagnosed with Charcot-Marie-Tooth type 2 (CMT2) which comprises:
administering to said patient an amount of an ASO-T-IGHMBP2 compound effective to treat such disease.
25
18. The method of claim 17, wherein the patient is diagnosed with Charcot-Marie-Tooth type 2S (CMT2S).
19. The method of claim 18, wherein the ASO-T-IGHMBP2 compound is
30 targeted to SEQ ID NO 7.
20. The method of claim 19, wherein the ASO-T-IGHMBP2 compound comprises a nucleotide sequence including SEQ ID NO 9.
- 35 21. The method of claim 20, wherein the ASO-T-IGHMBP2 compound comprises a nucleotide sequence including SEQ ID NO 8.

22. The method of claim 20, wherein the ASO-T-IGHMBP2 compound comprises a nucleotide sequence selected from a group consisting of: SEQ ID NO 10, SEQ ID NO 11, and SEQ ID NO 12.
- 5 23. The method of claim 20, wherein the ASO-T-IGHMBP2 compound comprises a nucleotide sequence 13 to 35 nucleotides in length within SEQ ID NO 15.
24. A method of inhibiting expression of a mutated IGHMBP2 gene in an
10 individual carrying a C31401A mutation of the IGHMBP2 gene, the method comprising:
administering to the individual an amount of an ASO-T-IGHMBP2 compound effective to inhibit expression of the mutant IGHMBP2 gene in the individual.
- 15 25. The method of claim 24, wherein the ASO-T-IGHMBP2 compound is targeted to SEQ ID NO 7.
26. The method of claim 25, wherein the ASO-T-IGHMBP2 compound
20 comprises a nucleotide sequence including SEQ ID NO 9.
27. The method of claim 26, wherein the ASO-T-IGHMBP2 compound comprises a nucleotide sequence including SEQ ID NO 8.
- 25 28. The method of claim 26, wherein the ASO-T-IGHMBP2 compound comprises a nucleotide sequence selected from a group consisting of: SEQ ID NO 10, SEQ ID NO 11, and SEQ ID NO 12.
29. The method of claim 26, wherein the ASO-T-IGHMBP2 compound
30 comprises a nucleotide sequence 13 to 35 nucleotides in length within SEQ ID NO 15.
30. An antisense oligonucleotide (ASO) targeted to a nucleic acid molecule encoding a mutated IGHMBP2 gene.
- 35 31. The ASO of claim 30, wherein the ASO is targeted to SEQ ID NO 7.

32. The ASO of claim 31 comprising a nucleotide sequence including SEQ ID NO 9.
33. The ASO of claim 32 comprising a nucleotide sequence including SEQ ID NO 8.
34. The ASO of claim 32 comprising a nucleotide sequence selected from a group consisting of: SEQ ID NO 10, SEQ ID NO 611 and SEQ ID NO 12.
35. The ASO of claim 32 comprising a nucleotide sequence 13 to 35 nucleotides in length within SEQ ID NO 15.
36. A pharmaceutical composition which comprises:
the ASO of claim 30; and
a pharmaceutically acceptable diluent, carrier, adjuvant, or combination thereof.
37. The pharmaceutical composition of claim 36 which further comprises a sterile parenteral dosage form.

FIG. 1

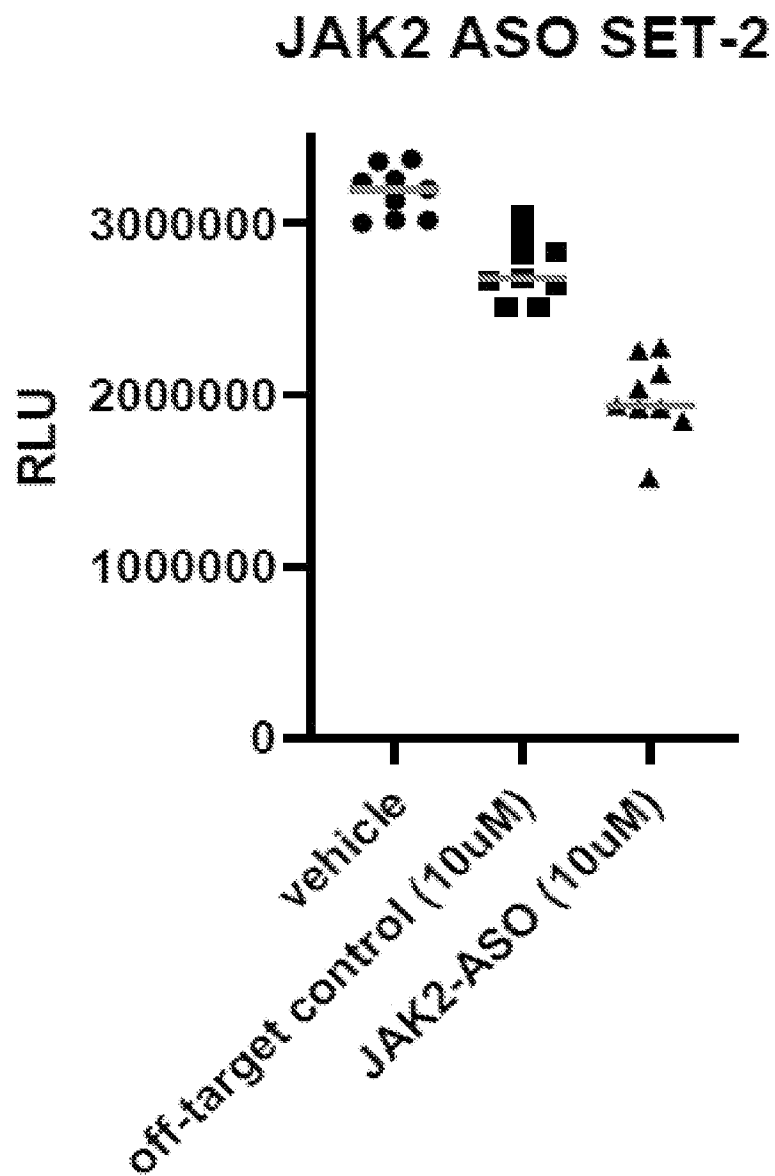


FIG. 2A

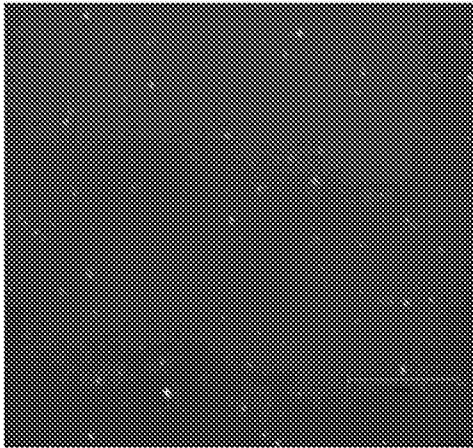


FIG. 2B

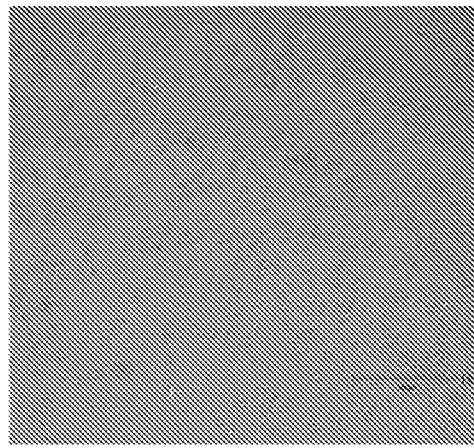


FIG. 3A

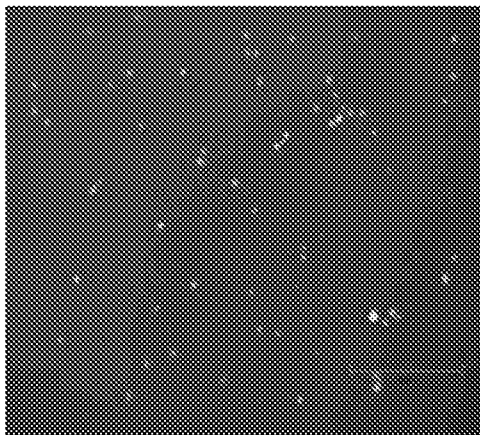


FIG. 3B

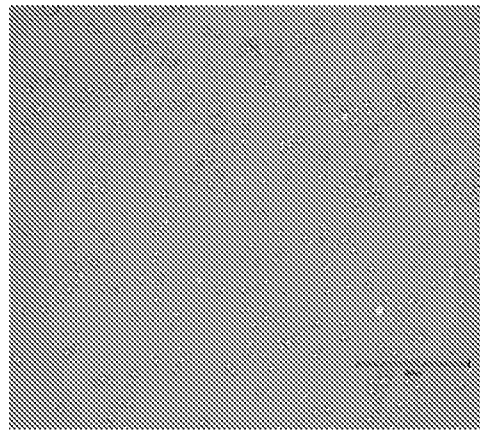


FIG. 4A



FIG. 4B

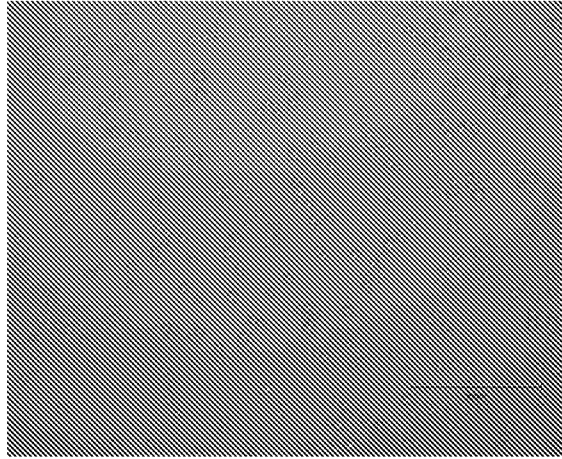


FIG. 5A

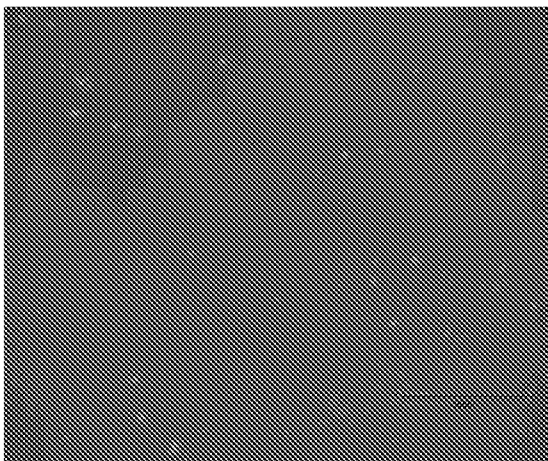


FIG. 5B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/073668

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K31/7088 C12N15/113 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 C12N		
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, WPI Data, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, FSTA		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAKASHIMA OSAMU ET AL: "Inducible Nitric Oxide Synthase Can Be Induced in the Absence of Active Nuclear Factor-[kappa]B in Rat Mesangial Cells : Involvement of the Janus Kinase 2 Signaling Pathway", JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY, vol. 10, no. 4, 1 April 1999 (1999-04-01), pages 721-729, XP055965948, US ISSN: 1046-6673, DOI: 10.1681/ASN.V104721 Retrieved from the Internet: URL:https://jasn.asnjournals.org/content/jnephrol/10/4/721.full.pdf?with-ds=yes>	12
Y	page 722 ----- -/--	1-4, 9-16
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
25 October 2022		03/01/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Piret, Bernard

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/073668

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Zhu Kui ET AL: "Mechanism by which H-2g, a glucose analog of blood group H antigen, mediates angiogenesis", , 15 March 2005 (2005-03-15), XP055965929, DOI: 10.1182/blood-2004-08- Retrieved from the Internet: URL:https://watermark.silverchair.com/zh800605002343.pdf?token=AQECAHi208BE49Oan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAA9YwggPSBgkqhkiG9w0BBwagggPDMIIDvwIBADCCA7gGCSqGSIB3DQEhATAeBglghkgBZQMEAS4wEQQMeTcgbekUVV2RyJo_AgEQgIIDiSe8m6K2Xhs6ZQQuiAMCN6GFqic6o52aQ7wUiZuOL8uhcYLLnxX7iF0KQgGST_27vBzUjhUJen-Y4zq3cYzCt [retrieved on 2022-09-28]	12
Y	page 2344 -----	1-4, 9-16
X	BITTORF T ET AL: "Requirement for JAK2 in Erythropoietin-Induced Signalling Pathways", CELLULAR SIGNALLING, ELSEVIER SCIENCE LTD, GB, vol. 9, no. 1, 1 January 1997 (1997-01-01), pages 85-89, XP027373282, ISSN: 0898-6568 [retrieved on 1997-01-01]	12
Y	page 86 -----	1-4, 9-16
X	YAKUBENKO VALENTIN P. ET AL: "From Macrophage Interleukin-13 Receptor to Foam Cell Formation", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 288, no. 4, 1 January 2013 (2013-01-01), pages 2778-2788, XP055967658, US ISSN: 0021-9258, DOI: 10.1074/jbc.M112.381343	12
Y	page 2780 -----	1-4, 9-16
X	WO 2014/161046 A1 (INST MEDICAL W & E HALL [AU]) 9 October 2014 (2014-10-09) claims 1-7, 20-22 -----	1, 4, 9, 12, 15, 16
X	WO 2017/214463 A1 (UNIV CALIFORNIA [US]) 14 December 2017 (2017-12-14)	1, 4, 9, 12, 15, 16
Y	page 28 - page 29; claims 1-5 -----	1-4, 9-16
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/073668

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KURODA JUNYA ET AL: "NS-018, a selective JAK2 inhibitor, preferentially inhibits CFU-GM colony formation by bone marrow mononuclear cells from high-risk myelodysplastic syndrome patients", LEUKEMIA RESEARCH, vol. 38, no. 5, 1 May 2014 (2014-05-01), pages 619-624, XP055967575, US ISSN: 0145-2126, DOI: 10.1016/j.leukres.2014.03.001 -----	1-4, 9-16
A	BOEHRER SIMONE ET AL: "Erlotinib exhibits antineoplastic off-target effects in AML and MDS: a preclinical study", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 111, no. 4, 15 February 2008 (2008-02-15), pages 2170-2180, XP086509639, ISSN: 0006-4971, DOI: 10.1182/BLOOD-2007-07-100362 [retrieved on 2020-10-31] -----	1-4, 9-16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/073668

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/073668

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1-4 (completely) ; 9-16 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4 (completely); 9-16 (partially)

A method of treating a patient diagnosed with myelodysplastic syndrome (MDS) which comprises: administering to said patient an amount of an ASO-T-JAK2 compound effective to treat such disease.

2. claims: 5-8 (completely); 9-16 (partially)

A method of treating a patient diagnosed with polycythemia vera (PV) which comprises: administering to the patient an amount of an ASO-T-JAK2 compound effective to treat such disease.

3. claims: 17-37

An antisense oligonucleotide (ASO) targeted to a nucleic acid molecule encoding a mutated IGHMBP2 gene; methods and products derived therefrom.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/073668

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014161046 A1	09-10-2014	AU 2014246667 A1	19-11-2015
		EP 2981252 A1	10-02-2016
		US 2016045498 A1	18-02-2016
		WO 2014161046 A1	09-10-2014

WO 2017214463 A1	14-12-2017	US 2019247413 A1	15-08-2019
		WO 2017214463 A1	14-12-2017
