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(71) Applicant: **SANTARIS PHARMA A/S** [DK/DK]; Fremtidsvej 3, DK-2970 Hørsholm (DK).

(72) Inventors: **HEDTJÄRN, Maj**; Kenny Drews vej 83, 2. Lejl. 1, DK-2450 Copenhagen SV (DK). **NIELSEN, Niels Fisker**; Stengårdsvænge 134, DK-2800 Kgs. Lyngby (DK).

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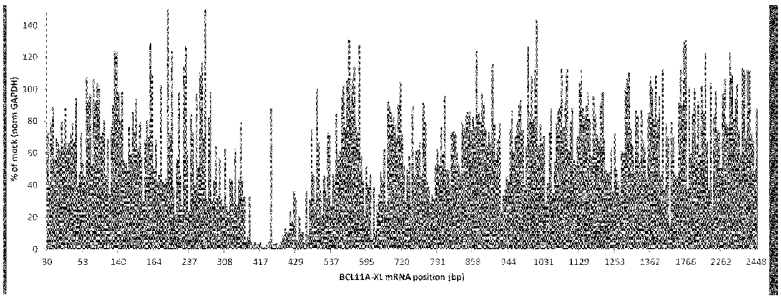


Figure 3

(57) Abstract: The present invention provides, among other things, oligonucleotide modulators (e.g., inhibitors) of B cell lymphoma/leukemia 11A (BCL11A) and improved methods and composition for treating BCL11A-related diseases, disorders or conditions based on such modulators.

OLIGONUCLEOTIDE MODULATORS OF B-CELL CLL/LYMPHOMA 11A (BCL11A) AND USES THEREOF

BACKGROUND

Hemoglobinopathies are diseases that relate to the dysfunction of the hemoglobin protein. Typically, these diseases involve either a lack of or malfunctioning hemoglobin protein, which originate from genetic mutations in globin genes (*e.g.*, alpha, beta, *etc.*; Figure 1). Hemoglobinopathies are one group of a broad spectrum of red blood cell associated disorders that are characterized by single-gene inherited disorders that, in most cases, are autosomal co-dominant traits. It is estimated that about 7% of the world's population are carriers. Hereditary hemoglobinopathies manifest in one of three forms: thalassemia (alpha, beta, delta), sickle-cell disease and hereditary persistence of fetal hemoglobin (HbF). Sickle cell disease (SCD) and beta-thalassemia are the most common forms of hemoglobinopathies and are major causes of morbidity and mortality world-wide. SCD, as the name implies, involves changes in the structure of a globin protein arising from a mutation and results in a malfunctioning hemoglobin protein, while thalassemias are associated with mutations in globin genes that yield an underproduction of normal globin proteins. This can occur through mutations in regulatory proteins. Anemia, in some cases sever, is a common result of hemoglobin dysfunction.

Various treatments for hemoglobinopathies have been explored over time. A major focus has typically been on restoring hemoglobin function, for example, by increasing the level of fetal hemoglobin (HbF). However, not many effective treatments have been successfully developed. Recently, the understanding of mechanisms that regulate HbF has been an area of much research. It was reported that BCL11A is expressed in adult erythroid precursor cells in the bone marrow and functions to repress γ-globin production (Sankaran et al. 2008 Science vol 322 page 1839-1842).

WO 2010/030963 describes modulation of BCL11A using a pool of siRNA samples against 4 target sequences. There is no indication as to whether the individual target sequences are able to down regulate BCL11A.

WO 2012/079046 describes double-stranded ribonucleic acid (dsRNA) compositions targeting the BCL11A gene.

SUMMARY

The present invention provides, among other things, antisense oligonucleotide modulators (e.g., inhibitors) of BCL11A and methods and compositions for treating BCL11A-related diseases, disorders or conditions based on such modulators. It is contemplated that antisense oligonucleotides provided by the present invention are particularly useful for treating hemoglobinopathies, such as sickle cell disease and β-thalassemias.

In one aspect, the present invention provides an antisense oligonucleotide capable of down-regulating or decreasing expression of human BCL11A having a sequence that is at least 80% (e.g. at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the reverse complement of a continuous sequence within a region selected from nucleotides 410 to 450 of the human BCL11A gene of SEQ ID NO 1 or a messenger RNA (mRNA) isoform of BCL11A, wherein the antisense oligonucleotide is a gapmer. In some embodiments, an antisense oligonucleotide of the present invention is less than 19 nucleotides in length. In some embodiments, an antisense oligonucleotide of the present invention is less than 18 nucleotides in length.

In one aspect, the present invention provides an antisense oligonucleotide capable of down-regulating or decreasing expression of human BCL11A having less than 18 nucleotides (e.g, less than 17, 16, 15, 14, 13, or 12) in length and a sequence that is at least about 80% (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the reverse complement of a continuous sequence within a region selected from nucleotides 410 to 450 of the human BCL11A gene of SEQ ID NO 1 or an messenger RNA (mRNA) isoform of BCL11A.

In one aspect, the present invention provides an antisense oligonucleotide capable of down-regulating or decreasing expression of human BCL11A having a sequence at least about 80% (e.g, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the reverse complement of a continuous sequence within a region selected from nucleotides 410 to 450 of the human BCL11A gene of SEQ ID NO 1 or a messenger RNA (mRNA) isoform of BCL11A and is represented by the formula X_a-Y_b-$X_{a'}$, wherein X is a nucleotide analogue; Y is a continuous sequence of DNA; *a* is 1, 2, 3, 4 or 5; *a'* is 1, 2, 3, 4 or 5; and *b* is an integer number between 5 and 15. In some embodiments, the nucleotide analogue is a locked nucleic acid (LNA).

In some embodiments, *a* and *a'* are different. In some embodiments, *a* and *a'* are the same. In some embodiments, *a* and/or *a'* is 1. In some embodiments, *a* and/or *a'* is 2. In some embodiments, *a* and/or *a'* is 3. In some embodiments, *a* and/or *a'* is 4. In some embodiments, *a* and/or *a'* is 5.

In some embodiments, *b* is an integer number between 5 and 15, inclusive. In some embodiments, *b* is an integer number between 5 and 10, inclusive. In some embodiments, *b* is an integer number between 7 and 11, inclusive. In some embodiments, *b* is an integer number selected from the group consisting of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15.

In some embodiments, the isoform of BCL11A is selected from the group consisting of XL, L, M, S and XS or homologs or orthologs thereof. In some embodiments, an antisense oligonucleotide of the present invention is capable of down-regulating or decreasing the expression of the mouse BCL11A gene.

In some embodiments, antisense oligonucleotides of the present invention comprises or contains at least one, at least two, at least three, at least four, at least five, at least six, at least

seven, at least eight or more nucleoside analogues. In some embodiments, an antisense oligonucleotide of the present invention comprises from 3-8 nucleotide analogues, *e.g.* 6 or 7 nucleotide analogues. In some embodiments, at least one of said nucleotide analogues is a locked nucleic acid (LNA); for example at least 3 or at least 4, or at least 5, or at least 6, or at least 7, or 8, of the nucleotide analogues may be LNA. In some embodiments, all the nucleotides analogues may be LNA.

In some embodiments, an oligonucleotide of the present invention comprises or contain at least one, at least two, at least three, at least, four, at least five, at least six, at least seven, at least eight or more LNA units. In some embodiments, the one or more LNA units are located at the 5' and/or '3 ends of the antisense oligonucleotide. In some embodiments, an antisense oligonucleotide of the present invention comprises at least one, at least two or at least three LNA units at the 5' and/or '3 ends. In some embodiments, an antisense oligonucleotide of the present invention comprises at least one, at least two or at least three LNA units internally.

In some embodiments, the LNA unit(s) is a beta-D-oxy-LNA nucleotide. In some embodiment, an antisense oligonucleotide of the present invention comprises one or more additional chemical modifications. In some embodiments, an antisense oligonucleotide of the present invention comprises one or more additional chemical modifications that include a 2'O-methyl modification and/or a phosphorothioate linkage. In some embodiments, an antisense oligonucleotide of the present invention comprises at least one LNA unit that is a LNA 5-methylcytosine nucleotide.

In some embodiments, an antisense oligonucleotide of the present invention has 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 11-17, 11-16, 11-15, 11-14, 11-13, 12-17, 12-16, 12-15, or 12-14 nucleotides in length. In some embodiments, an antisense oligonucleotide of the present invention has 12-16 nucleotides in length.

In some embodiments, an antisense oligonucleotide of the present invention has a sequence that is identical to the reverse complement of a continuous sequence within a region selected from nucleotides 410 to 450 of the human BCL11A gene of SEQ ID NO 1or an messenger RNA (mRNA) isoform of human BCL11A.

An alternative aspect of the present invention is an antisense oligonucleotide capable of decreasing expression of human BCL11A comprising a sequence that is at least 80% identical to the reverse complement of a continuous sequence within a region selected from nucleotides 1-283 (Exon 1), nucleotides 284 – 613 (Exon 2), or nucleotides 614 – 715 (Exon 3) of the human BCL11A gene.

In some embodiments, a continuous sequence according to the present invention is within nucleotides 410 – 450 of the human BCL11A mRNA isoform XL.

In some embodiments, a continuous sequence according to the present invention is within nucleotides 415 – 436 of the human BCL11A mRNA isoform XL.

In some embodiments, a continuous sequence according to the present invention is within nucleotides 420 – 450 of the human BCL11A mRNA isoform XL.

In some embodiments, the oligonucleotide of the invention comprises or consists a sequence motif selected from the group consisting of 5'- ATTGCATTGTTTCCG-3' (SEQ ID NO: 63), 5'- GTTTGTGCTCGAT-3' (SEQ ID NO: 64), 5'- CATTGCATTGTTTCCG-3'(SEQ ID NO: 65), 5'- CGTTTGTGCTCGAT-3'(SEQ ID NO: 66), 5'- CGTTTGTGCTCGATAA-3'(SEQ ID NO: 67), 5'- CCGTTTGTGCTCGA-3'(SEQ ID NO: 68), 5'- CGTTTGTGCTCGA-3' (SEQ ID NO: 69), 5'- TTTGTGCTCGATAA-3'(SEQ ID NO: 70), 5'- TTGTGCTCCATAA-3' (SEQ ID NO: 71) and 5'- TTTCCGTTTGTGCTCG (SEQ ID NO: 72), 5'- ATTGCATTGTTTCCGT-3' (SEQ ID NO: 73), 5'-CGTTTGTGCTCGATA-3' (SEQ ID NO: 74).

In some embodiments, an antisense oligonucleotide of the present invention has a sequence selected from Table 2.

In some embodiments, an antisense oligonucleotide of the present invention has a sequence selected from SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 32, SEQ ID NO: 21, SEQ ID NO: 34, SEQ ID NO:10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO:27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30 SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 60, SEQ ID NO: 61 or SEQ ID NO: 62,

In some embodiments, an antisense oligonucleotide of the present invention is selected from 5'- $^{m}C_s{}^{o}$ $A_s{}^{o}$ $T_s{}^{o}$ t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s{}^{o}$ $^{m}C_s{}^{o}$ G^{o} -3' (SEQ ID NO: 11), 5' - **$^{m}C_s{}^{o}$ $G_s{}^{o}$ $T_s{}^{o}$** t_s t_s g_s t_s g_s c_s t_s $^{m}c_s g_s a_s$ **$T_s{}^{o}A_s{}^{o}A^{o}$**- 3' (SEQ ID NO: 15) or 5' - **$^{m}C_s{}^{o}$ $G_s{}^{o}$ $T_s{}^{o}$** t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s **$A_s{}^{o}$ $T_s{}^{o}$ A^{o}-** 3' (SEQ ID NO: 32), 5' - **$T_s{}^{o}$ $T_s{}^{o}$ $G_s{}^{o}$** t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s **$A_s{}^{o}$ $A_s{}^{o}$ A^{o}** – 3' (SEQ ID NO: 14) and 5' - **$^{m}C_s{}^{o}$ $G_s{}^{o}$ $T_s{}^{o}$** t_s t_s g_s t_s g_s c_s t_s c_s **$G_s{}^{o}$ $A_s{}^{o}$ T^{o}** – 3' (SEQ ID NO: 35), wherein upper case letters indicate locked nucleic acid (LNA) units, subscript "s" represents phosphorothioate linkage, and lower case letters represent deoxyribonucleotide (DNA) units, "^{m}C" represents 5' methyl-cytosine LNA unit, and "^{m}c" represents 5' methyl-cytosine DNA unit.

In some embodiments, an antisense oligonucleotide of the present invention is 5'- $^{m}C_s{}^{o}$ $A_s{}^{o}$ $T_s{}^{o}$ t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s{}^{o}$ $^{m}C_s{}^{o}$ G^{o}-3'(SEQ ID NO: 11).

In another aspect, the present invention provides an antisense oligonucleotide capable of down-regulating or decreasing the expression of the human BCL11A gene having a sequence at least 80% (*e.g.,* 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an oligonucleotide sequence selected from Table 2.

In some embodiments, an antisense oligonucleotide according to the present invention has a sequence at least 80% (*e.g.,* 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an oligonucleotide sequence selected from 5'- $^{m}C_s^{o}$ A_s^{o} T_s^{o} t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s^{o}$ $^{m}C_s^{o}$ G^{o}-3' (SEQ ID NO: 11), 5' - **$^{m}C_s^{o}$ G_s^{o} T_s^{o}** t_s t_s g_s t_s g_s c_s t_s $^{m}c_s g_s a_s$ **$T_s^{o} A_s^{o} A^{o}$**- 3' (SEQ ID NO: 15) or 5' - **$^{m}C_s^{o}$ G_s^{o} T_s^{o}** t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s **A_s^{o} T_s^{o} A^{o}-** 3' (SEQ ID NO: 32) 5' - **$^{m}C_s^{o}$ G_s^{o} T_s^{o}** t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s **A_s^{o} T_s^{o} A^{o}** – 3' (SEQ ID NO: 32), 5' - **$^{m}C_s^{o}$ G_s^{o} T_s^{o}** t_s t_s g_s t_s g_s c_s t_s c_s **G_s^{o} A_s^{o} T^{o}** – 3' (SEQ ID NO: 35), 5' - **T_s^{o} T_s^{o} G_s^{o}** t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s **A_s^{o} A_s^{o} A^{o}** – 3' (SEQ ID NO: 14) and 5' - **$^{m}C_s^{o}$ G_s^{o} T_s^{o}** t_s t_s g_s t_s g_s c_s t_s c_s **G_s^{o} A_s^{o} T^{o}** – 3' (SEQ ID NO: 35), wherein upper case letters indicate locked nucleic acid (LNA) units, subscript "s" represents phosphorothioate linkage, and lower case letters represent deoxyribonucleotide (DNA) units, "^{m}C" represents 5' methyl-cytosine LNA unit, and "^{m}c" represents 5' methyl-cytosine DNA unit.

In some embodiments, a pharmaceutical composition comprising an antisense oligonucleotide as described herein and a pharmaceutically acceptable carrier is provided.

Among other things, the present invention provides a method of inhibiting BCL11A in a subject comprises administering to a subject in need of treatment an antisense oligonucleotide or a pharmaceutical composition as described herein.

Among other things , the present invention provides an antisense oligonucleotide for use in a method of inhibiting BCL11A comprising a step of administering to a subject in need of treatment an antisense oligonucleotide or a pharmaceutical composition as described herein.

In some embodiments, the present invention provides use of an antisense oligonucleotide or pharmaceutical composition of the present invention in the manufacture of a medicament for the treatment of an anemic disease, disorder or condition, such as sickle cell disease or β-thalassemia. In particular in the manufacture of a medicament for inhibiting BCL11A comprising administering an antisense oligonucleotide or pharmaceutical composition as described herein to a subject.

In some embodiments, the present invention provides, the antisense oligonucleotide according pharmaceutical composition of the present invention, for use as a medicament, such as for the treatment of an anemic disease, disorder or condition, such as sickle cell disease or β-thalassemia.

In some embodiments, the present invention provides a method of treating an anemic disease, disorder or condition in a subject comprises administering to a subject in need of treatment an antisense oligonucleotide or a pharmaceutical composition as described herein.

In some embodiments, the present invention provides an antisense oligonucleotide for use in the treatment of a disease or disorder such as those referred to herein, such as a hemoglobinopathie, such as an anemic disease, disorder or condition, such as thalassemia (α, β, δ), sickle-cell disease and hereditary persistence of fetal hemoglobin (HbF.)

In some embodiments, the present invention provides an antisense oligonucleotide for use in a method of treating an anemic disease, disorder or condition in a subject comprising

administering to a subject in need of treatment an antisense oligonucleotide or a pharmaceutical composition as described herein.In some embodiments, treatment methods of the present invention further comprise administering a second agent to a subject for the treatment of a disease or disorder, such as for the treatment of an anemic disease, disorder or condition.

In some embodiments, the anemic disease, disorder or condition treated by a method of the present invention is sickle cell disease.

In some embodiments, the anemic disease, disorder or condition treated by a method of the present invention is β-thalassemia.

In some embodiments, the administering of the antisense oligonucleotide or the pharmaceutical composition results in reduced expression of BCL11A in one or more target tissues. In some embodiments, the administering of the antisense oligonucleotide or the pharmaceutical composition results in increased γ-globin expression in one or more target tissues. In some embodiments, the administering of the antisense oligonucleotide or the pharmaceutical composition results in increased fetal hemoglobin production in one or more target tissues. In some embodiments, one or more target tissues are selected from bone marrow, liver, kidney, spleen, plasma cells, thymus, tonsillar epithelium, erythroid progenitor cells, pluripotent stem cells, dendritic cells and/or peripheral blood B-cells. In some embodiments, an antisense oligonucleotide or pharmaceutical composition is administered intravenously. In some embodiments, an antisense oligonucleotide or pharmaceutical composition is administered subcutaneously.

In some embodiments, the present invention provides a container comprising an antisense oligonucleotide or pharmaceutical composition as described herein. In some embodiments, an antisense oligonucleotide or pharmaceutical compostion of the present invention is provided in a single dosage form. In some embodiments, an antisense oligonucleotide or pharmaceutical composition of the present invention is provided in multiple (e.g, two, three, four, five or more) dosage form. In some embodiments, an antisense oligonucleotide or pharmaceutical composition of the present invention is provided in lyophilized form. In some embodiments, an antisense oligonucleotide or pharmaceutical composition of the present invention is provided in liquid form.

In some embodiments, the container is selected from an ampule, a vial, a cartridge, a reservoir, a lyo-ject, and a pre-filled syringe. In some embodiments, the container is a pre-filled syringe and is optionally selected from a borsilicate glass syringe with baked silicone coating, a borosilicate glass syringe with sprayed silicone, and a plastic resin syringe without silicone.

As used in this application, the terms “about” and “approximately” are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF DRAWINGS

The drawings are for illustration purposes only, not for limitation.

Figure 1 shows an exemplary illustration of the percent of total hemoglobin (y-axis) versus gestational and postnatal age in weeks (x-axis) for different globin chains in normal individuals (left) and individuals with beta globin chain dysfunction (right). Adapted and modified from Figure 167-2, Chapter 167. Cecil Medicine 23rd ed., Lee W. Goldman, Dennis A. Ausiello.
W.B. Saunders Elsevier 2008.

Figure 2 shows a schematic illustration, not to scale, of the three major isoforms of human BCL11A (S, L, and XL). Exons are labeled as they are found in each isoform from 5' to 3'.

Figure 3 shows exemplary inhibition of BCL11A-XL mRNA expression in human REH cells by 401 antisense oligonucleotides targeting BCL11A at a concentration of 25 μM.

Figure 4 shows exemplary inhibition of BCL11A-XL mRNA expression in human REH cells by selected antisense oligonucleotides targeting BCL11A at oligonucleotide concentrations ranging from 0.0064 to 20 μM.

Figure 5 shows exemplary inhibition of BCL11A-XL mRNA expression in human REH cells by selected antisense oligonucleotides designed from oligo 4 (top) and 5 (bottom) targeting BCL11A at oligonucleotide concentrations ranging from 0.25 to 60 μM.

Figure 6 shows exemplary inhibition of the major isoforms (S, L, and XL) of BCL11A mRNA expression in human REH cells by selected antisense oligonucleotides at concentrations ranging from 0.25 to 60 μM. Measurements of mRNA of BCL11A XL, L and S isoforms are shown in the left, middle and right columns for each concentration within each treatment group, respectively.

Figure 7 shows exemplary inhibition of mouse BCL11A mRNA expression in mouse MPC-11 cells by selected antisense oligonucleotides at concentrations ranging from 0.08 to 20 μM. Measurement of all isoforms (BCL11A-All) and isoform L (BCL11A-L) of mouse BCL11A are shown in the left and right columns for each concentration within each treatment group, respectively.

Figure 8 shows exemplary inhibition of BCL11A mRNA expression in the bone marrow (top) and spleen (bottom) of groups of female NMRI mice dosed with 15 mg/kg of selected antisense oligonucleotides targeting BCL11A. Measurements for bone marrow include all

isoforms (left column) and isoform L (right column) for each antisense oligonucleotide treatment group.

Figure 9 shows exemplary inhibition of BCL11A mRNA in bone marrow of wild-type C57BL/6 mice four weeks after administration with 25 or 15 mg/kg of selected antisense oligonucleotides targeting BCL11A.

Figure 10 shows exemplary inhibition of BCL11A mRNA in bone marrow of wild-type C57BL/6 mice eight weeks after administration with 25 or 15 mg/kg of selected antisense oligonucleotides targeting BCL11A.

Figure 11 shows exemplary inhibition of BCL11A mRNA in bone marrow of human β-YAC transgenic mice eight weeks post administration with 15 mg/kg of selected antisense oligonucleotides targeting BCL11A.

Figure 12 shows exemplary inhibition of BCL11A mRNA in $Ter119^+$ and $CD19^+$ bone marrow cell populations of human β-YAC transgenic mice eight weeks post administration with 15 mg/kg of selected antisense oligonucleotides targeting BCL11A.

Figure 13 shows exemplary total hemoglobin (g/L) in peripheral blood of non-human primate animals in phlebotomized (phleb.) and non-phlebotomized treatment groups at various treatment days. Vehicle control (saline) and candidate oligonucleotide 4 (10 and 20 mg/kg) treatment groups are indicated.

Figure 14 shows exemplary percent of reticulocytes in peripheral blood of non-human primate animals in phlebotomized (phleb.) and non-phlebotomized treatment groups at various treatment days. Vehicle control (saline) and candidate oligonucleotide 4 (10 and 20 mg/kg) treatment groups are indicated.

Figure 15 shows exemplary expression of BCL11A normalized to GAPDH in humerus bone marrow of phlebotomized non-human primate animals dosed with vehicle control (saline) or candidate oligonucleotide 4 at 20 mg/kg at week seven of a study. Measurements for isoform XL (left column) and all isoforms (right column) is shown for each treatment animal.

Figure 16 shows exemplary γ- and β-globin mRNA expression normalized to GAPDH in humerus bone marrow of phlebotomized non-human primate animals dosed with vehicle control (saline) or candidate oligonucleotide 4 at 20 mg/kg at week seven of a study. Measurements of human γ-globin (Gamma A+G ,column 1), *Macaca mulatta* γ-globin (HBG2, column 2), *Macaca mulatta* β-globin (HBB, column 3; HBB_mH, column 4) are shown for each animal within each treatment group.

Figure 17 shows exemplary expression of BCL11A mRNA normalized to GAPDH in humerus (top) and femur (bottom) bone marrow of phlebotomized non-human primate animals for control (saline) and candidate oligonucleotide 4 (10 and 20 mg/kg) treatment groups at week 17 of a study. Measurements of isoform XL (left column) and all isoforms (right column) are shown for each animal within each treatment group.

Figure 18 shows exemplary expression of γ-globin mRNA normalized to GAPDH in humerus (top) and femur (bottom) bone marrow in phlebotomized non-human primate animals for control (saline) and candidate oligonucleotide 4 (10 and 20 mg/kg) treatment groups at week 17 of a study. Measurements of human γ-globin (Gamma A+G, left column) and *Macaca mulatta* γ-globin (HBG2, right column) are shown for each animal within each treatment group.

Figure 19 shows exemplary expression of γ- and β-globin mRNA normalized to GAPDH in humerus bone marrow in control (saline) and candidate oligonucleotide 4 (10 and 20 mg/kg) treatment groups of phlebotomized non-human primate animals at week 17. Columns from left to right for each animal within each treatment group measurements of human γ-globin (Gamma A+G), *Macaca mulatta* γ-globin (HBG2), *Macaca mulatta* β-globin (RhHBB), and *Macaca mulatta* β-globin (RhHBB_mH), respectively.

Figure 20 shows exemplary expression of γ- and β-globin mRNA normalized to GAPDH in femur bone marrow in control (saline) and candidate oligonucleotide 4 (10 and 20 mg/kg) treatment groups of phlebotomized non-human primate animals at week 17. Columns from left to right for each animal within each treatment group measurements of human γ-globin (Gamma A+G), *Macaca mulatta* γ-globin (HBG2), *Macaca mulatta* β-globin (RhHBB), and *Macaca mulatta* β-globin (RhHBB_mH), respectively.

Figure 21 shows exemplary average expression of BCL11A (top) and γ-globin (bottom) mRNA normalized to GAPDH in humerus bone marrow of phlebotomized non-human primate animals in control (saline) and candidate oligonucleotide 4 (10 and 20 mg/kg) treatment groups at week 17. Measurements of isoform XL (left column) and all isoforms (right column) of BCL11A are shown for each animal within each treatment group. Measurements of human γ-globin (Gamma A+G, left column) and *Macaca mulatta* γ-globin (HBG2, right column) are shown for each animal within each treatment group.

Figure 22 shows exemplary average expression of BCL11A (top) and γ-globin (bottom) mRNA normalized to GAPDH in femur bone marrow of phlebotomized non-human primate animals in control (saline) and candidate oligonucleotide 4 (10 and 20 mg/kg) treatment groups at week 17. Measurements of isoform XL (left column) and all isoforms (right column) of BCL11A are shown for each animal within each treatment group. Measurements of human γ-globin (Gamma A+G, left column) and *Macaca mulatta* γ-globin (HBG2, right column) are shown for each animal within each treatment group.

Figure 23 shows exemplary fraction (‰) of F-cells in bone marrow for phlebotomized non-human primate animals at full scale (left) and zoomed-in scale (right).

Figure 24 shows exemplary fraction (‰) of F-cells in peripheral blood for phlebotomized non-human primate animals at full scale (left) and zoomed-in scale (right).

Figure 25 shows exemplary measurements of γ-globin protein in peripheral blood of non-human primate animals in control (top), 10 mg/kg (middle), and 20 mg/kg (bottom) treatment groups at various weeks after first dose.

Figure 26 shows exemplary measurements of γ-globin protein in peripheral blood of non-human primate animals as a percent of control at a respective time point of a γ-globin peak ("peak 1" or "peak 2") in control treated groups.

Figure 27 shows exemplary measurements of γ-globin protein in peripheral blood of non-human primate animals as a percent of control at a respective time point of a γ-globin peak ("peak 1" or "peak 2") in 10 mg/kg dose groups.

Figure 28 shows exemplary measurements of γ-globin protein in peripheral blood of non-human primate animals as a percent of control at a respective time point of a γ-globin peak ("peak 1" or "peak 2") in 20 mg/kg dose groups.

Figure 29 shows exemplary measurements of plasma concentration of antisense oligonucleotide 4 over time in wild-type mice.

Figure 30 shows exemplary measurements of concentration of antisense oligonucleotide 4 in various tissues over time from wild-type mice.

Figure 31 shows exemplary measurements of tissue concentration of antisense oligonucleotide 4 in various tissues over time from wild-type mice.

Figure 32 shows an exemplary model of predicted concentration of an antisense oligonucleotide of the present invention in bone marrow based on a single dose pharmacokinetic study.

DEFINITIONS

In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

Approximately or about: As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

Biologically active: As used herein, the phrase "biologically active" refers to a characteristic of any agent that has activity in a biological system, *in vitro* or *in vivo* (e.g., in an organism). For instance, an agent that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular embodiments, where a protein or polypeptide is biologically active, a portion of that protein or polypeptide that

shares at least one biological activity of the protein or polypeptide is typically referred to as a "biologically active" portion.

Improve, increase, reduce or inhibit: As used herein, the terms "improve," "increase," "reduce" or "inhibit" or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A "control individual" is an individual afflicted with the same form of disease as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

Individual, subject, patient: As used herein, the terms "subject," "individual" or "patient" refer to a human or a non-human mammalian subject. The individual (also referred to as "patient" or "subject") being treated is an individual (fetus, infant, child, adolescent, or adult human) suffering from a disease.

Locked Nucleic Acid (LNA): As used herein, the term "LNA" or "Locked Nucleic Acid" refers to a bicyclic nucleotide analogue, preferably a bicyclic nucleotide analogue with a bridge between the 2' and 4' position in the ribose ring (2' to 4' bicyclic nucleotide analogue). LNA is in the literature sometimes referred to as BNA (bridged nucleic acid or bicyclic nucleic acid). It may refer to an LNA monomer, or when used in the context of an "LNA oligonucleotide" refers to an oligonucleotide containing one or more such bicyclic nucleotide analogues.

Nucleotide: As used herein, the term "nucleotide", refers to a glycoside comprising a sugar moiety, a base moiety and a covalently linked phosphate group and covers both naturally occurring nucleotides, such as DNA or RNA, preferably DNA, and non-naturally occurring nucleotides comprising modified sugar and/or base moieties, which are also referred to as "nucleotide analogues" herein. In some embodiments, non-naturally occurring nucleotides include nucleotides which have modified sugar moieties, such as bicyclic nucleotides or 2' modified nucleotides, such as 2' substituted nucleotides. In some embodiments, non-naturally occurring nucleotides include locked nucleic acid (LNA).

Substantial homology: The phrase "substantial homology" is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "substantially homologous" if they contain homologous residues in corresponding positions. Homologous residues may be identical residues. Alternatively, homologous residues may be non-identical residues with appropriately similar structural and/or functional characteristics. For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as "hydrophobic" or "hydrophilic" amino acids, and/or as having "polar" or "non-polar" side chains. Substitution of one amino acid for another of the same type may often be considered a "homologous" substitution.

As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, *et al.*, Basic local alignment search tool, *J. Mol. Biol.*, 215(3): 403-410, 1990; Altschul, *et al.*, *Methods in Enzymology*; Altschul, *et al.*, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis, *et al.*, *Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, *et al.*, (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying homologous sequences, the programs mentioned above typically provide an indication of the degree of homology. In some embodiments, two sequences are considered to be substantially homologous if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are homologous over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 9, 10, 11, 12, 13, 14, 15, 16, 17 or more residues. In some embodiments, the relevant stretch includes contiguous residues along a complete sequence. In some embodiments, the relevant stretch includes discontinuous residues along a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more residues.

Substantial identity: The phrase “substantial identity” is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be “substantially identical” if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences as well as EMBOSS needel for global alignments or EMBOSS Water for local alignments. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, *J. Mol. Biol.*, 215(3): 403-410, 1990; Altschul, *et al.*, *Methods in Enzymology*; Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis *et al.*, *Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is the complete

sequence of the oligonucleotide. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more residues. *Target tissues*: As used herein , the term "target tissues" refers to any tissue that is affected by the defects in or lower than desired activity from protein subunits, or globin chains, that make up hemoglobin, especially in the liver, spleen and bone marrow. In some embodiments, target tissues include those tissues in which there is an abnormality in the expression of the globin chains, *e.g.*, alpha, beta or gamma. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature. As used herein, a target tissue may be a liver target tissue, a spleen target tissue and/or a bone marrow target tissue. Exemplary target tissues are described in detail below.

Therapeutically effective amount: As used herein, the term "therapeutically effective amount" refers to an amount of a therapeutic agent which confers a therapeutic effect on the treated subject, at a reasonable benefit/risk ratio applicable to any medical treatment. The therapeutic effect may be objective (*i.e.*, measurable by some test or marker) or subjective (*i.e.*, subject gives an indication of or feels an effect). In particular, the "therapeutically effective amount" refers to an amount of a therapeutic agent or composition effective to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease. A therapeutically effective amount is commonly administered in a dosing regimen that may comprise multiple unit doses. For any particular therapeutic agent, a therapeutically effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration, on combination with other pharmaceutical agents. Also, the specific therapeutically effective amount (and/or unit dose) for any particular patient may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific pharmaceutical agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and/or rate of excretion or metabolism of the specific agent employed; the duration of the treatment; and like factors as is well known in the medical arts.

Treatment: As used herein, the term "treatment" (also "treat" or "treating") refers to any administration of a therapeutic agent (*e.g.*, oligonucleotide) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of a particular disease, disorder, and/or condition (*e.g.*, hemoglobin dysfunction or deficiency, sickle cell disease, thalassemia). Such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits

one or more established signs of the relevant disease, disorder and/or condition. Exemplary signs of a relevant disease as described herein include anemia, which may range from moderate to serve depending on the patient who manifests the signs.

DETAILED DESCRIPTION

The present invention provides, among other things, improved compositions and methods for modulating B-cell CLL/Lymphoma 11A (BCL11A) activity and for treatment of a disease, disorder or condition associated with BCL11A. It is contemplated that reducing or inhibiting BCL11A activity results in increased expression of globin genes, *e.g.,* gamma globin. Therefore, the present invention is particularly useful for treating hemoglobinopathies, such as sickle cell disease and β-thalassemias. In particular, the present invention is based on antisense oligonucleotide modulators of BCL11A that reduce or inhibit BCL11A activity by down-regulating or decreasing expression of BCL11A. In some embodiments, an oligonucleotide capable of down-regulating or decreasing the expression of the human BCL11A gene target a region of the human BCL11A gene or an messenger RNA (mRNA) isoform of BCL11A (e.g., XL, L, M, S or XS). In some embodiments, an oligonucleotide capable of down-regulating or decreasing the expression of the human BCL11A gene has a sequence based on the reverse complement of a continuous sequence of the human BCL11A gene or an messenger RNA (mRNA) isoform of BCL11A.

Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

BCL11A and Related Diseases and Conditions

The human BCL11A gene encodes a C_2H_2 zinc finger protein having similarity to the mouse BCL11A protein. BCL11A is a lymphoid transcription factor that functions in B cells, and, up until recently, was unknown to have a role in erythropoiesis. BCL11A is now understood to have a role in globin gene regulation and expression appears to correlate with developmental expression of globin genes. BCL11A is expressed in adult erythroid precursor cells in the bone marrow and functions in an inverse relationship with gamma globin genes, *i.e.*, BCL11A functions as a repressor of gamma globin production.

BCL11A is represented in several isoforms. Figure 2 sets forth three major isoforms of BCL11A, which differ in the usage of two potential 3' terminal exons. BCL11A is known to associate with other proteins to form complexes that function to regulate the fetal-to-adult hemoglobin switch. BCL11A is implicated in disease associated with hemoglobin dysfunction. In particular, inhibition of BCL11A upregulates gamma globin expression and, as a result, production of fetal hemoglobin, which can compensate for globin gene dysfunction encountered in hemoglobinopathies, such as sickle cell disease, β-thalassemias, and the like.

Modulators of BCL11A

As discussed in the Examples below, the present inventors have successfully identified antisense oligonucleotide modulators that target one or more isoforms of BCL11A. In some embodiments, modulators according to the present invention target a region common to the three major isoforms depicted in Figure 2. Specifically the present inventors have identified a specific region within Exon 2 from nucleotides 410 to 450 of the human BCL11A gene that very efficiently downregulates BCL11A. The corresponding region in the mouse BCL11A gene (e.g., XL, L or S) range from nucleotides 517 to 557. Figure 3 clearly shows that across Exons 1, 2, 3 and 4, this region is a hotspot in terms of designing single stranded oligonucleotides capable of decreasing the expression of BCL11A. The knowledge of such a hotspot increases the likelihood of success in designing an oligonucleotide with good potency and which is well tolerated by the subject to be treated.

Design of antisense oligonucleotides

Among other things, the present invention provides antisense oligonucleotides useful for modulation of nucleic acid molecules encoding human BCL11A. In particular, an antisense oligonucleotide suitable for the present invention includes any oligonucleotide that is capable of down-regulating or decreasing, reducing or inhibiting BCL11A expression or activity.

Typically, an oligonucleotide capable of down-regulating or decreasing the expression of the human BCL11A gene may be designed based on the sequence of the human BCL11A gene or an messenger RNA (mRNA) isoform of BCL11A (e.g., XL, L or S). For example, an oligonucleotide capable of down-regulating or decreasing the expression of the human BCL11A gene may have a sequence that is substantially identical to the reverse complement of a continuous sequence of the human BCL11A gene or an messenger RNA (mRNA) isoform of BCL11A. In some embodiments, an oligonucleotide according to the present invention has a sequence at least about 50% (e.g., at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the reverse complement of a continuous sequence of the human BCL11A gene or an messenger RNA (mRNA) isoform of BCL11A. Since the human BCL11A gene and mouse BCL11A gene share high sequence identity, an oligonucleotide according to the present invention may also be designed based on the sequence of the mouse BCL11A gene or an messenger RNA (mRNA) isoform of BCL11A. In some embodiments, an oligonucleotide according to the present invention has a sequence at least about 50% (e.g., at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the reverse complement of a continuous sequence of the mouse BCL11A gene or an messenger RNA (mRNA) isoform of BCL11A.

Alternatively, an oligonucleotide capable of down-regulating or decreasing the expression of the human BCL11A gene is capable of hybridizing or binding to a target region of one or more isoforms of BCL11A mRNA. In some embodiments, an oligonucleotide capable of decreasing the expression of the human BCL11A gene is capable of hybridizing or binding to a

target region of BCL11A mRNA that is found in an exon (*e.g.,* exon 1, exon 2, exon 3, exon 4, or exon 5). In some embodiments, an oligonucleotide capable of decreasing the expression of the human BCL11A gene is capable of hybridizing or binding to a target region of human or mouse BCL11A.

It will be appreciated that hybridization of an antisense oligonucleotide to a target region of BCL11A mRNA may be performed *in vitro* or *in vivo*. Hybridization may be performed under low, medium, and/or stringent hybridization conditions, as is well known in the art. In general, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are used to identify molecules having complementary nucleic acid sequences. Stringent hybridization conditions typically permit binding between nucleic acid molecules having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more nucleic acid sequence identity. Standard conditions are disclosed, for example, in Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Labs Press, the contents of which is incorporated herein by reference in its entirety. Formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting 50%, 40%, 30%, 20%, 10%, 5% or less mismatch of nucleotides are available in the art, for example, in Meinkoth *et al.*, 1984, Anal. Biochem. 138, 267-284; the contents of which is incorporated herein by reference in its entirety. It will be appreciated that hybrids between oligonucleotides (14-20 bp) and immobilized DNA show decreased stability and should be taken into account when defining optimal conditions for their hybridization.

Hybridization condition stringency can be affected by buffer ionic strength, base composition of the nucleotide, the length of the shortest chain in the duplex (n), and the concentration of helix destabilizing agents such as formamide. For example, hybridization stringency can be altered by adjusting the salt and/or formamide concentrations and/or by changing the temperature. The stringency can be adjusted either during the hybridization step, or in post hybridization washes. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or Northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of stringent wash conditions is a 0.2X SSC wash at 65°C. for 15 minutes. In some embodiments, a high stringency wash is preceded by a low stringency wash to remove back-ground probe signal. An example medium stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 100X SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 4X SSC at 40°C. for 15 minutes. In general, a signal to noise ratio of 2X (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

Sequences of BCL11A mRNA Isoforms

As described above, Figure 2 sets forth the three major human BCL11A mRNA isoforms, i.e., isoform XL, L and S. Similarly, there three major mouse BCL11A mRNA isoforms, i.e., isoform XL, L and S. For both mouse and human, other BCL11A mRNA isoforms have been identified. For example, in humans several isoforms based on alternative splice variants are described in the Ensembl genebuild assemblies (European Bioinformatics Institute and Wellcome Trust Sanger Institute), which are identified by the following transcript identification numbers: ENST00000358510, ENST00000538214, ENST00000537768, ENST00000477659, ENST00000489516, ENST00000409351, ENST00000479026, ENST00000492272, ENST00000489183. Sequences of exemplary human and mouse isoforms of BCL11A are set forth in the sequence list with indication of exons:

SEQ ID NO: 1 = Human BCL11A-XL NCBI accession number NM_022893

SEQ ID NO: 2 = Human BCL11A-L NCBI accession number NM_018014

SEQ ID NO: 3 = Human BCL11A-S NCBI accession number NM_138559

SEQ ID NO: 4 = Mouse BCL11A-XL NCBI accession number NM_001242934

SEQ ID NO: 5 = Mouse BCL11A-L NCBI accession number NM_016707

SEQ ID NO: 6 = Mouse BCL11A-S L NCBI accession number NM_001159289

SEQ ID NO: 7= Mouse BCL11A-XS NCBI accession number NM_001159290

In some embodiments, provided antisense oligonucleotides bind to a region within one or more isoforms of a human or mouse BCL11A as shown in SEQ ID NO: 1 to 7. In some embodiments, provided antisense oligonucleotides bind to a region within an exon of a human or mouse BCL11A isoform as shown in SEQ ID NO: 1 to 7. In some embodiments, provided antisense oligonucleotides bind to a region within an exon of an isoform of human BCL11A, mouse BCL11A, or a combination thereof. In some embodiments, provided antisense oligonucleotides bind to a region within nucleotides 1-283, 284 – 613, or 614 – 715 of a human BCL11A. Preferably, nucleotides 1-283, 284 – 613, or 614 – 715 of SEQ ID NO: 1. In some embodiments, provided antisense oligonucleotides bind to a region within nucleotides 250 – 500, 259 – 438, 284 – 613, 415 – 445, 415 – 436, 716 – 5946, 716 – 2458, 2459 – 3958, or nucleotides 859 – 2358 of a human BCL11A. In various embodiments, a human BCL11A is selected from isoforms XL, L or S as shown in SEQ ID NO: 1 to 3. In various embodiments, a mouse BCL11A is selected from isoforms XL, L or S as shown in SEQ ID NO: 4 to 7.

In some embodiments, an antisense oligonucleotide of the present invention has a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to the reverse complement of a continuous sequence of a human or mouse BCL11A gene or a messenger RNA (mRNA) isoform of a human or mouse BCL11A. In some embodiments, an oligonucleotide of the present invention has a sequence that is identical to the reverse complement of a continuous sequence of the human or mouse BCL11A gene or an messenger RNA (mRNA) isoform of human or mouse BCL11A.

In some embodiments, a continuous sequence according to the present invention is within a region selected from nucleotides 1-283 (Exon 1), nucleotides 284 – 613 (Exon 2), or nucleotides 614 – 715 (Exon 3) of the human BCL11A gene.

In some embodiments, a continuous sequence according to the present invention is within nucleotides of a human BCL11A mRNA isoform XL (SEQ ID NO: 1). In some embodiments, a continuous sequence according to the present invention is within nucleotides 200 – 620, 410 – 450, 415 – 436, 415 - 446, 420 – 450, or within nucleotides 716 – 5946 (exon 4) of a human BCL11A mRNA isoform XL (SEQ ID NO: 1).

In some embodiments, a continuous sequence according to the present invention is within nucleotides of a human BCL11A mRNA isoform L (SEQ ID NO: 2). In some embodiments, a continuous sequence according to the present invention is within nucleotides 716 – 2458 (exon 4) or nucleotides 2459 – 3958 (exon 5) of a human BCL11A mRNA isoform L.

In some embodiments, a continuous sequence according to the present invention is within nucleotides of a human BCL11A mRNA isoform S (SEQ ID NO: 3). In some embodiments, a continuous sequence according to the present invention is within nucleotides 716 – 858 (exon 4) or nucleotides 859 – 2358 (exon 5) of a human BCL11A mRNA isoform S.

In some embodiments, provided antisense oligonucleotides bind to a target region that is substantially identical to the corresponding region of the human or mouse BCL11A as shown in SEQ ID NO: 1 to 7. For example, provided antisense oligonucleotides may bind to a target region that has a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to that of the corresponding region (e.g., exon 1, 2, 3, 4, or 5) of the human or mouse BCL11A as shown in SEQ ID NO: 1 to 7. Exemplary regions are described throughout the specification.

The Oligonucleotide

The term “oligonucleotide” in the context of the present invention, refers to a molecule formed by covalent linkage of two or more nucleotides. The term is used interchangeably with the term oligomer. Herein, a single nucleotide (unit) may also be referred to as a monomer or unit. In some embodiments, the terms “nucleoside”, “nucleotide”, “unit” and “monomer” are used interchangeably. It will be recognized that when referring to a sequence of nucleotides or monomers, what is referred to is the sequence of bases, such as A, T, G, C or U.

The oligonucleotide of the invention is capable of decreasing expression of human BCL11A comprising a sequence that is at least 80% identical to the reverse complement of a continuous sequence within a region selected from nucleotides 410 to 450 of the human BCL11A gene or an messenger RNA (mRNA) isoform of BCL11A.

In some embodiments, the oligonucleotide of the invention comprises or consists a sequence motif selected from the group shown in Table 1. Sequence motifs are essentially a

nucleotide sequence that can be used as the basis for generating oligonucleotides that essentially comprise or contain the same sequence but varies for example in the number of nucleotide analouges, length or internucleotide linkages.

Table 1. Sequence motifs that can be used to design specific oligonucleotides.

Sequence (5'-3')	
ATTGCATTGTTTCCG	SEQ ID NO: 63
GTTTGTGCTCGAT	SEQ ID NO: 64
CATTGCATTGTTTCCG	SEQ ID NO: 65
CGTTTGTGCTCGAT	SEQ ID NO: 66
CGTTTGTGCTCGATAA	SEQ ID NO: 67
CCGTTTGTGCTCGA	SEQ ID NO: 68
CGTTTGTGCTCGA	SEQ ID NO: 69
TTTGTGCTCGATAA	SEQ ID NO: 70
TTGTGCTCCATAA	SEQ ID NO: 71
TTTCCGTTTGTGCTCG	SEQ ID NO: 72
ATTGCATTGTTTCCGT	SEQ ID NO: 73
CGTTTGTGCTCGATA	SEQ ID NO: 74

In some embodiments, the oligonucleotide sequence motif is not TCCGTTTGTGCTCGATAAA (SEQ ID NO: 75) or not TTTGTGCTCGATAAAAATA (SEQ ID NO: 76), or not ATTGTTTCCGTTTGTGCTC (SEQ ID NO: 77).

In preferred embodiments, the oligonucleotide of the invention comprises or is a gapmer.

In some embodiments, the oligonucleotide is less than 19 nucleotides in length, preferably less than 18, more preferably less than 17 nucleotides in length.

In some embodiments, the oligonucleotide of the invention comprises affinity enhancing nucleotide analogues.

In some embodiments, the nucleotide analogues are sugar modified nucleotides, such as sugar modified nucleotides independently or dependently selected from the group consisting of: 2'-O-alkyl-RNA units, 2'-OMe-RNA units, 2'-O-alkyl-DNA, 2'-amino-DNA units, 2'-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, HNA units, INA units and 2'MOE units.

In some embodiments, the nucleotide analogues comprise or consist of Locked Nucleic Acid (LNA) units.

In preferred embodiments, the oligomer is a single stranded molecule. In some embodiments, the oligonucleotide does not comprise short regions of, for example, at least 3, 4 or 5 contiguous nucleotides, which are complementary to equivalent regions within the same oligonucleotide (*i.e.* duplexes or hairpins). The oligonucleotide, in some embodiments, may be

not (essentially) double stranded. In some embodiments, the oligonucleotide is essentially not double stranded, such as is not a siRNA.

Exemplary Antisense Oligonucleotides

Exemplary antisense oligonucleotides of the present invention are listed in Table 2.

TABLE 2

Oligo #	Sequence (5'-3')	
1	$^{m}C_s$° T_s° A_s° t_s g_s t_s g_s t_s t_s c_s c_s T_s° G_s° T°	SEQ ID NO: 8
2	G_s° A_s° G_s° a_s c_s a_s t_s g_s g_s t_s g_s g_s g_s $^{m}C_s$° T_s° G°	SEQ ID NO: 9
3	A_s° T_s° T_s° g_s c_s a_s t_s t_s g_s t_s t_s t_s c_s ^{m}Cs°Gs°T°	SEQ ID NO: 10
4	$^{m}C_s$° A_s° T_s° t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 11
5	A_s° T_s° T_s° g_s $^{m}c_s$ a_s t_s t_s g_s t_s t_s t_s $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 12
6	A_s° T_s° T_s° g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 13
7	T_s° T_s° G_s° t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s A_s° A_s° A°	SEQ ID NO: 14
8	$^{m}C_s$° G_s° T_s° t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$$g_s$$a_s$$T_s$°$A_s$°A°	SEQ ID NO: 15
9	$^{m}C_s$° $^{m}C_s$° G_s° t_s t_s t_s g_s t_s g_s c_s t_s $^{m}C_s$° G_s° A°	SEQ ID NO: 16
10	T_s° T_s° g_s t_s g_s c_s t_s $^{m}c_s$ c_s a_s T_s°A_s°A°	SEQ ID NO: 17
11	T_s° T_s° T_s° c_s $^{m}c_s$ g_s t_s t_s t_s g_s t_s g_s c_s T_s° $^{m}C_s$° G°	SEQ ID NO: 18
12	$^{m}C_s$° A_s° T_s° t_s g_s $^{m}c_s$ a_s t_s t_s g_s t_s t_s t_s $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 19
13	$^{m}C_s$° A_s° t_s° t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 20
14	$^{m}C_s$° A_s° T_s° t_s g_s c_s a_s t_s t_s g_s t_s t_s T_s° $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 21
15	$^{m}C_s$° A_s° T_s° T_s° g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 22
16	$^{m}C_s$° A_s° T_s° T_s° g_s c_s a_s t_s t_s g_s t_s t_s T_s° $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 23
17	$^{m}C_s$° A_s° T_s° t_s g_s c_s a_s t_s t_s g_s t_s T_s° T_s° $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 24
18	$^{m}C_s$° A_s° T_s° T_s° G_s° c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s$° $^{m}C_s$° G°	SEQ ID NO: 25
19	G_s° T_s° T_s° t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s A_s° A_s° A°	SEQ ID NO: 26
20	T_s° T_s° T_s° g_s t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s A_s° A_s° A°	SEQ ID NO: 27
21	T_s° T_s° T_s° g_s t_s g_s c_s t_s $^{m}c_s$ g_s a_s T_s° A_s° A_s°	SEQ ID NO: 28
22	G_s° T_s° T_s° t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s a_s T_s° A_s° A_s°	SEQ ID NO: 29
23	G_s° T_s° T_s° t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s A_s° T_s° A_s°	SEQ ID NO: 30
24	$^{m}C_s$° $^{m}C_s$° G_s° t_s t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s A_s°T_s°A°	SEQ ID NO: 31
25	$^{m}C_s$° G_s° T_s° t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s A_s° T_s° A°	SEQ ID NO: 32
26	G_s° T_s° t_s t_s g_s t_s g_s c_s t_s c_s G_s° A_s° T°	SEQ ID NO: 33
27	$^{m}C_s$° G_s° T_s° t_s t_s g_s t_s g_s c_s t_s c_s G_s° A_s° T°	SEQ ID NO: 34
28	$^{m}C_s$° $^{m}C_s$° G_s° t_s t_s t_s g_s t_s g_s c_s t_s c_s G_s° A_s° T°	SEQ ID NO: 35
29	T_s° $^{m}C_s$° $^{m}C_s$° g_s t_s t_s t_s g_s t_s g_s c_s t_s c_s G_s° A_s° T°	SEQ ID NO: 36
30	$^{m}C_s$° G_s° t_s t_s t_s g_s t_s g_s c_s t_s $^{m}C_s$° G_s° A°	SEQ ID NO: 37
31	T_s° T_s° $^{m}C_s$° $^{m}c_s$ g_s t_s t_s t_s g_s t_s g_s c_s t_s $^{m}C_s$° G_s° A°	SEQ ID NO: 38

Oligo #	Sequence (5'-3')	
32	**T_s° ^mC_s° ^mC_s°** g_s t_s t_s t_s g_s t_s g_s c_s t_s **^mC_s° G_s° A°**	SEQ ID NO: 39
33	**T_s° ^mC_s° ^mC_s°** g_s t_s t_s t_s g_s t_s g_s c_s **T_s° ^mC_s° G°**	SEQ ID NO: 40
34	**T_s° T_s° ^mC_s°** ^mc_s g_s t_s t_s t_s g_s t_s g_s c_s **T_s° ^mC_s° G°**	SEQ ID NO: 41
35	**G_s° T_s° T_s°** t_s c_s ^mc_s g_s t_s t_s t_s g_s t_s g_s **^mC_s° T_s° ^mC°**	SEQ ID NO: 42
36	**T_s° T_s° T_s°** c_s ^mc_s g_s t_s t_s t_s g_s t_s g_s **^mC_s° T_s° ^mC°**	SEQ ID NO: 43
37	**^mC_s° G_s°** t_s t_s t_s g_s t_s g_s c_s t_s ^mc_s g_s a_s **T_s°A_s°A°**	SEQ ID NO: 44
38	**^mC_s° G_s° T_s°** t_s t_s g_s t_s g_s c_s t_s ^mc_s g_s **A_s°T_s°A_s°A°**	SEQ ID NO: 45
39	**^mC_s° G_s° T_s° T_s°** t_s g_s t_s g_s c_s t_s ^mc_s g_s a_s **T_s°A_s°A°**	SEQ ID NO: 46
40	**^mC_s° G_s° T_s° T_s°** t_s g_s t_s g_s c_s t_s ^mc_s g_s **A_s° T_s°A_s°A°**	SEQ ID NO: 47
41	**^mC_s° G_s° T_s°** t_s t_s g_s t_s g_s c_s t_s ^mc_s **G_s° A_s° T_s°A_s°A°**	SEQ ID NO: 48
42	**^mC_s° G_s° T_s° T_s° T_s°** g_s t_s g_s c_s t_s ^mc_s g_s a_s **T_s°A_s°A°**	SEQ ID NO: 49
43	**^mC_s° G_s°** t_s t_s t_s g_s t_s g_s c_s t_s ^mc_s g_s **A_s° T_s° A°**	SEQ ID NO: 50
44	**^mC_s° G_s° T_s°** t_s t_s g_s t_s g_s c_s t_s ^mc_s g_s a_s **T_s° A°**	SEQ ID NO: 51
45	**^mC_s° G_s° T_s° T_s°** t_s g_s t_s g_s c_s t_s ^mc_s g_s a_s **T_s° A°**	SEQ ID NO: 52
46	**^mC_s° G_s°** t_s t_s t_s g_s t_s g_s c_s t_s ^mc_s **G_s° A_s° T_s° A°**	SEQ ID NO: 53
47	**^mC_s° G_s° T_s° T_s°** t_s g_s t_s g_s c_s t_s ^mc_s g_s **A_s° T_s° A°**	SEQ ID NO: 54
48	**^mC_s° G_s° T_s°** t_s t_s g_s t_s g_s c_s t_s ^mc_s **G_s° A_s° T_s° A°**	SEQ ID NO: 55
49	**^mC_s° G_s° T_s°** t_s t_s g_s t_s g_s c_s t_s ^mc_s **G_s° A_s° T°**	SEQ ID NO: 56
50	**^mC_s° G_s°** t_s t_s t_s g_s t_s g_s c_s t_s ^mc_s g_s **A_s° T°**	SEQ ID NO: 57
51	**^mC_s° G_s°** t_s° t_s t_s g_s t_s g_s c_s t_s c_s **G_s° A_s° T°**	SEQ ID NO: 58
52	**^mC_s° G_s° T_s°** t_s t_s g_s t_s g_s c_s t_s ^mc_s g_s **A_s° T°**	SEQ ID NO: 59
53	**^mC_s° G_s° T_s° T_s°** t_s g_s t_s g_s c_s t_s ^mc_s g_s **A_s° T°**	SEQ ID NO: 60
54	**^mC_s° G_s°** t_s t_s t_s g_s t_s g_s c_s t_s **^mC_s° G_s° A_s° T°**	SEQ ID NO: 61
55	**^mC_s° G_s° T_s° T_s°** t_s g_s t_s g_s c_s t_s **^mC_s° G_s° A_s° T°**	SEQ ID NO: 62

In various embodiments, antisense oligonucleotides according to the present invention include those oligonucleotides having a sequence at least 50% (*e.g.,* 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to 12 or more (e.g., 13, 14, 15, 16, 17, or 18) contiguous nucleotides that appear in an antisense oligonucleotide sequence selected from Table 2.

In various embodiments, antisense oligonucleotides according to the present invention include those oligonucleotides having a sequence at least 50% (*e.g.,* 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to the nucleotide sequence of an antisense oligonucleotide selected from Table 2.

Length

In will be appreciated that an antisense oligonucleotide in accordance with the present invention may be of any appropriate length. An antisense oligonucleotide of the present

invention may comprise or consist of a contiguous nucleotide sequence of a total of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleotides in length. In some embodiments, an antisense oligonucleotide comprises or consists of a contiguous nucleotide sequence of a total of 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 11-17, 11-16, 11-15, 11-14, 11-13, 12-17, 12-16, 12-15, or 12-14 nucleotides in length. In some embodiments, an antisense oligonucleotide of the present invention is 10 - 16 or 12 – 16 nucleotides in length In some embodiments, an antisense oligonucleotide of the present invention consists of no more than 22 nucleotides, such as no more than 20 nucleotides, such as no more than 19 nucleotides, such as 15, 16, 17 or 18 nucleotides. In some embodiments, an antisense oligonucleotide of the present invention comprises less than 20 nucleotides In some embodiments, an antisense oligonucleotide of the present invention is less than 18 nucleotides in length. Without wishing to be bound by theory, it should be understood that when a range is given for an antisense oligonucleotide of the present invention, or contiguous nucleotide sequence length, it includes the lower an upper lengths provided in the range, for example from (or between) 10 – 30, includes both 10 and 30.

"Percent (%) nucleic acid sequence identity" with respect to the nucleotide sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. The percentage sequence identity may be calculated by counting the number of aligned nucleic acid that are identical between the 2 sequences, dividing by the total number of monomers in the oligomer, and multiplying by 100. In such a comparison, if gaps exist, it is preferable that such gaps are merely mismatches rather than an area where a number of nucleic acid within the gap differs between the aligned sequences, e.g. between the oligonucleotide of the invention and the target region. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN or Megalign (DNASTAR) software as well as EMBOSS needel for global alignments or EMBOSS Water for local alignments. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Preferably, the WU-BLAST-2 software is used to determine amino acid sequence identity (Altschul *et al.*, Methods in Enzymology, 266, 460-480 (1996); http://blast.wustl/edu/blast/README.html). WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, world threshold (T)=11. HSP score (S) and HSP S2 parameters are dynamic values and are established by the program itself, depending upon the composition of the particular sequence, however, the minimum values may be adjusted and are set as indicated above.

Nucleosides and Nucleoside analogues

In some embodiments, the terms "nucleoside analogue" and "nucleotide analogue" are used interchangeably.

The term "nucleotide" as used herein, refers to a glycoside comprising a sugar moiety, a base moiety and a covalently linked group (linkage group), such as a phosphate or phosphorothioate internucleotide linkage group, and covers both naturally occurring nucleotides, such as DNA or RNA, and non-naturally occurring nucleotides comprising modified sugar and/or base moieties, which are also referred to as "nucleotide analogues" herein. Herein, a single nucleotide (unit) may also be referred to as a monomer or nucleic acid unit.

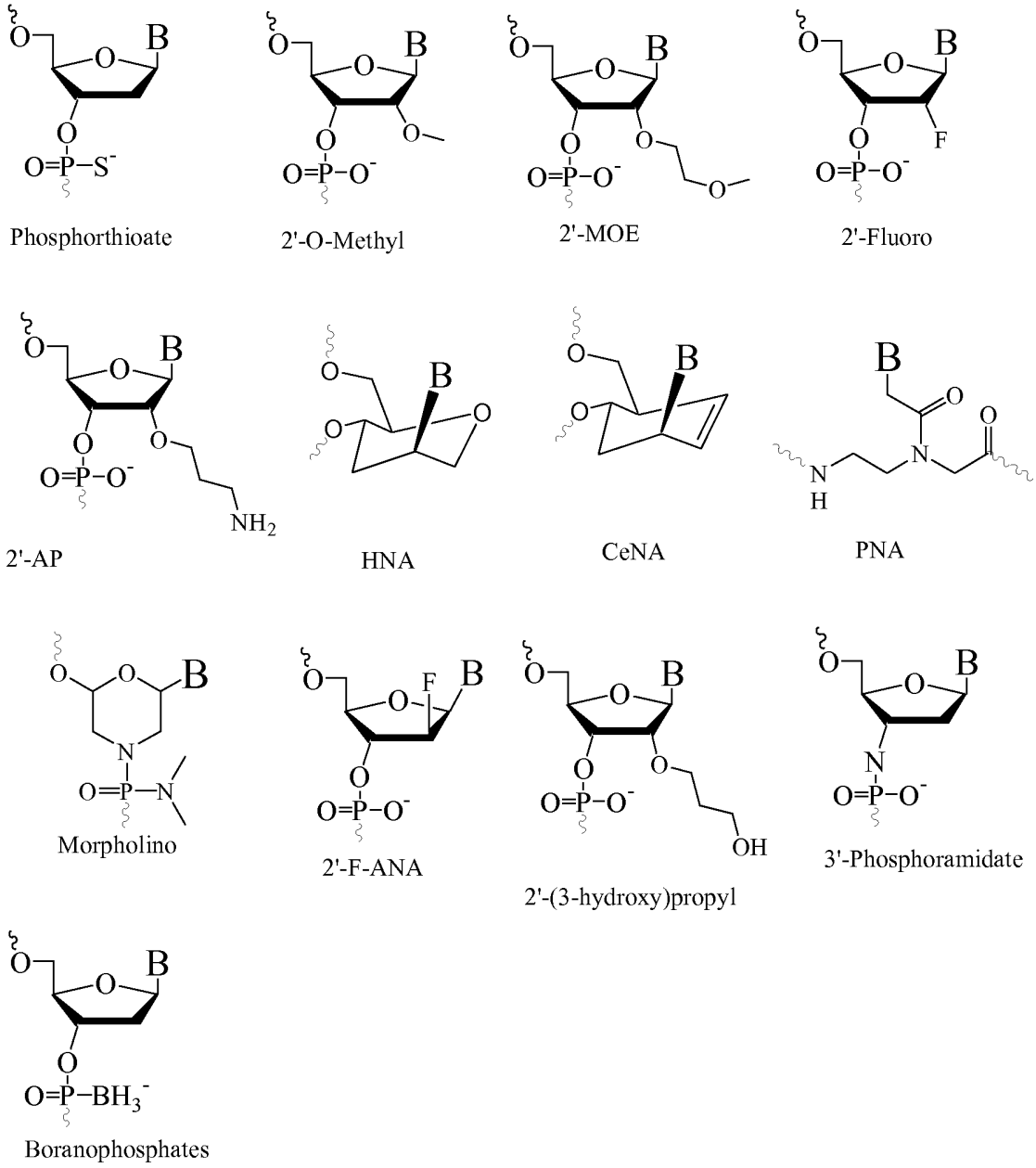
In the field of biochemistry, the term "nucleoside" is commonly used to refer to a glycoside comprising a sugar moiety and a base moiety, and may therefore be used when referring to the nucleotide units, which are covalently linked by the internucleotide linkages between the nucleotides of an oligonucleotide. In the field of biotechnology, the term "nucleotide" is often used to refer to a nucleic acid monomer or unit, and as such in the context of an oligonucleotide may refer to the base – such as the "nucleotide sequence", typically refer to the nucleobase sequence (*i.e.* the presence of the sugar backbone and internucleoside linkages are implicit). Likewise, particularly in the case of oligonucleotides where one or more of the internucleoside linkage groups are modified, the term "nucleotide" may refer to a "nucleoside" for example the term "nucleotide" may be used, even when specifying the presence or nature of the linkages between the nucleosides.

As one of ordinary skill in the art would recognise, the 5' terminal nucleotide of an oligonucleotide does not comprise a 5' internucleotide linkage group, although may or may not comprise a 5' terminal group.

Non-naturally occurring nucleotides include nucleotides which have modified sugar moieties, such as bicyclic nucleotides or 2' modified nucleotides, such as 2' substituted nucleotides.

"Nucleotide analogues" are variants of natural nucleotides, such as DNA or RNA nucleotides, by virtue of modifications in the sugar and/or base moieties. Analogues could in principle be merely "silent" or "equivalent" to the natural nucleotides in the context of the oligonucleotide, *i.e.* have no functional effect on the way the oligonucleotide works to inhibit target gene expression. Such "equivalent" analogues may nevertheless be useful if, for example, they are easier or cost effective to manufacture, or are more stable to storage or manufacturing conditions, or represent a tag or label. Preferably, however, the analogues will have a functional effect on the way in which the oligonucleotide works to inhibit expression; for example by producing increased binding affinity to the target and/or increased resistance to intracellular nucleases and/or increased ease of transport into the cell. Specific examples of nucleoside analogues are described by *e.g.* Freier & Altmann; *Nucl. Acid Res.*, 1997, 25, 4429-

4443 and Uhlmann; *Curr. Opinion in Drug Development*, 2000, 3(2), 293-213, and in Scheme 1 and in the section "Locked Nucleic Acid (LNA)"



Scheme 1

An oligonucleotide may thus comprise or consist of a simple sequence of natural occurring nucleotides – preferably 2'-deoxynucleotides (referred to here generally as "DNA"), but also possibly ribonucleotides (referred to here generally as "RNA"), or a combination of such naturally occurring nucleotides and one or more non-naturally occurring nucleotides, *i.e.* nucleotide analogues. Such nucleotide analogues may suitably enhance the affinity of the oligomer for the target sequence. Examples of suitable and preferred nucleotide analogues are provided by WO2007/031091 or are referenced therein.

Incorporation of affinity-enhancing nucleotide analogues in the oligomer, such as LNA or 2'-substituted sugars, can allow the size of the specifically binding oligomer to be reduced, and

may also reduce the upper limit to the size of the oligonucleotide before non-specific or aberrant binding takes place.

In some embodiments, an antisense oligonucleotide of the present invention comprises at least 1 nucleoside analogue. In some embodiments, an antisense oligonucleotide of the present invention comprises at least 2 nucleotide analogues. In some embodiments, an antisense oligonucleotide of the present invention comprises from 3-8 nucleotide analogues, *e.g.* 6 or 7 nucleotide analogues. In some embodiments, at least one of said nucleotide analogues is a locked nucleic acid (LNA); for example at least 3 or at least 4, or at least 5, or at least 6, or at least 7, or 8, of the nucleotide analogues may be LNA. In some embodiments, all the nucleotides analogues may be LNA.

It will be recognized by persons of skill upon reading this disclosure that when referring to a preferred nucleotide sequence motif or nucleotide sequence, which consists of only nucleotides, an antisense oligonucleotide of the present invention which are defined by that sequence may comprise a corresponding nucleotide analogue in place of one or more of the nucleotides present in said sequence, such as LNA units or other nucleotide analogues, which raise the duplex stability/T_m of the oligomer/target duplex (*i.e.* affinity enhancing nucleotide analogues).

In some embodiments, any mismatches between the nucleotide sequence of the oligomer and the target sequence are preferably found in regions outside the affinity enhancing nucleotide analogues, such as region B or Y as referred to in the section “Gapmer Design”, and/or region D as referred to in the section “Gapmer Design”, and/or at the site of non modified such as DNA nucleotides in the oligonucleotide, and/or in regions which are 5’ or 3’ to the contiguous nucleotide sequence.

Examples of such modification of the nucleotide include modifying the sugar moiety to provide a 2’-substituent group or to produce a bicyclic structure which enhances binding affinity and may also provide increased nuclease resistance.

A preferred nucleotide analogue is LNA, such as oxy-LNA (such as beta-D-oxy-LNA, and alpha-L-oxy-LNA), and/or amino-LNA (such as beta-D-amino-LNA and alpha-L-amino-LNA) and/or thio-LNA (such as beta-D-thio-LNA and alpha-L-thio-LNA) and/or ENA (such as beta-D-ENA and alpha-L-ENA). Most preferred is beta-D-oxy-LNA.

In some embodiments, nucleotide analogues present within an antisense oligonucleotide of the present invention (such as in regions A and C mentioned in the section “Gapmer Design”) are independently selected from, for example: 2’-O-alkyl-RNA units, 2’-OMe-RNA units, 2’-O-alkyl-DNA, 2’-amino-DNA units, 2’-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2’-fluoro-ANA units, HNA units, INA (intercalating nucleic acid -Christensen, 2002. Nucl. Acids. Res. 2002 30: 4918-4925, hereby incorporated by reference) units and 2’MOE units.

In some embodiments, there is only one of the above types of nucleotide analogues present in an antisense oligonucleotide of the present invention, or contiguous nucleotide sequence thereof.

In some embodiments, nucleotide analogues are 2'-O-methoxyethyl-RNA (2'MOE), 2'-fluoro-DNA monomers or LNA nucleotide analogues, and as such an antisense oligonucleotide of the present invention may comprise nucleotide analogues which are independently selected from these three types of analogue, or may comprise only one type of analogue selected from the three types. In some embodiments at least one of said nucleotide analogues is 2'-MOE-RNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-MOE-RNA nucleotide units. In some embodiments, at least one of said nucleotide analogues is 2'-fluoro DNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-fluoro-DNA nucleotide units.

In some embodiments, an antisense oligonucleotide of the present invention comprises at least one Locked Nucleic Acid (LNA) unit, such as 1, 2, 3, 4, 5, 6, 7, or 8 LNA units, such as from 3 – 7 or 4 to 8 LNA units, or 3, 4, 5, 6 or 7 LNA units. In some embodiments, all the nucleotide analogues are LNA. In some embodiments, an antisense oligonucleotide of the present invention may comprise both beta-D-oxy-LNA, and one or more of the following LNA units: thio-LNA, amino-LNA, oxy-LNA, 5'-methyl-LNA and/or ENA in either the beta-D or alpha-L configurations or combinations thereof. In some embodiments, all LNA cytosine units are 5'-methyl-Cytosine.

In some embodiments, an antisense oligonucleotide of the present invention may comprise both nucleotide analogues (preferably LNA) and DNA units. Preferably the combined total of nucleotide analogues (preferably LNA) and DNA units is 10-25, such as 10 – 24, preferably 10-20, such as 10 – 18, even more preferably 12-16. In some embodiments, the nucleotide sequence of an antisense oligonucleotide of the present invention, such as the contiguous nucleotide sequence, consists of at least one nucleotide analogues (preferably LNA) and the remaining nucleotide units are DNA units. In some embodiments, an antisense oligonucleotide of the present invention comprises only LNA nucleotide analogues and naturally occurring nucleotides (such as RNA or DNA, most preferably DNA nucleotides), optionally with modified internucleotide linkages such as phosphorothioate.

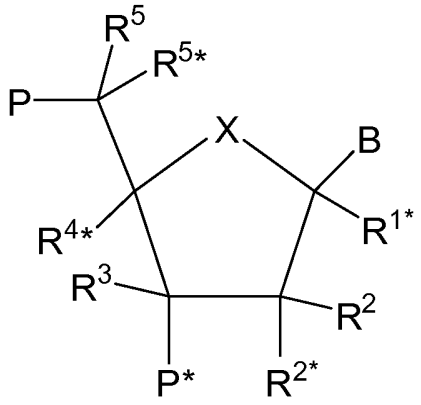
The term "nucleobase" refers to the base moiety of a nucleotide and covers both naturally occurring a well as non-naturally occurring variants. Thus, "nucleobase" covers not only the known purine and pyrimidine heterocycles but also heterocyclic analogues and tautomeres thereof.

Examples of nucleobases include, but are not limited to adenine, guanine, cytosine, thymidine, uracil, xanthine, hypoxanthine, 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

In some embodiments, at least one of the nucleobases present in the oligomer is a modified nucleobase selected from the group consisting of 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

Locked Nucleic Acid (LNA)

The term "LNA" refers to a bicyclic nucleoside analogue, known as "Locked Nucleic Acid". It may refer to an LNA monomer, or, when used in the context of an "LNA oligonucleotide", LNA refers to an oligonucleotide containing one or more such bicyclic nucleotide analogues. LNA nucleotides are characterised by the presence of a linker group (such as a bridge) between C2' and C4' of the ribose sugar ring – for example as shown as the biradical R^{4*} - R^{2*} as described below. The LNA used in an antisense oligonucleotide of the present invention preferably has the structure of the general formula I:



Formula I

wherein for all chiral centers, asymmetric groups may be found in either R or S orientation;

wherein X is selected from -O-, -S-, -N(R^{N*})-, -C(R^6R^{6*})-, such as, in some embodiments –O-;

B is selected from hydrogen, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, nucleobases including naturally occurring and nucleobase analogues, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; preferably, B is a nucleobase or nucleobase analogue;

P designates an internucleotide linkage to an adjacent monomer, or a 5'-terminal group, such internucleotide linkage or 5'-terminal group optionally including the substituent R^5 or equally applicable the substituent R^{5*};

P* designates an internucleotide linkage to an adjacent monomer, or a 3'-terminal group;

R^{4*} and R^{2*} together designate a bivalent linker group consisting of 1 - 4 groups/atoms selected from -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)$_2$-, -S-, -SO_2-, -N(R^a)-, and >C=Z,

wherein Z is selected from -O-, -S-, and -N(R^a)-, and R^a and R^b each is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, optionally substituted C_{1-12}-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2), wherein for all chiral centers, asymmetric groups may be found in either *R* or *S* orientation, and;

each of the substituents R^{1*}, R^2, R^3, R^5, R^{5*}, R^6 and R^{6*}, which are present is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene; ; wherein R^N is selected from hydrogen and C_{1-4}-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C_{1-4}-alkyl; and basic salts and acid addition salts thereof. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, R^{4*} and R^{2*} together designate a biradical consisting of a groups selected from the group consisting of C(R^aR^b)-C(R^aR^b)-, C(R^aR^b)-O-, C(R^aR^b)-NR^a-, C(R^aR^b)-S-, and C(R^aR^b)-C(R^aR^b)-O-, wherein each R^a and R^b may optionally be independently selected. In some embodiments, R^a and R^b may be, optionally independently selected from the group consisting of hydrogen and $_{C1-6}$alkyl, such as methyl, such as hydrogen.

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-CH(CH_2OCH_3)- (2'O-methoxyethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem) – in either the R- or S-configuration.

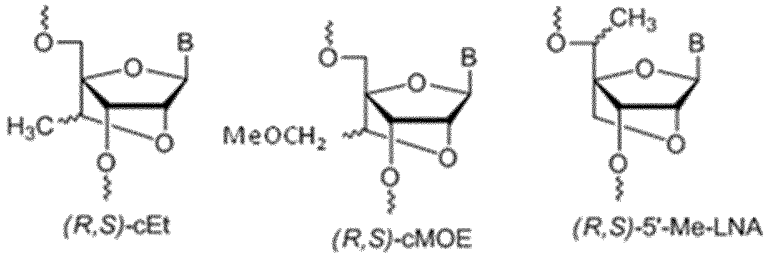
In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-CH(CH_2CH_3)- (2'O-ethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem). – in either the R- or S- configuration.

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-CH(CH_3)-. – in either the R- or S- configuration.

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-CH_2-O-CH_2- - (Seth at al., 2010, J. Org. Chem).

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-NR-CH_3- - (Seth at al., 2010, J. Org. Chem) .

In some embodiments, the LNA units have a structure selected from the following group:



In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen.

In some embodiments, R^{1*}, R^2, R^3 are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, R^{1*}, R^2, R^3 are hydrogen.

In some embodiments, R^5 and R^{5*} are each independently selected from the group consisting of H, –CH_3, -CH_2-CH_3,- CH_2-O-CH_3, and -CH=CH_2. Suitably in some embodiments, either R^5 or R^{5*} are hydrogen, whereas the other group (R^5 or R^{5*} respectively) is selected from the group consisting of C_{1-5} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl or substituted acyl (-C(=O)-); wherein each substituted group is mono or poly substituted with substituent groups independently selected from halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, OJ_1, SJ_1, NJ_1J_2, N_3, $COOJ_1$, CN, O-C(=O)NJ_1J_2, N(H)C(=NH)NJ,J_2 or N(H)C(=X)N(H)J_2 wherein X is O or S; and each J_1 and J_2 is, independently, H, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-}

$_{6}$ alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} aminoalkyl, substituted C_{1-6} aminoalkyl or a protecting group. In some embodiments either R^5 or R^{5*} is substituted C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is substituted methylene wherein preferred substituent groups include one or more groups independently selected from F, NJ_1J_2, N_3, CN, OJ_1, SJ_1, O-C(=O)NJ_1J_2, N(H)C(=NH)NJ, J_2 or N(H)C(O)N(H)J_2. In some embodiments each J_1 and J_2 is, independently H or C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is methyl, ethyl or methoxymethyl. In some embodiments either R^5 or R^{5*} is methyl. In a further embodiment either R^5 or R^{5*} is ethylenyl. In some embodiments either R^5 or R^{5*} is substituted acyl. In some embodiments either R^5 or R^{5*} is C(=O)NJ_1J_2. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such 5' modified bicyclic nucleotides are disclosed in WO 2007/134181, which is hereby incorporated by reference in its entirety.

In some embodiments B is a nucleobase, including nucleobase analogues and naturally occurring nucleobases, such as a purine or pyrimidine, or a substituted purine or substituted pyrimidine, such as a nucleobase referred to herein, such as a nucleobase selected from the group consisting of adenine, cytosine, thymine, adenine, uracil, and/or a modified or substituted nucleobase, such as 5-thiazolo-uracil, 2-thio-uracil, 5-propynyl-uracil, 2'thio-thymine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, and 2,6-diaminopurine.

In some embodiments, R^{4*} and R^{2*} together designate a biradical selected from -C(R^aR^b)-O-, -C(R^aR^b)-C(R^cR^d)-O-, -C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-O-, -C(R^aR^b)-O-C(R^cR^d)-, -C(R^aR^b)-O-C(R^cR^d)-O-, -C(R^aR^b)-C(R^cR^d)-, -C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-, -C(R^a)=C(R^b)-C(R^cR^d)-, -C(R^aR^b)-N(R^c)-, -C(R^aR^b)-C(R^cR^d)- N(R^e)-, -C(R^aR^b)-N(R^c)-O-, and -C(R^aR^b)-S-, -C(R^aR^b)-C(R^cR^d)-S-, wherein R^a, R^b, R^c, R^d, R^e, and R^f each is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2). For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, R^{4*} and R^{2*} together designate a biradical (bivalent group) selected from -CH_2-O-, -CH_2-S-, -CH_2-NH-, -CH_2-N(CH_3)-, -CH_2-CH_2-O-, -CH_2-CH(CH_3)-, -CH_2-CH_2-S-, -CH_2-CH_2-NH-, -CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-O-, -CH_2-CH_2-CH(CH_3)-, -CH=CH-CH_2-, -CH_2-O-CH_2-O-, -CH_2-NH-O-, -CH_2-N(CH_3)-O-, -CH_2-O-CH_2-, -CH(CH_3)-O-, and –CH(CH_2-O-

CH_3)-O-, and/or, -CH_2-CH_2-, and -CH=CH- For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, R^{4*} and R^{2*} together designate the biradical C(R^aR^b)-N(R^c)-O-, wherein R^a and R^b are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen, and; wherein R^c is selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

In some embodiments, R^{4*} and R^{2*} together designate the biradical C(R^aR^b)-O-C(R^cR^d) -O-, wherein R^a, R^b, R^c, and R^d are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

In some embodiments, R^{4*} and R^{2*} form the biradical –CH(Z)-O-, wherein Z is selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio; and wherein each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, OJ_1, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ^3C(=X)NJ_1J_2$ and CN, wherein each J_1, J_2 and J_3 is, independently, H or C_{1-6} alkyl, and X is O, S or NJ_1. In some embodiments Z is C_{1-6} alkyl or substituted C_{1-6} alkyl. In some embodiments Z is methyl. In some embodiments Z is substituted C_{1-6} alkyl. In some embodiments said substituent group is C_{1-6} alkoxy. In some embodiments Z is CH_3OCH_2-. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in US 7,399,845 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some some embodiments, R^{1*}, R^2, R^{3*} are hydrogen, and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181.

In some embodiments, R^{4*} and R^{2*} together designate a biradical which comprise a substituted amino group in the bridge such as consist or comprise of the biradical –CH_2-N(R^c)-, wherein R^c is C_{1-12} alkyloxy. In some embodiments R^{4*} and R^{2*} together designate a biradical –Cq_3q_4-NOR -, wherein q_3 and q_4 are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl; wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, OJ_1, SJ_1, NJ_1J_2, $COOJ_1$, CN, O-C(=O)NJ_1J_2, N(H)C(=NH)N J_1J_2 or N(H)C(=X=N(H)J_2 wherein X is O

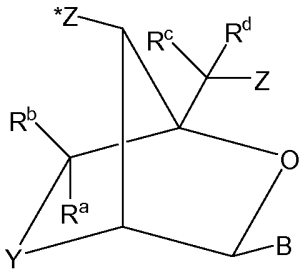
or S; and each of J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or a protecting group. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in WO2008/150729 which is hereby incorporated by reference in its entirity. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some embodiments, R^{1*}, R^2, R^3 are hydrogen and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181. In some embodiments R^{4*} and R^{2*} together designate a biradical (bivalent group) $C(R^aR^b)$-O-, wherein R^a and R^b are each independently halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_1 SJ_1, SOJ_1, SO_2J_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O\text{-}C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; or R^a and R^b together are =C(q3)(q4); q_3 and q_4 are each, independently, H, halogen, C_1-C_{12}alkyl or substituted C_1-C_{12} alkyl; each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2- C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, OJ_1, SJ_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O\text{-}C(=O)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$. and; each J_1 and J_2 is, independently, H, C1-C_6 alkyl, substituted C1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, C1-C_6 aminoalkyl, substituted C1-C_6 aminoalkyl or a protecting group. Such compounds are disclosed in WO2009006478A, hereby incorporated in its entirety by reference.

In some embodiments, R^{4*} and R^{2*} form the biradical - Q -, wherein Q is $C(q_1)(q_2)C(q_3)(q_4)$, $C(q_1)=C(q_3)$, $C[=C(q_1)(q_2)]\text{-}C(q_3)(q_4)$ or $C(q_1)(q_2)\text{-}C[=C(q_3)(q_4)]$; q_1, q_2, q_3, q_4 are each independently. H, halogen, C_{1-12} alkyl, substituted C_{1-12} alkyl, C_{2-12} alkenyl, substituted C_{1-12} alkoxy, OJ_1, SJ_1, SOJ_1, SO_2J_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)\text{-}NJ_1J_2$, $C(=O)J_1$, $\text{-}C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; each J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or a protecting group; and, optionally wherein when Q is $C(q_1)(q_2)(q_3)(q_4)$ and one of q_3 or q_4 is CH_3 then at least one of the other of q_3 or q_4 or one of q_1 and q_2 is other than H. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in WO2008/154401 which is hereby incorporated by reference in its entirity. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6}

aminoalkyl. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some embodiments, R^{1*}, R^2, R^3 are hydrogen and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181 or WO2009/067647 (alpha-L-bicyclic nucleic acids analogs).

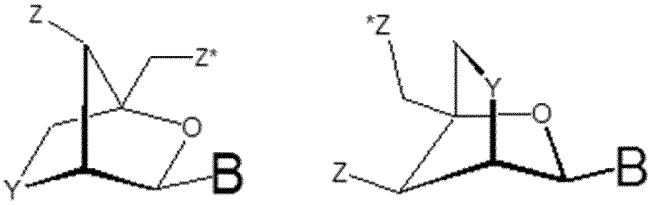
Further bicyclic nucleoside analogues and their use in antisense oligonucleotides are disclosed in WO2011/115818, WO2011/085102, WO2011/017521, WO09100320, WO2010/036698, WO2009/124295 and WO2009/006478. Such nucleoside analogues may in some aspects be useful in the compounds of present invention.

In some embodiments, the LNA used in an antisense oligonucleotide of the present invention preferably has the structure of the general formula II:

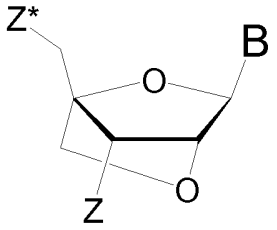


Formula II

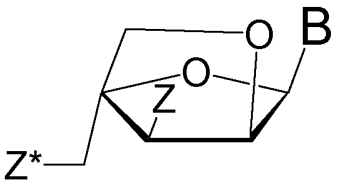
wherein Y is selected from the group consisting of -O-, -CH_2O-, -S-, -NH-, N(R^e) and/or –CH_2-; Z and Z* are independently selected among an internucleotide linkage, R^H, a terminal group or a protecting group; B constitutes a natural or non-natural nucleotide base moiety (nucleobase), and R^H is selected from hydrogen and C_{1-4}-alkyl; R^a, R^b R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2); and R^H is selected from hydrogen and C_{1-4}-alkyl. In some embodiments R^a, R^b R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen and C_{1-6} alkyl, such as methyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation, for example, two exemplary stereochemical isomers include the beta-D and alpha-L isoforms, which may be illustrated as follows:



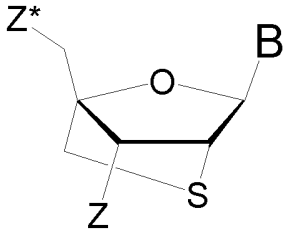
Specific exemplary LNA units are shown below:



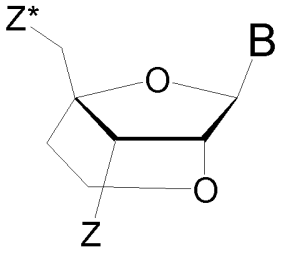
β-D-oxy-LNA



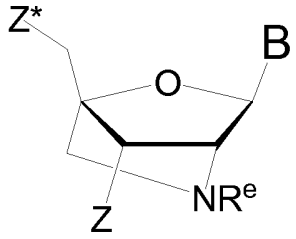
α-L-Oxy-LNA



β-D-thio-LNA



β-D-ENA



β-D-amino-LNA

The term "thio-LNA" comprises a locked nucleotide in which Y in the general formula above is selected from S or $-CH_2-S-$. Thio-LNA can be in both beta-D and alpha-L-configuration.

The term "amino-LNA" comprises a locked nucleotide in which Y in the general formula above is selected from -N(H)-, N(R)-, $CH_2-N(H)-$, and $-CH_2-N(R)-$ where R is selected from hydrogen and C_{1-4}-alkyl. Amino-LNA can be in both beta-D and alpha-L-configuration.

The term "oxy-LNA" comprises a locked nucleotide in which Y in the general formula above represents –O-. Oxy-LNA can be in both beta-D and alpha-L-configuration.

The term "ENA" comprises a locked nucleotide in which Y in the general formula above is $-CH_2-O-$ (where the oxygen atom of $–CH_2-O-$ is attached to the 2'-position relative to the base B). R^e is hydrogen or methyl.

In some exemplary embodiments, LNA is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA and beta-D-thio-LNA, in particular beta-D-oxy-LNA.

Gapmer Design

The oligonucleotide of the present invention is preferably a gapmer. A gapmer oligonucleotide is an oligonucleotide which comprises a contiguous stretch of nucleotides which is capable of recruiting an RNAse, such as RNAseH, such as a region of at least 6 or 7 DNA nucleotides, referred to herein in as region B or region Y_b. The length of the RNAseH recruiting region can be indicated by an integer number $_b$ between 5 and 15. Region B or Y is flanked both 5' and 3' by regions of affinity enhancing nucleotide analogues, such as between 1-6 nucleotide analogues 5' and 3' to the contiguous stretch of nucleotides which is capable of recruiting RNAse. These regions are referred to as regions A or X and C or $X_{a'}$ respectively. The number of the nucleotide analogues can be indicated by $_a$ or $_{a'}$ and is between 1 and 6, preferably 1, 2, 3, 4 or 5.

EP 1 222 309 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH. An oligomer is deemed capable of recruiting RNase H if, when provided with the complementary RNA target, it has an initial rate, as measured in pmol/l/min, of at least 1 %, such as at least 5%, such as at least 10% or ,more than 20% of the of the initial rate determined using DNA only oligonucleotide, having the same base sequence but containing only DNA monomers, with no 2' substitutions, with phosphorothioate linkage groups between all monomers in the oligonucleotide, using the methodology provided by Example 91 - 95 of EP 1 222 309.

In some embodiments, an oligomer is deemed essentially incapable of recruiting RNaseH if, when provided with the complementary RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is less than 1%, such as less than 5%,such as less than 10% or less than 20% of the initial rate determined using the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Example 91 - 95 of EP 1 222 309.

In other embodiments, an oligomer is deemed capable of recruiting RNaseH if, when provided with the complementary RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is at least 20%, such as at least 40 %, such as at least 60 %, such as at least 80 % of the initial rate determined using the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Example 91 - 95 of EP 1 222 309 (hereby incorporated by reference).

In some embodiments, the monomers which are capable of recruiting RNAse are selected from the group consisting of DNA monomers, alpha-L-LNA monomers, C4' alkylayted DNA monomers (see WO2009/090182 and Vester *et al.*, Bioorg. Med. Chem. Lett. 18 (2008) 2296 – 2300, hereby incorporated by reference), and UNA (unlinked nucleic acid) nucleotides (see Fluiter *et al.*, Mol. Biosyst., 2009, 10, 1039 hereby incorporated by reference). UNA is

unlocked nucleic acid, typically where the C2 – C3 C-C bond of the ribose has been removed, forming an unlocked "sugar" residue.

In some embodiments, a gapmer comprises a (poly)nucleotide sequence of formula (5' to 3'), A-B-C or X_a-Y_b-$X_{a'}$, or optionally A-B-C-D or D-A-B-C or X_a-Y_b-$X_{a'}$-*D or D-* X_a-Y_b-$X_{a'}$, wherein; region A or X_a (5' region) consists or comprises of at least one nucleotide analogue, such as at least one locked nucleic acid (LNA) unit, such as from 1-6 nucleotide analogues, such as LNA units, and; region B or Y consists or comprises of at least five consecutive nucleotides which are capable of recruiting RNAse (when formed in a duplex with a complementary RNA molecule, such as the mRNA target), such as DNA nucleotides, and; region C or $X_{a'}$ (3' region) consists or comprises of at least one nucleotide analogue, such as at least one LNA unit, such as from 1-6 nucleotide analogues, such as LNA units, and; region D, when present consists or comprises of 1, 2 or 3 nucleotide units, such as DNA nucleotides.

In some embodiments, region A or X_a includes or consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as LNA units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 3 or 4 nucleotide analogues, such as 3 or 4 LNA units; and/or region C or $X_{a'}$ includes or consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as LNA units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 3 or 4 nucleotide analogues, such as 3 or 4 LNA units.

In some embodiments, B or Y includes or consists or comprises of 5, 6, 7, 8, 9, 10, 11 or 12 consecutive nucleotides which are capable of recruiting RNAse, or from 5-15, or from 6-10, or from 7-9, such as 8 consecutive nucleotides which are capable of recruiting RNAse. In some embodiments, region B or Y consists or comprises at least one DNA nucleotide unit, such as 1-12 DNA units, preferably from 4-12 DNA units, more preferably from 6-10 DNA units, such as from 7-10 DNA units, most preferably 8, 9 or 10 DNA units.

In some embodiments, region A or X_a includes or consists of 3 or 4 nucleotide analogues, such as described in the "Nucleosides and Nucleoside analogues" section, preferably the analogue is LNA. Region B includes or consists of 7, 8, 9 or 10 DNA units, and region C or $X_{a'}$ includes or consists of 3 or 4 nucleotide analogues, such as described in the "Nucleosides and Nucleoside analogues" section, preferably the analogue is LNA. Such designs include, for example, (A-B-C or X_a-Y_b-$X_{a'}$) 2-11-3, 2-10-2, 2-8-4, 2-9-3, 2-9-4, 3-10-3, 3-10-4, 4-10-3, 3-9-3, 3-9-4, 4-9-3, 3-8-3, 3-8-4, 4-8-3, 3-7-3, 3-7-4, 4-7-3, and may further include region D, which may have one or 2 nucleotide units, such as DNA units. Examples of gapmer designs are shown in WO2004/046160 and are hereby incorporated by reference. In some embodiments, a gapmer antisense oligonucleotide of the present invention may be a shortmer gapmer as described in U.S. Provisional Patent Application No. 60/977,409 and are hereby incorporated by reference.

In some embodiments, an oligonucleotide of the present invention comprises a contiguous nucleotide sequence of a total of 10, 11, 12, 13, 14, 15, 16, 17 or 18 nucleotide

units, wherein the contiguous nucleotide sequence is of formula (5'-3'), A-B-C or X_a-Y_b-$X_{a'}$, or optionally A-B-C-D or D-A-B-C or X_a-Y_b-$X_{a'}$-*D or D*- X_a-Y_b-$X_{a'}$, wherein; A or $X_{a'}$ consists of 1, 2, 3 or 4 nucleotide analogue units, such as LNA units; B or Y consists of 7, 8, 9, 10 or 11 contiguous nucleotide units which are capable of recruiting RNAse when formed in a duplex with a complementary RNA molecule (such as a mRNA target); and C or $X_{a'}$ consists of 1, 2, 3 or 4 nucleotide analogue units, such as LNA units. When present, D consists of a single DNA unit.

In some embodiments, A or X_a consists of 1 LNA unit. In some embodiments, A or X_a consists of 2 LNA units. In some embodiments, A or X_a consists of 3 LNA units. In some embodiments, A or X_a consists of 4 LNA units. In some embodiments, C or $X_{a'}$ consists of 1 LNA unit. In some embodiments, C or $X_{a'}$ consists of 2 LNA units. In some embodiments, C or $X_{a'}$ consists of 3 LNA units. In some embodiments, C or $X_{a'}$ consists of 4 LNA units. In some embodiments, B or Yconsists of 7 nucleotide units. In some embodiments, B or Y consists of 8 nucleotide units. In some embodiments, B or Yconsists of 9 nucleotide units. In certain embodiments, region B consists of 10 nucleoside monomers. In certain embodiments, region B or Y comprises 1 – 10 DNA monomers. In some embodiments, B consists of 10 nucleotide units. In some embodiments, B or Y consists of 11 nucleotide units. In some embodiments, B or Y comprises of between 1-11 DNA units, inclusive, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 DNA units. In some embodiments, B or Y consists of DNA units. In some embodiments B or Y comprises of at least one LNA unit which is in the alpha-L configuration, such as 2, 3, 4, 5, 6, 7, 8 or 9 LNA units in the alpha-L-configuration. In some embodiments, B or Y comprises of at least one alpha-L-oxy LNA unit or wherein all the LNA units in the alpha-L-configuration are alpha-L-oxy LNA units. In some embodiments, the number of nucleotides present in A-B-C or X_a-Y_b-$X_{a'}$ are selected from the group consisting of (nucleotide analogue units--region B or Y--nucleotide analogue units): 1-8-1, 1-8-2, 2-8-1, 2-8-2, 3-8-3, 2-8-3, 3-8-2, 4-8-1, 4-8-2, 1-8-4, 2-8-4, or; 1-9-1, 1-9-2, 2-9-1, 2-9-2, 2-9-3, 3-9-2, 1-9-3, 3-9-1, 4-9-1, 1-9-4, or; 1-10-1, 1-10-2, 2-10-1, 2-10-2, 1-10-3, 3-10-1, or; 1-11-1, 1-11-2, 2-11-1, 2-11-2, 2-11-3, 3-11-2, 4-11-1, 1-11-4. In some embodiments, the number of nucleotides in A-B-C are selected from the group consisting of 3-8-3, 3-10-3, 3-9-3, 2-8-3, 2-11-3, 3-9-4, 4-9-3, 4-8-4, 3-8-5, 5-8-3, 2-10-3, 3-10-2, 4-9-2, 2-9-4, 4-8-3, 3-8-4, 2-10-2, 2-9-3, 3-9-2, 4-8-2, 2-8-4 and 4-7-4. In certain embodiments, each of regions A and C or X_a and $X_{a'}$ consists of three LNA monomers, and region B or Y consists of 8 or 9 or 10 nucleoside monomers, preferably DNA monomers. In some embodiments, both A and C consists of two, three or four LNA units each, and B consists of 8, 9, 10 or 11 nucleotide units, preferably DNA units.

In various embodiments, other gapmer designs include those where regions A and/or C or X_a and/or $X_{a'}$ consists of 3, 4, 5 or 6 nucleoside analogues, such as monomers containing a 2'-O-methoxyethyl-ribose sugar (2'-MOE) or monomers containing a 2'-fluoro-deoxyribose sugar, and region B consists of 8, 9, 10, 11 or 12 nucleosides, such as DNA monomers, where

regions A-B-C have 3-9-3, 3-10-3, 5-10-5 or 4-12-4 monomers. Further gapmer designs are disclosed in WO2007/146511, hereby incorporated by reference.

Internucleotide Linkages

Monomers of an antisense oligonucleotide as described herein are coupled together via linkage groups. Suitably, each monomer is linked to the 3' adjacent monomer via a linkage group.

Upon reading the present disclosure, persons of ordinary skill in the art would understand that the 5' monomer at the end of an antisense oligonucleotide of the present invention does not comprise a 5' linkage group, although it may or may not comprise a 5' terminal group.

The terms "linkage group" or "internucleotide linkage" are intended to mean a group capable of covalently coupling together two nucleotides. Specific and preferred examples include phosphate groups and phosphorothioate groups. An antisense oligonucleotide of the present or contiguous nucleotides sequence thereof are coupled together via linkage groups. Suitably, each nucleotide is linked to the 3' adjacent nucleotide via a linkage group. Exemplary internucleotide linkages include those described in WO2007/031091, hereby incorporated by reference.

In some embodiments, an internucleotide linkage may be modified from its normal phosphodiester to one that is more resistant to nuclease attack, such as phosphorothioate or boranophosphate--these two, being cleavable by RNase H, also allow that route of antisense inhibition in reducing the expression of the target gene.

Suitable sulphur (S) containing internucleotide linkages as provided herein may be preferred. Phosphorothioate internucleotide linkages are also preferred, particularly for the gap region (B or Y) of gapmers. Phosphorothioate linkages may also be used for the flanking regions (A/X_a and C/ $X_{a'}$, and for linking A/X_a or C/ $X_{a'}$ to D, and within region D, as appropriate).

Regions A or X_a, B or Y and C or $X_{a'}$, may however comprise internucleotide linkages other than phosphorothioate, such as phosphodiester linkages, particularly, for instance when the use of nucleotide analogues protects the internucleotide linkages within regions A or X_a and C or X_a from endo-nuclease degradation,such as when regions A or X_a and C or $X_{a'}$ comprise LNA nucleotides.

Internucleotide linkages of an oligonucleotide of the present invention may be phosphodiester, phosphorothioate or boranophosphate to allow RNase H cleavage of targeted RNA. Phosphorothioate is preferred, for improved nuclease resistance and other reasons, such as ease of manufacture. In some embodiments, nucleotides and/or nucleotide analogues of an oligonucleotide of the present invention are linked to each other by means of phosphorothioate groups. In a preferred embodiment of the invention the oligonucleotide comprise at least one phosphorothioate linkage.

It is recognized that the inclusion of phosphodiester linkages, such as one or two linkages, into an otherwise phosphorothioate oligonucleotide, particularly between or adjacent to nucleotide analogue units (typically in region A or X_a and or C or $X_{a'}$) can modify the bioavailability and/or bio-distribution of an oligonucleotide as described in WO2008/053314, hereby incorporated by reference.

In some embodiments, such as the embodiments referred to above, where suitable and not specifically indicated, all remaining linkage groups are either phosphodiester or phosphorothioate, or a mixture thereof.

In some embodiments, all the internucleotide linkage groups of the oligonucleotide are phosphorothioate. When referring to specific gapmer oligonucleotide sequences, such as those provided herein it will be understood that, in various embodiments, when the linkages are phosphorothioate linkages, alternative linkages, such as those disclosed herein may be used, for example phosphate (phosphodiester) linkages may be used, particularly for linkages between nucleotide analogues, such as LNA, units. Likewise, when referring to specific gapmer oligonucleotide sequences, such as those provided herein, when the C nucleotide residues are annotated as 5' methyl modified cytosine, in various embodiments, one or more of the C nucleotides present in the oligonucleotide may be unmodified C residues.

Pharmaceutical compositions

The present invention further provides pharmaceutical compositions comprising therapeutic actives in accordance with the invention (*e.g.*, antisense oligonucleotides), together with one or more pharmaceutically acceptable excipients. Such pharmaceutical compositions may optionally comprise one or more additional therapeutically-active substances.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation.

Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a diluent or another excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's *The Science and Practice of Pharmacy*, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

In some embodiments, a pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use in humans and for veterinary use. In some embodiments, an excipient is approved by United States Food and Drug Administration. In some embodiments, an excipient is pharmaceutical grade. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in pharmaceutical formulations. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in *Remington: The Science and Practice of Pharmacy* 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

In some embodiments, liposomes may be used to deliver antisense oligonucleotides described herein. As used herein, a liposome is an artificially-prepared vesicle composed of a lipid bilayer. Liposomes can be prepared by disrupting biological membranes (such as by sonication). Liposomes may include natural phospholipids, or mixed lipid chains with surfactant properties (*e.g.*, egg phosphatidylethanolamine). A liposome design may employ surface ligands for targeting desired target tissues.

Administration

The present invention provides methods of administering an effective amount of a therapeutic active described herein (*e.g.*, an antisense oligonucleotide) to a subject in need of treatment.

Antisense oligonucleotides described herein may be administered through various administration routes including, but not limited to, intravenous, subcutaneous, intramuscular, parenteral, transdermal, or transmucosal (*e.g.*, oral or nasal). In some embodiments, antisense oligonucleotides described herein may be administered through intravenous administration. In some embodiments, antisense oligonucleotides described herein may be administered through subcutaneous administration. In some embodiments, a dosage regime for an oligonucleotide may be repeated after an initial dosage regime, indeed the dosage regime may be repeated as necessary in order to treat or prevent the progression of a disease. In some embodiments, antisense oligonucleotides described herein may be administered daily, twice a week, once a week, bi-weekly, monthly, once every two months, once every three months, once every four months, once every six months, or at variable intervals.

Applications

Antisense oligonucleotides of the present invention may be utilized as research reagents for, for example, diagnostics, therapeutics and prophylaxis.

In research, an antisense oligonucleotide of the present invention may be used to specifically inhibit the synthesis of BCL11A protein (typically by degrading or inhibiting the mRNA and thereby prevent protein formation) in cells and experimental animals thereby facilitating functional analysis of the target or an appraisal of its usefulness as a target for therapeutic intervention.

In diagnostics, an antisense oligonucleotide of the present invention may be used to detect and quantitate BCL11A expression in cell and tissues by northern blotting, *in-situ* hybridisation or similar techniques.

For therapeutics, an animal or a human, suspected of having a disease or disorder, which can be treated by modulating the expression of BCL11A is treated by administering an

antisense oligonucleotide of the present invention. Further provided are methods of treating a mammal, such as treating a human, suspected of having or being prone to a disease or condition, associated with expression of BCL11A by administering a therapeutically or prophylactically effective amount of one or more of an antisense oligonucleotide or composition of the present invention. An antisense oligonucleotide, a conjugate or a pharmaceutical composition of the present invention is typically administered in an effective amount.

Antisense oligonucleotides of the present invention are suitable for the manufacture of a medicament for the treatment of a disorder as referred to herein, or for a method of the treatment of as a disorder as referred to herein.

A method for treating a disorder as referred to herein is provided, said method comprising administering an antisense oligonucleotide as described herein, and/or a conjugate, and/or a pharmaceutical composition to a patient in need thereof.

The present invention provides a method of treating an anemic disease, disorder or condition comprising administering to a subject in need of treatment an oligonucleotide according to the invention or a pharmaceutical composition of the invention.

In one embodiment the anemic disease, disorder or condition is sickle cell disease.

In another embodiment the anemic disease, disorder or condition is β-thalassemia.

When applied in a method of treatment the administeration of an oligonucleotide of the invention or the pharmaceutical composition of the invention results in reduced expression of BCL11A in one or more target tissues. Preferably, the administration of the oligonucleotide of the inventionor the pharmaceutical composition of the invention results in increased γ-globin expression in one or more target tissues. The administion of the oligonucleotide of the invention or the pharmaceutical composition of the invention may result in increased fetal hemoglobin production in one or more target tissues. Preferably, the target tissues are selected from bone marrow, liver, kidney, spleen and/or plasma cells, peripheral blood B-cells, dendritic cells, erythroid progenitor cells, pluripotent stem cells, thymus, tonsillar epithelium.

Therapeutic uses

Antisense oligo nucleotide modulators of BCL11A described herein may be used to treat various BCL11A related diseases, disorders and conditions.

Sickle Cell Disease (SCD)

Sickle Cell Disease, or sickle cell anemia is an inherited genetic disorder characterized by red blood cells having an abnormal, rigid, sickle shape, which reduces the flexibility of the cell. This results from a mutation in a beta globin chain gene and is manifested in an autosomal recessive manner with overdominance. SCD is associated with various severe complications, such as reduced life expectancy, and causes a pathological condition that can lead to death. However, due to genetic polymorphism of mutations, not all inherited hemoglobin variants are detrimental.

SCD is more commonly reported in populations from tropical and sub-tropical sub-Saharan regions. These are also regions where malaria is commonly observed. Interestingly, carriers of SCD (*i.e.,* having one copy of the mutation) are found to be more resistant to infection and show less severe symptoms when infected.

Antisense oligonucleotide modulators of BCL11A described herein may be used to treat SCD. The terms, "treat" or "treatment," as used herein, refers to amelioration of one or more symptoms associated with the disease, prevention or delay of the onset of one or more symptoms of the disease, and/or lessening of the severity or frequency of one or more symptoms of the disease.

In some embodiments, treatment refers to partially or completely alleviation, amelioration, relief, inhibition, delaying onset, reducing severity and/or incidence of one or more symptoms in a SCD patient, including, but not limited to, anemia; yellowing of the eyes; paleness, coldness and/or yellowing of the skin; shortness of breath; muscular weakness; intestinal changes (*e.g.,* changes in stool color); fatigue; dizziness; fainting; changes to blood vessels (*e.g.,* low blood pressure); changes affecting the heart (*e.g.,* heart palpitations, rapid heart rate, chest pain, angina, heart attack), and organ enlargement (*e.g.,* spleen).

In some embodiments, treatment refers to reduced symptoms of anemia in a subject in need of treatment. In certain embodiments, the amount of symptoms of anemia may be reduced by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more as compared to a pre-treatment or no-treatment control (*e.g.,* the amount of symptoms of anemia by a control subject with similar diseased or developmental stage but without treatment).

In some embodiments, treatment refers to increased gamma globin expression (*e.g.,* total expression, percent expression increase per week, per month, per two months, per six months, *etc.*). In various embodiments, increased gamma globin expression compensates for a lack of or reduced expression of beta globulin in a SCD patient.

Thalassemia (α, β and δ)

Thalassemia, like SCD, is an inherited genetic disorder that affects the blood. Thalassemia manifests as an autosomal recessive condition and leads to weakening and destruction of red blood cells. Thalassemia is caused by mutations or deletions of genes that affect how the body makes hemoglobin, which is the protein within red blood cells that is responsible for carrying oxygen. Individuals suffering from thalassemia are characterized by low hemoglobin production and have fewer circulating red blood cells than normal, which results in mild or severe anemia. Thalassemia originated in the Mediterranean region.

Thalassemia can cause significant complications, including pneumonia, iron overload, bone deformities and cardiovascular sickness. However, like SCD, this disease has been observed to confer a degree of protection against malaria for those that are carriers of the disease.

Hemoglobin is composed of four protein chains, two alpha and two beta globin chains, which are arranged in a heterodimer. In humans, the beta globin chains are encoded by a single gene on chromosome 11, while the alpha globin chains are encoded by two gene on chromosome 16 that are linked. This sets up a genetic situation where normal individuals contain two beta chain loci and four alpha chain loci. In patients with thalassemia, mutations in either the alpha or beta chain, which gives rise to the low and/or abnormal production of red blood cells. As a result, thalassemias are categorized according to which chain has a mutation. Alpha and beta thalassemias are common in African, Asian, Greek and Italian ethnic groups.

Alpha thalassemas (mutations in the alpha chain) concern the HBA1 and HBA2 genes, and result in decreased production of alpha globin. This creates a situation where there is an increase in beta globin production in adults and increase gamma globin production in infants. The increase in beta globin production leads to the formation of tetramers that are unstable and have an impaired ability to dissociate with oxygen.

Beta thalassemias (mutations in the beta chain) concern the HBB gene, and the severity of the disease that results is dependent on the mutation. Some mutations prevent the formation of beta chains, which the most severe form of the disease, while others allow some formation of beta chains, albeit at a reduced level. As a result of beta chain mutation, there is an excess of alpha chain production, which do not form tetramers as in the case of alpha thalassemias. Alternatively, the excess alpha chains bind to the membranes of red blood cells and result in damage to the membrane, and can be toxic if the alpha chains aggregate.

Although at a low frequency, delta thalassemias can occur. Similarly, they result from mutations in delta globin chain genes and result in an abnormal production of these chains. It has been reported that about 3% of hemoglobin of adults is made of alpha and delta chains.

Antisense oligonucleotide modulators of BCL11A described herein may be used to treat thalassemias, *e.g.*, alpha, beta and/or delta thalassemas. The terms, “treat” or “treatment,” as used herein, refers to amelioration of one or more symptoms associated with the disease, prevention or delay of the onset of one or more symptoms of the disease, and/or lessening of the severity or frequency of one or more symptoms of the disease.

In some embodiments, treatment refers to partial or complete alleviation, amelioration, relief, inhibition, delaying onset, reducing severity and/or incidence of one or more symptoms in a thalassemia patient, including, but not limited to, pneumonia, iron overload, bone deformities and cardiovascular sickness. In some embodiments, treatment refers to partial or complete alleviation, amelioration, relief, inhibition, delaying onset, reducing severity and/or incidence of one or more symptoms in a thalassemia patient, including, but not limited to, anemia; yellowing of the eyes; paleness, coldness and/or yellowing of the skin; shortness of breath; muscular weakness; intestinal changes (*e.g.,* changes in stool color); fatigue; dizziness; fainting; changes to blood vessels (*e.g.,* low blood pressure); changes affecting the heart (*e.g.,* heart palpitations, rapid heart rate, chest pain, angina, heart attack), and organ enlargement (*e.g.,* spleen).

In some embodiments, treatment refers to reduced symptoms of anemia in a subject in need of treatment. In certain embodiments, the amount of symptoms of anemia may be reduced by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more as compared to a pre-treatment or no-treatment control (*e.g.,* the amount of symptoms of anemia by a control subject with similar diseased or developmental stage but without treatment).

In some embodiments, treatment refers to increased gamma globin expression (*e.g.,* total expression, percent expression increase per week, per month, per two months, per six months, *etc.*). In various embodiments, increased gamma globin expression compensates for a lack of or reduced expression of alpha, beta or delta globulin in a thalassemia patient.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature citations are incorporated by reference.

EXAMPLES

Example 1. Design and synthesis of oligonucleotides that target BCL11A

This example illustrates exemplary methods of designing and synthesis of LNA oligonucleotides that can effectively down-regulate BCL11A expression and activity. In this example, the primary target region is the overlapping regions among the XL, L and S isoforms (Figure 2).

A total of 401 LNA oligonucleotides were designed and synthesized in seven libraries based on the sequences of the three major isoforms of human BCL11A (*i.e.*, XL, L or S; Table 3) resulting in oligonucleotides of various specificities, lengths (*e.g.*, 12-16 mers) and LNA designs.

TABLE 3

BCL11A	Isoform	Accession No.	
Human	XL	NM_022893	SEQ ID NO: 1
	L	NM_018014	SEQ ID NO: 2
	S	NM_138559	SEQ ID NO: 3
Mouse	XL	NM_001242934	SEQ ID NO: 4
	L	NM_016707	SEQ ID NO: 5
	S	NM_001159289	SEQ ID NO: 6

Exemplary methods for designing LNA units are described in Wahlestedt, C. *et al.* 2000, PNAS 91(10):5633-5638, which is incorporated herein by reference. Exemplary LNA oligonucleotides are shown in Table 4.

TABLE 4

Oligo #	BCL11A-XL Position	Length	LNA Design	Sequence (5'-3')
1	597	14	3-8-3	$^{m}C_s^\circ$ T_s° A_s° t_s g_s t_s g_s t_s t_s c_s c_s T_s° G_s° T°
2	220	16	3-10-3	G_s° A_s° G_s° a_s c_s a_s t_s g_s g_s t_s g_s g_s g_s $^{m}C_s^\circ$ T_s° G°
3	429	16	3-10-3	A_s° T_s° T_s° g_s c_s a_s t_s t_s g_s t_s t_s t_s c_s $^{m}Cs^\circ Gs^\circ T^\circ$
4	430	16	3-10-3	$^{m}C_s^\circ$ A_s° T_s° t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
5	430	15	3-9-3	A_s° T_s° T_s° g_s $^{m}c_s$ a_s t_s t_s g_s t_s t_s t_s $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
6	430	15	3-9-3 ^{m}c	A_s° T_s° T_s° g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
7	415	14	3-8-3 ^{m}c	T_s° T_s° G_s° t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s A_s° A_s° A°
8	416	16	3-10-3	$^{m}C_s^\circ$ G_s° T_s° t_s t_s g_s t_s g_s c_s t_s $^{m}c_s g_s a_s T_s^\circ A_s^\circ A^\circ$
9	419	14	3-8-3	$^{m}C_s^\circ$ $^{m}C_s^\circ$ G_s° t_s t_s t_s g_s t_s g_s c_s t_s $^{m}C_s^\circ$ $G_s^\circ A^\circ$
10	416	13	2-8-3 ^{m}c	T_s° T_s° g_s t_s g_s c_s t_s $^{m}c_s$ c_s a_s $T_s^\circ A_s^\circ A^\circ$
11	420	16	3-10-3 ^{m}C	T_s° T_s° T_s° c_s $^{m}c_s$ g_s t_s t_s t_s g_s t_s g_s c_s T_s° $^{m}C_s^\circ$ G°
12	430	16	3-10-3 ^{m}C	$^{m}C_s^\circ$ A_s° T_s° t_s g_s $^{m}c_s$ a_s t_s t_s g_s t_s t_s t_s $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
13	430	16	2-11-3	$^{m}C_s^\circ$ A_s° t_s° t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
14	430	16	3-9-4	$^{m}C_s^\circ$ A_s° T_s° t_s g_s c_s a_s t_s t_s g_s t_s t_s T_s° $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
15	430	16	4-9-3	$^{m}C_s^\circ$ A_s° T_s° T_s° g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
16	430	16	4-8-4	$^{m}C_s^\circ$ A_s° T_s° T_s° g_s c_s a_s t_s t_s g_s t_s t_s T_s° $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
17	430	16	3-8-5	$^{m}C_s^\circ$ A_s° T_s° t_s g_s c_s a_s t_s t_s g_s t_s T_s° T_s° $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
18	430	16	5-8-3	$^{m}C_s^\circ$ A_s° T_s° T_s° G_s° c_s a_s t_s t_s g_s t_s t_s t_s $^{m}C_s^\circ$ $^{m}C_s^\circ$ G°
19	415	16	3-10-3 ^{m}C	G_s° T_s° T_s° t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s A_s° A_s° A°
20	415	15	3-9-3 ^{m}c	T_s° T_s° T_s° g_s t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s A_s° A_s° A°
21	416	14	3-8-3 ^{m}c	T_s° T_s° T_s° g_s t_s g_s c_s t_s $^{m}c_s$ g_s a_s T_s° A_s° A_s°
22	416	15	3-9-3 ^{m}c	G_s° T_s° T_s° t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s a_s T_s° A_s° A_s°
23	417	14	3-8-3 ^{m}c	G_s° T_s° T_s° t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s A_s° T_s° A_s°
24	417	16	3-10-3 ^{m}C	$^{m}C_s^\circ$ $^{m}C_s^\circ$ G_s° t_s t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s $A_s^\circ T_s^\circ A^\circ$
25	417	15	3-9-3	$^{m}C_s^\circ$ G_s° T_s° t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s A_s° T_s° A°
26	418	13	2-8-3	G_s° T_s° t_s t_s g_s t_s g_s c_s t_s c_s G_s° A_s° T°
27	418	14	3-8-3	$^{m}C_s^\circ$ G_s° T_s° t_s t_s g_s t_s g_s c_s t_s c_s G_s° A_s° T°

Oligo #	BCL11A-XL Position	Length	LNA Design	Sequence (5'-3')
28	418	15	3-9-3	$^{m}\mathbf{C}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ c_s\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$
29	418	16	3-10-3	$\mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{°}\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ c_s\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$
30	419	13	2-8-3	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{A°}$
31	419	16	3-10-3 ^{m}C	$\mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{°}\ ^{m}c_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{A°}$
32	419	15	3-9-3	$\mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{o}\ ^{m}\mathbf{C}_s\mathbf{°}\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{A°}$
33	420	14	3-8-3	$\mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{°}\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ \mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G°}$
34	420	15	3-9-3 ^{m}c	$\mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{Cs}\mathbf{°}\ ^{m}c_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ \mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G°}$
35	421	16	3-10-3 ^{m}C	$\mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ c_s\ ^{m}c_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{C°}$
36	421	15	3-9-3 ^{m}c	$\mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ c_s\ ^{m}c_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ ^{m}\mathbf{C°}$
37	416	16	2-11-3	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ \mathbf{T}_s\mathbf{°}\mathbf{A}_s\mathbf{°}\mathbf{A°}$
38	416	16	3-9-4	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ \mathbf{A}_s\mathbf{°}\mathbf{T}_s\mathbf{°}\mathbf{A}_s\mathbf{°}\mathbf{A°}$
39	416	16	4-9-3	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ \mathbf{T}_s\mathbf{°}\mathbf{A}_s\mathbf{°}\mathbf{A°}$
40	416	16	4-8-4	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ \mathbf{A}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\mathbf{A}_s\mathbf{°}\mathbf{A°}$
41	416	16	3-8-5	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\mathbf{A}_s\mathbf{°}\mathbf{A°}$
42	416	13	5-8-3	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ \mathbf{T}_s\mathbf{°}\mathbf{A}_s\mathbf{°}\mathbf{A°}$
43	417	15	2-10-3	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ \mathbf{A}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{A°}$
44	417	15	3-10-2	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ \mathbf{T}_s\mathbf{°}\ \mathbf{A°}$
45	417	15	4-9-2	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ \mathbf{T}_s\mathbf{°}\ \mathbf{A°}$
46	417	15	2-9-4	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{A°}$
47	417	15	4-8-3	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ \mathbf{A}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{A°}$
48	417	15	3-8-4	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{A°}$
49	418	14	3-8-3 ^{m}c	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$
50	418	14	2-10-2	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$
51	418	13	2-9-3	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ t_s^{o}\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ c_s\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$
52	418	13	3-9-2	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$
53	418	14	4-8-2	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$
54	418	14	2-8-4	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$
55	418	15	4-7-4	$^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ \mathbf{T}_s\mathbf{°}\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}\mathbf{C}_s\mathbf{°}\ \mathbf{G}_s\mathbf{°}\ \mathbf{A}_s\mathbf{°}\ \mathbf{T°}$

^{m}C denotes nucleotide monomer with a 5-methylcytosin-1-yl base; subscript "s" denotes a phosphorothioate linkage; Capital/bold base denotes a locked nucleic acid; superscript "o" denotes Oxy-LNA.

Example 2. In vitro screening and IC_{50} determination of BCL11A-specific oligonucleotides

The effect of the oligonucleotides on BCL11A nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. BCL11A can be expressed endogenously or by transient or stable transfection of a nucleic acid. The expression level of BCL11A nucleic acid can be routinely determined using, for example, Northern blot analysis, Quantitative PCR, Ribonuclease protection assays. In this example, oligonucleotides synthesized according to Example 1 that selectively target BCL11A were tested on human REH cells and BCL11A mRNA expression was measured. Other cell types can be routinely used, provided that the target is expressed in the cell type chosen. Cells were cultured in the appropriate medium as described below and maintained at 37°C. at 95-98% humidity and 5% CO_2. When cultured under hypoxia or anoxia, O_2 levels were kept at 1-2% or 0-0.5%, respectively. Cells were routinely passaged 2-3 times weekly.

Briefly, 401 oligonucleotides were employed in three-day mammalian cell culture experiments using human REH cells to determine the effect on expression of BCL11A mRNA. Antisense oligonucleotides were added to the cells at 5 and 25 μM without any additional reagents or uptake enhancers using gymnosis delivery technology (Stein, C.A. *et al.* 2010, Nucleic Acids Research 38(1):e3). BCL11A mRNA was measured by quantitative real-time RT-PCR (RT-qPCR). Exemplary results for inhibition of BCL11A by antisense oligonucleotides made in accordance with Example 1 is set forth in Figure 3.

As shown in Figure 3, antisense oligonucleotides made according to Example 1 were capable of inhibiting expression of BCL11A mRNA by targeting several different positions across BCL11A isoform XL, in particular, at positions overlapping among the XL, L and S isoforms (see Figure 2).

In another experiment, IC_{50} values and effect on expression of BCL11A mRNA at various concentrations (ranging from 0.0064 to 20 μM) for selected antisense oligonucleotides was determined using human REH cells as described above. Exemplary results are shown in Table 5 (IC_{50}) and Figure 4 (BCL11A mRNA). Oligo # 56: antisense oligonucleotide that does not target BCL11A mRNA.

TABLE 5

Oligo #	IC_{50} (μM)
4	1.5
7	1.5
8	0.3
9	0.3
10	0.8
11	0.8
19	1.0
20	6.0
21	0.8
22	0.6

Oligo #	IC_{50} (μM)
23	0.5
24	0.7
25	0.3
26	0.6
27	0.9
28	0.5
29	0.4
30	0.7
31	0.4
32	0.3
33	0.5
34	1.2
35	1.6
36	0.8

In a similar experiment, IC_{50} values and effect on expression of BCL11A mRNA at various concentrations (ranging from 0.25 to 60 μM) for different oligonucleotides designed from oligos 4 and 5 was determined using human REH cells as described above. Exemplary results are shown in Table 6 (IC_{50}) and Figure 5 (BCL11A mRNA). Oligo # 56: antisense oligonucleotide that does not target BCL11A mRNA; Mock: no antisense oligonucleotide added to cells.

TABLE 6

Oligo #	IC_{50} (μM)
4	3.9
12	6.2
13	5.1
14	2.0
15	7.1
16	12.6
17	5.1
18	74.8
5	3.2
6	3.4

In another experiment, IC_{50} values and effect on the expression of the different isoforms of BCL11A mRNA at various concentrations (ranging from 0.25 to 60 μM) for selected oligonucleotides was determined using human REH cells as described above. Exemplary results are shown in Table 7 (IC_{50}) and Figure 6 (isoform BCL11A mRNA). Oligo # 56: antisense oligonucleotide that does not target BCL11A mRNA; Mock: no antisense oligonucleotide added to cells.

TABLE 7

Oligo #	IC_{50} (μM)		
	XL	L	S
3-03	2.3	2.4	2.0
4-03	1.9	1.4	1.3
1	1.6	0.9	1.2

In another similar experiment, IC_{50} values and effect on expression of BCL11A mRNA at various concentrations (ranging from 0.08 – 20 µM) for selected oligonucleotides was determined using mouse MPC-11 cells using similar experimental conditions as described above for human REH cells. Exemplary results are shown in Table 8 (IC_{50}) and Figure 7 (BCL11A mRNA). Oligo # 56: antisense oligonucleotide that does not target BCL11A mRNA; Mock: no antisense oligonucleotide added to cells.

TABLE 8

Oligo #	IC50 (µM) BCL11A-All
4	0.8
14	1.0
8	0.2
25	0.3
27	0.8
5	0.7
6	1.4

In yet another experiment, IC_{50} values for different oligonucleotides designed from oligos 8, 25 and 27 were determined using human REH cells as described above. Typically, IC_{50} values were determined using six-point 5x dilutions ranging from 0.0064 to 20 µM. Exemplary results are shown in Table 9.

TABLE 9

Oligo #	Design	IC_{50} (µM)
8	3-10-3	1.0
37	2-11-3	2.0
38	3-9-4	1.3
39	4-9-3	0.6
40	4-8-4	1.1
41	3-8-5	0.9
42	5-8-3	1.8
25	3-9-3	0.7
43	2-10-3	0.9
44	3-10-2	0.3
45	4-9-2	0.3
46	2-9-4	0.4
47	4-8-3	0.3
48	3-8-4	0.6
27	3-8-3	0.5
49	3-8-3^{m}c	0.4
50	2-10-2	0.8
51	2-9-3	0.4
52	3-9-2	0.5
53	4-8-2	0.4
54	2-8-4	1.8
55	4-7-4	11.4

Taken together, these data show that antisense oligonucleotides provided by the present invention such as those described in Example 1 can effectively inhibit BCL11A with a typical

IC_{50} ranging between 0.25 μM-60 μM. In addition, selected antisense oligonucleotides provided by the present invention can effectively inhibit mouse BCL11A with a typical IC_{50} ranging between 0.10 μM-1.5 μM.

Example 3. In vivo tolerance of oligonucleotides

The oligonucleotides described in the prior examples were tested for their *in vivo* tolerability using NMRI mice.

Briefly, female NMRI mice (n=5 per group) were dosed at 0, 3, 7, 10 and 14 days with either saline (control) or a selected LNA oligonucleotide (15 mg/kg) via intravenous administration. Mice were sacrificed 48 hours after the final dose. The following parameters were recorded for each animal in each group: body weight (day 0, day 5, 6 or 7, and day 10, 13, 14 or 16), organ (liver, kidney and spleen) weight at sacrifice, serum alanine aminotransferase (ALT) activity, and BCL11A mRNA expression in whole bone marrow and spleen. Exemplary results are shown in Figures 8.

As shown in Figure 8 the ability of selected antisense oligonucleotides to inhibit BCL11A mRNA expression in mice was confirmed in harvested bone marrow and spleen. For example, oligos 8 and 25 demonstrated about 40% reduction of BCL11A mRNA in bone marrow, while oligos 8 and 20 demonstrated about the same reduction of BCL11A mRNA in spleen. Generally, inhibition of BCL11A mRNA expression in bone marrow ranged on average from about 10 – 50%, whereas inhibition of BCL11A mRNA expression in spleen ranged on average from about 10 – 40%. Typically, body and organ weights of treated animals were unaffected.

Taken together, these data show that antisense oligonucleotides provided by the present invention such as those described in Example 1 are well tolerate and can effectively inhibit BCL11A mRNA expression in various target tissues *in vivo*, including but not limited to, bone marrow, spleen.

In a similar experiment, selected antisense oligonucleotides were tested as described above for their *in vivo* tolerability using NMRI mice. Typically, body and organ weights of mice administered selected antisense oligonucleotides that target BCL11A were typically unaffected. Serum ALT levels for mice administered selected antisense oligonucleotides demonstrated similar results as compared to the saline group.

In a similar experiment, selected antisense oligonucleotides were tested for *in vivo* tolerability using Wistar rats. Briefly, male Wistar rats (n=5 per group) were dosed once per week (day 0, 7, 14, 21 and 28) with either saline (control) or a selected antisense oligonucleotide (25 mg/kg) via subcutaneous administration. Rats were sacrificed at day 30. The following parameters were recorded for each animal in each group: bodyweight during study, organ (liver, kidney and spleen) weight at sacrifice, liver and kidney histopathology, and clinical serum chemistry (alanine aminotransferase, asparatate aminotransferase, alkaline phosphatase, bilirubin, urea and creatinine).

Organ (e.g., liver, kidney and spleen) weights in Wistar rats administered selected antisense oligonucleotides that target BCL11A were typically unaffected. In addition, measurements for various clinical serum chemistry markers (e.g., ALT, AST, ALP, Bilirubin, Urea, Creatine) demonstrated results that were similar as compared to control groups.

Taken together, these data demonstrates that antisense oligonucleotides provided by the present invention such as those described in Example 1 are generally safe and well tolerated.

Example 4. In vivo efficacy in wild-type and β-YAC transgenic mice

Wild-type and transgenic mice transgenic for the human β-globin gene (β-YAC) were used to determine the *in vivo* efficacy of several LNA oligonucleotides made according to the previous Examples.

Briefly, wild-type and β-YAC transgenic mice were dosed (25 or 15 mg/kg) via subcutaneous route with selected antisense oligonucleotides according to one of two schedules: (1) dosing at day 0, 3, 6, 13, 20 and 27, with day 29 designated for necropsy (sacrifice) and (2) dosing at day 0, 3, 6, 13, 20, 27, 34, 41, 48, and 55, with day 57 designated for necropsy (sacrifice). For both dosing schedules, bleeds were take prior to day 0 and at necropsy (day 29 or 57, respectively). Endpoints used in this study included BCL11A knockdown in target tissue (*e.g.*, bone marrow) as well as blood chemistry and biodistribution of oligonucleotides.

Consistent with the results shown Example 3, there was no adverse effect on body weight up to 58 days of treatment with various antisense oligonucleotides for both wild-type and transgenic mice. No significant differences were observed in AST levels among treatment groups.

Exemplary results for knockdown of BCL11A mRNA expression in bone marrow of wild-type mice are set forth in Figure 9 (four weeks post administration) and 10 (eight weeks post administration). Exemplary results for knockdown of BCL11A mRNA expression in β-YAC transgenic mice are set forth in Figure 11. Exemplary results for knockdown of BCL11A mRNA expression in $Ter119^+$ and $CD19^+$ bone marrow cells of β-YAC transgenic mice eight weeks post administration are set forth in Figure 12.

As shown in the above results, knockdown of BCL11A mRNA expression was greater at eight weeks, however, candidate oligo 8 demonstrated the greatest decrease in BCL11A among the oligonucleotides tested. No difference in knockdown of BCL11A expression was observed for candidate oligo 4 when dosed at 15 or 25 mg/kg. Further, no differences in knockdown of BCL11A expression for the selected oligonucleotides was observed for either wild-type or transgenic mice when administered for eight weeks.

Taken together, this example demonstrates that antisense oligonucleotides provided by the present invention can effectively inhibit BCL11A expression in various target tissues *in vivo*, including but not limited to, bone marrow, spleen.

Example 5. Pharmacology Study in non-human primates

A pharmacological study employing an exemplary antisense oligonucleotides that specifically target one or more isoforms of BCL11A was performed to further confirm the *in* vivo safety and efficacy.

Briefly, the study covered a six to sixteen week time period, during which female cynomologus monkeys (ranging two to four years of age and 2.5 – 4 kg in weight) were administered six weekly doses at 20 mg/kg or twelve weekly doses at 10 mg/kg and 20 mg/kg of selected BCL11A-specific LNA antisense oligonucleotides or control (saline) via subcutaneous injection. The animals were sacrificed approximately seven days after the last dosage at approximately week seven or week 17 depending on the duration of the study as described above. As the study proceeds, half of the treatment groups were rendered moderately anemic due to repeated blood sampling during a pretesting phase of the study as well as throughout the dosing period to stimulate erythropoiesis. The experimental design is set forth in Table 10.

TABLE 10

Group	Dose Level (mg/kg)	No. of animals		Phlebotomy
		Interim sacrifice (week 7)	Final sacrifice (week 17)	
Control	0	4	4	-
Low dose	10	-	4	-
High dose	20	4	4	-
Control	0	4	4	+
Low dose	10	-	4	+
High dose	20	4	4	+

Weekly s.c. administration (6 or 16 doses)

Sacrifice seven days after last dose (week 7 or week 17)

Pharmacodynamic biomarkers: Peripheral blood (every second week) and bone marrow (at necropsy, week seven and 17 according to study design) were sampled and used for determination of BCL11A and γ-globin mRNA expression, as well as fetal hemoglobin (HbF) and γ-globin protein levels by an ELISA assay. HbF levels in bone marrow was also analyzed using high-performance liquid chromatography (HPLC). F-cells were measured using the Kleihauer method. Bone marrow sampled at week seven was sampled from the humerus via live bone marrow aspiration. Bone marrow sampled at week 17 was sampled from the humerus and femur bones using multiple methodology. Sampling at week 17 from the humerus bone was performed via live bone marrow aspiration. Sampling at week 17 from the femur was performed via flushing of bone marrow with buffer followed by centrifugation and analysis of the resulting pellet. Sampling at week 17 from the femur was also performed from whole frozen femur.

Hematology analysis included counts of red blood cells, reticulocytes and total hemoglobin measured from samples every two weeks.

Peripheral blood was sampled for pharmacokinetic analysis at two, four, eight, 24 and 48 hours post first and week 12 dose (only week 17 groups) of LNA antisense oligonucleotide in week groups following the 16 week study design only.

At necropsy (week 7 and week 17), liver, kidney and bone marrow were sampled for analysis and weight measurements. Clinical chemistry analysis was also performed at necropsy.

Exemplary total hemoglobin measurements from peripheral blood are shown in Figure 13. Exemplary percentage of reticulocytes in peripheral blood are shown in Figure 14. Exemplary measurements of BCL11A mRNA in humerus bone marrow by RT-qPCR at week seven are shown in Figure 15. Exemplary measurements of γ-globin and β-globin mRNA in humerus bone marrow by RT-qPCR at week seven are shown in Figure 16. Exemplary measurements of BCL11A mRNA in humerus (top) and femur (bottom) bone marrow by RT-qPCR at week 17 are shown in Figure 17. Exemplary measurements of γ-globin mRNA in humerus (top) and femur (bottom) bone marrow by RT-qPCR at week 17 are shown in Figure 18. Exemplary measurements of γ-globin and β-globin mRNA in humerus bone marrow by RT-qPCR at week 17 are shown in Figure 19. Exemplary measurements of γ-globin and β-globin mRNA in femur bone marrow by RT-qPCR at week 17 are shown in Figure 20. Exemplary average measurements of BCL11A (top) and γ-globin (bottom) mRNA in humerus bone marrow by RT-qPCR at week 17 are shown in Figure 21. Exemplary average measurements of BCL11A (top) and γ-globin (bottom) mRNA in femur bone marrow by RT-qPCR at week 17 are shown in Figure 22.

Exemplary measurements of fraction (‰) of F-cells in bone marrow for selected phlebotomized animals at full scale (left) and zoomed-in scale (right) are shown in Figure 23. Exemplary measurements of fraction (‰) of F-cells in peripheral blood for selected phlebotomized animals at full scale (left) and zoomed-in scale (right) are shown in Figure 24.

Exemplary measurements of γ-globin in peripheral blood in control (top), 10 mg/kg (middle), and 20 mg/kg (bottom) dose groups are shown in Figure 25. Exemplary measurements of γ-globin in peripheral blood as a percent of control at a respective time point of a γ-globin peak (“peak 1” or “peak 2”) for control, 10 mg/kg and 20 mg/kg dose groups are shown in Figures 26, 27 and 28, respectively.

As shown in the above results, about a two-fold higher expression of BCL11A was observed in bone marrow samples from femurs as compared to humerus bones. For γ-globin expression, a two- to three-fold higher expression was observed in bone marrow samples from humerus as compared to femurs. The greatest differences were observed in certain particular animals as described below.

For measurements of F-cells in bone marrow, animal I demonstrated about 10‰ F-cells at week 17 as compared to about 0.2‰ in control animals. For measurements of F-cells in peripheral blood, animal I demonstrated about 8‰ at week 17 as compared to about 0.3‰ in control animals. Further, F-cells in this animal began to increase at about week 15 in measurements from samples obtained from peripheral blood.

For measurements of γ-globin in peripheral blood, an increase was observed at week 15 for animal I with a further increase at week 17 in the 10 mg/kg dose group. In a similar fashion, animal Q demonstrated an increase at week 17 in the 20 mg/kg dose group.

In non-phlebotomy groups, no reduction in BCL11A mRNA expression was observed as compared to control groups. Likewise, no increase in F-cells or HbF (γ-globin) was observed for any of the animals.

In summary, experimental results described in this example demonstrate effective target engagement in animal I by greater than 85% knockdown of BCL11A mRNA in bone marrow (humerus and femur) as compared to control animals and by about 60% knockdown of BCL11A mRNA in animal Q in bone marrow (humerus and femur) as compared to control animals. Further, greater than 80-fold induction of γ-globin mRNA expression in bone marrow of animal I as compared to control animals, and a seven-fold increase in γ-globin protein in peripheral blood of animal I as compared to controls were recorded. Animal Q demonstrated about three-fold increase in γ-globin protein in peripheral blood as compared to control animals. Animal I also demonstrated an increase in F-cells in bone marrow and peripheral blood as compared to control animals.

Taken together, this example demonstrates that antisense oligonucleotides provided by the present invention can effectively inhibit BCL11A expression in various target tissues *in vivo* and increase γ-globin protein in peripheral blood by at least two-fold or more as compared to vehicle control.

Example 6. In vivo pharmacokinetics

This example determines the *in vivo* pharmacokinetics of selected LNA oligonucleotides made according to the previous Examples.

Briefly, wild-type mice were given a single dose (20 mg/kg) via subcutaneous route with selected antisense oligonucleotides. Sampling of plasma, liver, kidney and bone marrow were takine at several time points up to 28 days. The pharmacokinetic profile for each tissue sampled was determined. Exemplary results are shown in Figures 29-31.

The results demonstrated rapid distribution and observable distribution of antisense oligonucleotides to all sampled tissues, including bone marrow. The C_{max} was about 21 µg/mL at ten minutes post subcutaneous administration. Liver, kidney and plasma $t_{1/2\beta}$ was about ten days. In the bone marrow, $t_{1/2\beta}$ was about three days.

From this pharmacokinetic study, a predictive model for bone marrow exposure to antisense oligonucleotides was determined (Figure 32).

Taken together, this example demonstrates that antisense oligonucleotides provided by the present invention are effectively and safely absorbed by multiple tissues upon administration (e.g., subcutaneous). Further, antisense oligonucleotides provided by the present invention are distributed to multiple target tissues, including bone marrow, without any adverse effects.

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated that various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only and the invention is described in detail by the claims that follow.

Use of ordinal terms such as “first,” “second,” “third,” etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

The articles “a” and “an” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, (e.g., in Markush group or similar format) it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case

been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. The publications, websites and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

CLAIMS:

1. An antisense oligonucleotide capable of decreasing expression of human BCL11A comprising a sequence that is at least 80% identical to the reverse complement of a continuous sequence within a region selected from nucleotides 410 to 450 of the human BCL11A gene of SEQ ID NO 1 or a messenger RNA (mRNA) isoform of BCL11A, wherein the antisense oligonucleotide is a gapmer.

2. The antisense oligonucleotide according to claim 1, wherein the antisense oligonucleotide is represented by the formula X_a-Y_b-$X_{a'}$, wherein:

X is a nucleotide analogue;

Y is a continuous sequence of DNA;

a is 1, 2, 3, 4 or 5;

a' is 1, 2, 3, 4 or 5; and

b is an integer number between 5 and 15.

3. The antisense oligonucleotide according to claim 2, wherein *a* and/or *a'* is between 2 and 4.

4. The antisense oligonucleotide according to any one of claim 2 or 3, wherein b is an integer number between 7 and 10.

5. The antisense oligonucleotide according to any one of the preceding claims, wherein the antisense oligonucleotide is less than 19 nucleotides in length.

6. The antisense oligonucleotide according to claim 5, wherein the oligonucleotide is 10 to16 nucleotides in length.

7. The antisense oligonucleotide according to any one of the preceding claims, wherein the oligonucleotide comprises at least one nucleotide analogue selected from the group consisting of 2'-O-alkyl-RNA units, 2'-OMe-RNA units, 2'-O-alkyl-DNA, 2'-amino-DNA units, 2'-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, HNA units, INA units and 2'MOE units.

8. The antisense oligonucleotide according to claim 7, wherein the nucleotide analogue is a LNA unit selected from the group consisting of beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA, alpha-L-amino-LNA, beta-D-thio-LNA, alpha-L-thio-LNA, 5'-methyl-LNA, beta-D-ENA and alpha-L-ENA.

9. The antisense oligonucleotide according to any of the preceding claims, wherein the oligonucleotide comprise at least one phosphorothioate linkage.

10. The antisense oligonucleotide according to any of the preceding claims, wherein the oligonucleotide is capable of recruiting an RNAaseH.

11. The antisense oligonucleotide according to any one of the preceding claims, wherein the antisense oligonucleotide comprises an oligonucleotide sequence motif selected from the group consisting of SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66 SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74.

12. The antisense oligonucleotide according to any one of the preceding claims, wherein the antisense oligonucleotide has a sequence selected from SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 32, SEQ ID NO: 21, SEQ ID NO: 34, SEQ ID NO:10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO:27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30 SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 60, SEQ ID NO: 61 or SEQ ID NO:

13. The antisense oligonucleotide according to any one of the preceding claims, wherein the antisense oligonucleotide is 5'- $^{m}\mathbf{C}_{s}\mathbf{°}$ $\mathbf{A}_{s}\mathbf{°}$ $\mathbf{T}_{s}\mathbf{°}$ t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s $^{m}\mathbf{C}_{s}\mathbf{°}$ $^{m}\mathbf{C}_{s}\mathbf{°}$ $\mathbf{G}^{o}$-3' (SEQ ID NO: 11), 5' - $^{m}\mathbf{C}_{s}\mathbf{°}$ $\mathbf{G}_{s}\mathbf{°}$ $\mathbf{T}_{s}\mathbf{°}$ t_s t_s g_s t_s g_s c_s t_s $^{m}c_s g_s a_s \mathbf{T}_{s}\mathbf{°} \mathbf{A}_{s}\mathbf{°} \mathbf{A}\mathbf{°}$- 3' (SEQ ID NO: 15), 5' - $^{m}\mathbf{C}_{s}\mathbf{°}$ $\mathbf{G}_{s}\mathbf{°}$ $\mathbf{T}_{s}\mathbf{°}$ t_s t_s g_s t_s g_s c_s t_s $^{m}c_s$ g_s $\mathbf{A}_{s}\mathbf{°}$ $\mathbf{T}_{s}\mathbf{°}$ $\mathbf{A}\mathbf{°}$- 3' (SEQ ID NO: 32) 5' - $\mathbf{T}_{s}\mathbf{°}$ $\mathbf{T}_{s}\mathbf{°}$ $\mathbf{G}_{s}\mathbf{°}$ t_s g_s c_s t_s $^{m}c_s$ g_s a_s t_s $\mathbf{A}_{s}\mathbf{°}$ $\mathbf{A}_{s}\mathbf{°}$ $\mathbf{A}\mathbf{°}$ – 3' (SEQ ID NO: 14) or 5' - $^{m}\mathbf{C}_{s}\mathbf{°}$ $\mathbf{G}_{s}\mathbf{°}$ $\mathbf{T}_{s}\mathbf{°}$ t_s t_s g_s t_s g_s c_s t_s c_s $\mathbf{G}_{s}\mathbf{°}$ $\mathbf{A}_{s}\mathbf{°}$ $\mathbf{T}\mathbf{°}$ – 3' (SEQ ID NO: 35), wherein upper case letters indicate locked nucleic acid (LNA) units, subscript "s" represents phosphorothioate linkage, and lower case letters represent deoxyribonucleotide (DNA) units, "^{m}C" represents 5' methyl-cytosine LNA unit, and "^{m}c" represents 5' methyl-cytosine DNA unit.

14. A pharmaceutical composition comprising the antisense oligonucleotide according to any one of claims 1 to 13 and a pharmaceutically acceptable carrier.

15. The antisense oligonucleotide according to any one of claims 1 to 13 or pharmaceutical composition claim 14, for use as a medicament, such as for the treatment of an anemic disease, disorder or condition, such as sickle cell disease or β-thalassemia.

16. The use of an antisense oligonucleotide according to any one of claims 1 to 13 or pharmaceutical composition claim 14, for the manufacture of a medicament for the treatment of an anemic disease, disorder or condition, such as sickle cell disease or β-thalassemia.

17. A method of inhibiting BCL11A comprising administering to a subject in need of treatment an antisense oligonucleotide according to any one of claims 1 to 13 or a pharmaceutical composition of claim 14.

18. A method of treating an anemic disease, disorder or condition comprising administering to a subject in need of treatment an oligonucleotide according to any one of claims 1 to 13 or a pharmaceutical composition of claim 14.

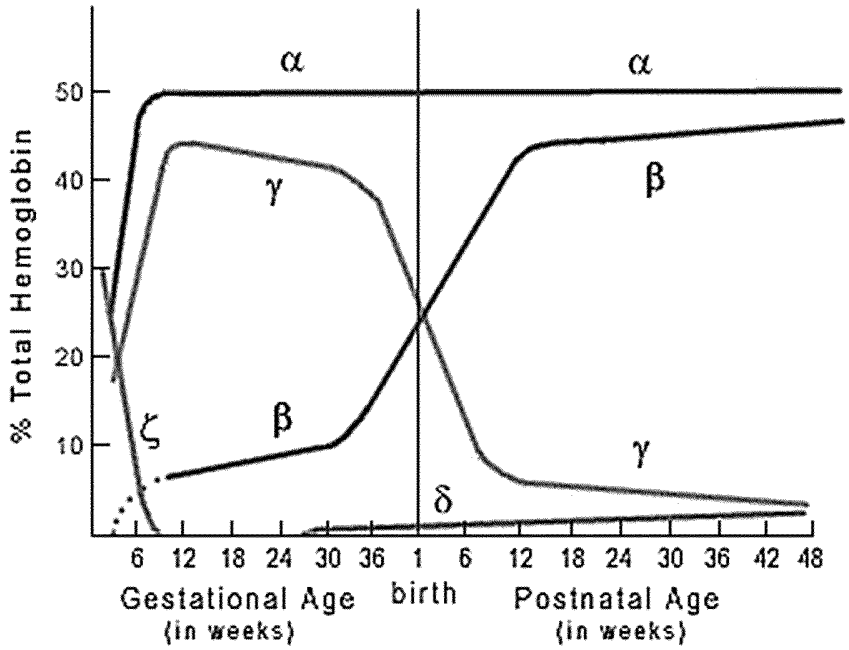
19. The method of claim18, wherein the anemic disease, disorder or condition is sickle cell disease.

20. The method of claim 18, wherein the anemic disease, disorder or condition is β-thalassemia.

FIGURES

Figure 1

Normal globin gene switching



Beta chain dysfunction

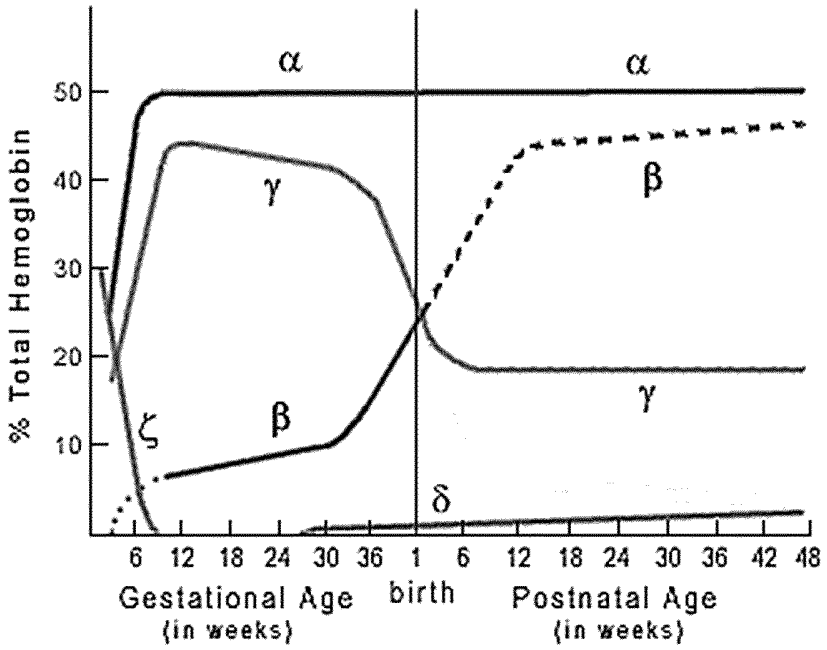


Figure 2

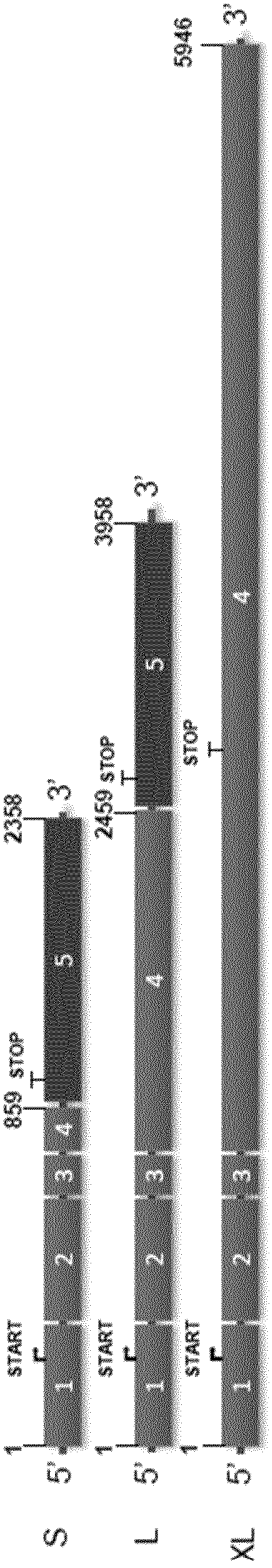


Figure 3

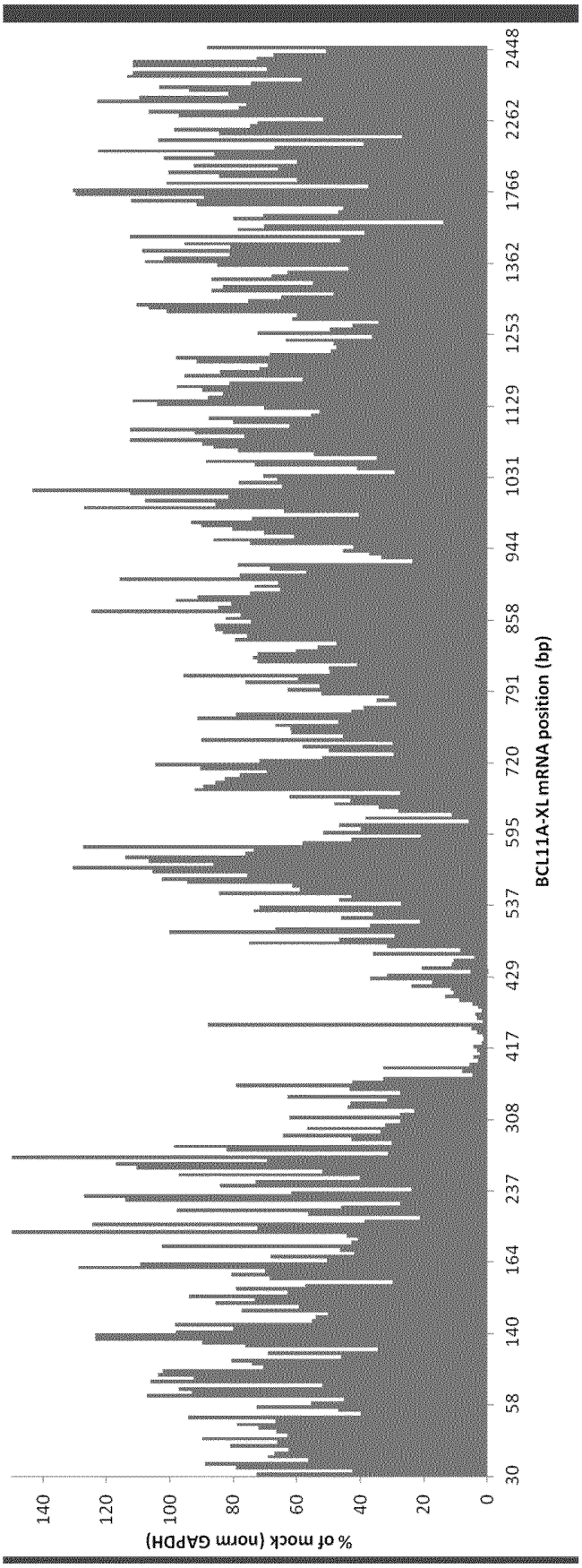

% of mock (norm GAPDH)
0
20
40
60
80
100
120
140
BCL11A-XL mRNA position (bp)
30
58
140
164
237
308
417
429
537
595
720
791
858
944
1031
1129
1253
1362
1766
2262
2448

Figure 4

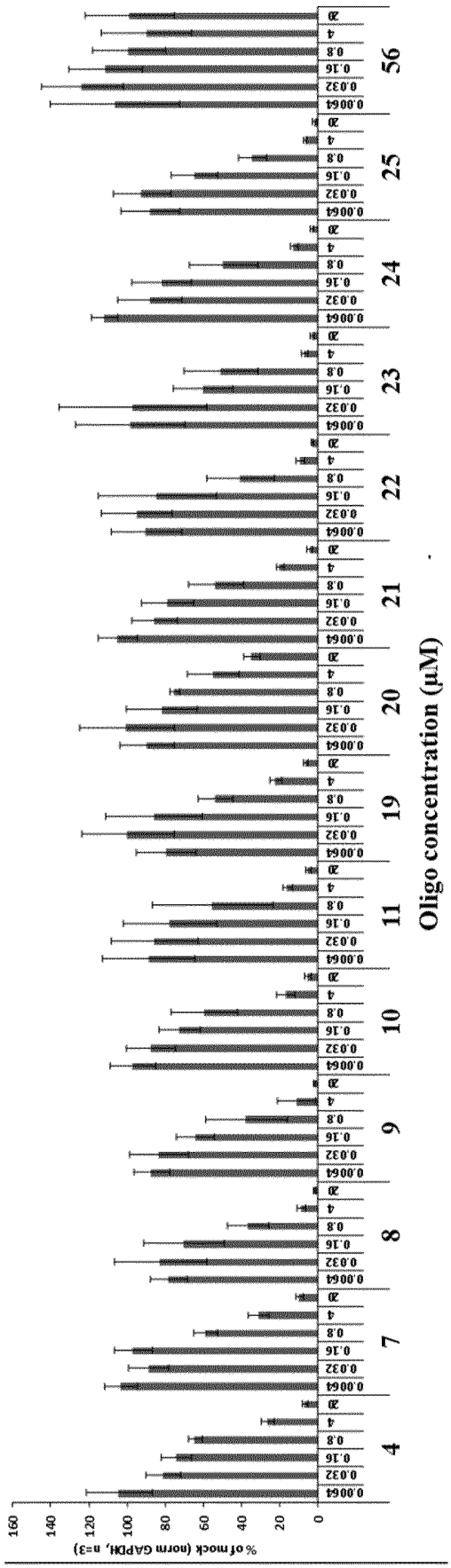

% of mock (norm GAPDH, n=3)
0
20
40
60
80
100
120
140
160
Oligo concentration (µM)
4
7
8
9
10
11
19
20
21
22
23
24
25
56
20
4
0.8
0.16
0.032
0.0064

Figure 4 (continued)

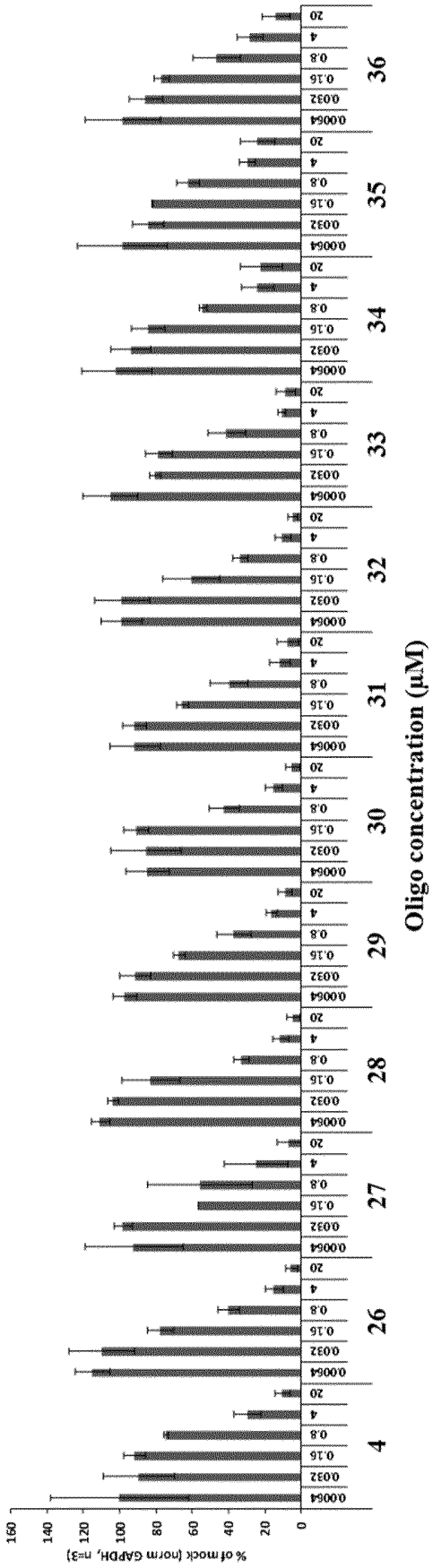


Figure 5

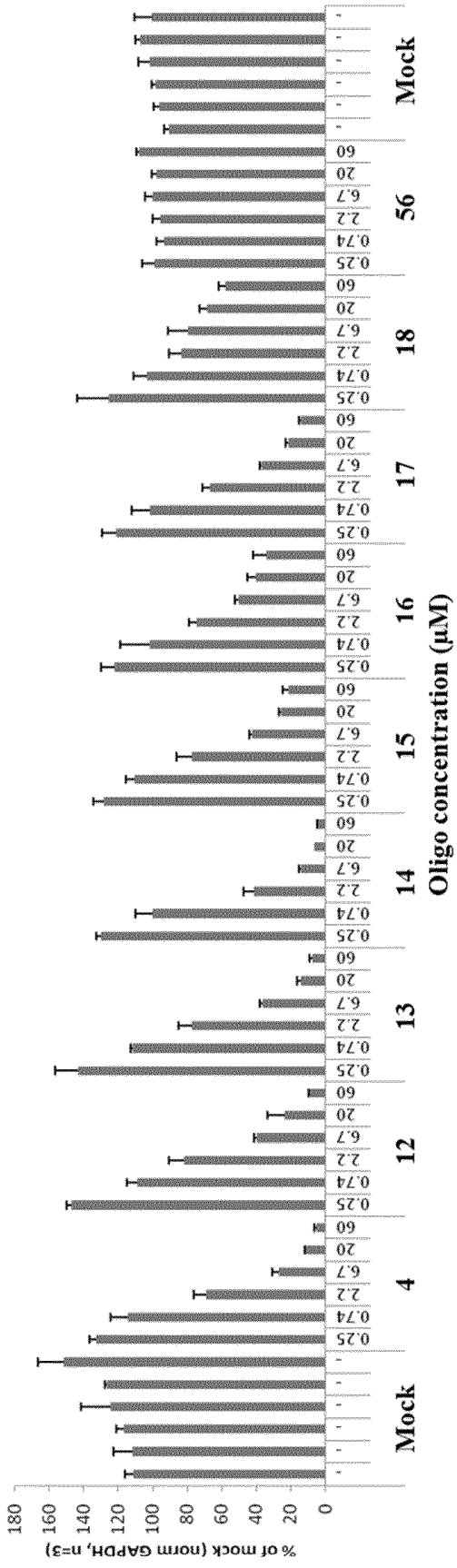

% of mock (norm GAPDH, n=3)
180
160
140
120
100
80
60
40
20
0
Mock
4
12
13
14
15
16
17
18
56
Mock
Oligo concentration (µM)
-
0.25
0.74
2.2
6.7
20
60

Figure 5 (continued)

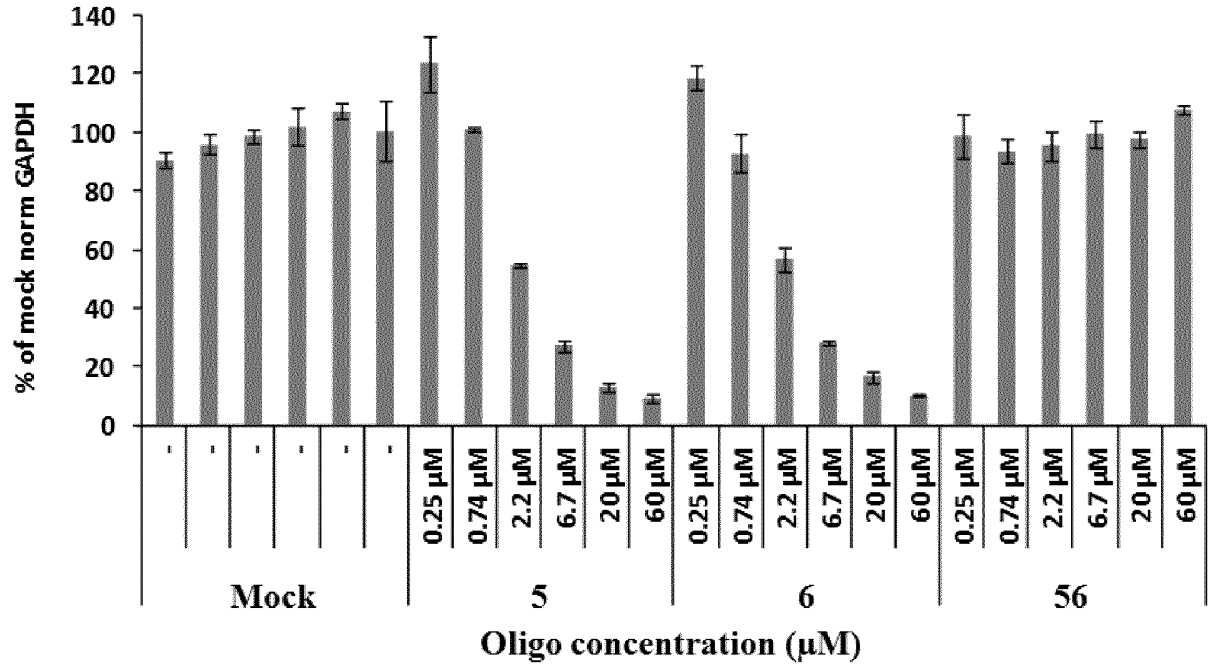


Figure 6

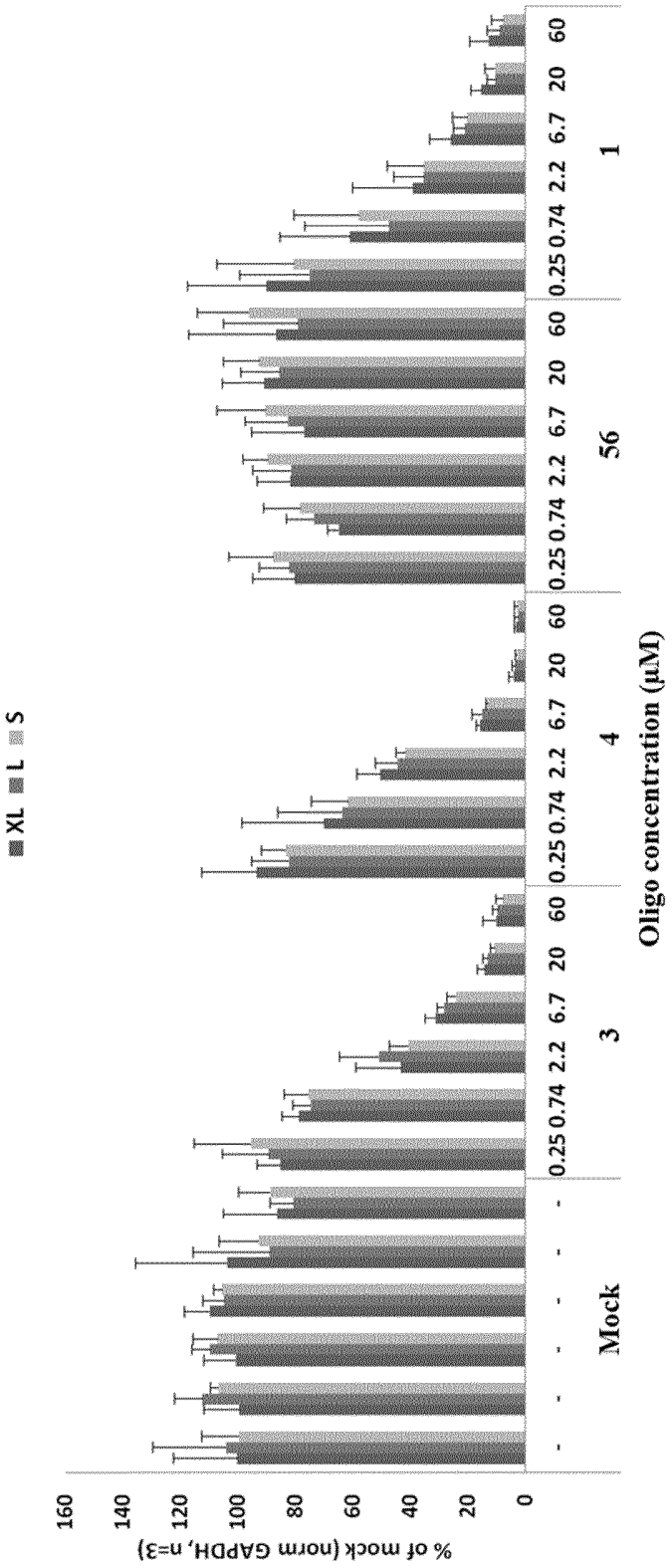

XL
L
S
% of mock (norm GAPDH, n=3)
0
20
40
60
80
100
120
140
160
Mock
3
4
56
1
Oligo concentration (µM)
-
0.25
0.74
2.2
6.7
20
60

Figure 7

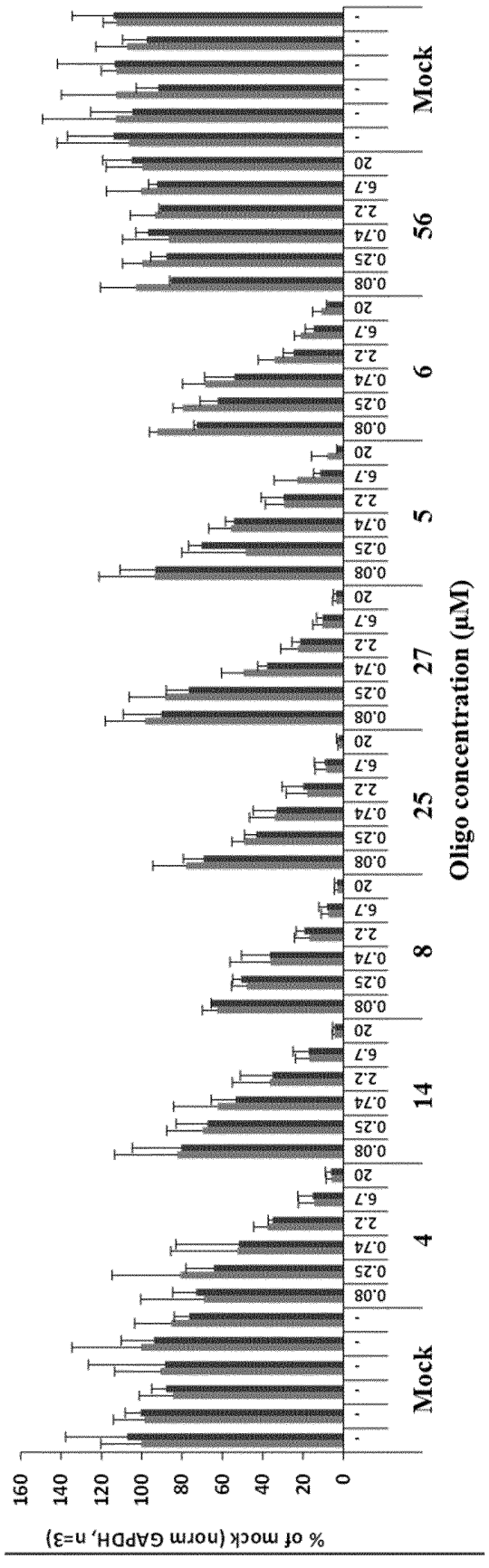

% of mock (norm GAPDH, n=3)
160
140
120
100
80
60
40
20
0
Oligo concentration (µM)
Mock
4
14
8
25
27
5
6
56
Mock
-
0.08
0.25
0.74
2.2
6.7
20

Figure 8

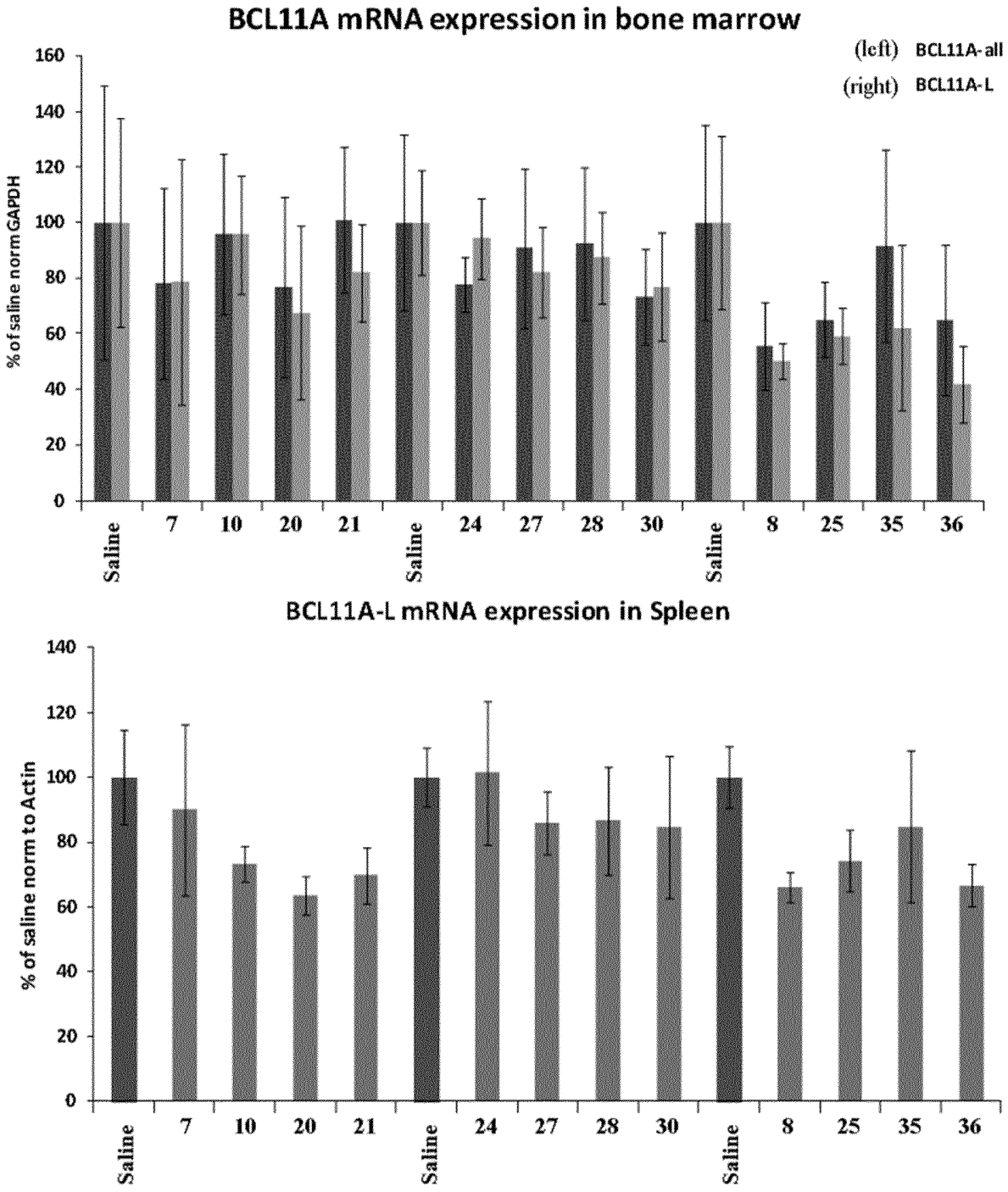

BCL11A mRNA expression in bone marrow
(left) BCL11A-all
(right) BCL11A-L
% of saline norm GAPDH
160
140
120
100
80
60
40
20
0
Saline
7
10
20
21
Saline
24
27
28
30
Saline
8
25
35
36
BCL11A-L mRNA expression in Spleen
% of saline norm to Actin
140
120
100
80
60
40
20
0
Saline
7
10
20
21
Saline
24
27
28
30
Saline
8
25
35
36

Figure 9

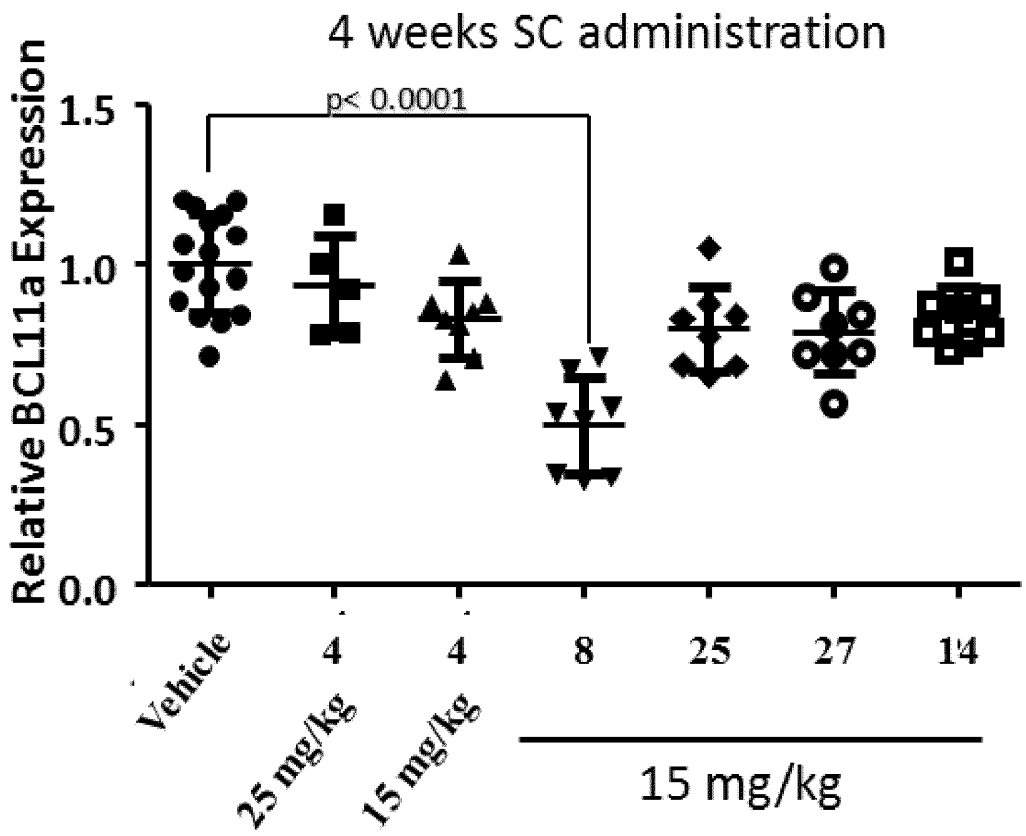

4 weeks SC administration
p< 0.0001
Relative BCL11a Expression
1.5
1.0
0.5
0.0
Vehicle
4
25 mg/kg
4
15 mg/kg
8
25
27
14
15 mg/kg

Figure 10

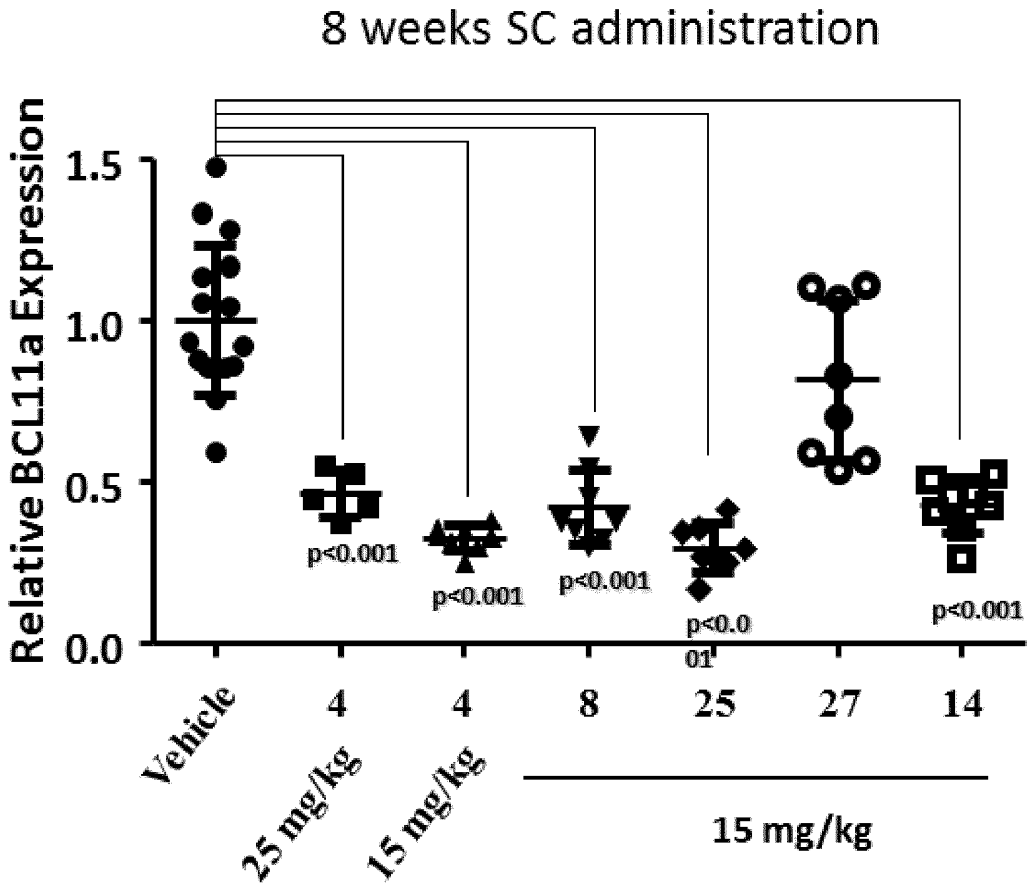

8 weeks SC administration
Relative BCL11a Expression
1.5
1.0
0.5
0.0
p<0.001
p<0.001
p<0.001
p<0.0
01
p<0.001
Vehicle
4
4
8
25
27
14
25 mg/kg
15 mg/kg
15 mg/kg

Figure 11

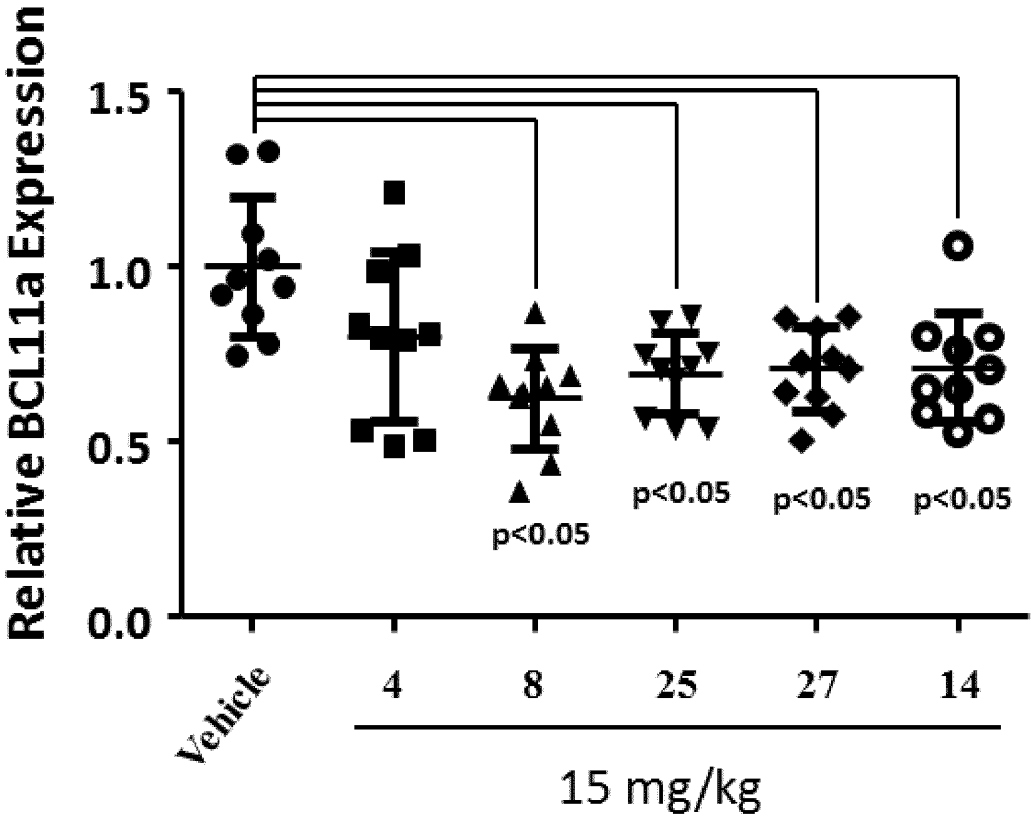

Relative BCL11a Expression
1.5
1.0
0.5
0.0
p<0.05
p<0.05
p<0.05
p<0.05
Vehicle
4
8
25
27
14
15 mg/kg

Figure 12

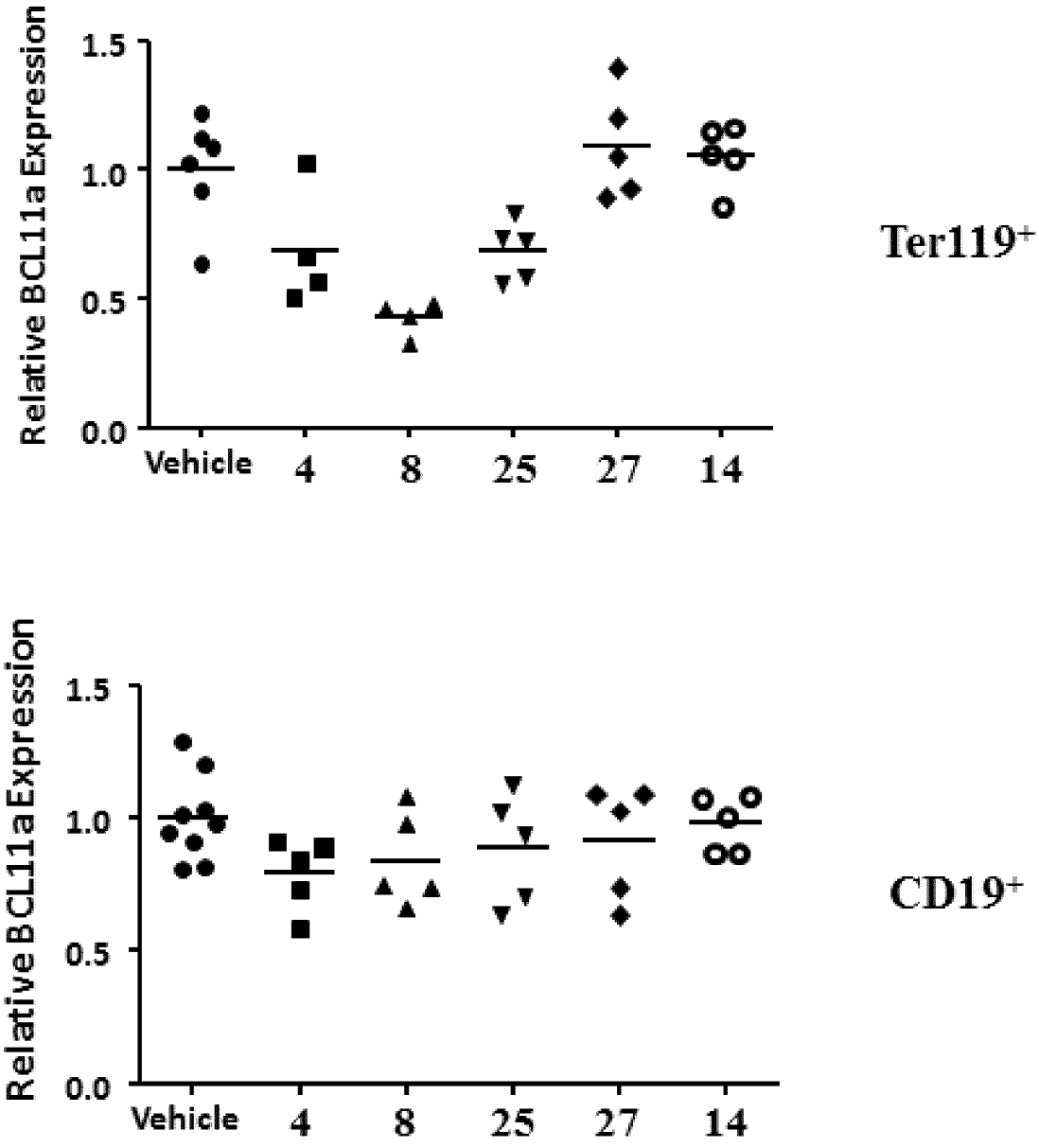

Relative BCL11a Expression
1.5
1.0
0.5
0.0
Vehicle
4
8
25
27
14
Ter119+
Relative BCL11a Expression
1.5
1.0
0.5
0.0
Vehicle
4
8
25
27
14
CD19+

Figure 13

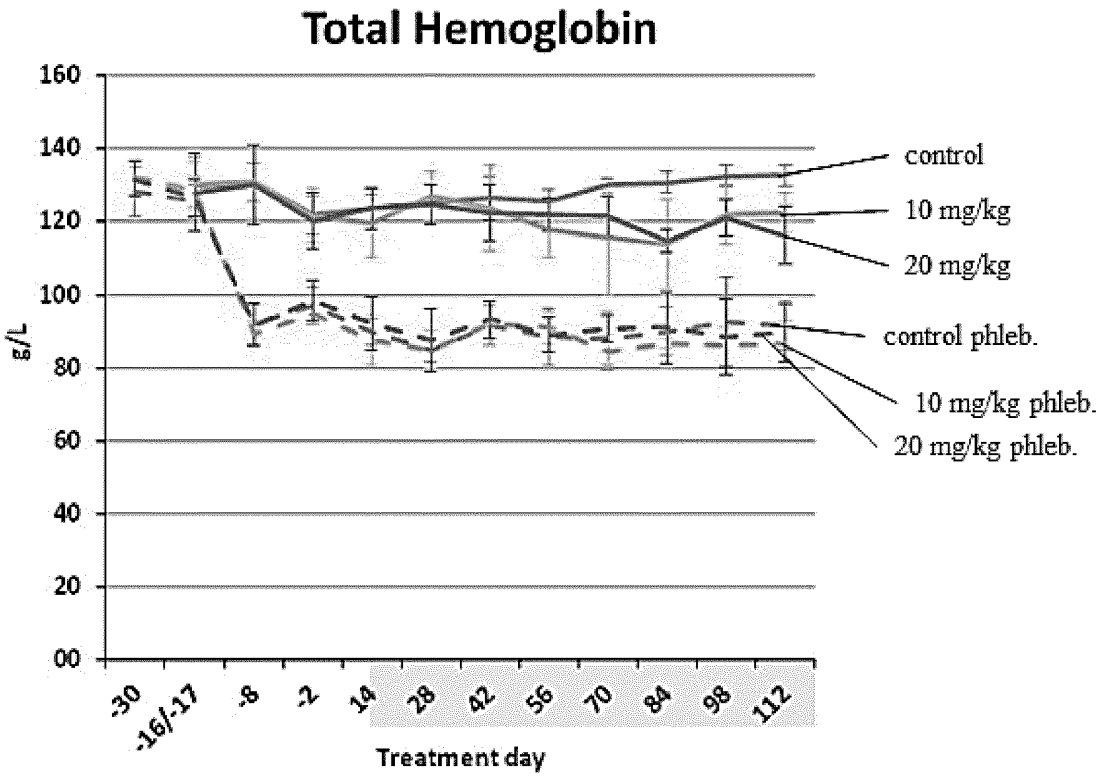

Total Hemoglobin
160
140
120
100
80
60
40
20
00
g/L
-30
-16/-17
-8
-2
14
28
42
56
70
84
98
112
Treatment day
control
10 mg/kg
20 mg/kg
control phleb.
10 mg/kg phleb.
20 mg/kg phleb.

Figure 14

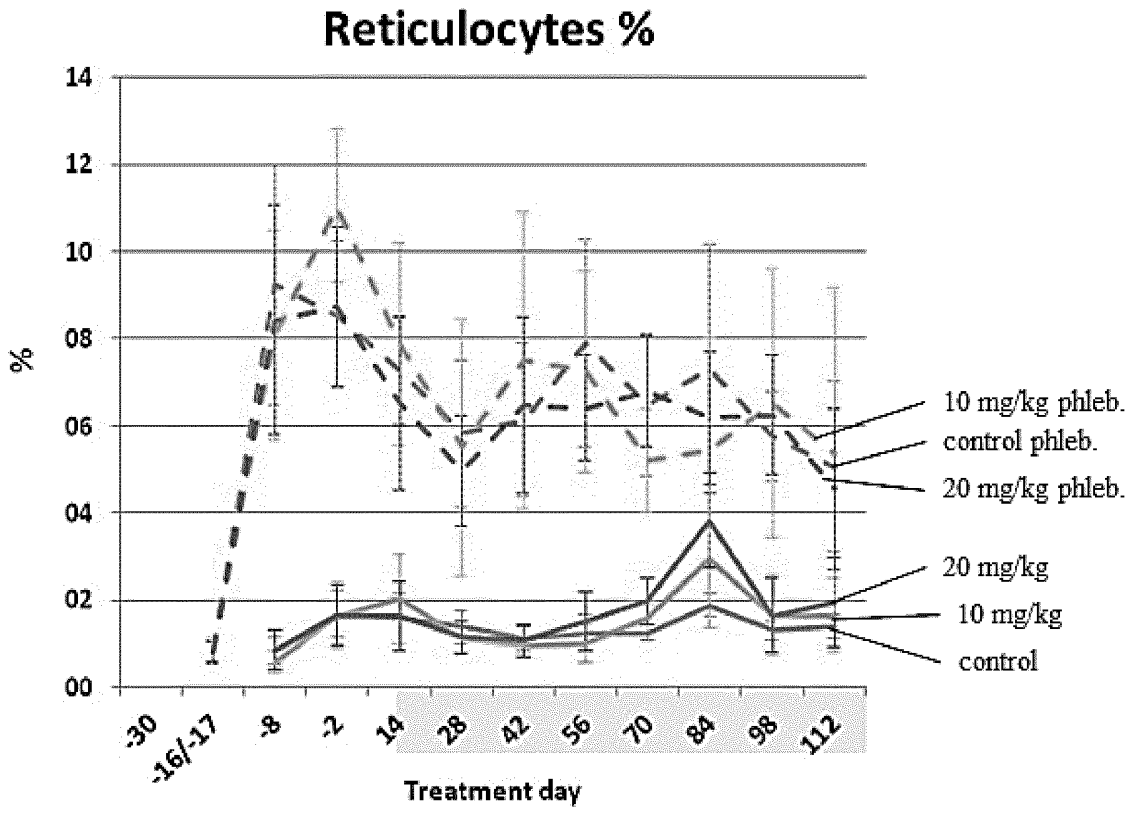

Reticulocytes %
%
14
12
10
08
06
04
02
00
-30
-16/-17
-8
-2
14
28
42
56
70
84
98
112
Treatment day
10 mg/kg phleb.
control phleb.
20 mg/kg phleb.
20 mg/kg
10 mg/kg
control

Figure 15

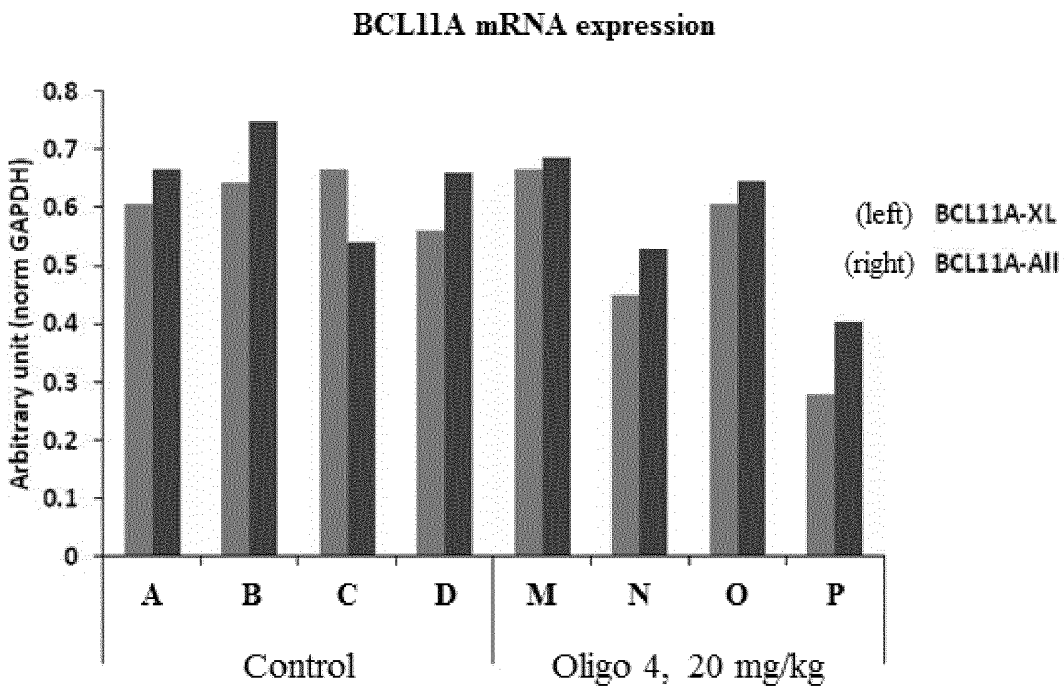

BCL11A mRNA expression
Arbitrary unit (norm GAPDH)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A
B
C
D
M
N
O
P
Control
Oligo 4, 20 mg/kg
(left) BCL11A-XL
(right) BCL11A-All

Figure 16

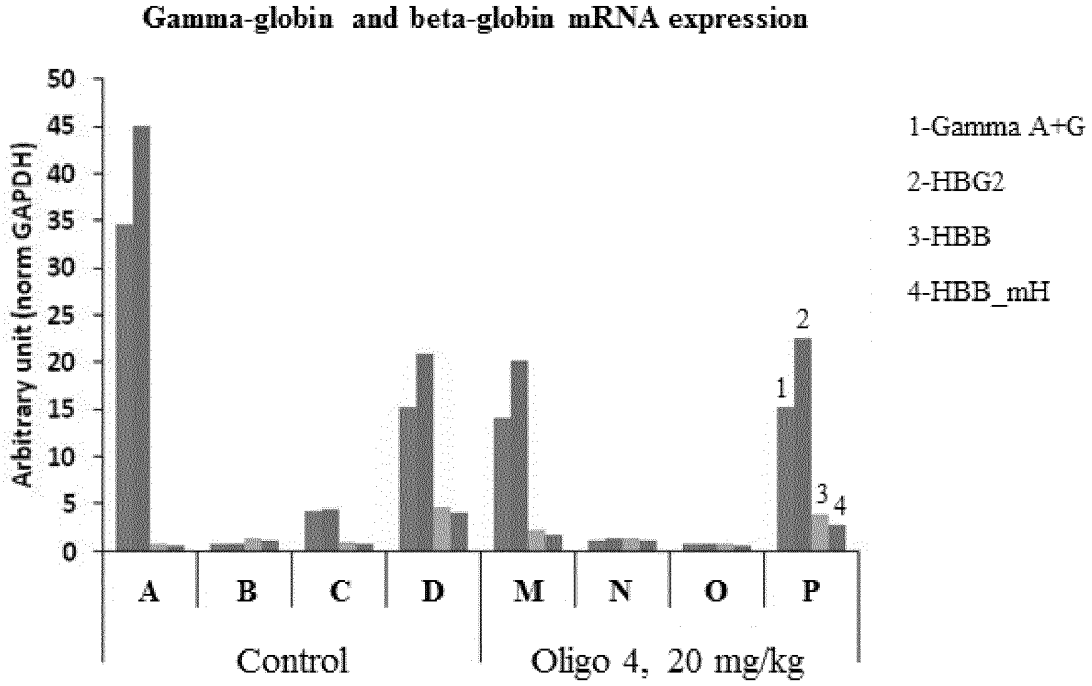

Gamma-globin and beta-globin mRNA expression
Arbitrary unit (norm GAPDH)
50
45
40
35
30
25
20
15
10
5
0
A
B
C
D
M
N
O
P
Control
Oligo 4, 20 mg/kg
1-Gamma A+G
2-HBG2
3-HBB
4-HBB_mH
1
2
3
4

Figure 17

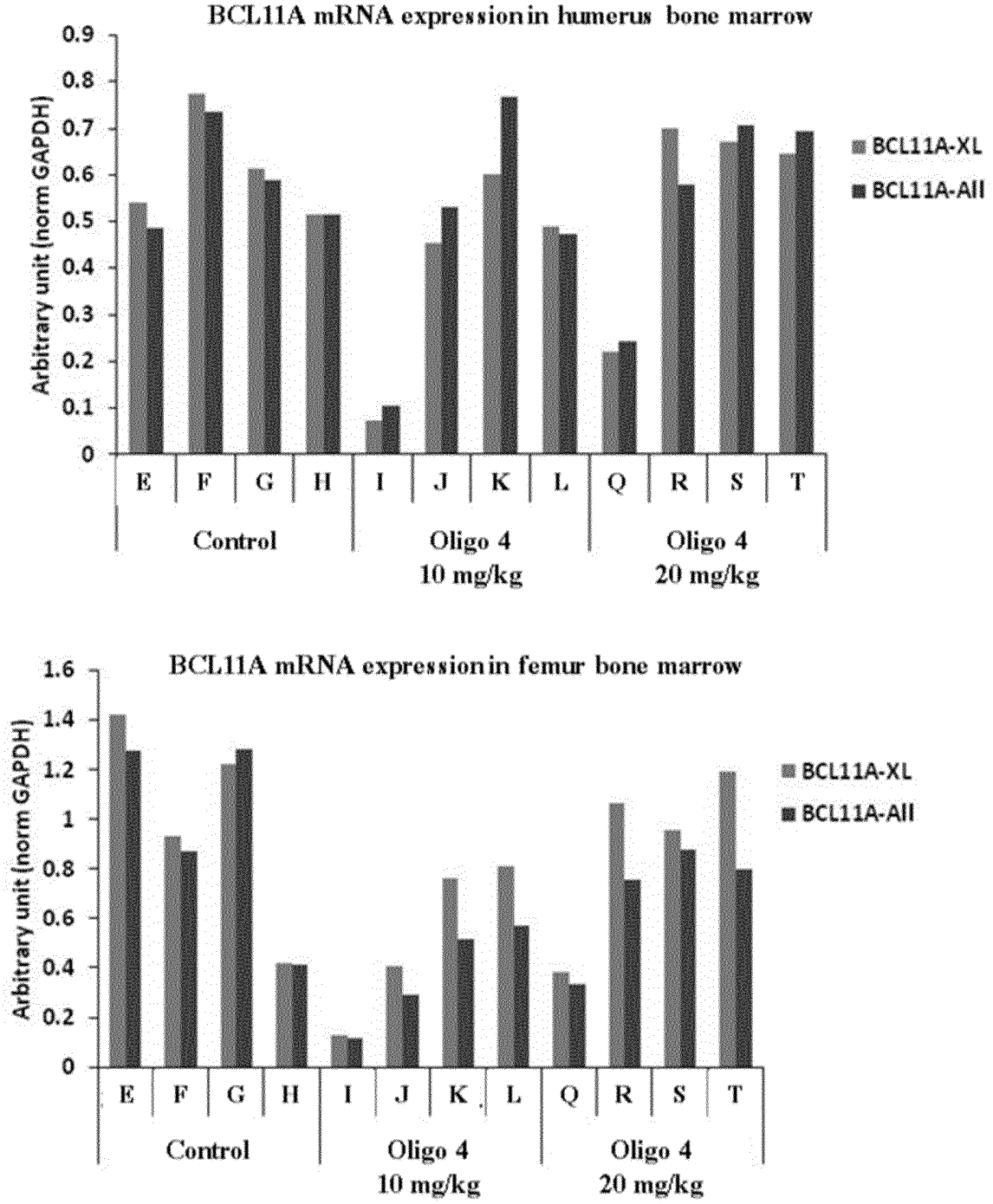

BCL11A mRNA expression in humerus bone marrow
Arbitrary unit (norm GAPDH)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BCL11A-XL
BCL11A-All
E F G H I J K L Q R S T
Control
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg
BCL11A mRNA expression in femur bone marrow
Arbitrary unit (norm GAPDH)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
BCL11A-XL
BCL11A-All
E F G H I J K L Q R S T
Control
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg

Figure 18

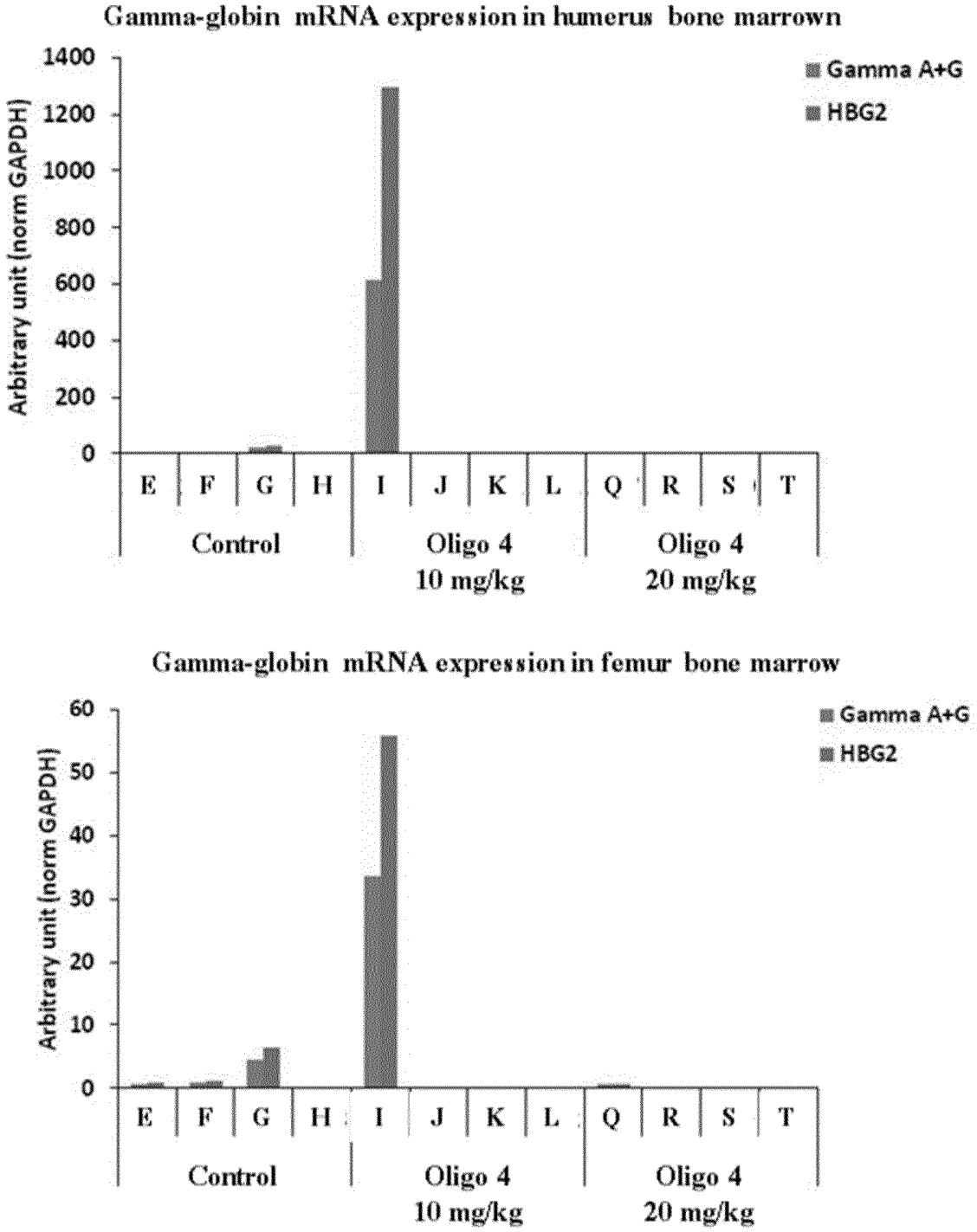

Gamma-globin mRNA expression in humerus bone marrown
Arbitrary unit (norm GAPDH)
1400
1200
1000
800
600
400
200
0
Gamma A+G
HBG2
E
F
G
H
I
J
K
L
Q
R
S
T
Control
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg
Gamma-globin mRNA expression in femur bone marrow
Arbitrary unit (norm GAPDH)
60
50
40
30
20
10
0
Gamma A+G
HBG2
E
F
G
H
I
J
K
L
Q
R
S
T
Control
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg

Figure 19

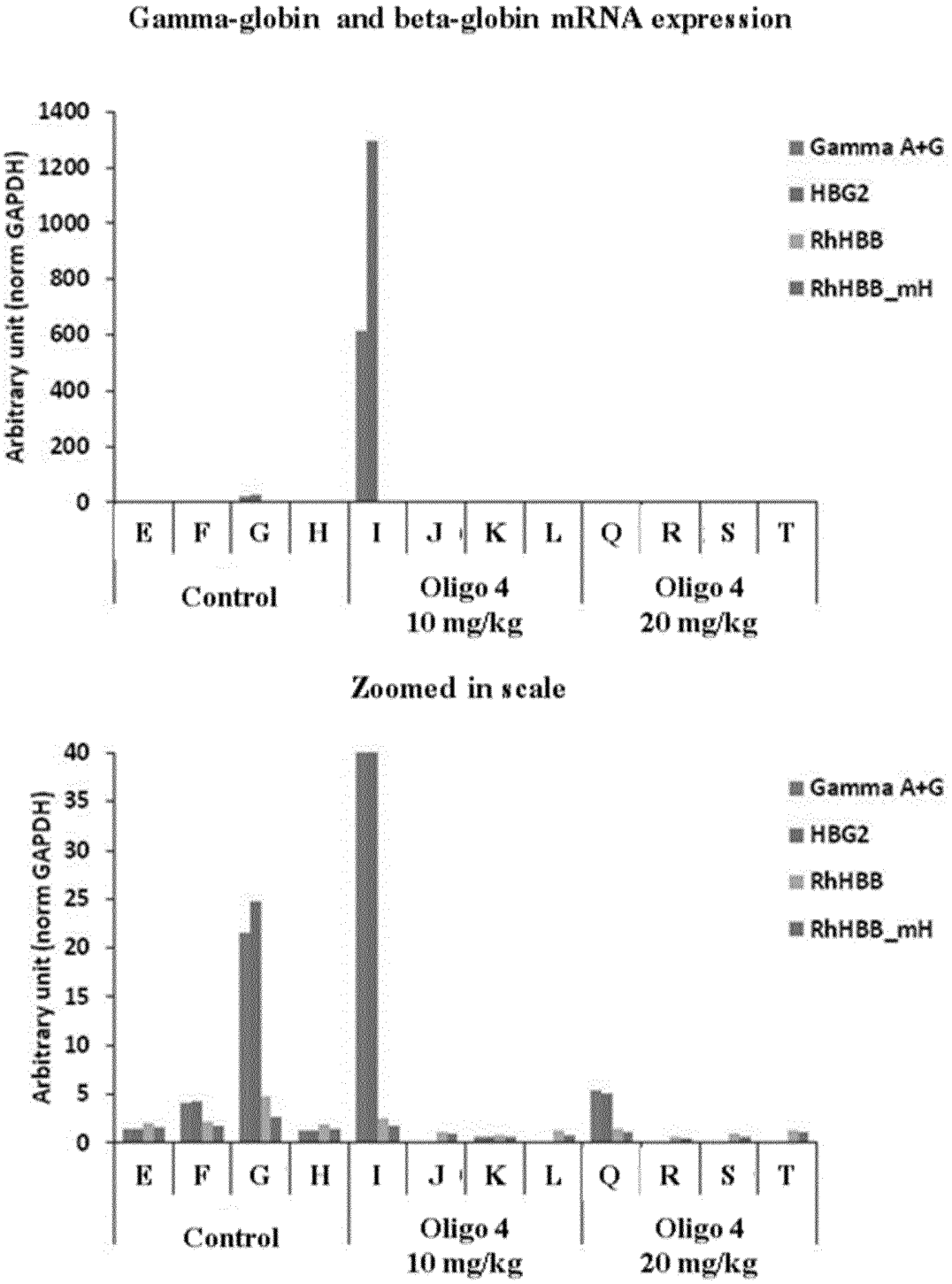

Gamma-globin and beta-globin mRNA expression
Arbitrary unit (norm GAPDH)
1400
1200
1000
800
600
400
200
0
Gamma A+G
HBG2
RhHBB
RhHBB_mH
E F G H I J K L Q R S T
Control
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg
Zoomed in scale
Arbitrary unit (norm GAPDH)
40
35
30
25
20
15
10
5
0
Gamma A+G
HBG2
RhHBB
RhHBB_mH
E F G H I J K L Q R S T
Control
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg

Figure 20

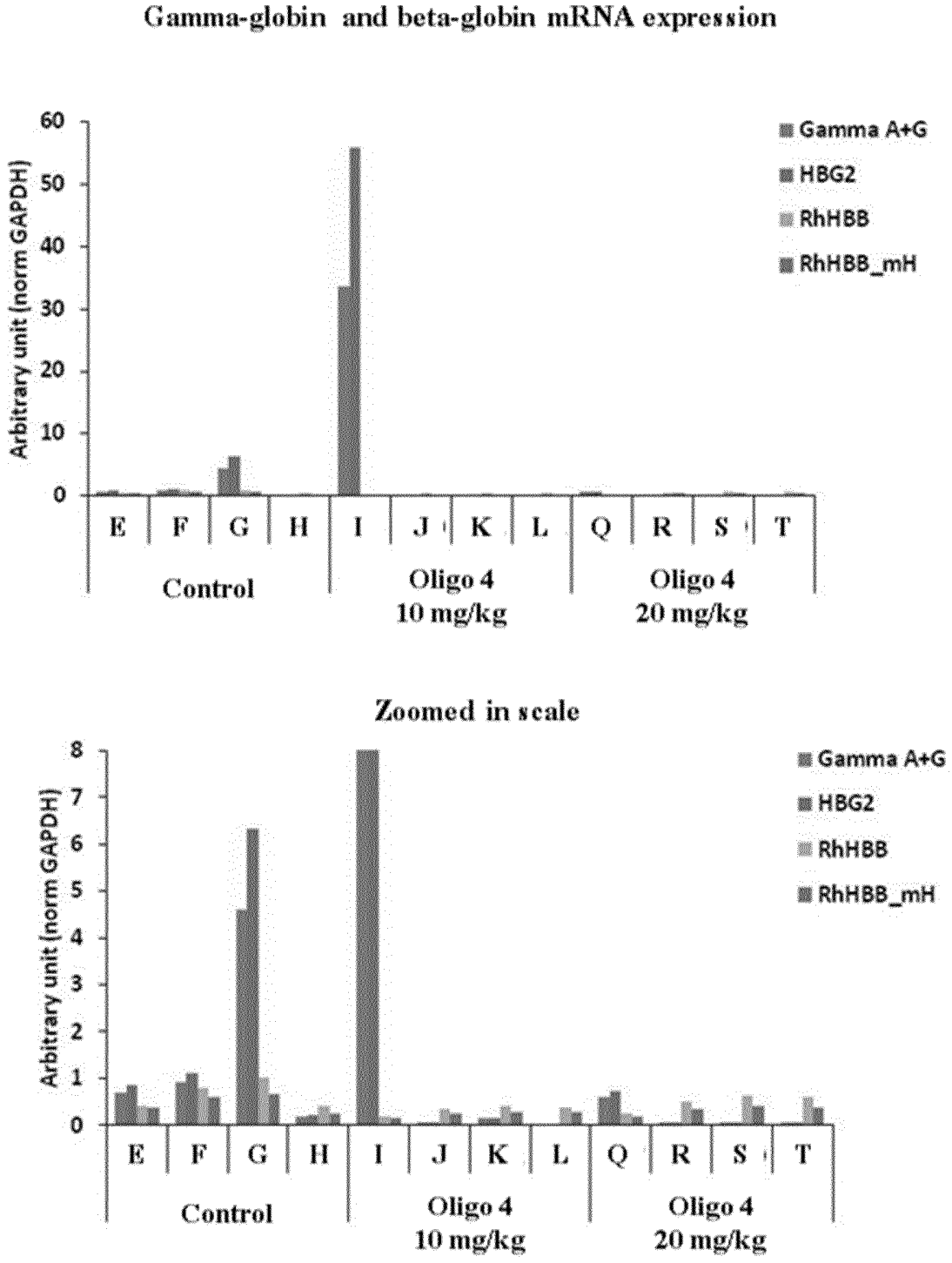

Gamma-globin and beta-globin mRNA expression
Arbitrary unit (norm GAPDH)
60
50
40
30
20
10
0
Gamma A+G
HBG2
RhHBB
RhHBB_mH
E
F
G
H
I
J
K
L
Q
R
S
T
Control
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg
Zoomed in scale
Arbitrary unit (norm GAPDH)
8
7
6
5
4
3
2
1
0
Gamma A+G
HBG2
RhHBB
RhHBB_mH
E
F
G
H
I
J
K
L
Q
R
S
T
Control
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg

Figure 21

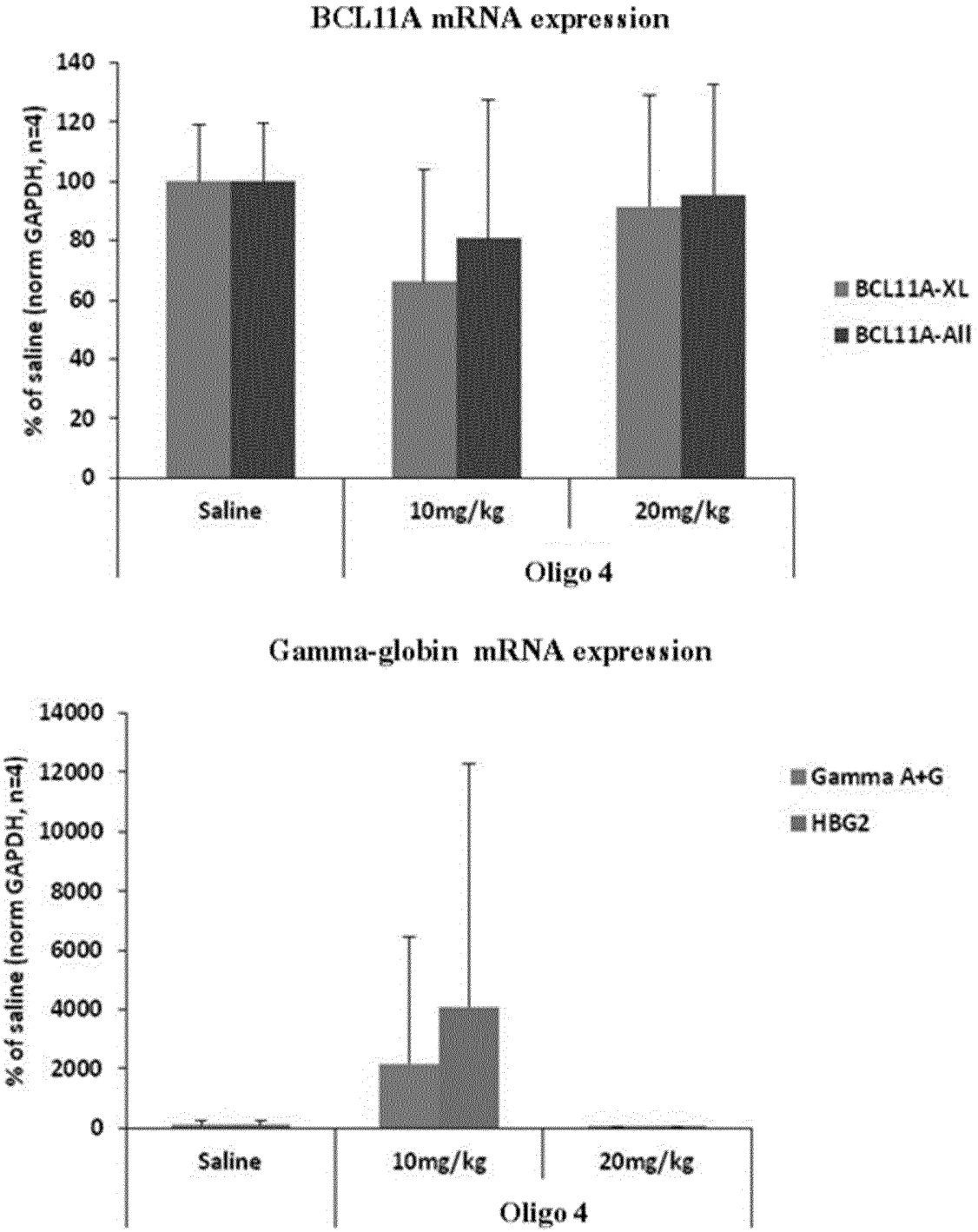

BCL11A mRNA expression
% of saline (norm GAPDH, n=4)
140
120
100
80
60
40
20
0
Saline
10mg/kg
20mg/kg
Oligo 4
BCL11A-XL
BCL11A-All
Gamma-globin mRNA expression
% of saline (norm GAPDH, n=4)
14000
12000
10000
8000
6000
4000
2000
0
Saline
10mg/kg
20mg/kg
Oligo 4
Gamma A+G
HBG2

Figure 22

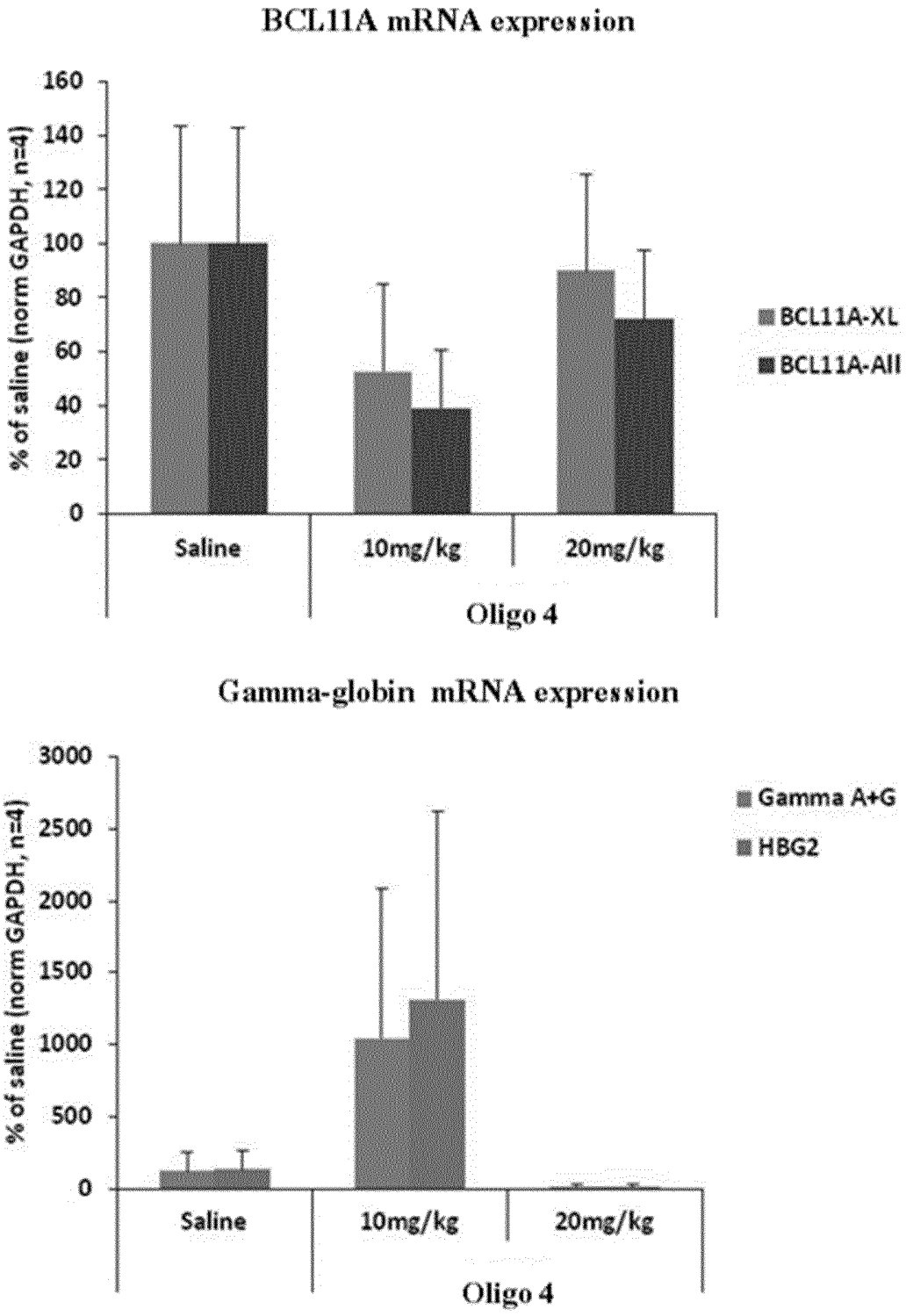

BCL11A mRNA expression
% of saline (norm GAPDH, n=4)
160
140
120
100
80
60
40
20
0
Saline
10mg/kg
20mg/kg
Oligo 4
BCL11A-XL
BCL11A-All
Gamma-globin mRNA expression
% of saline (norm GAPDH, n=4)
3000
2500
2000
1500
1000
500
0
Saline
10mg/kg
20mg/kg
Oligo 4
Gamma A+G
HBG2

Figure 23

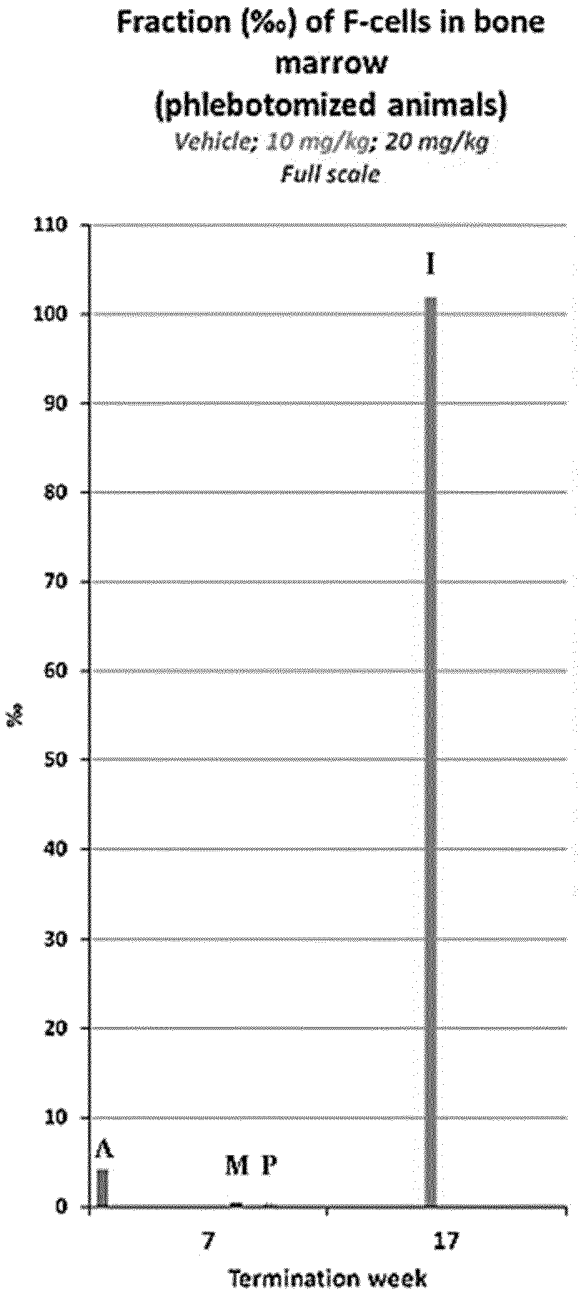

Fraction (‰) of F-cells in bone marrow
(phlebotomized animals)
Vehicle; 10 mg/kg; 20 mg/kg
Full scale
110
100
90
80
70
60
50
40
30
20
10
0
‰
A
M P
I
7
17
Termination week

Figure 23 (continued)

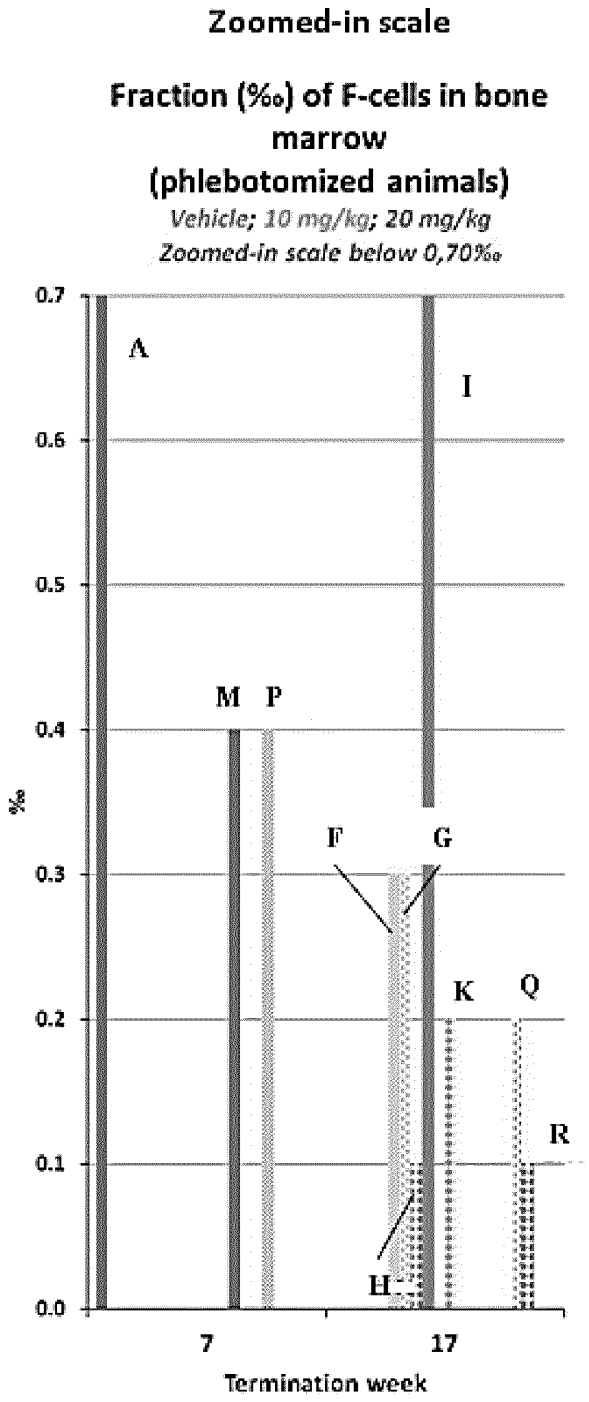


Figure 24

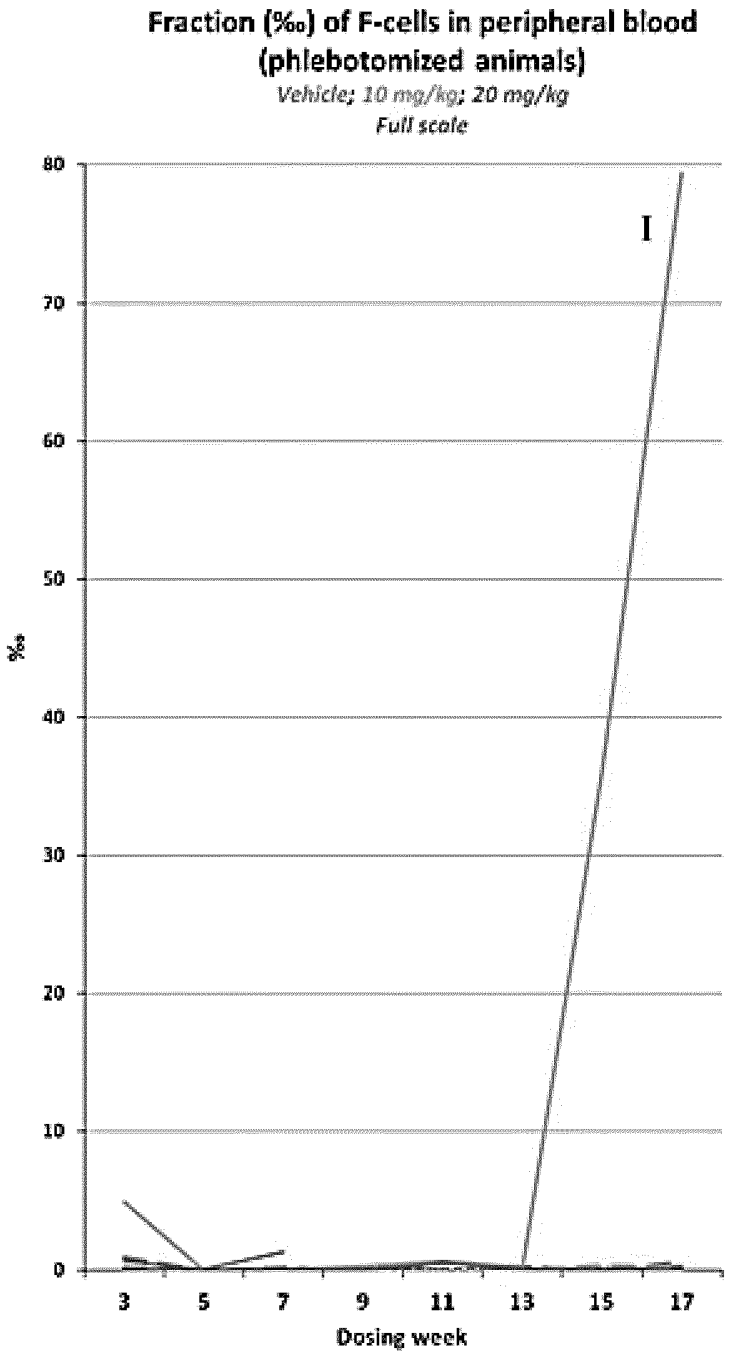

Fraction (‰) of F-cells in peripheral blood
(phlebotomized animals)
Vehicle; 10 mg/kg; 20 mg/kg
Full scale
80
70
60
50
40
30
20
10
0
‰
I
3
5
7
9
11
13
15
17
Dosing week

Figure 24 (continued)

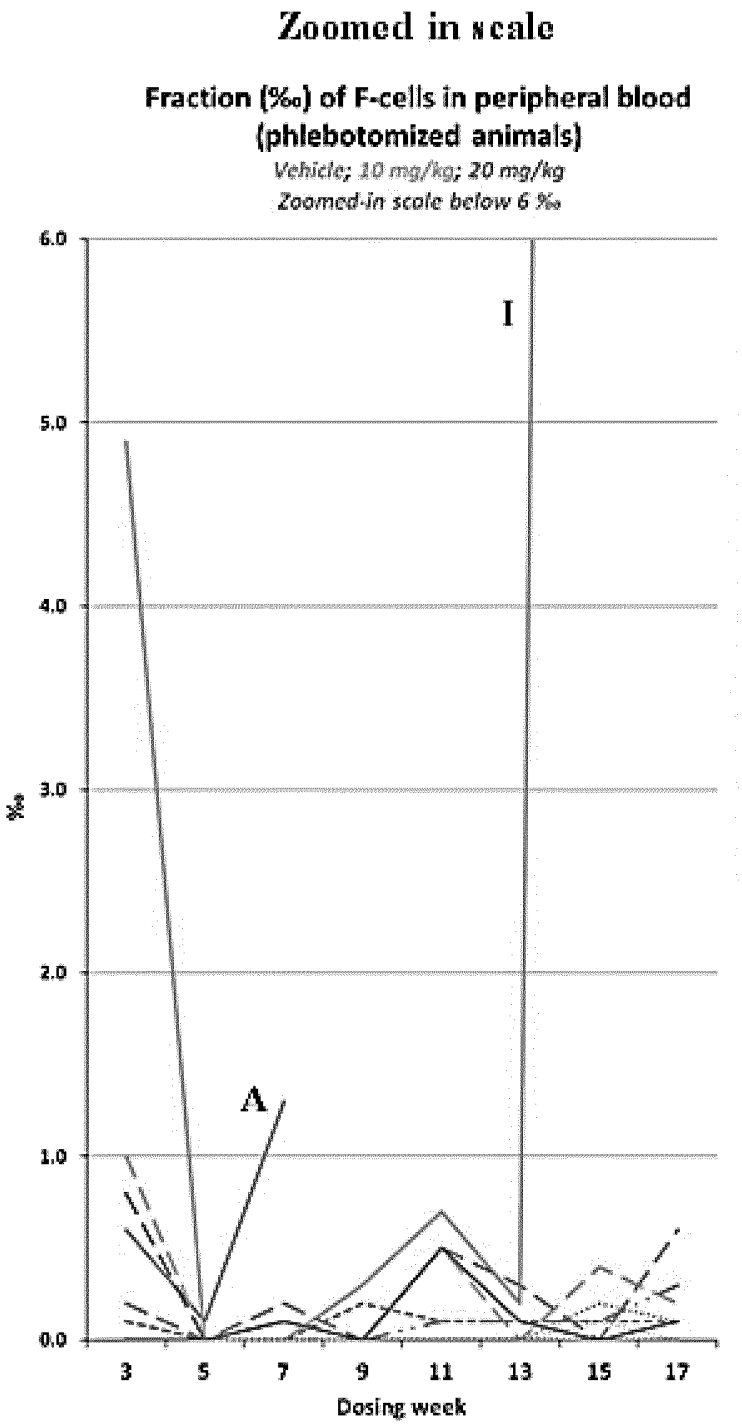
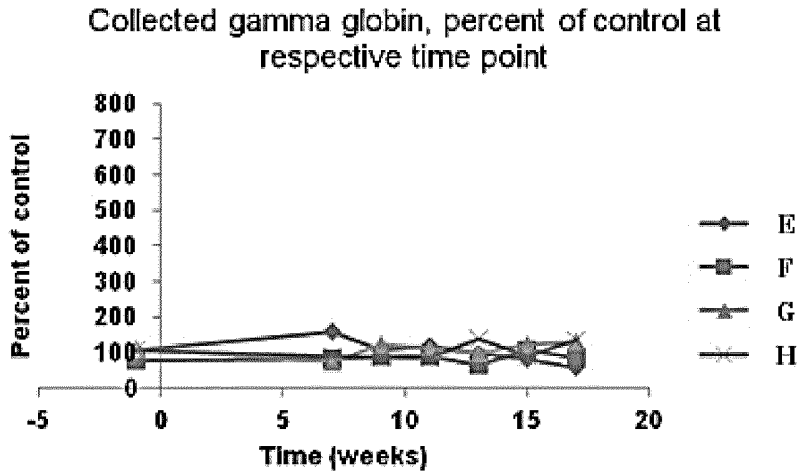
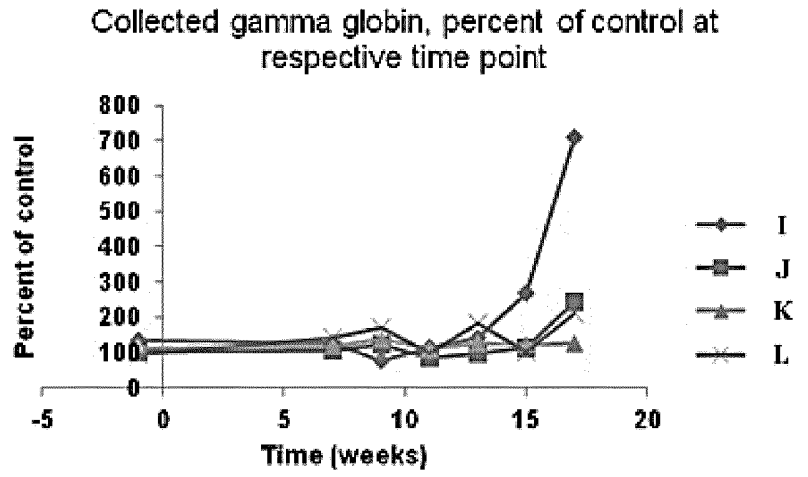


Figure 25


Collected gamma globin, percent of control at respective time point
Percent of control
800
700
600
500
400
300
200
100
0
-5
0
5
10
15
20
Time (weeks)
E
F
G
H


Collected gamma globin, percent of control at respective time point
Percent of control
800
700
600
500
400
300
200
100
0
-5
0
5
10
15
20
Time (weeks)
I
J
K
L

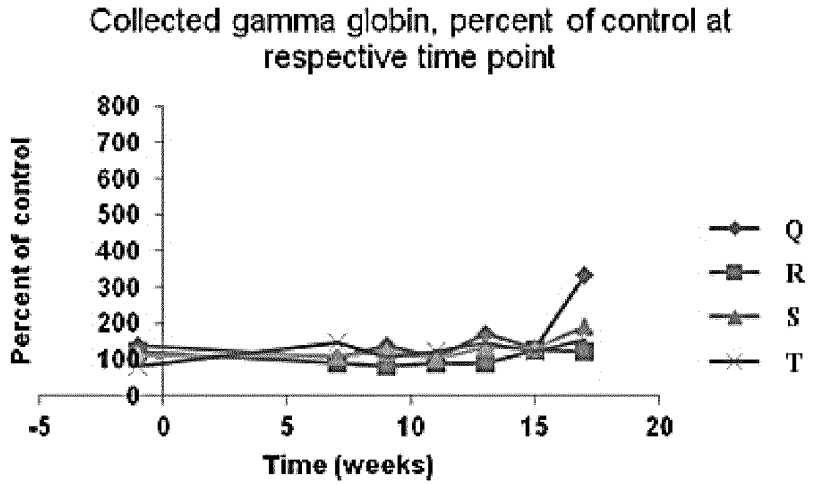

Collected gamma globin, percent of control at respective time point
Percent of control
800
700
600
500
400
300
200
100
0
-5
0
5
10
15
20
Time (weeks)
Q
R
S
T

Figure 26

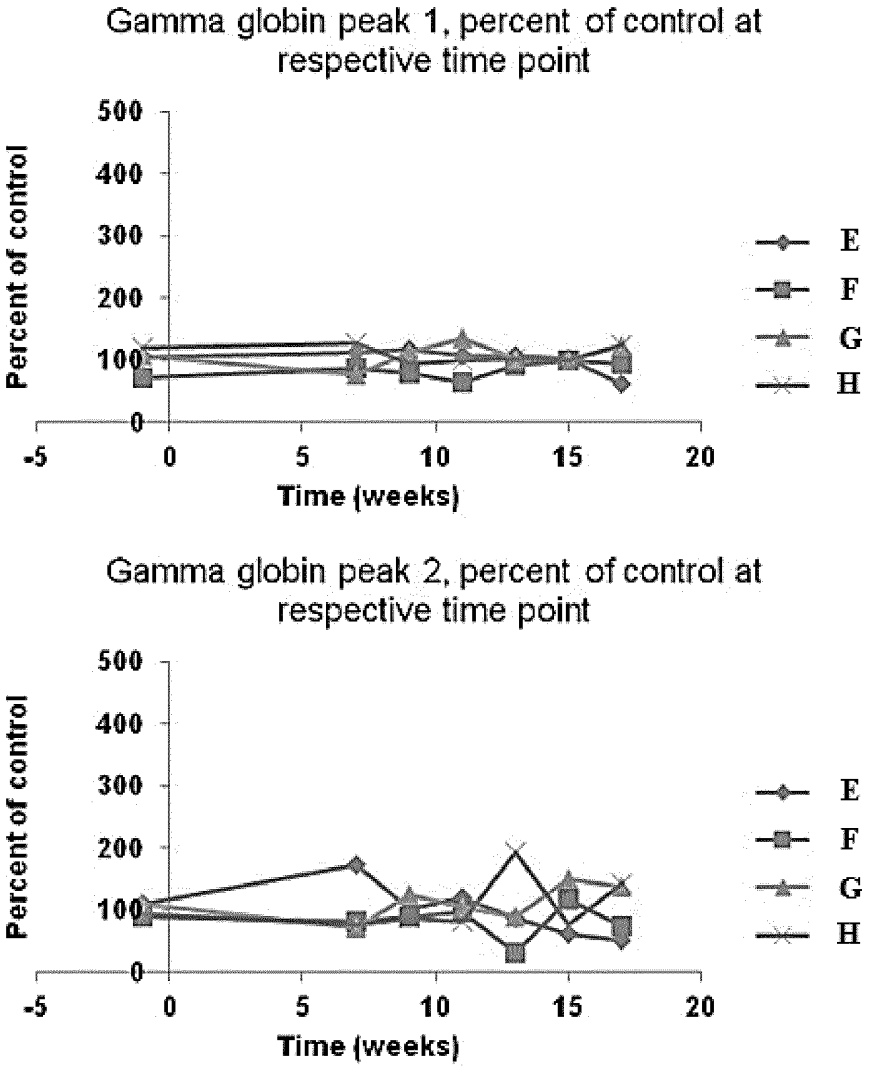

Gamma globin peak 1, percent of control at respective time point
Percent of control
500
400
300
200
100
0
-5
0
5
10
15
20
Time (weeks)
E
F
G
H
Gamma globin peak 2, percent of control at respective time point
Percent of control
500
400
300
200
100
0
-5
0
5
10
15
20
Time (weeks)
E
F
G
H

Figure 27

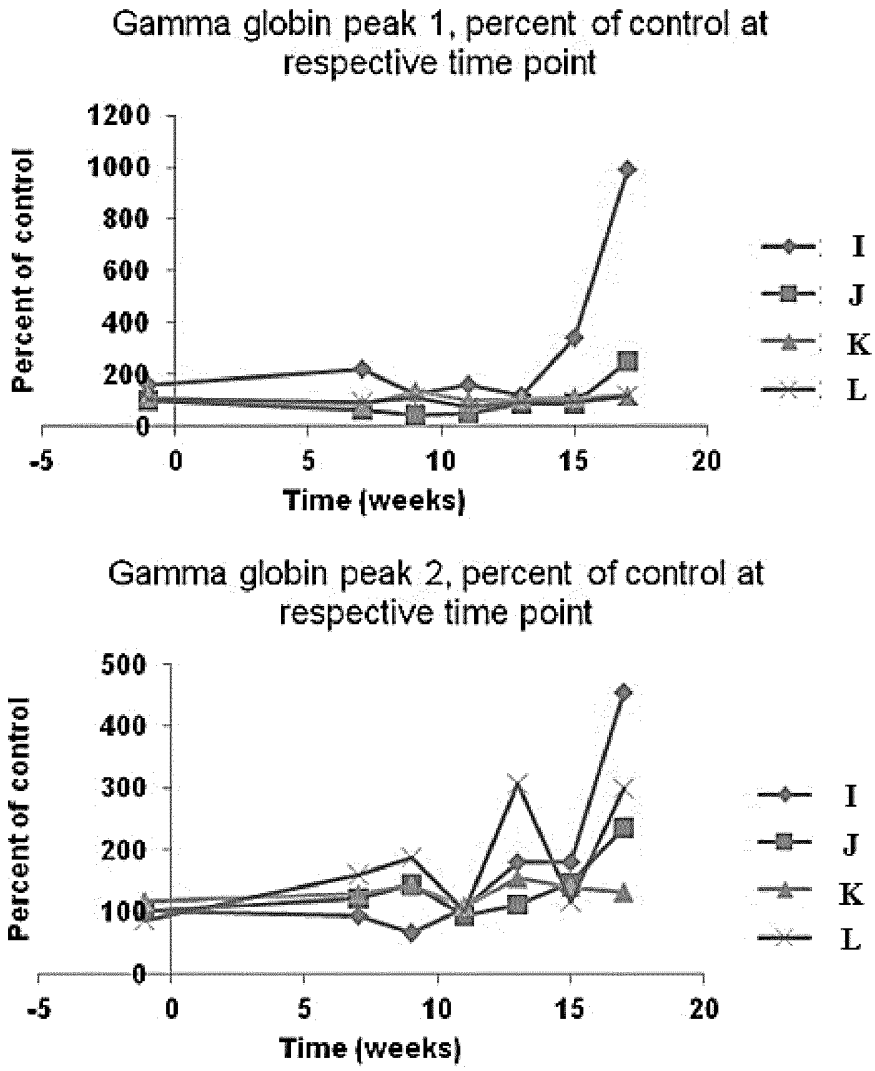

Gamma globin peak 1, percent of control at respective time point
Percent of control
1200
1000
800
600
400
200
0
-5
0
5
10
15
20
Time (weeks)
I
J
K
L
Gamma globin peak 2, percent of control at respective time point
Percent of control
500
400
300
200
100
0
-5
0
5
10
15
20
Time (weeks)
I
J
K
L

Figure 28

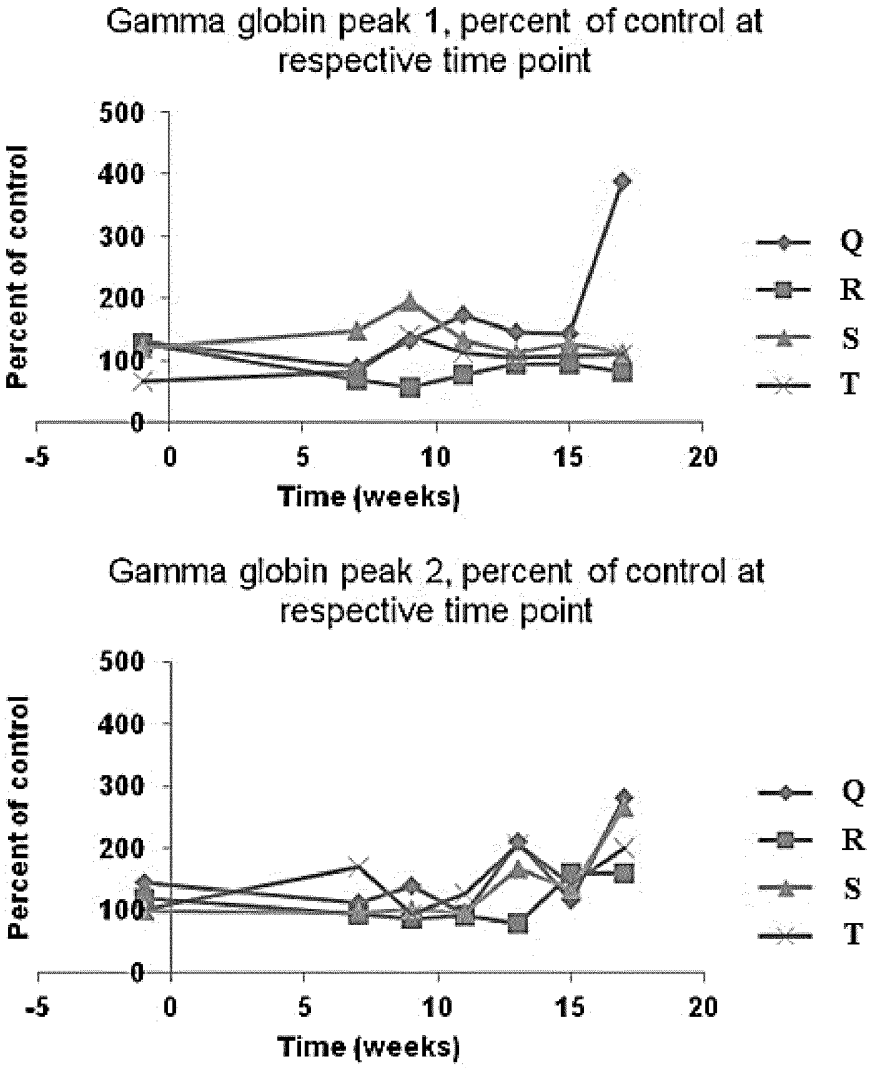

Gamma globin peak 1, percent of control at respective time point
Percent of control
500
400
300
200
100
0
-5
0
5
10
15
20
Time (weeks)
Q
R
S
T
Gamma globin peak 2, percent of control at respective time point
Percent of control
500
400
300
200
100
0
-5
0
5
10
15
20
Time (weeks)
Q
R
S
T

Figure 29

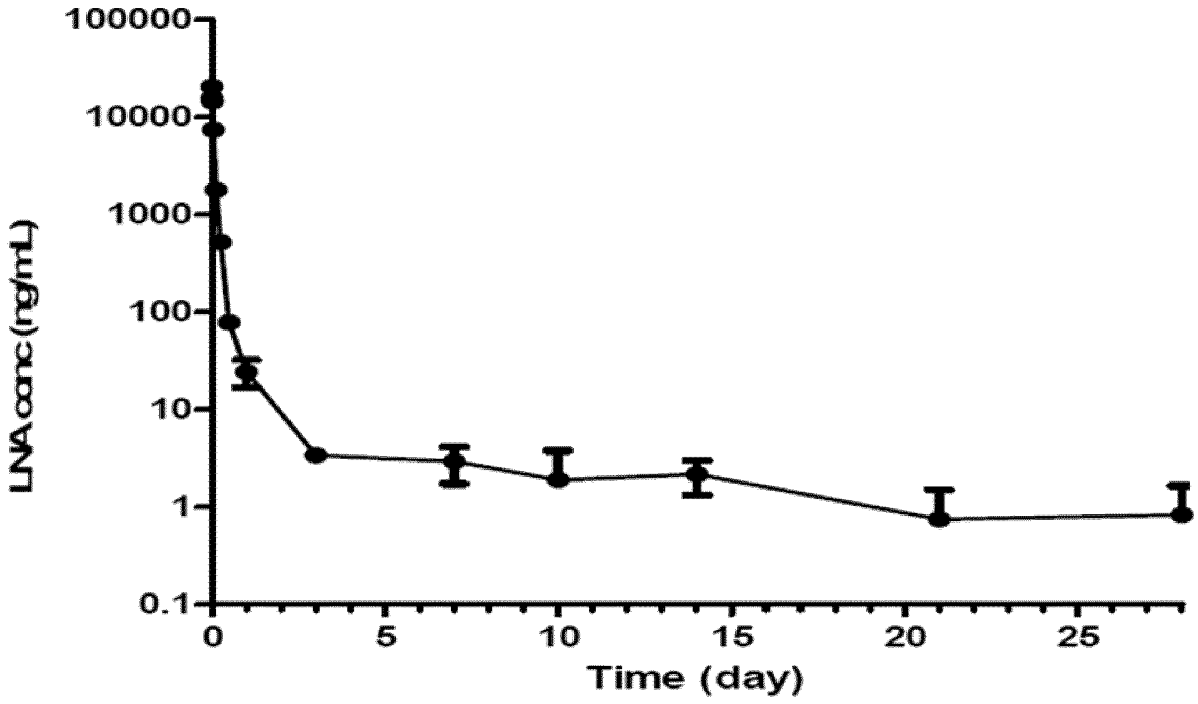


Figure 30

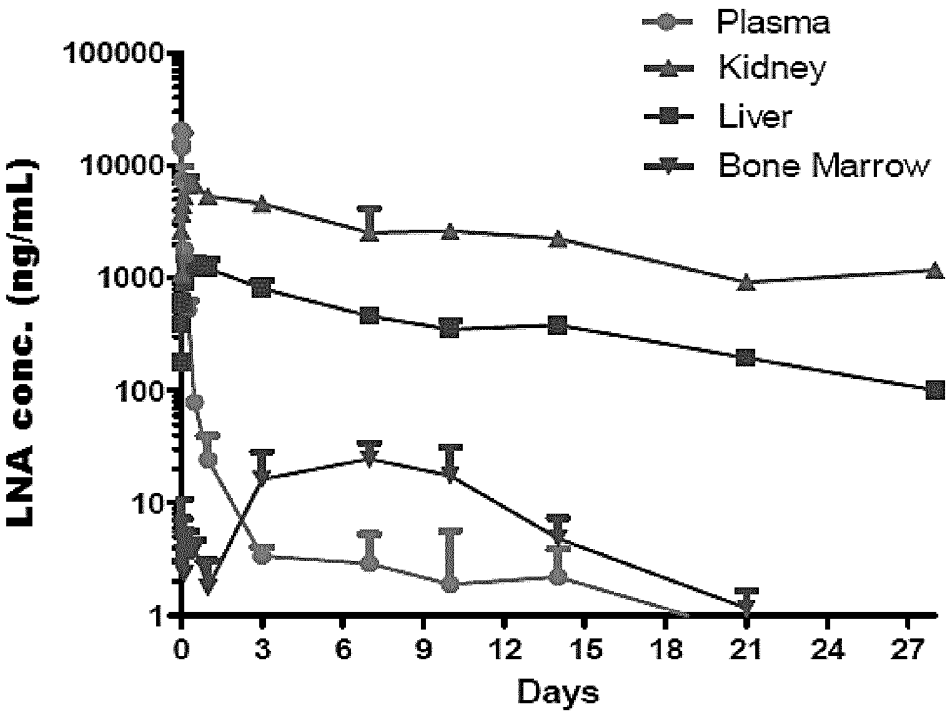
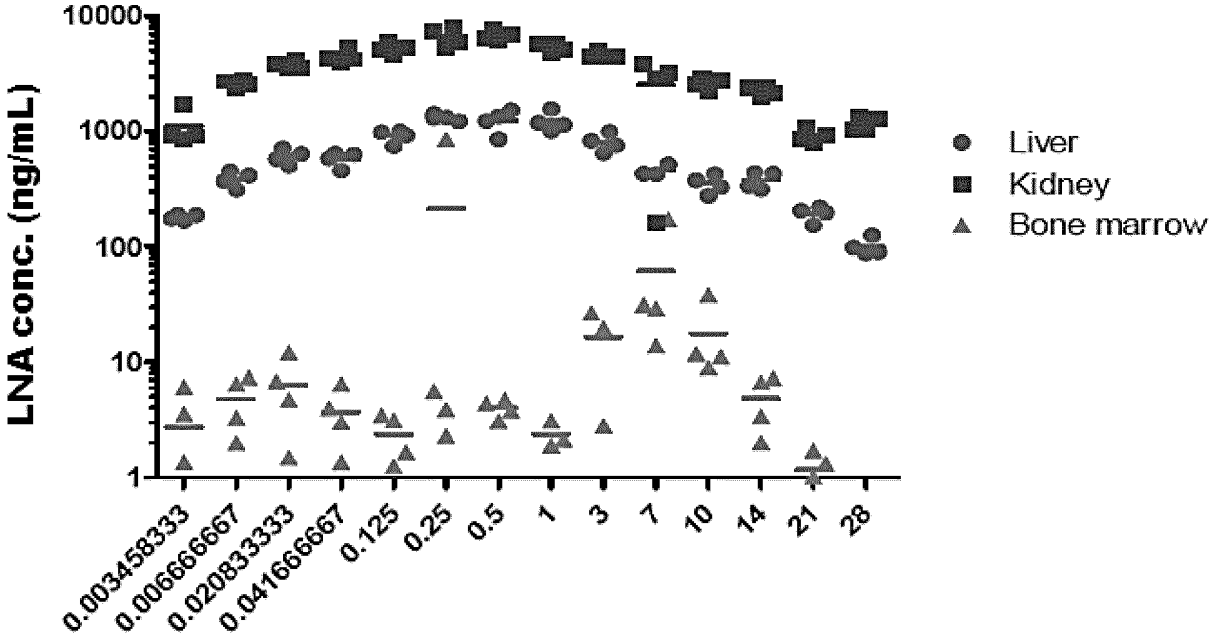
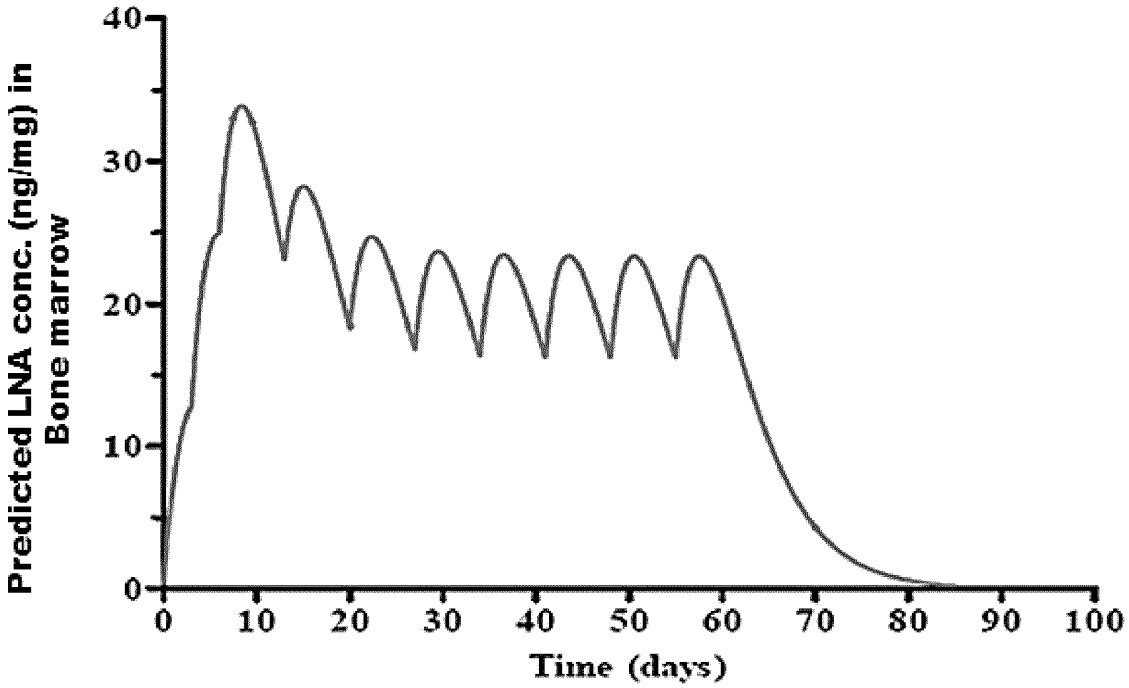


Figure 31



Tissue	$t_{1/2}$	C_{max}	AUC_{0-last}	$AUC_{0-\infty}$
	hr	ng/mg	hr*ng/mg	hr*ng/mg
Plasma	230	20,642	30,423	30,688
Kidney	269	6,746	1,583,640	2,042,285
Liver	219	1,320	274,935	306,785
Bone Marrow	68	25	5,615	5,731

Figure 32


Predicted LNA conc. (ng/mg) in Bone marrow
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Time (days)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/060813

A. CLASSIFICATION OF SUBJECT MATTER
INV. 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)
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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/030963 A2 (CHILDRENS MEDICAL CENTER [US]; ORKIN STUART H [US]; SANKARAN VIJAY G [) 18 March 2010 (2010-03-18) cited in the application the whole document	1-20
X	WO 2013/055985 A1 (CHILDRENS MEDICAL CENTER [US]; ORKIN STUART H [US]; XU JIAN [US]; CONG) 18 April 2013 (2013-04-18) cited in the application the whole document	1-20
A	WO 2012/079046 A2 (ALNYLAM PHARMACEUTICALS INC [US]; NOVOBRANTSEVA TATIANA [US]; BETTENCO) 14 June 2012 (2012-06-14) sequences 201,202,666,667	1

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 July 2014	01/08/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Smalt, Rolf

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ORAPAN SRIPICHAI ET AL: "HbF-Inducing Cytokines and BCL11A shRNA Have Combined Effects Upon Globin Gene Reprogramming In Adult Human Erythroblasts", BLOOD; 52ND ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); ORLANDO, FL, USA; DECEMBER 04 -07, 2010, AMERICAN SOCIETY OF HEMATOLOGY, US, [Online] vol. 116, no. 21, 19 November 2010 (2010-11-19), page 861, XP002722508, ISSN: 0006-4971 Retrieved from the Internet: URL:https://ash.confex.com/ash/2010/webprogram/Paper33417.html> the whole document -----	1
A	CN 102 329 794 A (UNIV JINAN) 25 January 2012 (2012-01-25) the whole document -----	1
A	CN 102 352 356 A (UNIV JINAN) 15 February 2012 (2012-02-15) the whole document -----	1
A	WO 2012/073047 A2 (GENOME RES LTD [GB]; LIU PENTAO [GB]; KHALED WALID [GB]; BURKE SHANNON) 7 June 2012 (2012-06-07) the whole document -----	1
A	WO 2008/113832 A2 (SANTARIS PHARMA AS [DK]; HANSEN BO [DK]; OERUM HENRIK [DK]; HANSEN HEN) 25 September 2008 (2008-09-25) the whole document -----	1
A	KAMMLER SUSANNE ET AL: "LNA ANTISENSE OLIGONUCLEOTIDES-A STRAIGHT-FORWARD CONCEPT FOR RNA THERAPEUTICS", NUCLEIC ACID THERAPEUTICS, vol. 21, no. 5, October 2011 (2011-10), page A16, XP002727548, & 7TH ANNUAL MEETING OF THE OLIGONUCLEOTIDE-THERAPEUTICS-SOCIETY; COPENHAGEN, DENMARK; SEPTEMBER 08 -10, 2011 the whole document -----	1

-/--

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	PERALTA RAECHEL ET AL: "Targeting BCL11A and KLF1 For The Treatment Of Sickle Cell Disease and beta-Thalassemia In Vitro using Antisense Oligonucleotides", BLOOD, vol. 122, no. 21, November 2013 (2013-11), page 1022, XP002727549, & 55TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY; NEW ORLEANS, LA, USA; DECEMBER 07 -10, 2013 the whole document -----	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/060813

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010030963 A2	18-03-2010	CA 2737180 A1	18-03-2010
		EP 2334794 A2	22-06-2011
		US 2011182867 A1	28-07-2011
		US 2013129629 A1	23-05-2013
		WO 2010030963 A2	18-03-2010
WO 2013055985 A1	18-04-2013	NONE	
WO 2012079046 A2	14-06-2012	EP 2648763 A2	16-10-2013
		US 2014018410 A1	16-01-2014
		WO 2012079046 A2	14-06-2012
CN 102329794 A	25-01-2012	NONE	
CN 102352356 A	15-02-2012	NONE	
WO 2012073047 A2	07-06-2012	NONE	
WO 2008113832 A2	25-09-2008	DK 2149605 T3	30-09-2013
		EP 2149605 A2	03-02-2010
		US 2010210712 A1	19-08-2010
		WO 2008113832 A2	25-09-2008

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(71)申请人 罗氏创新中心哥本哈根有限公司
地址 丹麦赫斯霍尔姆

(72)发明人 马伊·黑特耶恩
尼尔斯·菲斯克尔·尼尔森

(74)专利代理机构 中科专利商标代理有限责任公司 11021
代理人 张国梁 王旭

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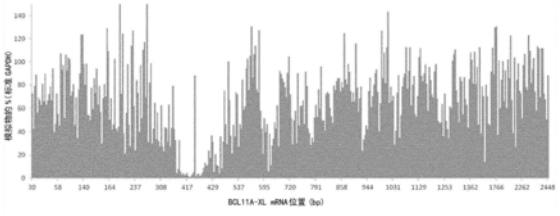
(54)发明名称

B-细胞CLL/淋巴瘤11A(BCL11A)的寡核苷酸调节剂及其用途

(57)摘要

本发明提供B细胞淋巴瘤/白血病11A(BCL11A)的寡核苷酸调节剂(例如,抑制剂)和基于这样的调节剂治疗BCL11A-相关疾病、病症或病况的改善的方法和组合物,以及其它事物。



CN 105247052 A

1.一种能够减少人 BCL11A 表达的反义寡核苷酸,所述反义寡核苷酸包含与选自 SEQ ID NO 1 的人 BCL11A 基因或 BCL11A 的信使 RNA(mRNA) 同种型的核苷酸 410 至 450 的区域内连续序列的反向互补物至少 80％相同的序列,其中所述反义寡核苷酸是 gapmer。

2.根据权利要求 1 所述的反义寡核苷酸,其中所述反义寡核苷酸由式 X_a-Y_b-$X_{a'}$ 表示,其中 :

X 是核苷酸类似物 ;

Y 是连续的 DNA 序列 ;

a 是 1,2,3,4 或 5 ;

a′ 是 1,2,3,4 或 5 ;并且

b 是 5 和 15 之间的整数。

3.根据权利要求 2 所述的反义寡核苷酸,其中 a 和 / 或 a′ 在 2 和 4 之间。

4.根据权利要求 2 或 3 中任一项所述的反义寡核苷酸,其中 b 是 7 和 10 之间的整数。

5.根据在前权利要求中任一项所述的反义寡核苷酸,其中所述反义寡核苷酸长度小于 19 个核苷酸。

6.根据权利要求 5 所述的反义寡核苷酸,其中所述寡核苷酸长度为 10 至 16 个核苷酸。

7.根据在前权利要求中任一项所述的反义寡核苷酸,其中所述寡核苷酸包含至少一个选自由以下组成的组的核苷酸类似物 :2’-O- 烷基 -RNA 单元、2’-OMe-RNA 单元、2’-O- 烷基 -DNA、2’- 氨基 -DNA 单元、2’- 氟 -DNA 单元、LNA 单元、阿糖核酸 (ANA) 单元、2’- 氟 -ANA 单元、HNA 单元、INA 单元和 2’MOE 单元。

8.根据权利要求 7 所述的反义寡核苷酸,其中所述核苷酸类似物是选自由以下组成的组的 LNA 单元 :β-D- 氧基 -LNA、α-L- 氧基 -LNA、β-D- 氨基 -LNA、α-L- 氨基 -LNA、β-D- 硫代 -LNA、α-L- 硫代 -LNA、5’- 甲基 -LNA、β-D-ENA 和 α-L-ENA。

9.根据在前权利要求中任一项所述的反义寡核苷酸,其中所述寡核苷酸包含至少一个硫代磷酸酯键。

10.根据在前权利要求中任一项所述的反义寡核苷酸,其中所述寡核苷酸能够募集 RNA 酶 H。

11.根据在前权利要求中任一项所述的反义寡核苷酸,其中所述反义寡核苷酸包含选自由以下组成的组的寡核苷酸序列基序 :SEQ ID NO :63、SEQ ID NO :64、SEQ ID NO :65、SEQ ID NO :66、SEQ ID NO :67、SEQ ID NO :68、SEQ ID NO :69、SEQ ID NO :70、SEQ ID NO :71、SEQ ID NO :72、SEQ ID NO :73 和 SEQ ID NO :74。

12.根据在前权利要求中任一项所述的反义寡核苷酸,其中所述反义寡核苷酸具有选自 SEQ ID NO :11、SEQ ID NO :15、SEQ ID NO :32、SEQ ID NO :21、SEQ ID NO :34、SEQ ID NO :10、SEQ ID NO :12、SEQ ID NO :13、SEQ ID NO :14、SEQ ID NO :16、SEQ ID NO :17、SEQ ID NO :18、SEQ ID NO :19、SEQ ID NO :20、SEQ ID NO :22、SEQ ID NO :23、SEQ ID NO :24、SEQ ID NO :25、SEQ ID NO :26、SEQ ID NO :27、SEQ ID NO :28、SEQ ID NO :29、SEQ ID NO :30、SEQ ID NO :31、SEQ ID NO :33、SEQ ID NO :35、SEQ ID NO :36、SEQ ID NO :37、SEQ ID NO :38、SEQ ID NO :39、SEQ ID NO :40、SEQ ID NO :41、SEQ ID NO :42、SEQ ID NO :43、SEQ ID NO :44、SEQ ID NO :45、SEQ ID NO :46、SEQ ID NO :47、SEQ ID NO :48、SEQ ID NO :49、SEQ ID NO :50、SEQ ID NO :51、SEQ ID NO :52、SEQ ID NO :53、SEQ ID NO :54、SEQ ID NO :

55、SEQ ID NO:56、SEQ ID NO:57、SEQ ID NO:58、SEQ ID NO:59、SEQ ID NO:60、SEQ ID NO:60、SEQ ID NO:61 或 SEQ ID NO:的序列。

13.根据在前权利要求中任一项所述的反义寡核苷酸,其中所述反义寡核苷酸是 5'-$^{m}C_s{}^{o}A_s{}^{o}T_s{}^{o}t_sg_sc_sa_st_st_sg_st_st_st_s{}^{m}C_s{}^{om}C_s{}^{o}G^{o}$-3'(SEQ ID NO:11),5'-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_s{}^{m}c_sg_sa_sT_s{}^{o}A_s{}^{o}A^{o}$-3'(SEQ ID NO:15),5'-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_s{}^{m}c_sg_sA_s{}^{o}T_s{}^{o}A^{o}$-3'(SEQ ID NO:32),5'-$T_s{}^{o}T_s{}^{o}G_s{}^{o}t_sg_sc_st_s{}^{m}c_sg_sa_st_sA_s{}^{o}A_s{}^{o}A^{o}$-3'(SEQ ID NO:14) 或 5'-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_sc_sG_s{}^{o}A_s{}^{o}T^{o}$-3'(SEQ ID NO:35),其中大写字母表示锁定核酸 (LNA) 单元,下标"s"表示硫代磷酸酯键,并且小写字母表示脱氧核糖核苷酸 (DNA) 单元,"^{m}C"表示 5′ 甲基 - 胞嘧啶 LNA 单元,并且"^{m}c"表示 5′ 甲基 - 胞嘧啶 DNA 单元。

14.一种药物组合物,所述药物组合物包含根据权利要求 1 至 0 中任一项所述的反义寡核苷酸和药用载体。

15.根据权利要求 1 至 13 中任一项所述的反义寡核苷酸或权利要求 14 所述的药物组合物,其用作药物,所述药物诸如用于治疗贫血疾病、病症或病况,如镰状细胞病或 β - 地中海贫血。

16.根据权利要求 1 至 13 中任一项所述的反义寡核苷酸或权利要求 14 所述的药物组合物用于制造药物的用途,所述药物用于治疗贫血疾病、病症或病况,如镰状细胞病或 β - 地中海贫血。

17.一种抑制 BCL11A 的方法,所述方法包括向需要治疗的受试者施用根据权利要求 1 至 13 中任一项所述的反义寡核苷酸或权利要求 14 所述的药物组合物。

18.一种治疗贫血疾病、病症或病况的方法,所述方法包括向需要治疗的受试者施用根据权利要求 1 至 13 中任一项所述的寡核苷酸或权利要求 14 所述的药物组合物。

19.权利要求 18 所述的方法,其中所述贫血疾病、病症或病况是镰状细胞病。

20.权利要求 18 所述的方法,其中所述贫血疾病、病症或病况是 β - 地中海贫血。

B- 细胞 CLL/ 淋巴瘤 11A(BCL11A) 的寡核苷酸调节剂及其用途

[0001] 背景

[0002] 血红蛋白病是与血红蛋白机能障碍相关的疾病。通常,这些疾病涉及血红蛋白的缺失或出现功能障碍的血红蛋白,其源自球蛋白基因(例如,α,β,等;图 1)的遗传突变。血红蛋白病是一组广谱的红血细胞相关病症,特征为在多数情况下为常染色体显性遗传的单基因遗传病症。据估计,约 7%的世界人口是携带者。遗传的血红蛋白病以三种形式之一显现:地中海贫血(α,β,δ)、镰状细胞病和遗传性胎儿血红蛋白持续存在症(HbF)。镰状细胞病(SCD)和 β- 地中海贫血是血红蛋白病的最常见形式并且是世界范围内发病和死亡的主要原因。SCD,如名称暗示的,涉及由突变形成的球蛋白结构的改变并且导致出现功能障碍的血红蛋白,而地中海贫血与导致正常球蛋白生产不足的球蛋白基因突变相关。这可以经由调节蛋白的突变发生。贫血,在一些情况下是血红蛋白机能障碍的常见结果。

[0003] 随时间,已经开发了血红蛋白病的各种治疗。主要焦点通常是在恢复血红蛋白功能,例如,通过增加胎儿血红蛋白(HbF)的水平。然而,尚未成功开发出很多有效的治疗。近来,关于调节 HbF 的机制的理解已经成为很多研究的领域。据报道,BCL11A 在骨髓中成人红细胞前体细胞中表达并行使功能,以抑制 γ- 球蛋白产生(Sankaran 等人 2008 Science 第 322 卷,第 1839-1842 页)。

[0004] WO 2010/030963 描述了使用针对 4 条靶序列的 siRNA 样品池(pool)调节 BCL11A。关于个体靶序列是否能够下调 BCL11A,没有表明。

[0005] WO 2012/079046 描述了靶向 BCL11A 基因的双链核糖核酸(dsRNA)组合物。

[0006] 概述

[0007] 本发明提供 BCL11A 的反义寡核苷酸调节剂(例如,抑制剂)和基于这样的调节剂治疗 BCL11A- 相关疾病、病症或病况的方法和组合物,以及其它事项。考虑到的是,本发明提供的反义寡核苷酸对于治疗血红蛋白病、如镰状细胞病和 β- 地中海贫血特别有用。

[0008] 在一个方面,本发明提供一种能够下调或减少人 BCL11A 表达的反义寡核苷酸,其具有与选自 SEQ ID NO 1 的人 BCL11A 基因或 BCL11A 的信使 RNA(mRNA)同种型的核苷酸 410 至 450 的区域内的连续序列的反向互补物至少 80%(例如至少约 85%,90%,95%,96%,97%,98%,或 99%)相同的序列,其中所述反义寡核苷酸是 gapmer。在一些实施方案中,本发明的反义寡核苷酸长度小于 19 个核苷酸。在一些实施方案中,本发明的反义寡核苷酸长度小于 18 个核苷酸。

[0009] 在一个方面,本发明提供一种能够下调或减少人 BCL11A 的表达的反义寡核苷酸,其具有小于 18 个核苷酸(例如,小于 17,16,15,14,13,或 12)的长度并且具有与选自 SEQ ID NO 1 的人 BCL11A 基因或 BCL11A 的信使 RNA(mRNA)同种型的核苷酸 410 至 450 的区域内的连续序列的反向互补物至少约 80%(例如,至少约 85%,90%,95%,96%,97%,98%,或 99%)相同的序列。

[0010] 在一个方面,本发明提供一种能够下调或减少人 BCL11A 表达的反义寡核苷酸,所述反义寡核苷酸具有与选自 SEQ ID NO 1 的人 BCL11A 基因或 BCL11A 的信使 RNA(mRNA)

同种型的核苷酸 410 至 450 的区域内的连续序列的反向互补物至少约 80％（例如，至少约 85％，90％，95％，96％，97％，98％，或 99％）相同的序列并且由式 X_a-Y_b-$X_{a'}$ 表示，其中 X 是核苷酸类似物；Y 是连续的 DNA 序列；a 是 1，2，3，4 或 5；a′ 是 1，2，3，4 或 5；并且 b 是 5 和 15 之间的整数。在一些实施方案中，所述核苷酸类似物是锁定核酸 (LNA)。

[0011] 在一些实施方案中，a 和 a′ 不同。在一些实施方案中，a 和 a′ 相同。在一些实施方案中，a 和 / 或 a′ 是 1。在一些实施方案中，a 和 / 或 a′ 是 2。在一些实施方案中，a 和 / 或 a′ 是 3。在一些实施方案中，a 和 / 或 a′ 是 4。在一些实施方案中，a 和 / 或 a′ 是 5。

[0012] 在一些实施方案中，b 是包括 5 和 15 之间的整数。在一些实施方案中，b 是包括 5 至 10 的整数。在一些实施方案中，b 是包括 7 至 11 的整数。在一些实施方案中，b 是选自由以下各项组成的组的整数：5，6，7，8，9，10，11，12，13，14 和 15。

[0013] 在一些实施方案中，BCL11A 的同种型选自由以下各项组成的组：XL，L，M，S 和 XS 或其同源物或直向同源物。在一些实施方案中，本发明的反义寡核苷酸能够下调或减少小鼠 BCL11A 基因的表达。

[0014] 在一些实施方案中，本发明的反义寡核苷酸包含或含有至少一个，至少两个，至少三个，至少四个，至少五个，至少六个，至少七个，至少八个或更多个核苷类似物。在一些实施方案中，本发明的反义寡核苷酸包含 3-8 个核苷酸类似物，例如 6 或 7 个核苷酸类似物。在一些实施方案中，至少一个所述核苷酸类似物是锁定核酸 (LNA)；例如至少 3 或至少 4，或至少 5，或至少 6，或至少 7，或 8 个核苷酸类似物可以是 LNA。在一些实施方案中，所有的核苷酸类似物可以是 LNA。

[0015] 在一些实施方案中，本发明的寡核苷酸包含或含有至少一个，至少两个，至少三个，至少四个，至少五个，至少六个，至少七个，至少八个或更多个 LNA 单元。在一些实施方案中，所述一个或多个 LNA 单元位于反义寡核苷酸的 5’ 和 / 或‘3 末端。在一些实施方案中，本发明的反义寡核苷酸在 5’ 和 / 或‘3 末端包含至少一个，至少两个或至少三个 LNA 单元。在一些实施方案中，本发明的反义寡核苷酸在内部包含至少一个，至少两个或至少三个 LNA 单元。

[0016] 在一些实施方案中，所述一个或多个 LNA 单元是 β -D- 氧基 -LNA 核苷酸。在一些实施方案中，本发明的反义寡核苷酸包含一种以上另外的化学修饰。在一些实施方案中，本发明的反义寡核苷酸包含一种以上另外的化学修饰，所述化学修饰包括 2’O- 甲基修饰和 / 或硫代磷酸酯键。在一些实施方案中，本发明的反义寡核苷酸包含至少一个 LNA 单元，所述 LNA 单元是 LNA 5- 甲基胞嘧啶核苷酸。

[0017] 在一些实施方案中，本发明的反义寡核苷酸具有 10-17，10-16，10-15，10-14，10-13，10-12，11-17，11-16，11-15，11-14，11-13，12-17，12-16，12-15，或 12-14 个核苷酸的长度。在一些实施方案中，本发明的反义寡核苷酸具有 12-16 个核苷酸的长度。

[0018] 在一些实施方案中，本发明的反义寡核苷酸具有与选自 SEQ ID NO 1 的人 BCL11A 基因或人 BCL11A 的信使 RNA(mRNA) 同种型的核苷酸 410 至 450 的区域内的连续序列的反向互补相同的序列。

[0019] 本发明的一个备选方面是一种能够减少人 BCL11A 表达的反义寡核苷酸，所述反义寡核苷酸包含与选自人 BCL11A 基因的核苷酸 1-283（外显子 1），核苷酸 284-613（外显

子 2)，或核苷酸 614-715（外显子 3）的区域内的连续序列的反向互补物至少 80％相同的序列。

[0020] 在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 XL 的核苷酸 410-450 内。

[0021] 在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 XL 的核苷酸 415-436 内。

[0022] 在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 XL 的核苷酸 420-450 内。

[0023] 在一些实施方案中，本发明的寡核苷酸包含选自由以下各项组成的组的序列基序或由选自由以下各项组成的组的序列基序组成：5’-ATTGCATTGTTTCCG-3’(SEQ ID NO：63)，5’-GTTTGTGCTCGAT-3’(SEQ ID NO：64)，5’-CATTGCATTGTTTCCG-3’(SEQ ID NO：65)，5’-CGTTTGTGCTCGAT-3’(SEQ ID NO：66)，5’-CGTTTGTGCTCGATAA-3’(SEQ ID NO：67)，5 ′ -CCGTTTGTGCTCGA-3’(SEQ ID NO：68)，5’-CGTTTGTGCTCGA-3’(SEQ ID NO：69)，5’-TTTGTGCTCGATAA-3’(SEQ ID NO：70)，5’-TTGTGCTCCATAA-3’(SEQ ID NO：71)和 5’-TTTCCGTTTGTGCTCG(SEQ ID NO：72)，5’-ATTGCATTGTTTCCGT-3’(SEQ ID NO：73)，5’-CGTTTGTGCTCGAIA-3’(SEQ ID NO：74)。

[0024] 在一些实施方案中，本发明的反义寡核苷酸具有选自表 2 的序列 s

[0025] 在一些实施方案中，本发明的反义寡核苷酸具有选自以下各项的序列：SEQ ID NO：11，SEQ ID NO：15，SEQ ID NO：32，SEQ ID NO：21，SEQ ID NO：34，SEQ ID NO：10，SEQ ID NO：12，SEQ ID NO：13，SEQ ID NO：14，SEQ ID NO：16，SEQ ID NO：17，SEQ ID NO：18，SEQ ID NO：19，SEQ ID NO：20，SEQ ID NO：22，SEQ ID NO：23，SEQ ID NO：24，SEQ ID NO：25，SEQ ID NO：26，SEQ ID NO：27，SEQ ID NO：28，SEQ ID NO：29，SEQ ID NO：30，SEQ ID NO：31，SEQ ID NO：33，SEQ ID NO：35，SEQ ID NO：36，SEQ ID NO：37，SEQ ID NO：38，SEQ ID NO：39，SEQ ID NO：40，SEQ ID NO：41，SEQ ID NO：42，SEQ ID NO：43，SEQ ID NO：44，SEQ ID NO：45，SEQ ID NO：46，SEQ ID NO：47，SEQ ID NO：48，SEQ ID NO：49，SEQ ID NO：50，SEQ ID NO：51，SEQ ID NO：52，SEQ ID NO：53，SEQ ID NO：54，SEQ ID NO：55，SEQ ID NO：56，SEQ ID NO：57，SEQ ID NO：58，SEQ ID NO：59，SEQ ID NO：60，SEQ ID NO：60，SEQ ID NO：61 或 SEQ ID NO：62，

[0026] 在一些实施方案中，本发明的反义寡核苷酸选自 5’-$^{m}C_s{}^{o}A_s{}^{o}T_s{}^{o}t_sg_sc_sa_st_st_sg_st_st_st_s{}^{m}C_s{}^{om}C_s{}^{o}G^{o}$-3’(SEQ ID NO：11)，5’-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_s{}^{m}c_sg_sa_sT_s{}^{o}A_s{}^{o}A^{o}$-3’(SEQ ID NO：15)或 5’-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_s{}^{m}c_sg_sA_s{}^{o}T_s{}^{o}A^{o}$-3’(SEQ ID NO：32)，5’-$T_s{}^{o}T_s{}^{o}G_s{}^{o}t_sg_sc_st_s{}^{m}c_sg_sa_st_sA_s{}^{o}A_s{}^{o}A^{o}$-3’(SEQ ID NO：14)和 5’-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_sc_sG_s{}^{o}A_s{}^{o}T^{o}$-3’(SEQ ID NO：35)，其中大写字母表示锁定核酸（LNA）单元，下标“s”表示硫代磷酸酯键，并且小写字母标示脱氧核糖核苷酸（DNA）单元，“^{m}C”表示 5′ 甲基－胞嘧啶 LNA 单元，并且“^{m}c”表示 5′ 甲基－胞嘧啶 DNA 单元。

[0027] 在一些实施方案中，本发明的反义寡核苷酸是 5’-$^{m}C_s{}^{o}A_s{}^{o}T_s{}^{o}t_sg_sc_sa_st_st_sg_st_st_st_s{}^{m}C_s{}^{om}C_s{}^{o}G^{o}$-3’(SEQ ID NO：11)。

[0028] 在另一个方面，本发明提供能够下调或减少人 BCL11A 基因表达的反义寡核苷酸，所述反义寡核苷酸具有与选自表 2 的寡核苷酸序列至少 80％（例如，85％，90％，91％，

92％,93％,94％,95％,96％,97％,98％,99％或更多）相同的序列。

[0029] 在一些实施方案中,根据本发明的反义寡核苷酸具有与选自以下各项的寡核苷酸序列至少 80％（例如,85％,90％,91％,92％,93％,94％,95％,96％,97％,98％,99％或更多）相同的序列 :5’-$^{m}C_s{}^{o}A_s{}^{o}T_s{}^{o}t_sg_sc_sa_st_st_sg_st_st_st_s{}^{m}C_s{}^{om}C_s{}^{o}G^{o}$-3’(SEQ ID NO :11),5’-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_s{}^{m}c_sg_sa_sT_s{}^{o}A_s{}^{o}A^{o}$-3’(SEQ ID NO :15) 或 5’-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_s{}^{m}c_sg_sg_sA_s{}^{o}T_s{}^{o}A^{o}$-3’(SEQ ID NO :32),5’-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_s{}^{m}c_sg_sA_s{}^{o}T_s{}^{o}A^{o}$-3’(SEQ ID NO :32),5’-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_sc_sG_s{}^{o}A_s{}^{o}T^{o}$-3’(SEQ ID NO :35),5’-$T_s{}^{o}T_s{}^{o}G_s{}^{o}t_sg_sc_st_s{}^{m}c_sg_sa_st_sA_s{}^{o}A_s{}^{o}A^{o}$-3’(SEQ ID NO :14) 和 5’-$^{m}C_s{}^{o}G_s{}^{o}T_s{}^{o}t_st_sg_st_sg_sc_st_sc_sG_s{}^{o}A_s{}^{o}T^{o}$-3’(SEQ ID NO :35),其中大写字母表示锁定核酸(LNA) 单元,下标“s”表示硫代磷酸酯键,并且小写字母标示脱氧核糖核苷酸 (DNA) 单元,“^{m}C”表示 5′ 甲基 - 胞嘧啶 LNA 单元,并且“^{m}c”表示 5′ 甲基 - 胞嘧啶 DNA 单元。

[0030] 在一些实施方案中,提供一种药物组合物,所述药物组合物包含本文所述的反义寡核苷酸和药用载体。

[0031] 除其它事项外,本发明提供抑制受试者中 BCL11A 的方法,所述方法包括向需要治疗的受试者施用本文所述的反义寡核苷酸或药物组合物。

[0032] 除其它事项外,本发明提供一种反义寡核苷酸,所述反义寡核苷酸用于抑制 BCL11A 的方法中,所述方法包括向需要治疗的受试者施用本文所述的反义寡核苷酸或药物组合物的步骤。

[0033] 在一些实施方案中,本发明提供本发明的反义寡核苷酸或药物组合物用于制造药物的用途,所述药物用于治疗贫血疾病、病症或病况,如镰状细胞病或 β - 地中海贫血。尤其在制造用于抑制 BCL11A 的药物方面,包括向受试者施用本文所述的反义寡核苷酸或药物组合物。

[0034] 在一些实施方案中,本发明提供根据本发明的药物组合物的反义寡核苷酸,其用作药物,如用于治疗贫血疾病、病症或病况,如镰状细胞病或 β - 地中海贫血。

[0035] 在一些实施方案中,本发明提供一种在受试者中治疗贫血疾病、病症或病况的方法,所述方法包括向需要治疗的受试者施用本文所述的反义寡核苷酸或药物组合物。

[0036] 在一些实施方案中,本发明提供一种反义寡核苷酸,其用于治疗疾病或病症,如本文提及的那些,如血红蛋白病 (hemoglobinopathie),如贫血疾病 \ 病症或病况,如地中海贫血（α，β，δ),镰状细胞病和遗传性胎儿血红蛋白持续存在症 (HbF)。

[0037] 在一些实施方案中,本发明提供一种反义寡核苷酸,所述反义寡核苷酸用于在受试者中治疗贫血疾病、病症或病况的方法,所述方法包括向需要治疗的受试者施用本文所述的反义寡核苷酸或药物组合物。在一些实施方案中,本发明的治疗方法还包括施用向受试者施用第二药剂,用于治疗疾病或病症,如用于治疗贫血疾病、病症或病况。

[0038] 在一些实施方案中,通过本发明的方法治疗的贫血疾病、病症或病况是镰状细胞病。

[0039] 在一些实施方案中,通过本发明的方法治疗的贫血疾病、病症或病况是 β - 地中海贫血。

[0040] 在一些实施方案中,施用所述反义寡核苷酸或所述药物组合物导致在一种以上靶组织中减少的 BCL11A 表达。在一些实施方案中,施用所述反义寡核苷酸或所述药物组合物导致一种以上靶组织中增加的 γ - 球蛋白表达。在一些实施方案中,施用所述反义寡核

苷酸或所述药物组合物导致一种以上靶组织中增加的胎儿血红蛋白产生。在一些实施方案中，一种以上靶组织选自骨髓、肝、肾、脾、浆细胞、胸腺、扁桃体上皮、红细胞系祖细胞、多能干细胞、树突状细胞和 / 或外周血 B- 细胞。在一些实施方案中，静脉内施用反义寡核苷酸或药物组合物。在一些实施方案中，皮下施用反义寡核苷酸或药物组合物。

[0041] 在一些实施方案中，本发明提供包含本文所述的反义寡核苷酸或药物组合物的容器。在一些实施方案中，以单一剂型提供本发明的反义寡核苷酸或药物组合物。在一些实施方案中，以多种（例如，两种、三种、四种、五种或更多种）剂型提供本发明的反义寡核苷酸或药物组合物。在一些实施方案中，以冻干形式提供本发明的反义寡核苷酸或药物组合物。在一些实施方案中，以液体形式提供本发明的反义寡核苷酸或药物组合物。

[0042] 在一些实施方案中，所述容器选自安瓿瓶、小瓶、筒、储器、二室注射器 (lyo-ject)、和预充式注射器。在一些实施方案中，所述容器是预充式注射器并且任选地选自带有烘过的硅酮涂层的硼硅酸盐玻璃注射器，喷涂有硅酮的硼硅酸盐玻璃注射器，和无硅酮的塑料树脂注射器。

[0043] 如本申请中使用的，术语“约 (about)”和“大约 (approximately)”等同使用。本申请中使用的任何数字，带有或不带有约 (about)/ 大约 (approximately)，意为覆盖相关领域中普通技术人源理解的任何正常波动。

[0044] 本发明的其它特征、目的和优势在接着的详细描述中显而易见。然而，应该理解该详细描述，在表明本发明的实施方案的同时，仅通过说明的方式给出，但不限制。从该详细描述，本发明范围内的各种改变和改进对于本领域技术人员将变得显而易见。

[0045] 附图简述

[0046] 附图仅用于说明的目的，不用于限制。

[0047] 图 1 显示对于正常个体（左）和具有 β 球蛋白链机能障碍的个体（右）中的不同球蛋白链，总血红蛋白 (y- 轴）相对妊娠和出生后周龄 (x- 轴）的百分数的典型的说明。从 Cecil Medicine 第 23 版，Lee W. Goldman，Dennis A. Ausiello. W. B. Saunders Elsevier 2008. 第 167 章 . 图 167-2 改变和改进的。

[0048] 图 2 显示三种主要的人 BCL11A 同种型 (S、L 和 XL) 的不按比例的示意图。如在各个同种型中发现的，从 5’至 3’标记外显子。

[0049] 图 3 显示由靶向 BCL11A 的 401 反义寡核苷酸以 25 μ M 的浓度在人 REH 细胞中对 BCL11A-XL mRNA 表达的典型的抑制。

[0050] 图 4 显示由选择的靶向 BCL11A 的反义寡核苷酸以范围从 0.0064 至 20 μ M 的寡核苷酸浓度在人 REH 细胞中对 BCL11A-XL mRNA 表达的典型的抑制。

[0051] 图 5 显示由选择的从靶向 BCL11A 的 oligo 4（顶部）和 5（底部）设计的反义寡核苷酸，以范围从 0.25 至 60 μ M 的寡核苷酸浓度在人 REH 细胞中对 BCL11A-XL mRNA 表达的典型的抑制。

[0052] 图 6 显示由选择的反义寡核苷酸以范围从 0.25 至 60 μ M 的浓度对人 REH 细胞中主要同种型 (S、L 和 XL) 的 BCL11AmRNA 表达的典型的抑制。对于各个治疗组中的各个浓度，BCL11AXL、L 和 S 同种型的 mRNA 的测量分别显示在左、中和右栏中。

[0053] 图 7 显示由选择的反义寡核苷酸以范围从 0.08 至 20 μ M 的浓度对小鼠 MPC-11 细胞中小鼠 BCL11A mRNA 表达的典型的抑制。对于各个治疗组内的各个浓度，所有同种型

(BCL11A-A11) 和同种型 L(BCL11A-L) 的小鼠 BCL11A 的测量分别显示在左和右栏中。

[0054] 图 8 显示用 15mg/kg 选择的靶向 BCL11A 的反义寡核苷酸剂量给药的雌性 NMRI 小鼠的组的骨髓（顶部）和脾（底部）中 BCL11A mRNA 表达的典型的抑制。骨髓的测量，对于各个反义寡核苷酸治疗组，包括所有同种型（左栏）和同种型 L（右栏）。

[0055] 图 9 显示用 25 或 15mg/kg 的选择的靶向 BCL11A 的反义寡核苷酸施用后四周，野生型 C57BL/6 小鼠骨髓中 BCL11A mRNA 的典型的抑制。

[0056] 图 10 显示施用以 25 或 15mg/kg 的选择的靶向 BCL11A 的反义寡核苷酸后八周，野生型 C57BL/6 小鼠的骨髓中 BCL11A mRNA 的典型的抑制。

[0057] 图 11 显示施用以 15mg/kg 的选择的靶向 BCL11A 的反义寡核苷酸后八周，人 β-YAC 转基因小鼠的骨髓中 BCL11AmRNA 的典型的抑制。

[0058] 图 12 显示施用以 15mg/kg 的选择的靶向 BCL11A 的反义寡核苷酸后八周，人 β-YAC 转基因小鼠的 $Ter119^+$ 和 $CD19^+$ 骨髓细胞群体中 BCL11A mRNA 的典型的抑制。

[0059] 图 13 显示在不同治疗日，执行静脉切开手术的 (phleb.) 和未执行静脉切开手术的治疗组中的非人灵长类动物的外周血中典型的总血红蛋白 (g/L)。表明载体对照（盐水）和候选寡核苷酸 4(10 和 20mg/kg) 治疗组。

[0060] 图 14 显示在不同治疗日，执行静脉切开手术的 (phleb.) 和未执行静脉切开手术的治疗组中的非人灵长类动物的外周血中网织红细胞的典型的百分数。表明载体对照（盐水）和候选寡核苷酸 4(10 和 20mg/kg) 治疗组。

[0061] 图 15 显示在研究的第七周，以 20mg/kg 用载体对照（盐水）或候选寡核苷酸 4 剂量给药的执行静脉切开手术的非人灵长类动物的肱骨骨髓中相对 GAPDH 标准化的典型的 BCL11A 表达。对于各个治疗动物，显示对于同种型 XL（左栏）和所有同种型（右栏）的测量。

[0062] 图 16 显示在研究的第七周以 20mg/kg 用载体对照（盐水）或候选寡核苷酸 4 剂量给药的执行静脉切开手术的非人灵长类动物的肱骨骨髓中相对 GAPDH 标准化的典型的 γ- 和 β- 球蛋白 mRNA 表达。对于各个治疗组内的各个动物显示人 γ- 球蛋白（γA+G，栏 1），猕猴 (Macaca mulatta) γ- 球蛋白 (HBG2，栏 2)，猕猴 β- 球蛋白 (HBB，栏 3 ;HBB_mH，栏 4) 的测量。

[0063] 图 17 显示在研究的第 17 周对于对照（盐水）和候选寡核苷酸 4(10 和 20mg/kg) 治疗组的执行静脉切开手术的非人灵长类动物的肱骨（顶部）和股骨（底部）骨髓中相对 GAPDH 标准化的 BCL11A mRNA 的典型的表达。对于各个治疗组内的各个动物，显示同种型 XL（左栏）和所有同种型（右栏）的测量。

[0064] 图 18 显示在研究的第 17 周对于对照（盐水）和候选寡核苷酸 4(10 和 20mg/kg) 治疗组的执行静脉切开手术的非人灵长类动物中肱骨（顶部）和股骨（底部）骨髓中相对 GAPDH 标准化的 γ- 球蛋白 mRNA 的典型的表达。对于各个治疗组中的各个动物显示人 γ- 球蛋白（γA+G，左栏）和猕猴 γ- 球蛋白 (HBG2，右栏）的测量。

[0065] 图 19 显示在第 17 周，执行静脉切开手术的非人灵长类动物的对照（盐水）和候选寡核苷酸 4(10 和 20mg/kg) 治疗组的肱骨骨髓中相对 GAPDH 标准化的 γ- 和 β- 球蛋白 mRNA 的典型的表达。栏从左至右分别是对于各个治疗组中的各个动物的人 γ- 球蛋白（γA+G)，猕猴 γ- 球蛋白 (HBG2)，猕猴 β- 球蛋白 (RhHBB)，和猕猴 β- 球蛋白 (RhHBB_mH)

的测量。

[0066] 图 20 显示在第 17 周，执行静脉切开手术的非人灵长类动物的对照（盐水）和候选寡核苷酸 4(10 和 20mg/kg) 治疗组中的股骨骨髓中相对 GAPDH 标准化的 γ- 和 β- 球蛋白 mRNA 的典型的表达。栏从左至右分别是对于各个治疗组中的各个动物人 γ- 球蛋白 (γA+G)，猕猴 γ- 球蛋白 (HBG2)，猕猴 β- 球蛋白 (RhHBB)，和猕猴 β- 球蛋白 (RhHBB_mH) 的测量。

[0067] 图 21 显示在第 17 周对照（盐水）和候选寡核苷酸 4(10 和 20mg/kg) 治疗组中执行静脉切开手术的非人灵长类动物中的肱骨骨髓中对于 GAPDH 标准化的 BCL11A（顶部）和 γ- 球蛋白（底部）mRNA 的典型的平均表达。对于各个治疗组中的各个动物，显示 BCL11A 的同种型 XL（左栏）和所有同种型（右栏）的测量。对于各个治疗组中的各个动物，显示人 γ- 球蛋白（γA+G，左栏）和猕猴 γ- 球蛋白 (HBG2，右栏）的测量。

[0068] 图 22 显示在第 17 周，对照（盐水）和候选寡核苷酸 4(10 和 20mg/kg) 治疗组中执行静脉切开手术的非人灵长类动物的股骨骨髓中相对 GAPDH 标准化的 BCL11A（顶部）和 γ- 球蛋白（底部）mRNA 的典型的平均表达。对于各个治疗组中的各个动物，显示 BCL11A 的同种型 XL（左栏）和所有同种型（右栏）的测量。对于各个治疗组中的各个动物，显示人 γ- 球蛋白（γA+G，左栏）和猕猴 γ- 球蛋白 (HBG2，右栏）的测量。

[0069] 图 23 显示对于执行静脉切开手术的非人灵长类动物，以全尺寸（左）和按比例放大（右）的骨髓中 F- 细胞的典型的分数 (‰)。

[0070] 图 24 显示对于执行静脉切开手术的非人灵长类动物，以全尺寸（左）和按比例放大（右）的外周血中 F- 细胞的典型的分数 (‰)。

[0071] 图 25 显示第一次剂量给药后不同周，对照（顶部）、10mg/kg（中间）、和 20mg/kg（底部）治疗组中非人灵长类动物的外周血中 γ- 球蛋白的典型的测量。

[0072] 图 26 显示在对照处理组中，在 γ- 球蛋白峰（“峰 1”或“峰 2”）的各自的时间点，非人灵长类动物的外周血中 γ- 球蛋白作为对照的百分数的典型的测量。

[0073] 图 27 显示在 10mg/kg 剂量组中，在 γ- 球蛋白峰（“峰 1”或“峰 2”）的各个时间点，非人灵长类动物的外周血中的 γ- 球蛋白作为对照的百分数的典型的测量。

[0074] 图 28 显示在 20mg/kg 剂量组中，在 γ- 球蛋白峰（“峰 1”或“峰 2”）的各个时间点，非人灵长类动物的外周血中的 γ- 球蛋白作为对照的百分数的典型的测量。

[0075] 图 29 显示在野生型小鼠中反义寡核苷酸 4 的血浆浓度随时间的典型的测量。

[0076] 图 30 显示来自野生型小鼠的各种组织中反义寡核苷酸 4 浓度随时间的典型的测量。

[0077] 图 31 显示来自野生型小鼠的各种组织中反义寡核苷酸 4 的组织浓度随时间的典型的测量。

[0078] 图 32 显示基于单剂量药代动力学研究的骨髓中本发明的反义寡核苷酸的预测浓度的典型的模型。

[0079] 定义

[0080] 为了让本发明更容易理解，首先在下文定义某些术语。对于以下术语和其它术语的另外的定义贯穿说明书列出。

[0081] 大约或约：如本文使用的，术语“大约”或“约”，在应用于一个以上研究的值时，是

指与规定的参考值类似的值。在某些实施方案中，术语“大约”或“约”是指落在规定的参考值的任意方向（大于或小于）的25％，20％，19％，18％，17％，16％，15％，14％，13％，12％，11％，10％，9％，8％，7％，6％，5％，4％，3％，2％，1％，或更小的值的范围，除非另有陈述或从上下文是明显的（除了这样的数值超过可能值的100％的情况）。

[0082] 生物活性的：如本文使用的，措辞“生物活性的”是指在生物体系中体外或体内（例如，在生物中）具有活性的任何化学剂的特性。例如，当施用于生物时，对该生物具有生物效应的试剂，被认为是有生物活性的。在特定实施方案中，在蛋白或多肽是有生物活性的情况下，该蛋白或多肽中贡献所述蛋白或多肽的至少一种生物活性的部分通常被称为“生物活性的”部分。

[0083] 改善，增加，减少或抑制：如本文使用的，术语“改善”、“增加”、“减少”或“抑制”或语法上的等同物，表示相对于基线测量值的值，所述基线测量值如在起始本文所述的治疗之前在相同个体中的测量值，或在缺少本文所述的治疗的情况下在对照个体（或多个对照个体）中的测量值。“对照个体”是受与治疗的个体相同形式的疾病折磨的个体，其与治疗的个体年龄大约相同（保证治疗的个体和一个或多个对照个体的疾病阶段可比）。

[0084] 个体，受试者，患者：如本文使用的，术语“受试者”、“个体”或“患者”是指人或非人哺乳动物受试者。治疗的个体（也称为“患者”或“受试者”）是患有疾病的个体（胎儿，婴儿，儿童，少年，或成人）。

[0085] 锁定核酸(LNA)：如本文使用的，术语“LNA”或“锁定核酸”是指双环核苷酸类似物，优选核糖环中2’和4’位置之间具有桥的双环核苷酸类似物(2’至4’双环核苷酸类似物)。LNA在文献中有时也称为BNA(桥接的核酸或双环核酸)。其可以是指LNA单体，或当在“LNA寡核苷酸”的情况下使用时是指含有一个以上这样的双环核苷酸类似物的寡核苷酸。

[0086] 核苷酸：如本文使用的，术语“核苷酸”，是指包含糖部分、碱基部分和共价连接的磷酸基团的糖苷并且覆盖天然存在的核苷酸，如DNA或RNA，优选DNA，和非天然存在的核苷酸，所述非天然存在的核苷酸包含修饰的糖和/或碱基部分，在本文中也称为“核苷酸类似物”。在一些实施方案中，非天然存在的核苷酸包括具有修饰的糖部分的核苷酸，如双环核苷酸或2′修饰的核苷酸，如2′取代的核苷酸。在一些实施方案中，非天然存在的核苷酸包括锁定核酸(LNA)。

[0087] 基本上同源：在本文使用措辞“基本上同源”，是指在氨基酸或核酸序列之间的比较。如本领域普通技术人员将理解的，如果两条序列在相应位置含有同源的残基，则它们通常被认为“基本上同源”。同源的残基可以是相同的残基。备选地，同源的残基可以是具有大致相似的结构和/或功能特性的不相同残基。例如，如本领域普通技术人员公知的，特定氨基酸通常归类为“疏水的”或“亲水的”氨基酸，和/或为具有“极性”或“非极性”侧链。将一种氨基酸置换为相同类型的另一种也可以被认为是“同源的”置换。

[0088] 如本领域公知的，可以使用多种算法中的任一种比较氨基酸或核酸序列，所述算法包括可在商业计算机程序中获得的那些如用于核苷酸序列的BLASTN和用于氨基酸序列的BLASTP、缺口BLAST和PSI-BLAST。典型的这种程序在Altschul，等人，Basic local alignment search tool，J.Mol.Biol.，215(3)：403-410，1990；Altschul，等人，Methods in Enzymology；Altschul，等人，″Gapped BLAST and PSI-BLAST：a new generation

of protein database search programs″，Nucleic Acids Res.25:3389-3402,1997；Baxevanis，等 人，Bioinformatics:A Practical Guide to the Analysis of Genes and Proteins，Wiley,1998；和Misener，等人，（编辑），Bioinformatics Methods and Protocols(Methods in Molecular Biology，第132卷），Humana Press,1999中描述。除了鉴定同源序列之外，上文提及的程序通常提供同源程度的指示。在一些实施方案中，如果在残基的相关延伸上两条序列的相应残基至少50％，55％，60％，65％，70％，75％，80％，85％，90％，91％，92％，93％，94％，95％，96％，97％，98％，99％或更多是同源的，则这两条序列被认为是基本上同源的。在一些实施方案中，所述相关链是完整序列。在一些实施方案中，所述相关延伸是至少9,10,11,12,13,14,15,16,17或更多个残基。在一些实施方案中，所述相关延伸包括沿完整序列的相邻残基。在一些实施方案中，所述相关延伸包括完整序列的不连续残基。在一些实施方案中，所述相关延伸是至少10,15,20,25,30,35,40,45,50，或更多个残基。

[0089] 基本相同：本文使用措辞“基本相同”是指氨基酸或核酸序列的比较。如本领域普通技术人员将理解的，如果两条序列在对应位置含有同一残基，它们通常被认为是“基本上相同的”。如本领域公知的，可以使用多种算法中的任一种比较氨基酸或核酸序列，所述算法包括可在商业计算机程序中获得的那些如用于核苷酸序列的BLASTN和用于氨基酸序列的BLASTP、缺口BLAST和PSI-BLAST以及用于总体比对的EMBOSS needel或局部比对的EMBOSS Water。典型的这种程序在Altschul，等人，Basic local alignment search tool，J.Mol.Biol.，215(3)：403-410,1990；Altschul，等人，Methods in Enzymology；Altschul 等 人，Nucleic Acids Res.25:3389-3402,1997；Baxevanis 等 人，Bioinformatics:A Practical Guide to the Analysis of Genes and Proteins,Wiley,1998；和Misener，等人，（编辑），Bioinformatics Methods and Protocols(Methods in Molecular Biology，第132卷），Humana Press,1999中描述。除了鉴定同一性序列之外，上文提及的程序通常提供同一性程度的指示。在一些实施方案中，如果在残基的相关延伸上，两条序列的对应残基的至少50％，55％，60％，65％，70％，75％，80％，85％，90％，91％，92％，93％，94％，95％，96％，97％，98％，99％或获更多是相同的，则两条序列被认为是基本上相同的。在一些实施方案中，所述相关延伸是寡核苷酸的完整序列。在一些实施方案中，所述相关延伸是至少10,15,20,25,30,35,40,45,50，或更多个残基。

[0090] 靶组织：如本文使用的，术语“靶组织”是指受尤其是肝、脾和骨髓中缺陷影响的或比来自尤其在肝、脾和骨髓中组成血红蛋白的蛋白亚单位，或球蛋白链的所需活性低的任何组织。在一些实施方案中，靶组织包括其中存在球蛋白链，例如，α，β 或 γ 表达异常的那些组织。在一些实施方案中，靶组织包括显示疾病相关病理、症状或特征的那些组织。如本文使用的，靶组织可以是肝靶组织、脾靶组织和/或骨髓靶组织。典型的靶组织在下文详述。

[0091] 治疗有效量：如本文使用的，术语“治疗有效量”是指以适用于任何医学治疗的合理利益/风险比对治疗的受试者赋予治疗效果的治疗剂的量。治疗效果可以客观的（即，可通过测试或标记测量）或主观的（即，受试者给出对效果的指示或感觉到效果）。尤其是，“治疗有效量”是指如通过改善与疾病相关的症状，预防或延迟疾病的开始，和/或还减轻疾病症状的严重度或频率，有效治疗、改善或预防预期的疾病或病况，或有效呈现可检测

的治疗或预防效果的治疗剂或组合物的量。治疗有效量通常以可以包含多个单位剂量的剂量给药方案施用。对于任何特定治疗剂，治疗有效量（和/或有效剂量给药方案内的适当的单位剂量）可以，例如，根据施用途径、与其它药剂组合而改变。此外，对于任何特定患者的特定治疗有效量（和/或单位剂量）可以取决于多种因素，包括要治疗的病症和病症的严重度；使用的特定药剂的活性；使用的特定组合物；年龄，体重，一般健康，性别和患者的膳食；施用时间，施用途径，和/或使用的特定药剂的排泄或代谢率；治疗持续时间；等医药领域公知的因素。

[0092] 治疗：如本文使用的，术语“治疗（treatment）”（也作“治疗（treat）”或“治疗（treating）”）是指任意施用部分或完全减轻、改善、缓解、抑制特定疾病、病症和/或病况（例如，血红蛋白机能障碍或缺陷，镰状细胞病，地中海贫血）的一种以上症状或特征，延迟特定疾病、病症和/或病况（例如，血红蛋白机能障碍或缺陷，镰状细胞病，地中海贫血）的一种以上症状或特征的开始，减小特定疾病、病症和/或病况（例如，血红蛋白机能障碍或缺陷，镰状细胞病，地中海贫血）的一种以上症状或特征的严重度和/或减少特定疾病、病症和/或病况（例如，血红蛋白机能障碍或缺陷，镰状细胞病，地中海贫血）的一种以上症状或特征的发生率的治疗剂（例如，寡核苷酸）。这种治疗可以是针对不呈现相关疾病、病症和/或病况的体征的受试者和/或仅呈现疾病、病症和/或病况的早期体征的受试者。备选地或此外，这种治疗可以是针对呈现相关疾病、病症和/或病况的一种以上确定的体征的受试者的。本文所述的相关疾病的典型体征包括贫血，其根据显现该体征的患者范围可以从中度到严重。

[0093] 详细描述

[0094] 除其它事项外，本发明提供用于调节B-细胞CLL/淋巴瘤11A(BCL11A)活性和用于治疗与BCL11A相关的疾病、病症或病况的改善的组合物和方法。考虑的是，降低或抑制BCL11A活性导致增加的球蛋白，例如，γ球蛋白基因的表达。因此，本发明对于治疗血红蛋白病，如镰状细胞病和β-地中海贫血是特别有用的。尤其是，本发明基于通过下调或减少BCL11A的表达减少或抑制BCL11A活性的BCL11A的反义寡核苷酸调节剂。在一些实施方案中，能够下调或减少人BCL11A基因表达的寡核苷酸靶向人BCL11A基因或BCL11A的信使RNA(mRNA)同种型（例如，XL，L，M，S或XS）的区域。在一些实施方案中，能够下调或减少人BCL11A基因表达的寡核苷酸具有基于人BCL11A基因或BCL11A的信使RNA(mRNA)同种型的连续序列的反向互补的序列。

[0095] 本发明的不同方面在以下部分详细描述。部分的使用不意在限制本发明。可以将各个部分应用于本发明的任意方面。在该应用中，“或”的使用意为“和/或”，除非另有说明。

[0096] BCL11A和相关疾病和病况

[0097] 人BCL11A基因编码C_2H_2锌指蛋白，其与小鼠BCL11A蛋白具有相似性。BCL11A是淋巴转录因子，在B细胞中行使功能，并且，直到现在仍不了解在红细胞生成中发挥作用。现在知道BCL11A在球蛋白基因调节中发挥作用并且表达似乎与球蛋白基因的发育表达相关。BCL11A在骨髓中的成人红细胞前体细胞中表达并且以与γ球蛋白基因的相反关系行使功能，即，BCL11A作为γ球蛋白产生的抑制剂行使功能。

[0098] BCL11A以多种同种型表示。图2列出了BCL11A的三种主要同种型，其在两个潜在

3’末端外显子的使用中不同。已知 BCL11A 与其它蛋白关联，形成复合体，所述复合体行使功能以调节胎儿－至－成人血红蛋白转换。BCL11A 涉及与血红蛋白机能障碍相关的疾病。尤其是，抑制 BCL11A 上调 γ 球蛋白表达并且，因此，产生胎儿血红蛋白，其可以补偿在血红蛋白病，如镰状细胞病，β－地中海贫血等中遭遇的球蛋白基因机能障碍。

[0099] BCL11A 的调节剂

[0100] 如下文实施例中讨论的，本发明人成功鉴定了靶向 BCL11A 的一种以上同种型的反义寡核苷酸调节剂。在一些实施方案中，根据本发明的调节剂靶向图 2 中描述的三种主要同种型的共有区域。具体地，本发明人已经鉴定了人 BCL11A 基因的核苷酸 410 至 450 的外显子 2 内非常有效地下调 BCL11A 的特定区域。小鼠 BCL11A 基因（例如，XL，L 或 S）中的相应区域范围从核苷酸 517 至 557。图 3 清楚地显示，在外显子 1,2,3 和 4 中，该区域是就设计能够减少 BCL11A 表达的单链寡核苷酸方面的热点。对该热点的知识增加了成功设计具有好的效力并且被治疗的受试者良好耐受的寡核苷酸的可能性。

[0101] 反义寡核苷酸的设计

[0102] 除其它事项外，本发明提供用于调节编码人 BCL11A 的核酸分子的反义寡核苷酸。尤其是，适于本发明的反义寡核苷酸包括任何能够下调或减少，降低或抑制 BCL11A 表达或活性的寡核苷酸。

[0103] 通常，能够下调或减少人 BCL11A 基因表达的寡核苷酸可以基于人 BCL11A 基因或 BCL11A 的信使 RNA(mRNA) 同种型（例如，XL，L 或 S）的序列设计。例如，能够下调或减少人 BCL11A 基因表达的寡核苷酸可以具有与人 BCL11A 基因或 BCL11A 的信使 RNA(mRNA) 同种型的连续序列的反向互补基本上相同的序列。在一些实施方案中，根据本发明的寡核苷酸具有与人 BCL11A 基因或 BCL11A 的信使 RNA(mRNA) 同种型的连续序列的反向互补物至少约 50％（例如，至少约 55％，60％，65％，70％，75％，80％，85％，90％，95％，96％，97％，98％，或 99％）相同的序列。因为人 BCL11A 基因和小鼠 BCL11A 基因共享高的序列同一性，根据本发明的寡核苷酸还可以根据小鼠 BCL11A 基因或 BCL11A 的信使 RNA(mRNA) 同种型的序列设计。在一些实施方案中，根据本发明的寡核苷酸具有与小鼠 BCL11A 基因或 BCL11A 的信使 RNA(mRNA) 同种型的连续序列的反向互补物至少约 50％（例如，至少约 55％，60％，65％，70％，75％，80％，85％，90％，95％，96％，97％，98％，或 99％）相同的序列。

[0104] 备选地，能够下调或减少人 BCL11A 基因表达的寡核苷酸能够与 BCL11A mRNA 的一种以上同种型的靶区域杂交或结合。在一些实施方案中，能够减少人 BCL11A 基因表达的寡核苷酸能够与在外显子（例如，外显子 1，外显子 2，外显子 3，外显子 4，或外显子 5）中发现的 BCL11A mRNA 的靶区域杂交或结合。在一些实施方案中，能够减少人 BCL11A 基因表达的寡核苷酸能够与人或小鼠 BCL11A 的靶区域杂交或结合。

[0105] 将理解，反义寡核苷酸与 BCL11AmRNA 的靶区域的杂交可以在体外或体内进行。杂交可以在低、中等和／或严格杂交条件下进行，如本领域公知的。通常，严格杂交条件是指标准杂交条件，在该条件下，使用核酸分子，包括寡核苷酸，鉴定具有互补核酸序列的分子。严格杂交条件通常允许具有至少约 50％，55％，60％，65％，70％，75％，80％，85％，90％，95％，或更高核酸序列同一性的核酸分子之间结合。标准条件例如，在 Sambrook 等人，1989，Molecular Cloning：A Laboratory Manual，Cold Spring Harbor Labs Press 中公开，其内容通过引用以其整体并入。在本领域中，计算合适的杂交和洗涤条件以实现允许

50％,40％,30％,20％,10％,5％或更少核苷酸错配的杂交的公式可在,例如,Meinkoth 等人,1984, Anal.Biochem.138,267-284 中获得 ;其内容通过引用以其整体并入。将理解,寡核苷酸 (14-20bp) 和固定的 DNA 之间的杂交显示减少稳定性并且应该在限定对于其杂交的情况时考虑。

[0106] 杂交条件严格度受缓冲液离子强度、核苷酸的碱基组合、双链中最短链的长度 (n),和螺旋去稳定剂如甲酰胺的浓度影响。例如,杂交严格度可以通过调整盐和 / 或甲酰胺浓度和 / 或通过改变温度来改变。严格度可以在杂交步骤过程中,或在杂交后洗涤中调整。用于 DNA 印迹或 RNA 印迹中滤膜上具有大于 100 个互补残基的互补核酸的杂交的严格杂交条件的实例是在 42℃,具有 1mg 肝素的 50％甲酰胺,将杂交进行过夜。严格洗涤条件的实例是在 65℃ 0.2XSSC 洗涤 15 分钟。在一些实施方案中,在高严格度洗涤之前,通过低严格度洗涤以去除背景探针信号。例如,多于 100 个核苷酸的双链的实例中等严格度洗涤是在 45℃ 100XSSC 15 分钟。对于例如,多于 100 个核苷酸双链的实例低严格度洗涤是在 40℃ 4X SSC 15 分钟。通常,特定杂交测定中对于不相关探针观察到的 2X(或更高) 的信噪比表示检测到特定杂交。

[0107] BCL11AmRNA 同种型的序列

[0108] 如上文所述,图 2 列出了三种主要人 BCL11A mRNA 同种型,即,同种型 XL、L 和 S。类似地,存在三种主要的小鼠 BCL11A mRNA 同种型,即,同种型 XL、L 和 S。对于小鼠和人,已经见顶了其他 BCL11A mRNA 同种型。例如,在人中,基于替代的剪接变体的多种同种型在 Ensembl 基因构建组装 (European Bioinformatics Institute and Wellcome Trust Sanger Institute) 中描述,其由以下转录本识别号鉴定 :ENST00000358510, ENST00000538214, ENST00000537768, ENST00000477659, ENST00000489516, ENST00000409351,ENST00000479026,ENST00000492272,ENST00000489183。典型的 BCL11A 的人小鼠同种型的序列列在序列表中,其中表明外显子 :

[0109] SEQ ID NO :1 ＝人 BCL11A-XL NCBI 登录号 NM_022893

[0110] SEQ ID NO :2 ＝人 BCL11A-L NCBI 登录号 NM_018014

[0111] SEQ ID NO :3 ＝人 BCL11A-S NCBI 登录号 NM_138559

[0112] SEQ ID NO :4 ＝小鼠 BCL11A-XL NCBI 登录号 NM_001242934

[0113] SEQ ID NO :5 ＝小鼠 BCL11A-LNCBI 登录号 NM_016707

[0114] SEQ ID NO :6 ＝小鼠 BCL11A-S LNCBI 登录号 NM_001159289

[0115] SEQ ID NO :7 ＝小鼠 BCL11A-XS NCBI 登录号 NM_001159290

[0116] 在一些实施方案中,提供的反义寡核苷酸结合 SEQ ID NO :1 至 7 中显示的人或小鼠 BCL11A 的一种以上同种型内的区域。在一些实施方案中,提供的反义寡核苷酸结合于 SEQ ID NO :1 至 7 中显示的人或小鼠 BCL11A 同种型的外显子内的区域。在一些实施方案中,提供的反义寡核苷酸结合人 BCL11A,小鼠 BCL11A 的同种型,或其组合的外显子内的区域。在一些实施方案中,提供的反义寡核苷酸结合人 BCL11A 的核苷酸 1-283,284-613,或 614-715 内的区域。优选地, SEQ ID NO :1 的核苷酸 1-283,284-613,或 614-715。在一些实施方案中,提供的反义寡核苷酸结合人 BCL11A 的核苷酸 250-500,259-438,284-613,415-445,415-436,716-5946,716-2458,2459-3958,或核苷酸 859-2358 内的区域。在多种实施方案中,人 BCL11A 选自 SEQ ID NO :1 至 3 中显示的同种型 XL, L 或 S。在多种实施方

案中，小鼠 BCL11A 选自 SEQ ID NO :4 至 7 中显示的同种型 XL，L 或 S。

[0117] 在一些实施方案中，本发明的反义寡核苷酸具有与人或小鼠 BCL11A 基因或人或小鼠 BCL11A 的信使 RNA(mRNA) 同种型的连续序列的反向互补物至少 50％，至少 55％，至少 60％，至少 65％，至少 70％，至少 75％，至少 80％，至少 85％，至少 90％，至少 95％，至少 97％，至少 98％，或至少 99％相同的序列。在一些实施方案中，本发明的寡核苷酸具有与人或小鼠 BCL11A 基因或人或小鼠 BCL11A 的信使 RNA(mRNA) 同种型的连续序列的反向互补相同的序列。

[0118] 在一些实施方案中，根据本发明的连续序列在选自人 BCL11A 基因的核苷酸 1-283(外显子 1)，核苷酸 284-613(外显子 2)，或核苷酸 614-715(外显子 3) 的区域内。

[0119] 在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 XL(SEQ ID NO :1) 的核苷酸内。在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 XL(SEQ ID NO :1) 的核苷酸 200-620，410-450，415-436，415-446，420-450 内，或在人 BCL11A mRNA 同种型 XL(SEQ ID NO :1) 的核苷酸 716-5946(外显子 4) 内。

[0120] 在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 L(SEQ ID NO :2) 的核苷酸内。在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 L 的核苷酸 716-2458(外显子 4) 或核苷酸 2459-3958(外显子 5) 内。

[0121] 在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 S(SEQ ID NO :3) 的核苷酸内。在一些实施方案中，根据本发明的连续序列在人 BCL11A mRNA 同种型 S 的核苷酸 716-858(外显子 4) 或核苷酸 859-2358(外显子 5)。

[0122] 在一些实施方案中，提供的反义寡核苷酸结合与 SEQ ID NO :1 至 7 中显示的人或小鼠 BCL11A 的相应区域基本上相同的靶区域。例如，提供的反义寡核苷酸可以结合具有 SEQ ID NO :1 至 7 中显示的人或小鼠 BCL11A 的相应区域 (例如，外显子 1，2，3，4，或 5) 的序列至少 50％，55％，60％，65％，70％，75％，80％，85％，90％，91％，92％，93％，94％，95％，96％，97％，98％，99％或更多同一性的序列的靶区域。典型的区域在整个说明书中描述。

[0123] 寡核苷酸

[0124] 在本发明范围内的术语“寡核苷酸”，是指两个以上核苷酸通过共价键形成的分子。该术语可与术语寡聚体交替使用。在本文中，单个核苷酸 (单位) 也可以被称为单体或单元。在一些实施方案中，术语“核苷”，“核苷酸”，“单位”和“单体”可交替使用。将认识到，当提及核苷酸或单体的序列时，指的是碱基如 A，T，G，C 或 U 的序列。

[0125] 本发明的寡核苷酸能够减少包含与选自人 BCL11A 基因或 BCL11A 的信使 RNA(mRNA) 同种型的核苷酸 410 至 450 的区域内的连续序列的反向互补物至少 80％相同的序列的人 BCL11A 的表达。

[0126] 在一些实施方案中，本发明的寡核苷酸包含选自表 1 中显示的组的序列基序或由选自表 1 中显示的组的序列基序组成。序列基序本质上是可以用作用于产生基本上包含或含有相同序列但例如核苷酸类似物的数量、长度或核苷酸间连接不同的寡核苷酸的基础的核苷酸序列。

[0127] 表 1. 可以用于设计特定寡核苷酸的序列基序。

[0128]

[0129]

序列 (5'-3')	
ATTGCATTGTTTCCG	SEQ ID NO：63
GTTTGTGCTCGAT	SEQ ID NO：64
CATTGCATTGTTTCCG	SEQ ID NO：65
CGTTTGTGCTCGAT	SEQ ID NO：66
CGTTTGTGCTCGATAA	SEQ ID NO：67
CCGTTTGTGCTCGA	SEQ ID NO：68
CGTTTGTGCTCGA	SEQ ID NO：69
TTTGTGCTCGATAA	SEQ ID NO：70
TTGTGCTCCATAA	SEQ ID NO：71
TTTCCGTTTGTGCTCG	SEQ ID NO：72
ATTGCATTGTTTCCGT	SEQ ID NO：73
CGTTTGTGCTCGATA	SEQ ID NO：74

[0130] 在一些实施方案中,所述寡核苷酸序列基序不是 TCCGTTTGTGCTCGATAAA(SEQ ID NO :75) 或不是 TTTGTGCTCGATAAAAATA(SEQ ID NO :76),或不是 ATTGTTTCCGTTTGTGCTC(SEQ ID NO :77)。

[0131] 在优选的实施方案中,本发明的寡核苷酸包含或是 gapmer。

[0132] 在一些实施方案中,所述寡核苷酸长度小于 19 个核苷酸,优选长度小于 18 个,更优选小于 17 个核苷酸。

[0133] 在一些实施方案中,本发明的寡核苷酸包含增强亲和力的核苷酸类似物。

[0134] 在一些实施方案中,所述核苷酸类似物是糖修饰的核苷酸,如独立地或从属地选自由以下各项组成的组的糖修饰的核苷酸 :2'-O- 烷基 -RNA 单元,2'-OMe-RNA 单元,2'-O- 烷基 -DNA,2'- 氨基 -DNA 单元,2'- 氟 -DNA 单元,LNA 单元,阿糖核酸 (ANA) 单元,2'- 氟 -ANA 单元,HNA 单元,INA 单元和 2'MOE 单元。

[0135] 在一些实施方案中,所述核苷酸类似物包含锁定核酸 (LNA) 单元或由锁定核酸 (LNA) 单元组成。

[0136] 在优选的实施方案中,所述寡聚体是单链分子。在一些实施方案中,所述寡核苷酸不包含与相同寡核苷酸内相当的区域互补的,例如,至少 3,4 或 5 个相邻核苷酸的短区域 (即双链或发夹)。所述寡核苷酸,在一些实施方案中,可以 (基本上) 不是双链的。在一些实施方案中,所述寡核苷酸基本上不是双链的,如不是 siRNA。

[0137] 典型的反义寡核苷酸

[0138] 典型的本发明的反义寡核苷酸列在表 2 中。

[0139] 表 2

[0140]

Oligo #	序列 (5'-3')	
1	$^{m}C_s^\circ T_s^\circ A_s^\circ t_s g_s t_s g_s t_s t_s c_s c_s T_s^\circ G_s^\circ T^\circ$	SEQ ID NO: 8
2	$G_s^\circ A_s^\circ G_s^\circ a_s c_s a_s t_s g_s g_s t_s g_s g_s g_s {}^{m}C_s^\circ T_s^\circ G^\circ$	SEQ ID NO: 9
3	$A_s^\circ T_s^\circ T_s^\circ g_s c_s a_s t_s t_s g_s t_s t_s t_s c_s {}^{m}Cs^\circ Gs^\circ T^\circ$	SEQ ID NO: 10
4	$^{m}C_s^\circ A_s^\circ T_s^\circ t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 11
5	$A_s^\circ T_s^\circ T_s^\circ g_s {}^{m}c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 12
6	$A_s^\circ T_s^\circ T_s^\circ g_s c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 13
7	$T_s^\circ T_s^\circ G_s^\circ t_s g_s c_s t_s {}^{m}c_s g_s a_s t_s A_s^\circ A_s^\circ A^\circ$	SEQ ID NO: 14
8	$^{m}C_s^\circ G_s^\circ T_s^\circ t_s t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s T_s^\circ A_s^\circ A^\circ$	SEQ ID NO: 15
9	$^{m}C_s^\circ {}^{m}C_s^\circ G_s^\circ t_s t_s t_s g_s t_s g_s c_s t_s {}^{m}C_s^\circ G_s^\circ A^\circ$	SEQ ID NO: 16
10	$T_s^\circ T_s^\circ g_s t_s g_s c_s t_s {}^{m}c_s c_s a_s T_s^\circ A_s^\circ A^\circ$	SEQ ID NO: 17
11	$T_s^\circ T_s^\circ T_s^\circ c_s {}^{m}c_s g_s t_s t_s t_s g_s t_s g_s c_s T_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 18
12	$^{m}C_s^\circ A_s^\circ T_s^\circ t_s g_s {}^{m}c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 19
13	$^{m}C_s^\circ A_s^\circ t_s^\circ t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 20
14	$^{m}C_s^\circ A_s^\circ T_s^\circ t_s g_s c_s a_s t_s t_s g_s t_s t_s T_s^\circ {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 21
15	$^{m}C_s^\circ A_s^\circ T_s^\circ T_s^\circ g_s c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 22
16	$^{m}C_s^\circ A_s^\circ T_s^\circ T_s^\circ g_s c_s a_s t_s t_s g_s t_s t_s T_s^\circ {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 23
17	$^{m}C_s^\circ A_s^\circ T_s^\circ t_s g_s c_s a_s t_s t_s g_s t_s T_s^\circ T_s^\circ {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 24
18	$^{m}C_s^\circ A_s^\circ T_s^\circ T_s^\circ G_s^\circ c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$	SEQ ID NO: 25
19	$G_s^\circ T_s^\circ T_s^\circ t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s t_s A_s^\circ A_s^\circ A^\circ$	SEQ ID NO: 26
20	$T_s^\circ T_s^\circ T_s^\circ g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s t_s A_s^\circ A_s^\circ A^\circ$	SEQ ID NO: 27
21	$T_s^\circ T_s^\circ T_s^\circ g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s T_s^\circ A_s^\circ A_s^\circ$	SEQ ID NO: 28
22	$G_s^\circ T_s^\circ T_s^\circ t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s T_s^\circ A_s^\circ A_s^\circ$	SEQ ID NO: 29
23	$G_s^\circ T_s^\circ T_s^\circ t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s A_s^\circ T_s^\circ A_s^\circ$	SEQ ID NO: 30
24	$^{m}C_s^\circ {}^{m}C_s^\circ G_s^\circ t_s t_s t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s A_s^\circ T_s^\circ A^\circ$	SEQ ID NO: 31
25	$^{m}C_s^\circ G_s^\circ T_s^\circ t_s t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s A_s^\circ T_s^\circ A^\circ$	SEQ ID NO: 32
26	$G_s^\circ T_s^\circ t_s t_s g_s t_s g_s c_s t_s c_s G_s^\circ A_s^\circ T^\circ$	SEQ ID NO: 33
27	$^{m}C_s^\circ G_s^\circ T_s^\circ t_s t_s g_s t_s g_s c_s t_s c_s G_s^\circ A_s^\circ T^\circ$	SEQ ID NO: 34
28	$^{m}C_s^\circ {}^{m}C_s^\circ G_s^\circ t_s t_s t_s g_s t_s g_s c_s t_s c_s G_s^\circ A_s^\circ T^\circ$	SEQ ID NO: 35
29	$T_s^\circ {}^{m}C_s^\circ {}^{m}C_s^\circ g_s t_s t_s t_s g_s t_s g_s c_s t_s c_s G_s^\circ A_s^\circ T^\circ$	SEQ ID NO: 36
30	$^{m}C_s^\circ G_s^\circ t_s t_s t_s g_s t_s g_s c_s t_s {}^{m}C_s^\circ G_s^\circ A^\circ$	SEQ ID NO: 37
31	$T_s^\circ T_s^\circ {}^{m}C_s^\circ {}^{m}c_s g_s t_s t_s t_s g_s t_s g_s c_s t_s {}^{m}C_s^\circ G_s^\circ A^\circ$	SEQ ID NO: 38

[0141]

Oligo #	序列 (5'-3')	
32	$T_s^\circ\ {}^mC_s^\circ\ {}^mC_s^\circ\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mC_s^\circ\ G_s^\circ\ A^\circ$	SEQ ID NO: 39
33	$T_s^\circ\ {}^mC_s^\circ\ {}^mC_s^\circ\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ T_s^\circ\ {}^mC_s^\circ\ G^\circ$	SEQ ID NO: 40
34	$T_s^\circ\ T_s^\circ\ {}^mC_s^\circ\ {}^mc_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ T_s^\circ\ {}^mC_s^\circ\ G^\circ$	SEQ ID NO: 41
35	$G_s^\circ\ T_s^\circ\ T_s^\circ\ t_s\ c_s\ {}^mc_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ {}^mC_s^\circ\ T_s^\circ\ {}^mC^\circ$	SEQ ID NO: 42
36	$T_s^\circ\ T_s^\circ\ T_s^\circ\ c_s\ {}^mc_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ {}^mC_s^\circ\ T_s^\circ\ {}^mC^\circ$	SEQ ID NO: 43
37	$^mC_s^\circ\ G_s^\circ\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ a_s\ T_s^\circ\ A_s^\circ\ A^\circ$	SEQ ID NO: 44
38	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ A_s^\circ\ T_s^\circ\ A_s^\circ\ A^\circ$	SEQ ID NO: 45
39	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ T_s^\circ\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ a_s\ T_s^\circ\ A_s^\circ\ A^\circ$	SEQ ID NO: 46
40	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ T_s^\circ\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ A_s^\circ\ T_s^\circ\ A_s^\circ\ A^\circ$	SEQ ID NO: 47
41	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ G_s^\circ\ A_s^\circ\ T_s^\circ\ A_s^\circ\ A^\circ$	SEQ ID NO: 48
42	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ T_s^\circ\ T_s^\circ\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ a_s\ T_s^\circ\ A_s^\circ\ A^\circ$	SEQ ID NO: 49
43	$^mC_s^\circ\ G_s^\circ\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ A_s^\circ\ T_s^\circ\ A^\circ$	SEQ ID NO: 50
44	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ a_s\ T_s^\circ\ A^\circ$	SEQ ID NO: 51
45	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ T_s^\circ\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ a_s\ T_s^\circ\ A^\circ$	SEQ ID NO: 52
46	$^mC_s^\circ\ G_s^\circ\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ G_s^\circ\ A_s^\circ\ T_s^\circ\ A^\circ$	SEQ ID NO: 53
47	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ T_s^\circ\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ A_s^\circ\ T_s^\circ\ A^\circ$	SEQ ID NO: 54
48	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ G_s^\circ\ A_s^\circ\ T_s^\circ\ A^\circ$	SEQ ID NO: 55
49	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ G_s^\circ\ A_s^\circ\ T^\circ$	SEQ ID NO: 56
50	$^mC_s^\circ\ G_s^\circ\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ A_s^\circ\ T^\circ$	SEQ ID NO: 57
51	$^mC_s^\circ\ G_s^\circ\ t_s^\circ\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ c_s\ G_s^\circ\ A_s^\circ\ T^\circ$	SEQ ID NO: 58
52	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ A_s^\circ\ T^\circ$	SEQ ID NO: 59
53	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ T_s^\circ\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mc_s\ g_s\ A_s^\circ\ T^\circ$	SEQ ID NO: 60
54	$^mC_s^\circ\ G_s^\circ\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mC_s^\circ\ G_s^\circ\ A_s^\circ\ T^\circ$	SEQ ID NO: 61
55	$^mC_s^\circ\ G_s^\circ\ T_s^\circ\ T_s^\circ\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ {}^mC_s^\circ\ G_s^\circ\ A_s^\circ\ T^\circ$	SEQ ID NO: 62

[0142] 在多种实施方案中,根据本发明的反义寡核苷酸包括具有与选自表 2 的反义寡核苷酸序列中出现的 12 或更多(例如,13,14,15,16,17,或 18)个相邻核苷酸至少 50%(例如,50%,55%,60%,65%,70%,75%,80%,85%,90%,91%,92%,93%,94%,95%,96%,97%,98%,99%或更多)相同的序列的那些寡核苷酸。

[0143] 在多种实施方案中,根据本发明的反义寡核苷酸包括与选自表 2 的反义寡核苷酸的核苷酸序列至少 50%(例如,50%,55%,60%,65%,70%,75%,80%,85%,90%,91%,92%,93%,94%,95%,96%,97%,98%,99%或更多)相同的序列的那些寡核苷酸。

[0144] 长度

[0145] 将理解,根据本发明的反义寡核苷酸可以有任何适当的长度。本发明的反义寡核苷酸可以包含总共 10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29 或 30 个相邻核苷酸长度的相邻核苷酸序列或由总共 10,11,12,13,14,15,16,17,18,19,

20,21,22,23,24,25,26,27,28,29 或 30 个相邻核苷酸长度的相邻核苷酸序列组成。在一些实施方案中,反义寡核苷酸包含总共 10-18,10-17,10-16,10-15,10-14,10-13,10-12,11-17,11-16,11-15,11-14,11-13,12-17,12-16,12-15,或 12-14 核苷酸长度的相邻核苷酸序列或由总共 10-18,10-17,10-16,10-15,10-14,10-13,10-12,11-17,11-16,11-15,11-14,11-13,12-17,12-16,12-15,或 12-14 核苷酸长度的相邻核苷酸序列组成。在一些实施方案中,本发明的反义寡核苷酸长度是 10-16 或 12-16 个核苷酸。在一些实施方案中,本发明的反义寡核苷酸由不多于 22 个核苷酸,如不多于 20 个核苷酸,如不多于 19 个核苷酸,如 15,16,17 或 18 个核苷酸组成。在一些实施方案中,本发明的反义寡核苷酸包含小于 20 个核苷酸。在一些实施方案中,本发明的反义寡核苷酸长度小于 18 个核苷酸。不希望受理论限制,应该理解,当对于本发明的反义寡核苷酸,或相邻核苷酸序列长度给定范围时,其包括范围,例如从 10-30(或在 10-30 之间)中提供的较低和较高长度,包括 10 和 30 二者。

[0146] 就本文中鉴定的核苷酸序列而言,“核酸序列同一性百分数(%)”定义为在比对序列并引入缺口(如果需要)以实现最大百分数序列同一性后,候选序列中与参考序列中核苷酸相同的核苷酸的百分数。百分数序列同一性可以通过将 2 条序列之间相同的比对的核酸的数量计数,除以寡聚体中单体的总数,并乘以 100 来计算。在这样的比较中,如果缺口存在,优选这样的缺口仅仅是错配,而不是其中缺口内的很多核酸在比对的序列之间,例如本发明的寡核苷酸和靶区域之间不同的区域。为了确定百分数核酸序列同一性的比对可以以不同方式实现,这些方式在本领域技术内,例如,使用公众可获得的计算机软件如 BLAST,ALIGN 或 Megalign(DNASTAR) 软件以及用于总体比对的 EMBOSS needel 或用于局部比对的 EMBOSS Water。本领域技术人员可以确定用于比对的适当参数,包括比较的序列的全长的最大比对所需的任何算法。优选地,WU-BLAST-2 软件用于确定氨基酸序列同一性(Altschul 等人,Methods in Enzymology,266,460-480(1996) ;http ://blast.wustl/edu/blast/README.html)。WU-BLAST-2 使用多个检索参数,其多数设置为缺省值。可调整的参数以以下值设置 :重叠间隔 = 1,重叠分数 = 0.125,世界阈值 (T) = 11。HSP 得分 (S) 和 HSP S2 参数是动态值并且根据特定序列的组合通过程序本身确立,然而,可以调整最小值并且设置为上文表示的。

[0147] 核苷和核苷类似物

[0148] 在一些实施方案中,术语“核苷类似物”和“核苷酸类似物”可交替使用。

[0149] 如本文使用的术语“核苷酸”,是指包含糖部分,碱基部分和共价连接的基团(连接基团),如磷酸酯或硫代磷酸酯核苷酸间连接基团的糖苷,并且包括天然存在的核苷酸,如 DNA 或 RNA,和非天然存在的核苷酸,包含修饰的糖和 / 或碱基部分,其在本文中也称为“核苷酸类似物”。在本文中,单核苷酸(单元)也可以称为单体或核酸单元。

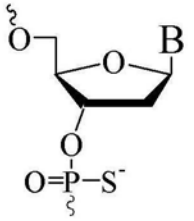
[0150] 在生物化学领域,通常使用术语“核苷”指包含糖部分和碱基部分的糖苷,并且可以因此在提及核苷酸单元时使用,其通过寡核苷酸的核苷酸之间的核苷酸连接共价连接。在生物化学领域,常使用术语“核苷酸”指核酸单体或单元,并且本身在寡核苷酸的情况下可以指碱基 - 如“核苷酸序列”,通常是指核碱基序列(即糖骨架和核苷酸间连接的存在是暗含的)。同样,特别是在一种以上核苷间连接基团被修饰的寡核苷酸的情况下,术语“核苷酸”可以是指“核苷”,例如甚至当指定核苷间的连接的存在和性质时,可以使用术语“核苷酸”。

[0151] 如本领域普通技术人员将认识到的，寡核苷酸的 5’末端核苷酸不包含 5’核苷酸间连接基团，尽管可以或可以不包含 5’末端基团。

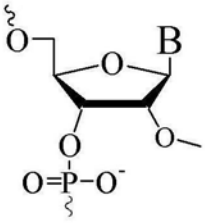
[0152] 非天然存在的核苷酸包括具有修饰的糖部分的核苷酸，如双环核苷酸或 2’修饰的核苷酸，如 2’取代的核苷酸。

[0153] “核苷酸类似物”是由于糖和 / 或碱基部分中的修饰导致的天然核苷酸，如 DNA 或 RNA 核苷酸的变体。原则上，在寡核苷酸的情况下，类似物相对天然核苷酸可以仅是“沉默的”或“相当的”，即对寡核苷酸发挥作用以抑制靶基因表达的方式无功能性影响。然而，这样的“相当的”类似物可以是有用的，如果，例如，它们更容易或低成本地制造，或储存或制造条件下更稳定，或表示标签或标记。然而，优选地，所述类似物将对寡核苷酸发挥作用以抑制表达的方式具有功能性影响 ；例如通过产生对靶的增加的结合亲和力和 / 或增加的对细胞内核酸酶的抗性和 / 或增加的转运至细胞内的容易程度。核苷酸类似物的具体实例由例 如 Freier&Altmann ；Nucl. Acids Res.，1997，25，4429-4443 和 Uhlmann ；Curr. Opinion in Drug Development，2000，3(2)，293-213 描述，并且在方案 1 和“锁定核酸 (LNA)”部分描述。

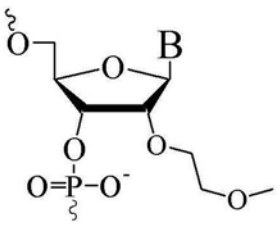
[0154]



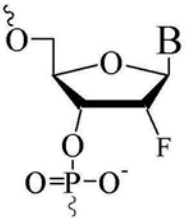
硫代磷酸酯



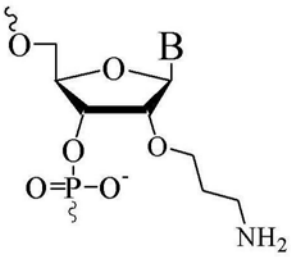
2'-O- 甲基



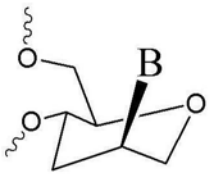
2'-MOE



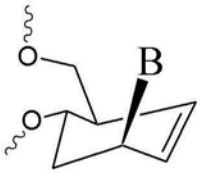
2'- 氟



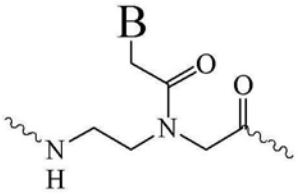
2'-AP



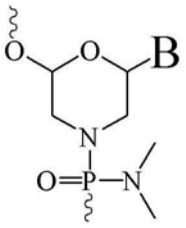
HNA



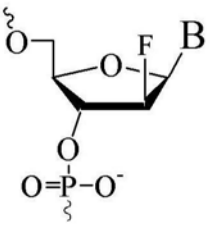
CeNA



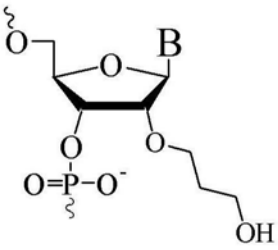
PNA



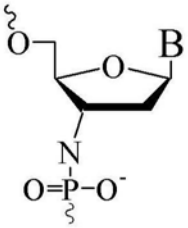
吗啉基



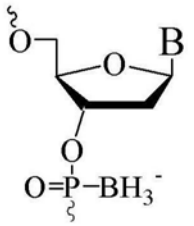
2'-F-ANA



2'-(3-羟基)丙基



3'- 氨基磷酸酯



硼磷酸酯

[0155] 方案 1

[0156] 因此，寡核苷酸可以包含以下各项或由以下各项组成：天然存在的核苷酸的简单序列－优选 2'－脱氧核苷酸（这里通常称为“DNA”），但也可能是核糖核苷酸（这里通常称为“RNA”），或这样的天然存在的核苷酸和一种以上非天然存在的核苷酸，即核苷酸类似物的组合。这样的核苷酸类似物可以适当地增强寡聚体对靶序列的亲和力。适当的和优选的核苷酸类似物的实例由 WO2007/031091 提供或在其中引用。

[0157] 在寡聚体中并入增强亲和力的核苷酸类似物，如 LNA 或 2'－取代的糖，可能使得特异结合的寡聚体的尺寸减少，并且还可以减少非特异或异常结合发生之前寡核苷酸尺寸的上限。

[0158] 在一些实施方案中，本发明的反义寡核苷酸包含至少 1 种核苷酸类似物。在一些实施方案中，本发明的反义寡核苷酸包含至少 2 种核苷酸类似物。在一些实施方案中，本发明的反义寡核苷酸包含 3-8 种核苷酸类似物，例如 6 或 7 种核苷酸类似物。在一些实施方

案中，至少一个所述核苷酸类似物是锁定核酸 (LNA)；例如至少 3 或至少 4，或至少 5，或至少 6，或至少 7，或 8 种所述核苷酸类似物可以是 LNA。在一些实施方案中，所有所述核苷酸类似物可以是 LNA。

[0159] 在阅读本公开内容后技术人员将意识到，当提及仅由核苷酸组成的优选的核苷酸序列基序或核苷酸序列时，由该序列限定的本发明的反义寡核苷酸可以包含替代所述序列中存在的一种以上核苷酸的相应核苷酸类似物，如 LNA 单元或其它核苷酸类似物，其提升寡聚体 / 靶双链的双链稳定性 /T_m（即增强亲和力的核苷酸类似物）。

[0160] 在一些实施方案中，寡聚体和靶序列的核苷酸序列之间的任何错配优选在增强亲和力的核苷酸类似物的外部区域发现，如“Gapmer 设计”部分中提到的区域 B 或 Y，和 / 或“Gapmer 设计”部分提到的区域 D，和 / 或在寡核苷酸中未修饰的如 DNA 核苷酸的位点，和 / 或在相邻核苷酸序列的 5’ 或 3’ 的区域中发现。

[0161] 这种核苷酸修饰的实例包括修饰糖部分以提供 2’- 取代基或产生双环结构，其增强结合亲和力并且还可以提供增加的核酸酶抗性。

[0162] 优选的核苷酸类似物是 LNA，如氧基 -LNA（如 β-D- 氧基 -LNA，和 α-L- 氧基 -LNA），和 / 或氨基 -LNA（如 β-D- 氨基 -LNA 和 α-L- 氨基 -LNA）和 / 或硫代 -LNA（如 β-D- 硫代 -LNA 和 α-L- 硫代 -LNA）和 / 或 ENA（如 β-D-ENA 和 α-L-ENA）。最优选的是 β-D- 氧基 -LNA。

[0163] 在一些实施方案中，本发明的反义寡核苷酸内存在的核苷酸类似物（如“Gapmer 设计”部分提到的区域 A 和 C 中）独立地选自，例如 :2’-O- 烷基 -RNA 单元，2’-OMe-RNA 单元，2’-O- 烷基 -DNA，2’- 氨基 -DNA 单元，2’- 氟 -DNA 单元，LNA 单元，阿糖核酸 (ANA) 单元，2’- 氟 -ANA 单元，HNA 单元，INA（插入核酸 -Christensen，2002.Nucl.Acids.Res.200230 : 4918-4925，在此通过引用并入本文）单元和 2’MOE 单元。

[0164] 在一些实施方案中，在本发明的反义寡核苷酸，或其相邻核苷酸序列中仅存在一种上述类型的核苷酸类似物

[0165] 在一些实施方案中，核苷酸类似物是 2’-O- 甲氧基乙基 -RNA(2’MOE)，2’- 氟 -DNA 单体或 LNA 核苷酸类似物，并且这样本发明的反义寡核苷酸可以包含独立地选自这三种类型的类似物的核苷酸类似物，或可以仅包含选自所述三种类型中的一种类型的类似物。在一些实施方案中至少一个所述核苷酸类似物是 2’-MOE-RNA，如 2，3，4，5，6，7，8，9 或 10 个 2’-MOE-RNA 核苷酸单元。在一些实施方案中，至少一个所述核苷酸类似物是 2’- 氟 DNA，如 2，3，4，5，6，7，8，9 或 10 个 2’- 氟 -DNA 核苷酸单元。

[0166] 在一些实施方案中，本发明的反义寡核苷酸包含至少一个锁定核酸 (LNA) 单元，如 1，2，3，4，5，6，7，或 8 个 LNA 单元，如 3-7 或 4 至 8 个 LNA 单元，或 3，4，5，6 或 7 个 LNA 单元。在一些实施方案中，所有所述核苷酸类似物是 LNA。在一些实施方案中，本发明的反义寡核苷酸可以既包含 β-D- 氧基 -LNA，也包含一种以上的以下 LNA 单元 :硫代 -LNA，氨基 -LNA，氧基 -LNA，5’- 甲基 -LNA 和 / 或 β-D 或 α-L 构型的 ENA 或其组合。在一些实施方案中，所述 LNA 胞嘧啶单元是 5’- 甲基 - 胞嘧啶。

[0167] 在一些实施方案中，本发明的反义寡核苷酸可以既包含核苷酸类似物（优选 LNA）也包含 DNA 单元。优选合并的总核苷酸类似物（优选 LNA）和 DNA 单元是 10-25，如 10-24，优选 10-20，如 10-18，甚至更优选 12-16 个。在一些实施方案中，本发明的反义寡核苷酸的

核苷酸序列，如相邻核苷酸序列，由至少一个核苷酸类似物（优选 LNA）组成并且剩余的核苷酸单元是 DNA 单元。在一些实施方案中，本发明的反义寡核苷酸仅包含 LNA 核苷酸类似物和天然存在的核苷酸（如 RNA 或 DNA，最优选 DNA 核苷酸），任选地具有修饰的核苷酸间连接，如硫代磷酸酯。

[0168] 术语“核碱基”是指核苷酸的碱基部分并且包括天然存在的以及非天然存在的变体。因此，“核碱基”不仅包括已知的嘌呤和嘧啶杂环，而且还包括杂环类似物和其互变异构体。

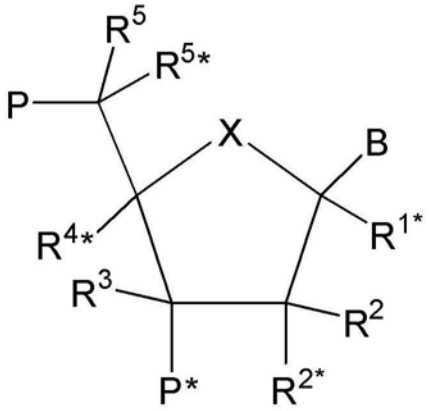
[0169] 核碱基的实例包括，但不限于腺嘌呤，鸟嘌呤，胞嘧啶，胸腺嘧啶，尿嘧啶，黄嘌呤，次黄嘌呤，5- 甲基胞嘧啶，异胞嘧啶，假异胞嘧啶，5- 溴尿嘧啶，5- 丙炔基尿嘧啶，6- 氨基嘌呤，2- 氨基嘌呤，肌苷，二氨基嘌呤，和 2- 氯 -6- 氨基嘌呤。

[0170] 在一些实施方案中，寡聚体中存在的至少一个核碱基是选自由以下各项组成的组的修饰的核碱基：5- 甲基胞嘧啶，异胞嘧啶，假异胞嘧啶，5- 溴尿嘧啶，5- 丙炔基尿嘧啶，6- 氨基嘌呤，2- 氨基嘌呤，肌苷，二氨基嘌呤，和 2- 氯 -6- 氨基嘌呤。

[0171] 锁定核酸 (LNA)

[0172] 术语“LNA”是指双环核苷酸类似物，称为“锁定核酸”。其可以是指 LNA 单体，或，当在“LNA 寡核苷酸”的情况下使用时，LNA 是指含有一个以上这种双环核苷酸类似物的寡核苷酸。LNA 核苷酸特征为在核糖糖环的 C2’和 C4’之间 - 例如下文所述的二元基 R^{4*}-R^{2*} 中所示，存在接头基团（如桥）。用于本发明的反义寡核苷酸的 LNA 优选具有通式 I 的结构：

[0173]



式 I

[0174] 其中对于所有手性中心，不对称基团可以在 R 或 S 取向中找到；

[0175] 其中 X 选自 -O-，-S-，$-N(R^{N*})-$，$-C(R^{6}R^{6*})-$，如，在一些实施方案中 -O-：

[0176] B 选自氢，任选地取代的 C_{1-4}- 烷氧基，任选地取代的 C_{1-4}- 烷基，任选地取代的 C_{1-4}- 酰氧基，包括天然存在的和核碱基类似物的核碱基，DNA 插入剂，光化学活性基团，热化学活性基团，螯合基团，报告基团，和配体；优选地，B 是核碱基或核碱基类似物；

[0177] P 指示与相邻单体的核苷酸间连接，或 5′ - 末端基团，这样的核苷酸间连接或 5′ - 末端基团任选地包括取代基 R^{5} 或同样适用取代基 R^{5*}；

[0178] P* 指示与相邻单体的核苷酸间连接，或 3′ - 末端基团；

[0179] R^{4*}和 R^{2*}一起指示由 1-4 个选自以下各项的基团 / 原子组成的二价接头基团 :-C(R^aR^b)-,-C(R^a) = C(R^b)-,-C(R^a) = N-,-O-,-Si(R^a)$_2$-,-S-,-SO_2-,-N(R^a)-,和 > C = Z,其中 Z 选自 -O-,-S-,和 -N(R^a)-,并且 R^a和 R^b各自独立地选自氢,任选地取代的 C_{1-12}- 烷基,任选地取代的 C_{2-12}- 烯基,任选地取代的 C_{2-12}- 炔基,羟基,任选地取代的 C_{1-12}- 烷氧基,C_{2-12}- 烷氧基烷基,C_{2-12}- 烯氧基,羧基,C_{1-12}- 烷氧基羰基,C_{1-12}- 烷基羰基,甲酰基,芳基,芳氧基 - 羰基,芳氧基,芳基羰基,杂芳基,杂芳氧基 - 羰基,杂芳氧基,杂芳基羰基,氨基,单 - 和二 (C_{1-6}- 烷基) 氨基,氨甲酰基,单 - 和二 (C_{1-6}- 烷基)- 氨基 - 羰基,氨基 -C_{1-6}- 烷基 - 氨基羰基,单 - 和二 (C_{1-6}- 烷基) 氨基 -C_{1-6}- 烷基 - 氨基羰基,C_{1-6}- 烷基 - 羰基氨基,脲基,C_{1-6}- 烷酰氧基,磺基 (sulphono),C_{1-6}- 烷基磺酰氧基,硝基,叠氮基,硫烷基,C_{1-6}- 烷基硫代,卤素,DNA 插入剂,光化学活性基团,热化学活性基团,螯合基团,报告基团,和配体,其中芳基和杂芳基可以任选地被取代并且其中两个成对的取代基 R^a和 R^b一起可以指示任选地取代的亚甲基 (= CH_2),其中对于所有手性中心,不对称基团可以在 R 或 S 取向上发现,并且 ;

[0180] 存在的 R^{1*},R^2,R^3,R^5,R^{5*},R^6和 R^{6*}各个取代基独立地选自氢,任选地取代的 C_{1-12}- 烷基,任选地取代的 C_{2-12}- 烯基,任选地取代的 C_{2-12}- 炔基,羟基,C_{1-12}- 烷氧基,C_{2-12}- 烷氧基烷基,C_{2-12}- 烯氧基,羧基,C_{1-12}- 烷氧基羰基,C_{1-12}- 烷基羰基,甲酰基,芳基,芳氧基 - 羰基,芳氧基,芳基羰基,杂芳基,杂芳氧基 - 羰基,杂芳氧基,杂芳基羰基,氨基,单 - 和二 (C_{1-6}- 烷基) 氨基,氨甲酰基,单 - 和二 (C_{1-6}- 烷基)- 氨基 - 羰基,氨基 -C_{1-6}- 烷基 - 氨基羰基,单 - 和二 (C_{1-6}- 烷基) 氨基 -C_{1-6}- 烷基 - 氨基羰基,C_{1-6}- 烷基 - 羰基氨基,脲基,C_{1-6}- 烷酰氧基,磺基 (sulphono),C_{1-6}- 烷基磺酰氧基,硝基,叠氮基,硫烷基,C_{1-6}- 烷基硫代,卤素,DNA 插入剂,光化学活性基团,热化学活性基团,螯合基团,报告基团,和配体,其中芳基和杂芳基可以任选地被取代,并且其中两个成对的取代基一起可以指示氧代,硫代,亚氨基,或任选地取代的亚甲基 ;其中 R^N选自氢和 C_{1-4}- 烷基,并且其中两个相邻的 (非成对的) 取代基可以指示导致双键的另外的键 ;并且 R^{N*},当存在并且不涉及二元基时,选自氢和 C_{1-4}- 烷基 ;以及其碱性盐和酸加成盐。对于所有手性中心,不对称基团可以在 R 或 S 取向上找到。

[0181] 在一些实施方案中,R^{4*}和 R^{2*}一起指示由选自由以下各项组成的组的基团组成的二元基 :C(R^aR^b)-C(R^aR^b)-,C(R^aR^b)-O-,C(R^aR^b)-NR^a-,C(R^aR^b)-S-,和 C(R^aR^b)-C(R^aR^b)-O-,其中各个 R^a和 R^b可以任选地独立选择。在一些实施方案中,R^a和 R^b可以,任选地独立地选自由以下各项组成的组 :氢和 C_{1-6}烷基,如甲基,如氢。

[0182] 在一些实施方案中,R^{4*}和 R^{2*}一起指示二元基 -O-CH(CH_2OCH_3)-(2’O- 甲氧基乙基双环核酸 -Seth 等人,2010,J.Org.Chem)- 在 R- 或 S- 构型中。

[0183] 在一些实施方案中,R^{4*}和 R^{2*}一起指示二元基 -O-CH(CH_2CH_3)-(2’O- 乙基双环核酸 -Seth 等人,2010,J.Org.Chem).- 在 R- 或 S- 构型中。

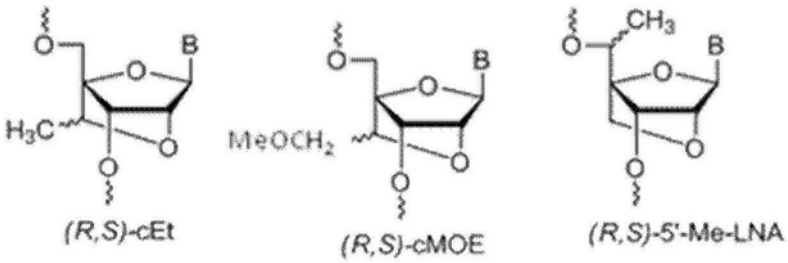
[0184] 在一些实施方案中,R^{4*}和 R^{2*}一起指示二元基 -O-CH(CH_3)-.- 在 R- 或 S- 构型中。

[0185] 在一些实施方案中,R^{4*}和 R^{2*}一起指示二元基 -O-CH_2-O-CH_2--(Seth 等人,2010,J.Org.Chem)。

[0186] 在一些实施方案中,R^{4*}和 R^{2*}一起指示二元基 -O-NR-CH_3--(Seth 等人,2010,J.Org.Chem)。

[0187] 在一些实施方案中,LNA 单元具有选自以下组的结构 :

[0188]



[0189] 在一些实施方案中，R^{1*}，R^2，R^3，R^5，R^{5*}独立地选自由以下各项组成的组：氢，卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基或取代的 C_{2-6}炔基，C_{1-6}烷氧基，取代的 C_{1-6}烷氧基，acyl，取代的酰基，C_{1-6}氨基烷基或取代的 C_{1-6}氨基烷基。对于所有手性中心，不对称基团可以在 R 或 S 取向上找到。

[0190] 在一些实施方案中，R^{1*}，R^2，R^3，R^5，R^{5*}是氢。

[0191] 在一些实施方案中，R^{1*}，R^2，R^3独立地选自由以下各项组成的组：氢，卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基或取代的 C_{2-6}炔基，C_{1-6}烷氧基，取代的 C_{1-6}烷氧基，酰基，取代的酰基，C_{1-6}氨基烷基或取代的 C_{1-6}氨基烷基。对于所有手性中心，不对称基团可以在 R 或 S 取向上找到。

[0192] 在一些实施方案中，R^{1*}，R^2，R^3是氢。

[0193] 在一些实施方案中，R^5和 R^{5*}各自独立地选自由以下各项组成的组：H，$-CH_3$，$-CH_2-CH_3$，$-CH_2-O-CH_3$，和 $-CH=CH_2$。适当地，在一些实施方案中，R^5或 R^{5*}中任一个是氢，而另一个集团（分别是 R^5或 R^{5*}）选自由以下各项组成的组：C_{1-5}烷基，C_{2-6}烯基，C_{2-6}炔基，取代的 C_{1-6}烷基，取代的 C_{2-6}烯基，取代的 C_{2-6}炔基或取代的酰基（$-C(=O)-$）；其中各个取代的基团被独立地选自以下各项的取代基单或多取代：卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基，取代的 C_{2-6}炔基，OJ_1，SJ_1，NJ_1J_2，N_3，$COOJ_1$，CN，$O-C(=O)NJ_1J_2$，$N(H)C(=NH)NJ$，J_2或 $N(H)C(=X)N(H)J_2$其中 X 是 O 或 S；并且各个 J_1和 J_2，独立地是 H，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基，取代的 C_{2-6}炔基，C_{1-6}氨基烷基，取代的 C_{1-6}氨基烷基或保护基团。在一些实施方案中，R^5或 R^{5*}之一是取代的 C_{1-6}烷基。在一些实施方案中，R^5或 R^{5*}之一是取代的亚甲基，其中优选的取代基包括一个以上独立地选自 F，NJ_1J_2，N_3，CN，OJ_1，SJ_1，$O-C(=O)NJ_1J_2$，$N(H)C(=NH)NJ$，J_2或 $N(H)C(O)N(H)J_2$的基团。在一些实施方案中，各个 J_1和 J_2独立地是 H 或 C_{1-6}烷基。在一些实施方案中，R^5或 R^{5*}之一是甲基，乙基或甲氧基甲基。在一些实施方案中，R^5或 R^{5*}之一是甲基。在进一步实施方案中，R^5或 R^{5*}之一是乙烯基。在一些实施方案中，R^5或 R^{5*}之一是取代的酰基。在一些实施方案中，R^5或 R^{5*}之一是 $C(=O)NJ_1J_2$。对于所有手性中心，不对称基团可以在 R 或 S 取向上找到。这样的 5'修饰的双环核苷酸在 WO2007/134181 中公开，其在此通过引用以其整体并入。

[0194] 在一些实施方案中，B 是核碱基，包括核碱基类似物和天然存在的核碱基，如嘌呤或嘧啶，或取代的嘌呤或取代的嘧啶，如本文提及的核碱基，如选自由以下各项组成的组的核碱基：腺嘌呤，胞嘧啶，胸腺嘧啶，腺嘌呤，尿嘧啶，和/或修饰的或取代的核碱基，如 5- 噻唑并 - 尿嘧啶，2- 硫代 - 尿嘧啶，5- 丙炔基 - 尿嘧啶，2' 硫代 - 胸腺嘧啶，5- 甲基胞嘧啶，5- 噻唑并 - 胞嘧啶，5- 丙炔基 - 胞嘧啶，和 2，6- 二氨基嘌呤。

[0195] 在一些实施方案中，R^{4*}和 R^{2*}一起指示选自以下各项的二元基：$-C(R^aR^b)-O-$，-C

$(R^aR^b)-C(R^cR^d)-O-$，$-C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-O-$，$-C(R^aR^b)-O-C(R^cR^d)-$，$-C(R^aR^b)-O-C(R^cR^d)-O-$，$-C(R^aR^b)-C(R^cR^d)-$，$-C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-$，$-C(R^a)=C(R^b)-C(R^cR^d)-$，$-C(R^aR^b)-N(R^c)-$，$-C(R^aR^b)-C(R^cR^d)-N(R^e)-$，$-C(R^aR^b)-N(R^c)-O-$，和 $-C(R^aR^b)-S-$，$-C(R^aR^b)-C(R^cR^d)-S-$，其中 R^a，R^b，R^c，R^d，R^e，和 R^f各自独立地选自氢，任选地取代的 C_{1-12}- 烷基，任选地取代的 C_{2-12}- 烯基，任选地取代的 C_{2-12}- 炔基，羟基，C_{1-12}- 烷氧基，C_{2-12}- 烷氧基烷基，C_{2-12}- 烯氧基，羧基，C_{1-12}- 烷氧基羰基，C_{1-12}- 烷基羰基，甲酰基，芳基，芳氧基 - 羰基，芳氧基，芳基羰基，杂芳基，杂芳氧基 - 羰基，杂芳氧基，杂芳基羰基，氨基，单 - 和二 (C_{1-6}- 烷基) 氨基，氨甲酰基，单 - 和二 (C_{1-6}- 烷基)- 氨基 - 羰基，氨基 -C_{1-6}- 烷基 - 氨基羰基，单 - 和二 (C_{1-6}- 烷基) 氨基 -C_{1-6}- 烷基 - 氨基羰基，C_{1-6}- 烷基 - 羰基氨基，脲基，C_{1-6}- 烷酰氧基，磺基 (sulphono)，C_{1-6}- 烷基磺酰氧基，硝基，叠氮基，硫烷基，C_{1-6}- 烷基硫代，卤素，DNA 插入剂，光化学活性基团，热化学活性基团，螯合基团，报告基团，和配体，其中芳基和杂芳基可以任选地被取代并且两个成对的取代基 R^a和 R^b一起可以指示任选地取代的亚甲基 (= CH_2)。对于所有手性中心，不对称基团可以在 R 或 S 取向上找到。

[0196] 在一些实施方案中，R^{4*}和 R^{2*}一起指示选自以下各项的二元基 (二价基团)-CH_2-O-，$-CH_2-S-$，$-CH_2-NH-$，$-CH_2-N(CH_3)-$，$-CH_2-CH_2-O-$，$-CH_2-CH(CH_3)-$，$-CH_2-CH_2-S-$，$-CH_2-CH_2-NH-$，$-CH_2-CH_2-CH_2-$，$-CH_2-CH_2-CH_2-O-$，$-CH_2-CH_2-CH(CH_3)-$，$-CH=CH-CH_2-$，$-CH_2-O-CH_2-O-$，$-CH_2-NH-O-$，$-CH_2-N(CH_3)-O-$，$-CH_2-O-CH_2-$，$-CH(CH_3)-O-$，和 $-CH(CH_2-O-CH_3)-O-$，和 / 或，$-CH_2-CH_2-$，和 $-CH=CH-$。对于所有手性中心，不对称基团可以在 R 或 S 取向上找到。

[0197] 在一些实施方案中，R^{4*}和 R^{2*}一起指示二元基 $C(R^aR^b)-N(R^c)-O-$，其中，R^a和 R^b独立地选自由以下各项组成的组 :氢，卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基或取代的 C_{2-6}炔基，C_{1-6}烷氧基，取代的 C_{1-6}烷氧基，酰基，取代的酰基，C_{1-6}氨基烷基或取代的 C_{1-6}氨基烷基，如氢，并且 ;其中 R^c选自由以下各项组成的组 :氢，卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基或取代的 C_{2-6}炔基，C_{1-6}烷氧基，取代的 C_{1-6}烷氧基，酰基，取代的酰基，C_{1-6}氨基烷基或取代的 C_{1-6}氨基烷基，如氢。

[0198] 在一些实施方案中，R^{4*}和 R^{2*}一起指示二元基 $C(R^aR^b)-O-C(R^cR^d)-O-$，其中 R^a，R^b，R^c，和 R^d独立地选自由以下各项组成的组 :氢，卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基或取代的 C_{2-6}炔基，C_{1-6}烷氧基，取代的 C_{1-6}烷氧基，酰基，取代的酰基，C_{1-6}氨基烷基或取代的 C_{1-6}氨基烷基，如氢。

[0199] 在一些实施方案中，R^{4*}和 R^{2*}形成二元基 -CH(Z)-O-，其中 Z 选自由以下各项组成的组 :C_{1-6}烷基，C_{2-6}烯基，C_{2-6}炔基，取代的 C_{1-6}烷基，取代的 C_{2-6}烯基，取代的 C_{2-6}炔基，酰基，取代的酰基，取代的酰胺，硫醇或取代的硫基 ;并且其中各个取代的基团，独立地被独立地选自以下各项的任选地保护的取代基单或取代取代 :卤素，氧代，羟基，OJ_1，NJ_1J_2，SJ_1，N_3，$OC(=X)J_1$，$OC(=X)NJ_1J_2$，$NJ^3C(=X)NJ_1J_2$和 CN，其中各个 J_1，J_2和 J_3独立地是 H 或 C_{1-6}烷基，并且 X 是 O，S 或 NJ_1。在一些实施方案中，Z 是 C_{1-6}烷基或取代的 C_{1-6}烷基。在一些实施方案中，Z 是甲基。在一些实施方案中，Z 是取代的 C_{1-6}烷基。在一些实施方案中，所述取代基是 C_{1-6}烷氧基。在一些实施方案中，Z 是 CH_3OCH_2-。对于所有手性中心，不对称基团可以在 R 或 S 取向中找到。这样的双环核苷酸在 US 7,399,845 中公开，其在此通过引用以其整体并入。在一些实施方案中，R^{1*}，R^2，R^3，R^5，R^{5*}是氢。在一些实施方案中，R^{1*}，R^2，R^{3*}是氢，并且 R^5，R^{5*}中的一个或二者可以是除了氢之外的如上文和在 WO 2007/134181 中提及

的。

[0200] 在一些实施方案中，R^{4*}和R^{2*}一起指示在桥接中包含取代的氨基的二元基，如由二元基 $-CH_2-N(R^c)-$ 组成，或包含二元基 $-CH_2-N(R^c)-$，其中 R^c是 C_{1-12}烷基氧基。在一些实施方案中，R^{4*}好 R^{2*}一起指示二元基 $-Cq_3q_4-NOR-$，其中 q_3和 q_4独立地选自由以下各项组成的组：氢，卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基或取代的 C_{2-6}炔基，C_{1-6}烷氧基，取代的 C_{1-6}烷氧基，酰基，取代的酰基，C_{1-6}氨基烷基或取代的 C_{1-6}氨基烷基；其中各个取代的基团，独立地被独立地选自以下各项的取代基单或多取代：卤素，OJ_1，SJ_1，NJ_1J_2，$COOJ_1$，CN，$O-C(=O)NJ_1J_2$，$N(H)C(=NH)N\ J_1J_2$或 $N(H)C(=X=N(H)J_2$，其中 X 是 O 或 S；并且各个 J_1和 J_2独立地是 H，C_{1-6}烷基，C_{2-6}烯基，C_{2-6}炔基，C_{1-6}氨基烷基或保护基团。对于所有手性中心，不对称基团可以在 R 或 S 取向中找到。这样的双环核苷酸在 WO2008/150729 中公开，其在此通过引用以其整体并入。在一些实施方案中，R^{1*}，R^2，R^3，R^5，R^{5*}独立地选自由以下各项组成的组：氢，卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基或取代的 C_{2-6}炔基，C_{1-6}烷氧基，取代的 C_{1-6}烷氧基，酰基，取代的酰基，C_{1-6}氨基烷基或取代的 C_{1-6}氨基烷基。在一些实施方案中，R^{1*}，R^2，R^3，R^5，R^{5*}是氢。在一些实施方案中，R^{1*}，R^2，R^3是氢并且 R^5，R^{5*}中的一个或两个可以是除氢之外的如上文和 WO2007/134181 中提及的。在一些实施方案中，R^{4*}和 R^{2*}一起指示二元基（二价基团）$C(R^aR^b)-O-$，其中 R^a和 R^b各自独立地是卤素，C_1-C_{12}烷基，取代的 C_1-C_{12}烷基，C_2-C_{12}烯基，取代的 C_2-C_{12}烯基，C_2-C_{12}炔基，取代的 C_2-C_{12}炔基，C_1-C_{12}烷氧基，取代的 C_1-C_{12}烷氧基，OJ_1SJ_1，SOJ_1，SO_2J_1，NJ_1J_2，N_3，CN，$C(=O)OJ_1$，$C(=O)NJ_1J_2$，$C(=O)J_1$，$O-C(=O)NJ_1J_2$，$N(H)C(=NH)NJ_1J_2$，$N(H)C(=O)NJ_1J_2$或 $N(H)C(=S)NJ_1J_2$；或 R^a和 R^b一起是 $=C(q3)(q4)$；q_3和 q_4各自独立地是，H，卤素，C_1-C_{12}烷基或取代的 C_1-C_{12}烷基；各个取代的基团独立地被独立地选自以下各项的取代基单或多取代：卤素，C_1-C_6烷基，取代的 C_1-C_6烷基，C_2-C_6烯基，取代的 C_2-C_6烯基，C_2-C_6炔基，取代的 C_2-C_6炔基，OJ_1，SJ_1，NJ_1J_2，N_3，CN，$C(=O)OJ_1$，$C(=O)NJ_1J_2$，$C(=O)J_1$，$O-C(=O)NJ_1J_2$，$N(H)C(=O)NJ_1J_2$或 $N(H)C(=S)NJ_1J_2$。并且；各个 J_1和 J_2独立地是，H，C1-C_6烷基，取代的 C1-C_6烷基，C_2-C_6烯基，取代的 C_2-C_6烯基，C_2-C_6炔基，取代的 C_2-C_6炔基，C1-C_6氨基烷基，取代的 C1-C_6氨基烷基或保护基团。这样的化合物在 WO2009006478A 中公开，其在此通过引用以其整体并入。

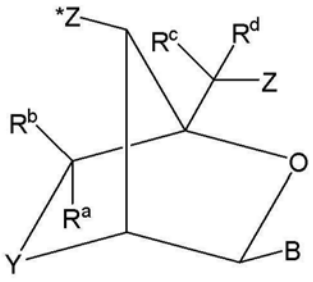
[0201] 在一些实施方案中，R^{4*}和 R^{2*}形成二元基 -Q-，其中 Q 是 $C(q_1)(q_2)C(q_3)(q_4)$，$C(q_1)=C(q_3)$，$C[=C(q_1)(q_2)]-C(q_3)(q_4)$ 或 $C(q_1)(q_2)-C[=C(q_3)(q_4)]$；$q_1$，$q_2$，$q_3$，$q_4$各自独立地是 H，卤素，$C_{1-12}$烷基，取代的 C_{1-12}烷基，C_{2-12}烯基，取代的 C_{1-12}烷氧基，OJ_1，SJ_1，SOJ_1，SO_2J_1，NJ_1J_2，N_3，CN，$C(=O)OJ_1$，$C(=O)-NJ_1J_2$，$C(=O)J_1$，$-C(=O)NJ_1J_2$，$N(H)C(=NH)NJ_1J_2$，$N(H)C(=O)NJ_1J_2$或 $N(H)C(=S)NJ_1J_2$；各个 J_1和 J_2独立地是 H，C_{1-6}烷基，C_{2-6}烯基，C_{2-6}炔基，C_{1-6}氨基烷基或保护基团；并且，任选地其中当 Q 是 $C(q_1)(q_2)(q_3)(q_4)$ 并且 q_3或 q_4中的一个是 CH_3时，则 q_3或 q_4中的另一个或 q_1和 q_2中的一个中的至少一个不是 H。在一些实施方案中，R^{1*}，R^2，R^3，R^5，R^{5*}是氢。对于所有手性中心，不对称基团可以在 R 或 S 取向中找到。这样的双环核苷酸在 WO2008/154401 中公开，其在此通过引用以其整体并入。在一些实施方案中，R^{1*}，R^2，R^3，R^5，R^{5*}独立地选自由以下各项组成的组：氢，卤素，C_{1-6}烷基，取代的 C_{1-6}烷基，C_{2-6}烯基，取代的 C_{2-6}烯基，C_{2-6}炔基或取代的 C_{2-6}炔基，C_{1-6}烷氧基，取代的 C_{1-6}烷氧基，酰基，取代的酰基，C_{1-6}氨基烷基或取代的 C_{1-6}氨基烷基。在一些实施方案中，

R^{1*}，R^2，R^3，R^5，R^{5*}是氢。在一些实施方案中，R^{1*}，R^2，R^3是氢并且 R^5，R^{5*}中的一个或两个可以是除氢之外的如上文和在 WO 2007/134181 或 WO2009/067647 中提及的（α-L-双环核酸类似物）。

[0202] 进一步的双环核苷酸类似物和其在反义寡核苷酸中的用途在 WO2011/115818，WO2011/085102，WO2011/017521，WO009100320，WO2010/036698，WO2009/124295 和 WO2009/006478 中公开。这样的核苷酸类似物在一些方面可以用于本发明的化合物。

[0203] 在一些实施方案中，用于本发明的反义寡核苷酸的 LNA 优选具有通式 II 的结构：

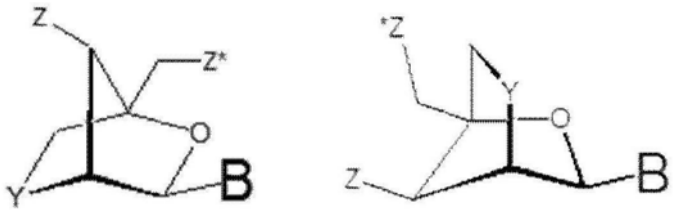
[0204]



式 II

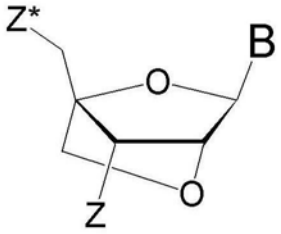
[0205] 其中 Y 选自由以下各项组成的组：-O-，$-CH_2O-$，-S-，-NH-，$N(R^e)$ 和 / 或 $-CH_2-$；Z 和 Z* 独立地选自核苷酸间连接，R^H，末端基团或保护基团；B 构成天然或非天然核苷酸碱基部分（核碱基），并且 R^H选自氢和 C_{1-4}- 烷基；R^a，R^bR^c，R^d和 R^e任选地独立地，选自由以下各项组成的组：氢，任选地取代的 C_{1-12}- 烷基，任选地取代的 C_{2-12}- 烯基，任选地取代的 C_{2-12}- 炔基，羟基，C_{1-12}- 烷氧基，C_{2-12}- 烷氧基烷基，C_{2-12}- 烯氧基，羧基，C_{1-12}- 烷氧基羰基，C_{1-12}- 烷基羰基，甲酰基，芳基，芳氧基 - 羰基，芳氧基，芳基羰基，杂芳基，杂芳氧基 - 羰基，杂芳氧基，杂芳基羰基，氨基，单 - 和二（C_{1-6}- 烷基）氨基，氨甲酰基，单 - 和二（C_{1-6}- 烷基）- 氨基 - 羰基，氨基 $-C_{1-6}$- 烷基 - 氨基羰基，单 - 和二（C_{1-6}- 烷基）氨基 $-C_{1-6}$- 烷基 - 氨基羰基，C_{1-6}- 烷基 - 羰基氨基，脲基，C_{1-6}- 烷酰氧基，磺基（sulphono），C_{1-6}- 烷基磺酰氧基，硝基，叠氮基，硫烷基，C_{1-6}- 烷基硫代，卤素，DNA 插入剂，光化学活性基团，热化学活性基团，螯合基团，报告基团，和配体，其中芳基和杂芳基可以任选地被取代并且其中两个成对的取代基 R^a和 R^b一起可以指示任选地取代的亚甲基（＝ CH_2）；并且 R^H选自氢和 C_{1-4}- 烷基。在一些实施方案中，R^a，R^bR^c，R^d和 R^e任选地独立地，选自由以下各项组成的组：氢和 C_{1-6}烷基，如甲基。对于所有手性中心，不对称基团可在 R 或 S 取向中找到，例如，两种典型的立体化学异构体包括 β-D 和 α-L 同种型，其可以如下表明：

[0206]

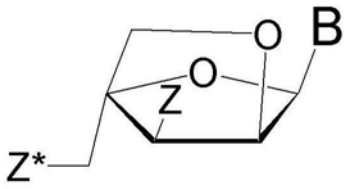


[0207] 具体的典型 LNA 单元表示如下：

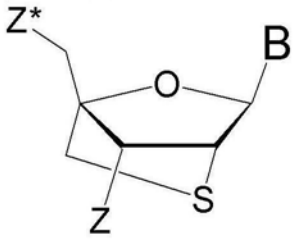
[0208]



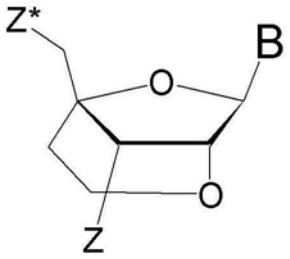
β-D-氧基-LNA



α-L-氧基-LNA

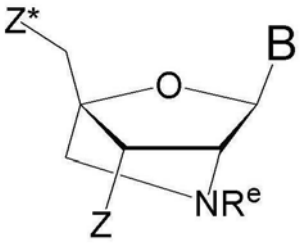


β-D-硫代-LNA



β-D-ENA

[0209]



β-D-氨基-LNA

[0210] 术语″硫代-LNA″包含锁定的核苷酸,其中以上通式中的Y选自S或-CH_2-S-。硫代-LNA可以是β-D和α-L-构型二者。

[0211] 术语″氨基-LNA″包含锁定的核苷酸,其中以上通式中的Y选自-N(H)-,N(R)-,CH_2-N(H)-,和-CH_2-N(R)-,其中R选自氢和C_{1-4}-烷基。氨基-LNA可以是β-D和α-L-构型二者。

[0212] 术语″氧基-LNA″包含锁定的核苷酸,其中以上通式中的Y表示-O-。氧基-LNA可以是β-D和α-L-构型二者。

[0213] 术语″ENA″包含锁定的核苷酸,其中以上通式中的Y是-CH_2-O-(其中-CH_2-O-的氧原子连接于相对于碱基B的2'-位置)。R^e是氢或甲基。

[0214] 在一些典型的实施方案中,LNA选自β-D-氧基-LNA,α-L-氧基-LNA,β-D-氨基-LNA和β-D-硫代-LNA,尤其是β-D-氧基-LNA。

[0215] Gapmer设计

[0216] 本发明的寡核苷酸优选为gapmer。gapmer寡核苷酸是包含一段相邻的能够募集RNA酶,如RNA酶H的核苷酸(如至少6或7DNA核苷酸的区域,在本文中称为区域B或区域Yb)的寡核苷酸。RNA酶H募集区域的长度可以由5和15之间的整数$_b$表示。区域B或Y在5′和3′侧翼有增强亲和力的核苷酸类似物的区域,如能够募集RNA酶的相邻段核苷酸的5′和3′端的1-6个核苷酸类似物。这些区域分别被称为区域A或X和C或X_a。所述

核苷酸类似物的数量可以由 $_{a}$或 $_{a,}$表示并且在 1 至 6 之间，优选 1,2,3,4 或 5。

[0217] EP 1 222 309 提供用于确定 RNA 酶 H 活性的体外方法，其可以用于确定募集 RNA 酶 H 的能力。如果当提供以互补 RNA 靶时，使用 EP 1 222 309 的实施例 91-95 提供的方法，其具有这样的起始速率：以 pmol/l/min 测量的，使用仅 DNA 的寡核苷酸（具有相同碱基序列但仅含有 DNA 单体，不具有 2’取代，在所述寡核苷酸的所有单体之间具有硫代磷酸酯键基团）确定的起始速率至少 1％，如至少 5％，如至少 10％或大于 20％，则寡聚体被认为能够募集 RNA 酶 H。

[0218] 在一些实施方案中，如果当提供以互补 RNA 靶和 RNA 酶 H 时，使用 EP 1 222 309 的实施例 91-95 提供的方法，RNA 酶 H 起始速率（如以 pmol/l/min 测量的）小于使用相当的仅 DNA 寡核苷酸（没有 2’取代，在寡核苷酸的所有核苷酸之间具有硫代磷酸酯键）确定的起始速率的 1％，如小于 5％，如小于 10％或小于 20％，则认为寡聚体基本上能够募集 RNA 酶 H。

[0219] 在其他实施方案中，如果当提供以互补 RNA 靶和 RNA 酶 H 时，使用 EP 1 222 309 的实施例 91-95 提供的方法（通过引用并入本文），RNA 酶 H 起始速率（如以 pmol/l/min 测量的）是相当的仅 DNA 寡核苷酸（没有 2’取代，在寡核苷酸的所有核苷酸之间具有硫代磷酸酯键）确定的起始速率的至少 20％，如至少 40％，如至少 60％，如至少 80％，则认为寡聚体基本上能够募集 RNA 酶 H。

[0220] 在一些实施方案中，能够募集 RNA 酶的单体选自由以下各项组成的组：DNA 单体，α-L-LNA 单体，C4’烷基化的 DNA 单体（参见 WO2009/090182 和 Vester 等人，Bioorg.Med.Chem.Lett.18(2008)2296-2300，在此通过引用并入本文），和 UNA（不连接的核酸）核苷酸（参见 Fluiter 等人，Mol.Biosyst.，2009,10,1039 在此通过引用并入本文）。UNA 是未锁定核酸，通常其中核糖的 C2-C3C-C 键被去除，形成未锁定的“糖”残基。

[0221] 在一些实施方案中，gapmer 包含式（5′至 3′）的（多）核苷酸序列，A-B-C 或 X_a-Y_b-$X_{a'}$，或任选地 A-B-C-D 或 D-A-B-C 或 X_a-Y_b-$X_{a'}$-D 或 D-X_a-Y_b-$X_{a'}$，其中；区域 A 或 X_a(5′区域）由至少一个核苷酸类似物，如至少一个锁定核酸（LNA）单元，如 1-6 个核苷酸类似物，如 LNA 单元组成，或包含至少一个核苷酸类似物，如至少一个锁定核酸（LNA）单元，如 1-6 个核苷酸类似物，如 LNA 单元，并且；区域 B 或 Y 由能够募集 RNA 酶（当与互补 RNA 分子，如 mRNA 靶形成双链体（duplex）时），如 DNA 核苷酸的至少五个连续的核苷酸组成，或包含能够募集 RNA 酶（当与互补 RNA 分子，如 mRNA 靶形成双链体（duplex）时），如 DNA 核苷酸的至少五个连续的核苷酸，并且；区域 C 或 $X_{a'}$（3′区域）由至少一个核苷酸类似物，如至少一个 LNA 单元，如 1-6 个核苷酸类似物，如 LNA 单元组成，或包含至少一个核苷酸类似物，如至少一个 LNA 单元，如 1-6 个核苷酸类似物，如 LNA 单元，并且；区域 D，当存在时由 1,2 或 3 个核苷酸单元，如 DNA 核苷酸组成，或包含 1,2 或 3 个核苷酸单元，如 DNA 核苷酸。

[0222] 在一些实施方案中，区域 A 或 X_a包括 1,2,3,4,5 或 6 个核苷酸类似物，如 LNA 单元，如 2-5 个核苷酸类似物，如 2-5 个 LNA 单元，如 3 或 4 个核苷酸类似物，如 3 或 4 个 LNA 单元或由 1,2,3,4,5 或 6 个核苷酸类似物，如 LNA 单元，如 2-5 个核苷酸类似物，如 2-5 个 LNA 单元，如 3 或 4 个核苷酸类似物，如 3 或 4 个 LNA 单元组成；和/或区域 C 或 $X_{a'}$ 包括 1,2,3,4,5 或 6 个核苷酸类似物，如 LNA 单元，如 2-5 个核苷酸类似物，如 2-5 个 LNA 单元，如 3 或 4 个核苷酸类似物，如 3 或 4 个 LNA 单元或由 1,2,3,4,5 或 6 个核苷酸类似物，如 LNA 单

元，如 2-5 个核苷酸类似物，如 2-5 个 LNA 单元，如 3 或 4 个核苷酸类似物，如 3 或 4 个 LNA 单元组成。

[0223] 在一些实施方案中，B 或 Y 包括能够募集 RNA 酶的 5,6,7,8,9,10,11 或 12 个连续的核苷酸，或能够募集 RNA 酶的 5-15 个，或 6-10 个，或 7-9 个，如 8 个连续的核苷酸，或 B 或 Y 由能够募集 RNA 酶的 5,6,7,8,9,10,11 或 12 个连续的核苷酸，或能够募集 RNA 酶的 5-15 个，或 6-10 个，或 7-9 个，如 8 个连续的核苷酸组成，或 B 或 Y 由能够募集 RNA 酶的 5,6,7,8,9,10,11 或 12 个连续的核苷酸，或能够募集 RNA 酶的 5-15 个，或 6-10 个，或 7-9 个，如 8 个连续的核苷酸构成。在一些实施方案中，区域 B 或 Y 由至少一个 DNA 核苷酸单元，如 1-12 个 DNA 单元，优选 4-12 个 DNA 单元，更优选 6-10 个 DNA 单元，如 7-10 个 DNA 单元，最优选 8,9 或 10 个 DNA 单元组成，或包含至少一个 DNA 核苷酸单元，如 1-12 个 DNA 单元，优选 4-12 个 DNA 单元，更优选 6-10 个 DNA 单元，如 7-10 个 DNA 单元，最优选 8,9 或 10 个 DNA 单元。

[0224] 在一些实施方案中，区域 A 或 X_a 包括 3 或 4 个核苷酸类似物，如“核苷和核苷类似物”部分所述的或由 3 或 4 个核苷酸类似物，如“核苷和核苷类似物”部分所述的组成，优选所述类似物是 LNA。区域 B 包括 7,8,9 或 10 个 DNA 单元或由 7,8,9 或 10 个 DNA 单元组成，并且区域 C 或 $X_{a'}$ 包括 3 或 4 个核苷酸类似物，如“核苷和核苷类似物”部分所述的或由 3 或 4 个核苷酸类似物，如“核苷和核苷类似物”部分所述的组成，优选所述类似物是 LNA。这样的设计包括，例如，(A-B-C 或 X_a-Y_b-$X_{a'}$)2-11-3,2-10-2,2-8-4,2-9-3,2-9-4,3-10-3,3-10-4,4-10-3,3-9-3,3-9-4,4-9-3,3-8-3,3-8-4,4-8-3,3-7-3,3-7-4,4-7-3，并且还可以包括区域 D，所述区域 D 可以具有一个或 2 个核苷酸单元，如 DNA 单元。gapmer 设计的实例显示在 WO2004/046160 中并且在此通过引用并入本文。在一些实施方案中，本发明的 gapmer 反义寡核苷酸可以是 shortmer gapmer，如美国临时专利申请号 60/977,409 中所述的并且在此通过引用并入本文。

[0225] 在一些实施方案中，本发明的寡核苷酸包含总共 10,11,12,13,14,15,16,17 或 18 个核苷酸单元的相邻核苷酸序列，其中所述相邻核苷酸序列是式 (5′ -3′)，A-B-C 或 X_a-Y_b-$X_{a'}$ ，或任选地 A-B-C-D 或 D-A-B-C 或 X_a-Y_b-$X_{a'}$ -D 或 D-X_a-Y_b-$X_{a'}$ 的，其中 ;A 或 $X_{a'}$ 由 1,2,3 或 4 个核苷酸类似物单元，如 LNA 单元组成 ;B 或 Y 由当与互补 RNA 分子（如 mRNA 靶）形成双链体时能够募集 RNA 酶的 7,8,9,10 或 11 个相邻核苷酸单元组成 ;并且 C 或 $X_{a'}$ 由 1,2,3 或 4 个核苷酸类似物单元，如 LNA 单元组成。当存在时，D 由单个 DNA 单元组成。

[0226] 在一些实施方案中，A 或 X_a 由 1 个 LNA 单元组成。在一些实施方案中，A 或 X_a 由 2 个 LNA 单元组成。在一些实施方案中，A 或 X_a 由 3 个 LNA 单元组成。在一些实施方案中，A 或 X_a 由 4 个 LNA 单元组成。在一些实施方案中，C 或 $X_{a'}$ 由 1 个 LNA 单元组成。在一些实施方案中，C 或 $X_{a'}$ 由 2 个 LNA 单元组成。在一些实施方案中，C 或 $X_{a'}$ 由 3 个 LNA 单元组成。在一些实施方案中，C 或 $X_{a'}$ 由 4 个 LNA 单元组成。在一些实施方案中，B 或 Y 由 7 个核苷酸单元组成。在一些实施方案中，B 或 Y 由 8 个核苷酸单元组成。在一些实施方案中，B 或 Y 由 9 个核苷酸单元组成。在某些实施方案中，区域 B 由 10 个核苷酸单体组成。在某些实施方案中，区域 B 或 Y 包含 1-10 个 DNA 单体。在一些实施方案中，B 由 10 个核苷酸单元组成。在一些实施方案中，B 或 Y 由 11 个核苷酸单元组成。在一些实施方案中，B 或 Y 包含 1-11 个 DNA 单元，包括，如 2,3,4,5,6,7,8,9,10，或 11 个 DNA 单元。在一些实施方案中，B 或 Y 由 DNA 单元组成。在一些实施方案中，B 或 Y 包含 α-L 构型的至少一个 LNA 单元，如 α-L- 构

型的2,3,4,5,6,7,8或9个LNA单元。在一些实施方案中,B或Y包含至少一个 α-L-氧基LNA单元或其中所有 α-L-构型的LNA单元是 α-L-氧基LNA单元。在一些实施方案中,存在于A-B-C或X_a-Y_b-$X_{a'}$中的核苷酸数选自由以下各项组成的组:(核苷酸类似物单元--区域B或Y--核苷酸类似物单元):1-8-1,1-8-2,2-8-1,2-8-2,3-8-3,2-8-3,3-8-2,4-8-1,4-8-2,1-8-4,2-8-4, 或;1-9-1,1-9-2,2-9-1,2-9-2,2-9-3,3-9-2,1-9-3,3-9-1,4-9-1,1-9-4, 或;1-10-1,1-10-2,2-10-1,2-10-2,1-10-3,3-10-1, 或;1-11-1,1-11-2,2-11-1,2-11-2,2-11-3,3-11-2,4-11-1,1-11-4。在一些实施方案中,A-B-C中的核苷酸数选自由以下各项组成的组:3-8-3,3-10-3,3-9-3,2-8-3,2-11-3,3-9-4,4-9-3,4-8-4,3-8-5,5-8-3,2-10-3,3-10-2,4-9-2,2-9-4,4-8-3,3-8-4,2-10-2,2-9-3,3-9-2,4-8-2,2-8-4 和 4-7-4。在某些实施方案中,区域A和C或X_a和$X_{a'}$中的每个由三个LNA单体组成,并且区域B或Y由8或9或10个核苷酸单体,优选DNA单体组成。在一些实施方案中,A和C个自由两个、三个或四个LNA单元组成,并且B由8,9,10或11个核苷酸单元,优选DNA单元组成。

[0227] 在多种实施方案中,其它gapmer设计包括这样的那些:其中区域A和/或C或X_a和/或$X_{a'}$由3,4,5或6个核苷类似物,如含有2'-O-甲氧基乙基-核糖(2'-MOE)的单体或含有2'-氟-脱氧核糖的单体组成,并且区域B由8,9,10,11或12个核苷,如DNA单体组成,其中区域A-B-C具有3-9-3,3-10-3,5-10-5或4-12-4单体。其它gapmer设计在WO2007/146511中公开,其在此通过引用并入本文。

[0228] 核苷酸间连接

[0229] 本文所述的反义寡核苷酸的单体通过连接基团偶联。适当地,各个单体通过连接基团与3'相邻单体连接。

[0230] 在阅读本公开内容时,本领域普通技术人员将理解,本发明的反义寡核苷酸末端的5'单体不包含5'连接基团,尽管其可以或可以不包含5'末端基团。

[0231] 术语"连接基团"或"核苷酸间连接"意在表示能够将两个核苷酸共价偶联的基团。具体和优选的实例包括磷酸酯基团和硫代磷酸酯基团。本发明的反义寡核苷酸或其相邻核苷酸序列通过连接基团偶联。适当地,各个核苷酸通过连接基团与3′相邻核苷酸连接。典型的核苷酸间连接包括WO2007/031091中描述的那些,其在此通过引用并入本文。

[0232] 在一些实施方案中,核苷酸间连接可以从其正常的磷酸二酯修饰为对核酸酶攻击更具抗性的连接,如硫代磷酸酯或硼磷酸酯(boranophosphate)--这两种,可由RNA酶H裂开,还使得减少靶基因的表达的反义抑制途径。

[0233] 本文提供的适当的含硫(S)核苷酸间连接可以是优选的。硫代磷酸酯核苷酸间连接也是优选的,尤其对于gapmer的缺口(gap)区域(B或Y)。硫代磷酸酯键还可以用在区域(A/X_a和C/$X_{a'}$,并且对于将A/X_a或C/$X_{a'}$与D连接,并且在区域D内,视情况而定)侧翼。

[0234] 然而区域A或X_a,B或Y和C或$X_{a'}$,可以包含核苷酸间连接,而不是硫代磷酸酯,如磷酸二酯连接,尤其是,例如当使用核苷酸类似物保护区域A或X_a和C或$X_{a'}$内的核苷酸间连接免受内切核酸酶降解,如在区域A或X_a和C或$X_{a'}$包含LNA核苷酸时。

[0235] 本发明寡核苷酸的核苷酸间连接可以是磷酸二酯,硫代磷酸酯或硼磷酸酯,以允许RNA酶H裂开靶RNA。由于改善的核酸酶抗性和其它原因,如容易制造,硫代磷酸酯是优选的。在一些实施方案中,本发明的寡核苷酸的核苷酸和/或核苷酸类似物通过硫代磷酸酯基团的方式彼此连接。在本发明的优选实施方案中,所述寡核苷酸包含至少一个硫代磷

酸酯键。

[0236] 认识到，将磷酸二酯连接，如一个或两个连接，包括在另外的硫代磷酸酯寡核苷酸中，尤其是核苷酸类似物单元之间或与核苷酸类似物单元相邻（通常在区域 A 或 X_a和或 C 或 $X_{a'}$中）可以改进 WO2008/053314 中描述的寡核苷酸的生物利用度和 / 或生物分布，WO2008/053314 在此通过引用并入本文。

[0237] 在一些实施方案中，如上文引用的实施方案，在合适且未具体指出的情况下，所有其余的连接基团是磷酸二酯或硫代磷酸酯，或其混合物。

[0238] 在一些实施方案中，寡核苷酸的所有核苷酸间连接是硫代磷酸酯。当涉及具体的 gapmer 寡核苷酸序列，如本文中提供的那些，将理解的是，在多种实施方案中，当连接是硫代磷酸酯键时，可以使用替代的连接，如本文公开的那些，例如，尤其是对于核苷酸类似物，如 LNA，单元之间的连接，可以使用磷酸酯（磷酸二酯）连接。同样，当涉及具体 gapmer 寡核苷酸序列，如本文提供的那些时，当 C 核苷酸残基被注释为 5′ 甲基修饰的胞嘧啶时，在多种实施方案中，存在于所述寡核苷酸中的一个以上的 C 核苷酸可以是未修饰的 C 残基。

[0239] 药物组合物

[0240] 本发明还提供药物组合物，所述药物组合物包含根据本发明的治疗活性剂（例如，反义寡核苷酸），连同一种以上药用赋形剂。这样的药物组合物可以任选地包含一种以上另外的治疗活性物质。

[0241] 尽管本文提供的药物组合物的描述主要针对适于凭处方 (ethical) 施用于人的药物组合物，技术人员将理解，这样的组合物通常适于施用于所有种类的动物。适于施用于人的药物组合物的改进从而使得该组合物适于施用于不同动物被很好的理解，并且普通的熟练兽医药理学家可以仅以普通的（如果有）实验设计和 / 或进行这种改进。

[0242] 本文描述的药物组合物的制剂可以通过已知的任何方法制备或之后在药理学领域中开发。通常，这样的制备方法包括将活性成分与稀释剂或赋形剂和 / 或一种以上其它附加成分关联，和随后，如果必要和 / 或需要，将产物成形和 / 或包装为所需的单剂量或多剂量单元的步骤。

[0243] 根据本发明的药物组合物可以以单个单位剂量，和 / 或多个单个单位剂量制备、包装和 / 或出售。如本文使用的，“单位剂量”是包含预定量的活性成分的药物组合物的个别量。活性成分的量通常等于将施用于受试者的活性成分的剂量和 / 或这样的剂量的方便的级分，如，例如，这样的剂量的二分之一或三分之一。

[0244] 根据本发明的药物组合物中的活性成分、药用赋形剂和 / 或任何其它成分的相对量将根据同一性、大小和 / 或治疗的受试者的病况并且进一步根据施用组合物的途径而改变。通过实施例的方式，组合物可以包含 0.1％至 100％ (w/w) 的活性成分。

[0245] 药物制剂可以另外包含药用赋形剂，如本文使用的，其包括任何和所有溶剂、分散介质、稀释剂或其它液体载体、分散或悬浮辅剂、表面活性剂、等渗剂、增稠剂或乳化剂、防腐剂、固体粘合剂、润滑剂等，如适于所需的特定剂型的。Remington’s The Science and Practice of Pharmacy， 第 21 版，A.R.Gennaro(Lippincott，Williams&Wilkins，Baltimore，MD,2006 ;通过引用并入本文）公开了用于配制药物组合物的各种赋形剂和用于其制备的已知技术。除了任何常规赋形剂介质与物质或其衍生物不相容（如通过产生任何不想要的生物效应或另外以有害的方式与药物组合物的其它一种或多种成分相互作

用），其用途被考虑在本发明的范围内。

[0246] 在一些实施方案中，药用赋形剂为至少 95％，至少 96％，至少 97％，至少 98％，至少 99％，或 100％纯度。在一些实施方案中，赋形剂被批准用于人和兽医使用。在一些实施方案中，赋形剂由美国食品和药品监督管理局批准。在一些实施方案中，赋形剂药用级的。在一些实施方案中，赋形剂满足美国药典 (USP)，欧洲药典 (EP)，英国药典，和 / 或国际药典的标准。

[0247] 用于制造药物组合物的药用赋形剂包括，但不限于，惰性稀释剂、分散剂和 / 或成粒剂、表面活性剂和 / 或乳化剂、崩解剂、粘合剂、防腐剂、缓冲剂、润滑剂和 / 或油。这样的赋形剂可以任选地包括在药物制剂中。根据配制者的判断，赋形剂如椰油和栓剂蜡、着色剂、包覆剂、甜味剂、调味剂和 / 或芳香剂可以存在于组合物中。

[0248] 药剂的配制和 / 或制造中的通常考虑可以，例如，在 Remington :The Science and Practice of Pharmacy 第 21 版，Lippincott Williams&Wilkins,2005（通过引用并入本文）中找到。

[0249] 在一些实施方案中，脂质体可以用于递送本文所述的反义寡核苷酸。如本文使用的，脂质体是人工制备的由脂双层组成的囊泡。脂质体可以通过破坏生物膜（如通过超声）来制备。脂质体可以包括天然磷脂，或具有表面活性剂性质的脂链（例如，卵磷脂酰乙醇胺）。脂质体设计可以使用靶向所需靶组织的表面配体。

[0250] 施用

[0251] 本发明提供向需要治疗的受试者施用有效量的本文所述的治疗活性剂（例如，反义寡核苷酸）的方法。

[0252] 本文所述的反义寡核苷酸可以通过给药途径施用，所述给药途径包括但不限于，静脉内、皮下、肌肉内、肠胃外、经皮或经粘膜（例如，口服或经鼻）。在一些实施方案中，本文所述的反义寡核苷酸可以通过静脉内给药施用。在一些实施方案中，本文所述的反义寡核苷酸可以通过皮下给药施用。在一些实施方案中，用于寡核苷酸的剂量给药方案可以在初始剂量给药方案后重复，实际上，所述剂量给药方案可以根据需要重复，从而治疗或防止疾病进展。在一些实施方案中，本文所述的反义寡核苷酸可以每日、一周两次、一周一次、每两周、每月、每两月一次、每三月一次、每四月一次、每六月一次，或以不定间隔施用。

[0253] 应用

[0254] 本发明的反义寡核苷酸可以用作研究试剂，用于例如，诊断、治疗和预防。

[0255] 在研究中，本发明的反义寡核苷酸可以用于特异地抑制细胞和实验动物中 BCL11A 蛋白的合成（通常通过降解或抑制 mRNA 并从而阻止蛋白形成），从而有利于靶的功能性分析或其作为用于治疗干预的靶的有用性的评价。

[0256] 在诊断中，本发明的反义寡核苷酸可以用于通过 RNA 印迹、原位杂交和类似技术检测和定量细胞和组织中 BCL11A 表达。

[0257] 对于治疗，怀疑患有疾病或病症，可以通过调节 BCL11A 的表达来治疗的动物或人，通过施用本发明的反义寡核苷酸来治疗。进一步提供的是通过施用治疗或预防有效量的一种以上本发明的反义寡核苷酸或组合物治疗怀疑患有或易于患有与 BCL11A 表达相关的疾病或病况的哺乳动物，如人的方法。反义寡核苷酸，本发明的缀合物或药物组合物通常以有效量施用。

[0258] 本发明的反义寡核苷酸适于制造用于治疗本文提及的病症的药物，或适于治疗本文提及的病症的方法。

[0259] 提供用于治疗本文提及的病症的方法，所述方法包括向需要其的患者施用本文所述的反义寡核苷酸，和 / 或缀合物，和 / 或药物组合物。

[0260] 本发明提供治疗贫血疾病、病症或病况的方法，所述方法包括向需要治疗的受试者施用根据本发明的寡核苷酸或本发明的药物组合物。

[0261] 在一个实施方案中，所述贫血疾病、病症或病况是镰状细胞病。

[0262] 在另一个实施方案中，所述贫血疾病、病症或病况是 β - 地中海贫血。

[0263] 当应用于治疗方法时，本发明寡核苷酸或本发明药物组合物的施用导致一种以上靶组织中 BCL11A 表达的减少。优选地，本发明的寡核苷酸或本发明的药物组合物的施用导致一种以上靶组织中 γ - 球蛋白表达的增加。本发明的寡核苷酸或本发明药物组合物的施用可以导致一种以上靶组织中胎儿血红蛋白的增加。优选地，所述靶组织选自骨髓、肝、肾、脾和 / 或浆细胞、外周血 B- 细胞、树突状细胞，红细胞祖先细胞、多能干细胞、胸腺、扁桃体上皮。

[0264] 治疗用途

[0265] 本文所述的 BCL11A 的反义寡核苷酸调节剂可以用于处理各种 BCL11A 相关疾病、病症和病况。

[0266] 镰状细胞病 (SCD)

[0267] 镰状细胞病 (Sickle Cell Disease)，或镰状细胞贫血 (sickle cell) 是遗传的基因病症，其特征为具有异常的、僵硬的、镰刀状红血细胞，其减少细胞的柔韧性。这由 β 球蛋白链基因的突变导致并且以常染色体隐性的方式以超显性显现。SCD 与各种严重的并发症相关，如减少的预期寿命，并且引起可以导致死亡的生理病况。然而，由于突变的遗传多态性，不是所有的遗传性血红蛋白变体都是有害的。

[0268] SCD 更常在热带和亚热带撒哈拉以南的区域的群体中报道。这些也是常观察到疟疾的区域。有趣的是，发现 SCD 的携带者 (即，具有一个拷贝的突变) 对感染更具抗性并且在感染时显出不那么严重的症状。

[0269] 本文所述的 BCL11A 的反义寡核苷酸调节剂可以用于治疗 SCD。术语，如本文使用的“治疗 (treat)”或“治疗 (treatment)”是指减轻一种以上与疾病相关的症状，防止或延迟疾病的一种以上症状的开始，和 / 或减小疾病的一种以上症状的严重度或频率。

[0270] 在一些实施方案中，治疗是指部分或完全缓解、减轻、免除、抑制 SCD 患者的一种以上症状，延迟 SCD 患者的一种以上症状的开始、减小 SCD 患者的一种以上症状的严重度和 / 或发病率，所述症状包括但不限于，贫血 ；眼睛变黄 ；皮肤苍白、冰冷和 / 或变黄 ；气短 ；肌肉无力 ；肠改变 (例如，粪便颜色改变) ；疲劳 ；头晕 ；昏倒 ；血管的改变 (例如，低血压) ；影响心脏的改变 (例如，心悸，心率快速，胸痛，心绞痛，心脏病发作)，和器官扩大 (例如，脾)。

[0271] 在一些实施方案中，治疗是指需要治疗的受试者中减少的贫血症状。在某些实施方案中，贫血症状的量可以以与治疗前或无治疗的对照 (例如，患类似疾病的或类似发展阶段，但未治疗的对照受试者的贫血症状量) 相比至少约 10％，20％，30％，40％，50％，60％，70％，80％，90％，95％，或更多减少。

[0272] 在一些实施方案中，治疗是指增加的 γ 球蛋白表达（例如，总表达，每周、每月、每两月、每六月等的百分数表达增加）。在多种实施方案中，增加的球蛋白表达考虑了 SCD 患者中 β 球蛋白的缺少或减少的表达。

[0273] 地中海贫血（α，β 和 δ）

[0274] 地中海贫血，如 SCD，是影响血液的遗传的基因病症。地中海贫血以常染色体隐性情况显示并且导致红血细胞的弱化和破坏。地中海贫血由影响身体制造血红蛋白的基因的突变或删除导致，所述血红蛋白是红血细胞内负责携带氧的蛋白。患有地中海贫血的个体特征为低血红蛋白生产并且比正常的具有更少的循环红血细胞，其导致轻度或重度贫血。地中海贫血起源于地中海区域。

[0275] 地中海贫血可以导致显著的并发症，包括肺炎、铁超负荷、骨畸形和心血管疾病。然而，如 SCD，已经观察到该疾病对于是该疾病的载体的那些人而言赋予一定程度的保护。

[0276] 血红蛋白由四条蛋白链组成，两条 α 球蛋白链和两条 β 球蛋白链，其以异二聚体排列。在人中，β 球蛋白链由染色体 11 上的单个基因编码，而 α 球蛋白链由染色体 16 上关联的两个基因编码。这产生这样的遗传状态，其中正常个体含有两个 β 链基因座和四个 α 链基因座。在患有地中海贫血的患者中，α 或 β 链中的突变，导致红血细胞的低和 / 或异常产生。因此，根据哪条链突变来将地中海贫血分类。α 和 β 地中海贫血在非洲、亚洲、希腊和意大利种族中常见。

[0277] α 地中海贫血（α 链中突变）涉及 HBA1 和 HBA2 基因，并且导致减少的 α 球蛋白产生。这形成了成人中 β 球蛋白的产生增加和婴儿中 γ 球蛋白产生增加的状况。β 球蛋白产生的增加导致四聚体的形成，该四聚体不稳定并且具有受损的与氧解离的能力。

[0278] β 地中海贫血（β 链中突变）涉及 HBB 基因，并且其导致的疾病严重度取决于突变。一些突变阻止 β 链的形成，其是疾病的最严重形式，同时其它的允许一些 β 链形成（尽管以减少的水平）。作为 β 链突变的结果，存在过量的 α 链产生，其在 α 地中海贫血的情况下不形成四聚体。备选地，过量的 α 链结合红血细胞膜并且导致膜损伤，并且如果 α 链聚集则可以是有毒的。

[0279] 尽管 δ 地中海贫血以低频率发生。类似地，它们由 δ 球蛋白链基因的突变造成并且导致这些链的异常产生。已经报道，成人血红蛋白的约 3％由 α 和 δ 链构成。

[0280] 本文所述的 BCL11A 的反义寡核苷酸调节剂可以用于治疗地中海贫血，例如，α，β 和 / 或 δ 地中海贫血。如本文使用的术语，“治疗 (treat)”或“治疗 (treatment)”，是指减轻一种以上与疾病相关的症状，防止或延迟疾病的一种以上症状的发生，和 / 或减小疾病的一种以上症状的严重度或频率。

[0281] 在一些实施方案中，治疗是指部分或完全缓解、减轻、免除、抑制地中海贫血患者中一种以上症状、延迟地中海贫血患者中一种以上症状的开始，减少地中海贫血患者中一种以上症状的严重度和 / 或发生率，所述症状包括但不限于肺炎、铁超负荷、骨畸形和心血管病。在一些实施方案中，治疗是指部分或完全缓解、减轻、免除、抑制地中海贫血患者的一种以上症状，延迟地中海贫血患者的一种以上症状的发生，减少地中海贫血患者的一种以上症状的严重度和 / 或发生率，包括但不限于，贫血；眼睛变黄；皮肤苍白、冰冷和 / 或变黄；气短；肌肉无力；肠改变（例如，粪便颜色改变）；疲劳；头晕；昏倒；血管的改变（例如，低血压）；影响心脏的改变（例如，心悸，心率快速，胸痛，心绞痛，心脏病发作），和器官扩大

（例如，脾）。

[0282] 在一些实施方案中，治疗是指需要治疗的受试者中减少的贫血症状。在某些实施方案中，贫血症状的量可以以与治疗前或无治疗的对照（例如，患类似疾病的或类似发展阶段，但未治疗的对照受试者的贫血症状量）相比至少约 10％，20％，30％，40％，50％，60％，70％，80％，90％，95％，或更多减少。

[0283] 在一些实施方案中，治疗是指增加的 γ 球蛋白表达（例如，总表达，每周、每月、每两月、每六月等的百分数表达增加）。在多种实施方案中，增加的 γ 球蛋白表达考虑了地中海贫血患者中 α，β 或 δ 球蛋白的缺少或减少的表达。

[0284] 通过参考以下实施例，本发明将被更全面理解。然而，它们不应该被理解为限制本发明的范围。所有引用通过引用并入本文。

实施例

[0285] 实施例 1. 靶向 BCL11A 的寡核苷酸的设计和合成

[0286] 本实施例说明了设计和合成可以有效下调 BCL11A 表达和活性的 LNA 寡核苷酸的典型方法。在该实施例中，主要的靶区域是 XL，L 和 S 同种型中的重叠区域（图 2）。

[0287] 基于人 BCL11A 的三种主要同种型（即，XL，L 或 S；表 3）的序列，在七个文库中设计和合成总共 401 种 LNA 寡核苷酸，得到不同特异性长度（例如，12-16 聚体）和 LNA 设计的寡核苷酸。

[0288] 表 3

[0289]

BCL11A	同种型	登录号	
	XL	NM_022893	SEQ ID NO：1
人	L	NM_018014	SEQ ID NO：2
	S	NM_138559	SEQ ID NO：3
	XL	NM_001242934	SEQ ID NO：4
鼠	L	NM_016707	SEQ ID NO：5
	S	NM_001159289	SEQ ID NO：6

[0290] 设计 LNA 单元的典型方法在 Wahlestedt，C. 等人 2000，PNAS91(10)：5633-5638 中描述，其通过引用并入本文。典型的 LNA 寡核苷酸在表 4 中显示。

[0291] 表 4

[0292]

Oligo #	BCL11A-XL 位置	长度	LNA 设计	序列 (5'-3')
1	597	14	3-8-3	$^{m}C_s^\circ T_s^\circ A_s^\circ t_s g_s t_s g_s t_s t_s c_s c_s T_s^\circ G_s^\circ T^\circ$
2	220	16	3-10-3	$G_s^\circ A_s^\circ G_s^\circ a_s c_s a_s t_s g_s g_s t_s g_s g_s g_s {}^{m}C_s^\circ T_s^\circ G^\circ$
3	429	16	3-10-3	$A_s^\circ T_s^\circ T_s^\circ g_s c_s a_s t_s t_s g_s t_s t_s t_s c_s {}^{m}Cs^\circ Gs^\circ T^\circ$
4	430	16	3-10-3	$^{m}C_s^\circ A_s^\circ T_s^\circ t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
5	430	15	3-9-3	$A_s^\circ T_s^\circ T_s^\circ g_s {}^{m}c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
6	430	15	3-9-3 ^{m}c	$A_s^\circ T_s^\circ T_s^\circ g_s c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
7	415	14	3-8-3 ^{m}c	$T_s^\circ T_s^\circ G_s^\circ t_s g_s c_s t_s {}^{m}c_s g_s a_s t_s A_s^\circ A_s^\circ A^\circ$
8	416	16	3-10-3	$^{m}C_s^\circ G_s^\circ T_s^\circ t_s t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s T_s^\circ A_s^\circ A^\circ$
9	419	14	3-8-3	$^{m}C_s^\circ {}^{m}C_s^\circ G_s^\circ t_s t_s t_s g_s t_s g_s c_s t_s {}^{m}C_s^\circ G_s^\circ A^\circ$
10	416	13	2-8-3 ^{m}c	$T_s^\circ T_s^\circ g_s t_s g_s c_s t_s {}^{m}c_s c_s a_s T_s^\circ A_s^\circ A^\circ$
11	420	16	3-10-3 ^{m}c	$T_s^\circ T_s^\circ T_s^\circ c_s {}^{m}c_s g_s t_s t_s t_s g_s t_s g_s c_s T_s^\circ {}^{m}C_s^\circ G^\circ$
12	430	16	3-10-3 ^{m}c	$^{m}C_s^\circ A_s^\circ T_s^\circ t_s g_s {}^{m}c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
13	430	16	2-11-3	$^{m}C_s^\circ A_s^\circ t_s^\circ t_s g_s c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
14	430	16	3-9-4	$^{m}C_s^\circ A_s^\circ T_s^\circ t_s g_s c_s a_s t_s t_s g_s t_s t_s T_s^\circ {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
15	430	16	4-9-3	$^{m}C_s^\circ A_s^\circ T_s^\circ T_s^\circ g_s c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
16	430	16	4-8-4	$^{m}C_s^\circ A_s^\circ T_s^\circ T_s^\circ g_s c_s a_s t_s t_s g_s t_s t_s T_s^\circ {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
17	430	16	3-8-5	$^{m}C_s^\circ A_s^\circ T_s^\circ t_s g_s c_s a_s t_s t_s g_s t_s T_s^\circ T_s^\circ {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
18	430	16	5-8-3	$^{m}C_s^\circ A_s^\circ T_s^\circ T_s^\circ G_s^\circ c_s a_s t_s t_s g_s t_s t_s t_s {}^{m}C_s^\circ {}^{m}C_s^\circ G^\circ$
19	415	16	3-10-3 ^{m}c	$G_s^\circ T_s^\circ T_s^\circ t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s t_s A_s^\circ A_s^\circ A^\circ$
20	415	15	3-9-3 ^{m}c	$T_s^\circ T_s^\circ T_s^\circ g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s t_s A_s^\circ A_s^\circ A^\circ$
21	416	14	3-8-3 ^{m}c	$T_s^\circ T_s^\circ T_s^\circ g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s T_s^\circ A_s^\circ A_s^\circ$
22	416	15	3-9-3 ^{m}c	$G_s^\circ T_s^\circ T_s^\circ t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s a_s T_s^\circ A_s^\circ A_s^\circ$
23	417	14	3-8-3 ^{m}c	$G_s^\circ T_s^\circ T_s^\circ t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s A_s^\circ T_s^\circ A_s^\circ$
24	417	16	3-10-3 ^{m}c	$^{m}C_s^\circ {}^{m}C_s^\circ G_s^\circ t_s t_s t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s A_s^\circ T_s^\circ A^\circ$
25	417	15	3-9-3	$^{m}C_s^\circ G_s^\circ T_s^\circ t_s t_s g_s t_s g_s c_s t_s {}^{m}c_s g_s A_s^\circ T_s^\circ A^\circ$
26	418	13	2-8-3	$G_s^\circ T_s^\circ t_s t_s g_s t_s g_s c_s t_s c_s G_s^\circ A_s^\circ T^\circ$
27	418	14	3-8-3	$^{m}C_s^\circ G_s^\circ T_s^\circ t_s t_s g_s t_s g_s c_s t_s c_s G_s^\circ A_s^\circ T^\circ$

[0293]

Oligo #	BCL11A-XL 位置	长度	LNA 设计	序列 (5'-3')
28	418	15	3-9-3	$^{m}C_s°\ ^{m}C_s°\ G_s°\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ c_s\ G_s°\ A_s°\ T°$
29	418	16	3-10-3	$T_s°\ ^{m}C_s°\ ^{m}C_s°\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ c_s\ G_s°\ A_s°\ T°$
30	419	13	2-8-3	$^{m}C_s°\ G_s°\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}C_s°\ G_s°\ A°$
31	419	16	3-10-3 ^{m}c	$T_s°\ T_s°\ ^{m}C_s°\ ^{m}c_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}C_s°\ G_s°\ A°$
32	419	15	3-9-3	$T_s°\ ^{m}C_so\ ^{m}C_s°\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}C_s°\ G_s°\ A°$
33	420	14	3-8-3	$T_s°\ ^{m}C_s°\ ^{m}C_s°\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ T_s°\ ^{m}C_s°\ G°$
34	420	15	3-9-3 ^{m}c	$T_s°\ T_s°\ ^{m}Cs°\ ^{m}c_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ T_s°\ ^{m}C_s°\ G°$
35	421	16	3-10-3 ^{m}c	$G_s°\ T_s°\ T_s°\ t_s\ c_s\ ^{m}c_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ ^{m}C_s°\ T_s°\ ^{m}C°$
36	421	15	3-9-3 ^{m}c	$T_s°\ T_s°\ T_s°\ c_s\ ^{m}c_s\ g_s\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ ^{m}C_s°\ T_s°\ ^{m}C°$
37	416	16	2-11-3	$^{m}C_s°\ G_s°\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ T_s°A_s°A°$
38	416	16	3-9-4	$^{m}C_s°\ G_s°\ T_s°\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ A_s°T_s°A_s°A°$
39	416	16	4-9-3	$^{m}C_s°\ G_s°\ T_s°\ T_s°\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ T_s°A_s°A°$
40	416	16	4-8-4	$^{m}C_s°\ G_s°\ T_s°\ T_s°\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ A_s°\ T_s°A_s°A°$
41	416	16	3-8-5	$^{m}C_s°\ G_s°\ T_s°\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ G_s°\ A_s°\ T_s°A_s°A°$
42	416	13	5-8-3	$^{m}C_s°\ G_s°\ T_s°\ T_s°\ T_s°\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ T_s°A_s°A°$
43	417	15	2-10-3	$^{m}C_s°\ G_s°\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ A_s°\ T_s°\ A°$
44	417	15	3-10-2	$^{m}C_s°\ G_s°\ T_s°\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ T_s°\ A°$
45	417	15	4-9-2	$^{m}C_s°\ G_s°\ T_s°\ T_s°\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ a_s\ T_s°\ A°$
46	417	15	2-9-4	$^{m}C_s°\ G_s°\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ G_s°\ A_s°\ T_s°\ A°$
47	417	15	4-8-3	$^{m}C_s°\ G_s°\ T_s°\ T_s°\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ A_s°\ T_s°\ A°$
48	417	15	3-8-4	$^{m}C_s°\ G_s°\ T_s°\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ G_s°\ A_s°\ T_s°\ A°$
49	418	14	3-8-3 ^{m}c	$^{m}C_s°\ G_s°\ T_s°\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ G_s°\ A_s°\ T°$
50	418	14	2-10-2	$^{m}C_s°\ G_s°\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ A_s°\ T°$
51	418	13	2-9-3	$^{m}C_s°\ G_s°\ t_s°\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ c_s\ G_s°\ A_s°\ T°$
52	418	13	3-9-2	$^{m}C_s°\ G_s°\ T_s°\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ A_s°\ T°$
53	418	14	4-8-2	$^{m}C_s°\ G_s°\ T_s°\ T_s°\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}c_s\ g_s\ A_s°\ T°$
54	418	14	2-8-4	$^{m}C_s°\ G_s°\ t_s\ t_s\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}C_s°\ G_s°\ A_s°\ T°$
55	418	15	4-7-4	$^{m}C_s°\ G_s°\ T_s°\ T_s°\ t_s\ g_s\ t_s\ g_s\ c_s\ t_s\ ^{m}C_s°\ G_s°\ A_s°\ T°$

[0294] ^{m}C 表示具有 5- 甲基胞嘧啶 -1- 基碱基的核苷酸单体；下标“s”表示硫代磷酸酯键；大写 / 加粗碱基表示锁定核酸；下标“o”表示氧基 -LNA。

[0295] 实施例 2.BCL11A- 特异寡核苷酸的体外筛选和 IC_{50}确定

[0296] 寡核苷酸对 BCL11A 核酸表达的影响可以在多种细胞类型中的任一种中测试，条件是靶核酸以可测量水平存在。BCL11A 可以内源表达或通过瞬时或稳定转染核酸来表达。BCL11A 核酸的表达水平可以使用例如，RNA 印迹、定量 PCR、核糖核酸酶保护测定常规确定。在该实施例中，根据实施例 1 合成的，选择性靶向 BCL11A 的寡核苷酸在人 REH 细胞上测试

并且测量BCL11A mRNA表达。可以常规使用其它细胞类型，条件是靶在选择的细胞类型中表达。将细胞培养在如下文所述的适当培养基中，并维持在37℃，95-98％湿度和5％ CO_2下。当在缺氧或氧缺乏下培养时，将O_2水平分别保持在1-2％或0-0.5％。每周将细胞常规传代2-3次。

[0297] 简言之，使用人REH细胞将401种寡核苷酸用于三天哺乳动物细胞培养实验，用于确定对BCL11A mRNA表达的影响。使用剥裸(gymnosis)递送技术将反义寡核苷酸以5和25μM添加到细胞中而无任何其它试剂或摄取促进剂(Stein，C.A.等人2010，Nucleic Acids Research 38(1)：e3)。通过定量实时RT-PCR(RT-qPCR)测量BCL11A mRNA。根据实施例1制备的反义寡核苷酸抑制BCL11A的典型结果列在图3中。

[0298] 如图3中所示，通过靶向BCL11A同种型XL中的不同位置，尤其是，XL，L和S同种型间的重叠位置(参见图2)，根据实施例1制备的反义寡核苷酸能够抑制BCL11A mRNA的表达。

[0299] 在其它实验中，对于选择的反义寡核苷酸，不同浓度(范围从0.0064至20μM)的IC_{50}值和对BCL11A mRNA表达的效果使用上文所述人REH细胞确定。典型的结果在表5(IC_{50})和图4(BCL11AmRNA)中显示。Oligo#56：不靶向BCL11A mRNA的反义寡核苷酸。

[0300] 表5

[0301]

Oligo #	IC_{50} (μM)
4	1.5
7	1.5
8	0.3
9	0.3
10	0.8
11	0.8
19	1.0
20	6.0

[0302]

Oligo #	IC_{50} (μM)
21	0.8
22	0.6
23	0.5
24	0.7
25	0.3
26	0.6
27	0.9
28	0.5
29	0.4
30	0.7
31	0.4
32	0.3
33	0.5
34	1.2
35	1.6
36	0.8

[0303] 在类似实验中,对于从 oligos 4 和 5 设计的不同寡核苷酸,不同浓度(范围从 0.25 至 60μM)IC_{50}值和对 BCL11A mRNA 表达的效果使用上文所述人 REH 细胞确定。典型的结果显示在表 6(IC_{50})和图 5(BCL11A mRNA)中。Oligo#56:不靶向 BCL11A mRNA 的反义寡核苷酸;模拟物(Mock):无反义寡核苷酸添加至细胞中。

[0304] 表 6

[0305]

Oligo #	IC_{50} (μM)
4	3.9
12	6.2
13	5.1
14	2.0

[0306]

Oligo #	IC_{50} (μM)
15	7.1
16	12.6
17	5.1
18	74.8
5	3.2
6	3.4

[0307] 在另一实验中，对于选择的寡核苷酸，不同浓度（范围从 0.25 至 60 μM）的 IC_{50} 值和对 BCL11A mRNA 的不同同种型的表达的影响使用上文所述人 REH 细胞确定。典型的结果显示在表 7（IC_{50}）和图 6（同种型 BCL11A mRNA）中。Oligo#56：不靶向 BCL11AmRNA 的反义寡核苷酸；模拟物：无反义寡核苷酸添加至细胞中。

[0308] 表 7

[0309]

Oligo #	IC_{50} (μM)		
	XL	L	S
3-03	2.3	2.4	2.0
4-03	1.9	1.4	1.3
1	1.6	0.9	1.2

[0310] 在另一类似实验中，对于选择的寡核苷酸，不同浓度（范围从 0.08-20 μM）的 IC_{50} 值和对 BCL11A mRNA 表达的影响，使用小鼠 MPC-11 细胞，使用与上文对于人 REH 细胞所述的类似的实验条件确定。典型的结果显示在表 8（IC_{50}）和图 7（BCL11A mRNA）中。Oligo#56：不靶向 BCL11A mRNA 的反义寡核苷酸；模拟物：无反义寡核苷酸添加至细胞中。

[0311] 表 8

[0312]

Oligo #	IC50 (μM) BCL11A-All
4	0.8
14	1.0
8	0.2

[0313]

Oligo #	IC50 (μM) BCL11A-All
25	0.3
27	0.8
5	0.7
6	1.4

[0314] 在另一实验中，对于从 oligos 8,25 和 27 设计的不同寡核苷酸的 IC_{50} 值使用上文所述人 REH 细胞确定。通常，使用范围从 0.0064 至 20 μM 的六点 5x 稀释确定 IC_{50} 值。典型的结果显示在表 9 中。

[0315] 表 9

[0316]

Oligo #	设计	IC_{50} (μM)
8	3-10-3	1.0
37	2-11-3	2.0
38	3-9-4	1.3
39	4-9-3	0.6
40	4-8-4	1.1
41	3-8-5	0.9
42	5-8-3	1.8
25	3-9-3	0.7
43	2-10-3	0.9
44	3-10-2	0.3
45	4-9-2	0.3
46	2-9-4	0.4
47	4-8-3	0.3
48	3-8-4	0.6
27	3-8-3	0.5
49	3-8-3^{m}c	0.4
50	2-10-2	0.8
51	2-9-3	0.4

[0317]

Oligo #	设计	IC_{50} (μM)
52	3-9-2	0.5
53	4-8-2	0.4
54	2-8-4	1.8
55	4-7-4	11.4

[0318] 综合考虑，这些数据显示，本发明提供的反义寡核苷酸，如实施例 1 中描述的那些可以以范围从 0.25μM-60μM 的典型 IC_{50}有效抑制 BCL11A。此外，本发明提供的选择的反义寡核苷酸可以以范围从 0.10μM-1.5μM 的典型 IC_{50}有效抑制小鼠 BCL11A。

[0319] 实施例 3. 寡核苷酸的体内耐受

[0320] 使用 NMRI 小鼠对在前实施例中所述的寡核苷酸测试其体内耐受度。

[0321] 简言之，将雌性 NMRI 小鼠（n ＝ 5/ 组）在 0,3,7,10 和 14 天用盐水（对照）或选择的 LNA 寡核苷酸 (15mg/kg) 通过静脉内施用剂量给药。最后一次剂量给药后 48 小时处死小鼠。对于各组中动物记录以下参数 :体重（第 0 天，第 5,6 或 7 天，和第 10,13,14 或 16 天），处死时器官（肝、肾和脾）重，血清丙氨酸氨基转移酶 (ALT) 活性，和全骨髓和脾中的 BCL11A mRNA 表达。典型的结果显示在图 8 中。

[0322] 如图 8 中所示，选择的反义寡核苷酸抑制小鼠中 BCL11A mRNA 表达的能力在收获的骨髓和脾中确认。例如，在骨髓中 oligos 8 和 25 显示出约 40％的 BCL11A mRNA 的减少，而在脾中 oligos 8 和 20 显示约相同的 BCL11A mRNA 减少。通常，抑制骨髓中 BCL11A mRNA 表达范围平均从约 10-50％，而抑制脾中 BCL11AmRNA 表达范围平均从约 10-40％。通常，治疗的动物的体重和器官重不受影响。

[0323] 综合考虑，这些数据表明，本发明提供的反义寡核苷酸如实施例 1 中描述的那些是良好耐受的并且可以有效抑制各种靶组织体内 BCL11A mRNA 表达，包括但不限于，骨髓，脾。

[0324] 在类似实验中，如上文所述，使用 NMRI 小鼠对选择的反义寡核苷酸测试其体内耐受度。通常，施用靶向 BCL11A 的选择的反义寡核苷酸的小鼠的体重和器官重通常不受影响。施用选择的反义寡核苷酸的小鼠的血清 ALT 水平呈现与盐水组相比相似的结果。

[0325] 在类似的实验中，使用 Wistar 大鼠对选择的反义寡核苷酸测试体内耐受度。简言之，每周（第 0,7,14,21 和 28 天）经皮下施用对雄性 Wistar 大鼠 (n ＝ 5/ 组）以盐水（对照）或选择的反义寡核苷酸 (25mg/kg) 剂量给药一次。在第 30 天处死大鼠。对于各组的动物记录以下参数 :研究期间体重，处死时的器官（肝，肾和脾）重，肝和肾组织病理，和临床血清化学（丙氨酸氨基转移酶，天冬氨酸氨基转移酶，碱性磷酸酶，胆红素，尿素和肌酸酐）。

[0326] 施用靶向 BCL11A 的选择的反义寡核苷酸的 Wistar 大鼠中的器官（例如，肝，肾和脾）重通常不受影响。此外，对各种临床血清化学标记（例如，ALT,AST,ALP,胆红素，尿素，肌酸）的测量呈现与对照组相比类似的结果。

[0327] 综合考虑，这些数据表明，本发明提供的反义寡核苷酸如实施例 1 中描述的那些通常是安全和良好耐受的。

[0328] 实施例 4. 野生型和 β-YAC 转基因小鼠中的体内效力

[0329] 野生型和对人 β- 球蛋白基因（β-YAC) 为转基因的小鼠用于确定根据在前实施例制备的多种 LNA 寡核苷酸的体内效力。

[0330] 简言之，根据以下两个时间安排之一经皮下途径向野生型和 β-YAC 转基因小鼠剂量给药以 (25 或 15mg/kg) 选择的反义寡核苷酸 :(1) 在第 0,3,6,13,20 和 27 天剂量给药，指定第 29 天尸检（处死）和 (2) 在第 0,3,6,13,20,27,34,41,48，和 55 天剂量给药，指定第 57 天尸检（处死）。对于两个剂量给药时间表，在第 0 天和尸检（分别是第 29 或 57)

之前采血。本研究中使用的终点包括靶组织（例如，骨髓）中的 BCL11A 敲除以及寡核苷酸的血液化学和生物分布。

[0331] 与实施例 3 显示的结果一致，对于野生型和转基因小鼠，直至用各种反义寡核苷酸治疗的第 58 天，也没有对体重的副作用。在治疗组间在 AST 水平未观察到显著差异。

[0332] 野生型小鼠骨髓中 BCL11A mRNA 表达的敲除的典型结果在图 9（施用后四周）和 10（施用后八周）中陈述。β-YAC 转基因小鼠中 BCL11AmRNA 表达敲除的典型结果在图 11 中陈述。施用后八周 β-YAC 转基因小鼠的 $Ter119^+$ 和 $CD19^+$ 骨髓细胞的 BCL11A mRNA 表达敲除的典型结果在图 12 中陈述。

[0333] 如以上结果显示的，BCL11AmRNA 表达的敲除在第八周更强，然而，在测试的寡核苷酸中，候选 oligo 8 呈现更弱的 BCL11A 的减少。对于候选 oligo 4，当以 15 或 25mg/kg 剂量给药时，未观察到 BCL11A 表达敲除的差异。此外，对于野生型或转基因小鼠，当施用八周时，对于选择的寡核苷酸未观察到 BCL11A 表达敲除的差异。

[0334] 综合考虑，该实施例表明，本发明提供的反义寡核苷酸可以有效抑制各个靶组织中体内 BCL11A 表达，包括但不限于骨髓，脾。

[0335] 实施例 5. 非人灵长类中的药理学研究

[0336] 进行使用特异靶向一种以上同种型的 BCL11A 的典型反义寡核苷酸的药理学研究以进一步证明体内安全和效力。

[0337] 简言之，研究包括六至十六周的时期，在此期间，经皮下注射给雌性食蟹猴（年龄二至四岁并且体重 2.5-4kg）以 20mg/kg 每六周的剂量或以 10mg/kg 和 20mg/kg 每十二周的剂量施用选择的 BCL11A- 特异的 LNA 反义寡核苷酸或对照（盐水）。在最后一次给药（大约第七周或第 17 周，取决于上述研究的持续时间）后大约七天处死动物。随研究进展，由于研究的预试阶段期间的重复取血以及刺激红细胞生成的整个剂量给药期，治疗组中的一半造成中度贫血。实验设计在表 10 中陈述。

[0338] 表 10

[0339]

组	剂量水平 (mg/kg)	动物的数量		
		中期处死 (第 7 周)	最终处死 (第 17 周)	执行静脉切开手术
对照	0	4	4	-
低剂量	10	-	4	-
高剂量	20	4	4	-
对照	0	4	4	+
低剂量	10	-	4	+
高剂量	20	4	4	+

[0340] 每周皮下施用（6 或 16 剂量）

[0341] 最后一次剂量给药（第 7 周或第 17 周）后七天处死

[0342] 药效学生物标记：对外周血（每两周）和骨髓（根据研究设计，在尸检时，第七周

和第 17 周）取样并用于通过 ELISA 测定确定 BCL11A 和 γ-球蛋白 mRNA 表达，以及胎儿血红蛋白 (HbF) 和 γ-球蛋白水平。还使用高效液相色谱 (HPLC) 分析骨髓中 HbF 水平。使用 Kleihauer 方法测量 F- 细胞。在第七周通过活骨髓抽吸从肱骨取样骨髓。使用多种方法从肱骨和股骨取样第 17 周取样的骨髓。通过活骨髓抽吸进行在第 17 周从肱骨的取样。在第 17 周从股骨的取样通过用缓冲液冲骨髓接着离心和分析得到的沉淀来进行。在第 17 周从股骨的取样也从完全冷冻股骨进行。

[0343] 血液学分析包括每两周计数从样品测量的红血细胞、网织红细胞和总血红蛋白。

[0344] 对外周血进行取样用于在仅按照 16 周研究设计的周组中，在第一次和第 12 周剂量给药（仅第 17 周组）LNA 反义寡核苷酸后两、四、八 24 和 48 小时的药代动力学分析。

[0345] 在尸检时（第 7 周和第 17 周），对肝，肾和骨髓进行取样，用于分析和重量测量。在尸检时还进行临床化学分析。

[0346] 来自外周血的典型的总血红蛋白测量在图 13 中显示。外周血中网织红细胞的典型的百分数在图 14 中显示。通过 RT-qPCR 在第七周对肱骨骨髓中 BCL11AmRNA 的典型的测量在图 15 中显示。通过 RT-qPCR 在第七周对肱骨骨髓中 γ-球蛋白和 β-球蛋白 mRNA 典型的测量在图 16 中显示。通过 RT-qPCR 在第 17 周对肱骨（顶部）和股骨（底部）骨髓中 BCL11A mRNA 的典型的测量在图 17 中显示。通过 RT-qPCR 在第 17 周对肱骨（顶部）和股骨（底部）骨髓中 γ-球蛋白 mRNA 的典型的测量在图 18 中显示。通过 RT-qPCR 在第 17 周对肱骨骨髓中 γ-球蛋白和 β-球蛋白 mRNA 的典型的测量在图 19 中显示。通过 RT-qPCR 在第 17 周对股骨骨髓中 γ-球蛋白和 β-球蛋白 mRNA 的典型的测量在图 20 中显示。通过 RT-qPCR 在第 17 周对肱骨骨髓中 BCL11A（顶部）和 γ-球蛋白（底部）mRNA 的典型的平均测量在图 21 中显示。通过 RT-qPCR 在第 17 周对股骨骨髓中 BCL11A（顶部）和 γ-球蛋白（底部）mRNA 的典型的平均测量在图 22 中显示。

[0347] 对于选择的执行静脉切开手术的动物，骨髓中 F- 细胞的分数 (‰) 的典型的测量（全尺寸（左）和按比例放大（右））在图 23 中显示。对于选择的执行静脉切开手术的动物，外周血中 F- 细胞的分数 (‰) 的典型的测量（全尺寸（左）和按比例放大（右））在图 24 中显示。

[0348] 对照（顶部），10mg/kg（中间），和 20mg/kg（底部）剂量组中外周血中 γ-球蛋白的典型的测量在图 25 中显示。对于对照，10mg/kg 和 20mg/kg 剂量组，外周血中 γ-球蛋白（作为对照的百分数）在 γ-球蛋白峰（“峰 1”或“峰 2”）的各个时间点的典型的测量分别在图 26，27 和 28 中显示。

[0349] 如以上结果所显示的，与肱骨相比，在来自股骨的骨髓样品中观察到约高两倍的 BCL11A 表达。对于 γ-球蛋白表达，与股骨相比，在来自肱骨的骨髓样品中观察到高两倍至三倍的表达。在如下文所述的某些特定动物中观察到最大的不同。

[0350] 对于骨髓中 F- 细胞的测量，与对照动物中的约 0.2‰相比，在第 17 周动物 I 呈现约 10‰ F- 细胞。对于外周血中 F- 细胞的测量，与对照动物中的约 0.3‰相比，在第 17 周动物 I 呈现约 8‰。此外，在来自获白外周血的样品的测量中，该动物中的 F- 细胞在约第 15 周开始增加。

[0351] 对于外周血中 γ-球蛋白的测量，对于动物 I 在第 15 周观察到增加，此外在 10mg/kg 剂量组中在第 17 周观察到增加。以类似的方式，动物 Q 在 20mg/kg 剂量组在第 17 周呈

现增加。

[0352] 在未执行静脉切开手术组中，与对照组相比未观察到BCL11A mRNA表达的减少。同样，对于任何动物都未观察到F-细胞或HbF（γ-球蛋白）的增加。

[0353] 总之，本实施例中所述的实验结果表明与对照动物相比，在动物I中以在骨髓（肱骨和股骨）中大于85％的BCL11A mRNA敲除有效靶向结合，并且与对照动物相比，在动物Q中以在骨髓（肱骨和股骨）中约60％的BCL11A mRNA敲除有效靶向结合。此外，记录到与对照动物相比在动物I骨髓中大于80-倍的γ-球蛋白mRNA表达引入，和与对照相比，动物I外周血中七倍的γ-球蛋白增加。与对照动物相比，动物Q呈现出外周血中γ-球蛋白约三倍的增加。与对照动物相比，动物I还呈现骨髓和外周血中F-细胞的增加。

[0354] 综合考虑，本实施例证明，与载体对照相比，本发明提供的反义寡核苷酸可以有效抑制各个靶组织体内BCL11A表达并且以至少两倍或更高倍数增加外周血中的γ-球蛋白。

[0355] 实施例6. 体内药代动力学

[0356] 本实施例确定选择的根据之前的实施例制备的LNA寡核苷酸的体内药代动力学。

[0357] 简言之，通过皮下途径给予野生型小鼠单剂量(20mg/kg)的选择的反义寡核苷酸。在直至28天的多个时间点进行血浆，肝，肾和骨髓的取样。确定对于各个取样的组织的药代动力学特性。典型的结果显示在图29-31中。

[0358] 结果表明反义寡核苷酸快速分散和可观察地分散到所有取样的组织中，包括骨髓。在皮下施用后十分钟C_{max}为约21μg/mL。肝，肾和血浆$t_{1/2\beta}$为约十天。在骨髓中，$t_{1/2\beta}$为约三天。

[0359] 从该药代动力学研究，对于暴露于反义寡核苷酸的骨髓确定预测模型（图32）。

[0360] 综合考虑，该实施例表明，在施用（例如，皮下）时，本发明提供的反义寡核苷酸被多个组织有效和安全吸收。此外，本发明提供的反义寡核苷酸分散到多个靶组织，包括骨髓，而没有任何副作用。

[0361] 这样描述了本发明的至少一个实施方案的多个方面，将理解，对于本领域技术人员而言，各种改变、改进和改善将容易发生。这种改变、改进和改善意在为本公开的部分，并且意在本发明的精神和范围内。因此，在前说明书和附图仅是通过实施例的方式并且本发明以接着的权利要求详细描述。

[0362] 在权利要求中使用序数术语如“第一”、“第二”、“第三”等来修饰权利要求要素，其本身不隐含一个权利要求要素相对另一个的任何优先、优选或顺序或进行方法的行动的临时顺序，而是仅用作标签来将具有某名称的权利要求要素与具有相同名称的另一个要素相区分，（但对于序号术语的使用）以区分权利要求要素。

[0363] 冠词“一个”（“a”和“an”）用在本文中在说明书和权利要求书中，除非清楚地相反指明，应该理解为包括复数引用物。如果一个、多于一个或所有的组成员存在、用于或以除此之外的方式与给定的产物或方法相关，则认为在一个或多个组成员之间包括“或”的权利要求或描述是满足的，除非相反指明或者从上下文中另外清楚可见。本发明包括其中仅一个组成员存在、用于或以除此之外的方式与给定的产物或方法相关的实施方案。本发明还包括其中多于一个或整个组成员存在、用于或以除此之外的方式与给定的产物或方法相关的实施方案。此外，应该理解，本发明包括所有的变化、组合和改变，其中来自一个或多个所列的权利要求的一个或多个限制、要素、从句、描述性术语等被引入到从属于该主权利要

求的另一项权利要求中（或者，相关的、任意其他的权利要求），除非另外指明或者除非本领域普通技术人员明显看出将引起矛盾或不一致。在存在列举的要素（例如，在马库什组或类似的形式中时），应该理解，也公开了该要素的每个亚组，并且可以从该组中去除任意一个或多个要素。应该理解，通常，当提及本发明或本发明的方面包括具体的要素、特征等时，本发明的某些实施方案或本发明的方法由所述要素、特征等组成或基本上由所述要素、特征等组成。为了简化的目的，这些实施方案在每种情形中在本文中没有具体地以如此多的语言进行描述。还应该理解，本发明的任意实施方案或方面可以明确地被权利要求排除在外，而不管说明书中是否引用该具体的排除。本文引用的，描述本发明的背景和就其实施提供其它详细内容的出版物、网页和其它参考材料在此通过引用并入本文。

[0001]

序列表

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aagagcagca gtatctatgt ctgtgctccc tggaagttgt acttcatttc ttttccatac 4320

actgtgtgct atttgtgtta acattgaaga ggatttgttt ttattttgtt ttattctttc 4380

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gagtataaaa taaacctgct cagataggac aattaagtgc actgtacagt tttcccagtt 4560

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atgacgattg tttatcaacg tcatctagag gaatttgccc caaacaggaa cacatagcag 720

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cccagcactt aagcaaacgg gaattctcgc ccgaacctct tgaagccatt cttacagatg 420

atgaaccaga ccatggcccg ttgggagctc cagaagggga ccacgacctt ctcacctgtg 480

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[0035]

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[0036]

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[0037]

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[0038]

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[0039]

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[0040]

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[0041]

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<400> 65
cattgcattg tttccg 16

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[0042]

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cgtttgtgct cga 13

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tttgtgctcg ataa 14

<210> 71
<211> 13
<212> DNA
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<400> 71
ttgtgctcca taa 13

<210> 72
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[0043]

<210> 73
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cgtttgtgct cgata 15

<210> 75
<211> 19
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attgtttccg tttgtgctc 19

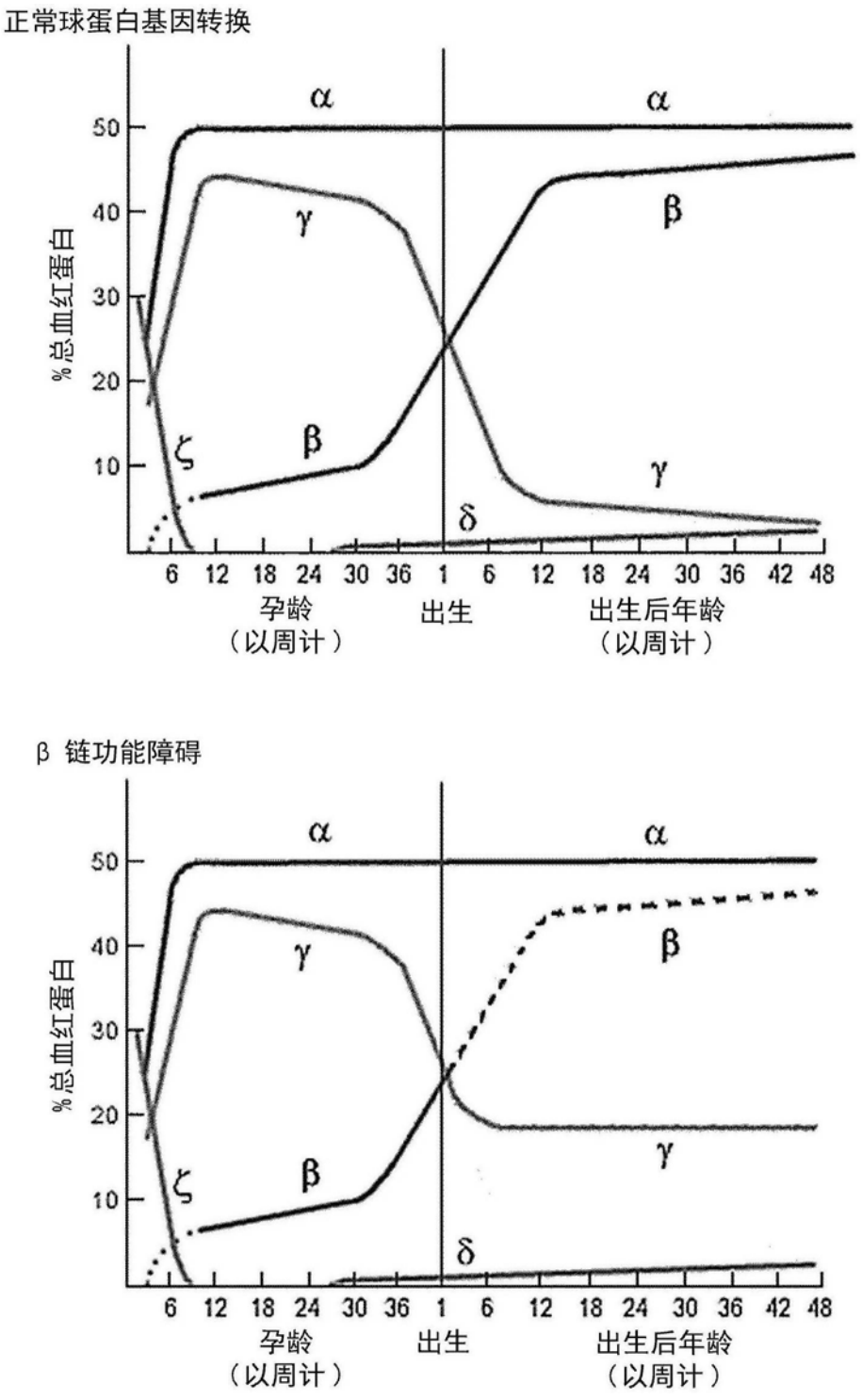

正常球蛋白基因转换
%总血红蛋白
α
α
γ
β
ζ
β
γ
δ
6 12 18 24 30 36 1 6 12 18 24 30 36 42 48
孕龄
（以周计）
出生
出生后年龄
（以周计）
β 链功能障碍
%总血红蛋白
α
α
γ
β
ζ
β
γ
δ
6 12 18 24 30 36 1 6 12 18 24 30 36 42 48
孕龄
（以周计）
出生
出生后年龄
（以周计）

图 1

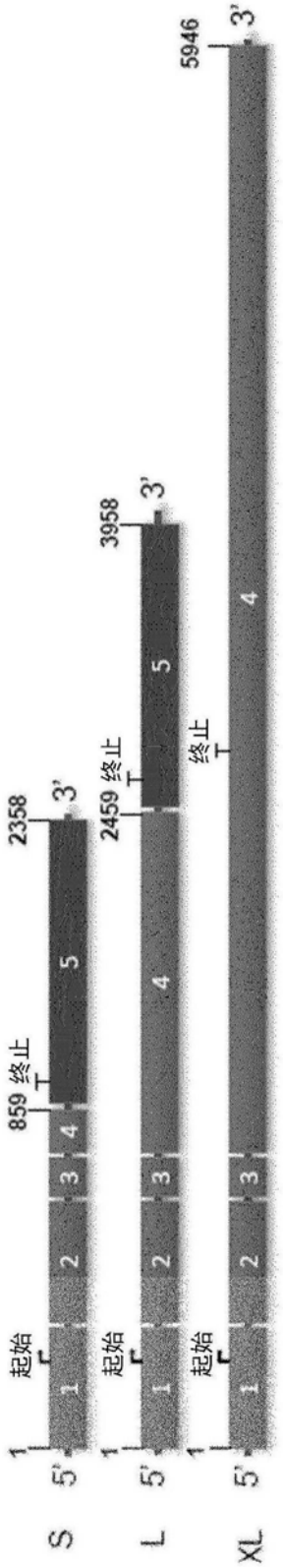

5946
3'
3958
2459
终止
2358
859
终止
起始
5'
S
L
XL

图 2

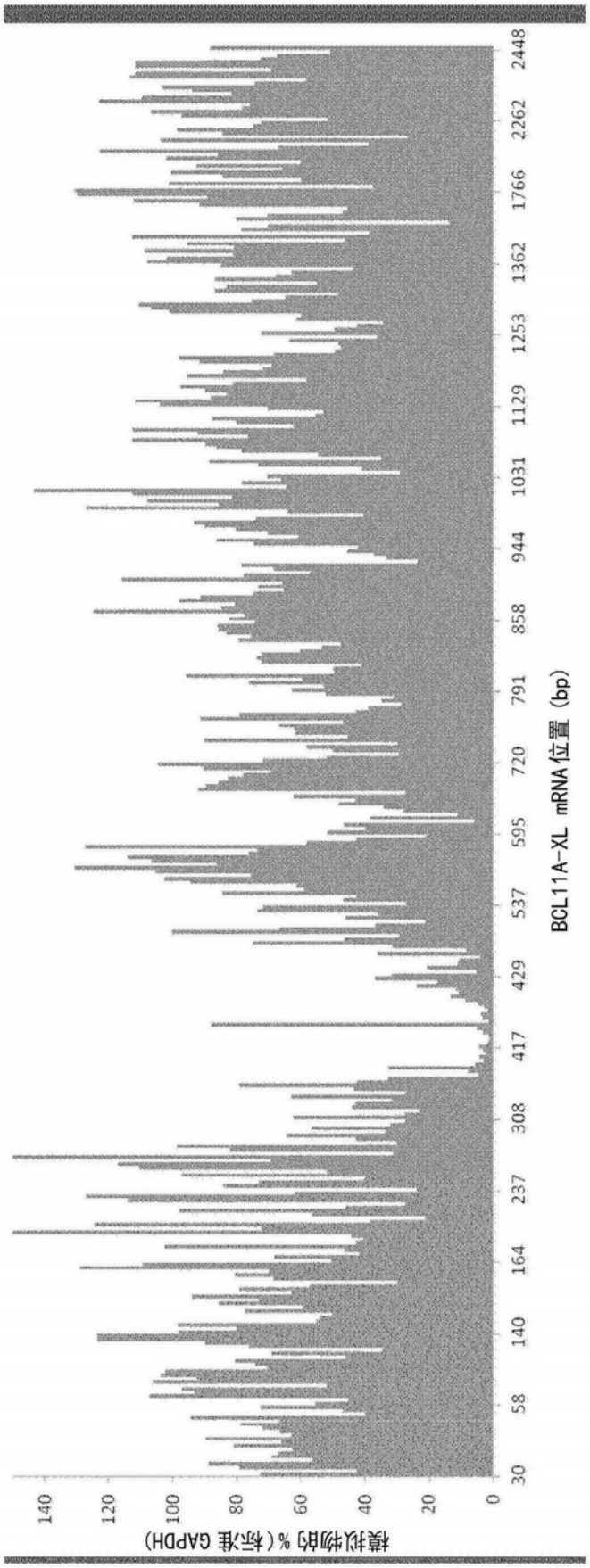

模拟物的 %（标准 GAPDH）
0
20
40
60
80
100
120
140
30
58
140
164
237
308
417
429
537
595
720
791
858
944
1031
1129
1253
1362
1766
2262
2448
BCL11A-XL mRNA位置（bp）

图 3

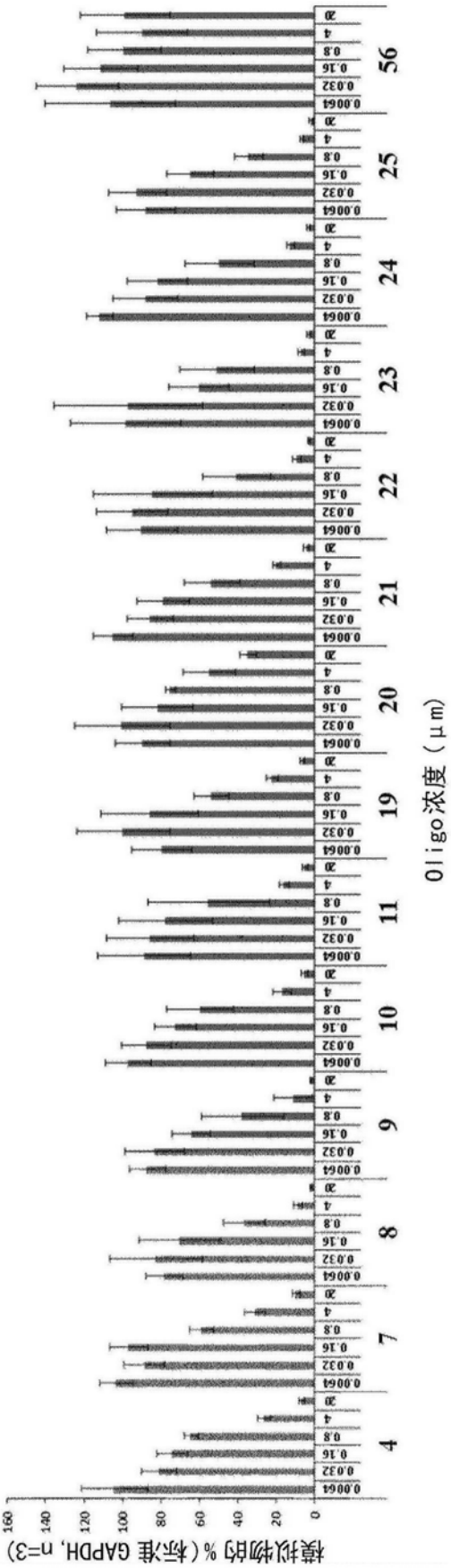

模拟物的 %（标准 GAPDH，n=3）
0
20
40
60
80
100
120
140
160
4
7
8
9
10
11
19
20
21
22
23
24
25
56
Oligo浓度（μm）

图 4

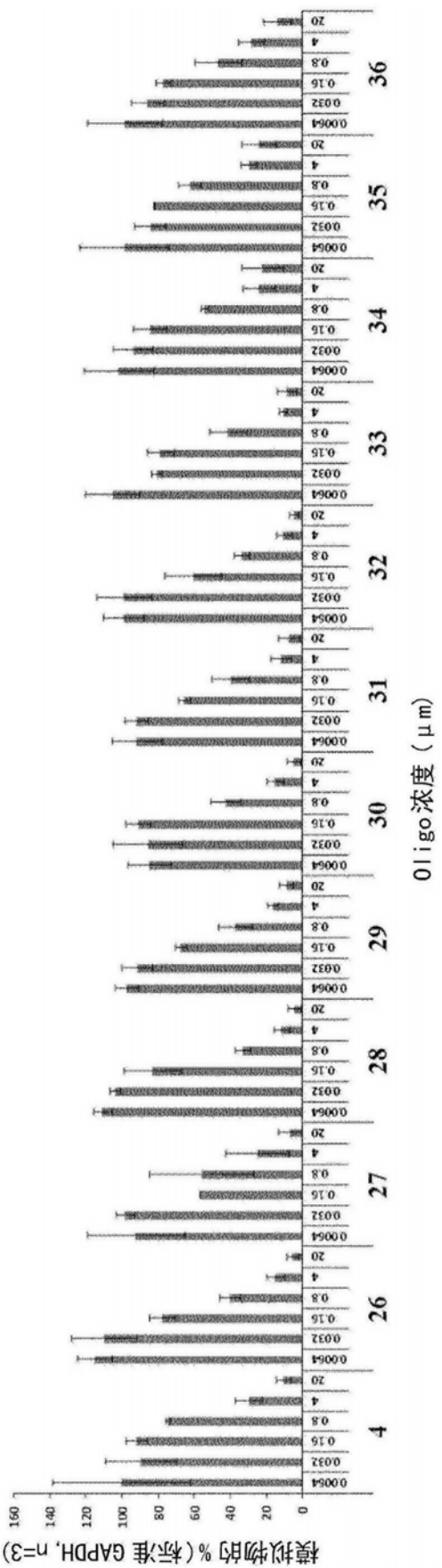

模拟物的 %（标准 GAPDH，n=3）
160
140
120
100
80
60
40
20
0
0.0064
0.032
0.16
0.8
4
20
4
26
27
28
29
30
31
32
33
34
35
36
Oligo浓度（μm）

图 4（续）

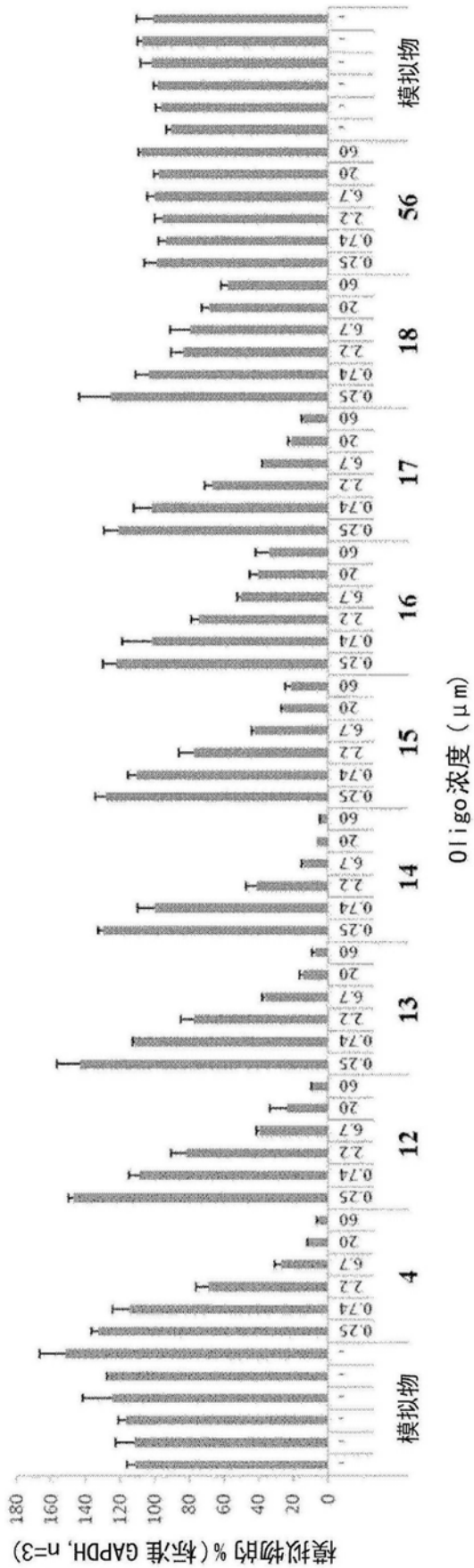

模拟物的 %（标准 GAPDH，n=3）
180
160
140
120
100
80
60
40
20
0
模拟物
0.25
0.74
2.2
6.7
20
60
4
12
13
14
15
16
17
18
56
Oligo浓度（μm）

图 5

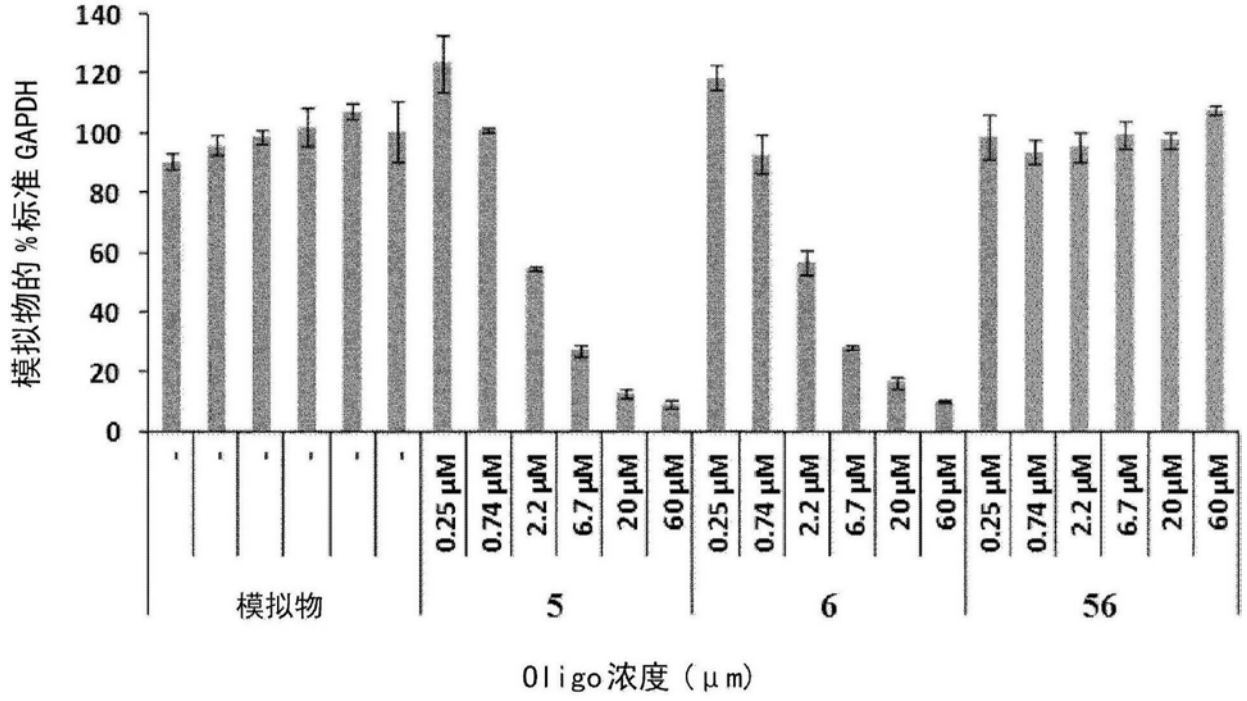

模拟物的％标准 GAPDH
140
120
100
80
60
40
20
0
-
-
-
-
-
-
0.25 μM
0.74 μM
2.2 μM
6.7 μM
20 μM
60 μM
0.25 μM
0.74 μM
2.2 μM
6.7 μM
20 μM
60 μM
0.25 μM
0.74 μM
2.2 μM
6.7 μM
20 μM
60 μM
模拟物
5
6
56
Oligo浓度（μm）

图 5（续）

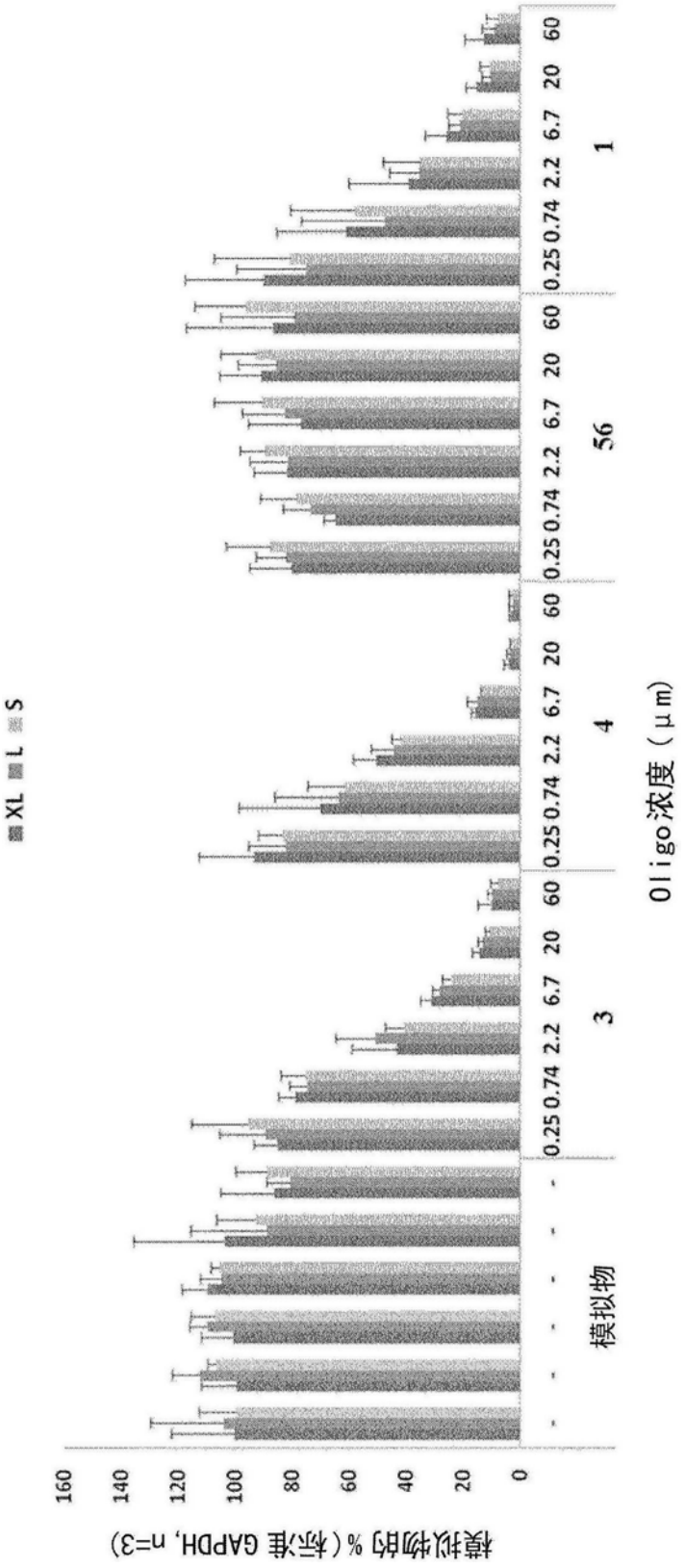

模拟物的%(标准 GAPDH, n=3)
160
140
120
100
80
60
40
20
0
XL
L
S
模拟物
3
4
56
1
Oligo浓度(μm)
-
0.25
0.74
2.2
6.7
20
60

图 6

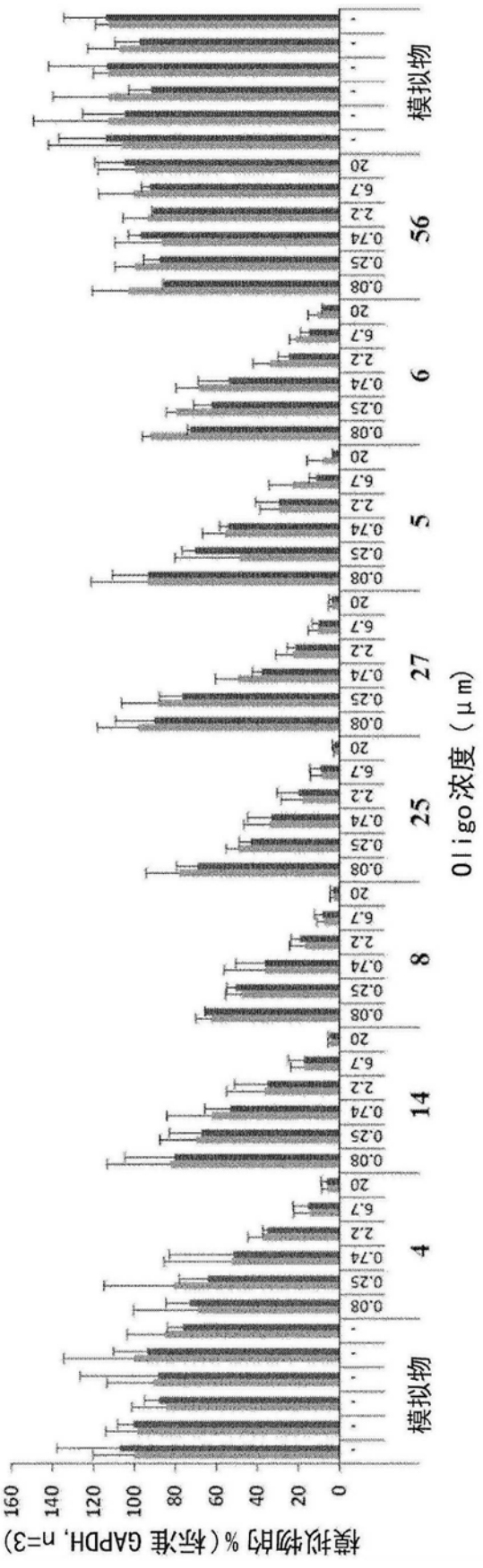

模拟物的%(标准 GAPDH, n=3)
160
140
120
100
80
60
40
20
0
模拟物
-
-
-
-
-
0.08
0.25
0.74
2.2
6.7
20
4
14
8
25
27
5
6
56
Oligo浓度（μm）

图 7

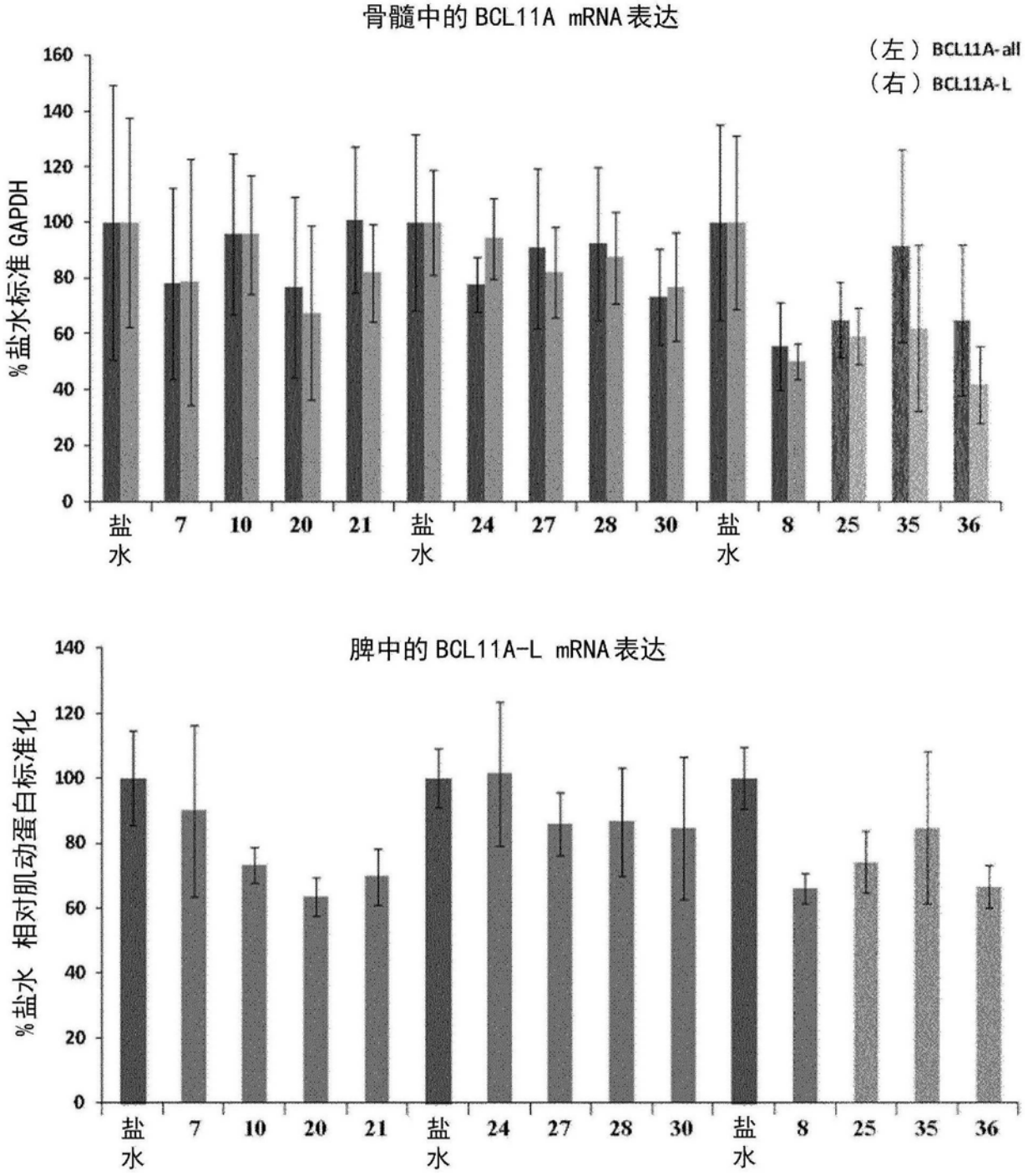

骨髓中的 BCL11A mRNA 表达
（左）BCL11A-all
（右）BCL11A-L
%盐水标准 GAPDH
160
140
120
100
80
60
40
20
0
盐水
7
10
20
21
盐水
24
27
28
30
盐水
8
25
35
36
脾中的 BCL11A-L mRNA 表达
%盐水 相对肌动蛋白标准化
140
120
100
80
60
40
20
0
盐水
7
10
20
21
盐水
24
27
28
30
盐水
8
25
35
36

图 8

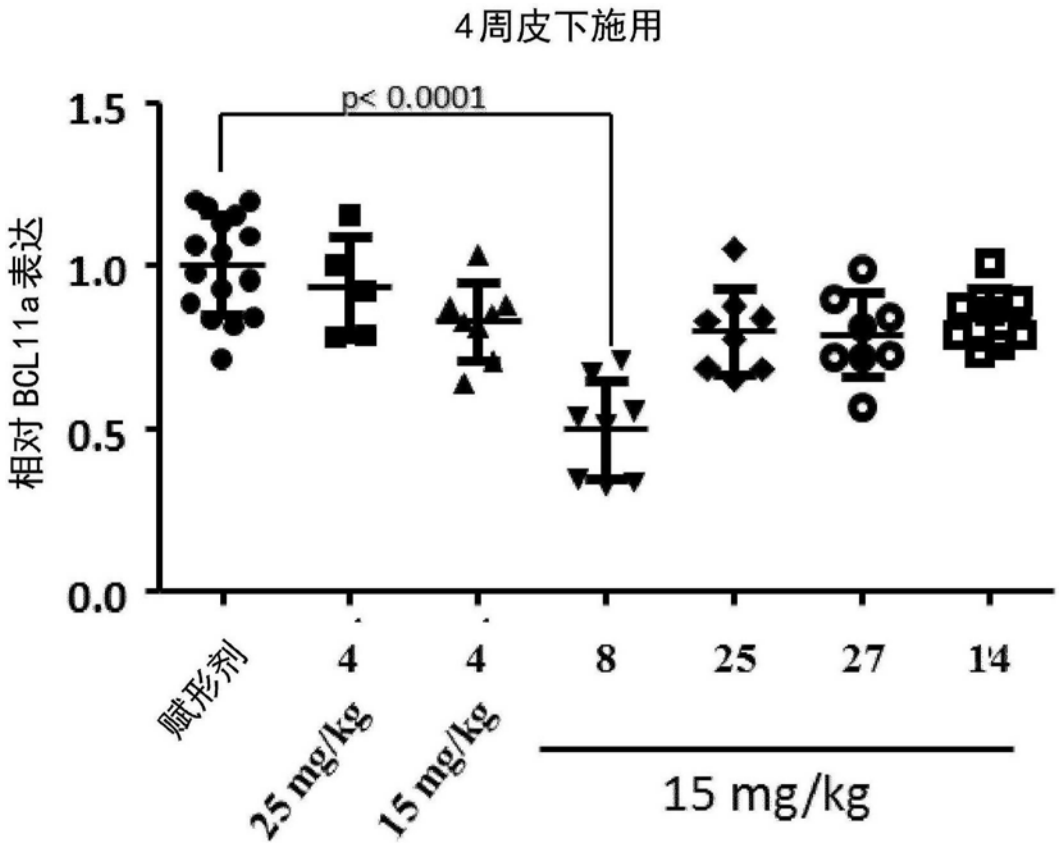

4周皮下施用
p< 0.0001
相对 BCL11a 表达
1.5
1.0
0.5
0.0
赋形剂
4
25 mg/kg
4
15 mg/kg
8
25
27
14
15 mg/kg

图 9

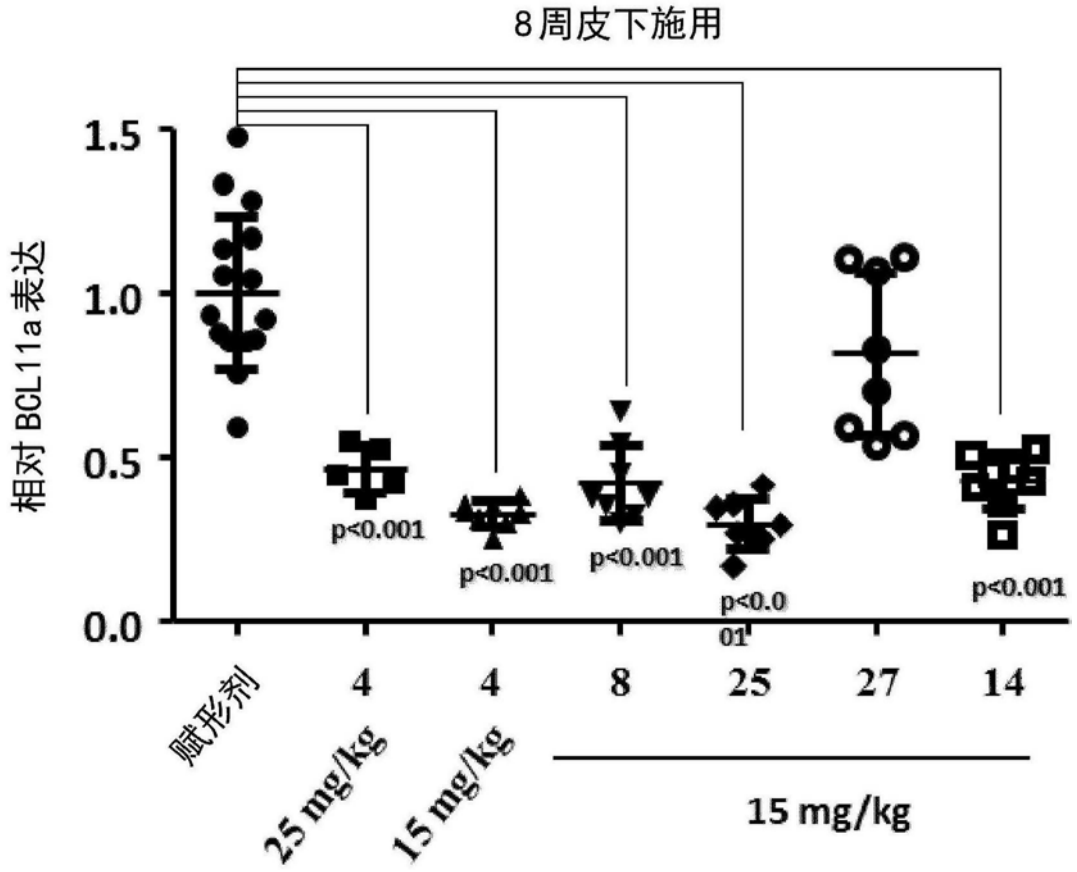

8周皮下施用
相对 BCL11a 表达
1.5
1.0
0.5
0.0
p<0.001
p<0.001
p<0.001
p<0.0
01
p<0.001
赋形剂
4
4
8
25
27
14
25 mg/kg
15 mg/kg
15 mg/kg

图 10

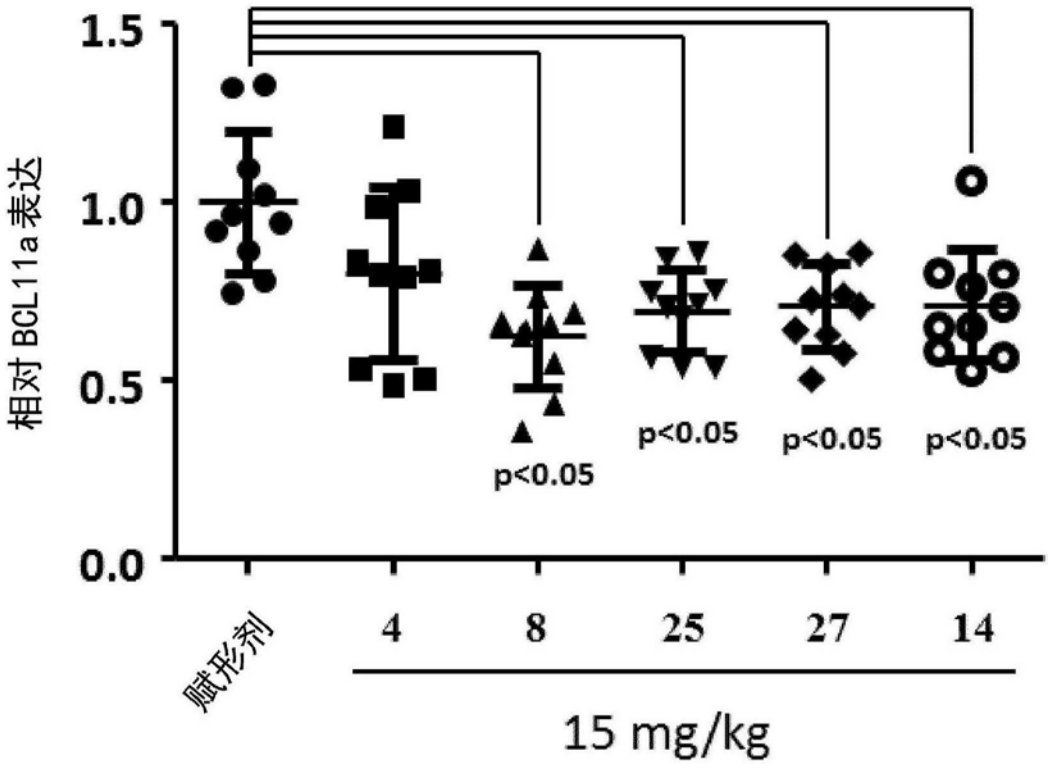

1.5
1.0
0.5
0.0
相对 BCL11a 表达
p<0.05
p<0.05
p<0.05
p<0.05
赋形剂
4
8
25
27
14
15 mg/kg

图 11

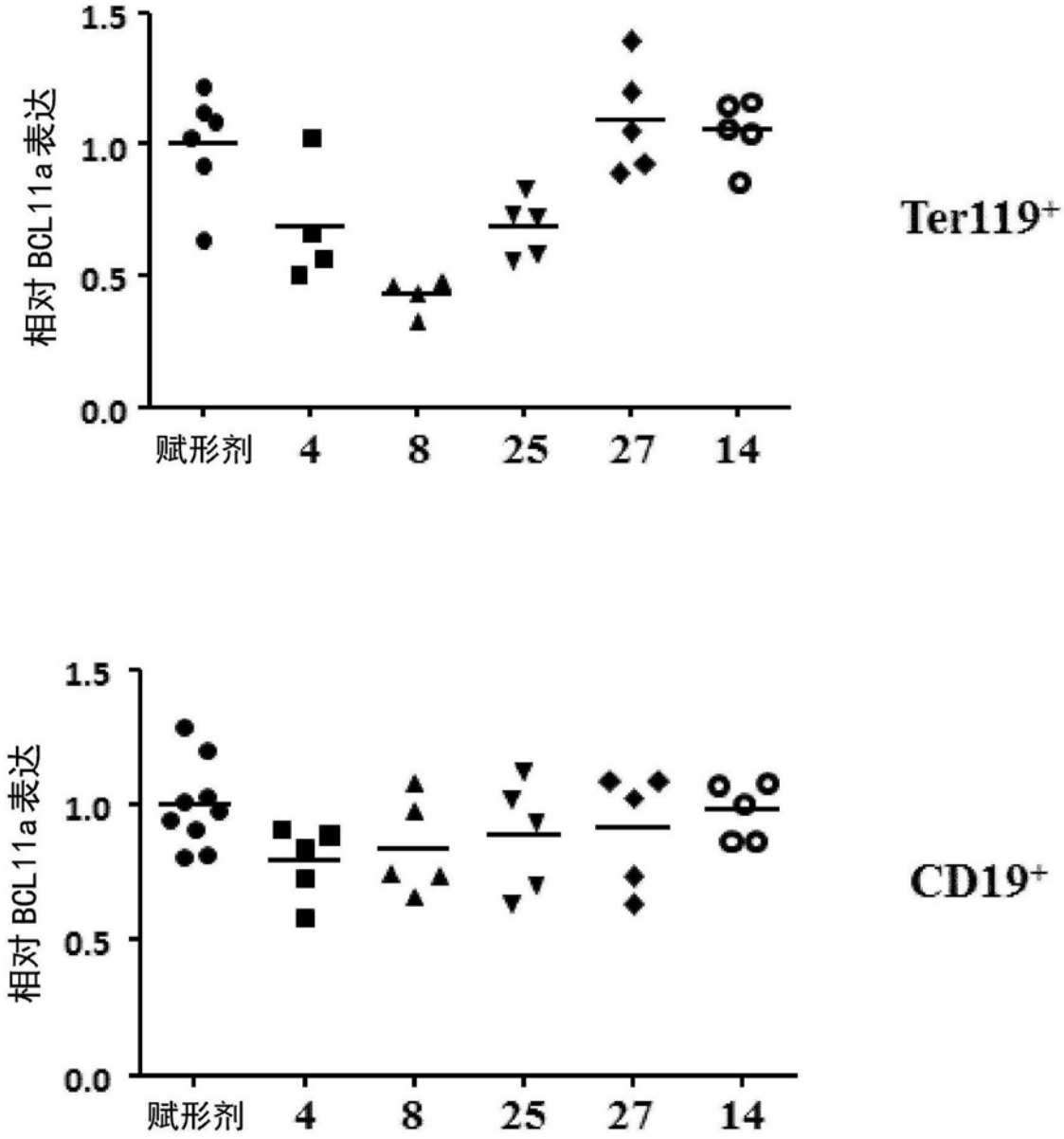

1.5
1.0
0.5
0.0
相对 BCL11a 表达
赋形剂 4 8 25 27 14
Ter119+
1.5
1.0
0.5
0.0
相对 BCL11a 表达
赋形剂 4 8 25 27 14
CD19+

图 12

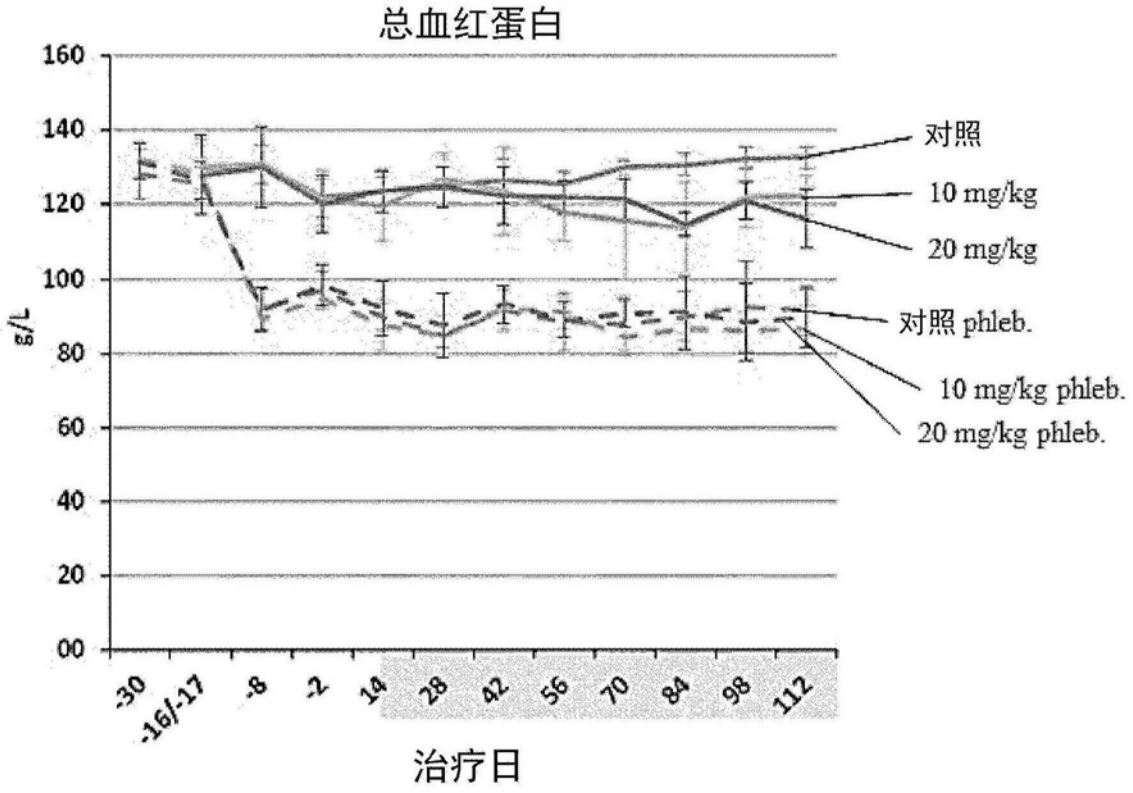

总血红蛋白
160
140
120
100
80
60
40
20
00
g/L
-30
-16/-17
-8
-2
14
28
42
56
70
84
98
112
治疗日
对照
10 mg/kg
20 mg/kg
对照 phleb.
10 mg/kg phleb.
20 mg/kg phleb.

图 13

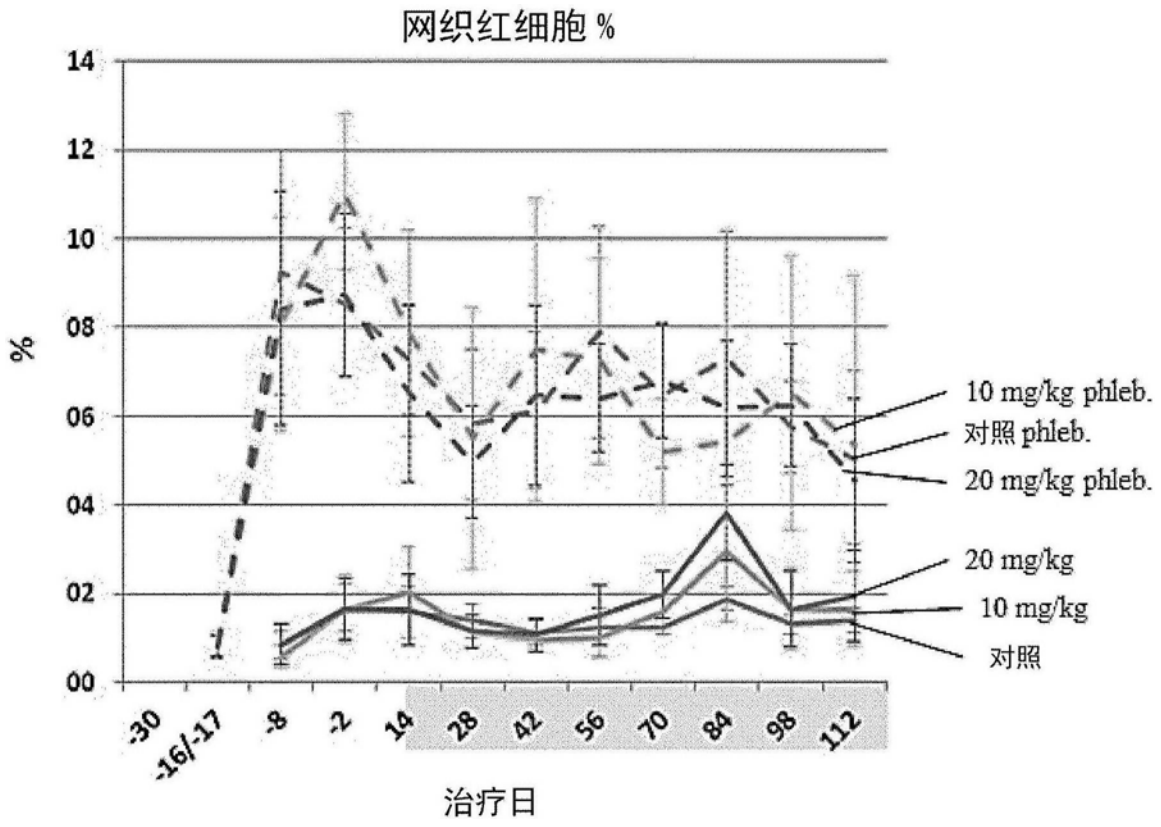

网织红细胞 %
14
12
10
08
06
04
02
00
%
-30
-16/-17
-8
-2
14
28
42
56
70
84
98
112
治疗日
10 mg/kg phleb.
对照 phleb.
20 mg/kg phleb.
20 mg/kg
10 mg/kg
对照

图 14

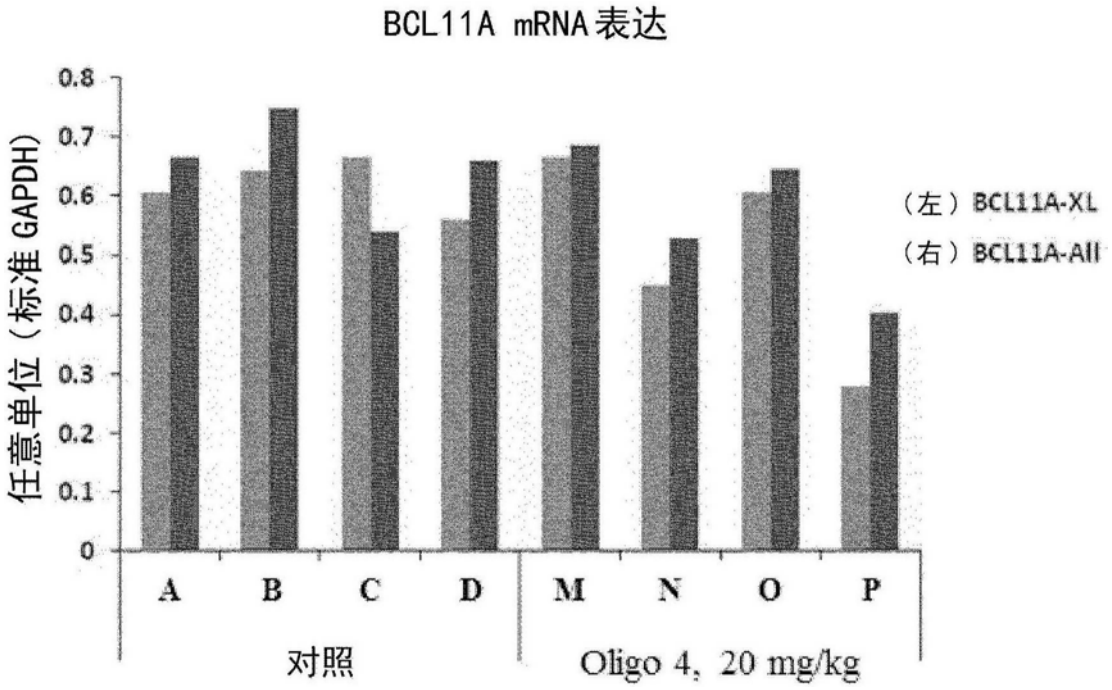

BCL11A mRNA表达
任意单位（标准 GAPDH）
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A
B
C
D
M
N
O
P
对照
Oligo 4, 20 mg/kg
（左）BCL11A-XL
（右）BCL11A-All

图 15

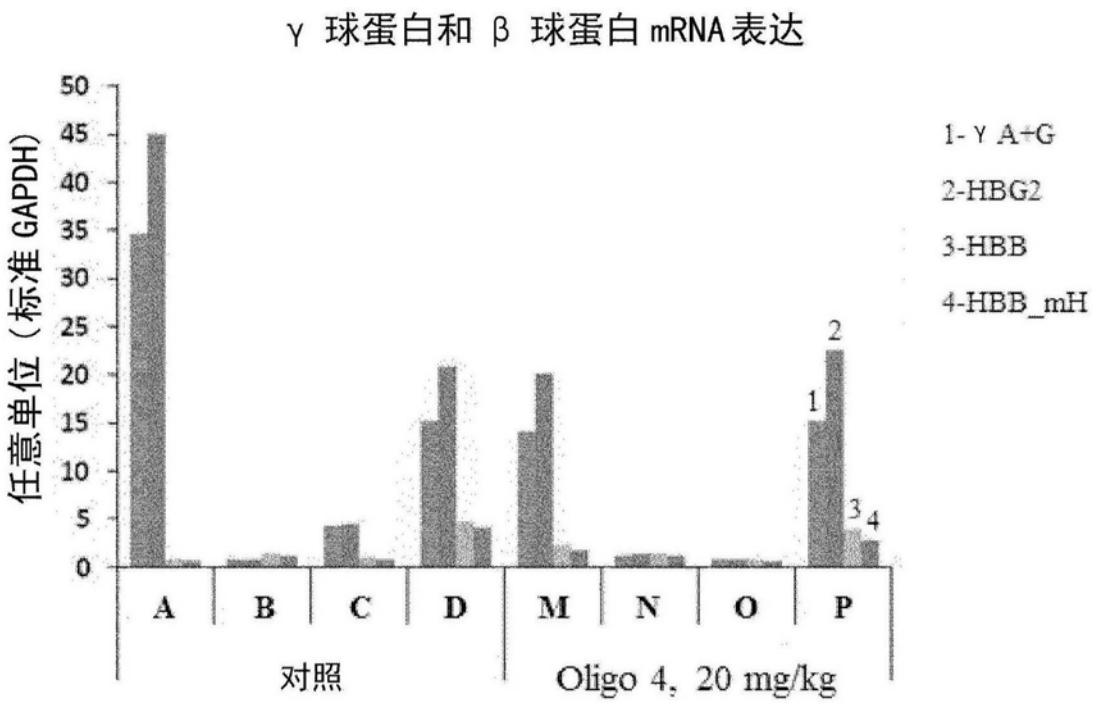

γ 球蛋白和 β 球蛋白 mRNA 表达
任意单位（标准 GAPDH）
50
45
40
35
30
25
20
15
10
5
0
A
B
C
D
M
N
O
P
对照
Oligo 4, 20 mg/kg
1-γ A+G
2-HBG2
3-HBB
4-HBB_mH
1
2
3
4

图 16

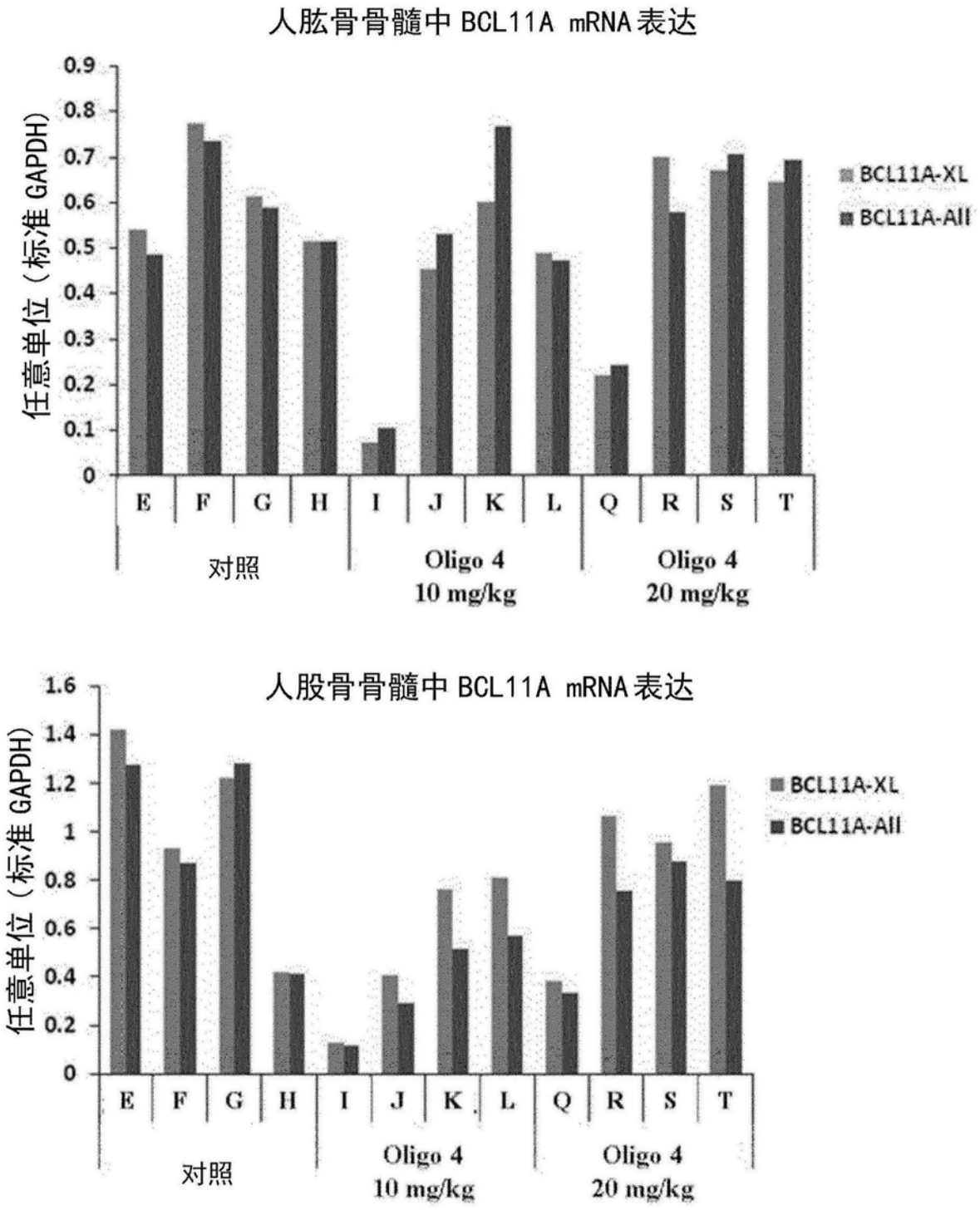

人肱骨骨髓中 BCL11A mRNA 表达
任意单位（标准 GAPDH）
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BCL11A-XL
BCL11A-All
E F G H I J K L Q R S T
对照
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg
人股骨骨髓中 BCL11A mRNA 表达
任意单位（标准 GAPDH）
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
BCL11A-XL
BCL11A-All
E F G H I J K L Q R S T
对照
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg

图 17

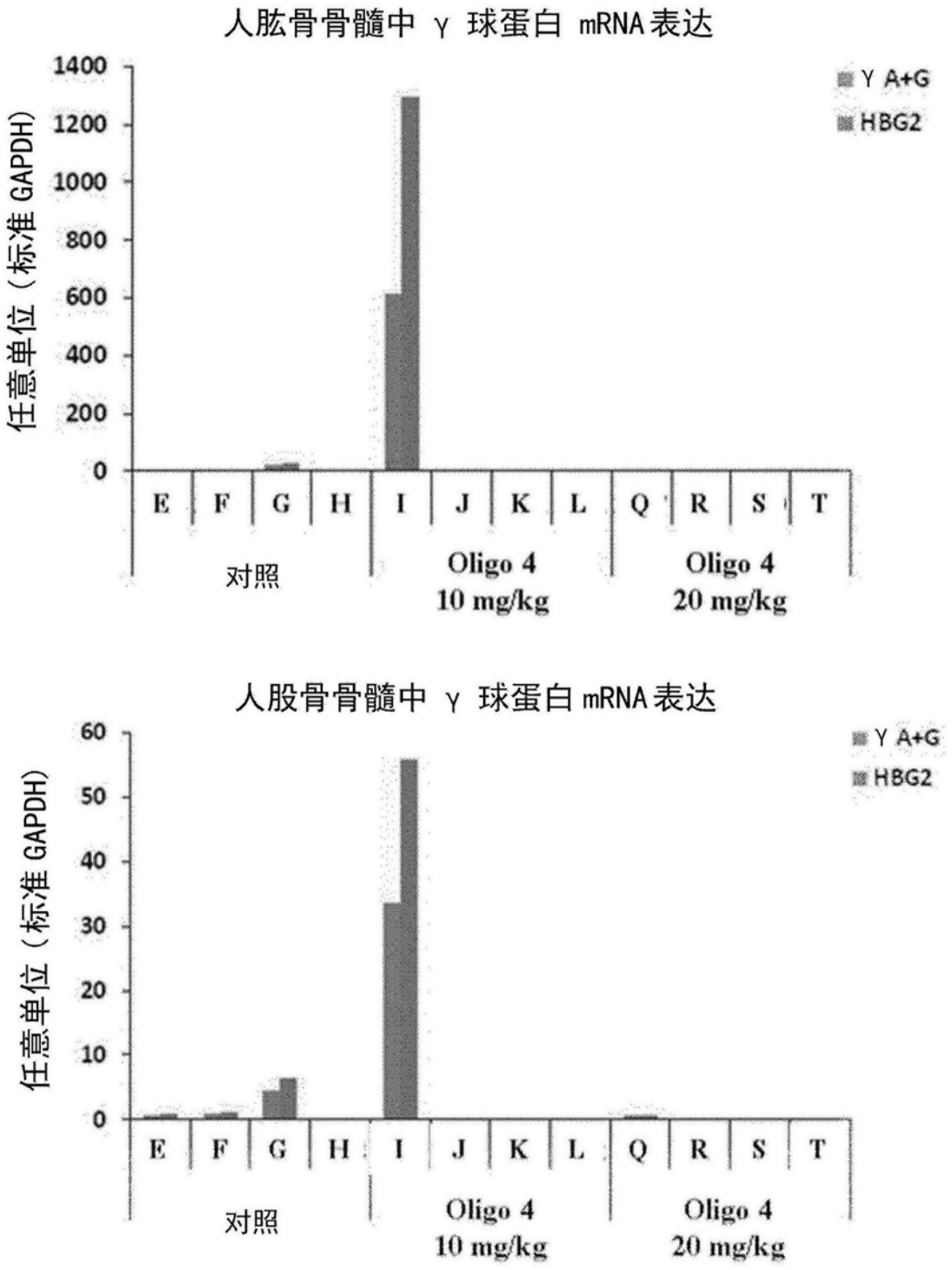

人肱骨骨髓中 γ 球蛋白 mRNA 表达
任意单位（标准 GAPDH）
1400
1200
1000
800
600
400
200
0
γ A+G
HBG2
E F G H I J K L Q R S T
对照
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg
人股骨骨髓中 γ 球蛋白 mRNA 表达
任意单位（标准 GAPDH）
60
50
40
30
20
10
0
γ A+G
HBG2
E F G H I J K L Q R S T
对照
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg

图 18

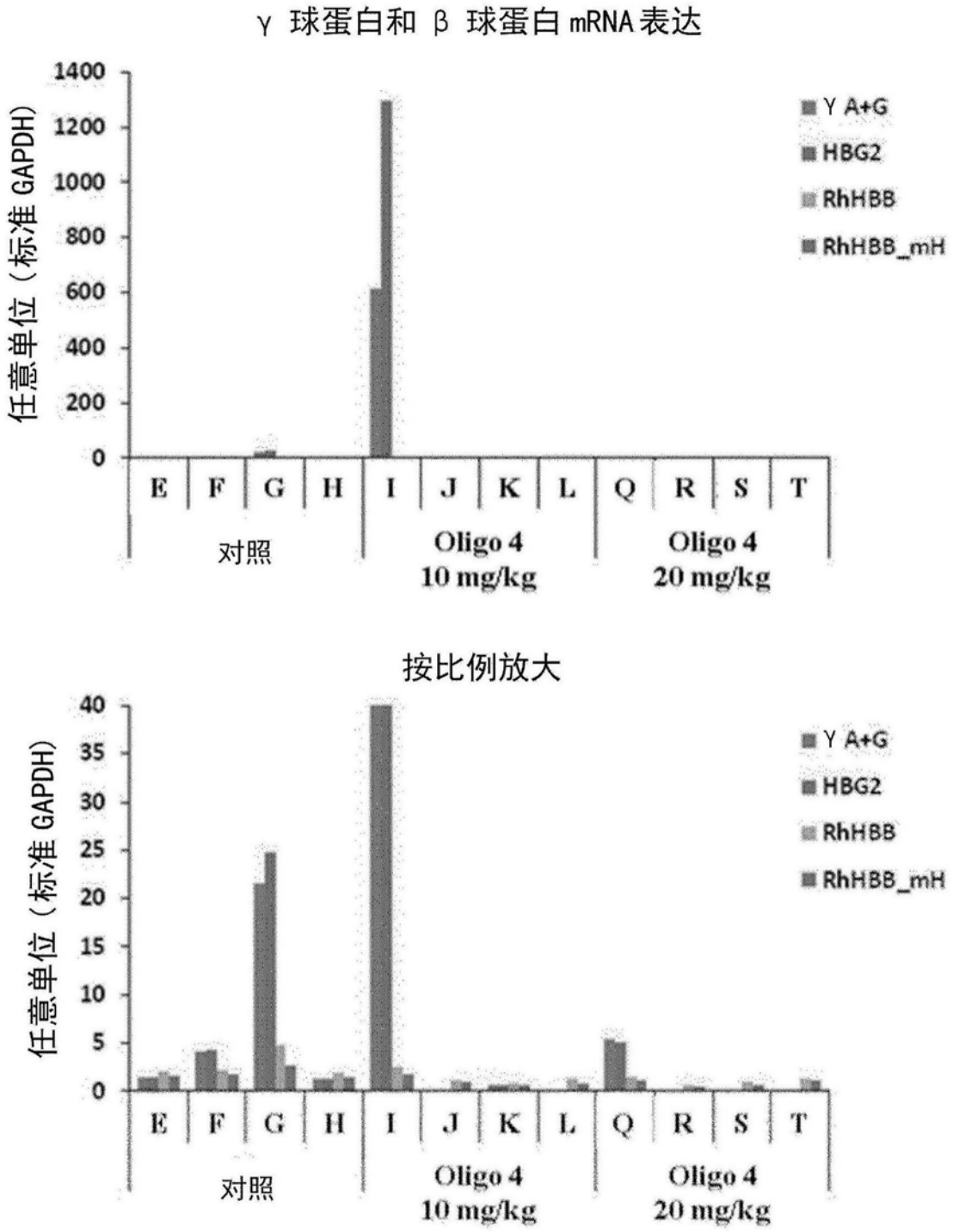

γ 球蛋白和 β 球蛋白 mRNA 表达
任意单位（标准 GAPDH）
1400
1200
1000
800
600
400
200
0
γ A+G
HBG2
RhHBB
RhHBB_mH
E F G H I J K L Q R S T
对照
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg
按比例放大
任意单位（标准 GAPDH）
40
35
30
25
20
15
10
5
0
γ A+G
HBG2
RhHBB
RhHBB_mH
E F G H I J K L Q R S T
对照
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg

图 19

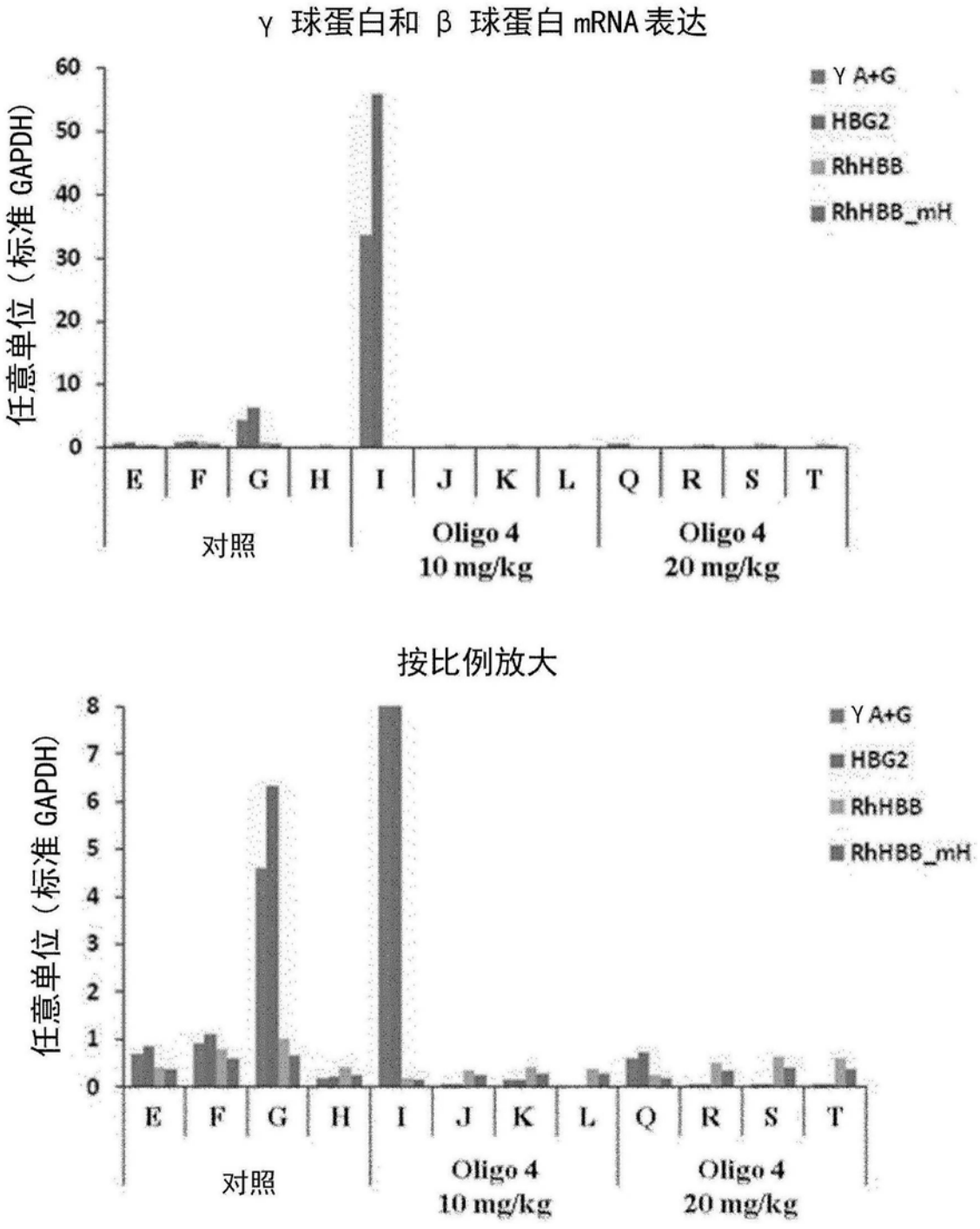

γ 球蛋白和 β 球蛋白 mRNA 表达
任意单位（标准 GAPDH）
60
50
40
30
20
10
0
γ A+G
HBG2
RhHBB
RhHBB_mH
E F G H I J K L Q R S T
对照
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg
按比例放大
任意单位（标准 GAPDH）
8
7
6
5
4
3
2
1
0
γ A+G
HBG2
RhHBB
RhHBB_mH
E F G H I J K L Q R S T
对照
Oligo 4
10 mg/kg
Oligo 4
20 mg/kg

图 20

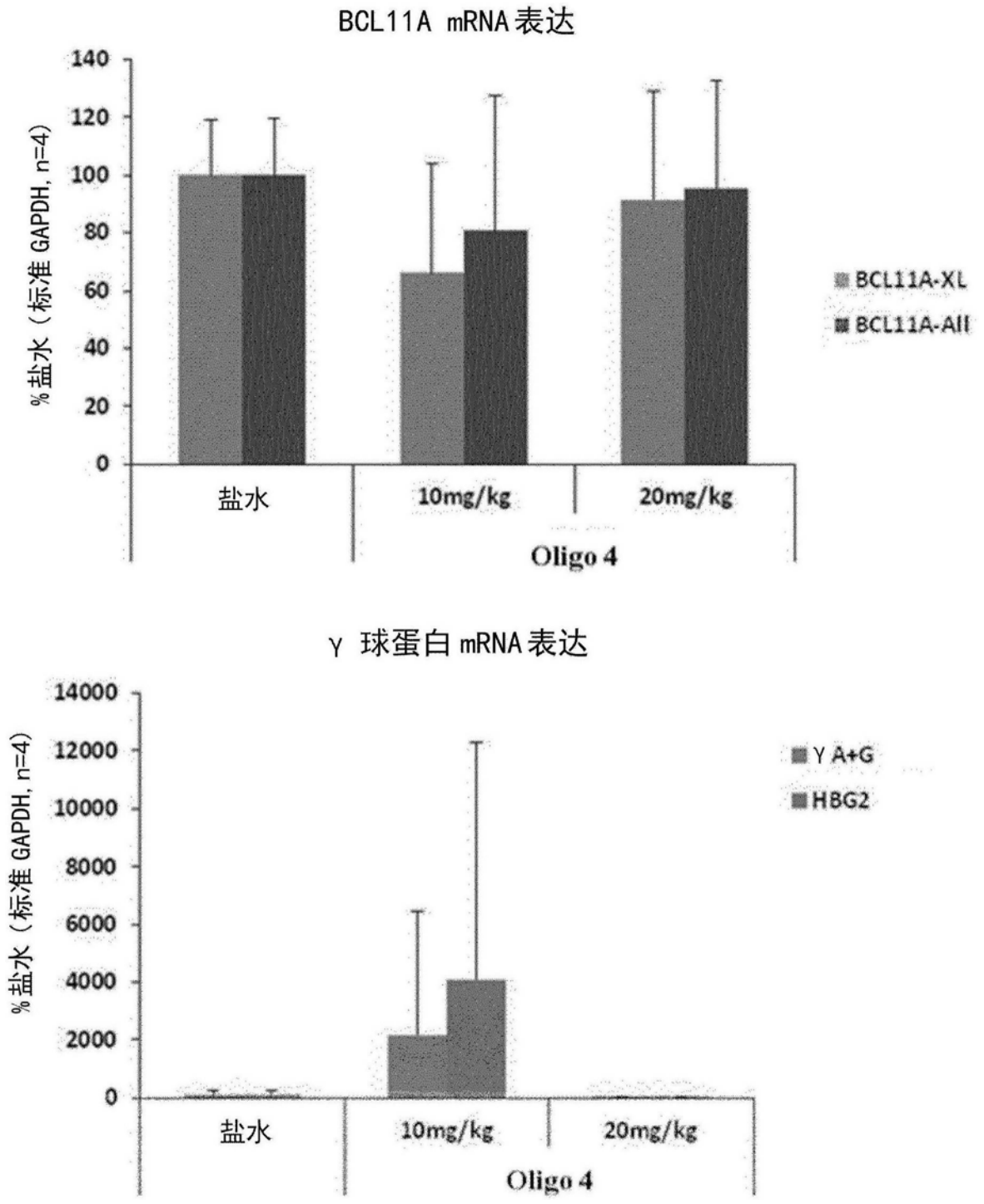

BCL11A mRNA表达
% 盐水（标准 GAPDH, n=4）
140
120
100
80
60
40
20
0
盐水
10mg/kg
20mg/kg
Oligo 4
BCL11A-XL
BCL11A-All
γ 球蛋白 mRNA表达
% 盐水（标准 GAPDH, n=4）
14000
12000
10000
8000
6000
4000
2000
0
盐水
10mg/kg
20mg/kg
Oligo 4
γ A+G
HBG2

图 21

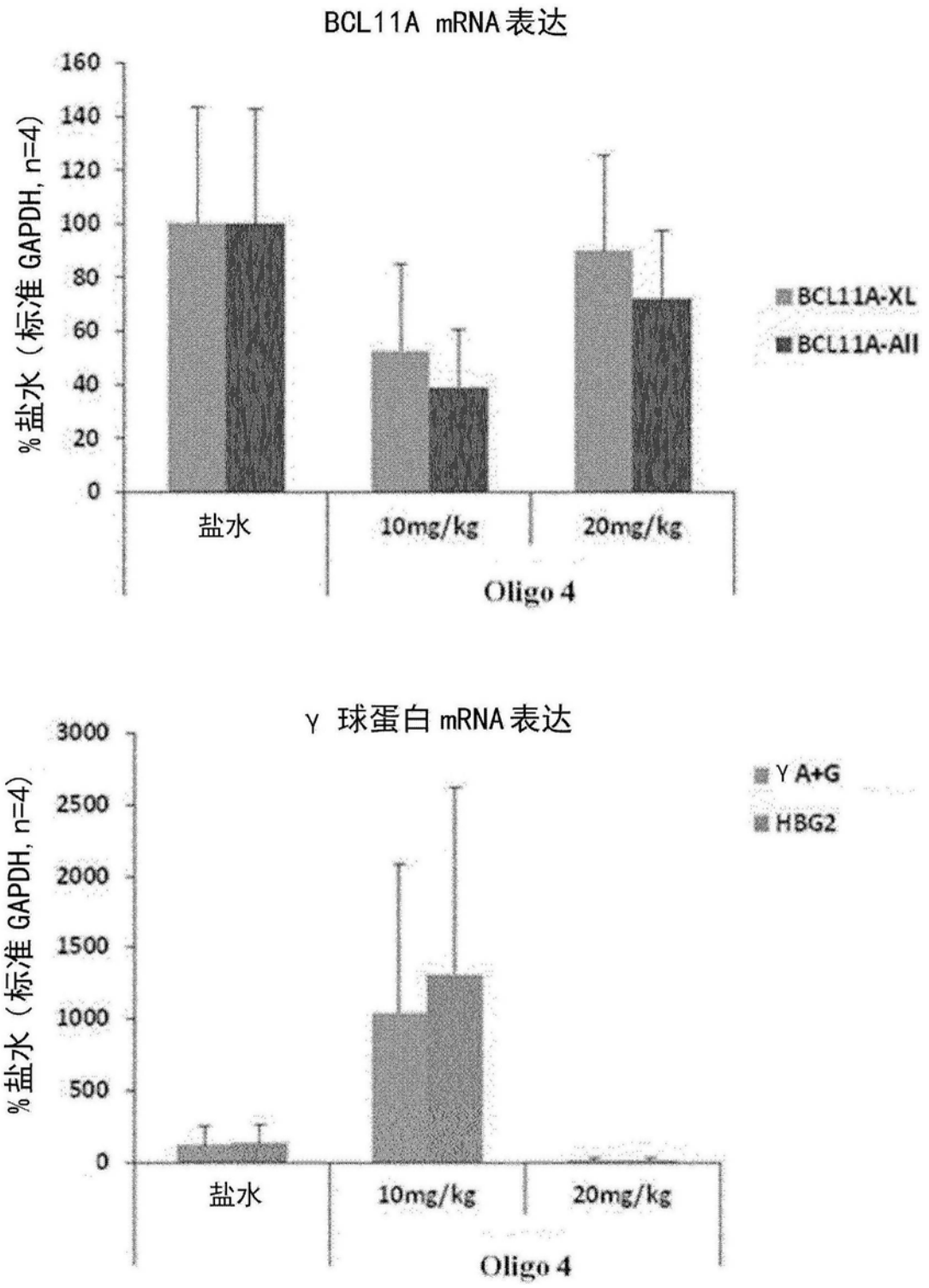

BCL11A mRNA表达
% 盐水（标准 GAPDH, n=4）
160
140
120
100
80
60
40
20
0
盐水
10mg/kg
20mg/kg
Oligo 4
BCL11A-XL
BCL11A-All
γ 球蛋白 mRNA表达
% 盐水（标准 GAPDH, n=4）
3000
2500
2000
1500
1000
500
0
盐水
10mg/kg
20mg/kg
Oligo 4
γ A+G
HBG2

图 22

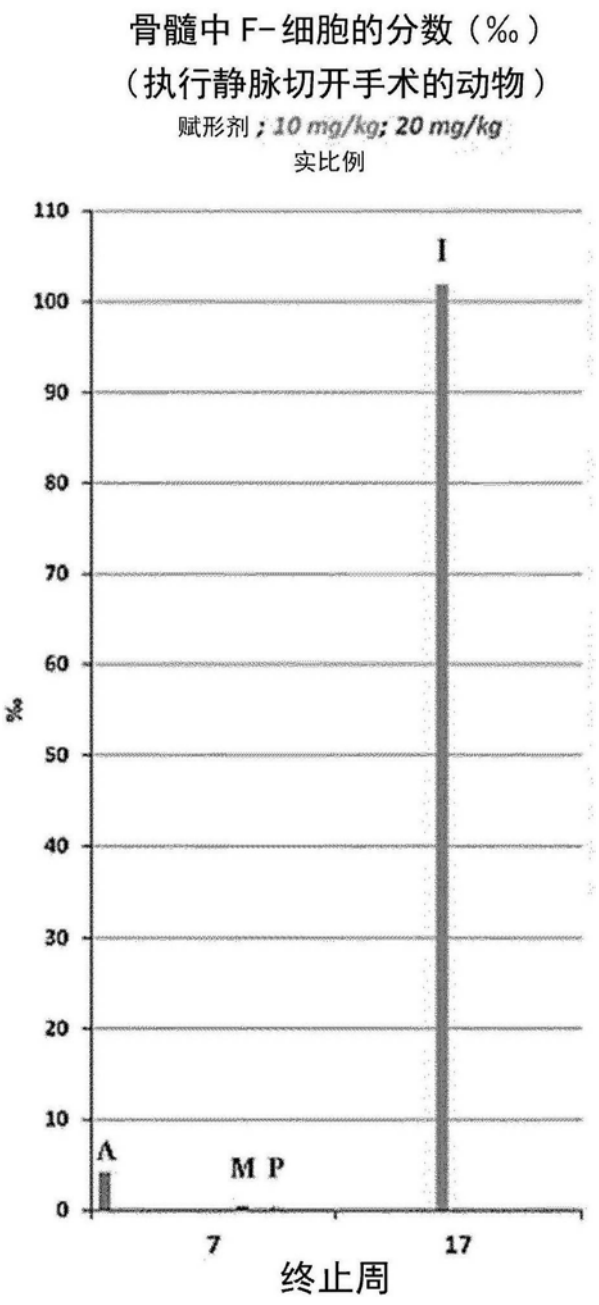

骨髓中 F-细胞的分数（‰）
（执行静脉切开手术的动物）
赋形剂；10 mg/kg; 20 mg/kg
实比例
110
100
90
80
70
60
50
40
30
20
10
0
‰
A
M P
I
7
17
终止周

图 23

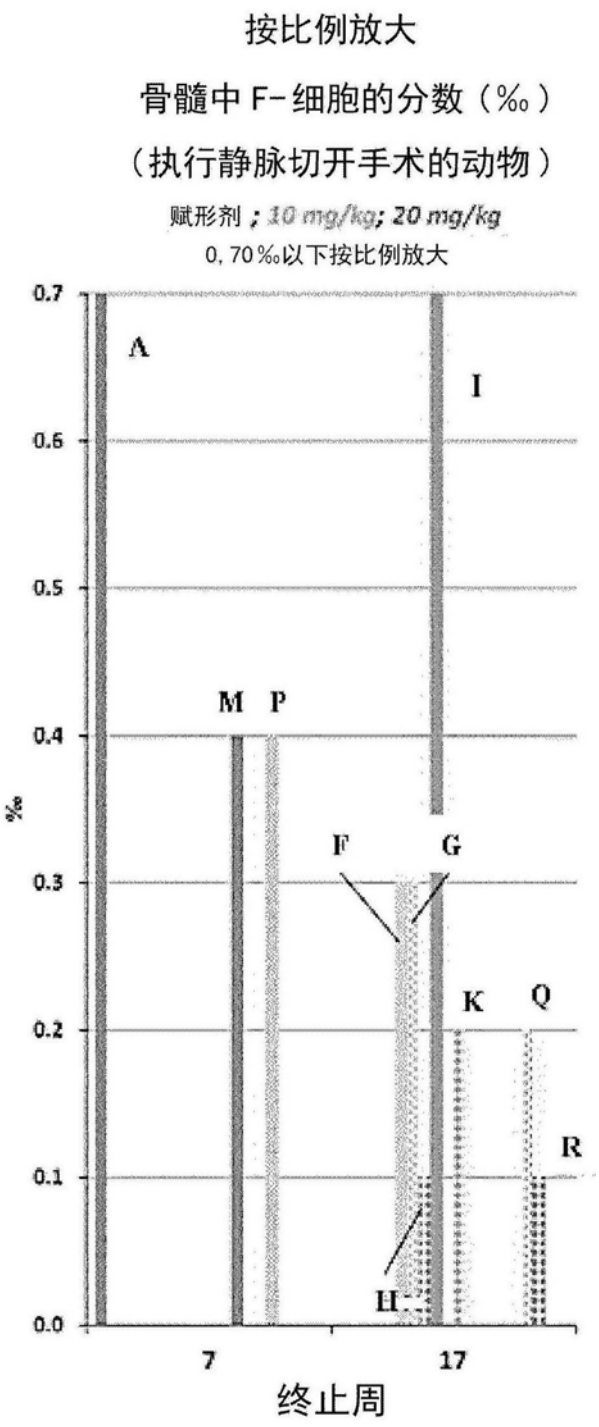

按比例放大
骨髓中 F-细胞的分数（‰）
（执行静脉切开手术的动物）
赋形剂；10 mg/kg; 20 mg/kg
0, 70‰以下按比例放大
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
‰
A
I
M P
F
G
K
Q
R
H
7
17
终止周

图 23（续）

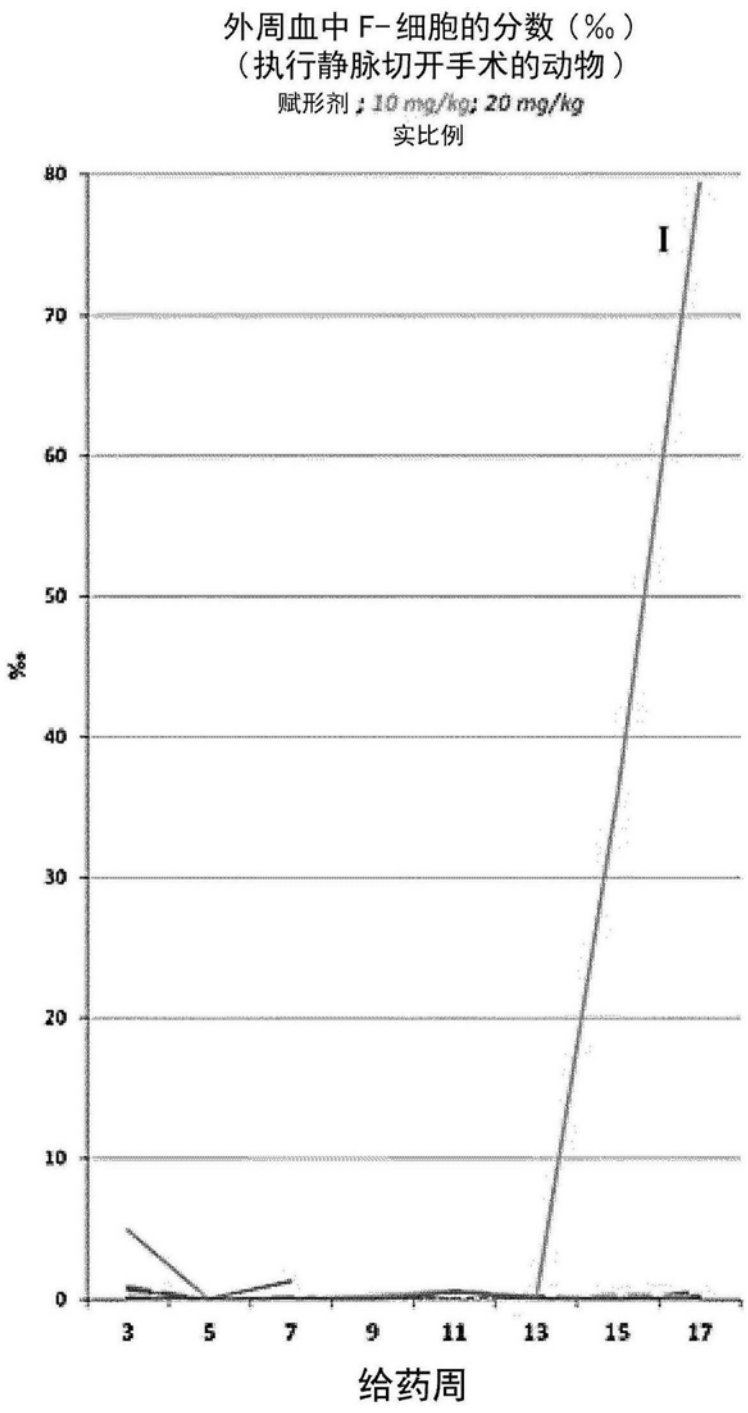

外周血中 F-细胞的分数（‰）
（执行静脉切开手术的动物）
赋形剂；10 mg/kg; 20 mg/kg
实比例
80
70
60
50
40
30
20
10
0
‰
I
3
5
7
9
11
13
15
17
给药周

图 24

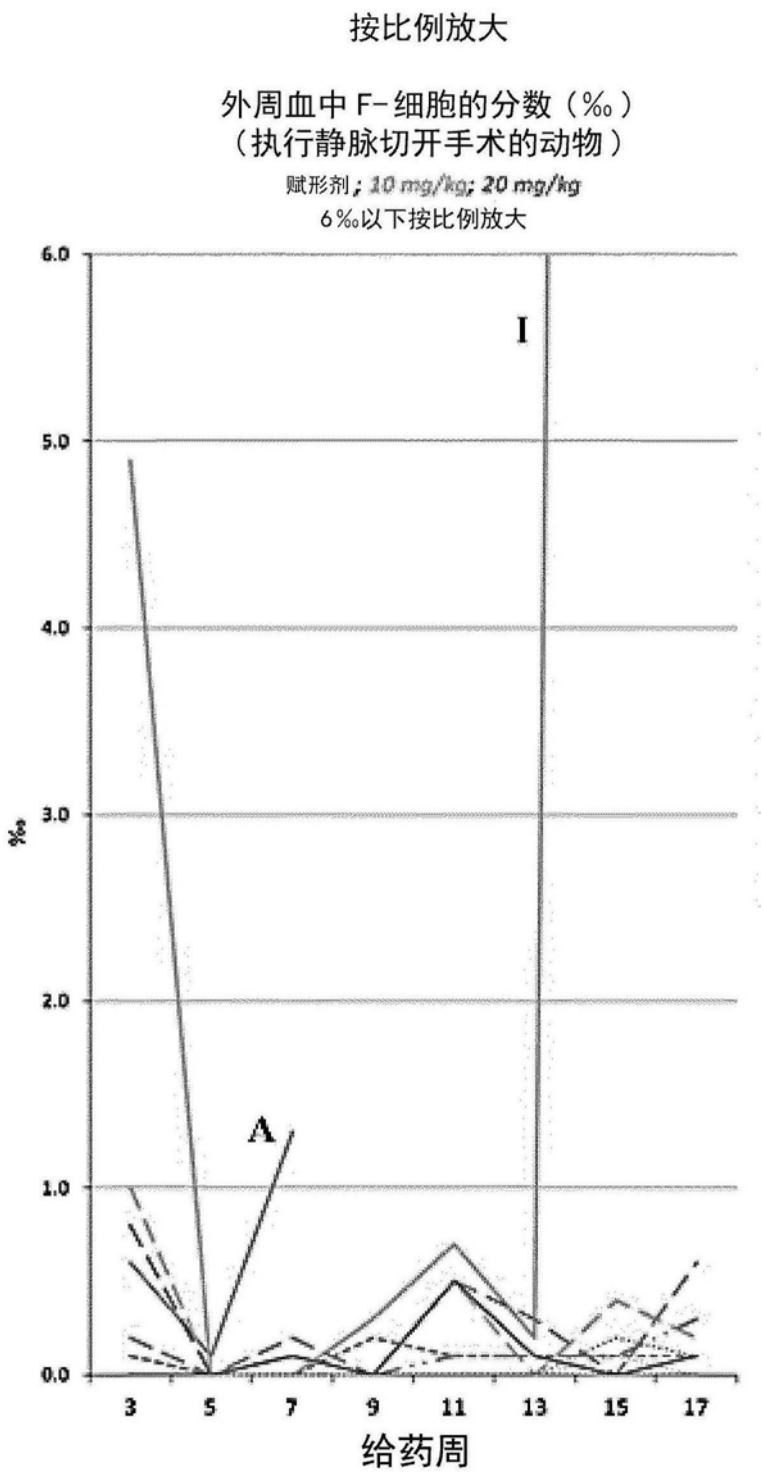

按比例放大
外周血中 F-细胞的分数（‰）
（执行静脉切开手术的动物）
赋形剂；10 mg/kg；20 mg/kg
6‰以下按比例放大
6.0
5.0
4.0
3.0
2.0
1.0
0.0
‰
I
A
3
5
7
9
11
13
15
17
给药周

图 24（续）

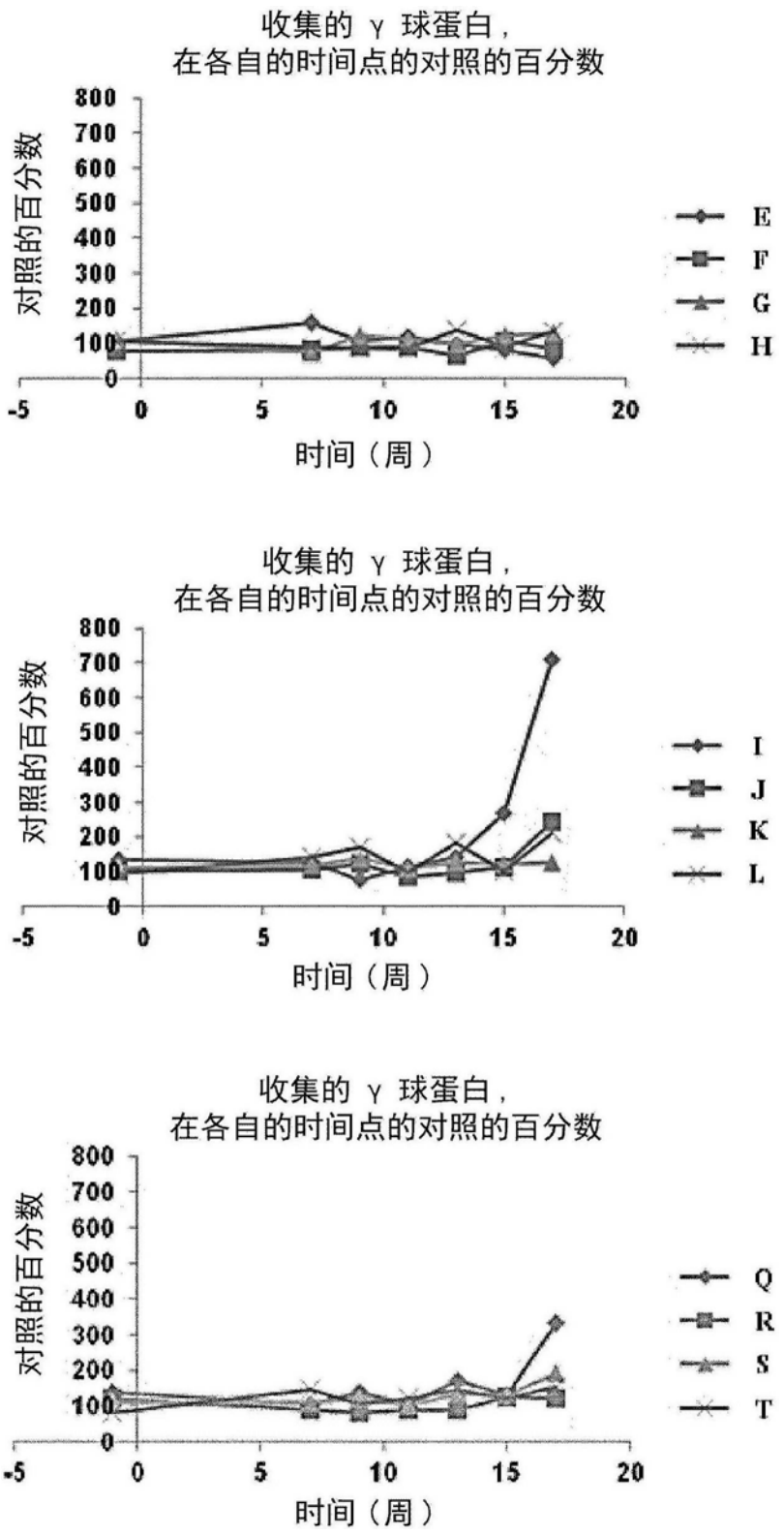

收集的 γ 球蛋白，
在各自的时间点的对照的百分数
对照的百分数
800
700
600
500
400
300
200
100
0
-5
0
5
10
15
20
时间（周）
E
F
G
H
收集的 γ 球蛋白，
在各自的时间点的对照的百分数
对照的百分数
时间（周）
I
J
K
L
收集的 γ 球蛋白，
在各自的时间点的对照的百分数
对照的百分数
时间（周）
Q
R
S
T

图 25

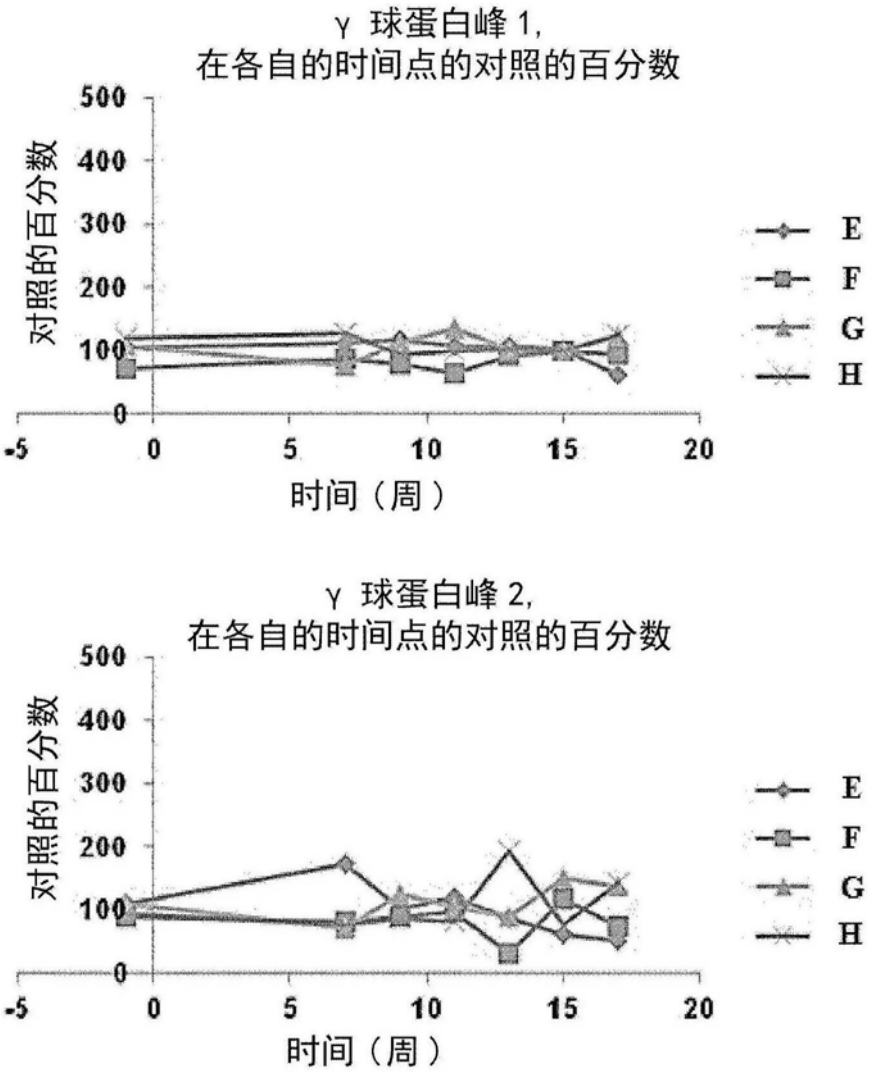

γ 球蛋白峰 1,
在各自的时间点的对照的百分数
对照的百分数
500
400
300
200
100
0
-5
0
5
10
15
20
时间（周）
E
F
G
H
γ 球蛋白峰 2,
在各自的时间点的对照的百分数
对照的百分数
500
400
300
200
100
0
-5
0
5
10
15
20
时间（周）
E
F
G
H

图 26

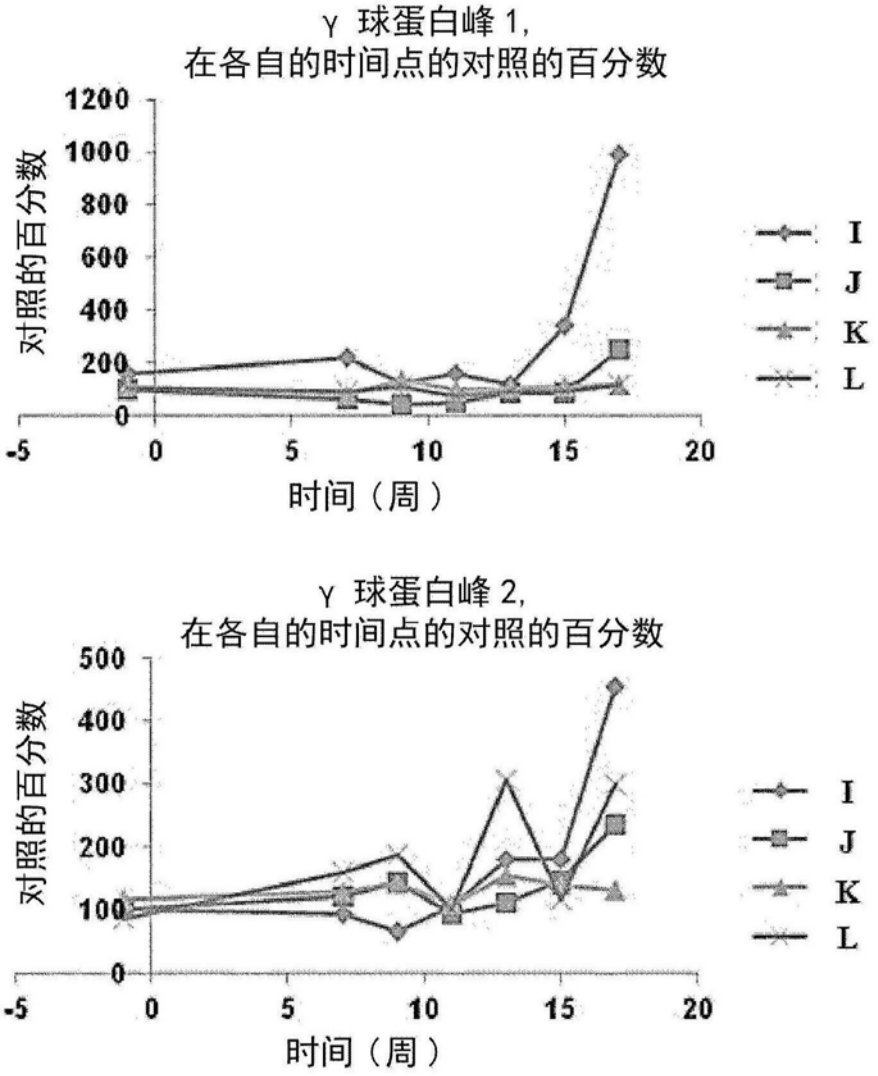

γ 球蛋白峰 1,
在各自的时间点的对照的百分数
对照的百分数
1200
1000
800
600
400
200
0
-5
0
5
10
15
20
时间（周）
I
J
K
L
γ 球蛋白峰 2,
在各自的时间点的对照的百分数
对照的百分数
500
400
300
200
100
0
-5
0
5
10
15
20
时间（周）
I
J
K
L

图 27

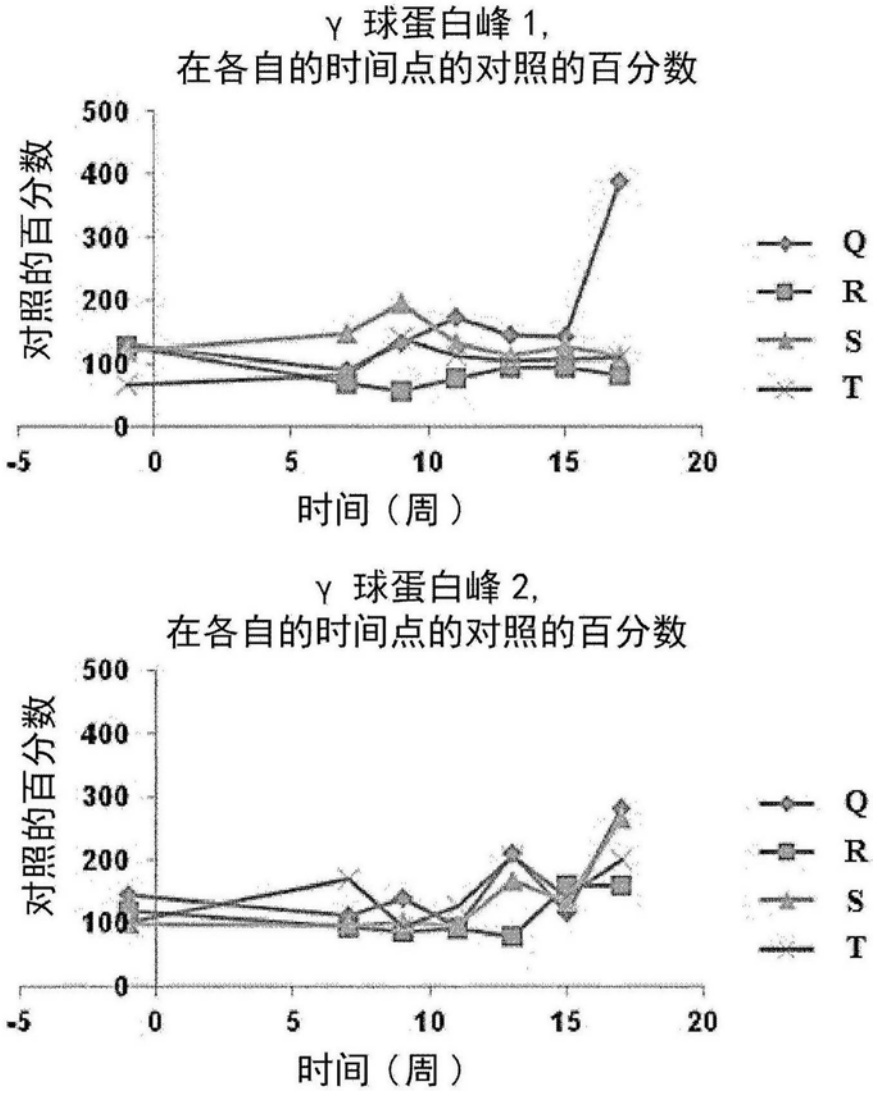

γ 球蛋白峰 1,
在各自的时间点的对照的百分数
对照的百分数
500
400
300
200
100
0
-5
0
5
10
15
20
时间（周）
Q
R
S
T
γ 球蛋白峰 2,
在各自的时间点的对照的百分数
对照的百分数
500
400
300
200
100
0
-5
0
5
10
15
20
时间（周）
Q
R
S
T

图 28

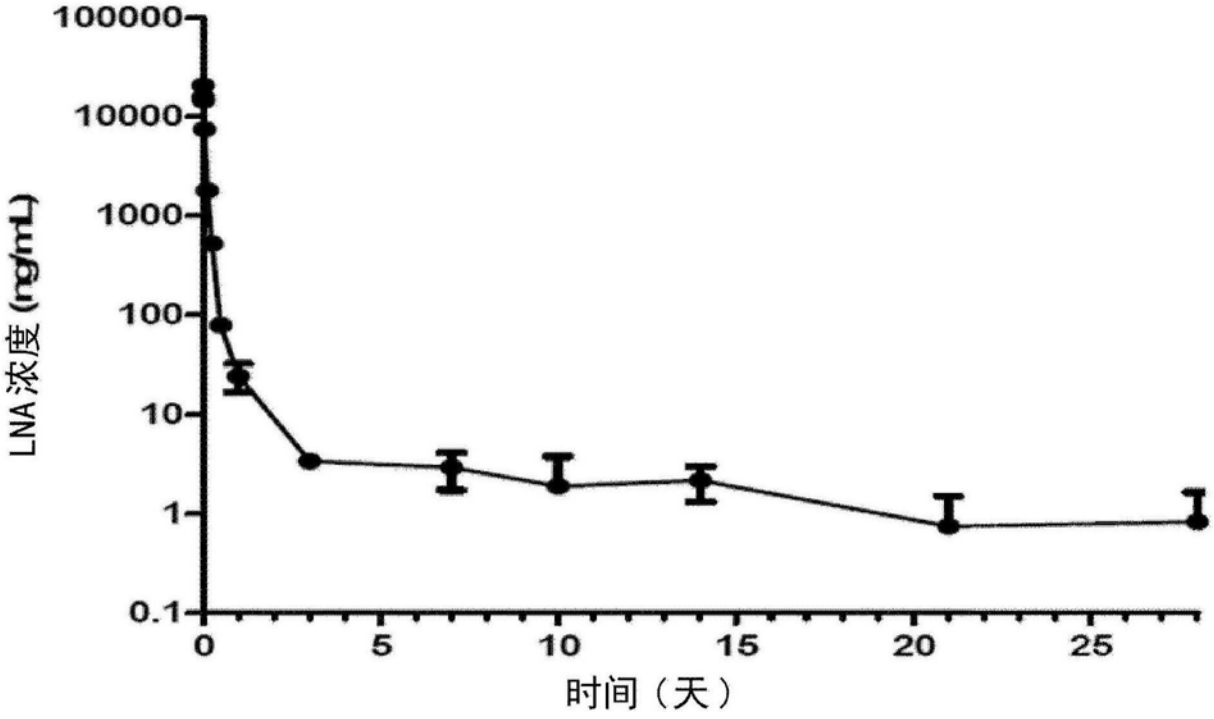

100000
10000
1000
100
10
1
0.1
LNA浓度 (ng/mL)
0
5
10
15
20
25
时间（天）

图 29

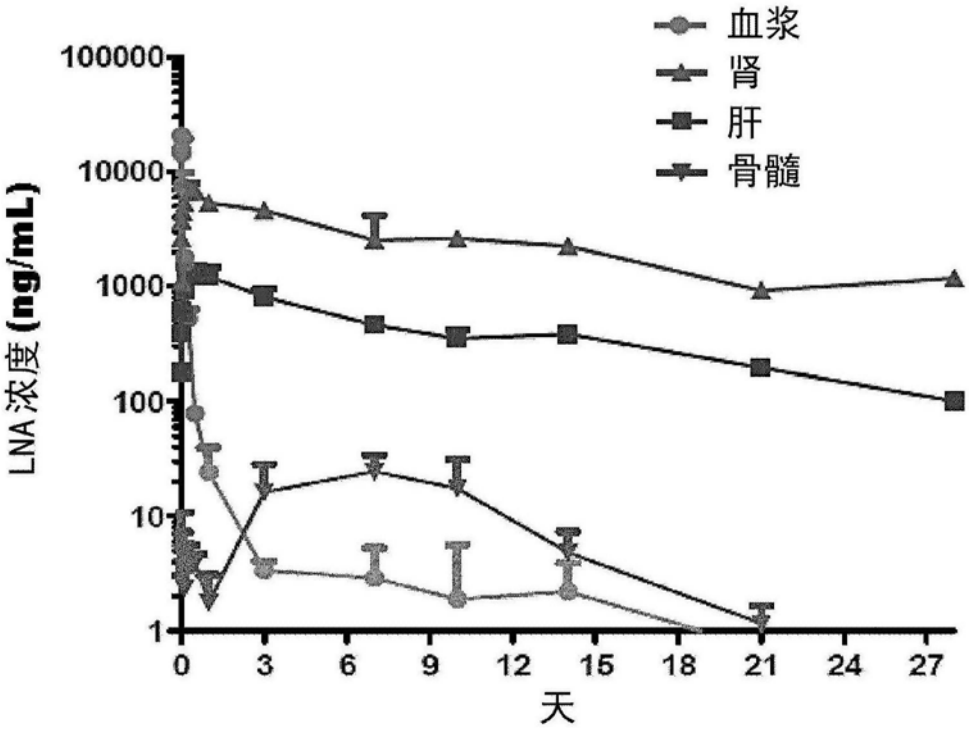
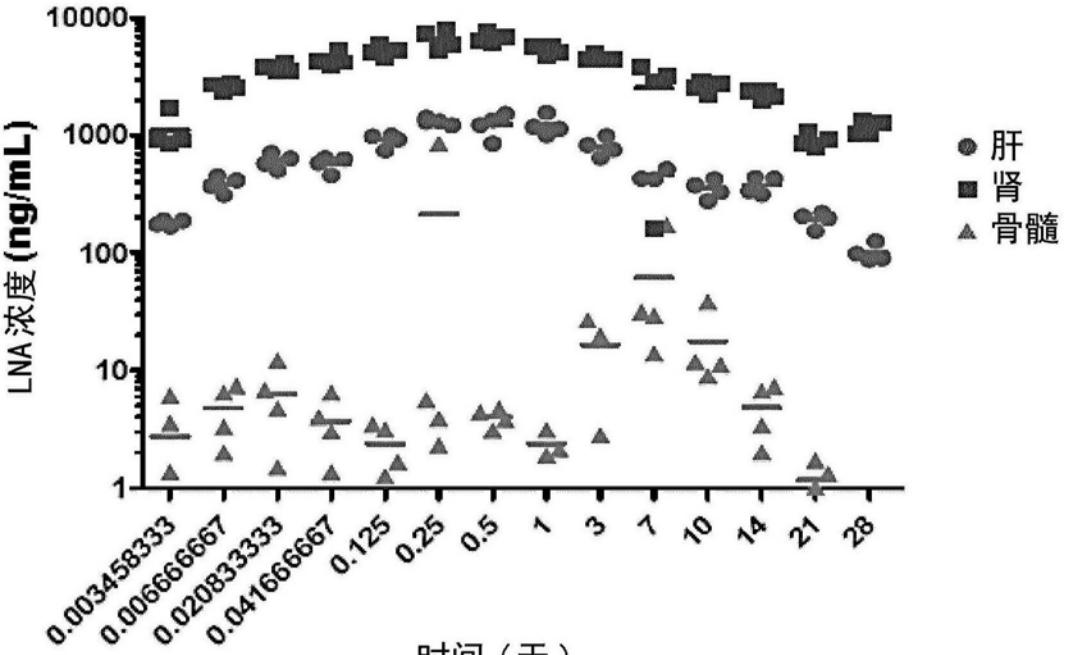

血浆
肾
肝
骨髓
100000
10000
1000
100
10
1
LNA浓度 (ng/mL)
0
3
6
9
12
15
18
21
24
27
天

图 30



组织	$t_{1/2}$ hr	C_{max} ng/mg	AUC_{0-last} hr*ng/mg	$AUC_{0-\infty}$ hr*ng/mg
血浆	230	20,642	30,423	30,688
肾	269	6,746	1,583,640	2,042,285
肝	219	1,320	274,935	306,785
骨髓	68	25	5,615	5,731

图 31

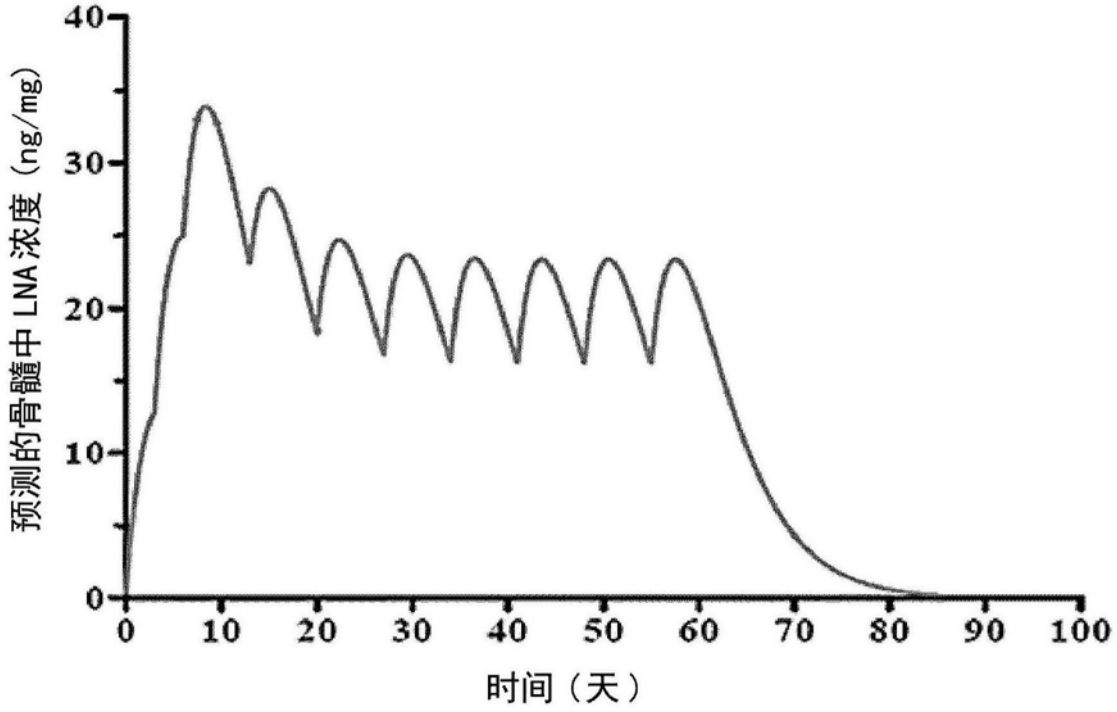


图 32