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(54) **Title: TREATMENT OF HEMOGLOBIN (HBF/HBG) RELATED DISEASES BY INHIBITION OF NATURAL ANTISENSE TRANSCRIPT TO HBF/HBG**

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Antisense compounds modulate expression and/or function of globin genes. Methods for treating diseases associated with globins comprise administering one or more antisense compounds to patients.

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(54) Title: TREATMENT OF HEMOGLOBIN (HBF/HBG) RELATED DISEASES INHIBITION OF NATURAL ANTISENSE TRANSCRIPT TO HBF/HBG

(57) Abstract: Antisense compounds modulate expression and/or function of globin genes. Methods for treating diseases associated with globins comprise administering one or more antisense compounds to patients.

TREATMENT OF HEMOGLOBIN (HBF/HBG) RELATED DISEASES BY INHIBITION OF NATURAL ANTISENSE TRANSCRIPT TO HBF/HBG

FIELD OF THE INVENTION

[0001] Embodiments of the invention comprise oligonucleotides modulating expression and/or function of globin molecules.

BACKGROUND

[0002] DNA-RNA and RNA-RNA hybridization are important to many aspects of nucleic acid function including DNA replication, transcription, and translation. Hybridization is also central to a variety of technologies that either detect a particular nucleic acid or alter its expression. Antisense nucleotides, for example, disrupt gene expression by hybridizing to target RNA, thereby interfering with RNA splicing, transcription, translation, and replication. Antisense DNA has the added feature that DNA-RNA hybrids serve as a substrate for digestion by ribonuclease H, an activity that is present in most cell types. Antisense molecules can be delivered into cells, as is the case for oligodeoxynucleotides (ODNs), or they can be expressed from endogenous genes as RNA molecules. The FDA recently approved an antisense drug, VITRA VENE™ (for treatment of cytomegalovirus retinitis), reflecting that antisense has therapeutic utility.

SUMMARY

[0003] This Summary is provided to present a summary of the invention to briefly indicate the nature and substance of the invention. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims.

[0004] In a preferred embodiment, a composition comprises one or more antisense oligonucleotides which bind to sense and/or antisense globin polynucleotides.

[0005] In another preferred embodiment, the oligonucleotides comprise one or more modified or substituted nucleobases.

[0006] In another preferred embodiment, the oligonucleotides comprise one or more modified bonds.

[0007] In yet another embodiment, the modified nucleobases comprise modified bases comprising phosphorothioate, methylphosphonate, peptide nucleic acids, or locked nucleic acid (LNA) molecules. Preferably, the modified nucleobases are locked nucleic acid molecules, including α-L-LNA.

[0008] In another preferred embodiment, the oligonucleotides are administered to a patient subcutaneously, intra-muscularly, intra- venously or intra-peritoneally.

[0009] In another preferred embodiment, the oligonucleotides are administered in a pharmaceutical composition. A treatment regimen comprises administering the antisense compounds at least once to patient, however, this treatment can be modified to include multiple doses over a period of time. The treatment can be combined with one or more other types of therapies.

[0010] In another preferred embodiment, the oligonucleotides are encapsulated in a liposome.

[0011] Other aspects are described *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Figure 1 is a graph of real time PCR results showing that the levels of HBF mRNA in HepG2 cells were significantly increased 48 h after treatment with two of the siRNAs designed to HBF antisense Hs.702397.

DETAILED DESCRIPTION

[0013] Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. The present invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders

and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention.

Definitions

[0014] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[0015] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0016] As used herein, the term "mRNA" means the presently known mRNA transcript(s) of a targeted gene, and any further transcripts which may be elucidated.

[0017] By "antisense oligonucleotides" or "antisense compound" is meant an RNA or DNA molecule that binds to another RNA or DNA (target RNA, DNA). For example, if the oligonucleotide is an RNA and the target is an RNA then the RNA oligonucleotide binds to the RNA target by means of RNA-RNA interactions and alters the activity of that target RNA (Eguchi *et al.*, 1991 *Ann. Rev. Biochem.* 60, 631-652). An antisense oligonucleotide can upregulate or downregulate expression and/or function of a particular polynucleotide. The definition is meant to include any foreign RNA or DNA molecule which is useful from a

therapeutic, diagnostic, or other viewpoint. Such molecules include, for example, antisense RNA or DNA molecules, interference RNA (RNAi), micro RNA, decoy RNA molecules, siRNA, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

[0018] In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. The term "oligonucleotide", also includes linear or circular oligomers of natural and/or modified monomers or linkages, including deoxyribonucleosides, ribonucleosides, substituted and alpha-anomeric forms thereof, peptide nucleic acids (PNA), ed nucleic acids (LNA), phosphorothioate, methylphosphonate, and the like. Oligonucleotides are capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick type of base pairing, Hoögsteen or reverse Hoögsteen types of base pairing, or the like.

[0019] The antisense oligonucleotide may be "chimeric", that is, composed of different regions. In the context of this invention "chimeric" compounds are oligonucleotides, which contain two or more chemical regions, for example, DNA region(s), RNA region(s), PNA region(s) etc. Each chemical region is made up of at least one monomer unit, *i.e.*, a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically comprise at least one region wherein the oligonucleotide is modified in order to exhibit one or more desired properties. The desired properties of the oligonucleotide include, but are not limited, for example, to increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. Different regions of the oligonucleotide may therefore have different properties. The chimeric oligonucleotides of the present invention can be formed as mixed structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide analogs as described above.

[0020] The oligonucleotide can be composed of regions that can be linked in "register", that is, when the monomers are linked consecutively, as in native DNA, or linked via spacers. The spacers are intended to constitute a covalent "bridge" between the regions and have in preferred cases a length not exceeding about 100 carbon atoms. The spacers may carry different functionalities, for example, having positive or negative charge, carry special nucleic acid binding properties (intercalators, groove binders, toxins, fluorophors etc.), being lipophilic, inducing special secondary structures like, for example, alanine containing peptides that induce alpha-helices.

[0021] As used herein "HBF/HBG1" is inclusive of mutants, variants, alleles, sense and antisense polynucleotide strands, etc. of HBF/HBG1 genes.

[0022] As used herein, the term "oligonucleotide specific for" or "oligonucleotide targets" refers to an oligonucleotide having a sequence (i) capable of forming a stable complex with a portion of the targeted gene, or (ii) capable of forming a stable duplex with a portion of a mRNA transcript of the targeted gene.

[0023] As used herein, the terms "target nucleic acid" and encompass DNA, RNA (comprising pre-mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds, which specifically hybridize to it, is generally referred to as "antisense". The functions of DNA to be interfered include, for example, replication and transcription. The functions of RNA to be interfered, include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of an encoded product or oligonucleotides.

[0024] RNA interference "RNAi" is mediated by double stranded RNA (dsRNA) molecules that have sequence-specific homology to their "target" nucleic acid sequences (Caplen, N. J., *et al., Proc. Natl. Acad. Sci.* USA 98:9742-9747 (2001)). In certain embodiments of the present invention, the mediators of RNA-dependent gene silencing are 21-25 nucleotide "small

interfering" RNA duplexes (siRNAs). The siRNAs are derived from the processing of dsRNA by an RNase enzyme known as Dicer (Bernstein, E., *et al.*, *Nature* 409:363-366 (2001)). siRNA duplex products are recruited into a multi-protein siRNA complex termed RISC (RNA Induced Silencing Complex). Without wishing to be bound by any particular theory, a RISC is then believed to be guided to a target nucleic acid (suitably mRNA), where the siRNA duplex interacts in a sequence-specific way to mediate cleavage in a catalytic fashion (Bernstein, E., *et al.*, *Nature* 409:363-366 (2001); Boutla, A., *et al.*, *Curr. Biol.* 11:1776-1780 (2001)). Small interfering RNAs that can be used in accordance with the present invention can be synthesized and used according to procedures that are well known in the art and that will be familiar to the ordinarily skilled artisan. Small interfering RNAs for use in the methods of the present invention suitably comprise between about 0 to about 50 nucleotides (nt). In examples of nonlimiting embodiments, siRNAs can comprise about 5 to about 40 nt, about 5 to about 30 nt, about 10 to about 30 nt, about 15 to about 25 nt, or about 20-25 nucleotides.

[0025] Selection of appropriate RNAi is facilitated by using computer programs that automatically align nucleic acid sequences and indicate regions of identity or homology. Such programs are used to compare nucleic acid sequences obtained, for example, by searching databases such as GenBank or by sequencing PCR products. Comparison of nucleic acid sequences from a range of species allows the selection of nucleic acid sequences that display an appropriate degree of identity between species. In the case of genes that have not been sequenced, Southern blots are performed to allow a determination of the degree of identity between genes in target species and other species. By performing Southern blots at varying degrees of stringency, as is well known in the art, it is possible to obtain an approximate measure of identity. These procedures allow the selection of RNAi that exhibit a high degree of complementarity to target nucleic acid sequences in a subject to be controlled and a lower degree of complementarity to corresponding nucleic acid sequences in other species. One skilled in the art will realize that there is considerable latitude in selecting appropriate regions of genes for use in the present invention.

[0026] By "enzymatic RNA" is meant an RNA molecule with enzymatic activity (Cech, 1988 *J. American. Med. Assoc.* 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of a

enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

[0027] By "decoy RNA" is meant an RNA molecule that mimics the natural binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA (Sullenger *et al.*, 1990, *Cell*, 63, 601-608). This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

[0028] As used herein, the term "monomers" typically indicates monomers linked by phosphodiester bonds or analogs thereof to form oligonucleotides ranging in size from a few monomeric units, *e.g.*, from about 3-4, to about several hundreds of monomeric units. Analogs of phosphodiester linkages include: phosphorothioate, phosphorodithioate, methylphosphornates, phosphoroselenoate, phosphoramidate, and the like, as more fully described below.

[0029] In the present context, the terms "nucleobase" and "nucleotides" or "nucleosides" are used interchangeably herein and the terms cover naturally occurring nucleobases as well as non-naturally occurring nucleobases. It should be clear to the person skilled in the art that various nucleobases which previously have been considered "non-naturally occurring" have subsequently been found in nature. Thus, "nucleobase" includes not only the known purine and pyrimidine heterocycles, but also heterocyclic analogues and tautomers thereof. Illustrative examples of nucleobases are adenine, guanine, thymine, cytosine, uracil, purine, xanthine, diaminopurine, 8-oxo-N^6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N^4,N^4-ethanocytosin, N^6,N^6-ethano-2,6-diaminopurine, 5-methylcytosine, 5-(C^3-C^6)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine, isoguanin, inosine and the "non-naturally occurring" nucleobases described in Benner *et al.*, U.S. Pat No. 5,432,272. The term "nucleobase" is intended to cover every and all of these examples as well as analogues and

tautomers thereof. Especially interesting nucleobases are adenine, guanine, thymine, cytosine, and uracil, which are considered as the naturally occurring nucleobases in relation to therapeutic and diagnostic application in humans. Nucleoside includes the natural nucleosides, including 2'-deoxy and 2'-hydroxyl forms, *e.g.*, as described in Kornberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992).

[0030] "Analogs" in reference to nucleosides includes synthetic nucleosides having modified base moieties and/or modified sugar moieties, *e.g.*, described generally by Scheit, Nucleotide Analogs, John Wiley, New York, 1980; Freier & Altmann, *Nucl. Acid. Res.*, 1997, 25(22), 4429-4443, Toulmé, J.J., *Nature Biotechnology* 19:17-18 (2001); Manoharan M., *Biochemica et Biophysica Acta* 1489:117-139(1999); Freier S. M., *Nucleic Acid Research*, 25:4429-4443 (1997), Uhlman, E., *Drug Discovery & Development*, 3: 203-213 (2000), Herdewin P., *Antisense & Nucleic Acid Drug Dev.*, 10:297-310 (2000),); 2'-O, 3`-C-linked [3.2.0] bicycloarabinonucleosides (see *e.g.* N.K Christiensen., *et al*, *J. Am. Chem. Soc.*, 120: 5458-5463 (1998). Such analogs include synthetic nucleosides designed to enhance binding properties, *e.g.*, duplex or triplex stability, specificity, or the like.

[0031] As used herein, "hybridization" means the pairing of substantially complementary strands of oligomeric compounds. One mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoögsteen or reversed Hoögsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleobases) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

[0032] An antisense compound is "specifically hybridizable" when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, *i.e.*, under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and under conditions in which assays are performed in the case of *in vitro* assays.

[0033] As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a compound of the invention will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances and in the context of this invention, "stringent conditions" under which oligomeric compounds hybridize to a target sequence are determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated. In general, stringent hybridization conditions comprise low concentrations (<0.15M) of salts with inorganic cations such as Na^{++} or K^{++} (*i.e.*, low ionic strength), temperature higher than 20°C - 25° C. below the Tm of the oligomeric compound:target sequence complex, and the presence of denaturants such as formamide, dimethylformamide, dimethyl sulfoxide, or the detergent sodium dodecyl sulfate (SDS). For example, the hybridization rate decreases 1.1% for each 1% formamide. An example of a high stringency hybridization condition is 0.1X sodium chloride-sodium citrate buffer (SSC)/0.1% (w/v) SDS at 60° C. for 30 minutes.

[0034] "Complementary," as used herein, refers to the capacity for precise pairing between two nucleobases on one or two oligomeric strands. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, said target nucleic acid being a DNA, RNA, or oligonucleotide molecule, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligomeric compound and the further DNA, RNA, or oligonucleotide molecule are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleobases which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleobases such that stable and specific binding occurs between the oligomeric compound and a target nucleic acid.

[0035] It is understood in the art that the sequence of an oligomeric compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligonucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (*e.g.*, a loop structure, mismatch or hairpin

structure). The oligomeric compounds of the present invention comprise at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403-410; Zhang and Madden, *Genome Res.*, 1997, 7, 649-656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, 1981, 2, 482-489).

[0036] As used herein, the term "Thermal Melting Point (Tm)" refers to the temperature, under defined ionic strength, pH, and nucleic acid concentration, at which 50% of the oligonucleotides complementary to the target sequence hybridize to the target sequence at equilibrium. As the target sequences are generally present in excess, at Tm, 50% of the oligonucleotides are occupied at equilibrium). Typically, stringent conditions will be those in which the salt concentration is at least about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30ºC. for short oligonucleotides (*e.g.*, 10 to 50 nucleotide). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

[0037] As used herein, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene.

[0038] The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to a wild type gene. This definition may also include, for example, "allelic," "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. Of particular utility in the invention are variants of wild type gene products. Variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes that give rise to variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

[0039] The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs,) or single base mutations in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population with a propensity for a disease state, that is susceptibility versus resistance.

[0040] Derivative polynucleotides include nucleic acids subjected to chemical modification, for example, replacement of hydrogen by an alkyl, acyl, or amino group. Derivatives, *e.g.*, derivative oligonucleotides, may comprise non-naturally-occurring portions, such as altered sugar moieties or inter-sugar linkages. Exemplary among these are phosphorothioate and other sulfur containing species which are known in the art. Derivative nucleic acids may also contain labels, including radionucleotides, enzymes, fluorescent agents, chemiluminescent agents, chromogenic agents, substrates, cofactors, inhibitors, magnetic particles, and the like.

[0041] A "derivative" polypeptide or peptide is one that is modified, for example, by glycosylation, pegylation, phosphorylation, sulfation, reduction/alkylation, acylation, chemical

coupling, or mild formalin treatment. A derivative may also be modified to contain a detectable label, either directly or indirectly, including, but not limited to, a radioisotope, fluorescent, and enzyme label.

[0042] As used herein, the term "animal" or "patient" is meant to include, for example, humans, sheep, elks, deer, mule deer, minks, mammals, monkeys, horses, cattle, pigs, goats, dogs, cats, rats, mice, birds, chicken, reptiles, fish, insects and arachnids.

[0043] "Mammal" covers warm blooded mammals that are typically under medical care (*e.g.*, humans and domesticated animals). Examples include feline, canine, equine, bovine, and human, as well as just human.

[0044] "Treating" or "treatment" covers the treatment of a disease-state in a mammal, and includes: (a) preventing the disease-state from occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it; (b) inhibiting the disease-state, *e.g.*, arresting it development; and/or (c) relieving the disease-state, *e.g.*, causing regression of the disease state until a desired endpoint is reached. Treating also includes the amelioration of a symptom of a disease (*e.g.*, lessen the pain or discomfort), wherein such amelioration may or may not be directly affecting the disease (*e.g.*, cause, transmission, expression, etc.).

Polynucleotide and Oligonucleotide Compositions and Molecules

[0045] In preferred embodiments, antisense oligonucleotides are used to prevent or treat diseases or disorders associated with abnormal globin gene expression or function. The gamma globin genes (HBG1 and HBG2) are normally expressed in the fetal liver, spleen and bone marrow. Two gamma chains together with two alpha chains constitute fetal hemoglobin (HbF) which is normally replaced by adult hemoglobin (HbA) at birth. In some beta-thalassemias and related conditions, gamma chain production continues into adulthood. The two types of gamma chains differ at residue 136 where glycine is found in the G-gamma product (HBG2) and alanine is found in the A-gamma product (HBG1). The former is predominant at birth. The order of the genes in the beta-globin cluster is: 5'-epsilon -- gamma-G -- gamma-A -- delta -- beta--3'.

[0046] Diseases or disorders associated with abnormal globin expression and/or function include, for example, anemias, such as for example sickle cell anemia, thalassemia, and the like. Sickle cell disease is a systemic disorder that is caused by a mutation (Glu6Val) in the gene that encodes β globin. The sickle hemoglobin molecule (HbS) is a tetramer of two α-globin chains and two sickle β-globin chains, and has the tendency to polymerize when deoxygenated. HbS facilitates abnormal interactions between the sickle erythrocyte and leukocytes and endothelial cells, which trigger a complex pathobiology. This multifaceted pathophysiology provides the opportunity to interrupt the disease at multiple sites, including polymerization of HbS, erythrocyte density and cell–cell interactions. For example, it is possible to induce higher concentrations of fetal hemoglobin, which disrupts the pathology-initiating step of HbS polymerization. In some embodiments, treatment of a patient comprises administration of one or more antisense oligonucleotides to a patient. The treatment can be combined with one or more therapies. For example, improving the hydration of sickle erythrocytes and agents to counteract the endothelial, inflammatory and oxidative abnormalities of sickle cell disease.

[0047] An antisense compound is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired. Such conditions include, *i.e.*, physiological conditions in the case of *in vivo* assays or therapeutic treatment, and conditions in which assays are performed in the case of *in vitro* assays.

[0048] An antisense compound, whether DNA, RNA, chimeric, substituted etc, is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarily to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, *i.e.*, under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and in the case of *in vitro* assays, under conditions in which the assays are performed.

[0049] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in

the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

[0050] In embodiments of the present invention oligomeric antisense compounds, particularly oligonucleotides, bind to target nucleic acid molecules and modulate the expression and/or function of molecules encoded by a target gene. The functions of DNA to be interfered comprise, for example, replication and transcription. The functions of RNA to be interfered comprise all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The functions may be up-regulated or inhibited depending on the functions desired.

[0051] The antisense compounds, include, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

[0052] Targeting an antisense compound to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. This target nucleic acid may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target nucleic acid encodes HBF/HBG.

[0053] The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, *e.g.*, modulation of expression, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic

acid. "Sites," as used in the present invention, are defined as positions within a target nucleic acid.

[0054] In a preferred embodiment, the antisense oligonucleotides bind to the natural antisense sequences of HBF/HBG and modulates the expression of the globin gene. For example fetal hemoglobin. For example, a natural antisense sequence of HBF comprises SEQ ID NO: 2 and variants thereof.

[0055] In another preferred embodiment, the antisense oligonucleotides bind to one or more segments of HBF/HBG polynucleotides. The segments comprise at least five consecutive nucleobases of the HBF/HBG sense or antisense polynucleotides.

[0056] Fetal hemoglobin (HBF) is the main oxygen transport protein in the fetus during the last seven months of development in the uterus and in the newborn until roughly 6 months old. Functionally, fetal hemoglobin differs most from adult hemoglobin in that it is able to bind oxygen with greater affinity than the adult form, giving the developing fetus better access to oxygen from the mother's bloodstream. In newborns, fetal hemoglobin is nearly completely replaced by adult hemoglobin by approximately the twelfth week of postnatal life. In adults, fetal hemoglobin production can be reactivated by the compositions described herein, which is useful in the treatment of such diseases as sickle-cell disease.

[0057] When fetal hemoglobin production is switched off after birth, normal children begin producing adult hemoglobin (HbA) but children with sickle-cell disease instead begin producing a defective form of hemoglobin called hemoglobin S. This variety of hemoglobin aggregates, forming filaments and so causes red blood cells to change their shape from round to sickle-shaped, which have a greater tendency to stack on top of one another and crowd blood vessels. These invariably lead to so-called painful vaso-occlusive episodes, which are a hallmark of the disease. If fetal hemoglobin remains the predominant form of hemoglobin after birth, however, the number of painful episodes decreases in patients with sickle cell anemia. Hydroxyurea, used also as an anti-cancer drug, is a viable treatment for sickle cell anemia, as it promotes the production of fetal hemoglobin while inhibiting sickling due to hemoglobin S polymerization.

[0058] Thus in some embodiments, treatment of anemia comprises administration of antisense oligonucleotides to elevate hemoglobin F levels and to promote the development of HbF-containing F-cells.

[0059] Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes has a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG; and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function *in vivo*. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). Eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate translation of an mRNA transcribed from a gene encoding globin molecules, regardless of the sequence(s) of such codons. A translation termination codon (or "stop codon") of a gene may have one of three sequences, *i.e.*, 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively).

[0060] The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (*i.e.*, 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (*i.e.*, 5' or 3') from a translation termination codon. Consequently, the "start codon region" (or "translation initiation codon region") and the "stop codon region" (or "translation termination codon region") are all regions that may be targeted effectively with the antisense compounds of the present invention.

[0061] The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is

also a region which may be targeted effectively. Within the context of the present invention, a targeted region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

[0062] Another target region includes the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene). Still another target region includes the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap site. Another target region for this invention is the 5' cap region.

[0063] Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. In one embodiment, targeting splice sites, *i.e.*, intron-exon junctions or exon-intron junctions, is particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular splice product is implicated in disease. An aberrant fusion junction due to rearrangement or deletion is another embodiment of a target site. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources are known as "fusion transcripts". Introns can be effectively targeted using antisense compounds targeted to, for example, DNA or pre-mRNA.

[0064] In another preferred embodiment, the antisense oligonucleotides bind to coding and/or non-coding regions of a target polynucleotide and modulate the expression and/or function of the target molecule.

[0065] In another preferred embodiment, the antisense oligonucleotides bind to natural antisense polynucleotides and modulate the expression and/or function of the target molecule.

[0066] In another preferred embodiment, the antisense oligonucleotides bind to sense polynucleotides and modulate the expression and/or function of the target molecule.

[0067] Alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants". More specifically, "pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence.

[0068] Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller "mRNA variants". Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants". If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

[0069] Variants can be produced through the use of alternative signals to start or stop transcription. Pre-mRNAs and mRNAs can possess more that one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. Within the context of the invention, the types of variants described herein are also embodiments of target nucleic acids.

[0070] The locations on the target nucleic acid to which the antisense compounds hybridize are defined as at least a 5-nucleobase portion of a target region to which an active antisense compound is targeted.

[0071] While the specific sequences of certain exemplary target segments are set forth herein, one of skill in the art will recognize that these serve to illustrate and describe particular embodiments within the scope of the present invention. Additional target segments are readily identifiable by one having ordinary skill in the art in view of this disclosure.

[0072] Target segments 5-100 nucleobases in length comprising a stretch of at least five (5) consecutive nucleobases selected from within the illustrative preferred target segments are considered to be suitable for targeting as well.

[0073] Target segments can include DNA or RNA sequences that comprise at least the 5 consecutive nucleobases from the 5'-terminus of one of the illustrative preferred target segments (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately upstream of the 5'-terminus of the target segment and continuing until the DNA or RNA contains about 5 to about 100 nucleobases). Similarly preferred target segments are represented by DNA or RNA sequences that comprise at least the 5 consecutive nucleobases from the 3'-terminus of one of the illustrative preferred target segments (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the DNA or RNA contains about 5 to about 100 nucleobases). One having skill in the art armed with the target segments illustrated herein will be able, without undue experimentation, to identify further preferred target segments.

[0074] Once one or more target regions, segments or sites have been identified, antisense compounds are chosen which are sufficiently complementary to the target, *i.e.*, hybridize sufficiently well and with sufficient specificity, to give the desired effect.

[0075] In embodiments of the invention the oligonucleotides bind to an antisense strand of a particular target. The oligonucleotides are at least 5 nucleotides in length and can be synthesized so each oligonucleotide targets overlapping sequences such that oligonucleotides are synthesized so as to cover the entire length of the target polynucleotide. The targets also include coding as well as non coding regions.

[0076] In one embodiment, it is preferred to target specific nucleic acids by antisense oligonucleotides. Targeting an antisense compound to a particular nucleic acid, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a non coding polynucleotide such as for example, non coding RNA (ncRNA).

[0077] RNAs can be classified into (1) messenger RNAs (mRNAs), which are translated into proteins, and (2) non-protein-coding RNAs (ncRNAs). ncRNAs comprise microRNAs, antisense transcripts and other Transcriptional Units (TU) containing a high density of stop codons and lacking any extensive "Open Reading Frame". Many ncRNAs appear to start from initiation sites in 3' untranslated regions (3'UTRs) of protein-coding loci. ncRNAs are often rare and at least half of the ncRNAs that have been sequenced by the FANTOM consortium seem not to be polyadenylated. Most researchers have for obvious reasons focused on polyadenylated mRNAs that are processed and exported to the cytoplasm. Recently, it was shown that the set of non-polyadenylated nuclear RNAs may be very large, and that many such transcripts arise from so-called intergenic regions (Cheng, J. *et al.* (2005) Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. *Science* 308 (5725), 1149-1154; Kapranov, P. *et al.* (2005). Examples of the complex architecture of the human transcriptome revealed by RACE and high-density tiling arrays. *Genome Res* 15 (7), 987-997). The most common mechanism by which ncRNAs regulate gene expression is by base-pairing with target transcripts. The RNAs that function by base pairing can be grouped into (1) cis-encoded RNAs that are encoded at the same genetic location, but on the opposite strand to the RNAs they act upon and therefore display perfect complementarity to their target, and (2) trans-encoded RNAs that are encoded at a chromosomal location distinct from the RNAs they act upon and generally do not exhibit perfect base-pairing potential with their targets.

[0078] Without wishing to be bound by theory, perturbation of an antisense polynucleotide by the antisense oligonucleotides or RNA compounds described herein, can alter the expression of the corresponding sense messenger RNAs. However, this regulation can either be discordant (antisense knockdown results in sense transcript elevation) or concordant (antisense knockdown results in concomitant sense transcript reduction). In these cases, antisense oligonucleotides can

be targeted to overlapping or non-overlapping parts of the antisense strand resulting in knockdown of the target. Coding as well as non-coding antisense can be targeted in an identical manner and that either category is capable of regulating the corresponding sense transcripts – either in a concordant or disconcordant manner. The strategies that are employed in identifying new oligonucleotides for use against a target can be based on the knockdown of antisense RNA transcripts by antisense oligonucleotides or any other means for modulating the desired target.

[0079] *Strategy 1*: In the case of discordant regulation, knocking down the antisense transcript elevates the expression of the conventional (sense) gene. Should that latter gene encode for a known or putative drug target, then knockdown of its antisense counterpart could conceivably mimic the action of a receptor agonist or an enzyme stimulant.

[0080] *Strategy 2*: In the case of concordant regulation, one could concomitantly knock down both antisense and sense transcripts and thereby achieve synergistic reduction of the conventional (sense) gene expression. If, for example, an antisense oligonucleotide is used to achieve knockdown, then this strategy can be used to apply one antisense oligonucleotide targeted to the sense transcript and another antisense oligonucleotide to the corresponding antisense transcript, or a single energetically symmetric antisense oligonucleotide that simultaneously targets overlapping sense and antisense transcripts.

[0081] According to the present invention, antisense compounds include antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially

double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleobase positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines, however, in some embodiments, the gene expression or function is up regulated. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

[0082] Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect cleavage or other modification of the target nucleic acid or may work via occupancy-based mechanisms. In general, nucleic acids (including oligonucleotides) may be described as "DNA-like" (*i.e.*, generally having one or more 2'-deoxy sugars and, generally, T rather than U bases) or "RNA-like" (*i.e.*, generally having one or more 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-like structure are "DNA-like" and those which have A-form-like structure are "RNA-like." In some (chimeric) embodiments, an antisense compound may contain both A- and B-form regions.

[0083] In another preferred embodiment, the desired oligonucleotides or antisense compounds, comprise at least one of: antisense RNA oligonucleotides; antisense DNA oligonucleotides; chimeric antisense oligonucleotides; antisense oligonucleotides comprising modified linkages; interference RNA (RNAi); short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA);

small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof.

[0084] dsRNA can also activate gene expression, a mechanism that has been termed "small RNA-induced gene activation" or RNAa. dsRNAs targeting gene promoters induce potent transcriptional activation of associated genes. RNAa was demonstrated in human cells using synthetic dsRNAs, termed "small activating RNAs" (saRNAs). It is currently not known whether RNAa is conserved in other organisms.

[0085] Small double-stranded RNA (dsRNA), such as small interfering RNA (siRNA) and microRNA (miRNA), have been found to be the trigger of an evolutionary conserved mechanism known as RNA interference (RNAi). RNAi invariably leads to gene silencing via remodeling chromatin to thereby suppress transcription, degrading complementary mRNA, or blocking protein translation. dsRNAs may also act as small activating RNAs (saRNA). Without wishing to be bound by theory, by targeting sequences in gene promoters, saRNAs would induce target gene expression in a phenomenon referred to as dsRNA-induced transcriptional activation (RNAa).

[0086] In a further embodiment, the "preferred target segments" identified herein may be employed in a screen for additional compounds that modulate the expression of HBF/HBG polynucleotides. "Modulators" are those compounds that decrease or increase the expression of a nucleic acid molecule encoding HBF/HBG and which comprise at least a 5-nucleobase portion that is complementary to a preferred target segment. The screening method comprises the steps of contacting a preferred target segment of a nucleic acid molecule encoding HBF/HBG polynucleotides with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the expression of a nucleic acid molecule encoding HBF/HBG polynucleotides. Once it is shown that the candidate modulator or modulators are capable of modulating (*e.g.* either decreasing or increasing) the expression of a nucleic acid molecule encoding HBF/HBG polynucleotides, the modulator may then be employed in further investigative studies of the function of HBF/HBG polynucleotides, or for use as a research, diagnostic, or therapeutic agent in accordance with the present invention.

[0087] The preferred target segments of the present invention may be also be combined with their respective complementary antisense compounds of the present invention to form stabilized double-stranded (duplexed) oligonucleotides.

[0088] Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation as well as RNA processing via an antisense mechanism. Moreover, the double-stranded moieties may be subject to chemical modifications (Fire *et al.*, *Nature*, 1998, 391, 806-811; Timmons and Fire, *Nature* 1998, 395, 854; Timmons *et al.*, *Gene*, 2001, 263, 103-112; Tabara *et al.*, *Science*, 1998, 282, 430-431; Montgomery *et al.*, *Proc. Natl. Acad. Sci.* USA, 1998, 95, 15502-15507; Tuschl *et al.*, *Genes Dev.*, 1999, 13, 3191-3197; Elbashir *et al.*, *Nature*, 2001, 411, 494-498; Elbashir *et al.*, *Genes Dev.* 2001, 15, 188-200). For example, such double-stranded moieties have been shown to inhibit the target by the classical hybridization of antisense strand of the duplex to the target, thereby triggering enzymatic degradation of the target (Tijsterman *et al.*, *Science*, 2002, 295, 694-697).

[0089] In a preferred embodiment, an antisense oligonucleotide targets HBF/HBG polynucleotides (*e.g.* accession number NM_000559), variants, alleles, isoforms, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense molecule.

[0090] In accordance with embodiments of the invention, the target nucleic acid molecule is not limited to HBF/HBG polynucleotides alone but extends to any of the isoforms, homologs and the like of globin family members.

[0091] In another preferred embodiment, an RNA oligonucleotide targets a natural antisense sequence of HBF/HBG polynucleotides, for example, polynucleotides set forth as SEQ ID NO: 2, and any variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Examples of antisense oligonucleotides are set forth as SEQ ID NOS: 3 to 5.

[0092] In one embodiment, the oligonucleotides are complementary to or bind to nucleic acid sequences of HBF/HBG antisense, including without limitation noncoding sequences associated with globin polynucleotides and modulate expression and/or function of HBF/HBG polynucleotides.

[0093] In another preferred embodiment, the oligonucleotides are complementary to or bind to nucleic acid sequences of HBF/HBG natural antisense, set forth as SEQ ID NO: 2 and the oligonucleotides modulate expression and/or function of HBF/HBG polynucleotides.

[0094] In a preferred embodiment, oligonucleotides comprise sequences of at least 5 consecutive nucleobases of SEQ ID NOS: 1 to 5.

[0095] The polynucleotide targets comprise globin genes, including family members thereof, variants of HBF/HBG; mutants of HBF/HBG, including SNPs; noncoding sequences of HBF/HBG; alleles of HBF/HBG; species variants, fragments and the like. Preferably the oligonucleotide is an antisense molecule.

[0096] In another preferred embodiment, the oligonucleotide binding to HBF/HBG polynucleotides, comprise: antisense RNA, interference RNA (RNAi), short interfering RNA (siRNA); micro interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); or, small activating RNA (saRNA).

[0097] In another preferred embodiment, targeting of globin polynucleotides, *e.g.* SEQ ID NOS: 1 to 3, NM_001110, modulates the expression or function of these targets. In one embodiment, expression or function is up-regulated as compared to a control. In another preferred embodiment, expression or function is down-regulated as compared to a control.

[0098] In another preferred embodiment, antisense compounds comprise polynucleotides set forth as SEQ ID NOS: 2 to 5. These oligonucleotides can comprise one or more modified nucleobases, shorter or longer fragments, modified bonds and the like.

[0099] In another preferred embodiment, SEQ ID NOS: 2 to 5 comprise one or more LNA nucleobases.

[00100] The modulation of a desired target nucleic acid can be carried out in several ways known in the art. For example, antisense oligonucleotides, siRNA etc. Enzymatic nucleic acid molecules (*e.g.*, ribozymes) are nucleic acid molecules capable of catalyzing one or more of a variety of reactions, including the ability to repeatedly cleave other separate nucleic acid molecules in a nucleotide base sequence-specific manner. Such enzymatic nucleic acid

molecules can be used, for example, to target virtually any RNA transcript (Zaug *et al.*, 324, *Nature* 429 1986; Cech, 260 *JAMA* 3030, 1988; and Jefferies *et al.*, 17 *Nucleic Acids Research* 1371, 1989).

[00101] Because of their sequence-specificity, trans-cleaving enzymatic nucleic acid molecules show promise as therapeutic agents for human disease (Usman & McSwiggen, 1995 *Ann. Rep. Med. Chem.* 30, 285-294; Christoffersen and Marr, 1995 *J. Med. Chem.* 38, 2023-2037). Enzymatic nucleic acid molecules can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the mRNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited.

[00102] In general, enzymatic nucleic acids with RNA cleaving activity act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

[00103] Several approaches such as *in vitro* selection (evolution) strategies (Orgel, 1979, *Proc. R. Soc.* London, B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing a variety of reactions, such as cleavage and ligation of phosphodiester linkages and amide linkages, (Joyce, 1989, *Gene*, 82, 83-87; Beaudry *et al.*, 1992, *Science* 257, 635-641; Joyce, 1992, Scientific American 267, 90-97; Breaker *et al.*, 1994, *TIBTECH* 12, 268; Bartel *et al.*, 1993, *Science* 261:1411-1418; Szostak, 1993, *TIBS* 17, 89-93; Kumar *et al.*, 1995, *FASEB J.*, 9, 1183; Breaker, 1996, *Curr. Op. Biotech.*, 7, 442).

[00104] The development of ribozymes that are optimal for catalytic activity would contribute significantly to any strategy that employs RNA-cleaving ribozymes for the purpose of regulating gene expression. The hammerhead ribozyme, for example, functions with a catalytic rate (k_{cat})

of about 1 min^{-1} in the presence of saturating (10 mM) concentrations of Mg^{2+} cofactor. An artificial "RNA ligase" ribozyme has been shown to catalyze the corresponding self-modification reaction with a rate of about 100 min^{-1}. In addition, it is known that certain modified hammerhead ribozymes that have substrate binding arms made of DNA catalyze RNA cleavage with multiple turn-over rates that approach 100 min^{-1}. Finally, replacement of a specific residue within the catalytic core of the hammerhead with certain nucleotide analogues gives modified ribozymes that show as much as a 10-fold improvement in catalytic rate. These findings demonstrate that ribozymes can promote chemical transformations with catalytic rates that are significantly greater than those displayed *in vitro* by most natural self-cleaving ribozymes. It is then possible that the structures of certain self-cleaving ribozymes may be optimized to give maximal catalytic activity, or that entirely new RNA motifs can be made that display significantly faster rates for RNA phosphodiester cleavage.

[00105] Intermolecular cleavage of an RNA substrate by an RNA catalyst that fits the "hammerhead" model was first shown in 1987 (Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600). The RNA catalyst was recovered and reacted with multiple RNA molecules, demonstrating that it was truly catalytic.

[00106] Catalytic RNAs designed based on the "hammerhead" motif have been used to cleave specific target sequences by making appropriate base changes in the catalytic RNA to maintain necessary base pairing with the target sequences (Haseloff and Gerlach, *Natur*e, 334, 585 (1988); Walbot and Bruening, *Nature*, 334, 196 (1988); Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600; Koizumi, M., Iwai, S. and Ohtsuka, E. (1988) *FEBS Lett.*, 228: 228-230). This has allowed use of the catalytic RNA to cleave specific target sequences and indicates that catalytic RNAs designed according to the "hammerhead" model may possibly cleave specific substrate RNAs *in vivo*. (see Haseloff and Gerlach, *Nature*, 334, 585 (1988); Walbot and Bruening, *Nature*, 334, 196 (1988); Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600).

[00107] RNA interference (RNAi) has become a powerful tool for blocking gene expression in mammals and mammalian cells. This approach requires the delivery of small interfering RNA (siRNA) either as RNA itself or as DNA, using an expression plasmid or virus and the coding sequence for small hairpin RNAs that are processed to siRNAs. This system enables efficient

transport of the pre-siRNAs to the cytoplasm where they are active and permit the use of regulated and tissue specific promoters for gene expression.

[00108] In a preferred embodiment, an oligonucleotide or antisense compound comprises an oligomer or polymer of ribonucleic acid (RNA) and/or deoxyribonucleic acid (DNA), or a mimetic, chimera, analog or homolog thereof. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often desired over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a target nucleic acid and increased stability in the presence of nucleases.

[00109] According to the present invention, the oligonucleotides or "antisense compounds" include antisense oligonucleotides (*e.g.* RNA, DNA, mimetic, chimera, analog or homolog thereof), ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, saRNA, aRNA, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleobase positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only

one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines (Hammond *et al.*, *Nat. Rev. Genet.*, 1991, 2, 110-119; Matzke *et al.*, *Curr. Opin. Genet. Dev.*, 2001, 11, 221-227; Sharp, *Genes Dev.*, 2001, 15, 485-490). When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

[00110] Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect cleavage or other modification of the target nucleic acid or may work via occupancy-based mechanisms. In general, nucleic acids (including oligonucleotides) may be described as "DNA-like" (*i.e.*, generally having one or more 2'-deoxy sugars and, generally, T rather than U bases) or "RNA-like" (*i.e.*, generally having one or more 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-like structure are "DNA-like" and those which have A-form-like structure are "RNA-like." In some (chimeric) embodiments, an antisense compound may contain both A- and B-form regions.

[00111] The antisense compounds in accordance with this invention comprise an antisense portion from about 5 to about 80 nucleobases (*i.e.* from about 5 to about 80 linked nucleosides) in length. This refers to the length of the antisense strand or portion of the antisense compound. In other words, a single-stranded antisense compound of the invention comprises from 5 to about 80 nucleobases, and a double-stranded antisense compound of the invention (such as a dsRNA, for example) comprises an antisense strand or portion of 5 to about 80 nucleobases in length. One of ordinary skill in the art will appreciate that this comprehends antisense portions of 5, 6, 7,8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleobases in length, or any range therewithin.

[00112] In one embodiment, the antisense compounds of the invention have antisense portions of 10 to 50 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligonucleotides having antisense portions of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleobases in length, or any range therewithin. In some embodiments, the oligonucleotides are 15 nucleobases in length.

[00113] In one embodiment, the antisense or oligonucleotide compounds of the invention have antisense portions of 12 or 13 to 30 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies antisense compounds having antisense portions of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleobases in length, or any range therewithin.

[00114] In a preferred embodiment, administration of at least one oligonucleotide targeting any one or more polynucleotides of HBF/HBG, prevents or treats diseases associated with abnormal expression or function of HBF/HBG polynucleotides and encoded products thereof, or other related diseases. Examples of diseases which can be treated with the antisense oligonucleotides comprise thalassemia, sickle cell disease, erythropoiesis, pernicious anemia, anemia's, leukemias, and the like. The oligonucleotides are also preventative in that a patient at risk of developing, for example thalassemia can be administered one or more antisense polynucleotides to prevent the disease or disorder. The oligonucleotides can also be administered with other agents as part of treatment.

[00115] In another preferred embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenosine, variants may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the antisense or dsRNA compounds. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

[00116] In some embodiments, homology, sequence identity or complementarity, between the antisense compound, for example SEQ ID NOS: 2 to 5 and target is from about 50% to about

60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

[00117] In another preferred embodiment, the antisense oligonucleotides, such as for example, nucleic acid molecules set forth in SEQ ID NOS: 3 to 5 comprise one or more substitutions or modifications. In one embodiment, the nucleobases are substituted with locked nucleic acids (LNA).

[00118] In another preferred embodiment, the oligonucleotides target one or more regions of the target polynucleotides The RNA oligonucleotides are also targeted to overlapping regions of SEQ ID NO: 1, HBF/HBG polynucleotides.

[00119] Certain preferred oligonucleotides of this invention are chimeric oligonucleotides. "Chimeric oligonucleotides" or "chimeras," in the context of this invention, are oligonucleotides which contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one region of modified nucleotides that confers one or more beneficial properties (such as, for example, increased nuclease resistance, increased uptake into cells, increased binding affinity for the target) and a region that is a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of antisense modulation of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art. In one preferred embodiment, a chimeric oligonucleotide comprises at least one region modified to increase target binding affinity, and, usually, a region that acts as a substrate for RNAse H. Affinity of an

oligonucleotide for its target (in this case, a nucleic acid encoding ras) is routinely determined by measuring the T_m of an oligonucleotide/target pair, which is the temperature at which the oligonucleotide and target dissociate; dissociation is detected spectrophotometrically. The higher the T_m, the greater the affinity of the oligonucleotide for the target.

[00120] Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures comprise, but are not limited to, US patent nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922.

[00121] In another preferred embodiment, the region of the oligonucleotide which is modified comprises at least one nucleotide modified at the 2' position of the sugar, most preferably a 2'-O-alkyl, 2'-O-alkyl-O-alkyl or 2'-fluoro-modified nucleotide. In other preferred embodiments, RNA modifications include 2'-fluoro, 2'-amino and 2' O-methyl modifications on the ribose of pyrimidines, abasic residues or an inverted base at the 3' end of the RNA. Such modifications are routinely incorporated into oligonucleotides and these oligonucleotides have been shown to have a higher T_m (*i.e.*, higher target binding affinity) than; 2'-deoxyoligonucleotides against a given target. The effect of such increased affinity is to greatly enhance RNAi oligonucleotide modulation of gene expression. RNAse H is a cellular endonuclease that cleaves the RNA strand of RNA:DNA duplexes; activation of this enzyme therefore results in cleavage of the RNA target, and thus can greatly enhance the efficiency of RNAi inhibition. Cleavage of the RNA target can be routinely demonstrated by gel electrophoresis. In another preferred embodiment, the chimeric oligonucleotide is also modified to enhance nuclease resistance. Cells contain a variety of exo- and endo-nucleases which can degrade nucleic acids. A number of nucleotide and nucleoside modifications have been shown to make the oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide. Nuclease resistance is routinely measured by incubating oligonucleotides with cellular extracts or isolated nuclease solutions and measuring the extent of intact oligonucleotide remaining over time, usually by gel electrophoresis. Oligonucleotides which have been modified to enhance their nuclease resistance

survive intact for a longer time than unmodified oligonucleotides. A variety of oligonucleotide modifications have been demonstrated to enhance or confer nuclease resistance. Oligonucleotides which contain at least one phosphorothioate modification are presently more preferred. In some cases, oligonucleotide modifications which enhance target binding affinity are also, independently, able to enhance nuclease resistance. Some desirable modifications can be found in De Mesmaeker *et al. Acc. Chem. Res.* 1995, 28:366-374.

[00122] Specific examples of some preferred oligonucleotides envisioned for this invention include those comprising modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, particularly CH_2 --NH--O--CH_2, CH,--N(CH_3)--O--CH_2 [known as a methylene(methylimino) or MMI backbone], CH_2 --O--N (CH_3)--CH_2, CH_2 --N (CH_3)--N (CH_3)--CH_2 and O--N (CH_3)--CH_2 --CH_2 backbones, wherein the native phosphodiester backbone is represented as O--P--O--CH,). The amide backbones disclosed by De Mesmaeker *et al. Acc. Chem. Res.* 1995, 28:366-374) are also preferred. Also preferred are oligonucleotides having morpholino backbone structures (Summerton and Weller, U.S. Pat. No. 5,034,506). In other preferred embodiments, such as the peptide nucleic acid (PNA) backbone, the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleobases being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone (Nielsen *et al. Science* 1991, 254, 1497). Oligonucleotides may also comprise one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH, SH, SCH_3, F, OCN, OCH_3 OCH_3, OCH_3 $O(CH_2)_n$ CH_3, $O(CH_2)_n$ NH_2 or $O(CH_2)_n$ CH_3 where n is from 1 to about 10; C_1 to C_{10} lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF_3 ; OCF_3; O--, S--, or N-alkyl; O--, S--, or N-alkenyl; $SOCH_3$; SO_2 CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy [2'-O-CH_2 CH_2 OCH_3, also known as 2'-O-(2-methoxyethyl)] (Martin *et al., Helv. Chim. Acta*, 1995, 78, 486). Other preferred modifications include 2'-methoxy (2'-O--CH_3),

2'-propoxy (2'-OCH_2 CH_2CH_3) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

[00123] Oligonucleotides may also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U). Modified nucleobases include nucleobases found only infrequently or transiently in natural nucleic acids, *e.g.*, hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, as well as synthetic nucleobases, *e.g.*, 2-aminoadenine, 2-(methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalklyamino)adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N_6 (6-aminohexyl)adenine and 2,6-diaminopurine. Kornberg, A., DNA Replication, W. H. Freeman & Co., San Francisco, 1980, pp75-77; Gebeyehu, G., *et al. Nucl. Acids Res.* 1987, 15:4513). A "universal" base known in the art, *e.g.*, inosine, may be included. 5-Me-C substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. (Sanghvi, Y. S., in Crooke, S. T. and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions.

[00124] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, a cholesteryl moiety (Letsinger *et al.*, *Proc. Natl. Acad. Sci.* USA 1989, 86, 6553), cholic acid (Manoharan *et al. Bioorg. Med. Chem. Let.* 1994, 4, 1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al. Ann. N.Y. Acad. Sci.* 1992, 660, 306; Manoharan *et al. Bioorg. Med. Chem. Let.* 1993, 3, 2765), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.* 1992, 20, 533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al. EMBO J.* 1991, 10, 111; Kabanov *et al. FEBS Lett.* 1990, 259, 327; Svinarchuk *et al. Biochimie* 1993, 75, 49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or

triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al. Tetrahedron Lett.* 1995, 36, 3651; Shea *et al. Nucl. Acids Res.* 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan *et al. Nucleosides & Nucleotides* 1995, 14, 969), or adamantane acetic acid (Manoharan *et al. Tetrahedron Lett.* 1995, 36, 3651). Oligonucleotides comprising lipophilic moieties, and methods for preparing such oligonucleotides are known in the art, for example, U.S. Pat. Nos. 5,138,045, 5,218,105 and 5,459,255.

[00125] It is not necessary for all positions in a given oligonucleotide to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single oligonucleotide or even at within a single nucleoside within an oligonucleotide. The present invention also includes oligonucleotides which are chimeric oligonucleotides as hereinbefore defined.

[00126] In another embodiment, the nucleic acid molecule of the present invention is conjugated with another moiety including but not limited to abasic nucleotides, polyether, polyamine, polyamides, peptides, carbohydrates, lipid, or polyhydrocarbon compounds. Those skilled in the art will recognize that these molecules can be linked to one or more of any nucleotides comprising the nucleic acid molecule at several positions on the sugar, base or phosphate group.

[00127] The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of one of ordinary skill in the art. It is also well known to use similar techniques to prepare other oligonucleotides such as the phosphorothioates and alkylated derivatives. It is also well known to use similar techniques and commercially available modified amidites and controlled-pore glass (CPG) products such as biotin, fluorescein, acridine or psoralen-modified amidites and/or CPG (available from Glen Research, Sterling VA) to synthesize fluorescently labeled, biotinylated or other modified oligonucleotides such as cholesterol-modified oligonucleotides.

[00128] In accordance with the invention, use of modifications such as the use of LNA monomers to enhance the potency, specificity and duration of action and broaden the routes of administration of oligonucleotides comprised of current chemistries such as MOE, ANA, FANA, PS etc (ref: Recent advances in the medical chemistry of antisense oligonucleotide by Uhlman, Current Opinions in Drug Discovery & Development 2000 Vol 3 No 2). This can be achieved by substituting some of the monomers in the current oligonucleotides by LNA monomers. The LNA modified oligonucleotide may have a size similar to the parent compound or may be larger or preferably smaller. It is preferred that such LNA-modified oligonucleotides contain less than about 70%, more preferably less than about 60%, most preferably less than about 50% LNA monomers and that their sizes are between about 5 and 25 nucleotides, more preferably between about 12 and 20 nucleotides.

[00129] Preferred modified oligonucleotide backbones comprise, but not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[00130] Representative United States patents that teach the preparation of the above phosphorus-containing linkages comprise, but are not limited to, US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5, 177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455, 233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563, 253; 5,571,799; 5,587,361; and 5,625,050.

[00131] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more

short chain heteroatomic or heterocyclic internucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[00132] Representative United States patents that teach the preparation of the above oligonucleosides comprise, but are not limited to, US patent nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264, 562; 5, 264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596, 086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623, 070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439.

[00133] In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds comprise, but are not limited to, US patent nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference . Further teaching of PNA compounds can be found in o Nielsen *et al.*, Science, 1991, 254, 1497-1500.

[00134] In another preferred embodiment of the invention the oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular-CH_2-NH-O-CH_2-,-CH_2-N (CH_3)-O-CH_2-known as a methylene (methylimino) or MMI backbone,-CH_2-O-N (CH_3)-CH_2-,-CH_2N(CH_3)-N(CH_3) CH_2-and-O-N(CH_3)-CH_2-CH_2- wherein the native phosphodiester backbone is represented as-O-P-O-CH_2- of the above referenced US

patent no. 5,489,677, and the amide backbones of the above referenced US patent no. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced US patent no. 5,034,506.

[00135] Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S-or N-alkynyl; or O alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C to CO alkyl or C_2 to CO alkenyl and alkynyl. Particularly preferred are O $(CH_2)_n$ O_mCH_3, $O(CH_2)_n$,OCH_3, $O(CH_2)_nNH_2$, $O(CH_2)nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_{2n}ON(CH_2)nCH_3)_2$ where n and m can be from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C to CO, (lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification comprises 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78, 486-504) *i.e.*, an alkoxyalkoxy group. A further preferred modification comprises 2'-dimethylaminooxyethoxy, *i.e.* , a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O-CH_2-O-CH_2-N $(CH_2)_2$.

[00136] Other preferred modifications comprise 2'-methoxy (2'-O CH_3), 2'-aminopropoxy (2'-O $CH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures comprise, but are not limited to, US patent nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514, 785; 5,519,134; 5,567,811;

5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646, 265; 5,658,873; 5,670,633; and 5,700,920.

[00137] Oligonucleotides may also comprise nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases comprise the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases comprise other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudo-uracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylquanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[00138] Further, nucleobases comprise those disclosed in United States Patent No. 3,687,808, those disclosed in 'The Concise Encyclopaedia of Polymer Science And Engineering', pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, 1990, those disclosed by Englisch *et al.*, 'Angewandle Chemie, International Edition', 1991, 30, page 613, and those disclosed by Sanghvi, Y.S., Chapter 15, 'Antisense Research and Applications', pages 289-302, Crooke, S.T. and Lebleu, B. ea., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These comprise 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, comprising 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds, 'Antisense Research and Applications', CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[00139] Representative United States patents that teach the preparation of the above noted modified nucleobases as well as other modified nucleobases comprise, but are not limited to, US patent nos. 3,687,808, as well as 4,845,205; 5,130,302; 5,134,066; 5,175, 273; 5, 367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,596,091; 5,614,617; 5,750,692, and 5,681,941.

[00140] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates, which enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide.

[00141] Such moieties comprise but are not limited to, lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acad. Sci.* USA, 1989, 86, 6553-6556), cholic acid (Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, 1994, 4, 1053-1060), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, *Ann. N. Y. Acad. Sci.*, 1992, 660, 306-309; Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, 1993, 3, 2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20, 533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Kabanov *et al.*, *FEBS Lett.*, 1990, 259, 327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75, 49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36, 3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Mancharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14, 969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36, 3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-t oxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.* , 1996, 277, 923-937).

[00142] Representative United States patents that teach the preparation of such oligonucleotide conjugates comprise, but are not limited to, US patent nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552, 538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486, 603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762, 779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082, 830; 5,112,963; 5,214,136; 5,082,830; 5,112,963;

5,214,136; 5, 245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391, 723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5, 565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599, 928 and 5,688,941.

[00143] *Drug discovery*: The compounds of the present invention can also be applied in the areas of drug discovery and target validation. The present invention comprehends the use of the compounds and preferred target segments identified herein in drug discovery efforts to elucidate relationships that exist between globin polynucleotides and a disease state, phenotype, or condition. These methods include detecting or modulating GLOBIN polynucleotides comprising contacting a sample, tissue, cell, or organism with the compounds of the present invention, measuring the nucleic acid or protein level of GLOBIN polynucleotides and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further compound of the invention. These methods can also be performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a particular disease, condition, or phenotype.

[00144] *Assessing Up-regulation or Inhibition of Gene Expression:* Transfer of an exogenous nucleic acid into a host cell or organism can be assessed by directly detecting the presence of the nucleic acid in the cell or organism. Such detection can be achieved by several methods well known in the art. For example, the presence of the exogenous nucleic acid can be detected by Southern blot or by a polymerase chain reaction (PCR) technique using primers that specifically amplify nucleotide sequences associated with the nucleic acid. Expression of the exogenous nucleic acids can also be measured using conventional methods including gene expression analysis. For instance, mRNA produced from an exogenous nucleic acid can be detected and quantified using a Northern blot and reverse transcription PCR (RT-PCR).

[00145] Expression of an RNA from the exogenous nucleic acid can also be detected by measuring an enzymatic activity or a reporter protein activity. For example, antisense modulatory activity can be measured indirectly as a decrease or increase in target nucleic acid

expression as an indication that the exogenous nucleic acid is producing the effector RNA. Based on sequence conservation, primers can be designed and used to amplify coding regions of the target genes. Initially, the most highly expressed coding region from each gene can be used to build a model control gene, although any coding or non coding region can be used. Each control gene is assembled by inserting each coding region between a reporter coding region and its poly(A) signal. These plasmids would produce an mRNA with a reporter gene in the upstream portion of the gene and a potential RNAi target in the 3' non-coding region. The effectiveness of individual antisense oligonucleotides would be assayed by modulation of the reporter gene. Reporter genes useful in the methods of the present invention include acetohydroxyacid synthase (AHAS), alkaline phosphatase (AP), beta galactosidase (LacZ), beta glucoronidase (GUS), chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), horseradish peroxidase (HRP), luciferase (Luc), nopaline synthase (NOS), octopine synthase (OCS), and derivatives thereof. Multiple selectable markers are available that confer resistance to ampicillin, bleomycin, chloramphenicol, gentamycin, hygromycin, kanamycin, lincomycin, methotrexate, phosphinothricin, puromycin, and tetracycline. Methods to determine modulation of a reporter gene are well known in the art, and include, but are not limited to, fluorometric methods (*e.g.* fluorescence spectroscopy, Fluorescence Activated Cell Sorting (FACS), fluorescence microscopy), antibiotic resistance determination.

Kits, Research Reagents, Diagnostics, and Therapeutics

[00146] The compounds of the present invention can be utilized for diagnostics, therapeutics, and prophylaxis, and as research reagents and components of kits. Furthermore, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes or to distinguish between functions of various members of a biological pathway.

[00147] For use in kits and diagnostics and in various biological systems, the compounds of the present invention, either alone or in combination with other compounds or therapeutics, are useful as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues.

[00148] As used herein the term "biological system" or "system" is defined as any organism, cell, cell culture or tissue that expresses, or is made competent to express products of the GLOBIN family member genes. These include, but are not limited to, humans, transgenic animals, cells, cell cultures, tissues, xenografts, transplants and combinations thereof.

[00149] As one nonlimiting example, expression patterns within cells or tissues treated with one or more antisense compounds are compared to control cells or tissues not treated with antisense compounds and the patterns produced are analyzed for differential levels of gene expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds that affect expression patterns.

[00150] Examples of methods of gene expression analysis known in the art include DNA arrays or microarrays (Brazma and Vilo, *FEBS Lett.*, 2000 480, 17-24; Celis, *et al.*, *FEBS Lett.*, 2000 480, 2-16), SAGE (serial analysis of gene expression)(Madden, *et al.*, *Drug Discov. Today*, 2000, 5, 415-425), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, *Methods Enzymol.*, 1999, 303, 258-72), TOGA (total gene expression analysis) (Sutcliffe, *et al.*, *Proc. Natl. Acad. Sci.* U.S.A., 2000, 97, 1976-81), protein arrays and proteomics (Celis, *et al.*, *FEBS Lett.*, 2000, 480, 2-16; Jungblut, *et al.*, *Electrophoresis*, 1999, 20, 2100-10), expressed sequence tag (EST) sequencing (Celis, *et al.*, *FEBS Lett.*, 2000, 480, 2-16; Larsson, *et al.*, *J. Biotechnol.*, 2000, 80, 143-57), subtractive RNA fingerprinting (SuRF) (Fuchs, *et al.*, *Anal. Biochem.*, 2000, 286, 91-98; Larson, *et al.*, *Cytometry*, 2000, 41, 203-208), subtractive cloning, differential display (DD) (Jurecic and Belmont, *Curr. Opin. Microbiol.*, 2000, 3, 316-21), comparative genomic hybridization (Carulli, *et al.*, *J. Cell Biochem.* Suppl., 1998, 31, 286-96), FISH (fluorescent *in situ* hybridization) techniques (Going and Gusterson, *Eur. J. Cancer*, 1999, 35, 1895-904) and mass spectrometry methods (To, *Comb. Chem. High Throughput Screen*, 2000, 3, 235-41).

[00151] The compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding globins. For example, oligonucleotides that hybridize with such efficiency and under such conditions as disclosed herein as to be

effective globin inhibitors are effective primers or probes under conditions favoring gene amplification or detection, respectively. These primers and probes are useful in methods requiring the specific detection of nucleic acid molecules encoding globins and in the amplification of said nucleic acid molecules for detection or for use in further studies of globins. Hybridization of the antisense oligonucleotides, particularly the primers and probes, of the invention with a nucleic acid encoding globins can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabeling of the oligonucleotide, or any other suitable detection means. Kits using such detection means for detecting the level of globins in a sample may also be prepared.

[00152] The specificity and sensitivity of antisense are also harnessed by those of skill in the art for therapeutic uses. Antisense compounds have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. Antisense oligonucleotide drugs have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that antisense compounds can be useful therapeutic modalities that can be configured to be useful in treatment regimes for the treatment of cells, tissues and animals, especially humans.

[00153] For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of globin polynucleotides is treated by administering antisense compounds in accordance with this invention. For example, in one non-limiting embodiment, the methods comprise the step of administering to the animal in need of treatment, a therapeutically effective amount of a globin inhibitor. The globin, e.g. HBF/HBG1 inhibitors of the present invention effectively inhibit the activity of the globin, e.g. HBF/HBG1 protein or inhibit the expression of the globin, e.g. HBF/HBG1 protein. In one embodiment, the activity or expression of HBF/HBG1 in an animal is inhibited by about 10%. Preferably, the activity or expression of HBF/HBG1 in an animal is inhibited by about 30%. More preferably, the activity or expression of HBF/HBG1 in an animal is inhibited by 50% or more. Thus, the oligomeric compounds modulate expression of HBF/HBG1 mRNA by at least 10%, by at least 20%, by at least 25%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100%.

[00154] For example, the reduction of the expression of HBF/HBG1 may be measured in serum, adipose tissue, liver or any other body fluid, tissue or organ of the animal. Preferably, the cells contained within said fluids, tissues or organs being analyzed contain a nucleic acid molecule encoding HBF/HBG1 protein.

[00155] The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of a compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the compounds and methods of the invention may also be useful prophylactically.

Conjugates

[00156] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugate groups include cholesterols, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve uptake, distribution, metabolism or excretion of the compounds of the present invention. Representative conjugate groups are disclosed in International Patent Application No. PCT/US92/09196, filed Oct. 23, 1992, and U.S. Pat. No. 6,287,860.

Conjugate moieties include, but are not limited to, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, *e.g.*, hexyl-5-tritylthiol, a thiocholesterol, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or

hexylamino-carbonyl-oxycholesterol moiety. Oligonucleotides of the invention may also be conjugated to active drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (S)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indomethicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

[00157] Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941.

Formulations

[00158] The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756.

[00159] Although, the antisense oligonucleotides do not need to be administered in the context of a vector in order to modulate a target expression and/or function, embodiments of the

invention relates to expression vector constructs for the expression of antisense oligonucleotides, comprising promoters, hybrid promoter gene sequences and possess a strong constitutive promoter activity, or a promoter activity which can be induced in the desired case.

[00160] In an embodiment, invention practice involves administering at least one of the foregoing antisense oligonucleotides with a suitable nucleic acid delivery system. In one embodiment, that system includes a non-viral vector operably linked to the polynucleotide. Examples of such non-viral vectors include the oligonucleotide alone (*e.g.* any one or more of SEQ ID NOS: 1 to 5) or in combination with a suitable protein, polysaccharide or lipid formulation.

[00161] Additionally suitable nucleic acid delivery systems include viral vector, typically sequence from at least one of an adenovirus, adenovirus-associated virus (AAV), helper-dependent adenovirus, retrovirus, or hemagglutinatin virus of Japan-liposome (HVJ) complex. Preferably, the viral vector comprises a strong eukaryotic promoter operably linked to the polynucleotide *e.g.*, a cytomegalovirus (CMV) promoter.

[00162] Additionally preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include Moloney murine leukemia viruses and HIV-based viruses. One preferred HIV-based viral vector comprises at least two vectors wherein the gag and pol genes are from an HIV genome and the *env* gene is from another virus. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector [Geller, A.I. *et al.*, *J. Neurochem*, 64: 487 (1995); Lim, F., *et al.*, in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); Geller, A.I. *et al.*, *Proc Natl. Acad. Sci.*: U.S.A.:90 7603 (1993); Geller, A.I., *et al.*, *Proc Natl. Acad. Sci* USA: 87:1149 (1990)], Adenovirus Vectors [LeGal LaSalle *et al.*, *Science*, 259:988 (1993); Davidson, *et al.*, *Nat. Genet.* 3: 219 (1993); Yang, *et al.*, *J. Virol.* 69: 2004 (1995)] and Adeno-associated Virus Vectors [Kaplitt, M.G., *et al.*, *Nat. Genet.* 8:148 (1994)].

[00163] The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon

administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof.

[00164] The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: *i.e.*, salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Pat. No. 6,287,860.

[00165] The present invention also includes pharmaceutical compositions and formulations that include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, *e.g.*, intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

[00166] The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active

ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[00167] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[00168] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

[00169] Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug that may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Pat. No. 6,287,860.

[00170] Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes that are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

[00171] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids. When incorporated into liposomes, these specialized lipids result in liposomes with enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860.

[00172] The pharmaceutical formulations and compositions of the present invention may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

[00173] In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860.

[00174] One of skill in the art will recognize that formulations are routinely designed according to their intended use, *i.e.* route of administration.

[00175] Preferred formulations for topical administration include those in which the oligonucleotides of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (*e.g.* dioleoyl-phosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (*e.g.* dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.* dioleoyltetramethylaminopropyl DOTAP and dioleoyl-phosphatidyl ethanolamine DOTMA).

[00176] For topical or other administration, oligonucleotides of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, oligonucleotides may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters, pharmaceutically acceptable salts thereof, and their uses are further described in U.S. Pat. No. 6,287,860.

[00177] Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which oligonucleotides of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts and fatty acids and their uses are further described in U.S. Pat. No. 6,287,860.

Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. Oligonucleotides of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. Oligonucleotide complexing agents and their uses are further described in U.S. Pat. No. 6,287,860.

[00178] Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[00179] Certain embodiments of the invention provide pharmaceutical compositions containing one or more oligomeric compounds and one or more other chemotherapeutic agents that function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide,

ifosfamide, cytosine arabinoside, bis-chloroethyl-nitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclo-phosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). When used with the compounds of the invention, such chemotherapeutic agents may be used individually (*e.g.*, 5-FU and oligonucleotide), sequentially (*e.g.*, 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (*e.g.*, 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Combinations of antisense compounds and other non-antisense drugs are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

[00180] In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. For example, the first target may be an HBF target, and the second target may be a region from another nucleotide sequence. Alternatively, compositions of the invention may contain two or more antisense compounds targeted to different regions of the same HBF nucleic acid target. Numerous examples of antisense compounds are illustrated herein and others may be selected from among suitable compounds known in the art. Two or more combined compounds may be used together or sequentially.

[00181] *Dosing*: The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the

disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50s} found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight, once or more daily, to once every 20 years.

[00182]

[00183]

By their citation of various references in this document, Applicants do not admit any particular reference is "prior art" to their invention. Embodiments of inventive compositions and methods are illustrated in the following examples.

EXAMPLES

[00184] The following non-limiting Examples serve to illustrate selected embodiments of the invention. It will be appreciated that variations in proportions and alternatives in elements of the components shown will be apparent to those skilled in the art and are within the scope of embodiments of the present invention.

Example 1: Modulation of globin polynucleotides

[00185] *Materials and Methods*:

[00186] *Treatment of HepG2 cells with antisense oligonucleotides:* HepG2 cells from ATCC (cat# HB-8065) were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35-011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO_2. One day before the experiment the cells were replated at the density of 1.5×10^5 cells/ml into 6 well plates and incubated at 37°C and 5% CO_2. On the day of the experiment the media in the 6 well plates was changed to fresh growth media.

[00187] All RNA oligonucleotides were diluted to the concentration of 20 μM. 2 μl of this solution was incubated with 400 μl of Opti-MEM media (Gibco cat#31985-070) and 4 μl of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min and applied to each well of the 6 well plates with HepG2 cells. Similar mixture including 2 μl of water instead of the oligonucleotide solution was used for the mock-transfected controls.

[00188] After 3-18 h of incubation at 37°C and 5% CO_2 the media was changed to fresh growth media. 48 h after addition of RNA oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturer's instructions.

[00189] 600 ng of RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI. The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using Mx4000 thermal cycler (Stratagene). Fold change in gene expression after treatment with RNA oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

[00190] *Results*:

[00191] Real time PCR results show that the levels of HBF mRNA in HepG2 cells are significantly increased 48 h after treatment with one oligonucleotide designed to HBF antisense Hs.702397 (Figure 1).

[00192] Gene Name: HBF/HBG1 (Accession Number: NM_000559):

[00193] ACACTCGCTTCTGGAACGTCTGAGGTTATCAATAAGCTCCTAGTCCAGAC
GCCATGGGTCATTTCACAGAGGAGGACAAGGCTACTATCACAAGCCTGTGGGGCAA
GGTGAATGTGGAAGATGCTGGAGGAGAAACCCTGGGAAGgtaggctctggtgaccaggacaaggg
agggaaggaaggaccctgtgcctggcaaaagtccaggtcgcttctcaggatttgtggcaccttctgactgtcaaactgttcttgtcaatctcac
agGCTCCTGGTTGTCTACCCATGGACCCAGAGGTTCTTTGACAGCTTTGGCAACCTGT
CCTCTGCCTCTGCCATCATGGGCAACCCCAAAGTCAAGGCACATGGCAAGAAGGTG
CTGACTTCCTTGGGAGATGCCACAAAGCACCTGGATGATCTCAAGGGCACCTTTGCC
CAGCTGAGTGAACTGCACTGTGACAAGCTGCATGTGGATCCTGAGAACTTCAAGgtga
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GGCAAAGAATTCACCCCTGAGGTGCAGGCTTCCTGGCAGAAGATGGTGACTGCAGT
GGCCAGTGCCCTGTCCTCCAGATACCACTGAGCTCACTGCCCATGATTCAGAGCTTT
CAAGGATAGGCTTTATTCTGCAAGCAATACAAATAATAAATCTATTCTGCTGAGAGA
TCAC (SEQ ID NO: 1).

[00194] Natural Antisense Sequence (Hs.702397):

GATTTATTAT TTGTATTGCT TGCAGAATAA AGCCTATCCT TGAAAGCTCT 50
GAnTCATGGG CAGTGAGCTC AGTGGnATCT GGnGGnCAGG GCACTGGCCA 100
CTGCAGTCAC CATCTTCTGC CAGGgnGCCT GCACCTCAGG GGTGAnTTCT 150
TTGCCGAAnT GGnTTGCCAA AnCGGTCACC AGCACATTTC CCAGGggCTT 200
GAAGTTCTCA GGnTCCACAT GCAGCTTGTC ACAGTGCAGT TCACTCAGCT 250
GGGCAAAGGT GCCnTTGAGA TCATCCgGGn GCTTTGTGGg nTCTCCCnAG 300
GgnGTCAGnA CCTTCTTGCC ATGTGCCTTG nCTTTGGGGg TTGCCCctgn 350
tgggcag (SEQ ID NO: 2).

[00195] *Antisense Oligonucleotides*:

[00196] HBF Hs.702397_3 :

5'-rGrUrC rArArG rGrCrA rCrArU rGrGrC rArArG rArArG rGrUrG rCrUrG-3' (SEQ ID NO: 3)

5'-rGrCrA rCrCrU rUrCrU rUrGrC rCrArU rGrUrG rCrCrU rUrGA C-3' (SEQ ID NO: 6)

[00197] HBF Hs.702397_2 :

5'-rCrCrU rGrGrC rArGrA rArGrA rUrGrG rUrGrA rCrUrG rCrArG rUrGrG-3' (SEQ ID NO: 4)

5'-rArCrU rGrCrA rGrUrC rArCrC rArUrC rUrUrC rUrGrC rCrAG G-3' (SEQ ID NO: 7)

[00198] HBF Hs.702397_1 :

5'-rCrUrU rUrCrA rArGrG rArUrA rGrGrC rUrUrU rArUrU rCrUrG rCrArA-3' (SEQ ID NO: 5)

5'-rGrCrA rGrArA rUrArA rArGrC rCrUrA rUrCrC rUrUrG rArAA G-3' (SEQ ID NO: 8)

[00199] *Detection Probes*: ABI Taqman Gene Expression Assay: Hs00361131_g1.

[00200] Although the invention has been illustrated and described with respect to one or more implementations, equivalent alterations and modifications will occur to others skilled in the art upon the reading and understanding of this specification and the annexed drawings. In addition, while a particular feature of the invention may have been disclosed with respect to only one of several implementations, such feature may be combined with one or more other features of the

other implementations as may be desired and advantageous for any given or particular application.

[00201] The Abstract of the disclosure will allow the reader to quickly ascertain the nature of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the following claims.

What is claimed is:

1. An oligonucleotide of 10-30 nucleotide in length, said oligonucleotide comprises 90% identity to a reverse complement of at least one globin polynucleotide as set forth in SEQ ID NO: 2, wherein the oligonucleotide increases the expression of the globin polynucleotide.

2. The oligonucleotide of claim 1, wherein said oligonucleotide comprises combinations of phosphorothioate internucleotide linkages and at least one internucleotide linkage selected from the group consisting of: alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, and carboxymethyl ester or a combination thereof.

3. The oligonucleotide of claim 1 or 2, wherein said oligonucleotide comprises at least one phosphorothioate internucleotide linkage.

4. The oligonucleotide of any one of claims 1-3, wherein said oligonucleotide comprises a backbone of phosphorothioate internucleotide linkages.

5. The oligonucleotide of any one of claims 1-4, wherein the oligonucleotide comprises at least one modified nucleobase.

6. The oligonucleotide of claim 5, wherein said modified nucleobase is a peptide nucleic acid, a locked nucleic acid (LNA) molecule, or a combination thereof.

7. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises a modified sugar moiety comprising a 2'-O-methoxyethyl modified sugar moiety, a 2'- methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, or a bicyclic sugar moiety.

8. The oligonucleotide of any one of claims 1-7, wherein the oligonucleotide is single stranded.

9. The oligonucleotide of any one of claims 1-8, wherein the oligonucleotide is a siRNA compound.

10. The oligonucleotide of any one of claims 1-9, wherein the oligonucleotide comprises at least one nucleotide sequence set forth as SEQ ID NOs: 3-8.

11. A composition comprising one or more oligonucleotides as defined in any one of claims 1-10, and a carrier.

12. The composition of claim 11, for increasing the expression of the globin polynucleotide in patient cells or tissues *in vivo* or *in vitro.*

13. The composition of claim 11 or 12, for treating a globin associated disease.

14. The composition of claim 13, wherein the globin associated disease is arthritis, inflammation, a neurological disease or disorder, an autoimmune disease, cancer, a bacterial disease, a viral disease, or a parasite.

15. The composition of 14, wherein said globin associated disease is a hematological disease or disorder, a red blood cell disease or disorder, a hematopoietic blood disease or disorder, anemia, Fanconi's anemia, thalassemia, beta-thalassemia, sickle cell disease, leukemia, cellular dyscrasia, dyserythropoiesis, anisocytosis, or poikilocytosis.

16. Use of at least one single stranded oligonucleotide of 10 to 30 nucleotides in length wherein said at least one oligonucleotide comprises at least 90% sequence identity to a reverse complement of a Hemoglobin polynucleotide as set forth in SEQ ID NO: 2; for increasing the function of and/or the expression of the Hemoglobin polynucleotide in patient cells or tissues *in vivo* or *in vitro.*

17. Use of at least one single stranded oligonucleotide 10 to 30 nucleotides in length wherein said at least one oligonucleotide comprises at least 90% sequence identity to a reverse complement of a Hemoglobin polynucleotide as set forth in SEQ ID NO: 2, in the manufacture of a medicament for increasing the function of and/or the expression of the Hemoglobin polynucleotide in patient cells or tissues *in vivo* or *in vitro.*

18. The use of claim 16 or 17, wherein the function of and/or the expression of the Hemoglobin polynucleotide is increased *in vivo* or *in vitro* with respect to a control.

19. Use of at least one short interfering RNA (siRNA) oligonucleotide 19 to 30 nucleotides in length, said at least one siRNA oligonucleotide hybridizes to an antisense polynucleotide of a Hemoglobin polynucleotide comprising SEQ ID NO: 2, for upregulating a function of and/or the expression of the Hemoglobin polynucleotide in mammalian cells or tissues *in vivo* or *in vitro.*

20. Use of at least one short interfering RNA (siRNA) oligonucleotide 19 to 30 nucleotides in length, said at least one siRNA oligonucleotide hybridizes to an antisense polynucleotide of a Hemoglobin polynucleotide comprising SEQ ID NO: 2, in the manufacture of a medicament for upregulating a function of and/or the expression of the Hemoglobin polynucleotide in mammalian cells or tissues *in vivo* or *in vitro*.

21. Use of at least one oligonucleotide of 10 to 30 nucleotides in length specific for noncoding and/or coding sequences of a sense and/or natural antisense strand of a Hemoglobin polynucleotide wherein said at least one oligonucleotide hybridizes to a reverse complement of the Hemoglobin polynucleotide as set forth in SEQ ID NO: 2, for upregulating the expression of the Hemoglobin polynucleotide in mammalian cells or tissues *in vivo* or *in vitro*.

22. Use of at least one oligonucleotide of 10 to 30 nucleotides in length specific for noncoding and/or coding sequences of a sense and/or natural antisense strand of a Hemoglobin polynucleotide wherein said at least one oligonucleotide hybridizes to a reverse complement of the Hemoglobin polynucleotide as set forth in SEQ ID NO: 2, in the manufacture of a medicament for upregulating the expression of the Hemoglobin polynucleotide in mammalian cells or tissues *in vivo* or *in vitro*.

23. The use of any one of claims 16-22, for treating a disease associated with said Hemoglobin polynucleotide and/or at least one encoded product thereof.

24. The use of claim 23, wherein the disease associated with the Hemoglobin polynucleotide is arthritis, inflammation, a neurological disease or disorder, an autoimmune disease, cancer, a bacterial disease, a viral disease, or a parasite.

25. The use of 23, wherein the disease associated with the Hemoglobin polynucleotide is selected from: a hematological disease or disorder, a red blood cell disease or disorder, a hematopoietic blood disease or disorder, anemia, Fanconi's anemia, thalassemia, beta-thalassemia, sickle cell disease, leukemia, cellular dyscrasia, dyserythropoiesis, anisocytosis or poikilocytosis.

26. The use of any one of claims 16-25, wherein said oligonucleotide comprises combinations of phosphorothioate internucleotide linkages and at least one internucleotide linkage selected from the group consisting of: alkylphosphonate, phosphorodithioate,

alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, and carboxymethyl ester or a combination thereof.

27. The use of any one of claims 16-26, wherein said oligonucleotide comprises at least one phosphorothioate internucleotide linkage.

28. The use of any one of claims 16-27, wherein said oligonucleotide comprises a backbone of phosphorothioate internucleotide linkages.

29. The use of any one of claims 16-28, wherein the oligonucleotide comprises at least one modified nucleobase.

30. The use of claim 29, wherein said modified nucleobase is a peptide nucleic acid, a locked nucleic acid (LNA) molecule, or a combination thereof.

31. The use of any one of claims 16-30, wherein the oligonucleotide comprises a modified sugar moiety comprising a 2'-O-methoxyethyl modified sugar moiety, a 2'- methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, or a bicyclic sugar moiety.

32. The use of any one of claims 16-31, wherein the oligonucleotide comprises at least one nucleotide sequence set forth as SEQ ID NOs: 3-8.

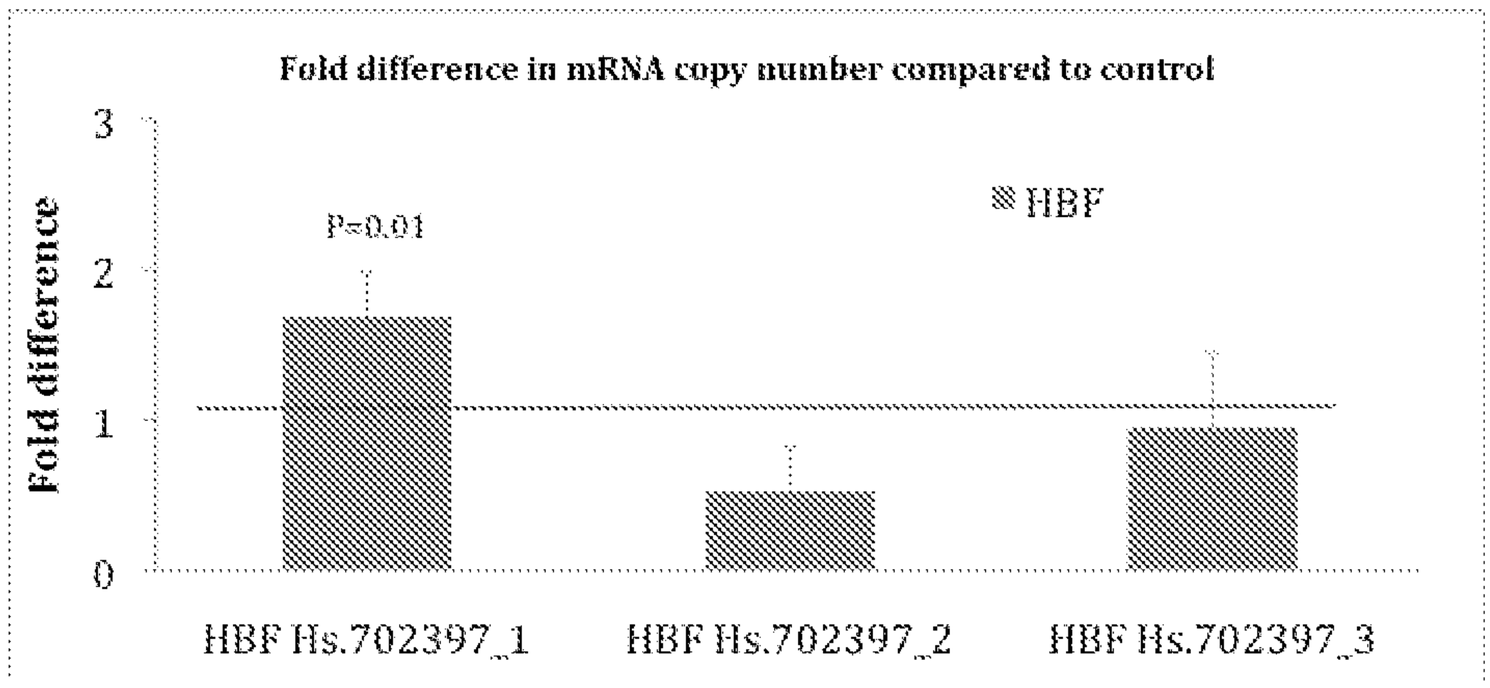
Fold difference in mRNA copy number compared to control
Fold difference
3
2
1
0
P=0.01
HBF
HBF Hs.702397_1
HBF Hs.702397_2
HBF Hs.702397_3

FIGURE 1