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(54) **Title**: METHODS FOR ADMINISTERING NUCLEIC ACID-BASED THERAPEUTICS

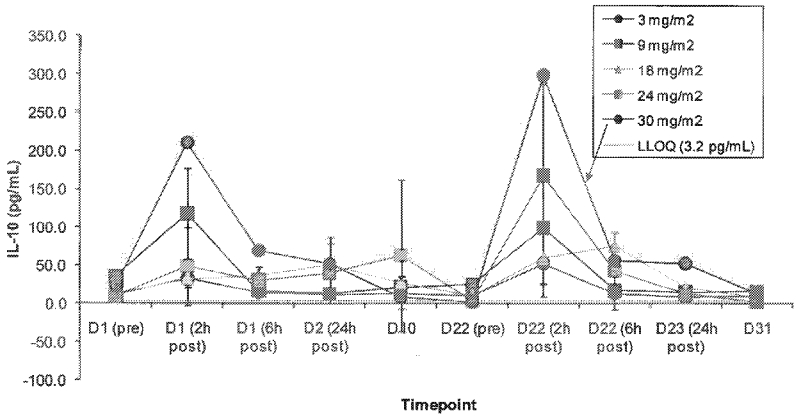

FIG. 1A

(57) **Abstract**: The present application relates to methods of administering a nucleic acid-based therapeutic, to treat a disease or disorder in a patient. The methods suitably comprise administering a first dose amount of a nucleic acid-based therapeutic during a first treatment cycle, and administering a second dose amount of the nucleic acid-based therapeutic during subsequent treatment cycles, wherein the first dose amount is lower than the second dose amount. In embodiments, the nucleic acid-based therapeutic is an siRNA that is part of a composition comprising a cyclodextrin polymer.

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METHODS FOR ADMINISTERING NUCLEIC ACID-BASED THERAPEUTICS

BACKGROUND OF THE INVENTION

Sequence Listing

[0001] The instant application contains a Sequence Listing that has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCI copy, created on June 8, 2012, is named 0052CA08.txt and is 41,726 bytes in size.

Field of the Invention

[0002] The present application relates to methods of administering a nucleic acid-based therapeutic to treat a disease or disorder in a patient. The methods suitably comprise administering a first dose amount of a nucleic acid-based therapeutic during a first treatment cycle, and administering a second dose amount of the nucleic acid-based therapeutic during subsequent treatment cycles, wherein the first dose amount is lower than the second dose amount. In embodiments, the nucleic acid-based therapeutic is an siRNA that is part of a composition comprising a cyclodextrin polymer.

Background of the Invention

[0003] Nucleic acid-based therapeutics have the potential to play a critical role in the treatment and prevention of numerous diseases and disorders, including various cancers, AIDS, neurological disorders, and cardiovascular disorders.

[0004] Nucleic acid-based therapy centers on the introduction of nucleic acids into the cells or tissue of a patient, followed by processing of the information coded by the nucleic acids introduced. Both DNA and RNA including plasmids containing transgenes for gene therapy, oligonucleotides for antisense and antigene applications, ribozymes, DNAzymes, aptamers,

miRNA, antisense RNA, dsRNA, mRNA and siRNA, have demonstrated potential as therapeutic candidates.

[0005] Various methods for introducing DNA and RNA into patients have been developed. These methods include the use of viral vectors, direct injection of DNA and RNA, and chemical methods to enhance delivery. Viral vectors include retroviruses, adenoviruses, and adeno-associated viruses. Chemical methods to enhance delivery include calcium phosphate transfection, the use of oligonucleotides, lipoplexes and polyplexes, and dendrimers. Direct injection of naked DNA and RNA has also been demonstrated. Injection can be performed directly with a syringe and needle into specific tissue, such as muscle, using particle bombardment (i.e., gene gun) techniques, electroporation and sonoporation. Direct injection techniques are simple and relatively safe but often result in poor efficiency of gene transfer, and a low level of stable integration of the nucleic acid.

[0006] One class of nucleic acid-based therapeutic is RNA interference (RNAi), including the use of dsRNA and RNAi constructs. dsRNA refers to a double stranded RNA molecule capable of RNA interference (RNAi), including short interfering RNA (siRNA) (*see for example*, Bass, 2001, *Nature, 411*, 428-429; Elbashir *et al.*, 2001, *Nature, 411*, 494-498; and Kreutzer *et al.*, PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, PCT Publication No. WO 01/36646; Fire, PCT Publication No. WO 99/32619; Plaetinck *et al.*, PCT Publication No. WO 00/01846; Mello and Fire, PCT Publication No. WO 01/29058; Deschamps-Depaillette, PCT Publication No. WO 99/07409; and Li *et al.*, PCT Publication No. WO 00/44914, the disclosures of each of which are incorporated by reference herein). RNAi provides a useful method of inhibiting or reducing gene expression *in vitro* or *in vivo*.

[0007] A challenge to the use of nucleic acid-based therapeutics (including RNAi) is the intracellular delivery to the specific tissues and organs that express the target gene. Upon introduction, the nucleic acid therapeutic must remain functional and be capable of reaching the cell nucleus, without a

significant reduction in efficiency. Additionally, care must be taken to prevent the nucleic acid from initiating an immune response in the patient. Once the therapeutic reaches the cell, integration into the host genome must be prevented in order to avoid influencing expression of the host genes and potentially triggering the expression of an oncogene or destruction of a tumor suppressor gene.

SUMMARY OF PREFERRED EMBODIMENTS

[0008] The needs identified above by met by the present application providing methods administering a nucleic acid-based therapeutic to treat a disease or disorder in a patient.

[0009] In embodiments, methods are provided of administering a nucleic acid-based therapeutic to treat a disease or disorder in a patient. The methods suitably comprise administering a first dose amount of a nucleic acid-based therapeutic during a first treatment cycle, and administering a second dose amount of the nucleic acid-based therapeutic during subsequent treatment cycles. Suitably, the first dose amount is lower than the second dose amount.

[00010] In exemplary embodiments, the first dose amount is about 9 mg/m^2 to about 27 mg/m^2, or about 15 mg/m^2 to about 20 mg/m^2, or more suitably about 18 mg/m^2. Suitably, the second dose amount is about 20 mg/m^2 to about 50 mg/m^2, more suitably about 25 mg/m^2 to about 40 mg/m^2. In embodiments, the first dose amount is about 20% to about 30% lower, about 30% to about 40% lower, about 40% to about 50% lower, about 50% to about 60% lower, about 60% to about 70% lower, about 70% to about 80% lower, about 80% to about 90% lower, or about 90% to about 99% lower than the second dose amount.

[00011] Suitably, the first and subsequent treatment cycles are 11 day to 49 day treatment cycles, including 21 day treatment cycles. In embodiments, the first and subsequent treatment cycles comprise administering on days 1, 3, 8, and 10 of the treatment cycles.

[00012] In embodiments, the nucleic acid-based therapeutic is an siRNA, dsRNA, miRNA, antisense RNA, aptamer, ribozyme, or an enzymatic nucleic acid. Suitably, the siRNA reduces the expression of a ribonucleotide reductase subunit 2 (R2) in a cell. In further embodiments, the siRNA reduces the expression of endothelial PAS domain protein 1 (EPAS1) in a cell.

[00013] In embodiments, the nucleic acid-based therapeutic is part of a composition comprising the nucleic acid-based therapeutic and a cyclodextrin-containing polymer. Suitably, the composition further comprises a stabilizing agent, for example, a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG). In embodiments, the composition further comprises a targeting agent, including a targeted agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand, such as transferrin.

[00014] In embodiments, the cyclodextrin-containing polymer is a β-cyclodextrin-containing polymer.

[00015] Suitably, the disease or disorder is selected from the group consisting of cystic fibrosis, Gaucher's disease, muscular dystrophy, AIDS, cancer, progressive heart failure, restenosis, hemophilia and neurological conditions. In embodiments, the cancer is multiple myeloma, leukemia, melanoma, or ovarian cancer.

[00016] Also provided are methods of administering an siRNA to treat a disease or disorder in a patient. The methods suitably comprise administering during a first treatment cycle a first dose amount of an siRNA composition comprising the siRNA, a cyclodextrin-containing polymer, a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG), and a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand. The methods further comprise administering a second dose amount of the siRNA composition during subsequent treatment cycles. Suitably, the first dose amount is lower than the second dose amount.

[00017] Exemplary dose amounts, treatment cycles and siRNAs are described throughout.

[00018] Also provided are methods of administering an siRNA to treat a disease or disorder in a patient. Suitably, the methods comprise administering during a first 21 day treatment cycle, a first dose of about 18 mg/m^2 of an siRNA composition comprising, the siRNA, a cyclodextrin-containing polymer, a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG), and a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and transferrin. The methods further comprise administering a second dose of about 25 mg/m^2 to about 40 mg/m^2 of the siRNA composition during subsequent 21 day treatment cycles.

[00019] In further embodiments, methods are provided of reducing an immune response to siRNA. The methods suitably comprise administering during a first treatment cycle a first dose amount of an siRNA composition comprising the siRNA, a cyclodextrin-containing polymer, a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG), and a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand. The methods further comprise administering a second dose amount of the siRNA composition during subsequent treatment cycles. Suitably, the first dose amount is lower than the second dose amount.

[00020] In embodiments, the methods reduce levels of IL-6 and/or TNF-α.

[00021] Further embodiments, features, and advantages of the embodiments, as well as the structure and operation of the various embodiments, are described in detail below with reference to accompanying drawings.

BRIEF DESCRIPTION OF THE FIGURES

[00022] FIGS. 1A-1C show average Cycle 1 (over Days 1-10) and Cycle 2 (over Days 22-31) plasma concentrations of IL-10, IL-6 and TNF-α by dose level for an siRNA-cyclodextrin composition.

[00023] FIG. 2 shows average maximum plasma concentration of IL-10, IL-6 and TNF-α during Cycle 1 ("C1") as a function of siRNA-cyclodextrin composition dose level.

[00024] FIG. 3 shows plasma concentrations of cytokines IL-6, TNF- α and IL-10 in a patient over Cycles 1-6.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[00025] It should be appreciated that the particular implementations shown and described herein are examples and are not intended to otherwise limit the scope of the application in any way.

[00026] The published patents, patent applications, websites, company names, and scientific literature referred to herein are hereby incorporated by reference in their entirety to the same extent as if each was specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the latter. Likewise, any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be resolved in favor of the latter.

[00027] As used in this specification, the singular forms "a," "an" and "the" specifically also encompass the plural forms of the terms to which they refer, unless the content clearly dictates otherwise. The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

[00028] Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present application pertains, unless otherwise defined. Reference is made herein to various methodologies and materials known to those of skill in the art.

[00029] In embodiments, methods are provided for administering a nucleic acid-based therapeutic to treat a disease or disorder in a patient. The methods suitably comprise administering a first dose amount of a nucleic acid-based therapeutic during a first treatment cycle, and administering a second dose amount of the nucleic acid-based therapeutic during subsequent treatment cycles. Suitably, the first dose amount is lower than the second dose amount.

[00030] As used herein, the term "nucleic acid-based therapeutic" refers to an siRNA, dsRNA, miRNA, antisense RNA, aptamer, ribozyme, or an enzymatic nucleic acid.

[00031] The term "dsRNA" as used herein refers to a double stranded RNA molecule capable of RNA interference (RNAi), including siRNA (see for example, Bass, 2001, Nature, 411, 428-429; Elbashir et al., 2001, Nature, 411, 494-498; and Kreutzer et al., PCT Publication No. WO 00/44895; Zernicka-Goetz et al., PCT Publication No. WO 01/36646; Fire, PCT Publication No. WO 99/32619; Plaetinck et al., PCT Publication No. WO 00/01846; Mello and Fire, PCT Publication No. WO 01/29058; Deschamps-Depaillette, PCT Publication No. WO 99/07409; and Li et al., PCT Publication No. WO 00/44914, the disclosures of each of which are incorporated by reference herein in their entireties). In addition, RNAi is a term initially applied to a phenomenon observed in plants and worms where double-stranded RNA (dsRNA) blocks gene expression in a specific and post-transcriptional manner. RNAi provides a useful method of inhibiting or reducing gene expression *in vitro* or *in vivo*.

[00032] The term "short interfering RNA," "siRNA," or "short interfering nucleic acid," as used herein, refers to any nucleic acid capable of mediating RNAi or gene silencing when processed appropriately by a cell. For example, the siRNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises complementarity to a target gene. The siRNA can be a single-stranded hairpin polynucleotide having self-complementary sense and antisense regions, wherein the antisense region comprises complementarity to

a target gene. The siRNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises complementarity to a target gene, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siRNA capable of mediating RNAi. The siRNA can also comprise a single stranded polynucleotide having complementarity to a target gene, wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez et al., 2002, Cell., 110, 563-574), or 5',3'-diphosphate. In certain embodiments, the siRNAs are non-enzymatic nucleic acids that bind to a target nucleic acid and alter the activity of the target nucleic acid. Binding and/or activity of the siRNA may be facilitated by interaction with one or more protein or protein complexes, such as the RNA Induced Silencing Complex (or RISC). In certain embodiments, the siRNAs comprise a sequence that is complementary to a target sequence along a single contiguous sequence of one strand of the siRNA molecule.

[00033] Optionally, the siRNAs of the application contain a nucleotide sequence that hybridizes under physiologic conditions (e.g., in a cellular environment) to the nucleotide sequence of at least a portion of the mRNA transcript for the gene to be inhibited (the "target" gene). The double-stranded RNA need only be sufficiently similar to natural RNA that it has the ability to mediate RNAi. Thus, the application has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism or evolutionary divergence. The number of tolerated nucleotide mismatches between the target sequence and the siRNA sequence is no more than 1 in 5 basepairs, or 1 in 10 basepairs, or 1 in 20 basepairs, or 1 in 50 basepairs. Mismatches in the center of the siRNA duplex are most critical and may essentially abolish cleavage of the target RNA. In contrast, nucleotides at the 3' end of the siRNA strand that is complementary to the target RNA do not significantly contribute to specificity of the target recognition. Sequence identity may be optimized by sequence comparison and

alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Greater than 90%, 95%, 96%, 97%, 98%, or 99% sequence identity, or even 100% sequence identity, between the siRNA and the portion of the target gene is preferred. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript under stringent conditions (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C. or 70°C. hybridization for 12-16 hours; followed by washing).

[00034] The double-stranded structure of dsRNA may be formed by a single self-complementary RNA strand, two complementary RNA strands, or a DNA strand and a complementary RNA strand. Optionally, RNA duplex formation may be initiated either inside or outside the cell. The RNA may be introduced in an amount which allows delivery of at least one copy per cell. Higher doses (e.g., at least 5, 10, 100, 500 or 1000 copies per cell) of double-stranded material may yield more effective inhibition, while lower doses may also be useful for specific applications. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for inhibition.

[00035] As described herein, siRNAs comprise a duplex region about 19-30 nucleotides in length, about 21-27 nucleotides in length, about 21-25 nucleotides in length, or about 21-23 nucleotides in length. The siRNAs are understood to recruit nuclease complexes and guide the complexes to the target gene transcript by pairing to the specific sequences. As a result, the target gene transcript is degraded by the nucleases in the protein complex. In certain embodiments, the siRNA molecules comprise a 3' hydroxyl group. In certain embodiments, the siRNA constructs can be generated by processing of longer double-stranded RNAs, for example, in the presence of the enzyme

dicer. In one embodiment, the Drosophila *in vitro* system is used. In this embodiment, dsRNA is combined with a soluble extract derived from Drosophila embryo, thereby producing a combination. The combination is maintained under conditions in which the dsRNA is processed to RNA molecules of about 21 to about 27 nucleotides. The siRNA molecules can be purified using a number of techniques known to those of skill in the art. For example, gel electrophoresis can be used to purify siRNAs. Alternatively, non-denaturing methods, such as non-denaturing column chromatography, can be used to purify the siRNA. In addition, chromatography (e.g., size exclusion chromatography), glycerol gradient centrifugation, affinity purification with antibody can be used to purify siRNAs.

[00036] Production of the subject dsRNAs (e.g., siRNAs) can be carried out by chemical synthetic methods or by recombinant nucleic acid techniques. Endogenous RNA polymerase of the treated cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription *in vitro*. As used herein, dsRNA or siRNA molecules of the application need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides.

[00037] The nucleic acid-based therapeutics (e.g., siRNA) may include modifications to either the phosphate-sugar backbone or the nucleoside, e.g., to reduce susceptibility to cellular nucleases, improve bioavailability, improve formulation characteristics, and/or change other pharmacokinetic properties. To illustrate, the phosphodiester linkages of natural RNA may be modified to include at least one of a nitrogen or sulfur heteroatom. Modifications in RNA structure may be tailored to allow specific genetic inhibition while avoiding a general response to dsRNA. Likewise, bases may be modified to block the activity of adenosine deaminase. The dsRNAs may be produced enzymatically or by partial/total organic synthesis, any modified ribonucleotide can be introduced by *in vitro* enzymatic or organic synthesis. Methods of chemically modifying RNA molecules can be adapted for modifying dsRNAs (see, e.g., Heidenreich et al. (1997) Nucleic Acids Res, 25:776-780; Wilson et al. (1994)

J Mol Recog 7:89-98; Chen et al. (1995) Nucleic Acids Res 23:2661-2668; Hirschbein et al. (1997) Antisense Nucleic Acid Drug Dev 7:55-61). Merely to illustrate, the backbone of an dsRNA or siRNA can be modified with phosphorothioates, phosphoramidate, phosphodithioates, chimeric methylphosphonate-phosphodiesters, peptide nucleic acids, 5-propynyl-pyrimidine containing oligomers or sugar modifications (e.g., 2'-substituted ribonucleosides, α-configuration). In certain cases, the dsRNAs of the application lack 2'-hydroxy (2'-OH) containing nucleotides. In certain embodiments, the siRNA molecules comprise a phosphorothioate sense strand. In certain embodiments, the siRNA molecules comprise a phosphodiester antisense strand.

[00038] In exemplary embodiments, the first dose amount of the nucleic acid-based therapeutic is in the range of about 5 mg/m^2 to about 40 mg/m^2. Those of ordinary skill in the art are readily able to determine a patient's body surface area (BSA) in square meters (m^2), for example, by utilizing methods as disclosed in DuBois *et al.*, *Arch. Int. Med 17*:863-871 (1916), so as to effectively compute the appropriate dose amount.

[00039] Suitably, the first dose amount of the nucleic acid-based therapeutic is about 7 mg/m^2 to about 30 mg/m^2, about 9 mg/m^2 to about 27 mg/m^2, about 11 mg/m^2 to about 27 mg/m^2, about 15 mg/m^2 to about 27 mg/m^2, about 15 mg/m^2 to about 25 mg/m^2, about 15 mg/m^2 to about 23 mg/m^2, about 15 mg/m^2 to about 20 mg/m^2, including any values in between these ranges, suitably about 15 mg/m^2, about 16 mg/m^2, about 17 mg/m^2, about 18 mg/m^2, about 19 mg/m^2, or about 20 mg/m^2.

[00040] In embodiments, the second dose amount of the nucleic acid-based therapeutic is about 10 mg/m^2 to about 50 mg/m^2, suitably about 15 mg/m^2 to about 50 mg/m^2, about 18 mg/m^2 to about 50 mg/m^2, about 20 mg/m^2 to about 50 mg/m^2, about 20 mg/m^2 to about 45 mg/m^2, about 20 mg/m^2 to about 40 mg/m^2, about 25 mg/m^2 to about 40 mg/m^2, including values between these ranges, suitably about 25 mg/m^2, about 26 mg/m^2, about 27 mg/m^2, about 28 mg/m^2, about 29 mg/m^2, about 30 mg/m^2, about 31 mg/m^2, about 32 mg/m^2,

about 33 mg/m^2, about 34 mg/m^2, about 35 mg/m^2, about 36 mg/m^2, about 37 mg/m^2, about 38 mg/m^2, about 39 mg/m^2, or about 40 mg/m^2.

[00041] In additional embodiments, the first dose amount of the nucleic acid-based therapeutic is in the range of at least about 5 mg/m^2 to at least about 40 mg/m^2. Suitably, the first dose amount of the nucleic acid-based therapeutic is at least about 7 mg/m^2 to about at least 30 mg/m^2, at least about 9 mg/m^2 to about at least 27 mg/m^2, at least about 11 mg/m^2 to at least about 27 mg/m^2, at least about 15 mg/m^2 to at least about 27 mg/m^2, at least about 15 mg/m^2 to about at least 25 mg/m^2, at least about 15 mg/m^2 to at least about 23 mg/m^2, at least about 15 mg/m^2 to at least about 20 mg/m^2, including any values in between these ranges, suitably at least about 15 mg/m^2, at least about 16 mg/m^2, at least about 17 mg/m^2, at least about 18 mg/m^2, at least about 19 mg/m^2, or at least about 20 mg/m^2.

[00042] In additional embodiments, the second dose amount of the nucleic acid-based therapeutic is at least about 10 mg/m^2 to at least about 50 mg/m^2, suitably at least about 15 mg/m^2 to at least about 50 mg/m^2, at least about 18 mg/m^2 to at least about 50 mg/m^2, at least about 20 mg/m^2 to at least about 50 mg/m^2, at least about 20 mg/m^2 to at least about 45 mg/m^2, at least about 20 mg/m^2 to at least about 40 mg/m^2, at least about 25 mg/m^2 to at least about 40 mg/m^2, including values between these ranges, suitably at least about 25 mg/m^2, at least about 26 mg/m^2, at least about 27 mg/m^2, at least about 28 mg/m^2, at least about 29 mg/m^2, at least about 30 mg/m^2, at least about 31 mg/m^2, at least about 32 mg/m^2, at least about 33 mg/m^2, at least about 34 mg/m^2, at least about 35 mg/m^2, at least about 36 mg/m^2, at least about 37 mg/m^2, at least about 38 mg/m^2, at least about 39 mg/m^2, or at least about 40 mg/m^2.

[00043] A person of ordinary skill in the art is readily able to determine the appropriate dosage amount based on the height, weight, age and other patient factors, when administering the nucleic acid-based therapeutics described herein.

[00044] Suitably, the first dose amount is about 1% to about 10% lower, about 10% to about 20% lower, about 20% to about 30% lower, about 30% to about 40% lower, about 40% to about 50% lower, about 50% to about 60% lower, about 60% to about 70% lower, about 70% to about 80% lower, about 80% to about 90% lower, or about 90% to about 99% lower than the second dose amount.

[00045] The first and/or second dose amounts can be administered to the patient via any suitable administration route, including, but not limited to, topical, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intrasternal injection and infusion, and intrahepatic arterial administration (including intrahepatic injection and intrahepatic infusion). In embodiments where an infusion is utilized (e.g., an intravenous infusion), the administration can take place over any suitable infusion time, for example, over the course of at least about 15 minutes, at least about 30 minutes, at least about 1 hour, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, etc.

[00046] As used herein, the term "treatment cycle" refers to the period over which the nucleic acid-based therapeutic is administered to a patient. The treatment cycle begins on the first day that the treatment is administered and ends on the day prior to beginning a new, subsequent treatment cycle, or ends on the last day of all administrations.

[00047] In exemplary embodiments, the treatment cycle is a 7 day to a 60 day treatment cycle, suitably a 7 day to 42 day treatment cycle, more suitably a 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 or 42 day treatment cycle. Other treatment cycles can be determined and utilized by a person of ordinary skill in the art.

[00048] In suitable embodiments, the first dose amount is administered to the patient on the first day of the first treatment cycle (i.e., day 1 of the first

treatment cycle). As used herein, "subsequent treatment days of the treatment cycle" refers to any day after day 1 of a treatment cycle. After administration of the first dose amount on day 1 of the first treatment cycle, the first dose is then suitably administered on subsequent treatment days of the first treatment cycle. For example, the first dose amount can be administered on days 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 etc., of the first treatment cycle, or on any individual day(s) or combination of day(s) of this first treatment cycle. For example, the first dose amount can be administered on day 1, day 3, day 8 and day 10 of the first treatment cycle (i.e., a 21 day treatment cycle), or day 1, day 3, day 8, day 10, day 14 of the first treatment cycle, or on every day, or any combination of days, of the first treatment cycle.

[00049] In embodiments, the second dose amount of the nucleic acid-based therapeutic is suitably administered during subsequent treatment cycles. Thus, following administration of the first dose amount during the first treatment cycle, the second dose amount is administered during subsequent treatment cycles, i.e., any or all subsequent treatment cycles following the first treatment cycle. For example, the first treatment cycle can comprise an 11 day to a 49 day treatment cycle, including a 21 day treatment cycle. Subsequent treatment cycles can also comprise 11 days to 49 days, following the end of the first treatment cycle. The total treatment regimen comprises all of the treatment cycles administered to a patient, i.e., the first treatment cycle and any and all subsequent treatment cycles. Suitably, the total treatment regimen will comprise a first treatment cycle, followed by any suitable number of subsequent treatment cycles, i.e., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc., subsequent treatment cycles.

[00050] In embodiments, the first treatment cycle and subsequent treatment cycles can comprise the same number of total days (i.e., 14, 21, 28, days, etc), or they can comprise different numbers of total days. For example, the first treatment cycle can comprise more total treatment days (i.e., 28 days/treatment cycle), and the subsequent treatment cycles can comprise less total treatments

days (i.e., 21 days/treatment cycle). In further embodiments, the first treatment cycle can comprise less total treatment days (i.e., 21 days/treatment cycle), and the subsequent treatment cycles can comprise more total treatments days (i.e., 28 days/treatment cycle). Other total numbers of treatment days, as described herein, can be utilized as well.

[00051] After administration of the second dose amount on day 1 of a subsequent treatment cycle, the second dose is then suitably administered on subsequent treatment days of the subsequent treatment cycles. For example, the second dose amount can be administered on days 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, etc., of the subsequent treatment cycle(s), or on any individual day(s), or combination of day(s) of the subsequent treatment cycle(s). For example, the second dose amount can be administered on day 1, day 3, day 8 and day 10 of the subsequent treatment cycle(s) (i.e., 21 day treatment cycles), or on day 1, day 3, day 8, day 10 and day 14, or on every day, or any combination of days, of the subsequent treatment cycles. In other embodiments, the second dose amount can be administered on any subsequent day of the subsequent treatment cycle(s), and can be repeated on any subsequent day or subsequent days of the subsequent treatment cycle(s).

[00052] In further embodiments, the first treatment cycle and subsequent treatment cycles can comprise administering on days 1, 8, 15, for every 28 days of a treatment cycle, on days 1 and 15 of every 28 days of a treatment cycle, or on days 1 and 8 of every 21 day treatment cycle, or on every day, or any combination of days, of a 21 or 28 day treatment cycle.

[00053] Suitably, the second dose amount is administered during any and all subsequent treatment cycles. However, in embodiments, the second dose amount can be varied from one subsequent treatment cycle to another subsequent treatment cycle, and can either be more or less than any other subsequent treatment cycle.

[00054] Suitably, a treatment regimen comprises a first treatment cycle followed by 1, 2, 3, 4 or 5 subsequent treatment cycles. In embodiments, the

first dose amount is administered during the first treatment cycle, and the second dose amount is administered during subsequent treatment cycles, e.g., during subsequent treatment cycles 2, 3, 4, 5, etc. In further embodiments, the first dose amount can be administered during the first treatment cycle, as well as any of the subsequent treatment cycles, to further increase the tolerance of the patient. For example, the first dose amount can be administered during the first treatment cycle, as well as during a second, third, fourth, etc., subsequent treatment cycle, and then the second dose amount can be administered during further subsequent treatment cycles.

[00055] In embodiments, the nucleic acid-based therapeutic is an siRNA. Suitably, the siRNA reduces the expression of a ribonucleotide reductase subunit 2 (R2) of a cell. Exemplary siRNA sequences that reduce the expression of R2 are known in the art, and disclosed for example, in U.S. Patent No. 7,427,605, the disclosure of which is incorporated by reference herein in its entirety.

[00056] Suitably, the siRNA comprises a first strand of 15 to 30 nucleotides in length comprising a sequence selected from the group consisting of SEQ ID NOs: 1-3 as set forth in Table 1 below, and (ii) a second strand of 15 to 30 nucleotides in length, wherein at least 12 nucleotides of the first and second strands are complementary to each other and form a double-stranded nucleic acid under physiological conditions.

Table 1

Core target sequences of R2.

Description	Sequence	SEQ ID NO
RRM2-444 Core	5' cgaguaccaug 3'	SEQ ID NO: 1
RRM2-632 Core	5' gauuuagccaa 3'	SEQ ID NO: 2
RRM2-928 Core	5' aagaaacgagg 3'	SEQ ID NO: 3

[00057] In further embodiments, the first strand comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22,

24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90 and 92 of Table 2, and the second strand comprises a sequence selected from the group consisting of SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91 and 93of Table 2.

Table 2

Description	Sequence	Strand	SEQ ID NO
siRRM2A (or RRM2-444)	5' cccaucgagua ccaugauauc 3'	Sense	SEQ ID NO: 4
	3' aggggguagcuc augguacuau 5'	Antisense	SEQ ID NO: 5
siRRM2B (or RRM2-632)	5' ggagcgauuua gccaagaagu 3'	Sense	SEQ ID NO: 6
	3' caccucgcuaa aucgguucuu 5'	Antisense	SEQ ID NO: 7
siRRM2C (or RRM2-928)	5' ggcucaagaaa cgaggacuga 3'	Sense	SEQ ID NO: 8
	3' gaccgaguucu uugcuccuga 5'	Antisense	SEQ ID NO: 9

Description	Sequence	Strand	SEQ ID NO
siRRM2A1 (or RRM2-444-27R)	5' [5'phos]cccau cgaguaccaugauauc ugGC 3'	Sense	SEQ ID NO: 10
	3' aggggguagcucau gguacuauagaccg 5'	Antisense	SEQ ID NO: 11
siRRM2A2" (or "RRM2-444-27L)	5' aucuuccccaucg aguaccaugauauc 3'	Sense	SEQ ID NO: 12
	3' TAgaaggggguagc ucaugguacuau[5' phos] 5'	Antisense	SEQ ID NO: 13
siRRM2B1 (or RRM2-632-27R)	5' [5'phos]ggagc gauuuagccaagaagu ucAG 3'	Sense	SEQ ID NO: 14
	3' caccucgcuaaau cgguucuucaaguc 5'	Antisense	SEQ ID NO: 15
siRRM2B2 (or RRM2-632-27L)	5' cuugguggagcga uuuagccaagaagu 3'	Sense	SEQ ID NO: 16
	3' GAaccaccucgcu aaaucgguucuu[5' phos] 5'	Antisense	SEQ ID NO: 17
siRRM2C1 (or RRM2-928-27R)	5' [5'phos]ggcuc aagaaacgaggacuga gaTG 3'	Sense	SEQ ID NO: 18
	3' gaccgaguucuuu gcuccugacucuac 5'	Antisense	SEQ ID NO: 19
siRRM2C2 (or RRM2-928-27L)	5' uauucuggcucaa gaaacgaggacuga 3'	Sense	SEQ ID NO: 20
	3' ATaagaccgaguu cuuugcuccuga[5' phos] 5'	Antisense	SEQ ID NO: 21

Description	Sequence	Strand	SEQ ID NO
siRRM2A - 5 (or RRM2-439)	5' ucuuccccauc gaguaccaug 3'	Sense	SEQ ID NO: 22
	3' guagaaggggu agcucauggu 5'	Antisense	SEQ ID NO: 23
siRRM2A - 4 (or RRM2-440)	5' cuuccccaucg aguaccauga 3'	Sense	SEQ ID NO: 24
	3' uagaaggggua gcucauggua 5'	Antisense	SEQ ID NO: 25
siRRM2A - 3 (or RRM2-441)	5' uuccccaucga guaccaugau 3'	Sense	SEQ ID NO: 26
	3' agaagggguag cucaugguac 5'	Antisense	SEQ ID NO: 27
siRRM2A - 2 (or RRM2-442)	5' uccccaucgag uaccaugaua 3'	Sense	SEQ ID NO: 28
	3' gaagggguagc ucaugguacu 5'	Antisense	SEQ ID NO: 29
siRRM2A - 1 (or RRM2-443)	5' ccccaucgagu accaugauau 3'	Sense	SEQ ID NO: 30
	3' aagggguagcu caugguacua 5'	Antisense	SEQ ID NO: 31
siRRM2A + 1 (or RRM2-445)	5' ccaucgaguac caugauaucu 3'	Sense	SEQ ID NO: 32
	3' gggguagcuca ugguacuaua 5'	Antisense	SEQ ID NO: 33
siRRM2A + 2 (or RRM2-446)	5' caucgaguacc augauaucug 3'	Sense	SEQ ID NO: 34
	3' ggguagcucau gguacuauag 5'	Antisense	SEQ ID NO: 35
siRRM2A + 3 (or RRM2-447)	5' aucgaguacca ugauaucugg 3'	Sense	SEQ ID NO: 36
	3' gguagcucaug guacuauaga 5'	Antisense	SEQ ID NO: 37
siRRM2A + 4 (or RRM2-448)	5' ucgaguaccau gauaucuggc 3'	Sense	SEQ ID NO: 38
	3' guagcucaugg uacuauagac 5'	Antisense	SEQ ID NO: 39
siRRM2A + 5 (or RRM2-449)	5' cgaguaccaug auaucuggca 3'	Sense	SEQ ID NO: 40
	3' uagcucauggu acuauagacc 5'	Antisense	SEQ ID NO: 41

Description	Sequence	Strand	SEQ ID NO
siRRM2B - 5 (or RRM2-627)	5' uugguggagcg auuuagccaa 3'	Sense	SEQ ID NO: 42
	3' ugaaccaccuc gcuaaaucgg 5'	Antisense	SEQ ID NO: 43
siRRM2B - 4 (or RRM2-628)	5' ugguggagcga uuuagccaag 3'	Sense	SEQ ID NO: 44
	3' gaaccaccucg cuaaaucggu 5'	Antisense	SEQ ID NO: 45
siRRM2B - 3 (or RRM2-629)	5' gguggagcgau uuagccaaga 3'	Sense	SEQ ID NO: 46
	3' aaccaccucgc uaaaucgguu 5'	Antisense	SEQ ID NO: 47
siRRM2B - 2 (or RRM2-630)	5' guggagcgauu uagccaagaa 3'	Sense	SEQ ID NO: 48

Description	Sequence	Strand	SEQ ID NO
	3' accaccucgcu aaaucgguuc 5'	Antisense	SEQ ID NO: 49
siRRM2B - 1 (or RRM2-631)	5' uggagcgauuu agccaagaag 3'	Sense	SEQ ID NO: 50
	3' ccaccucgcua aaucgguucu 5'	Antisense	SEQ ID NO: 51
siRRM2B + 1 (or RRM2-633)	5' gagcgauuuag ccaagaaguu 3'	Sense	SEQ ID NO: 52
	3' accucgcuaaa ucgguucuuc 5'	Antisense	SEQ ID NO: 53
siRRM2B + 2 (or RRM2-634)	5' agcgauuuagc caagaaguuc 3'	Sense	SEQ ID NO: 54
	3' ccucgcuaaau cgguucuuca 5'	Antisense	SEQ ID NO: 55
siRRM2B + 3 (or RRM2-635)	5' gcgauuuagcc aagaaguuca 3'	Sense	SEQ ID NO: 56
	3' cucgcuaaauc gguucuucaa 5'	Antisense	SEQ ID NO: 57
siRRM2B + 4 (or RRM2-636)	5' cgauuuagcca agaaguucag 3'	Sense	SEQ ID NO: 58
	3' ucgcuaaaucg guucuucaag 5'	Antisense	SEQ ID NO: 59
siRRM2B + 5 (or RRM2-637) (or siR2B + 5) (or siRRM2B + 521 mer)	5' gauuuagccaa gaaguucaga 3'	Sense	SEQ ID NO: 60
	3' cgcuaaaucgg uucuucaagu 5'	Antisense	SEQ ID NO: 61

Description	Sequence	Strand	SEQ ID NO
siRRM2C - 5 (or RRM2-923)	5' auucuggcuca agaaacgagg 3'	Sense	SEQ ID NO: 62
	3' uauaagaccga guucuuugcu 5'	Antisense	SEQ ID NO: 63
siRRM2C - 4 (or RRM2-924)	5' uucuggcucaa gaaacgagga 3'	Sense	SEQ ID NO: 64
	3' auaagaccgag uucuuugcuc 5'	Antisense	SEQ ID NO: 65
siRRM2C - 3 (or RRM2-925)	5' ucuggcucaag aaacgaggac 3'	Sense	SEQ ID NO: 66
	3' uaagaccgagu ucuuugcucc 5'	Antisense	SEQ ID NO: 67
siRRM2C - 2 (or RRM2-926)	5' cuggcucaaga aacgaggacu 3'	Sense	SEQ ID NO: 68
	3' aagaccgaguu cuuugcuccu 5'	Antisense	SEQ ID NO: 69
siRRM2C - 1 (or RRM2-927)	5' uggcucaagaa acgaggacug 3'	Sense	SEQ ID NO: 70
	3' agaccgaguuc uuugcuccug 5'	Antisense	SEQ ID NO: 71
siRRM2C + 1 (or RRM2-929)	5' gcucaagaaac gaggacugau 3'	Sense	SEQ ID NO: 72
	3' accgaguucuu ugcuccugac 5'	Antisense	SEQ ID NO: 73
siRRM2C + 2 (or RRM2-930)	5' cucaagaaacg aggacugaug 3'	Sense	SEQ ID NO: 74

Description	Sequence	Strand	SEQ ID NO
	3' ccgaguucuuu gcuccugacu 5'	Antisense	SEQ ID NO: 75
siRRM2C + 3 (or RRM2-931)	5' ucaagaaacga ggacugaugc 3'	Sense	SEQ ID NO: 76
	3' cgaguucuuug cuccugacua 5'	Antisense	SEQ ID NO: 77
siRRM2C + 4 (or RRM2-932)	5' caagaaacgag gacugaugcc 3'	Sense	SEQ ID NO: 78
	3' gaguucuuugc uccugacuac 5'	Antisense	SEQ ID NO: 79
siRRM2C + 5 (or RRM2-933)	5' aagaaacgagg acugaugccu 3'	Sense	SEQ ID NO: 80
	3' aguucuuugcu ccugacuacg 5'	Antisense	SEQ ID NO: 81

Description	Sequence	Strand	SEQ ID NO
siRRM2B + 5 27mer (or siR2B + 5-27)	5'[5'phos]gauu uagccaagaaguuc agauuAC 3'	Sense	SEQ ID NO: 82
	3' cgcuaaaucgg uucuucaagucuaa ug 5'	Antisense	SEQ ID NO: 83

Description	Sequence	Strand	SEQ ID NO
siRRM2B + 6 (or RRM2-638)	5' auuuagccaag aaguucagau 3'	Sense	SEQ ID NO: 84
(or siR2B + 6)	3' gcuaaaucggu ucuucaaguc 5'	Antisense	SEQ ID NO: 85
siRRM2B + 7 (or RRM2-639)	5' uuuagccaaga aguucagauu 3'	Sense	SEQ ID NO: 86
(or siR2B + 7)	3' cuaaaucgguu cuucaagucu 5'	Antisense	SEQ ID NO: 87
siRRM2B + 8 (or RRM2-640)	5' uuagccaagaa guucagauua 3'	Sense	SEQ ID NO: 88
(or siR2B + 8)	3' uaaaucgguuc uucaagucua 5'	Antisense	SEQ ID NO: 89
siRRM2B + 9 (or RRM2-641)	5' uagccaagaag uucagauuac 3'	Sense	SEQ ID NO: 90
(or siR2B + 9)	3' aaaucgguucu ucaagucuaa 5'	Antisense	SEQ ID NO: 91
siRRM2B + 10 (or RRM2-642)	5' agccaagaagu ucagauuaca 3'	Sense	SEQ ID NO: 92
(or siR2B + 10)	3' aaucgguucuu caagucuaau 5'	Antisense	SEQ ID NO: 93

[00058] Suitably, the first strand of the siRNA consists of SEQ ID NO: 60 and the second strand consists of SEQ NO: 61, as set forth in Table 2.

[00059] In still further embodiments, the siRNA reduces the expression of endothelial PAS domain protein 1 (EPAS1) in a cell. Exemplary siRNA sequences that reduce the expression of EPAS1 are well known in the art, including those disclosed in Published U.S. Patent Application No. 2010/0010071, the disclosure of which is incorporated by reference herein in its entirety.

[00060] In such embodiments, suitably the siRNA comprises: (i) a first strand of about 15 to about 30 nucleotides in length that comprises a sequence selected from SEQ ID NOs: 94-96 of Table 3, and (ii) a second strand of about

15 to about 30 nucleotides in length, wherein at least 12 nucleotides of the first and second strands are complementary to each other and form a double-stranded nucleic acid under physiological conditions.

Table 3

Examples of core target sequences of EPAS1 (H1F2α)

Description	Sense Sequence	SEQ ID NO
EPAS1-0677 Core	5' acacacaagcu 3'	SEQ ID NO: 94
EPAS1-2894 Core	5' gccacccagua 3'	SEQ ID NO: 95
EPAS1-4999 Core	5' ugucaacguaa 3'	SEQ ID NO: 96

[00061] In further embodiments, the first strand comprises a sequence selected from SEQ ID NOs: 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171 and 173 of Table 4; the second strand comprises a sequence selected from SEQ ID NO: 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172 and 174 of Table 4. Suitably, the first strand consists of SEQ ID NO: 121 of Table 4 and the second strand consists of SEQ ID NO: 122 of Table 4.

Table 4

Description	Sequence	Strand	SEQ ID NO
siEPAS1A	5' ugcgaacacacaagcuccucu 3'	Sense	SEQ ID NO: 97
(or EPAS1-0677)	3' ggacgcuugugugguucgaggga 5'	Antisense	SEQ ID NO: 98
siEPAS1B	5' gcuacgccacccaguaccagg 3'	Sense	SEQ ID NO: 99
(or EPAS1-2894)	3' cacgaugcggugggucauggu 5'	Antisense	SEQ ID NO: 100
siEPAS1C	5' cuaccugucaacguaacgauu 3'	Sense	SEQ ID NO: 101
(or EPAS1-4999)	3' gggauggacaguugcauugcu 5'	Antisense	SEQ ID NO: 102

Description	Sequence	Strand	SEQ ID NO
siEPAS1A1	5' [5'phos]ugcgaacacacaagcuccucuccTC 3'	Sense	SEQ ID NO 103
(or EPAS1-0677-27R)	3' ggacgcuugugugguucgaggagaggag 5'	Antisense	SEQ ID NO 104
siEPAS1A2	5' gcuuccugcgaacacacaagcuccucu 3'	Sense	SEQ ID NO 105
(or EPAS1-0677-27L)	3' CGaaggacgcuugugugguucgagga [5'phos] 5'	Antisense	SEQ ID NO 106
siEPAS1B1	5' [5'phos] gcuacgccacccaguaccaggacTA 3'	Sense	SEQ ID NO 107
(or EPAS1-2894-27R)	3' cacgaugcggugggucaugguccugau 5'	Antisense	SEQ ID NO 108
siEPAS1B2	5' cacagugcuacgccacccaguaccagg 3'	Sense	SEQ ID NO 109
(or EPAS1-2894-27L)	3' GTgucacgaugcggugggucauggu [5'phos] 5'	Antisense	SEQ ID NO 110
siEPAS1C1	5' [5'phos] cuaccugucaacguaacgauuucAT 3'	Sense	SEQ ID NO 111
(or EPAS1-4999-27R)	3' gggauggacaguugcauugcuaaagua 5'	Antisense	SEQ ID NO 112
siEPAS1C2	5' uuaacccuaccugucaacguaacgauu 3'	Sense	SEQ ID NO 113
(or EPAS1-4999-27L)	3' aauugggauggacaguugcauugcu [5'phos] 5'	Antisense	SEQ ID NO 114

Description	Sequence	Stand	SEQ ID NO
siEPAS1A-5	5' cuuccugcgaacacacaagcu 3'	Sense	SEQ ID NO: 115
(or EPAS1-0672)	3' ucgaaggacgcuuguguguuc 5'	Antisense	SEQ ID NO: 116
siEPAS1A-4	5' uuccugcgaacacacaagcuc 3'	Sense	SEQ ID NO: 117
(or EPAS1-0673)	3' cgaaggacgcuuguguguucg 5'	Antisense	SEQ ID NO: 118
siEPAS1A-3	5' uccugcgaacacacaagcucc 3'	Sense	SEQ ID NO: 119
(or EPAS1-0674)	3' gaaggacgcuuguguguucga 5'	Antisense	SEQ ID NO: 120
siEPAS1A-2	5' ccugcgaacacacaagcuccu 3'	Sense	SEQ ID NO: 121
(or EPAS1-0675)	3' aaggacgcuuguguguucgag 5'	Antisense	SEQ ID NO: 122
siEPAS1A-1	5' cugcgaacacacaagcuccuc 3'	Sense	SEQ ID NO: 123
(or EPAS1-0676)	3' aggacgcuuguguguucgagg 5'	Antisense	SEQ ID NO: 124
siEPAS1A + 1	5' gcgaacacacaagcuccucuc 3'	Sense	SEQ ID NO: 125
(or EPAS1-0678)	3' gacgcuuguguguucgaggag 5'	Antisense	SEQ ID NO: 126
siEPAS1A + 2	5' cgaacacacaagcuccucucc 3'	Sense	SEQ ID NO: 127
(or EPAS1-0679)	3' agcuuguguguucgaggaga 5'	Antisense	SEQ ID NO: 128
siEPAS1A + 3	5' gaacacacaagcuccucuccu 3'	Sense	SEQ ID NO: 129
(or EPAS1-0680)	3' cgcuuguguguucgaggagag 5'	Antisense	SEQ ID NO: 130
siEPAS1A + 4	5' aacacacaagcuccucuccuc 3'	Sense	SEQ ID NO: 131
(or EPAS1-0681)	3' gcuuguguguucgaggagagg 5'	Antisense	SEQ ID NO: 132
siEPAS1A + 5	5' acacacaagcuccucuccuca 3'	Sense	SEQ ID NO: 133
(or EPAS1-0682)	3' cuuguguguucgaggagagga 5'	Antisense	SEQ ID NO: 134

Description	Sequence	Stand	SEQ ID NO
siEPAS1B-5	5' acagugcuacgccacccagua 3'	Sense	SEQ ID NO 135
(or EPAS1-2889)	3' ggugucacgaugcggguggguc 5'	Antisense	SEQ ID NO 136
siEPAS1B-4	5' cagugcuacgccacccaguac 3'	Sense	SEQ ID NO 137
(or EPAS1-2890)	3' gugucacgaugcggguggguca 5'	Antisense	SEQ ID NO 138
siEPAS1B-3	5' agugcuacgccacccaguacc 3'	Sense	SEQ ID NO 139
or EPAS1-2891)	3' ugucacgaugcgggugggucau 5'	Antisense	SEQ ID NO 140
siEPAS1B-2	5' gugcuacgccacccaguacca 3'	Sense	SEQ ID NO 141
(or EPAS1-2892)	3' gucacgaugcgggugggucaug 5'	Antisense	SEQ ID NO 142
siEPAS1B-1	5' ugcuacgccacccaguaccag 3'	Sense	SEQ ID NO 143
(or EPAS1-2893)	3' ucacgaugcgggugggucaugg 5'	Antisense	SEQ ID NO 144
siEPAS1B + 1	5' cuacgccacccaguaccagga 3'	Sense	SEQ ID NO 145
(or EPAS1-2895)	3' acgaugcgggugggucaugguc 5'	Antisense	SEQ ID NO 146
siEPAS1B + 2	5' uacgccacccaguaccaggac 3'	Sense	SEQ ID NO 147
(or EPAS1-2896)	3' cgaugcgggugggucauggucc 5'	Antisense	SEQ ID NO 148
siEPAS1B + 3	5' acgccacccaguaccaggacu 3'	Sense	SEQ ID NO 149
(or EPAS1-2897)	3' gaugcgggugggucaugguccu 5'	Antisense	SEQ ID NO 150
siEPAS1B + 4	5' cgccacccaguaccaggacua 3'	Sense	SEQ ID NO 151
(or EPAS1-2898)	3' augcgggugggucaugguccug 5'	Antisense	SEQ ID NO 152
siEPAS1B + 5	5' gccacccaguaccaggacuac 3'	Sense	SEQ ID NO 153
(or EPAS1-2899)	3' ugcgggugggucaugguccuga 5'	Antisense	SEQ ID NO 154

Description	Sequence	Stand	SEQ ID NO
siEPAS1C-5	5' uaacccuaccugucaacguaa 3'	Sense	SEQ ID NO: 155
(or EPAS1-4994)	3' uaauugggauggacaguugca 5'	Antisense	SEQ ID NO: 156
siEPAS1C-4	5' aacccuaccugucaacguaac 3'	Sense	SEQ ID NO: 157
(or EPAS1-4995)	3' aauugggauggacaguugcau 5'	Antisense	SEQ ID NO: 158
siEPAS1C-3	5' acccuaccugucaacguaacg 3'	Sense	SEQ ID NO: 159
(or EPAS1-4996)	3' auugggauggacaguugcauu 5'	Antisense	SEQ ID NO: 160
siEPAS1C-2	5' cccuaccugucaacguaacga 3'	Sense	SEQ ID NO: 161
(or EPAS1-4997)	3' uugggauggacaguugcauug 5'	Antisense	SEQ ID NO: 162
siEPAS1C-1	5' ccuaccugucaacguaacgau 3'	Sense	SEQ ID NO: 163
(or EPAS1-4998)	3' ugggauggacaguugcauugc 5'	Antisense	SEQ ID NO: 164
siEPAS1C + 1	5' uaccugucaacguaacgauuu 3'	Sense	SEQ ID NO: 165
(or EPAS1-5000)	3' ggauggacaguugcauugcua 5'	Antisense	SEQ ID NO: 166
siEPAS1C + 2	5' accugucaacguaacgauuuc 3'	Sense	SEQ ID NO: 167
(or EPAS1-5001)	3' gauggacaguugcauugcuaa 5'	Antisense	SEQ ID NO: 168
siEPAS1C + 3	5' ccugucaacguaacgauuuca 3'	Sense	SEQ ID NO: 169
(or EPAS1-5002)	3' auggacaguugcauugcuaaa 5'	Antisense	SEQ ID NO: 170
siEPAS1C + 4	5' cugucaacguaacgauuucau 3'	Sense	SEQ ID NO: 171
(or EPAS1-5003)	3' uggacaguugcauugcuaaag 5'	Antisense	SEQ ID NO: 172
siEPAS1C + 5	5' ugucaacguaacgauuucaug 3'	Sense	SEQ ID NO: 173
(or EPAS1-5004)	3' ggacaguugcauugcuaaagu 5'	Antisense	SEQ ID NO: 174

[00062] In exemplary embodiments, the nucleic acid-based therapeutic is part of a composition comprising the nucleic acid-based therapeutic and a polymer.

[00063] Any type of polymer capable of forming a composition with the nucleic acid-based therapeutic may be used in the methods described herein. The polymer may be a linear or branched polymer. The polymer may be a homopolymer or a co-polymer. If a co-polymer is used, the co-polymer may be a random copolymer or a branched co-polymer. Suitably the polymer is water-dispersible and more preferably water soluble. For example, suitable polymers include, but are not limited to polysaccharides, polyesters, polyamides, polyethers, polycarbonates, polyacrylates, etc. For therapeutic pharmaceutical uses, the polymer should have a low toxicity profile and preferably are not toxic or cyctotoxic. As discussed below, a suitable polymer for use in a composition of the invention is a cyclodextrin-based polymer (i.e., to form an siRNA-cyclodextrin composition). Water soluble linear

cyclodextrin copolymers having molecular weights in the range of 3,000 to 100,000 are suitable and those having molecular weights of 3,000 to 50,000 are more suitable.

[00064] According to the invention, the polymer in the composition may be a single polymer or a mixture of two or more polymers, which can be the same or different polymers. Each polymer of the composition can further contain or may be further modified to contain a crosslinking group through which association of the polymers to form the particulate composite can be achieved.

[00065] At least one polymer of the compositions is suitable a polymer capable of forming an inclusion complex, as disclosed in U.S. Patent No. 7,166,302, the disclosure of which is incorporated by reference herein in its entirety. A "polymer capable of forming an inclusion complex" can be any polymer capable of one or more host-guest associations via nonbonding interactions (e.g. van der Waals forces, hydrogen bonding, dipole-dipole interactions, ion-paring, soluophobic interactions, etc.) with another compound (the complexing agent) or substituent on a compound. In other words, at least one polymer has host or guest functionality to form an inclusion complex with a complexing agent or a substituent on the complexing agent. The host or guest functionality may be part of the polymer backbone or may be present as a substituent or in a pendant or branched chain. An example of a polymer having host functionality in the polymer backbone is a linear cyclodextrin polymer. An example of a polymer having guest functionality not as part of the polymer backbone would be a polymer having pendant adamantane groups. Other examples of suitable "hosts" which may be employed with the polymer include, but are not limited to, carceronds, cavitands, crown ethers, cryptands, cucurbiturils, calixarenes, spherands and the like. Examples of inclusion guests suitable for such hosts include those known in the art such as, but not limited to, adamantane, diadamantane, naphthalene, and cholesterol.

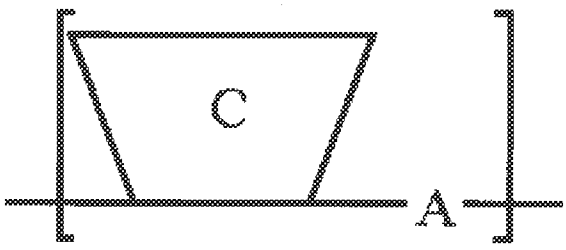
[00066] In exemplary embodiments, the polymer used to form the compositions is a cyclodextrin-containing polymer, more suitably a substantially linear cyclodextrin polymer. The polymer can also be a polyethyleneimine (PEI) or a

polymer having pendant cyclodextrins. A linear cyclodextrin copolymer is a polymer containing cyclodextrin moieties as an integral part of its polymer backbone. Polymers having pendant cyclodextrin moieties not a part of the main polymer chain but rather attached off the polymer backbone can also be used in the compositions described herein. A linear cyclodextrin-containing polymer can be any linear polymer containing at least one cyclodextrin moiety as part of the polymer backbone. The cyclodextrin-containing polymer is preferably water-soluble. More suitably, the linear cyclodextrin-containing polymer is a linear cyclodextrin copolymer or a linear oxidized cyclodextrin copolymer. The cyclodextrin groups within the polymer provide host functionality to the polymer allowing it to form inclusion complexes. The substantially linear polymer capable of inclusion complex formation can further contain or can be further modified to contain an additional functional group (e.g. thiol group).

[00067] In suitable embodiments, the polymer is a cyclodextrin-containing polymer. Exemplary cyclodextrin polymers for use in the methods described herein include β-cyclodextrin polymers. Examples of β-cyclodextrin polymers for use in the methods described herein are well known in the art, and are disclosed for example, in U.S. Patent Nos. 6,509,323; 6,884,789; 7,091,192 and 7,270,808, the disclosures of each of which are incorporated by reference herein in their entireties.

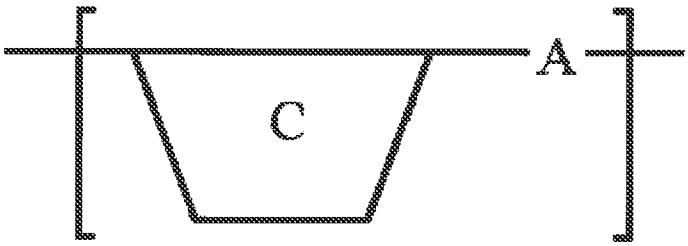
[00068] Suitably, the cyclodexrin polymer is a water-soluble, linear cyclodextrin copolymer comprising repeating units of formula Ia, Ib or both:

Ia



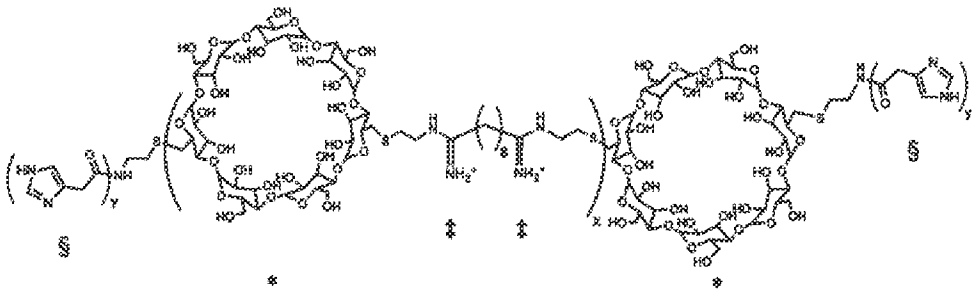
and

Ib



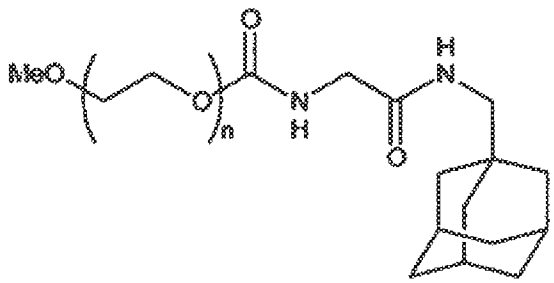
Suitably, C is a substituted or unsubstituted cyclodextrin monomer and A is a comonomer bound to cyclodextrin C. In embodiments, the cyclodextrin is a comonomer of an α, β, or γ-cyclodextrin, or combination thereof.

[00069] In exemplary embodiments, the β-cyclodexrin polymer of the compositions is represented below:



[00070] In embodiments, the composition further comprises a stabilizing agent. Exemplary stabilizing agents include those disclosed in U.S. Patent Nos. 7,018,609 and 7,166,302, the disclosures of which are incorporated by reference herein in their entireties. In U.S. Patent Nos. 7,018,609 and 7,166,302, exemplary stabilizing agents are often referred to as complexing agents.

[00071] Suitably, the stabilizing agent comprises an adamantane (AD) group and a polyethylene glycol (PEG). Such stabilizing agents include, for example the agent shown below.



[00072] Any suitable molecular weight PEG can be used in the stabilizing agents, including for example, PEG 500 to PEG 10,000, and particularly PEG 600, PEG 3400, and PEG 5000.

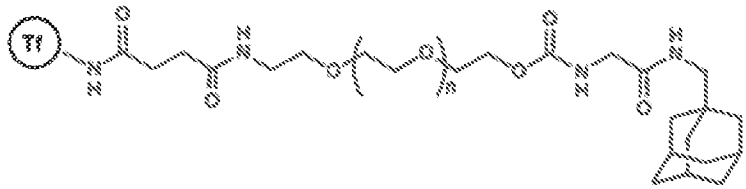
[00073] In further embodiments, compositions suitably also comprise a targeting agent. The targeting agent can be any agent (or multiple agents) that allows for targeting and/or binding to a desired cell. As would be understood by one of skill in the art, targeting and binding to a cell may include cell receptor attachment which in turn may lead to receptor mediated endocytosis.

[00074] Suitably, the targeting agent comprises an adamantane (AD) group, a polymer, such as a poly(ethylene glycol) (PEG) and a targeting ligand. Any suitable molecular weight PEG can be utilized in the targeting agents described herein.

[00075] Examples of suitable targeting ligands are known to those of ordinary skill in the art and include, but are not limited to, vitamins (e.g. folic acid), proteins (e.g. transferrin, and monoclonal antibodies), monosaccharides (e.g. galactose), peptides, and polysaccharides. The choice of ligand, as one of ordinary skill appreciates, may vary depending upon the type of delivery desired. If two or more ligands or targeting agents are attached, the ligands/targeting agents may be the same or different. As another example, the ligand may be membrane permeabilizing or membrane permeable agent such as the TAT protein from HIV-1. The TAT protein is a viral

transcriptional activation that is actively imported into the cell nucleus. Torchilin, V. P. et al, PNAS. 98, 8786 8791, (2001).

[00076] Exemplary targeting agents for use in the methods and compositions described herein are disclosed in U.S. Patent Nos. 7,166,302 and 7,018,609, the disclosures of each of which are incorporated by reference herein in their entireties. In suitable embodiments, the targeting agent is shown below:



[00077] In exemplary embodiments, as shown above, the targeting ligand is a transferrin (Tf) molecule.

[00078] The methods described herein can be utilized to treat any disease or disorder. Exemplary diseases and disorders that can be treated include, but are not limited to, cystic fibrosis, Gaucher's disease, muscular dystrophy, AIDS, cancer, progressive heart failure, restenosis, hemophilia and neurological conditions. In exemplary embodiments, the disease or disorder is cancer, including for example, multiple myeloma, leukemia, melanoma, or ovarian cancer.

[00079] In further embodiments, methods are provided of administering an siRNA to treat a disease or disorder in a patient. The methods suitably comprise administering during a first treatment cycle a first dose amount of an siRNA composition comprising the siRNA, a cyclodextrin-containing polymer, a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG), and a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand. A second dose amount of the siRNA composition is administered during subsequent treatment cycles. Suitably, the first dose amount is lower than the second dose amount.

[00080] Exemplary dose amounts for the first and second dose amounts are described throughout. In suitable embodiments, the first dose amount is about 18 mg/m^2 and the second dose amount is about 25 mg/m^2 to about 40 mg/m^2. In additional embodiments, the first dose amount is about 20% to about 30% lower, about 30% to about 40% lower, about 40% to about 50% lower, about 50% to about 60% lower, about 60% to about 70% lower, about 70% to about 80% lower, about 80% to about 90% lower, or about 90% to about 99% lower than the second dose amount.

[00081] Exemplary treatment cycles are described throughout, and include for example, a first and subsequent treatment cycles of 21 days. Suitably, the first dose amount and the second dose amount are administered on days 1, 3, 8, and/or 10 of the treatment cycles.

[00082] Exemplary siRNAs are described throughout, and include siRNAs that reduce the expression of a ribonucleotide reductase subunit 2 (R2) in a cell and siRNAs that reduce the expression of endothelial PAS domain protein 1 (EPAS1) in a cell. Suitable sequences for such siRNAs are described in the tables herein and in U.S. Patent No. 7,427,605 and Published U.S. Patent Application No. 2010/0010071.

[00083] As described herein, the targeting ligand of the targeting agent can be any suitable ligand, including for example, transferrin.

[00084] Exemplary structures of the cyclodextrin-containing polymer, stabilizing agent comprising and targeting agent are described throughout, and in U.S. Patent Nos. 7,018,609, 7,166,302 and 7,270,808, the disclosures of each of which are incorporated by reference herein in their entireties.

[00085] Exemplary diseases and disorders that can be treated using the disclosed methods are described herein.

[00086] In further embodiments, methods are provided for administering an siRNA to treat a disease or disorder in a patient. The methods suitably comprise administering during a first 21 day treatment cycle, a first dose of about 18 mg/m^2 of an siRNA composition. The siRNA composition suitably comprises the siRNA, a cyclodextrin-containing polymer, a stabilizing agent

comprising an adamantane (AD) group and a polyethylene glycol (PEG), and a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and transferrin. A second dose of about 25 mg/m^2 to about 40 mg/m^2 of the siRNA composition is administered during subsequent 21 day treatment cycles.

[00087] In exemplary embodiments, the first dose and second dose are administered on days 1, 3, 8, and/or 10 of the treatment cycles.

[00088] Suitable siRNAs are described herein and include siRNAs that reduce the expression of a ribonucleotide reductase subunit 2 (R2) in a cell and siRNAs that reduce the expression of endothelial PAS domain protein 1 (EPAS1) in a cell. Exemplary sequences for these siRNAs are described herein.

[00089] In further embodiments, methods are provided of reducing an immune response to siRNA. The methods suitably comprise, administering during a first treatment cycle a first dose amount of an siRNA composition comprising, the siRNA, a cyclodextrin-containing polymer, a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG), and a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand. The methods suitably comprise administering a second dose amount of the siRNA composition during subsequent treatment cycles. Suitably, the first dose amount is lower than the second dose amount.

[00090] Exemplary amounts of the first and second dose, as well as dosing schedules and treatment cycles, are described herein.

[00091] Suitably, the methods reduce the level of IL-6 or TNF-α produced by the patient. The levels of other cytokines (e.g., IL-10, IL-2, IL4, IFN-γ, IL-12p40 and IL12p70), can also be modified (i.e., increased or decreased) as a result of the methods described herein.

[00092] It will be readily apparent to one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein can be made without departing from the scope of

any of the embodiments. The following examples are included herewith for purposes of illustration only and are not intended to be limiting.

Examples

Example 1: Results of Clinical Administration of siRNA-cyclodextrin Compositions

[00093] The dosing schedule of an siRNA-cyclodextrin composition (a composition comprising an siRNA, a cyclodextrin-containing polymer, a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG), and a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand) has been performed, and an analysis of cytokine concentrations and adverse events reported for subjects who have received the composition. 20 subjects accrued to 5 subject cohorts were treated.

[00094] Correlative cytokine studies suggest that the siRNA-cyclodextrin composition causes a dose-dependent innate immune response in subjects that is primarily observed during initial infusions of the composition (i.e., Cycle 1). However, preliminary cytokine results also indicate that, following exposure and prolonged treatment with the composition, desensitization occurs. Based on this data, a protocol has been developed to treat patients at a lower dose for the first cycle followed by administration of a higher dose in subsequent cycles to increase tolerability.

[00095] Correlative studies measured select cytokines in subject plasma pre- and post- administration of the siRNA-cyclodextrin composition. Results revealed that cytokines IL-10, IL-6 and TNF-α, those associated with innate immunity, seem to have a strong temporal correlation with administration of the composition (FIGs. 1A-1C) while none of the other cytokines (IFN-γ, IL-2, IL-4, IL-12p40, and IL-12p70) show significant elevations during infusion or post-dosing. In addition, maximum cytokine concentrations for IL-10, IL-6 and TNF-α during Cycle 1 appear to be correlated with dose level of the

composition (FIG. 2) as well. In particular, elevations of pro-inflammatory cytokines IL-6 and TNF-α suggest the activation of an innate immune response to the siRNA-cyclodextrin composition.

[00096] Dose-dependent increases in IL-6 and TNF-α cytokine levels during Cycle 1 correlate with an increase in the frequency of adverse events across dose cohorts that are indicative of drug hypersensitivity such as fatigue (39%), chills (39%), nausea (28%), and pyrexia (22%), and the onset of an innate immune response. These events are generally self-limited (minutes to several days) and do not require medical intervention. The majority of treatment-emergent adverse events (TEAEs) reported during Cycle 1 at doses of 24 mg/m^2 and 30 mg/m^2 include chills and nausea (28%) as the most frequent. Diarrhea (17%) and vomiting (17%) have also been reported.

[00097] Only two events experienced by two subjects who received 30 mg/m^2, one event of ischemic colitis experienced by a male subject with a history of rectal bleeding, and one event of extreme fatigue reported another subject, were dose-limiting. Both of these dose limiting toxicities (DLT) occurred during the first cycle.

[00098] Furthermore, of the 18 subjects (18/20; 90%) who completed Cycle 1, a total of 63 TEAEs have been reported or an average of 3.5 TEAE/subject. Of the eight subjects who completed Cycle 2 and, in some cases, additional cycles, only a total of 10 TEAEs have been reported or an average of 1.25 TEAE/subject. Therefore, concomitant with changes in the cytokine profiles, subjects who received multiple cycles of the siRNA-cyclodextrin composition have experienced fewer TEAEs that are commonly associated with innate immune responses compared to the frequency of events reported for the same subjects during Cycle 1.

[00099] Cytokines have also been evaluated in subjects who have received multiple cycles of the siRNA-cyclodextrin composition (FIG 3 and Tables 6-8). A trend towards decreasing IL-6 and TNF-α levels and increasing IL-10 levels (FIG. 3) was demonstrated for one patient following treatment with several cycles of the siRNA-cyclodextrin composition at 30 mg/m^2. While IL-

6 and TNF-α are pro-inflammatory cytokines secreted in response to stimuli such as double stranded RNA, IL-10 is an anti-inflammatory cytokine able to suppress the synthesis of pro-inflammatory cytokines made by cells such as macrophages and regulatory T-cells. These results may indicate desensitization of this subject and a reduction of the innate immune response caused by the siRNA-cyclodextrin composition.

[000100] Changes in levels of pro-inflammatory IL-6, TNF- α and the anti-inflammatory IL-10 during Cycles 1 and 2 are summarized in Tables 6-8 for all subjects who received at least two cycles of the siRNA-cyclodextrin composition. At 3, 9 and 18 mg/m^2, levels of TNF-α and IL-6 were normal to moderately elevated during Cycle 1 with the exception of IL-6 for one patient treated at 18 mg/m^2 who had a significant increase in IL-6 during Cycle 1. This subject discontinued treatment due to an unrelated event before Cycle 2. However, no clear trends were seen when comparing Cycle 2 and Cycle 1 cytokine levels for these subjects. At 24 and 30 mg/m^2, Cycle 1 pro-inflammatory cytokine levels were consistently elevated but showed a decreasing trend towards a lower maximum concentration (c_{max}) in Cycle 2. In contrast, the anti-inflammatory cytokine IL-10 showed the opposite trend, with c_{max} increasing during Cycle 2 (vs. Cycle 1).

[000101] Table 6: Changes in TNF-α levels between Cycles 1 and 2 for subjects who received more than one cycle of the siRNA-cyclodextrin composition. Number of subjects who received 2 or more cycles (n) and total number of subjects per cohort (N) are listed in column "n/N".

Table 6

Dose [mg/m^2]	n/N	Average TNF-α c_{max} Cycle 1 [pg/mL]*	STDEV	Ratio of Cycle 2/1 c_{max}
3	2/3	17	12	94%
9	3/3	18	6	142%
18	1/3	24	14	97%
24	3/6	44	29	92%
30	1/3	74	83	57%

* Normal range maximum TNF-α value: 20.9 pg/mL

[000102] Table 7: Changes in IL-6 levels between Cycles 1 and 2 for subjects who received more than one cycle of the siRNA-cyclodextrin composition. Number of subjects who received 2 or more cycles (n) and total number of subjects per cohort (N) are listed in column "n/N".

Table 7

Dose [mg/m^2]	n/N	Average IL-6 c_{max} Cycle 1 [pg/mL]*	STDEV	Ratio of Cycle 2/1 c_{max}
3	2/3	23	12	100%
9	3/3	34	9	180%
18	1/3	555	652	130%
24	3/6	191	244	59%
30	1/3	267	273	46%

* Normal range maximum IL-6 value: 19.7 pg/mL

[000103] Table 8: Changes in IL-10 levels between Cycles 1 and 2 for subjects who received more than one cycle of the siRNA-cyclodextrin composition. Number of subjects who received 2 or more cycles (n) and total number of subjects per cohort (N) are listed in column "n/N".

Table 8

Dose [mg/m^2]	n/N	Average IL-10 c_{max} Cycle 1 [pg/mL]*	STDEV	Ratio of Cycle 2/1 c_{max}
3	2/3	32	10	143%
9	3/3	117	60	90%
18	1/3	49	23	212%
24	3/6	88	37	149%
30	1/3	210	89	164%

* Normal range maximum IL-10 value: 21.5 pg/mL

[000104] Though no wishing to be bound by any theory, these results indicate that frequent exposure of the siRNA-cyclodextrin composition can desensitize subjects, thereby reducing an innate immune response. Therefore, an administration protocol has been developed in which subjects will receive a

lower concentration of study composition in Cycle 1 (i.e., 18 mg/m^2), followed by a higher dose concentration for subsequent cycles (i.e., 27, 30 and 36 mg/m^2), as summarized in the table below. The dose schedule will be on Days 1, 3, 8 and 10 of a 21-day cycle as shown in Table 9. It is envisaged that this protocol will have improved tolerability and efficacy, as well as reduced toxicity, as compared to a dose schedule in which the dosage amount is the same at all dosage times during the treatment cycle.

Table 9

Cohort	Dose Level: Cycle 1*	Dose Level: Subsequent Cycles
1	18 mg/m^2	27 mg/m^2
2	18 mg/m^2	30 mg/m^2
3	18 mg/m^2	36 mg/m^2*

[000105] It is to be understood that while certain embodiments have been illustrated and described herein, the claims are not to be limited to the specific forms or arrangement of parts described and shown. In the specification, there have been disclosed illustrative embodiments and, although specific terms are employed, they are used in a generic and descriptive sense only and not for purposes of limitation. Modifications and variations of the embodiments are possible in light of the above teachings. It is therefore to be understood that the embodiments may be practiced otherwise than as specifically described.

WHAT IS CLAIMED IS:

1. A method of administering a nucleic acid-based therapeutic to treat a disease or disorder in a patient, the method comprising:

a) administering a first dose amount of a nucleic acid-based therapeutic during a first treatment cycle; and

b) administering a second dose amount of the nucleic acid-based therapeutic during subsequent treatment cycles,

wherein the first dose amount is lower than the second dose amount.

2. The method of claim 1, wherein the first dose amount is about 9 mg/m^2 to about 27 mg/m^2.

3. The method of claim 2, wherein the first dose amount is about 15 mg/m^2 to about 20 mg/m^2.

4. The method of claim 3, wherein the first dose amount is about 18 mg/m^2.

5. The method of claim 1, wherein the second dose amount is about 20 mg/m^2 to about 50 mg/m^2.

6. The method of claim 5, wherein the second dose amount is about 25 mg/m^2 to about 40 mg/m^2.

7. The method of claim 1, wherein the first dose amount is about 20% to about 30% lower, about 30% to about 40% lower, about 40% to about 50% lower, about 50% to about 60% lower, about 60% to about 70% lower, about 70% to about 80% lower, about 80% to about 90% lower, or about 90% to about 99% lower than the second dose amount.

8. The method of claim 1, wherein the first and subsequent treatment cycles are 11 day to 49 day treatment cycles.

9. The method of claim 8, wherein the first and subsequent treatment cycles are 21 day treatment cycles.

10. The method of claim 1, wherein the first and subsequent treatment cycles comprise administering on days 1, 3, 8, and 10 of the treatment cycles.

11. The method of claim 1, wherein the nucleic acid-based therapeutic is an siRNA, dsRNA, miRNA, antisense RNA, aptamer, ribozyme, or an enzymatic nucleic acid.

12. The method of claim 11, wherein the siRNA reduces the expression of a ribonucleotide reductase subunit 2 (R2) in a cell.

13. The method of claim 12, wherein the siRNA comprises a first strand of 15 to 30 nucleotides in length comprising a sequence selected from the group consisting of SEQ ID NOs: 1-3 of Table 1, and (ii) a second strand of 15 to 30 nucleotides in length, wherein at least 12 nucleotides of the first

and second strands are complementary to each other and form a double-stranded nucleic acid under physiological conditions.

14. The method of claim 13, wherein the first strand comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90 and 92 of Table 2, and the second strand comprises a sequence selected from the group consisting of SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91 and 93 of Table 2.

15. The method of claim 14, wherein the first strand consists of SEQ ID NO: 60 and the second strand consists of SEQ NO: 61 of Table 2.

16. The method of claim 12, wherein the siRNA reduces the expression of endothelial PAS domain protein 1 (EPAS1) in a cell.

17. The method of claim 16, wherein the siRNA comprises: (i) a first strand of about 15 to about 30 nucleotides in length that comprises a sequence selected from SEQ ID NOs: 94-96 of Table 3, and (ii) a second strand of about 15 to about 30 nucleotides in length, wherein at least 12 nucleotides of the first and second strands are complementary to each other and form a double-stranded nucleic acid under physiological conditions.

18. The method of claim 17, wherein the first strand comprises a sequence selected from SEQ ID NOs: 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171 and 173 of Table 4; the second strand comprises a sequence selected from SEQ ID NO: 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172 and 174 of Table 4.

19. The method of claim 18, wherein the first strand consists of SEQ ID NO: 121 and the second strand consists of SEQ ID NO: 122 of Table 4.

20. The method of claim 1, wherein nucleic acid-based therapeutic is part of a composition comprising the nucleic acid-based therapeutic and a cyclodextrin-containing polymer.

21. The method of claim 20, wherein the composition further comprises a stabilizing agent.

22. The method of claim 21, wherein the stabilizing agent comprises an adamantane (AD) group and a polyethylene glycol (PEG).

23. The method of claim 20, wherein the composition further comprises a targeting agent.

24. The method of 23, wherein the targeted agent comprises an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand.

25. The method of claim 24, wherein the targeting ligand is transferrin.

26. The method of claim 20, wherein the cyclodextrin-containing polymer is a β-cyclodextrin-containing polymer.

27. The method of claim 1, wherein the disease or disorder is selected from the group consisting of:

a) cystic fibrosis;

b) Gaucher's disease;

c) muscular dystrophy,

d) AIDS;

e) cancer;

f) progressive heart failure;

g) restenosis

h) hemophilia; and

i) neurological conditions.

28. The method according to claim 27, wherein the disease or disorder is cancer.

29. The method according to claim 28 wherein the cancer is multiple myeloma, leukemia, melanoma, or ovarian cancer.

30. A method of administering an siRNA to treat a disease or disorder in a patient, the method comprising:

a) administering during a first treatment cycle a first dose amount of an siRNA composition comprising:

i. the siRNA;

ii. a cyclodextrin-containing polymer;

iii. a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG); and

iv. a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand; and

b) administering a second dose amount of the siRNA composition during subsequent treatment cycles,

wherein the first dose amount is lower than the second dose amount.

31. The method of claim 30, wherein the first dose amount is about 18 mg/m^2.

32. The method of claim 30, wherein the second dose amount is about 25 mg/m^2 to about 40 mg/m^2.

33. The method of claim 30, wherein the first dose amount is about 20% to about 30% lower, about 30% to about 40% lower, about 40% to about 50% lower, about 50% to about 60% lower, about 60% to about 70% lower, about 70% to about 80% lower, about 80% to about 90% lower, or about 90% to about 99% lower than the second dose amount.

34. The method of claim 30, wherein the first and subsequent treatment cycles are 21 day treatment cycles.

35. The method of claim 34, wherein the first and subsequent treatment cycles comprise administering the second dose amount on days 1, 3, 8, and 10 of the treatment cycles.

36. The method of claim 30, wherein the siRNA reduces the expression of a ribonucleotide reductase subunit 2 (R2) in a cell.

37. The method of claim 36, wherein the first strand comprises a sequence selected from the group consisting of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90 and 92 of Table 2, and the second strand comprises a sequence selected from the group consisting of SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33,

35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91 and 93 of Table 2.

38. The method of claim 37, wherein the first strand consists of SEQ ID NO: 60 and the second strand consists of SEQ NO: 61 of Table 2.

39. The method of claim 30, wherein the siRNA reduces the expression of endothelial PAS domain protein 1 (EPAS1) in a cell.

40. The method of claim 39, wherein the first strand comprises a sequence selected from SEQ ID NOs: 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171 and 173 of Table 4; the second strand comprises a sequence selected from SEQ ID NO: 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172 and 174 of Table 4.

41. The method of claim 40, wherein the first strand consists of SEQ ID NO: 121 and the second strand consists of SEQ ID NO: 122 of Table 4.

42. The method of claim 30, wherein the targeting ligand is transferrin.

43. The method of claim 30, wherein the disease or disorder is selected from the group consisting of:

a) cystic fibrosis;

b) Gaucher's disease;

c) muscular dystrophy,

d) AIDS;

e) cancer;

f) progressive heart failure;

g) restenosis

h) hemophilia; and

i) neurological conditions.

44. The method according to claim 43, wherein the disease or disorder is cancer.

45. The method according to claim 44, wherein the cancer is multiple myeloma, leukemia, melanoma, or ovarian cancer.

46. A method of administering an siRNA to treat a disease or disorder in a patient, the method comprising:

a) administering during a first 21 day treatment cycle, a first dose of about 18 mg/m^2 of an siRNA composition comprising:

i. the siRNA;

ii. a cyclodextrin-containing polymer;

iii. a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG); and

iv. a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and transferrin; and

b) administering a second dose of about 25 mg/m^2 to about 40 mg/m^2 of the siRNA composition during subsequent 21 day treatment cycles.

47. The method of claim 46, wherein the first and subsequent treatment cycles comprise administering on days 1, 3, 8, and 10 of the treatment cycles.

48. The method of claim 46, wherein the siRNA reduces the expression of a ribonucleotide reductase subunit 2 (R2) in a cell.

49. The method of claim 48, wherein the first strand consists of SEQ ID NO: 60 and the second strand consists of SEQ NO: 61 of Table 2.

50. The method of claim 46, wherein the siRNA reduces the expression of endothelial PAS domain protein 1 (EPAS1) in a cell.

51. The method of claim 50, wherein the first strand consists of SEQ ID NO: 121 and the second strand consists of SEQ ID NO: 122 of Table 4.

52. The method of claim 46, wherein the disease or disorder is selected from the group consisting of:

a) cystic fibrosis;

b) Gaucher's disease;

c) muscular dystrophy,

d) AIDS;

e) cancer;

f) progressive heart failure;

g) restenosis

h) hemophilia; and

i) neurological conditions.

53. The method according to claim 52, wherein the disease or disorder is cancer.

54. The method according to claim 53, wherein the cancer is multiple myeloma, leukemia, melanoma, or ovarian cancer.

55. A method of reducing an immune response to siRNA, the method comprising:

a) administering during a first treatment cycle a first dose amount of an siRNA composition comprising:

i. the siRNA;

ii. a cyclodextrin-containing polymer;

iii. a stabilizing agent comprising an adamantane (AD) group and a polyethylene glycol (PEG); and

iv. a targeting agent comprising an adamantane (AD) group, a polyethylene glycol (PEG) and a targeting ligand; and

b) administering a second dose amount of the siRNA composition during subsequent treatment cycles,

wherein the first dose amount is lower than the second dose amount.

56. The method of claim 55, wherein the method reduces levels of IL-6.

57. The method of claim 55, wherein the method reduces levels of TNF-α.

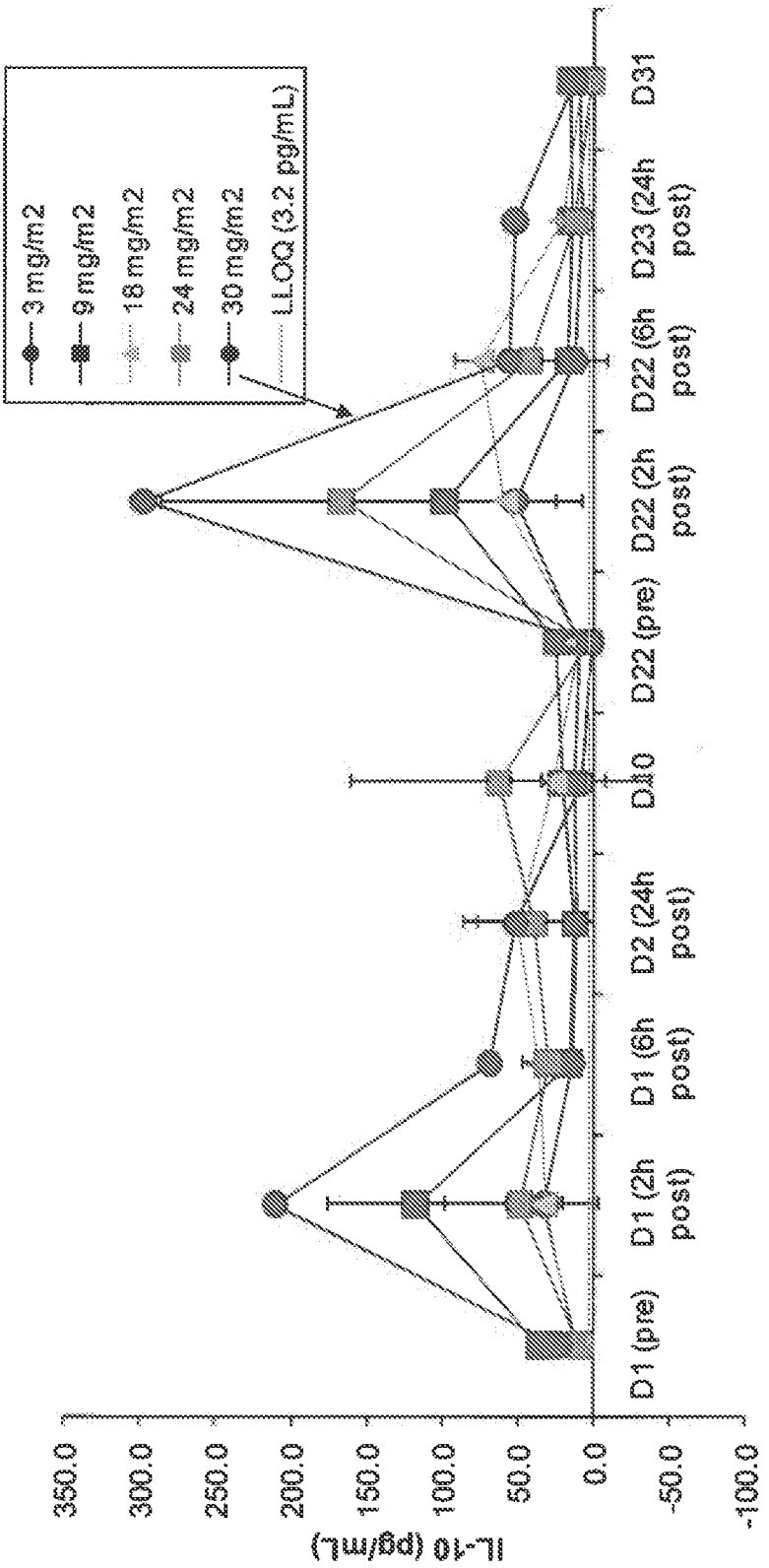

3 mg/m2
9 mg/m2
18 mg/m2
24 mg/m2
30 mg/m2
LLOQ (3.2 pg/mL)
IL-10 (pg/mL)
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
-50.0
-100.0
D1 (pre)
D1 (2h post)
D1 (6h post)
D2 (24h post)
D10
D22 (pre)
D22 (2h post)
D22 (6h post)
D23 (24h post)
D31
Timepoint

FIG. 1A

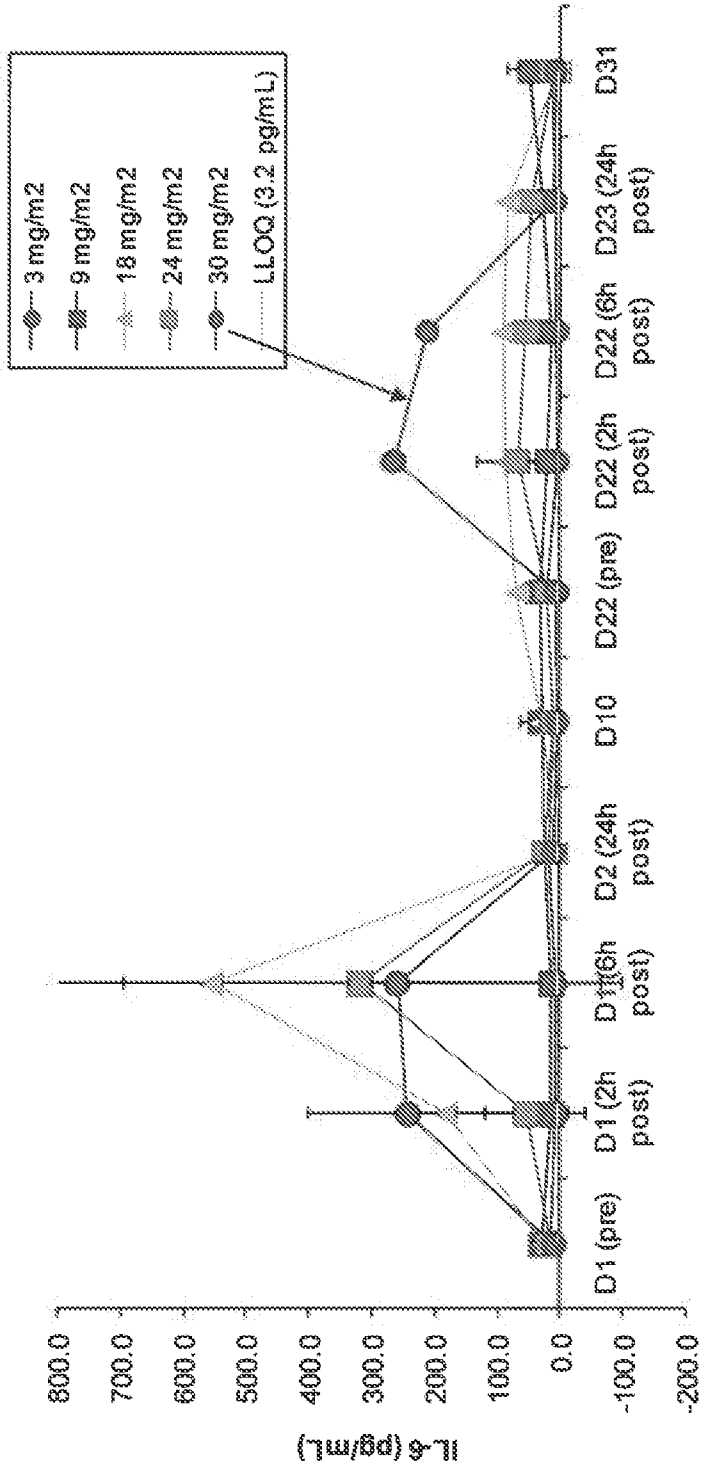

3 mg/m2
9 mg/m2
18 mg/m2
24 mg/m2
30 mg/m2
LLOQ (3.2 pg/mL)
IL-6 (pg/mL)
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
-100.0
-200.0
D1 (pre)
D1 (2h post)
D1 (6h post)
D2 (24h post)
D10
D22 (pre)
D22 (2h post)
D22 (6h post)
D23 (24h post)
D31
Timepoint

FIG. 1B

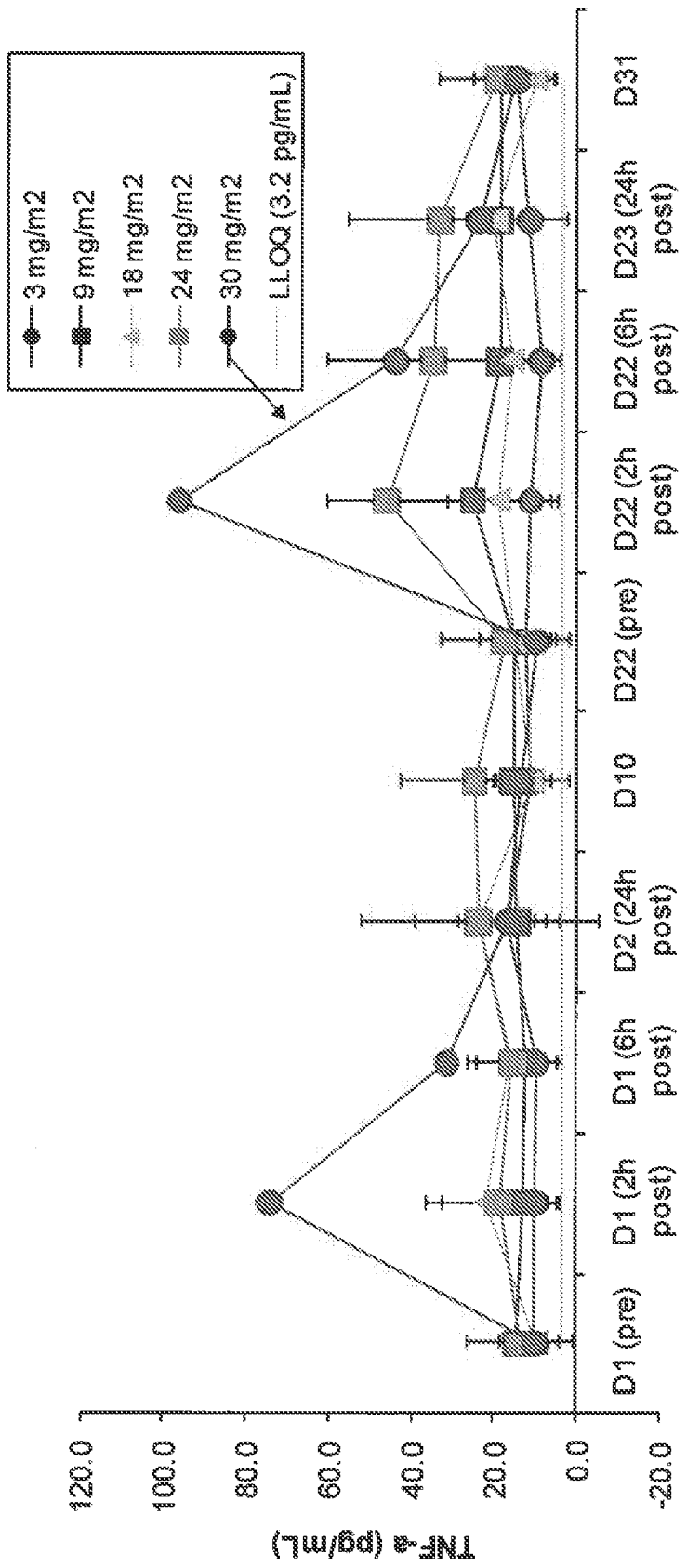

3 mg/m2
9 mg/m2
18 mg/m2
24 mg/m2
30 mg/m2
LLOQ (3.2 pg/mL)
TNF-a (pg/mL)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-20.0
D1 (pre)
D1 (2h post)
D1 (6h post)
D2 (24h post)
D10
D22 (pre)
D22 (2h post)
D22 (6h post)
D23 (24h post)
D31
Timepoint

FIG. 1C

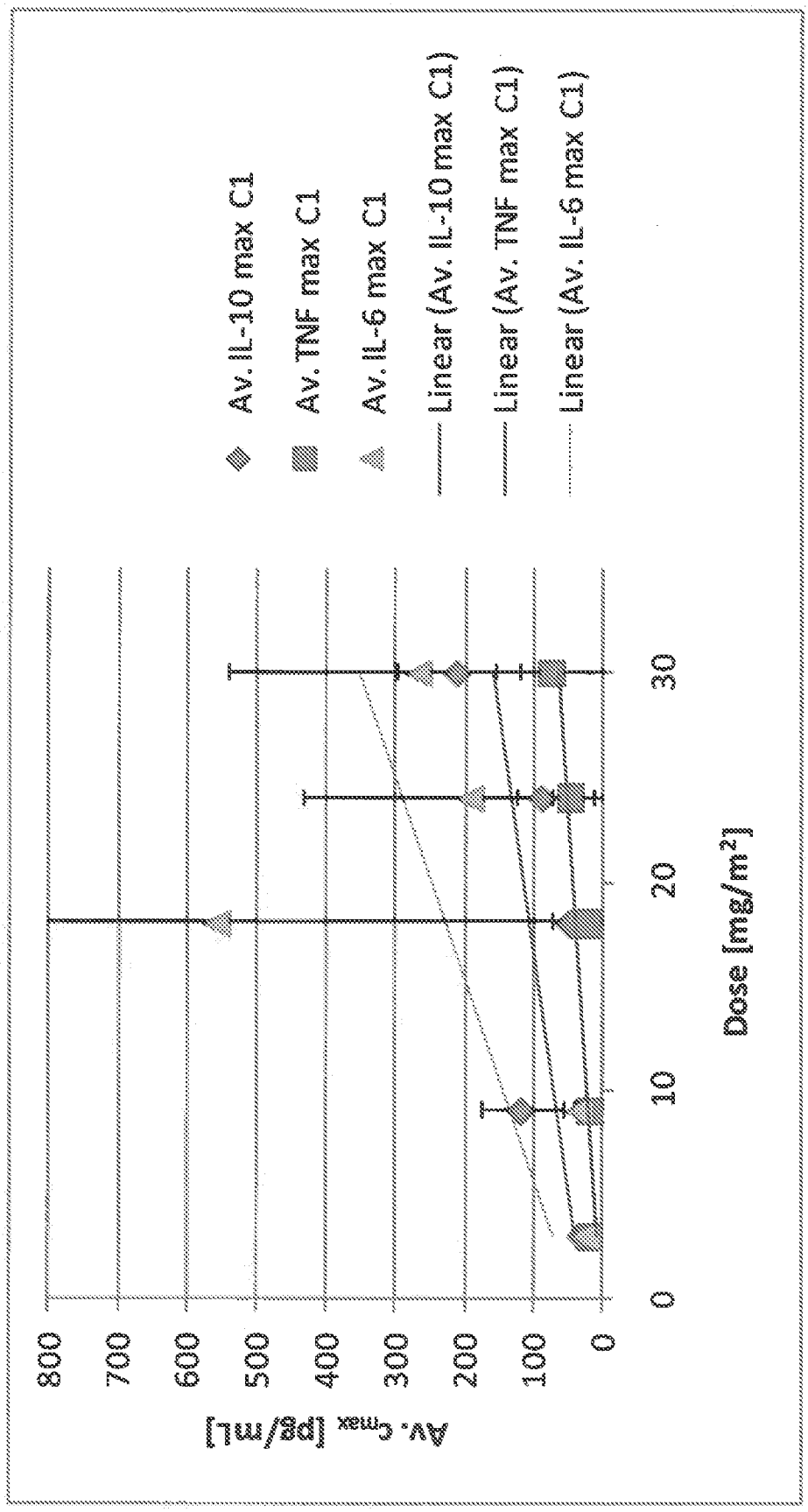

Av. IL-10 max C1
Av. TNF max C1
Av. IL-6 max C1
Linear (Av. IL-10 max C1)
Linear (Av. TNF max C1)
Linear (Av. IL-6 max C1)
Av. C_{max} [pg/mL]
800
700
600
500
400
300
200
100
0
0
10
20
30
Dose [mg/m^2]

FIG. 2

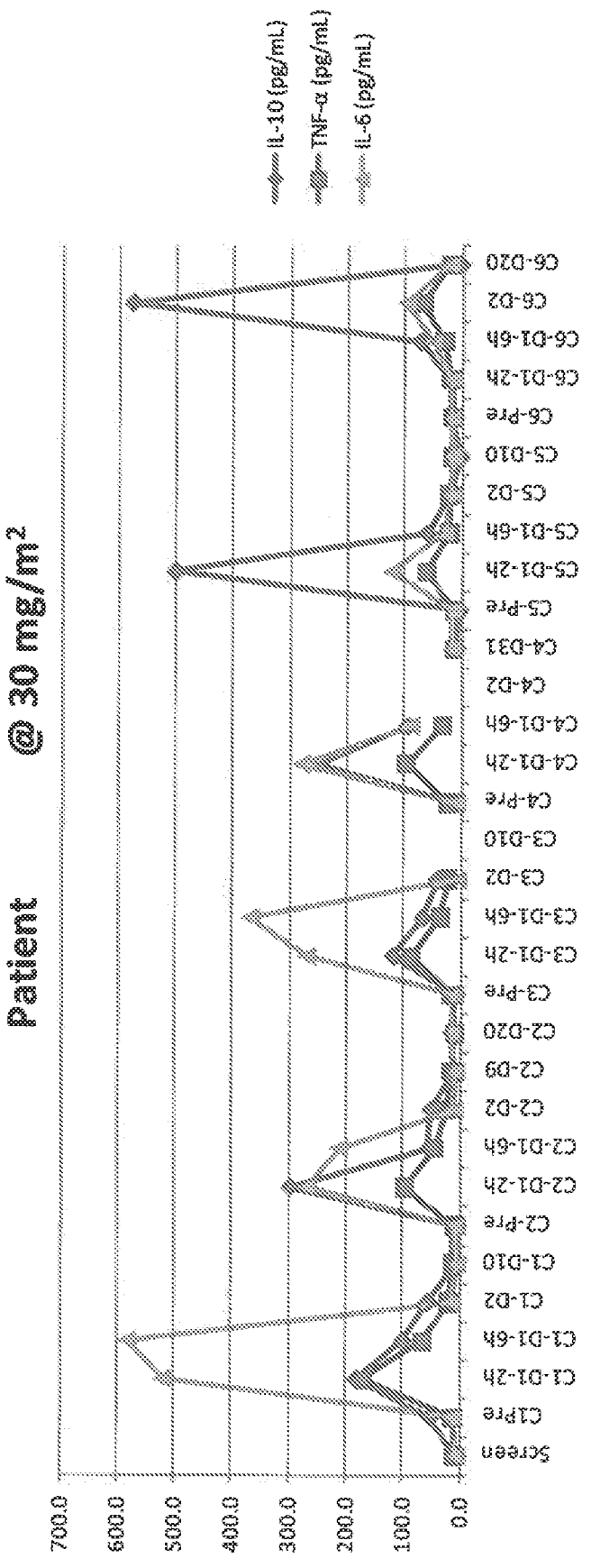

IL-10 (pg/mL)
TNF-α (pg/mL)
IL-6 (pg/mL)
Patient @ 30 mg/m²
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
Screen
C1Pre
C1-D1-2h
C1-D1-6h
C1-D2
C1-D10
C2-Pre
C2-D1-2h
C2-D1-6h
C2-D2
C2-D9
C2-D20
C3-Pre
C3-D1-2h
C3-D1-6h
C3-D2
C3-D10
C4-Pre
C4-D1-2h
C4-D1-6h
C4-D2
C4-D31
C5-Pre
C5-D1-2h
C5-D1-6h
C5-D2
C5-D10
C6-Pre
C6-D1-2h
C6-D1-6h
C6-D2
C6-D20

FIG. 3