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CA 2223769 C 2002/01/29

(11)(21) 2 223 769

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 1996/06/03
(87) Date publication PCT/PCT Publication Date: 1996/12/19
(45) Date de délivrance/Issue Date: 2002/01/29
(85) Entrée phase nationale/National Entry: 1997/12/05
(86) N° demande PCT/PCT Application No.: US 1996/008325
(87) N° publication PCT/PCT Publication No.: 1996/040266
(30) Priorité/Priority: 1995/06/07 (08/472,527) US

(51) Cl.Int.^{6}/Int.Cl.^{6} A61K 31/70, A61K 47/48, A61K 9/12

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(54) Titre : PROCEDE DE TRAITEMENT DE L'ASTHME
(54) Title: METHOD OF TREATMENT FOR ASTHMA

(57) Abrégé/Abstract:
A method of reducing bronchoconstriction in a subject in need of such treatment is disclosed. The method comprises administering to the subject an antisense oligonucleotide molecule directed against the A_1 or A_3 adenosine receptor in an amount effective to reduce bronchoconstriction. The method is useful for treating patients afflicted with asthma. Pharmaceutical formulations are also disclosed.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : **A61K 48/00, C07H 21/00** | **A1**

(11) International Publication Number: **WO 96/40266**

(43) International Publication Date: 19 December 1996 (19.12.96)

(21) International Application Number: PCT/US96/08325

(22) International Filing Date: 3 June 1996 (03.06.96)

(30) Priority Data:
08/472,527 7 June 1995 (07.06.95) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/472,527 (CON)
Filed on 7 June 1995 (07.06.95)

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(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHOD OF TREATMENT FOR ASTHMA

(57) Abstract

A method of reducing bronchoconstriction in a subject in need of such treatment is disclosed. The method comprises administering to the subject an antisense oligonucleotide molecule directed against the A_1 or A_3 adenosine receptor in an amount effective to reduce bronchoconstriction. The method is useful for treating patients afflicted with asthma. Pharmaceutical formulations are also disclosed.

METHOD OF TREATMENT FOR ASTHMA

This invention was made with Government support under grant RO1CA47217-06 from the National Cancer Institute. The Government has certain rights to this invention.

Field of the Invention

This application concerns a method of administering antisense oligonucleotides against the A_1 and A_3 Adenosine receptors as a treatment for asthma.

Background of the Invention

Asthma is one of the most common diseases in industrialized countries, and in the United States accounts for about 1% of all health care costs. K. Weiss et al., *New Engl. J. Med.* 326, 862-866 (1992). There has been reported an alarming increase in both the prevalence and mortality of asthma over the past decade, Asthma-United States, 1980-1990, *MMWR* 41, 733-735 (1992), and occupational asthma is predicted to be the preeminent occupational lung disease in the next decade. M. Chan-Yeung and J. Malo, *European Resp. J.* 7, 346-371 (1994) While the increasing mortality of asthma in industrialized countries could be attributable to the increased reliance upon beta agonists in the treatment of this disease, the underlying causes of asthma remain poorly understood. J. Gern and R. Lemanske, *In Immunology and Allergy Clinics of North America* 13, Bush, R.K. ed. W.B. Saunders Company, London, pp. 839-860 (1993).

Adenosine may constitute an important natural mediator of bronchial asthma. R. Pauwels et al., *Clinical & Exp. Allergy* 21 Suppl. 1, 48-55 (1991); S. Holgate et al., *Annals of the New York Acad. Sci.* 629, 227-236 (1991). The potential role of adenosine in human asthma is supported by the experimental finding that, in contrast to normal individuals, asthmatic individuals respond to aerosolized adenosine with marked bronchoconstriction. M. Church and S. Holgate, *Trends Pharmacol. Sci.* 7, 49-50 (1986); M. Cushley et al., *Br. J. Clin. Pharmacol.* 15, 161-165 (1983). Similarly, asthmatic rabbits produced using the dust mite allergic rabbit model of human asthma also were shown to respond to aerosolized adenosine with marked bronchoconstriction, while non asthmatic rabbits showed no response. S. Ali et al., *Agents Actions* 37, 165-176 (1992). Recent work using this model system has suggested that adenosine-mediated bronchoconstriction and bronchial hyperresponsiveness in asthma are mediated primarily through the stimulation of adenosine receptors. S. Ali et al., *J. Pharmacol. Exp. Ther.* 268, 1328-1334 (1994); S. Ali et al., *Am. J. Physiol* 266, L271-277 (1994).

Theophylline, an important drug in the treatment of asthma, is a known adenosine receptor antagonist (*see* M. Cushley et al., *Am. Rev. Resp. Dis.* 129, 380-384 (1984)) and was found to eliminate adenosine-mediated bronchoconstriction in asthmatic rabbits (Ali, et al., *supra*). Pretreatment of allergic rabbits with another A1-specific receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), potently inhibited adenosine-mediated bronchoconstriction and bronchial hyperresponsiveness in allergic rabbits. *Id.* The therapeutic potential, however, of currently available adenosine A_1 receptor-specific antagonists is limited by their toxicity. H. Klitgaard et al., *European J. Pharmacol.* 242, 221-228 (1993). Theophylline has been widely used in the treatment of asthma, but is associated

with frequent, significant toxicity resulting from its narrow therapeutic dose range. E. Powell et al., *Pediatric Emergency Care* 9, 129-133 (1993); S. Nasser and P. Rees, *Drug Safety* 8, 12-18 (1993); P. Epstein, *Annals of Internal Med.* 119, 1216-1217 (1993). The availability of an alternative strategy to downregulate adenosine-mediated bronchoconstriction would clearly be of therapeutic interest.

Summary of the Invention

A first aspect of the present invention is a method of reducing adenosine-mediated bronchoconstriction in a subject in need of such treatment. The method comprises administering an adenosine receptor antisense oligonucleotide to the lungs of the subject in an amount effective to reduce bronchoconstriction, where the adenosine receptor is selected from the group consisting of A_1 adenosine receptors and A_3 adenosine receptors.

A second aspect of the present invention is a method of treating asthma in a subject in need of such treatment. The method comprises administering an adenosine receptor antisense oligonucleotide to the lungs of the subject in an amount effective to treat asthma, where the adenosine receptor is selected from the group consisting of A_1 adenosine receptors and A_3 adenosine receptors.

A third aspect of the present invention is a pharmaceutical composition, comprising, together in a pharmaceutically acceptable carrier, an adenosine receptor antisense oligonucleotide in which the adenosine receptor is selected from the group consisting of A_1 adenosine receptors and A_3 adenosine receptors, in an amount effective to reduce adenosine-mediated bronchoconstriction.

A fourth aspect of the present invention is the use of an adenosine receptor antisense oligonucleotide as given above for the preparation of a medicament for **(a)**

reducing adenosine-mediated bronchoconstriction in a subject in need of such treatment, or **(b)** treating asthma in a subject in need of such treatment.

Antisense oligonucleotides have received considerable theoretical consideration as potentially useful pharmacologic agents in human disease. R. Wagner, *Nature* 372, 333-335 (1994). However, practical applications of these molecules in actual models of human disease have been elusive. One important consideration in the pharmacologic application of these molecules is route of administration. Most experiments utilizing antisense oligonucleotides *in vivo* have involved direct application to limited regions of the brain (see C. Wahlestedt, *Trends in Pharmacological Sciences* 15, 42-46 (1994); J. Lai et al., *Neuroreport* 5, 1049-1052 (1994); K. Standifer et al., *Neuron* 12, 805-810 (1994); A. Akabayashi et al., *Brain Research* 21, 55-61 (1994)), or to spinal fluid (see e.g. L. Tseng et al., *European J. Pharmacol*. 258, R1-3 (1994); R. Raffa et al., *European J. Pharmacol*. 258, R5-7 (1994); F. Gillardon et al., *European J. Neurosci*. 6, 880-884 (1994)). Such applications have limited clinical utility due to their invasive nature.

The systemic administration of antisense oligonucleotides also poses significant problems with respect to pharmacologic application, not the least of which is the difficulty in targeting disease-involved tissues. In contrast, the lung is an excellent potential target for antisense oligonucleotide application since it may be approached noninvasively and in a tissue-specific manner.

Brief Description of the Drawings

Figure 1 illustrates the effects of A_1 adenosine receptor antisense oligonucleotides and mismatch control antisense oligonucleotides on the dynamic compliance of the bronchial airway in a rabbit model. The two stars

represent significant difference at $p<0.01$, Student's t-test.

Figure 2 illustrates the specificity of A_1 adenosine receptor antisense oligonucleotides as indicated by the A_1 and A_2 adenosine receptor number present in airway tissue treated with A_1 adenosine receptor antisense oligonucleotides.

Detailed Description of the Invention

Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code, in accordance with 37 CFR §1.822 and established usage. *See, e.g., PatentIn User Manual*, 99-102 (Nov. 1990)(U.S. Patent and Trademark Office, Office of the Assistant Commissioner for Patents, Washington, D.C. 20231); U.S. Patent No. 4,871,670 to Hudson et al. at Col. 3 lines 20-43

The method of the present invention may be used to reduce adenosine-mediated bronchoconstriction in the lungs of a subject for any reason, including (but not limited to) asthma. Antisense oligonucleotides to the A_1 and A_3 receptors are shown to be effective in the downregulation of A_1 or A_3 in the cell. One novel feature of this treatment, as compared to traditional treatments for adenosine-mediated bronchoconstriction, is that administration is direct to the lungs. Additionally, a receptor protein itself is reduced in amount, rather than merely interacting with a drug, and toxicity is reduced.

As used herein, the term "treat" or "treating" asthma refers to a treatment which decreases the likelihood that the subject administered such treatment

will manifest symptoms of bronchoconstriction or asthma. The term "downregulate" refers to inducing a decrease in production, secretion or availability (and thus a decrease in concentration) of intracellular A_1 or A_3 adenosine receptor.

The present invention is concerned primarily with the treatment of human subjects but may also be employed for the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes.

In general, "antisense" refers to the use of small, synthetic oligonucleotides, resembling single-stranded DNA, to inhibit gene expression by inhibiting the function of the target messenger RNA (mRNA). Milligan, J.F. et al., *J. Med. Chem.* 36(14), 1923-1937 (1993). In the present invention, inhibition of gene expression of the A_1 or A_3 adenosine receptor is desired. Gene expression is inhibited through hybridization to coding (sense) sequences in a specific messenger RNA (mRNA) target by hydrogen bonding according to Watson-Crick base pairing rules. The mechanism of antisense inhibition is that the exogenously applied oligonucleotides decrease the mRNA and protein levels of the target gene or cause changes in the growth characteristics or shapes of the cells. *Id. See also* Helene, C. and Toulme, J., *Biochim. Biophys. Acta* 1049, 99-125 (1990); Cohen, J.S., Ed., *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression;* CRC Press:Boca Raton, FL (1987).

As used herein, "adenosine receptor antisense oligonucleotide" is defined as a short sequence of synthetic nucleotides that (1) hybridizes to any coding sequence in an mRNA which codes for the A_1 adenosine receptor or A_3 adenosine receptor, according to hybridization conditions described below, and (2) upon hybridization causes a decrease in gene expression of the A_1 or A_3 adenosine receptor.

The mRNA sequence of the A_1 or A_3 adenosine receptor is derived from the DNA base sequence of the gene expressing either the A_1 or A_3 adenosine receptor. The sequence of the genomic human A_1 adenosine receptor is known and is disclosed in U.S. Patent No. 5,320,963 to G. Stiles et al. The A_3 adenosine receptor has been cloned, sequenced and expressed in rat (*see* F. Zhou et al., *Proc. Nat'l Acad. Sci. USA* **89**:7432 (1992)) and human (*see* M.A. Jacobson et al., U.K. Patent Application No. 9304582.1 (1993)). Thus, antisense oligonucleotides that downregulate the production of the A_1 or A_3 adenosine receptor may be produced in accordance with standard techniques.

One aspect of this invention is an antisense oligonucleotide having a sequence capable of binding specifically with any sequence of an mRNA molecule which encodes a human A_1 adenosine receptor or A_3-adenosine receptor so as to prevent translation of the mRNA molecule. The antisense oligonucleotide may have a sequence disclosed herein in **SEQ ID NO:1**, **SEQ ID NO:3**, and **SEQ ID NO:5**.

Chemical analogs of oligonucleotides (e.g., oligonucleotides in which the phosphodiester bonds have been modified, e.g., to the methylphosphonate, the phosphotriester, the phosphorothioate, the phosphorodithioate, or the phosphoramidate, so as to render the oligonucleotide more stable *in vivo*) are also an aspect of the present invention. The naturally occurring phosphodiester linkages in oligonucleotides are susceptible to degradation by endogenously occurring cellular nucleases, while many analogous linkages are highly resistant to nuclease degradation. *See* Milligan et al., and Cohen, J.S., *supra*. Protection from degradation can be achieved by use of a "3'-end cap" strategy by which nuclease-resistant linkages are substituted for phosphodiester linkages at the 3' end of the oligonucleotide. *See* Tidd, D.M. and Warenius, H.M.,

Br. J. Cancer 60, 343-350 (1989); Shaw, J.P. et al., *Nucleic Acids Res.* 19, 747-750 (1991). Phosphoramidates, phosphorothioates, and methylphosphonate linkages all function adequately in this manner. More extensive modification of the phosphodiester backbone has been shown to impart stability and may allow for enhanced affinity and increased cellular permeation of oligonucleotides. *See* Milligan, et al., *supra*. Many different chemical strategies have been employed to replace the entire phosphodiester backbone with novel linkages. *Id.* Backbone analogues include phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, boranophosphate, phosphotriester, formacetal, 3'-thioformacetal, 5'-thioformacetal, 5'-thioether, carbonate, 5'-N-carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methylimino) (MMI) or methyleneoxy(methylimino) (MOMI) linkages. Phosphorothioate and methylphosphonate-modified oligonucleotides are particularly preferred due to their availability through automated oligonucleotide synthesis. *Id.* Where appropriate, the antisense oligonucleotides may be administered in the form of their pharmaceutically acceptable salts.

Antisense oligonucleotides may be of any suitable length (e.g., from about 10 to 60 nucleotides in length), depending on the particular target being bound and the mode of delivery thereof. Preferably the antisense oligonucleotide is directed to an mRNA region containing a junction between intron and exon. Where the antisense oligonucleotide is directed to an intron/exon junction, it may either entirely overlie the junction or may be sufficiently close to the junction to inhibit splicing out of the intervening exon during processing of precursor mRNA to mature mRNA (e.g., with the 3' or 5' terminus of the antisense oligonucleotide being is positioned within about, for example, 10, 5, 3, or 2

nucleotides of the intron/exon junction). Also preferred are antisense oligonucleotides which overlap the initiation codon.

When practicing the present invention, the antisense oligonucleotides administered may be related in origin to the species to which it is administered. When treating humans, human antisense may be used if desired.

Pharmaceutical compositions comprising an antisense oligonucleotide as given above effective to reduce expression of an A_1 or A_3 adenosine receptor by passing through a cell membrane and binding specifically with mRNA encoding an A_1 or A_3 adenosine receptor in the cell so as to prevent its translation are another aspect of the present invention. Such compositions are provided in a suitable pharmaceutically acceptable carrier (e.g., sterile pyrogen-free saline solution). The antisense oligonucleotides may be formulated with a hydrophobic carrier capable of passing through a cell membrane (e.g., in a liposome, with the liposomes carried in a pharmaceutically acceptable aqueous carrier). The oligonucleotides may also be coupled to a substance which inactivates mRNA, such as a ribozyme. Such oligonucleotides may be administered to a subject to inhibit the activation of A_1 or A_3 adenosine receptors, which subject is in need of such treatment for any of the reasons discussed herein. Furthermore, the pharmaceutical formulation may also contain chimeric molecules comprising antisense oligonucleotides attached to molecules which are known to be internalized by cells. These oligonucleotide conjugates utilize cellular uptake pathways to increase cellular concentrations of oligonucleotides. Examples of macromolecules used in this manner include transferrin, asialoglycoprotein (bound to oligonucleotides via polylysine) and streptavidin.

In the pharmaceutical formulation the antisense compound may be contained within a lipid particle or

vesicle, such as a liposome or microcrystal. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the antisense oligonucleotide is contained therein. Positively charged lipids such as N-[1-(2,3-dioleoyloxi)propyl]-N,N,N-trimethyl-ammoniumethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. *See, e.g.*, U.S. Patent Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen et al.;4,921,757 to Wheatley et al.; etc.

Subjects may be administered the active composition by any means which transports the antisense nucleotide composition to the lung. The antisense compounds disclosed herein may be administered to the lungs of a patient by any suitable means, but are preferably administered by generating an aerosol comprised of respirable particles, the respirable particles comprised of the antisense compound, which particles the subject inhales. The respirable particles may be liquid or solid. The particles may optionally contain other therapeutic ingredients.

Particles comprised of antisense compound for practicing the present invention should include particles of respirable size: that is, particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about .5 to 10 microns in size are respirable. Particles of non-respirable size which are included in the aerosol tend to deposit in the throat and be swallowed, and the quantity of non-respirable particles in the aerosol is preferably minimized. For nasal administration, a particle size in the range of 10-500 μm is preferred to ensure retention in the nasal cavity.

Liquid pharmaceutical compositions of active compound for producing an aerosol can be prepared by combining the antisense compound with a suitable vehicle, such as sterile pyrogen free water. Other therapeutic compounds may optionally be included.

Solid particulate compositions containing respirable dry particles of micronized antisense compound may be prepared by grinding dry antisense compound with a mortar and pestle, and then passing the micronized composition through a 400 mesh screen to break up or separate out large agglomerates. A solid particulate composition comprised of the antisense compound may optionally contain a dispersant which serves to facilitate the formation of an aerosol. A suitable dispersant is lactose, which may be blended with the antisense compound in any suitable ratio (e.g., a 1 to 1 ratio by weight). Again, other therapeutic compounds may also be included.

The dosage of the antisense compound administered will depend upon the disease being treated, the condition of the subject, the particular formulation, the route of administration, the timing of administration to a subject, etc. In general, intracellular concentrations of the oligonucleotide of from .05 to 50 μM, or more particularly .2 to 5 μM, are desired. For administration to a subject such as a human, a dosage of from about .01, .1, or 1 mg/Kg up to 50, 100, or 150 mg/Kg or more is typically employed. Depending on the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose administrations. Administration of the antisense compounds may be carried out therapeutically (i.e., as a rescue treatment) or prophylactically.

Aerosols of liquid particles comprising the antisense compound may be produced by any suitable means, such as with a nebulizer. *See, e.g.*, U.S. Patent No.

4,501,729. Nebulizers are commercially available devices which transform solutions or suspensions of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier, the active ingredient comprising up to 40% w/w of the formulation, but preferably less than 20% w/w. the carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl hydroxybenzoate, antioxidants, flavoring agents, volatile oils, buffering agents and surfactants.

Aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicaments to a subject produce particles which are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder (e.g., a metered dose thereof effective to carry out the treatments described herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened *in situ* and the powder delivered by air drawn through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active

ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 150 μl, to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation may additionally contain one or more co-solvents, for example, ethanol, surfactants, such as oleic acid or sorbitan trioleate, antioxidants and suitable flavoring agents.

The aerosol, whether formed from solid or liquid particles, may be produced by the aerosol generator at a rate of from about 10 to 150 liters per minute, more preferably from about 30 to 150 liters per minute, and most preferably about 60 liters per minute. Aerosols containing greater amounts of medicament may be administered more rapidly.

The following examples are provided to illustrate the present invention, and should not be construed as limiting thereon. In these examples, μM means micromolar, mL means milliliters, μm means micrometers, mm means millimeters, cm means centimeters, °C means degrees Celsius, μg means micrograms, mg means milligrams, g means grams, kg means kilograms, M means molar, and h means hours.

EXAMPLE 1

Design and synthesis of antisense oligonucleotides

The design of antisense oligonucleotides against the A_1 and A_3 adenosine receptors may require the solution of the complex secondary structure of the target A_1 receptor mRNA and the target A_3 receptor mRNA. After generating this structure, antisense nucleotides are designed which target regions of mRNA which might be construed to confer functional activity or stability to the mRNA and which optimally may overlap the initiation codon. Other target sites are readily usable. As a demonstration of specificity of the antisense effect, other oligonucleotides not totally complementary to the target mRNA, but containing identical nucleotide compositions on a w/w basis, are included as controls in antisense experiments.

Adenosine A_1 receptor mRNA secondary structure was analyzed and used as described above to design a phosphorothioate antisense oligonucleotide. The antisense oligonucleotide which was synthesized was designated **HAdA1AS** and had the following sequence:

5'-GAT GGA GGG CGG CAT GGC GGG-3' **(SEQ ID NO:1)**

As a control, a mismatched phosphorothioate antisense nucleotide designated **HAdA1MM** was synthesized with the following sequence:

5'-GTA GCA GGC GGG GAT GGG GGC-3' **(SEQ ID NO:2)**

Each oligonucleotide had identical base content and general sequence structure. Homology searches in GENBANK (release 85.0) and EMBL (release 40.0) indicated that the antisense oligonucleotide was specific for the human and rabbit adenosine A_1 receptor genes, and that the mismatched control was not a candidate for hybridization with any known gene sequence.

Adenosine A_3 receptor mRNA secondary structure was similarly analyzed and used as described above to design two phosphorothioate antisense oligonucleotides. The first antisense oligonucleotide (**HAdA3AS1**) synthesized had the following sequence:

5'-GTT GTT GGG CAT CTT GCC-3' **(SEQ ID NO:3)**

As a control, a mismatched phosphorothioate antisense oligonucleotide (**HAdA3MM1**) was synthesized, having the following sequence:

5'-GTA CTT GCG GAT CTA GGC-3' **(SEQ ID NO:4)**

A second phosphorothioate antisense oligonucleotide (**HAdA3AS2**) was also designed and synthesized, having the following sequence:

5'-GTG GGC CTA GCT CTC GCC-3' **(SEQ ID NO:5)**

Its control oligonucleotide (**HAdA3MM2**) had the sequence:

5'-GTC GGG GTA CCT GTC GGC-3' **(SEQ ID NO:6)**

Phosphorothioate oligonucleotides were synthesized on an Applied Biosystems Model 396 Oligonucleotide Synthesizer, and purified using NENSORB chromatography (DuPont, MD).

EXAMPLE 2

Testing of A1-Adenosine Receptor Antisense Oligonucleotides *in vitro*

The antisense oligonucleotide against the human A_1 receptor (**SEQ ID NO:1**) described above was tested for efficacy in an *in vitro* model utilizing lung adenocarcinoma cells HTB-54. HTB-54 lung adenocarcinoma cells were demonstrated to express the A_1 adenosine receptor using standard northern blotting procedures and

receptor probes designed and synthesized in the laboratory.

HTB-54 human lung adenocarcinoma cells (106/100 mm tissue culture dish) were exposed to 5.0 μM **HAdA1AS** or **HAdA1MM** for 24 hours, with a fresh change of media and oligonucleotides after 12 hours of incubation. Following 24 hour exposure to the oligonucleotides, cells were harvested and their RNA extracted by standard procedures. A 21-mer probe corresponding to the region of mRNA targeted by the antisense (and therefore having the same sequence as the antisense, but not phosphorothioated) was synthesized and used to probe northern blots of RNA prepared from **HAdA1AS**-treated, **HAdA1MM**-treated and non-treated HTB-54 cells. These blots showed clearly that **HAdA1AS** but not **HAdA1MM** effectively reduced human adenosine receptor mRNA by >50%. This result showed that **HAdA1AS** is a good candidate for an anti-asthma drug since it depletes intracellular mRNA for the adenosine A_1 receptor, which is involved in asthma.

EXAMPLE 3

Efficacy of A_1-Adenosine Receptor Antisense Oligonucleotides *in vivo*

A fortuitous homology between the rabbit and human DNA sequences within the adenosine A_1 gene overlapping the initiation codon permitted the use of the phosphorothioate antisense oligonucleotides initially designed for use against the human adenosine A_1 receptor in a rabbit model.

Neonatal New Zealand white Pasteurella-free rabbits were immunized intraperitoneally within 24 hours of birth with 312 antigen units/mL house dustmite (*D. farinae*) extract (Berkeley Biologicals, Berkeley, CA), mixed with 10% kaolin. Immunizations were repeated weekly for the first month and then biweekly for the next 2 months. At 3-4 months of age, eight sensitized rabbits were anesthetized and relaxed with a mixture of ketamine

hydrochloride (44 mg/kg) and acepromazine maleate (0.4 mg/kg) administered intramuscularly.

The rabbits were then laid supine in a comfortable position on a small molded, padded animal board and intubated with a 4.0-mm intratracheal tube (Mallinkrodt, Inc., Glens Falls, NY). A polyethylene catheter of external diameter 2.4 mm with an attached latex balloon was passed into the esophagus and maintained at the same distance (approximately 16 cm) from the mouth throughout the experiments. The intratracheal tube was attached to a heated Fleisch pneumotachograph (size 00; DOM Medical, Richmond, VA), and flow was measured using a Validyne differential pressure transducer (Model DP-45161927; Validyne Engineering Corp., Northridge, CA) driven by a Gould carrier amplifier (Model 11-4113; Gould Electronic, Cleveland, OH). The esophageal balloon was attached to one side of the differential pressure transducer, and the outflow of the intratracheal tube was connected to the opposite side of the pressure transducer to allow recording of transpulmonary pressure. Flow was integrated to give a continuous tidal volume, and measurements of total lung resistance (RL) and dynamic compliance (Cdyn) were calculated at isovolumetric and flow zero points, respectively, using an automated respiratory analyzer (Model 6; Buxco, Sharon, CT).

Animals were randomized and on Day 1 pretreatment values for PC50 were obtained for aerosolized adenosine. Antisense (**HAdA1AS**) or mismatched control (**HAdA1MM**) oligonucleotides were dissolved in sterile physiological saline at a concentration of 5000 ug (5 mg) per 1.0 ml. Animals were subsequently administered the aerosolized antisense or mismatch oligonucleotide via the intratracheal tube (approximately 5000 μg in a volume of 1.0 ml), twice daily for two days. Aerosols of either saline, adenosine, or antisense or mismatch oligonucleotides were generated by an ultrasonic

nebulizer (DeVilbiss, Somerset, PA), producing aerosol droplets 80% of which were smaller than 5 μm in diameter.

In the first arm of the experiment, four randomly selected allergic rabbits were administered antisense oligonucleotide and four the mismatched control oligonucleotide. On the morning of the third day, PC50 values (the concentration of aerosolized adenosine in mg/ml required to reduce the dynamic compliance of the bronchial airway 50% from the baseline value) were obtained and compared to PC50 values obtained for these animals prior to exposure to oligonucleotide.

Following a 1 week interval, animals were crossed over, with those previously administered mismatch control oligonucleotide now administered antisense oligonucleotide, and those previously treated with antisense oligonucleotide now administered mismatch control oligonucleotide. Treatment methods and measurements were identical to those employed in the first arm of the experiment. It should be noted that in six of the eight animals treated with antisense oligonucleotide, adenosine-mediated bronchoconstriction could not be obtained up to the limit of solubility of adenosine, 20 mg/ml. For the purpose of calculation, PC50 values for these animals were set at 20 mg/ml. The values given therefore represent a minimum figure for antisense effectiveness. Actual effectiveness was higher. The results of this experiment are illustrated in both **Figure 1** and **Table 1**.

TABLE 1. EFFECTS OF ADENOSINE A_1 RECEPTOR ANTISENSE OLIGONUCLEOTIDE UPON PC50 VALUES IN ASTHMATIC RABBITS.

Mismatch Control		A_1 receptor Antisense oligonucleotide	
Pre oligonucleotide	Post oligonucleotide	Pre oligonucleotide	Post oligonucleotide
3.56 ± 1.02	5.16 ± 1.93	2.36 ± 0.68	>19.5 ± 0.34**

Results are presented as the mean (N = 8) ± SEM. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected t test. **Significantly different from all other groups, $P < 0.01$.

In both arms of the experiment, animals receiving the antisense oligonucleotide showed an order of magnitude increase in the dose of aerosolized adenosine required to reduce dynamic compliance of the lung by 50%. No effect of the mismatched control oligonucleotide upon PC50 values was observed. No toxicity was observed in any animal receiving either antisense or control inhaled oligonucleotide.

These results show clearly that the lung has exceptional potential as a target for antisense oligonucleotide-based therapeutic intervention in lung disease. They further show, in a model system which closely resembles human asthma, that downregulation of the adenosine A_1 receptor largely eliminates adenosine-mediated bronchoconstriction in asthmatic airways. Bronchial hyperresponsiveness in the allergic rabbit model of human asthma is an excellent endpoint for antisense intervention since the tissues involved in this response lie near to the point of contact with aerosolized oligonucleotides, and the model closely simulates an important human disease.

EXAMPLE 4

Specificity of A_1-adenosine receptor Antisense oligonucleotide

At the conclusion of the crossover experiment of Example 3, airway smooth muscle from all rabbits was quantitatively analyzed for adenosine A_1 receptor number. As a control for the specificity of the antisense oligonucleotide, adenosine A_2 receptors, which should not have been affected, were also quantified.

Airway smooth muscle tissue was dissected from each rabbit and a membrane fraction prepared according to described methods (J. Kleinstein and H. Glossmann, *Naunyn-Schmiedeberg's Arch. Pharmacol.* 305, 191-200 (1978), with slight modifications. Crude plasma membrane preparations were stored at - 70°C until the time of

assay. Protein content was determined by the method of Bradford (M. Bradford, *Anal. Biochem.* 72, 240-254 (1976)). Frozen plasma membranes were thawed at room temperature and were incubated with 0.2 U/ml adenosine deaminase for 30 minutes at 37°C to remove endogenous adenosine. The binding of [^{3}H]DPCPX (A_1 receptor-specific) or [^{3}H]CGS-21680 (A_2 receptor-specific) was measured as previously described. S. Ali et al., *J. Pharmacol. Exp. Ther.* 268, 1328-1334 (1994); S. Ali et al., *Am. J. Physiol* 266, L271-277 (1994).

As illustrated in both **Figure 2** and **Table 2**, animals treated with adenosine A_1 antisense oligonucleotide in the crossover experiment had a nearly 75% decrease in A_1 receptor number compared to controls, as assayed by specific binding of the A_1-specific antagonist DPCPX. There was no change in adenosine A_2 receptor number, as assayed by specific binding of the A_2 receptor-specific agonist 2-[p-(2-carboxyethyl)-phenethylamino]-5'-(N-ethylcarboxamido) adenosine (CGS-21680).

TABLE 2. SPECIFICITY OF ACTION OF ADENOSINE A_1 RECEPTOR ANTISENSE OLIGONUCLEOTIDE.

	Mismatch Control oligonucleotide	A_1 Antisense oligonucleotide
A_1-Specific Binding	1105 ± 48**	293 ± 18
A_2-Specific Binding	302 ± 22	442 ± 171

Results are presented as the mean (N = 8) ± SEM. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected t test. **Significantly different from mismatch control, $P < 0.01$.

The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Nyce, Jonathan W.

(ii) TITLE OF INVENTION: METHOD OF TREATMENT FOR ASTHMA

(iii) NUMBER OF SEQUENCES: 6

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(C) CITY: Charlotte
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GATGGAGGGC GGCATGGCGG G 21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTAGCAGGCG GGGATGGGGG C 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTGTTGGGC ATCTTGCC 18

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTACTTGCGG ATCTAGGC 18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGGGCCTAG CTCTCGCC 18

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTCGGGGTAC CTGTCGGC 18

I CLAIM:

1. A pharmaceutical composition, comprising a pharmaceutically or veterinarilly acceptable carrier and at least one oligonucleotide(s) (oligo(s)) that when administered to a subject is effective for reducing levels of, or sensitivity to, adenosine, for reducing levels of adenosine receptor, or for alleviating bronchoconstriction, lung inflammation, allergies, asthma, or asthma, or diseases or conditions associated with either one, the oligo being anti-sense to the initiation codon region, the coding region, the 5' or 3' intron-exon junctions or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine receptor, or anti-sense to an adenosine receptor mRNA.

2. The composition of claim 1, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junctions or regions within 2 to 10 nucleotides of the junctions, of a gene encoding an adenosine A_1, A_{2b} or A_3 receptor or anti-sense to an adenosine A_1, A_{2b} or A_3 receptor mRNA; wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

3. The composition of claims 1-2, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a

gene encoding an adenosine A_1 receptor or anti-sense to an adenosine A_1 receptor mRNA.

4. The composition of claims 1-3, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junctions or regions within 2 to 10 nucleotides of the junctions, of a gene encoding an adenosine A_1 receptor or anti-sense to an adenosine A_1 receptor mRNA; wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

5. The composition of claims 1-4, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction, or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_1 receptor, wherein substantially all nucleotide substituents or linking residues are methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

6. The composition of claims 1-5, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a

gene encoding an adenosine A_{2b} receptor or anti-sense to an adenosine A_{2b} receptor mRNA.

7. The composition of claims 1-6, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junctions or regions within 2 to 10 nucleotides of the junctions, of a gene encoding an adenosine A_{2b} receptor or anti-sense to an adenosine A_{2b} receptor mRNA; wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

8. The composition of claims 1-7, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction, or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_{2b} receptor, wherein substantially all nucleotide substituents or linking residues are methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate residues.

9. The composition of claims 1-8, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a

gene encoding an adenosine A_3 receptor or anti-sense to an adenosine A_3 receptor mRNA.

10. The composition of claims 1-9, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_3 receptor, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

11. The composition of claims 1-10, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_3 receptor, wherein substantially all nucleotide substituents or linking residues are methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate.

12. The composition of claims 1-11, wherein the oligo(s) comprise(s) DNA.

13. The composition of claims 1-11, wherein the oligo(s) comprise(s) RNA.

14. The composition of claims 1-13, wherein the oligo(s) is(are) complementary to the coding region of a gene or anti-sense to the initiation codon of a mRNA.

15. The composition of claims 1-14, wherein the oligo(s) comprise(s) about 10 to about 60 mononucleotides.

16. The composition of claims 1-15, wherein the oligo(s) comprise(s) about 18 to about 21 mononucleotides.

17. The composition of claims 1-16, wherein the oligo(s) comprise(s) about 18 mononucleotides.

18. The composition of claims 1-17, wherein the oligo(s) comprise(s) 21 mononucleotides.

19. The composition of claims 1-18, wherein the oligo(s) comprises SEQ. ID NO.: 1, SEQ. ID NO:3, SEQ. ID NO:5, or SEQ. ID NO.: 1, SEQ. ID NO:3 or SEQ. ID NO:5, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate.

20. The composition of claims 1-19, wherein the oligo(s) comprise(s) SEQ. ID NO.: 1, or SEQ. ID NO.: 1, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine,

methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate.

21. The composition of claims 1-20, wherein the oligo(s) comprise(s) SEQ. ID NO.: 3, or SEQ. ID NO.: 3, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate.

22. The composition of claims 1-21, wherein the oligo(s) comprise(s) SEQ. ID NO.: 5, or SEQ. ID NO.: 5, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate residues.

23. The composition of claims 1-22, wherein the carrier is selected from solid or liquid carriers.

24. The composition of claims 1-23, further comprising an agent anti-oxidants, flavoring agents, volatile oils, buffering agents, dispersants, surfactants, propellants or preservatives.

25. The composition of claims 1-24, further comprising a surfactant.

26. The composition of claims 1-25, wherein the oligo(s) is(are) present in an amount of about 0.1 to about 100 w/w of the composition.

27. The composition of claims 1-26, wherein the oligo(s) is(are) present in an amount of about 0.1 up to about 40 w/w of the composition.

28. The composition of claims 1-27, wherein the oligo(s) is(are) present in an amount of about 0.1 up to about 20 w/w of the composition.

29. The composition of claims 1-28, wherein the carrier comprises a hydrophobic carrier.

30. The composition of claims 1-29, wherein the carrier comprises lipid particles or vesicles.

31. The composition of claims 1-30, comprising vesicles in the form of liposomes or particles in the form of microcrystals.

32. The composition of claims 1-31, comprising liposomes comprise the oligo.

33. The composition of claims 1-32, in the form of an aerosol.

34. The composition of claims 1-33, in the form of an aerosol comprising respirable or inhalable oligo particles of about 0.5 to about 10 microns in size, or nasal or intrapulmonary oligo particles of about 10 to about 500 microns in size.

35. The composition of claims 1-34, wherein the aerosol comprises liquid or solid oligo particles.

36. The composition of claims 1-35, which is provided in a capsule or cartridge.

37. The composition of claims 1-36, wherein the oligo(s) is(are) in the form of a suspension, solution or emulsion

38. The composition of claims 1-37, wherein the oligo(s) is(are) suspended, dissolved or emulsified in a solvent or mixtures of solvents.

39. The composition of claims 1-38, wherein the oligo(s) is(are) dissolved, suspended or emulsified in a chlorofluorocarbon(s) alone or with co-solvents; and the composition further comprises an agent selected surfactants, antioxidants or flavoring agents.

40. The composition of claims 1-39, provided in a capsule or cartridge that comprises a piercable or openable capsule or cartridge.

41. The composition of claims 1-40, which is effective for treating asthma.

42. The composition of claims 1-41, wherein the anti-sense oligo(s) is(are) operatively linked to a molecule that is internalized or taken up by living cells.

43. The composition of claims 1-42, wherein the oligo(s) is(are) linked to transferrin, asialoglycoprotein or streptavidin.

44. The composition of claims 1-43, further comprising a substance that inactivates mRNA.

45. The composition of claims 1-44, further comprising an enzyme that inactivates mRNA.

46. The composition of claims 1-45, further comprising a ribozyme.

47. A capsule or cartridge, comprising the composition of claim 1.

48. A diagnostic and therapeutic kit, comprising a delivery device and, in a separate container, the compositionor capsule or cartridge of claims 1-47.

49. The kit of claim 48, wherein the delivery device comprises a nebulizer that delivers individual pre-metered doses of the formulation.

50. The kit of claims 48-49, wherein the delivery device comprises an insufflator, and the kit further

comprises a piercable or openable capsule or cartridge with solid particles of the oligo(s) or the composition.

51. The kit of claims 48-50, wherein the delivery device comprises a pressurized inhaler, and the composition comprises a formulation of a suspension or solution of the oligo(s).

52. The kit of claims 48-51, wherein the oligo(s) is(are) suspended, dissolved or emulsified in a solvent, or mixtures of solvents.

53. The kit of claims 48-52, wherein the oligo(s) is dissolved, suspended or emulsified in a chlorofluorocarbon alone or with co-solvents; and the formulation further comprises an agent selected from surfactants, antioxidants or flavoring agents.

54. The kit of claims 48-53, wherein the composition is provided in a capsule or cartridge.

55. Use of the composition of claims 1-54, for the prevention or treatment of increased sensitivity to, or levels of, adenosine or adenosine receptor, bronchoconstriction, or lung inflammation, allergy(ies) or asthma, or a disease or condition associated with either one.

56. The use of claim 55, wherein the composition is formulated for administration directly into the respiration or airways in the form of an aerosol.

57. The use of claims 55-56, wherein the compositioin is formulated as an aerosol comprising respirable, inhalable, intrapulmonary or nasal solid or liquid particles.

58. The use of claims 55-57, for treating a disease or condition associated with lung inflammation.

59. The use of claims 55-58, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding

region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of an adenosine A_3 receptor gene or anti-sense to an adenosine A_3 receptor mRNA.

60. The use of claims 55-59, for treating a disease or condition associated with asthma.

61. The use of claims 55-60, for treating a respiratory disease or condition, where the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of an adenosine A_1 receptor gene or anti-sense to an adenosine A_1 receptor mRNA.

62. The use of claims 55-61, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of an adenosine A_{2b} receptor gene or anti-sense to an adenosine A_{2b} receptor mRNA.

63. The use of claims 55-62, wherein the oligo(s) is(are) to be administered in an amount of about 0.01 to about 150 mg/kg body weight.

64. The use of claims 55-63, wherein the oligo(s) is(are) to be administered in an amount of about 1 to about 100 mg/kg body weight.

65. The use of claims 55-64, wherein the oligo(s) is(are) to be administered in an amount of about 1 to up to about 50 mg/kg body weight.

66. The use of claims 55-65, for prophylactic or therapeutic use.

67. Use of at least one oligonucleotide(s) (oligo(s)) that is anti-sense to one or more target polydeoxyribonucleotide(s) encoding a polypeptide

associated with high sensitivity to or high levels of adenosine or adenosine receptors, or associated with bronchoconstriction, inflammation, allergy or asthma, or with a disease or condition associated with either one, the oligo(s) being anti-sense to the initiation codon, the coding region or the 5′ or 3′ intron-exon junctions of a gene encoding the polypeptide, or being anti-sense to the corresponding mRNA, for administration into the airways of a subject to reach and hybridize to the target polydeoxyribonucleotide(s), and reduce the production or availability, or to increase the degradation, of mRNA corresponding to the polydeoxyribonucleotide(s), or to reduce the amount of the target polypeptide present in the lungs.

68. The use of claim 67, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junctions or regions within 2 to 10 nucleotides of the junctions, of a gene encoding an adenosine A_1, A_{2b} or A_3 receptor or anti-sense to an adenosine A_1, A_{2b} or A_3 receptor mRNA; wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

69. The use of claims 67-68, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_1 receptor or anti-sense to an adenosine A_1 receptor mRNA.

70. The use of claims 67-69, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junctions or regions within 2 to 10 nucleotides of the junctions, of a gene encoding an adenosine A_1 receptor or anti-sense to an adenosine A_1 receptor mRNA; wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

71. The use of claims 67-70, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction, or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_1 receptor, wherein substantially all nucleotide substituents or linking residues are methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

72. The use of claims 67-71, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_{2b} receptor or anti-sense to an adenosine A_{2b} receptor mRNA.

73. The use of claims 67-72, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding

region, the 5' or 3' intron-exon junctions or regions within 2 to 10 nucleotides of the junctions, of a gene encoding an adenosine A_{2b} receptor or anti-sense to an adenosine A_{2b} receptor mRNA; wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

74. The use of claims 67-73, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction, or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_{2b} receptor, wherein substantially all nucleotide substituents or linking residues are methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate residues.

75. The use of claims 67-74, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_3 receptor or anti-sense to an adenosine A_3 receptor mRNA.

76. The use of claims 67-75, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a gene

encoding an adenosine A_3 receptor, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), methyleneoxy (methylimino) or phosphoramidate.

77. The use of claims 67-76, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5' or 3' intron-exon junction or regions within 2 to 10 nucleotides of the junctions of a gene encoding an adenosine A_3 receptor, wherein substantially all nucleotide substituents or linking residues are methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate.

78. The use of claims 76-77, wherein the oligo(s) comprise(s) DNA.

79. The use of claims 67-78, wherein the oligo(s) comprise(s) RNA.

80. The use of claims 67-79, wherein the oligo(s) is(are) complementary to the coding region of a gene or anti-sense to the initiation codon of a mRNA.

81. The use of claims 67-80, wherein the oligo(s) comprise(s) about 10 to about 60 mononucleotides.

82. The use of claims 67-81, wherein the oligo(s) comprise(s) about 18 to about 21 mononucleotides.

83. The use of claims 67-82, wherein the oligo(s) comprise(s) about 18 mononucleotides.

84. The use of claims 67-83, wherein the oligo(s) comprise(s) 21 mononucleotides.

85. The use of claims 67-84, wherein the oligo(s) comprises SEQ. ID NO.: 1, SEQ. ID NO:3, SEQ. ID NO:5, or SEQ. ID NO.: 1, SEQ. ID NO:3 or SEQ. ID NO:5, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate.

86. The use of claims 67-85, wherein the oligo(s) comprise(s) SEQ. ID NO.: 1, or SEQ. ID NO.: 1, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate.

87. The use of claims 67-86, wherein the oligo(s) comprise(s) SEQ. ID NO.: 3, or SEQ. ID NO.: 3, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene

(methyimino), methyleneoxy (methylimino) or phosphoramidate.

88. The use of claims 67-87, wherein the oligo(s) comprise(s) SEQ. ID NO.: 5, or SEQ. ID NO.: 5, wherein at least one nucleotide substituent or linking residue is methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene (methyimino), methyleneoxy (methylimino) or phosphoramidate residues.

89. The use of claims 67-88, wherein the carrier is selected from solid or liquid carriers.

90. The use of claims 67-89, further comprising an agent anti-oxidants, flavoring agents, volatile oils, buffering agents, dispersants, surfactants, propellants or preservatives.

91. The use of claims 67-90, further comprising a surfactant.

92. The use of claims 67-91, wherein the oligo(s) is(are) present in an amount of about 0.1 to about 100 w/w of the composition.

93. The use of claims 67-92, wherein the oligo(s) is(are) present in an amount of about 0.1 up to about 40 w/w of the composition.

94. The use of claims 67-93, wherein the oligo(s) is(are) present in an amount of about 0.1 up to about 20 w/w of the composition.

95. The use of claims 67-94, wherein the carrier comprises a hydrophobic carrier.

96. The use of claims 67-95, wherein the carrier comprises lipid particles or vesicles.

97. The use of claims 67-96, comprising vesicles in the form of liposomes or particles in the form of microcrystals.

98. The use of claims 67-97, comprising liposomes comprise the oligo.

99. The use of claims 67-98, wherein the aerosol comprises liquid or solid oligo particles.

100. The use of claims 67-99, wherein the composition is provided in a capsule or cartridge.

101. The use of claims 67-100, wherein the oligo(s) is(are) in the form of a suspension, solution or emulsion.

102. The use of claims 67-101, wherein the oligo(s) is(are) suspended, dissolved or emulsified in a solvent or mixtures of solvents.

103. The use of claims 67-102, wherein the oligo(s) is(are) dissolved, suspended or emulsified in a chlorofluorocarbon(s) alone or with co-solvents; and the composition further comprises an agent selected surfactants, antioxidants or flavoring agents.

104. The use of claims 67-103, provided in a capsule or cartridge that comprises a piercable or openable capsule or cartridge.

105. The use of claims 67-104, which is effective for treating asthma.

106. The use of claims 67-105, wherein the anti-sense oligo(s) is(are) operatively linked to a molecule that is internalized or taken up by living cells.

107. The use of claims 67-106, wherein the oligo(s) is(are) linked to transferrin, asialoglycoprotein or streptavidin.

108. The use of claims 67-107, further comprising a substance that inactivates mRNA.

109. The use of claims 67-108, further comprising an enzyme that inactivates mRNA.

110. The use of claims 67-109, further comprising a ribozyme.

111. The use of claims 67-110, placed in a capsule or cartridge.

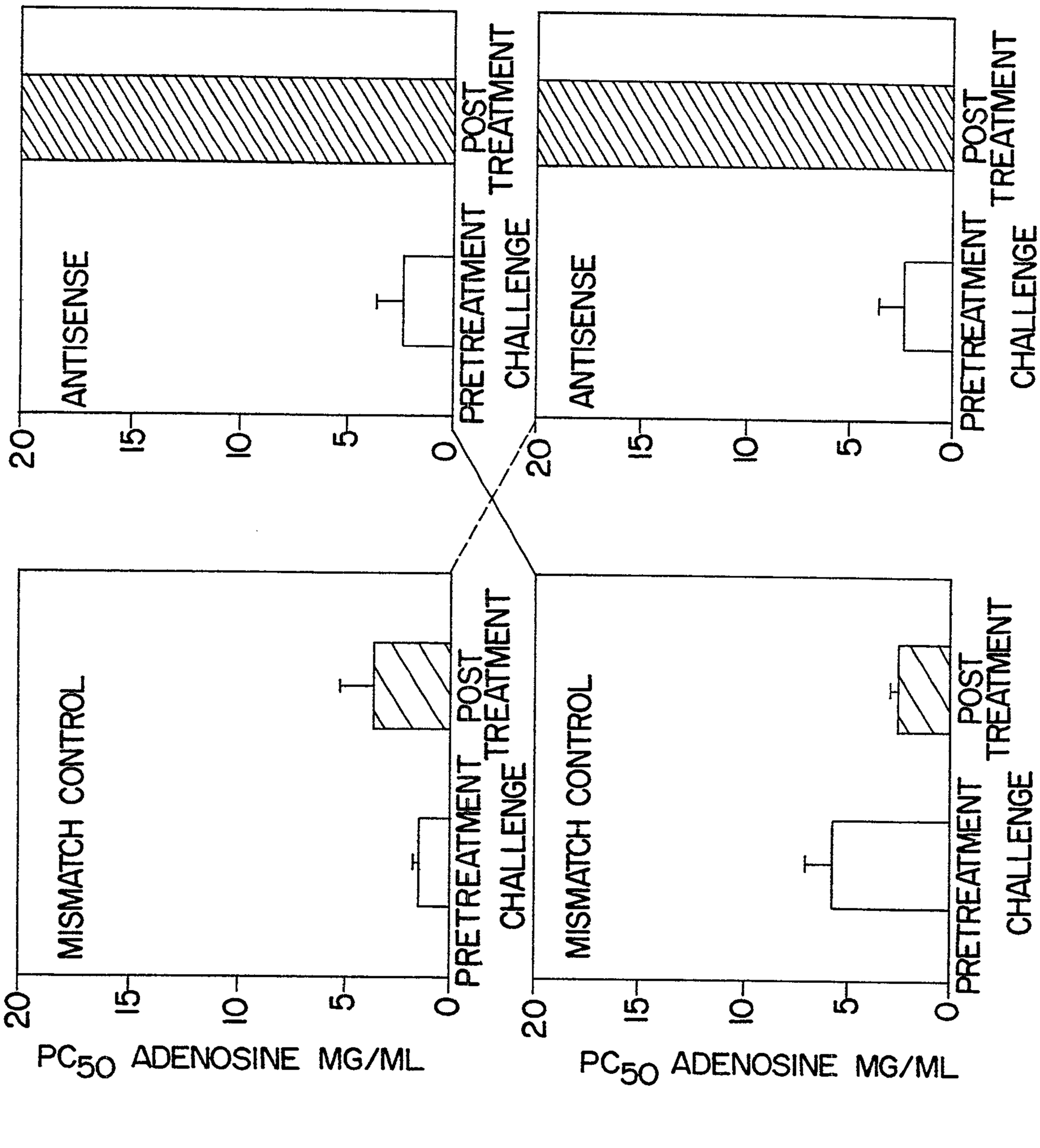
ANTISENSE
MISMATCH CONTROL
PRETREATMENT
CHALLENGE
POST TREATMENT
20
15
10
5
0
PC50 ADENOSINE MG/ML

FIG. 1.

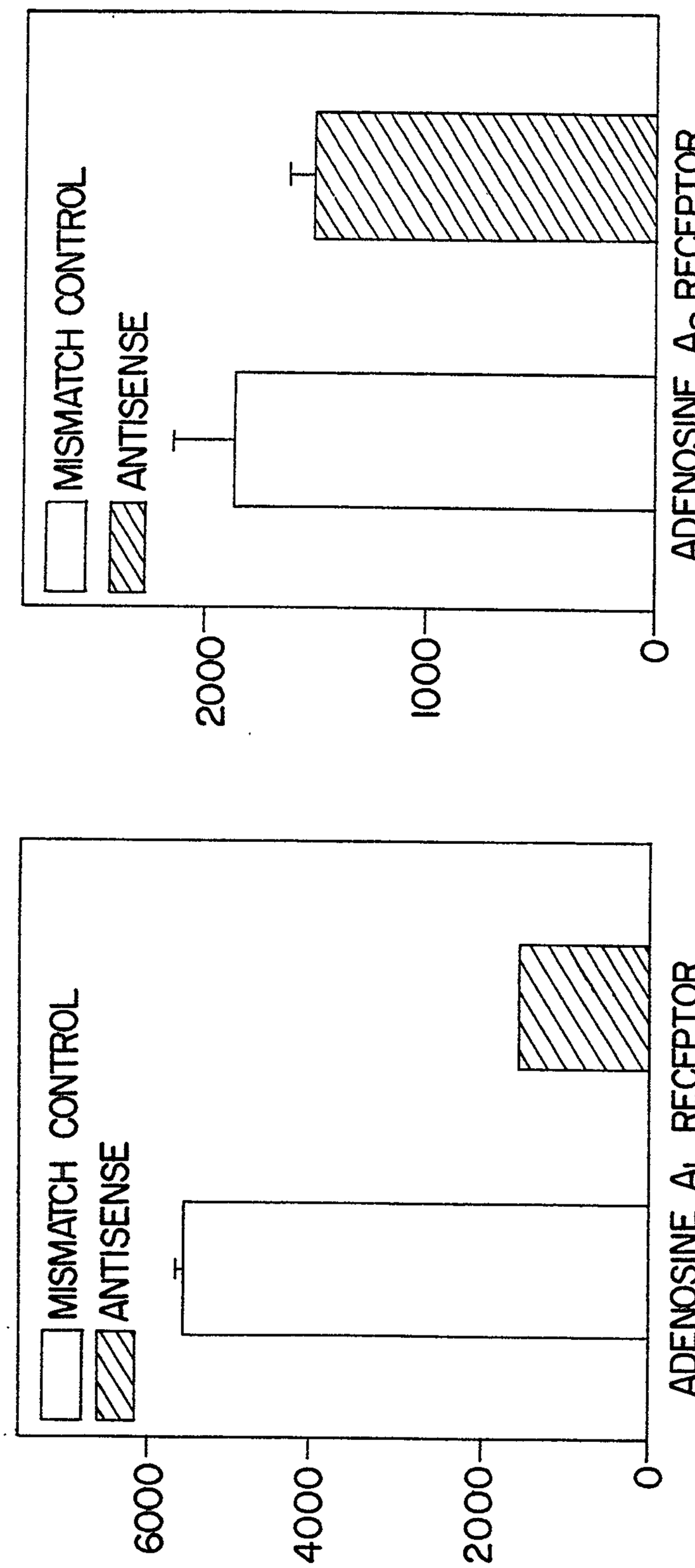
MISMATCH CONTROL
ANTISENSE
ADENOSINE A_2 RECEPTOR
2000
1000
0
MISMATCH CONTROL
ANTISENSE
ADENOSINE A_1 RECEPTOR
6000
4000
2000
0
SPECIFIC BINDING (CPM)/MG PROTEIN

FIG. 2.