

Sense and Antisense

Therapeutic Potential of Oligonucleotides and Interference RNA in Asthma and Allergic Disorders

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Abstract

Advances in our understanding of asthma pathogenesis and delineation of the human genome project are yielding novel candidate targets for therapeutic intervention. In parallel with target identification, the past decade has produced novel approaches to normalizing expression genes that are upregulated in disease processes. Single-stranded antisense oligonucleotides and double-stranded short-interfering RNA molecules, which specifically mark target transcripts for degradation, are being investigated for their ability to modulate disease processes. In both cases, the targets are RNA transcripts, and not protein; therefore, all types of molecular gene products can be inhibited, including historically undrugable species such as transcription factors and phosphatases. Various RNA interference strategies have been successfully tested in vitro and in animal models of disease, and data is beginning to accumulate from human clinical trials.

EPI-2010, a 21-mer phosphorothioate against the adenosine A1 receptor promoter region, has completed preclinical pharmacology testing and its initial clinical trials. The rationale for EPI-2010 is that overactivity of the adenosine signaling pathway in asthmatic lungs contributes to airway inflammation and hyperresponsiveness. Phase I/IIa clinical trials have shown EPI-2010 to be safe and well-tolerated, with modest indications of efficacy in patients with mild asthma.

Index Entries

Antisense; adenosine; airway hyperreactivity; asthma.

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Table 1
GINA Guidelines:
Goals of Long-Term Asthma Management

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- No symptoms or minor symptoms of asthma (symptoms include wheezing, coughing, shortness of breath, and chest tightness)
 - Sleeping through the night without asthma symptoms
 - No time off from school or work as a result of asthma
 - Full participation in physical activities
 - No emergency room visits or stays in the hospital
 - Little or no side effects from asthma medicine
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Background

Asthma afflicts at least 17 million Americans (150 million people worldwide), and more than 5000 Americans died from asthma in 2002 (1). There were approx 1.8 million asthma-related emergency visits/admissions in 2000 (Asthma Facts, Asthma and Allergy Foundation of America [2]). The Global Initiative for Asthma (GINA) guidelines and the National Asthma Education and Prevention Program have identified six goals of long-term asthma control (Table 1), and despite the increasing use of appropriate medicines, the objective of asthma control has not been met. Clearly, not all asthma targets have been addressed with currently available therapies, and novel disease targets need to be explored.

Novel Disease Targets

Advances in our understanding of the biochemistry of inflammation and hyperresponsiveness, genetics of asthma susceptibility, and the human genome project are yielding novel molecular targets for disease intervention. Whole-genome expression analysis approaches are being used extensively to identify genes and pathways in which expression patterns are altered in various model systems that are meant to approximate human asthma. Target identification programs use in vitro model systems

of inflammation, in vivo models of human asthma, and biopsy samples from human asthmatics as sources of messenger RNA (mRNA) for expression profiling. These types of studies have yielded hundreds of potential respiratory disease candidate genes for further evaluation because they are up- or downregulated in relation to the appropriate controls. For example, in an ovalbumin-sensitized mouse model of allergic asthma (3), analysis of 12,423 genes revealed a twofold or greater change in 3.41% (424) of the total genes; 2.74% (340) of the genes exhibited an increase in expression; and 0.67% (83) of the genes exhibited decreased expression. Interestingly, the data indicate that in this model, there is a fourfold difference between the upregulated and downregulated genes. Functional classification of differentially expressed genes showed a preponderance of upregulated immune-related murine genes (39%) compared to the downregulated genes (7%).

Two problems must be addressed in the development of new therapeutic agents for asthma. First, a system for target validation must be established to determine which novel targets are truly important in the asthma disease process. Second, once a target has been shown to be important, a therapeutic agent must be identified. In theory, use of antisense oligonucleotides (ASONS) and short-interfering (siRNA) should facilitate the process by addressing both problems. Unlike small molecules, ASONS and siRNA are designed from genomic information, which eliminates the need for screening large chemical libraries to find candidate molecules. Consequently, once a potential target is sequenced, single-stranded ASONS and duplex interfering RNA can be generated quickly for use in target validation. Following validation, the inhibitory oligonucleotides can be developed as a clinical candidate, circumventing many of the limitations in development of small molecule therapeutics.

Target Validation: Identifying Targets Involved in the Disease Process Using ASONs and siRNA

Research over the past decade has identified endogenous short, 20- to 24-nucleotide transcripts called microRNAs, which reduce mRNA levels and suppress translation of target genes. These effects can be mimicked by synthetic single-stranded ASONs and duplex oligonucleotides. In vitro studies have provided extensive evidence for the usefulness of these molecules in inhibiting specific gene expression and protein production (4–6). Duplex interfering RNAs are cleaved by an enzyme such as Dicer into small inhibitory RNAs with 3' overhanging ends. The duplex siRNA binds to a ribonucleoprotein silencing complex such as RNA-induced silencing complex, which directs the cleavage of the endogenous target mRNA or leads to attenuation of translation. ASONs act similarly by binding of the antisense sequence to its "sense" target, thereby preventing translation of mRNA. The blockade may involve simple hindrance of the translation machinery, degradation of the target mRNA by one or more of several intracellular ribonuclease enzymes, or other means. As single-stranded molecules, ASONs cross cell membranes more easily but are subject to degradation by endogenous ribonucleases once they are inside the cell. The ideal length of the ASONs ranges from 14 to 25 basepairs to maximize ability to cross cell membranes and provide the optimal target specificity. In contrast, duplex siRNA exhibits greater stability but has minimal ability to cross the plasma membrane—increasing the permeance of these molecules is a major focus of current research efforts in this field.

By its very nature, disease target validation often requires in vivo administration of validating agents in appropriate disease models. In 1997, a 21-mer phosphorothioate single-strand ASON (EPI-2010) designed to reduce adenosine A1 receptor expression was tested in an allergic rabbit model. A dose-dependent

functional inhibition of A1 receptor binding could be measured ex vivo for EPI-2010 but not for the corresponding mismatch control sequence (7). This effect was also specific for the target mRNA, because neither adenosine A2b receptor nor bradykinin B2 receptor expression was affected.

Since the first report, several animal studies (Table 2) have used antisense (or modification thereof) in which the role of certain signal transduction molecules and other protein targets would have been difficult to evaluate using small molecule approaches. The ASONs were delivered via aerosolization or intranasal administration. Table 2 does not include studies in which ASONs were administered by other routes or studies of non-antisense-based oligonucleotide modalities (e.g., CpG oligonucleotides) designed to modulate the immune system (8). As indicated in the footnotes to Table 2, either a mismatch or nonsense (random) oligonucleotides were used as a control for possible sequence-independent effects.

The following were the main conclusions from these studies:

1. Antisense effects have been shown in well-controlled studies against different target types, including transcription factors, tyrosine kinases, G protein-coupled receptors, cytokine receptors, growth factors, chemokines, and ion channels.
2. Airway-administered antisense can penetrate cell membrane and act in a variety of lung cell types.

Factors Contributing to the Effectiveness of Inhaled ASONs

The following anatomical and physiological properties of the respiratory tract may contribute to the effectiveness of inhaled ASONs in the treatment of pulmonary disease:

1. Aerosolization of antisense provides direct access to the target tissue.

Table 2
Summary of Effects of Airway Administered Antisense on Antigen Challenge Response

Target	Target type	Species	Route	Size/control	Effect	Cell type	Reference
GATA-3	Nuclear transcription factor	Mouse	Intranasal	18-mer ^{a,b}	↓protein ↓eosinophil infiltration ↓hyperresponsiveness	Th2 cells	24
c-kit (SCF)	Growth factor	Mouse	Intranasal	18-mer ^a	↓protein ↓IL-4 production ↓eosinophil infiltration	Cultured fibroblasts and keratinocytes Lung interstitium and epithelial cells	25
Syk tyrosine kinase	Kinase	Rat	Aerosol	60-mer liposome complex ^b	↓mRNA and protein ↓markers of pulm inflammation	Alveolar macrophages	26,27
B-chain of IL-3, IL-5, GM-CSF receptor	Receptors for cytokine/chemokines	BN Rat	Intratracheal	18-mer ^a	↓mRNA and protein ↓hyperresponsiveness	No cell type localization	28
Gob-5	Ca ²⁺ -dependent chloride channel	Mouse	Intratracheal	Adenovirus-antisense gob-5 ^c	↓mucus overproduction ↓hyperresponsiveness	Epithelia	29
Adenosine A1R	GPCR	Rabbit	Aerosol	21-mer ^a	↓protein ↓hyperresponsiveness	Smooth muscle	7
Bradykinin B2R	GPCR	Rabbit	Aerosol	21-mer	↓protein	Smooth muscle	7

^aMismatch control

^bNonsense control

^cNo effect of adenoviral vector alone

Abbreviations: GPCR, G protein-coupled receptor; IL, interleukin; mRNA, messenger RNA; BN, Brown-Norway; SCF, Stem cell factor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

2. A large surface area to surface liquid volume in the lungs facilitates absorption.
3. Cationic lipids, such as those found in pulmonary surfactant, facilitate cellular uptake.

Generation of aerosols with a specific range of particle size provides a simple, safe, and effective method for delivering ASONs to respiratory targets. Aerosol particles between 0.01 and 100 μ in diameter deposit at different locations within the respiratory tract, depending on their size. Particles larger than 5 μ in diameter deposit in the nasopharynx as a result of impaction, whereas particles with diameters between 1 and 5 μ deposit in the alveoli and small airways by sedimentation. Only particles less than 0.1 μ in diameter exhibit significant deposition in alveoli. Therefore, aerosolization of ASONs to a specific range of particle sizes provides a mechanism to maximize delivery to a target site within the respiratory tract.

The surface of the respiratory tract is covered by a thin film of aqueous liquid; on contact with the surface, the aerosol particle dissolves into this liquid layer. Continuous absorption of salt and water by the alveolar and airway epithelium limits the volume of this layer, ensuring that the airway remain patent. The small volume of liquid, coupled with the large surface area of the respiratory tract, facilitates delivery of ASONs to cells in and around the airways. In addition to achieving high concentrations at the airway/alveolar surface, the alveolar surface liquid and, to a lesser extent, airway surface liquid, contain surfactant, which is composed of cationic lipids similar to those used *in vitro* to facilitate uptake of naked oligonucleotides into cells. Facilitation of ASON uptake by surfactant can be demonstrated by measuring cellular accumulation of antisense in cell cultures that contain alveolar type II pneumocytes. Cellular uptake under these conditions is increased when the type II cells are stimulated to release surfactant (Nyce et al., unpublished data).

Optimal Models for Antisense Testing and Human Target Validation

An optimized approach to development of ASONs and their use in target validation for asthma includes development of an *in vitro* human cell expression system for screening a library of ASONs followed by *in vivo* testing in suitable animal models of asthma.

Several models of allergic asthma have been developed using mice, rats, guinea pigs, rabbits, sheep, and nonhuman primates (9). Generally, the models based on rodents and rabbits are less expensive and allow more rapid screening than the larger animal models. Also, mechanistic studies are more easily performed in the smaller animal models, because the animals generally are sacrificed following a certain time-course of modeling events, and multiple cell and tissue samples can be obtained. However, species differences in sequence homology and expression must be remembered when using nonprimate models of disease.

Compared to other animal models, the allergic monkey model of asthma (10) has a high positive predictive value for genetic-based therapies. This conclusion is based on the highly conserved genetic relationship between *Cynomolgus* monkeys and humans and outcome measurements of the model, which indicate many similarities with human asthma. Because of the genetic similarity to humans, this model has the advantage that the target sequence will likely be conserved, and test ASON or siRNA can be developed for clinical trials with efficacy in the model, without further sequence modification.

The ability to rapidly generate specific ASONs or siRNA to validate novel targets based on genomic information, coupled with the ability to rapidly progress those compounds directly into the clinics because of their specificity and low potential for toxicity, holds the potential to markedly reduce the time that is required to generate disease-relevant, target-specific drugs. To capitalize on this potential,

preclinical testing must be performed in animal models exhibiting target RNA sequences that are conserved in humans. This requirement may be best met through development of nonhuman primate models that accurately reproduce pulmonary diseases in humans.

What Are the Optimal Properties of Oligonucleotides for Clinical Therapeutics?

Many aspects of the research and development must be considered to develop an ASON for the clinic (Table 3). The choice of sequence that results in the desired inhibitory effect and minimizes side effects is beyond the scope of this article (11).

An enormous amount of data has been accumulated in recent years regarding the safety of ASONs in man, with supporting toxicology in numerous species (12). Less data are available for duplex RNA, but findings from ASONs likely are predictive of the safety of siRNA. ASONs have been studied most extensively as potential treatments for cancer (13,14). These studies generally have relied on intravenous infusion; consequently, some side effects related to backbone modifications and immune stimulation, such as complement activation, have been observed (Table 4). This has proved to be tolerable in these cases of life-threatening disease that have an acceptable risk–benefit ratio.

The local delivery of antisense represents one solution for achieving effective disease tissue concentrations yet minimizing systemic dose effects. This approach has been effectively used in the development of Fomivirsen™, the first antisense therapeutic to reach the market. Fomivirsen is an antisense treatment for acquired immune deficiency syndrome-associated cytomegalovirus retinitis and is delivered locally by intravitreal injection (15). Delivery of ASONs through inhalation may also avoid systemic side effects because little

or no ASON appears in the circulation following inhalation, even at high doses (Table 4).

Two key areas are discussed in further detail: distribution following airway administration (covering our experience with EPI-2010) and chemistry and metabolic stability.

Distribution

A study of the distribution of radiolabeled EPI-2010 in rabbit lungs indicated a fairly circumscribed delivery to the lung. Autoradiograph analysis indicated that the radiolabeled ASON EPI-2010 delivered by nebulization resulted in uniform deposition of the drug throughout the large and small airways of the rabbit lung. In contrast to studies involving parenteral delivery of antisense, where the highest concentrations of drug are found in the liver and kidney, the majority of EPI-2010-derived radioactivity was found in the target lung tissue. In fact, the combined EPI-2010-derived [³⁵S]-radioactivity found in the heart, liver, and kidney was only 6.9% of the administered dose (16). Additionally, postlabeling studies indicated that intact EPI-2010 could be detected only in the lung. These data provide additional evidence that inhaled ASONs do not effectively escape the lung as intact molecules. EPI-2010 was found to be relatively short-lived in the lung; our data demonstrate that only approx 3% of the delivered drug remained in the lung after 72 h and that its elimination half-life in the lung was approx 30 h (16). The studies described in this article measure EPI-2010-derived radioactivity; therefore, they provide data for EPI-2010 and its metabolites. The EPI-2010 elimination half-life is at the lower end of the range (20–120 h, depending on tissue or organ) reported for phosphorothioates that are delivered via parenteral routes (12).

Subsequent studies using fluorescent-labeled ASONs have demonstrated effective distribution throughout the mucosal and submucosal compartments. The ASONs are rapidly taken

Table 3
Key Points in Developing a Therapeutic Inhaled ASON From an Antisense Tool

Property	Goal	Proposed approaches
High potency and selectivity	Sufficient target inhibition without unacceptably large deliverable doses and nonspecific side effects	Choose targets with appropriate message, protein, and cell turnover rates. Increase binding affinity of ASON target-mRNA complex using newer chemistries. Screen <i>in silico</i> against whole-human genome sequence to avoid undesirable hybridization.
Formulation and delivery	Acceptable purity and shelf life Adequate delivery to cell target	Optimize chemistries and formulation. Confirm appropriate intracellular distribution.
Drug metabolism and pharmacokinetics	Appropriate physical and chemical properties conferring chemical and metabolic stability, and a duration of action consistent with clinically acceptable dose and dose regimen	Match particle size to desired lung distribution and target location. Use phosphorothioate backbone to increase stability and reduce metabolic clearance (Metabolism by RNase is sequence-independent: therefore, similar pharmacokinetic properties expected for all similarly-sized RASONS.).
Acceptable safety and toxicology	Acceptable safety margin between minimal effective therapeutic dose and those causing side effects	Investigate toxicology studies in species with conserved sequence to see target-dependent toxicities. Deliver by aerosol to minimize systemic levels and avoid side effects seen following intravenous route. Avoid CpG motifs in new molecules to avoid immune system stimulation.
Clinical benefit	Address those targets considered nontractable or nondrugable by conventional means	Explore new chemistries to reduce backbone related chemical toxicity. Select patients with appropriate diagnosis where current therapeutic options are limited with an acceptable risk-benefit ratio.

ASON, antisense oligonucleotide; RASON, respirable antisense oligonucleotide; RNase, ribonuclease; mRNA, messenger ribonucleic acid.

Table 4
Summary of Sequence-Dependent and -Independent Effects of Phosphorothioate ASONs

Effect	Sequence related?	Mechanism	Comment
Target inhibition in airways	Yes	Hybridization	Desired effect
Target inhibition in nonairway tissue	Yes	Hybridization	Avoided by airway administration of ASON
Inhibition of nonintended target containing homologous sequence	Yes	Hybridization	Minimized by in silico screening of humane genome sequence
CpG motif and G quartet effects: B-cell mitogen immune stimulation	Yes	Nonhybridization	Minimized by avoidance of such sequence motifs
Complement activation and hypotension prolongation of clotting time	No; nonspecific interactions related to negatively charged backbone	Nonhybridization	Minimized by selection of appropriate backbone chemistries and avoidance of intravenous route

ASON, antisense oligonucleotide.

up by diverse cell types, including epithelial and smooth muscle cells, macrophages, lymphocytes, eosinophils, and neutrophils and translocated to the nuclei. Consistent with decay of the radioactive signal in rabbits, the fluorescent signal is lost in most cell types over an 8-h period in vivo. Although inflammatory cells clearly take up the ASONs, the practicality of targeting a migratory cell is questionable because "untreated" cells continually enter the lungs from the circulation.

Phosphorothioate oligonucleotides do not appear in the circulation when orally administered. There is evidence that this is not related to poor solubility or metabolic stability but, rather, to poor absorption characteristics of the molecule across the gastrointestinal epithelium (17). It has been argued that this is expected based on the high molecular weight and hydrophilicity (log P ca. -3.5).

Chemistry Backbone and Metabolic Stability

Most antisense molecules in clinical trials are DNA (2'-deoxynucleotidic acid) oligonucleo-

tides with a phosphorothioate backbone (where one of the noncovalently bonded oxygen atoms is replaced by a sulfur atom), resulting in increased in vivo resistance to nucleases relative to standard phosphodiester oligonucleotides. However, the phosphorothioate backbone modification has been linked to toxic side effects when administered in high doses. Although local delivery of ASONs to the lung can minimize many of these side effects, it is desirable to evaluate new generation-specialized oligonucleotide chemistries that enhance hybridization to target mRNAs, reduce backbone related chemical toxicity, and inhibit the oligonucleotide susceptibility to nuclease degradation in vivo (18-20).

The metabolism of phosphorothioate oligonucleotides is by nucleases and not by cytochrome P-450 enzymes that metabolize small organic molecules. Drug interactions are a relatively common finding with small molecules because of the common metabolic pathway. Because of nuclease metabolism, this is not an anticipated problem with antisense drugs, and the lack of drug interactions represents a key potential drug class advantage.

Immunological Side Effects

Certain sequence motifs merit attention in the design of ASONs, probably because of their mimicry of DNA motifs that serve biological function. One example is the dinucleotide CpG (21,22). Greater than 90% of CpG motifs are methylated at C-5 in animal DNA; this is in contrast to bacterial DNA, in which CpG dinucleotides remain essentially unmethylated. This difference in C-5 methylation within CpG dinucleotides is important with regard to the animal immune system. Methylated CpGs are without consequence, whereas the detection of nonmethylated CpGs, a characteristic of bacterial DNA, induces a potent Th1-based immune response. Oligonucleotides that have unmethylated CpGs with certain sequence contexts surrounding them resemble bacterial DNA products and induce an immune response. This characteristic can be exploited therapeutically (8) but can also confound observations in the absence of appropriately designed control ASONs. Another example of confounding sequence motifs is G-rich oligonucleotides, which are capable of forming "G quartet" secondary structures that confer nonantisense-based mechanisms of activity (23).

Development of a Therapeutic ASON: Example of EPI-2010 for Asthma

Preclinical Pharmacology of EPI-2010 and the Adenosine A1 Receptor

EPI-2010 is a phosphorothioate oligonucleotide ([5' GAT GGA GGG CGG CAT GGC GGG 3']) directed to the promoter region of the adenosine A1 receptor, and the sequence is conserved in rabbit, monkey, and man. Based on the rationale that adenosine may contribute to airway inflammation, EPI-2010 was investigated in a rabbit model of allergen challenge. It reduced the pulmonary response (7), attenuating the fall in lung compliance and histamine hyperresponsiveness that is normally observed following allergen challenge. Ligand binding

studies performed following the removal of the tracheal smooth muscle from these rabbits indicated that A1 receptor expression was downregulated (7). The expression of the bradykinin B2 receptor was unaffected by EPI-2010, indicating the selectivity of effect. Also, a mismatch sequence of EPI-2010 (5' GTA GGT GGC GGG CAA GGC GGG 3') was without effect on adenosine A1 receptor expression, demonstrating the sequence dependency of the effect.

A role for the A1 receptor was further demonstrated in dust mite allergen-sensitized cynomolgus monkeys that exhibited adenosine sensitivity. In these animals, a single 5-mg dose of aerosolized EPI-2010, but not its mismatch control, reduced airway sensitivity to adenosine, increasing the PC30 to adenosine up to 245-fold (Fig. 1). The same dose of mismatch oligonucleotide had no effect, confirming the pharmacological specificity.

Clinical Efficacy of EPI-2010

In phase I studies, aerosolized doses of EPI-2010 did not affect any coagulation or cardiovascular parameters (which are known side effects of intravenous oligonucleotides). This supports the hypothesis that the local delivery ensures minimal systemic bioavailability. No evidence of systemic toxicity was observed in animal toxicology studies, and only a modest accumulation of pulmonary macrophages was noted in the lungs of treated animals. Based on the pattern of distribution of cells and drug, it is presumed that the pulmonary macrophages play a role in clearance of the oligonucleotide from the lung.

EPI-2010 was well tolerated in a series of single- and multiple-dose studies in normal subjects and patients with mild asthma. In a crossover study of 14 patients with mild asthma, adenosine monophosphate challenge was performed at baseline, day 4, and day 7 of each period. EPI-2010 induced a doubling in the geometric mean of adenosine monophosphate PC20 (fall in forced expiratory ventilation in 1

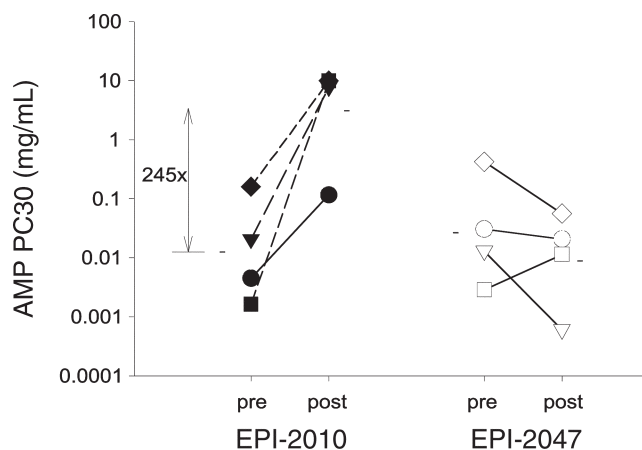


Fig. 1. EPI-2010 but not its mismatch control (EPI-2047) reduces airway sensitivity to adenosine in dust mite-challenged Cynomolgus monkey.

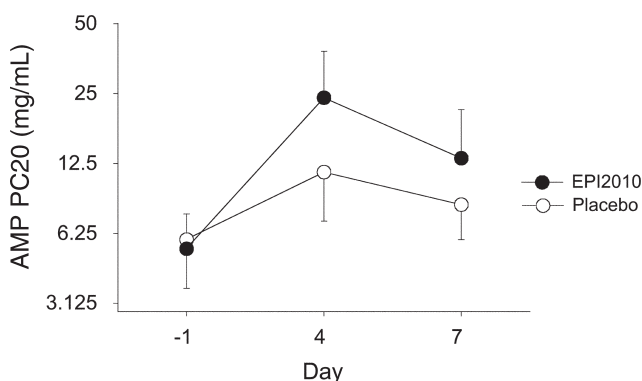


Fig. 2. Effects of a single inhaled dose of EPI-2010 on adenosine PC20 (FEV_{10}) in patients with mild asthma.

min [FEV_{10}]) measured at day 4, which did not reach statistical significance because of the apparent upward trend in the placebo-treated group. (Fig. 2)

There was also a reduction in the total numbers of episodes of wheezing from 56 (placebo) to 33 (Fig. 3A), and rescue with Salbutamol rescue was reduced in 11 out of 14 patients (1 unchanged; Fig. 3B) compared to placebo measured over the 7-d period after a single dose of EPI-2010.

Our early phase II trial of EPI-2010 was performed in patients with moderate persistent asthma who were on inhaled corticosteroids in

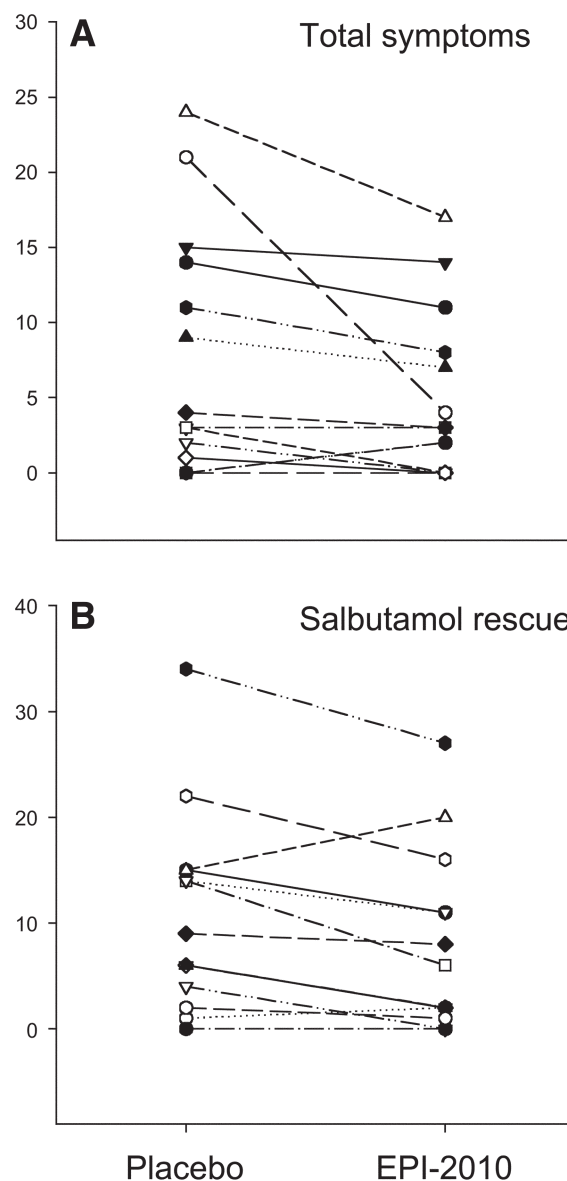


Fig. 3. A single inhaled dose of EPI-2010 reduces total airway symptom score (sum of diary-reported incidence of asthma symptoms) and salbutamol (sum of diary-reported 100 μ g equivalents) rescue over 7 d.

the United Kingdom and in steroid-naïve patients in the United States. Although the clinical data in patients with mild asthma and steroid-naïve patients were promising, EPI-2010 had an insufficient effect in patients on inhaled corticosteroids. Because EPI-2010 was ineffective in this group, an important part of the global asthma market was lost; therefore, we

discontinued further development of this molecule. However, we believe that the approach of using inhaled antisense to modulate disease targets is valid because it was possible to antagonize a specific receptor in animals and produce a modest mechanistically based result in humans. The degree to which the effects translate to a therapeutic benefit depends on the role of the target in the disease process.

Conclusion

We currently have a better understanding of which particular features of the development process are critical in developing a therapeutic respirable ASON from a laboratory antisense tool. This knowledge should facilitate development of siRNA as a pulmonary therapeutic agent. Clearly, this new broad class of drugs holds potential in addressing targets that are upregulated in pulmonary disease but are nontractable by conventional small molecules. It is hoped that this will yield new therapeutic options that will translate into improved asthma control for patients.

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References

1. Sears, M.R. (1995), *J Invest Allergol Clin Immunol* **5**, 66–72.
2. <http://www.aafa.org>. Accessed on Oct. 28, 2004.
3. Zimmermann, N., Moulton, E.A., Aronow, B.J., et al. (2002), *J Allergy Clin Immunol* **109**(1 suppl), S296,905.
4. Dove, A. (2002), *Nature Biotech* **20**, 121–124.
5. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998), *Nature* **391**(6669), 806–811.
6. Eric, G. (2001), *Current Biology* **11**, R772–R775.
7. Nyce, J.W., and Metzger, W.J. (1997), *Nature* **385**(6618), 721–725. Erratum in: (1997) *Nature* **390**(6658), 424.
8. Kandimalla, E.R., Yu, D., and Agrawal, S. (2002), *Curr Opin Mol Ther* **4**(2), 122–129.
9. Karol, M.H. (1994), *Eur Respir J* **7**(3), 555–568.
10. Zou, J., Young, S., Zhu, F., et al. (2002), *Genome Biol.* **3**(5), research0020. Epub 2002 Apr 11.
11. Agrawal, S. (1999), *Biochim Biophys Acta* **1489**(1), 53–68.
12. Levin, A.A. (1999), *Biochim Biophys Acta* **1489**(1), 69–84.
13. Cho-Chung, Y.S. (2002), *Curr Opin Investig Drugs* **3**(6), 934–939.
14. Tamm, I., Dorken, B., and Hartmann, G. (2001), *Lancet* **358**(9280), 489–497.
15. de Smet, M.D., Meenken, C.J., and van den Horn, G.J. (1999), *Ocul. Immunol. Inflamm.* **7**, 189–198.
16. Ali, S., Leonard, S.A., Kukoly, C.A., et al. (2001), *Am J Respir Crit Care Med* **163**, 989–993.
17. Nicklin, P.L., Bayley, D., Giddings, J., et al. (1998), *Pharm Res* **(4)**, 583–591.
18. Agrawal, S. (1999), *Biochim Biophys Acta* **1489**, 53–68.
19. Wang, H., Cai, Q., Zeng, X., Yu, D., et al. (1999), *Proc Natl Acad Sci USA* **96**, 13,989–13,994.
20. Crooke, S.T. (1999), *Antisense Nucleic Acid Drug Dev* **8**, 115–122.
21. Branch, A.D. (1996), *Hepatology* **24**, 1517–1529.
22. Coulson, J.M., Poyner, D.R., Chabtry, A., et al. (1996), *Mol Pharmacol* **50**, 314–325.
23. Dapic, V., Bates, P.J., Trent, J.O., et al. (2002), *Biochemistry* **41**(11), 3676–3685.
24. Finotto, S., De Sanctis, G.T., Lehr, H.A., et al. (2001), *J Exp Med* **193**(11), 1247–1260.
25. Finotto, S., Buerke, M., Lingnau, K., et al. (2001), *J Allergy Clin Immunol* **107**(2), 279–286.
26. Stenton, G.R., Kim, M.K., Noharo, O., et al. (2000), *J Immunol* **164**(10), 5532.
27. Stenton, G.R., Ulanova, M., Dery, R.E., et al. (2002), *J Immunol* **169**(2), 1028–1036.
28. Allakhverdi, Z., Allam, M., and Renzi, P.M. (2002), *Am J Respir Crit Care Med* **165**, 1015–1021.
29. Nakanishi, A., Morita, S., Iwashita, H., et al. (2001), *Proc Natl Acad Sci USA* **98**(9), 5175–5180.