Biodrugs 2006; 20 (1): 1-11
1173-8804/06/0001-0001/$39.95/0

The Future of Antisense Oligonucleotides in the Treatment of Respiratory Diseases

Marina Ulanova,[1,2] *Alan D. Schreiber*[3] and *A. Dean Befus*[1]

1 Department of Medicine, University of Alberta, Edmonton, Alberta, Canada
2 Northern Ontario School of Medicine, Thunder Bay, Ontario, Canada
3 University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

Abstract

Antisense oligonucleotides (ASO) are short synthetic DNA molecules designed to inhibit translation of a targeted gene to protein via interaction with messenger RNA. More recently, small interfering (si)RNA have been developed as potent tools to specifically inhibit gene expression. ASO directed against signaling molecules, cytokine receptors, and transcription factors involved in allergic immune and inflammatory responses, have been applied in experimental models of asthma and demonstrate potential as therapeutics. Several ASO-based drugs directed against oncogenes have been developed for therapy of lung cancer, and some have recently reached clinical trials. ASO and siRNA to respiratory syncytial virus infection have demonstrated good potential to treat this condition, particularly in combination with an antiviral drug. Although ASO-based therapeutics are promising for lung diseases, issues of specificity, identification of correct molecular targets, delivery and carrier systems, as well as potential adverse effects must be carefully evaluated before clinical application.

The original idea to use antisense oligonucleotides (ASO) to specifically inhibit gene expression was proposed over 25 years ago.[1] Advantages of ASO as a therapeutic tool were immediately obvious. In contrast to traditional drugs designed to inhibit disease-related proteins already synthesized, ASO prevent translation of a protein by interaction with its messenger (m)RNA. However, it took almost 20 years to develop this concept into the first (and currently only) ASO-based drug in clinical use. Fomivirsen (Vitravene®)[1], a cytomegalovirus (CMV)-directed ASO, is used topically to treat CMV retinitis, a severe complication of AIDS.[2] Presently, more than 30 ASO-based drugs are in different phases of clinical trials, and hundreds are in development and in preclinical studies.[3] Despite the attraction of the antisense concept, there remain several important issues relating to clinical application of ASO. This review will discuss these problems, summarize published data on ASO strategies in respiratory diseases, and emphasize recent developments and future prospects.

1. Principles of Antisense Oligonucleotides (ASO), Mechanisms of Action, and Related Issues

ASO are short synthetic DNA molecules, designed to interact by Watson-Crick base-pairing with mRNA encoding a target protein. When single-stranded DNA complementary to a target mRNA is introduced into a cell, it binds the mRNA and prevents translation of the protein. While this approach appears straightforward, initial attempts to introduce ASO into cells were unsuccessful because: (i) oligonucleotides are large molecules that are highly negatively charged and do not penetrate cell membranes well; and (ii) oligonucleotides are easily degraded by endo- and exonucleases before they can bind corresponding mRNA.

Thus, critical issues in the development of ASO-based therapy of respiratory diseases include:

1. target selection and ASO specificity;
2. ASO stability;
3. delivery of ASO to target organ/cells.

1 The use of trade names is for product identification purposes only and does not imply endorsement.

To overcome the problem of oligonucleotide degradation and to ensure efficient cellular delivery, several chemical modifications of ASO have been developed. The most commonly used and best studied is the phosphorothioate backbone modification (figure 1).

1.1 ASO Mechanisms of Action

Despite intensive studies, mechanisms of ASO action on mRNA are still incompletely understood. Current concepts suggest that the major mechanism of action of phosphorothioate ASO is activation of endonuclease RNAse H when ASO binds to mRNA.[4] This results in mRNA degradation and prevents translation of a specific protein. ASO binding to mRNA can also prevent assembly of the ribosomal complex (e.g. via steric blocking) or inhibit RNA splicing[5] (figure 2).

1.2 Different ASO Structures and Modifications: Stability Issues

The phosphorothioate backbone modification represents a replacement of a non-bridging oxygen by a sulfur atom at each phosphorus[6] (figure 1). This modification, commonly referred to as the 'first-generation', greatly increases resistance to nucleases. However, it can render undesired biological activity to some ASO, unrelated to their antisense properties (see section 1.7).

Several other antisense formulations, such as methyl-oligonucleotides, morpholino, peptide nucleic acids, locked nucleic acids, ribozymes, and more recently, small interfering (si)RNA, have been developed.[7-12] Some have improved stability against nucleases and increased binding affinity to mRNA, however, they can have drawbacks such as low cell penetrance and lack of RNAse H recruitment. Despite new generations of ASO, these disadvantages can be significant[3] and thus phosphorothioate-modified ASO are still commonly used.

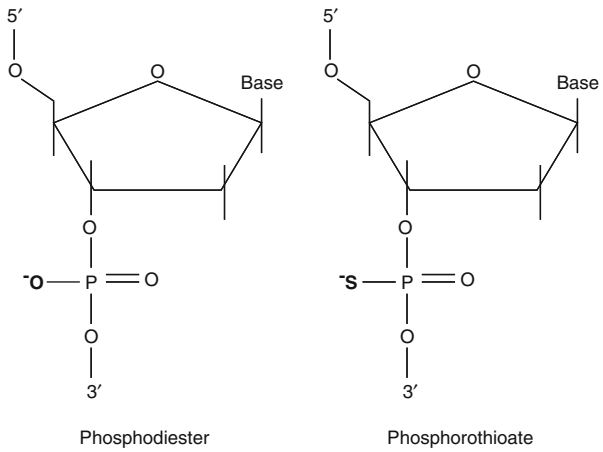

Fig. 1. Phosphorothioate backbone modification of oligonucleotides that inhibits nuclease degradation of antisense.

1.3 Small Interfering RNA

Since its discovery in 1998,[13] the natural phenomenon of RNA interference (RNAi) has been intensively studied. There is much enthusiasm about its potential to be a new, powerful therapeutic tool to specifically inhibit gene expression.[14] RNAi is a part of the innate antiviral defense in lower eukaryotes. It is induced by double-stranded (ds)RNA that is processed to 21–23 nucleotide siRNA (figure 3). RNAi results in degradation of homologous endogenous mRNA complementary to the antisense strand of siRNA. Although transfection of mammalian cells with dsRNA induces a strong interferon (IFN)-like response eventually leading to apoptosis, treatment with siRNA initiates RNAi without causing cell death.[15,16] siRNA has promise for therapy of genetic diseases, since siRNA can target single nucleotide polymorphisms, and thus specifically target selected oncogenes.[14]

Recent studies demonstrated that siRNA could selectively silence a disease-associated allele bearing a single mismatch.[17,18] However, clinical application of siRNA is still problematic because we do not fully understand mechanisms of RNAi action in higher eukaryotes. For example, exogenously applied siRNA may interfere with endogenous RNAi pathways and induce potentially dangerous off-target effects.[19,20] In addition, it is more difficult to ensure efficient delivery and cellular uptake of siRNA compared with ASO, because double-stranded siRNA do not bind plasma proteins and rapidly degrade in tissue environments.[19] Although published reports of siRNA use in *in vivo* in models of lung disease are limited to respiratory syncytial virus (RSV) and parainfluenza virus (PIV) infections,[21,22] siRNA-based approaches to inhibit oncogene expression, pro-inflammatory molecules or pro-fibrotic targets in lung disease are in development.

1.4 Target Selection and Specificity of ASO

Correct target selection is critical in development of ASO-based therapy of respiratory diseases. The targeted molecule must be important in disease pathogenesis and, as ASO can be extremely potent, it is essential to ensure both lung and disease specificity/selectivity of the target to avoid potential adverse effects.

Once a clinically relevant target protein has been selected, specificity of the ASO is a critical issue; it must inhibit expression of the target gene, but not other genes with similar sequences, i.e. the targeted mRNA sequence should not have homology to other

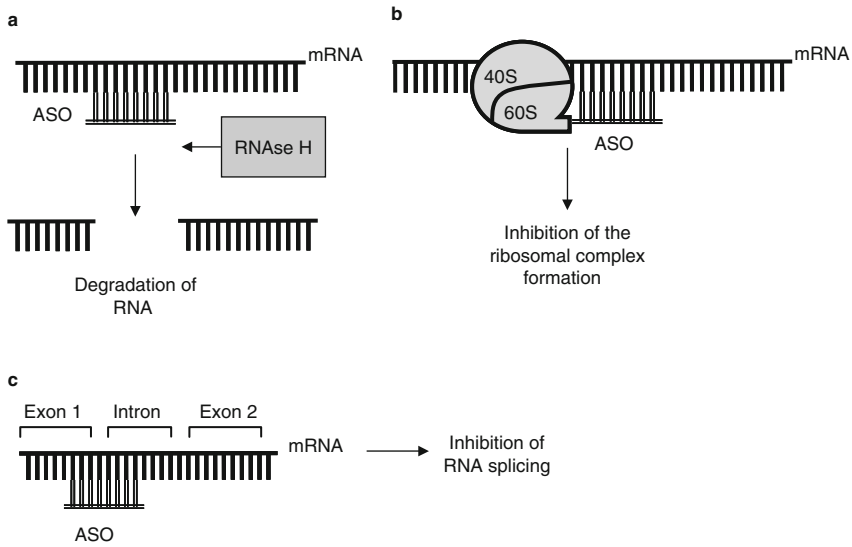

Fig. 2. Major mechanisms of action of antisense oligonucleotides (ASO). (**a**) Activation of endonuclease RNAse H, leading to messenger (m)RNA degradation. (**b**) Inhibition of the ribosomal complex formation via steric blocking. (**c**) Inhibition of mRNA splicing.

genes. In design of ASO, the genome should be carefully checked for possible hybridization of the ASO to sequences in non-targeted genes. Sequences common to several molecules of the same family or domains expressed in many genes must be avoided. Self-complementary regions, four or more contiguous guanine residues, or regions rich in guanines may form complexes with ASO or secondary structures that prevent efficient Watson-Crick hybridization to targeted mRNA and should be avoided.[23] The presence of immunostimulatory cytosine-guanine phosphate-linked dinucleotide (CpG) motifs within ASO is normally undesirable as they can stimulate Toll-like receptor (TLR) 9 on several cell types.[24] However, in some instances they may be included because of additional beneficial effects on the immune system. *In vitro* controls for ASO specificity, such as nonsense or mismatched oligonucleotide sequences, are important as they assess specificity of hybridization to the selected targeted sequence.

1.5 Delivery Issues

To ensure delivery of ASO to target cells, cationic liposomes are often used in complexes with ASO that can be internalized by pinocytosis/endocytosis.[25-27] Liposome delivery systems have been extensively used for intravenous and local application of ASO to the airways. Upon systemic application for cancer therapy, ASO-liposome complexes preferentially enter tumor tissues because of increased permeability of blood vessels in tumors.[28]

However, the role of cationic lipids in ASO delivery is not limited by their carrier function. Complexes of DNA oligonucleotides with cationic lipids can greatly enhance immunostimulatory properties of DNA.[29,30] These properties can provide an additional therapeutic effect, for example, in cancer. However, systemic release of high levels of pro-inflammatory cytokines tumor necrosis factor (TNF), interleukin (IL)-12 and IFNγ, as well as activation of natural killer (NK) cells following application of DNA-cationic lipid complexes can induce adverse effects.[29] A novel cationic cardiolipin analog-based liposome appeared to be less toxic and more effective for transfection of DNA and siRNA both *in vitro* and *in vivo*, compared with a commercially available DOTAP (1,2-dioleoyl-3-trimethylammonium-propane)-based liposome.[31]

In recent years, new carriers such as polyethylenimine (PEI) have been developed with enhanced efficiency of ASO delivery to target cells *in vitro* and *in vivo*.[32] Despite enhanced delivery to airway epithelial cells, PEI has toxicity and can be detrimental to lung function.[33] A new strategy using chitosan-DNA nanospheres for intranasal delivery of DNA recently showed advantages over lipid cationic carriers.[34-36] These nanospheres can protect DNA from nuclease degradation, and multiple compounds can be incorporated into nanospheres to achieve additional effects.[37] However, this delivery system has never been assessed for antisense treatment and requires further study.

Given carrier-related adverse effects, an attractive method for ASO delivery to the lung involves the use of a natural surfactant with cationic properties.[38] Several studies on local ASO application to the airways have relied on surfactant rather than using artificial carrier systems.[39-42] In a recent study, a single instillation of siRNA mixed with surfactant and elastase decreased ex-

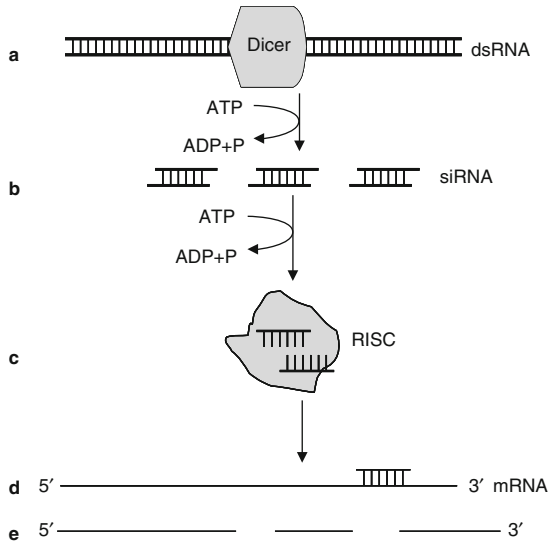

Fig. 3. Hypothetical model of RNA interference. (**a**) When introduced into a cell, double-stranded (ds)RNA is cleaved into small interfering (si)RNAs by a Dicer nuclease in an adenosine triphosphate (ATP)-dependent process. (**b**) Duplex siRNAs are recruited by several intracellular proteins, forming the RNA-induced silencing complex (RISC). (**c**) Unwinding of duplex siRNA occurs in an ATP-dependent manner. (**d**) The antisense strand of the siRNA binds to the messenger (m)RNA. (**e**) Activation of nuclease activity leads to degradation of the target mRNA. **ADP** = adenosine diphosphate; **P** = phosphate.

pression of a targeted protein in mouse lungs by 50–70% during 7 days of examination.[43]

1.6 ASO Pharmacokinetics

The pharmacokinetics and toxicology of phosphorothioate modified ASO have been intensively studied.[44,45] Effective doses of ASO for *in vivo* application depend both on the efficiency of the delivery system and mode of administration (systemic versus local). Following systemic application, phosphorothioate oligonucleotides bind to plasma proteins, ensuring their prolonged effect.[44] Various ASO doses for systemic (intravenous or subcutaneous) application in humans were carefully evaluated in several anti-cancer therapy trials, and no significant toxicity was observed at clinically relevant doses.[3]

The pharmacokinetic properties of aerosolized ASO were studied in several animal models; following ASO delivery to the lung, limited systemic distribution was detected.[46] Local delivery of ASO to the airways allows administration of lower doses compared with intravenous therapy of lung diseases. In a study of phosphorothioate ASO to the type 1 adenosine receptor (ADORA1), a single effective inhaled dose was 50 μg/kg and duration of the effect in the lung was 6.8 days.[46] In our studies, 250 μg/rat/day of ASO to spleen tyrosine kinase (SYK) was nebulized into an enclosed chamber on each of three consecutive days prior to allergen challenge (the precise dose each rat received was not determined). This exposure suppressed antigen-induced airway inflammation during the following 48 hours.[47] These examples demonstrate that the effective ASO dose and duration of its effect depend on the target characteristics, in addition to the importance of an efficient delivery system. Quantity and half-life of both target mRNA and encoded protein are critical determinants of ASO pharmacokinetics and pharmacodynamics in *in vivo* applications.

1.7 Toxicity Issues: Sources of Adverse Effects of Antisense Therapy

Adverse effects of ASO therapy (table I) can result from hybridization of ASO to nonspecific sequences in mRNA, rather than the targeted sequence. Assessing expression of the targeted gene at both mRNA and protein levels following ASO treatment is important to confirm the specificity of the ASO effect.

Upon DNA-RNA duplex formation, the endonuclease RNAse H is recruited to degrade the RNA in the duplex. As a result of this recruitment, there may be nonspecific activation of RNAse H, resulting in 'irrelevant cleavage' and effects on expression of other genes.[48]

A potential source of non-sequence-specific effects of ASO is the backbone modification of oligonucleotides. Phosphorothioate-modified oligonucleotides bind to a family of heparin-binding proteins including some growth factors and their receptors, extracellular matrix proteins and adhesion molecules.[49,50] This mechanism at least partially explains some adverse effects of systemic ASO application such as thrombocytopenia and hypotension.[51,52] This protein binding is due to the polyanionic nature of oligonucleotides, which is also responsible for their ability to activate complement.[44] As outlined in section 1.4, immunostimulatory CpG motifs in ASO sequences can also be an important source of adverse effects related to systemic cytokine release, such as fatigue, fever, and flu-like syndrome.[53] siRNA can also exert non-target-related biological effects, in particular, induction of pro-inflammatory cytokines. Such effects are related to the ability of dsRNA to bind TLRs present on immune cells and induce cellular activation,[54] and must be carefully assessed for each sequence used.

Local delivery of ASO offers advantages over systemic (intravenous) application because it allows lower doses to be used and thus minimizes systemic toxicity. An important consideration in using ASO in the airways is that adenosine can be released as an

oligonucleotide degradation product.[46] It can activate adenosine receptors that induce bronchoconstriction. Adenosine receptors are up-regulated in certain clinical conditions, particularly asthma,[55,56] and they themselves have been targeted in studies of ASO treatment of experimental asthma (see section 2.1.2).

Another source of potential adverse effects is related to ASO delivery systems. As discussed in section 1.5, enhanced immunostimulatory properties of ASO applied in cationic liposome complexes may release pro-inflammatory cytokines,[29,30] or cationic liposomes may affect cellular functions directly, e.g. by inhibiting TNF-induced endothelial vascular cell adhesion molecule-1 expression.[57] Cytotoxicity of cationic liposomes is dose-dependent and requires careful evaluation when liposomes are used.[58]

2. Antisense Treatment of Lung Disease

2.1 Experimental Asthma

As asthma is a complex heterogeneous disease, a major challenge is to identify appropriate molecular targets for ASO, and to identify delivery systems that target the lung, and minimize systemic distribution and related adverse effects. There are several examples of such approaches in experimental models.

2.1.1 Tyrosine Kinase Targets

The tyrosine kinase SYK mediates early signaling events important in the pathophysiology of allergic asthma and initiated by cross-linking high affinity receptors for IgE (FcεRI) on mast cells and basophils.[59-61] A 60 bp ASO directed against the *SYK* gene was constructed as a stem-loop structure, containing three phosphorothioate modifications,[62] and delivered by aerosolization *in vivo* using SYK ASO-liposome complexes combining cationic liposomes (1,2-dioleoyl-3-trimethylammonium-propane, DOTAP) with a neutral carrier lipid (dioleoylphosphatidyl-ethanol-amine, DOPE). Treatment of rats with nebulized SYK ASO-liposome complexes inhibited SYK mRNA and protein expression in alveolar macrophages.[63]

We studied the effects of aerosolized SYK ASO-liposome complexes in two models: (i) an infectious model of airway inflammation induced by the helminth *Nippostrongylus brasiliensis*; and (ii) IgE-mediated allergic inflammation induced by ovalbumin in sensitized Brown Norway rats, a model of allergic asthma. SYK ASO significantly inhibited inflammatory cell infiltration in the airways, lung eosinophilia and the rise in TNF in broncho-alveolar lavage induced by antigenic challenge. SYK ASO also suppressed antigen-induced tracheal contraction.[47,63] We have also aerosolized siRNA to SYK in rat ovalbumin-induced asthma and obtained promising down-regulation of inflammation (unpublished observations). Thus, aerosolized SYK ASO inhibited many central components of allergic asthma and inflammation.

Although SYK is a promising molecular target for ASO therapy of asthma and other inflammatory conditions such as acute lung injury, more studies are needed to assess potential risks related to SYK inhibition. For example, recent studies implicated *SYK* as a tumor suppressor gene in breast and gastric cancer.[64-66] Additionally, we established that SYK is abundantly expressed in lung

Table I. Adverse effects of antisense therapy

Type of effect	Mechanisms	Results
Sequence-specific	Hybridization of ASO to off-target sequences	Expression of other genes is affected
	Activation of RNAse H related to other genes	Expression of other genes is affected
	Four contiguous guanine residues ('guanine quartets') form higher-order structures	Non-specific biological effects
	Immunostimulatory CpG motifs	Immunoactivation, systemic cytokine release, hepatotoxicity
Non-sequence specific		
phosphorothioate backbone modification	Binding to heparin-binding proteins	Thrombocytopenia
polyanionic nature of ASO	Binding of various proteins	Complement activation
adenosine	Activation of adenosine receptors	Bronchoconstriction
Related to the delivery system	Cationic liposomes enhance immunostimulatory properties of ASO	Systemic cytokine release
	Cationic liposomes affect cellular function	Various effects due to interactions with plasma proteins and cellular receptors

ASO = antisense oligonucleotides; **CpG** = cytosine-guanine phosphate-linked dinucleotide.

epithelial cells and is involved in their production of pro-inflammatory molecules.[67] Thus, while SYK ASO may have advantages as a short-term local therapy of severe lung conditions, e.g. acute respiratory distress syndrome, long-term application of SYK ASO raises potential safety issues that must be further assessed.

Another signaling molecule that is a potential target for ASO therapy of asthma is LYN, a Src-family kinase.[68] LYN phosphorylation occurs as an immediate result of conformational changes in cytoplasmic domains of FcεRI after allergen-mediated cross-linking. LYN then interacts with SYK and induces its activation.[69,70] In eosinophils, LYN is associated with IL-5 receptor α subunit (IL5RA) and is important for IL-5-induced differentiation from bone marrow stem cells.[71] *In vitro* studies demonstrated that ASO directed against LYN blocked eosinophil differentiation from stem cells.[71] Although LYN ASO has never been applied in experimental models of asthma, the importance of LYN for eosinophil differentiation *in vivo* was confirmed in *LYN* knockout mice.[71]

2.1.2 Other Targets in Inflammatory Cell Activation

Recent studies of allergic asthma attempted to inhibit intracellular pathways involved in inflammatory cell activation. Inhaled ASO to p38α mitogen-activated protein kinase (MAPK14) down-regulated ovalbumin-induced pulmonary eosinophilia, mucus hypersecretion, and airway hyper-responsiveness (AHR) in a murine model of asthma.[72] ASO to the p65 subunit of the transcription factor NF-κB (RELA) that regulates expression of pro-inflammatory genes[73] applied intravenously significantly inhibited allergic responses in a mouse model.[74] Despite this proof of principle study, systemic application of ASO to NF-κB does not seem to be feasible for treatment of asthma given crucial involvement of this transcription factor in regulation of immune responses.

Given the important role of T helper type 2 (T_h2) cytokines and their receptors in allergic asthma, they have been targeted by ASO therapy. For example, ASO to IL-5, applied intravenously in a murine model of asthma, inhibited eosinophilia and AHR.[75] Allakhverdi and colleagues[42] used intratracheal injection of ASO to the common β chain of IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors and demonstrated significant reduction of eosinophilia and AHR in a rat model of asthma; this is moving forward to clinical testing. Intranasal application of ASO to stem cell factor (KIT ligand [KITLG]), essential for the development of mast cells,[76] decreased lung inflammation in a murine model of asthma.[40] ASO to the transcription factor GATA-3, essential for development of T_h2 responses,[77] also reduced lung inflammation and AHR.[41] In a recent study, the signal transducer and activator of transcription (STAT)-1 was targeted using intranasal application of decoy oligonucleotides in a mouse model of ovalbumin-induced asthma. This study demonstrated inhibition of antigen-induced airway inflammation and hyperreactivity.[78]

To directly target bronchial smooth muscle contraction in asthma, ASO to ADORA1 was developed and administered in aerosol form to rabbits. There was a significant reduction in both bronchoconstriction and airway inflammation.[39] This ASO-based therapy is currently in a phase II clinical trial.[79]

2.1.3 Alternative Approaches

All these ASO were applied as phosphorothioate-modified oligonucleotides in liposome delivery systems or as naked DNA. However, alternative approaches have recently been developed, including an adenoviral-mediated expression of ASO to Gob-5 (*CLCA1*) mRNA, a Ca^{2+}-dependent chloride channel,[80] in the bronchial epithelium in ovalbumin-sensitized mice. This approach decreased AHR and mucus production.[81] Using recombinant adenovirus for ASO delivery to target cells offers some advantages over other methods, such as selectivity to airway epithelium and prolonged expression of transfected genes (>1 week following instillation).[82,83] However, adenovirus-mediated gene delivery induces immune responses to adenovirus that preclude repeated applications[84] and there are several safety concerns such as the potential for oncogenic transformation.

Importantly, in animal models of asthma, ASO to various target molecules were applied prior to antigenic challenge. Whether or not ASO-mediated targeting of molecules involved in asthma pathogenesis will also be efficient during ongoing allergic inflammation requires further studies.

Recently, a ribozyme targeting the human IL-4 receptor α chain (IL4R, also known as IL4RA) was developed.[85] This approach offers some advantages over 'traditional' antisense because mechanisms of action of ribozymes rely on activation of RNAse P, which is ubiquitously present in cells. The construct can recycle after inducing the complementary mRNA cleavage, and therefore appears to act more efficiently than DNA-based antisense.[86] Another recent study used siRNA to silence gene expression of STAT6, a transcriptional regulator of T_h2 cytokines. *In vitro*-applied siRNA down-regulated STAT6 protein expression, as well as IL-4-dependent eotaxin production in human bronchial epithelial cells.[87]

Studies of the genetics of asthma have identified several potential asthma susceptibility genes, such as inflammatory mediators,[88] a disintegrin and metalloprotease domain 33 (ADAM33)[89] and G protein-coupled receptor for asthma susceptibility (GPRA),[90] which may be new targets for antisense therapy. Although biochemical mechanisms linking many of these candidate genes to asthma pathogenesis are poorly understood,[90] ASO strategies may help to elucidate such pathways.

2.2 Lung Cancer

In lung cancer, many oncogenes have been identified and studied as targets for antisense therapy. Several ASO-based drugs have reached phase II–III trials, and it is anticipated that some will soon be approved for clinical use (see Stahel and Zangemeister-Wittke[91] for review). In particular, the anti-apoptotic protein BCL-2 is a promising target for ASO therapy in non-small cell lung cancer.[92,93] ASO directed against the *BCL2* gene induces apoptosis of cancer cells and potentiates effects of chemotherapy.[91,94,95]

Other molecular targets for ASO therapy of lung cancer include signal transduction molecules: protein kinase C-α (PRKCA),[96] the regulatory subunit R1-α of protein kinase A (PRKAR1A),[97] RAF kinase (RAF1),[98] and the protein encoded by the *HRAS* oncogene.[99] Numerous pre-clinical studies are focused on targets in regulation of apoptosis, cell cycle progression, angiogenesis and metastasis, such as the apoptosis suppressor survivin (BIRC5);[100,101] the cytochrome *c* oxidase assembly protein COX17;[102] several growth factor receptors and receptor tyrosine kinases, as well as transcription factors.[103-107] ASO-based drugs in clinical trials in lung cancer are short oligonucleotide sequences (18-26-mer) with phosphorothioate backbone modifications. Recently, a locked nucleic acid-modified oligonucleotide with bispecific activity against BCL-2 and BCL-xL, another anti-apoptotic BCL protein, was developed and showed enhanced anti-tumor activity in cancer cells.[108]

Since human cancer cell lines preserve their RNAi machinery, use of siRNA to silence oncogenes involved in cancer pathogenesis has been suggested.[109] Indeed, siRNA against S-phase kinase-associated protein 2 (SKP2), a molecule involved in cell cycle regulation that is over-expressed in various cancers including small-cell lung carcinoma, was applied using lentiviral or adenoviral vectors. This strategy significantly inhibited tumor growth *in vitro* and *in vivo*.[110] Although there are several *in vitro* studies using siRNA to various target molecules potentially important in tumorigenesis,[111-114] siRNA-based strategies require further investigation with regard to efficiency, effectiveness and potential adverse effects.

When infused intravenously, ASO preparations showed moderate dose-dependent systemic toxicity such as thrombocytopenia, flu-like syndrome, hypotension and asthenia.[51] In some clinical trials, ASO-based therapeutics were combined with chemotherapeutic agents,[96] a strategy that also needs extensive study. Unfortunately, there are no published reports on local application of ASO in lung cancer.

2.3 Infectious Diseases

An antisense strategy intended to specifically cleave genomic RNA of RSV has recently been developed.[115] Application of ASO with 2′-5′ linked tetra-adenylates was shown to recruit the cellular nuclease RNAseL and successfully inhibit RSV replication.[116] Importantly, a combination of subtherapeutic doses of ASO with the antiviral drug ribavirin demonstrated a potent inhibitory effect.[116] A recent study described use of siRNA to RSV *in vivo*. Mice treated intranasally with siRNA nanoparticles to RSV protein NS1 before or after infection with RSV showed substantially decreased virus titers in the lung and decreased inflammation and airway hyper-reactivity compared with control animals.[21] Inhibition of both RSV and PIV following intranasal instillation of siRNA in the mouse was also demonstrated.[22] In addition, recent *in vitro* studies showed the ability of siRNA to inhibit replication of the newly discovered coronavirus SARS-CoV, the causative agent of severe acute respiratory syndrome (SARS).[117] This is the beginning of a potentially important research area, with opportunities to develop innovative ASO therapies for infectious diseases of the lung (table II).

3. Conclusions

ASO are promising therapeutic tools for various respiratory diseases ranging from infection to asthma, lung cancer, fibrosis and acute respiratory distress syndrome. A major advantage of ASO over conventional drugs is their capacity to specifically block synthesis of a disease-associated protein, thus preventing participation in pathogenesis.

ASO can be highly potent and specific and therefore it is essential that correct molecular targets be identified for therapy. Applying ASO in complex heterogeneous diseases such as asthma presents a major challenge, since this condition involves several pathways, numerous genes, and poorly understood gene-environment interactions. Some approaches to specifically target critical molecules in asthma have been successful, and development of

Table II. Clinically relevant targets of antisense-based therapy in respiratory diseases

Molecular target	Gene name	Disease	Mechanisms of action	References
Spleen tyrosine kinase	*SYK*	Asthma	Inhibition of intracellular signaling in allergic inflammation	47,62,63
Src-family kinase LYN	*LYN*	Asthma	Inhibition of intracellular signaling in allergic inflammation and eosinophil differentiation	71
p38 mitogen-activated protein kinase	*MAPK14*	Asthma	Inhibition of intracellular signaling in allergic inflammation	72
IL-5	*IL5*	Asthma	Inhibition of eosinophil-dependent component of asthma	75
Common β chain of IL-3, IL-5 and GM-CSF receptors	*CSF2RB*	Asthma	Down-regulation of effects of T_h2 cytokines	42
IL-4 receptor α chain	*IL4RA*	Asthma	Inhibition of development of T_h2 responses	85
Stem cell factor (KIT ligand)	*KITLG*	Asthma	Inhibition of mast cell development	40
Transcription factor GATA-3	*GATA3*	Asthma	Inhibition of development of T_h2 responses	41
Transcription factor STAT-1	*STAT1*	Asthma	Down-regulation of CD40 expression, important in T_h2 responses	78
Transcription factor STAT-6	*STAT6*	Asthma	Down-regulation of T_h2 responses	87
Type 1 adenosine receptor	*ADORA1*	Asthma	Regulation of bronchial smooth muscle contraction and inhibition of inflammation	39,79
Ca^{2+}-dependent chloride channel Gob-5	*CLCA1*	Asthma	Down-regulation of airway hyper-responsiveness and mucus production	81
Anti-apoptotic proteins: BCL-2, BCL-xL, Survivin	*BCL2*, *BCL2L1*, *BIRC5*	Lung cancer	Apoptosis of cancer cells	91-95,100,101,108
Signal transduction molecules, growth factor receptors, receptor tyrosine kinases, transcription factors	Examples: *PRKAR1A*, *RAF1*, *PRKCA*, *HRAS*, *COX17*, *SKP2*	Lung cancer	Inhibition of intracellular signaling involved in cancer development. Regulation of apoptosis, cell cycle progression, angiogenesis, metastasis	96-99,102-107,110
Viral proteins: RSV protein NS1, PIV, SARS-CoV		Infectious diseases	Anti-viral effect: inhibition of replication	21,22,115-117

GM-CSF = granulocyte-macrophage colony-stimulating factor; **IL** = interleukin; **PIV** = parainfluenza virus; **RSV** = respiratory syncytial virus; **SARS-CoV** = severe acute respiratory virus-associated coronavirus; **T_h2** = T helper type 2.

ASO-based drugs for clinical application can be anticipated in the near future. The genetics of asthma is a rapidly developing field and important discoveries of susceptibility genes will be important. Such genes, when targeted by antisense therapy, may provide an important contribution to the treatment of this common disease.

ASO therapy also has the potential to become a powerful tool against lung cancer. Successes are anticipated based on intensive molecular studies and discovery of causal oncogenes in lung cancer. Based on published observations, targeted delivery of ASO to the lung is feasible and has significant advantages over systemic application because it can minimize therapeutic doses and thus reduce systemic adverse effects.

There are several critical challenges in the development of ASO-based therapeutics. In addition to selection of the correct target protein, specificity of the ASO effect is essential and inhibition of other genes must be avoided. Both sequence-specific (e.g. CpG-mediated) and sequence-nonspecific (phosphorothioate-mediated) adverse effects should be carefully assessed. New formulations of ASO, such as siRNA, are promising for therapeutic application, but require more studies on mechanisms of action and safety. New methodology for delivery of ASO to selected target

cells and optimization of carrier systems are likely to provide important advances for therapeutic uses of ASO.

Acknowledgment

This work was supported by grants from the Canadian Institutes for Health Research, National Institutes of Health HL-27068 and HL-69498, and post-doctoral fellowships from the Canadian Society of Allergy and Clinical Immunology/Merck Frosst and the Alberta Heritage Foundation for Medical Research.

The authors have no conflicts of interest that are directly relevant to the content of this review.

References

1. Zamecnik PC, Stephenson ML. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. Proc Natl Acad Sci U S A 1978; 75 (1): 280-4
2. Crooke ST. Vitravene: another piece in the mosaic. Antisense Nucleic Acid Drug Dev 1998; 8 (4): vii-viii
3. Pirollo KF, Rait A, Sleer LS, et al. Antisense therapeutics: from theory to clinical practice. Pharmacol Ther 2003; 99 (1): 55-77
4. Dash P, Lotan I, Knapp M, et al. Selective elimination of mRNAs in vivo: complementary oligodeoxynucleotides promote RNA degradation by an RNase H-like activity. Proc Natl Acad Sci U S A 1987; 84 (22): 7896-900
5. Mercatante DR, Kole R. Control of alternative splicing by antisense oligonucleotides as a potential chemotherapy: effects on gene expression. Biochim Biophys Acta 2002; 1587 (2-3): 126-32
6. Matzura H, Eckstein F. A polyribonucleotide containing alternation P = O and P = S linkages. Eur J Biochem 1968; 3 (4): 448-52
7. Nielsen PE, Egholm M, Berg RH, et al. Peptide nucleic acids (PNAs): potential antisense and anti-gene agents. Anticancer Drug Des 1993; 8 (1): 53-63
8. Wahlestedt C, Salmi P, Good L, et al. Potent and nontoxic antisense oligonucleotides containing locked nucleic acids. Proc Natl Acad Sci U S A 2000; 97 (10): 5633-8
9. Doherty EA, Doudna JA. Ribozyme structures and mechanisms. Annu Rev Biochem 2000; 69: 597-615
10. Braasch DA, Corey DR. Novel antisense and peptide nucleic acid strategies for controlling gene expression. Biochemistry 2002; 41 (14): 4503-10
11. Heasman J. Morpholino oligos: making sense of antisense? Dev Biol 2002; 243 (2): 209-14
12. Stevenson M. Therapeutic potential of RNA interference. N Engl J Med 2004; 351 (17): 1772-7
13. Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 1998; 391 (6669): 806-11
14. Ichim TE, Li M, Qian H, et al. RNA interference: a potent tool for gene-specific therapeutics. Am J Transplant 2004; 4 (8): 1227-36
15. Caplen NJ, Mousses S. Short interfering RNA (siRNA)-mediated RNA interference (RNAi) in human cells. Ann N Y Acad Sci 2003; 1002: 56-62
16. Morris KV, Chan SW, Jacobsen SE, et al. Small interfering RNA-induced transcriptional gene silencing in human cells. Science 2004; 305 (5688): 1289-92
17. Miller VM, Xia H, Marrs GL, et al. Allele-specific silencing of dominant disease genes. Proc Natl Acad Sci U S A 2003; 100 (12): 7195-200
18. Ding H, Schwarz DS, Keene A, et al. Selective silencing by RNAi of a dominant allele that causes amyotrophic lateral sclerosis. Aging Cell 2003; 2 (4): 209-17
19. Hannon GJ, Rossi JJ. Unlocking the potential of the human genome with RNA interference. Nature 2004; 431 (7006): 371-8
20. Scacheri PC, Rozenblatt-Rosen O, Caplen NJ, et al. Short interfering RNAs can induce unexpected and divergent changes in the levels of untargeted proteins in mammalian cells. Proc Natl Acad Sci U S A 2004; 101 (7): 1892-7
21. Zhang W, Yang H, Kong X, et al. Inhibition of respiratory syncytial virus infection with intranasal siRNA nanoparticles targeting the viral NS1 gene. Nat Med 2005; 11 (1): 56-62
22. Bitko V, Musiyenko A, Shulyayeva O, et al. Inhibition of respiratory viruses by nasally administered siRNA. Nat Med 2005; 11 (1): 50-5
23. Dagle JM, Weeks DL. Oligonucleotide-based strategies to reduce gene expression. Differentiation 2001; 69 (2-3): 75-82
24. Krieg AM. CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol 2002; 20: 709-60
25. Bennett CF, Chiang MY, Chan H, et al. Cationic lipids enhance cellular uptake and activity of phosphorothioate antisense oligonucleotides. Mol Pharmacol 1992; 41 (6): 1023-33
26. Capaccioli S, Di Pasquale G, Mini E, et al. Cationic lipids improve antisense oligonucleotide uptake and prevent degradation in cultured cells and in human serum. Biochem Biophys Res Commun 1993; 197 (2): 818-25
27. Zelphati O, Szoka Jr FC. Intracellular distribution and mechanism of delivery of oligonucleotides mediated by cationic lipids. Pharm Res 1996; 13 (9): 1367-72
28. Gabizon A, Catane R, Uziely B, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res 1994; 54 (4): 987-92
29. Freimark BD, Blezinger HP, Florack VJ, et al. Cationic lipids enhance cytokine and cell influx levels in the lung following administration of plasmid: cationic lipid complexes. J Immunol 1998; 160 (9): 4580-6
30. Dow SW, Fradkin LG, Liggitt DH, et al. Lipid-DNA complexes induce potent activation of innate immune responses and antitumor activity when administered intravenously. J Immunol 1999; 163 (3): 1552-61
31. Chien PY, Wang J, Carbonaro D, et al. Novel cationic cardiolipin analogue-based liposome for efficient DNA and small interfering RNA delivery in vitro and in vivo. Cancer Gene Ther 2005; 12 (3): 321-8
32. Wiseman JW, Goddard CA, McLelland D, et al. A comparison of linear and branched polyethylenimine (PEI) with DCChol/DOPE liposomes for gene delivery to epithelial cells in vitro and in vivo. Gene Ther 2003; 10 (19): 1654-62
33. Chollet P, Favrot MC, Hurbin A, et al. Side-effects of a systemic injection of linear polyethylenimine-DNA complexes. J Gene Med 2002; 4 (1): 84-91
34. Kumar M, Behera AK, Lockey RF, et al. Intranasal gene transfer by chitosan-DNA nanospheres protects BALB/c mice against acute respiratory syncytial virus infection. Hum Gene Ther 2002; 13 (12): 1415-25
35. Ravi Kumar MN, Bakowsky U, Lehr CM. Preparation and characterization of cationic PLGA nanospheres as DNA carriers. Biomaterials 2004; 25 (10): 1771-7
36. Mohapatra SS. Mucosal gene expression vaccine: a novel vaccine strategy for respiratory syncytial virus. Pediatr Infect Dis J 2003; 22 (2 Suppl.): S100-3
37. Leong KW, Mao HQ, Truong L, et al. DNA-polycation nanospheres as non-viral gene delivery vehicles. J Control Release 1998; 53 (1-3): 183-93
38. Haitsma JJ, Lachmann U, Lachmann B. Exogenous surfactant as a drug delivery agent. Adv Drug Deliv Rev 2001; 47 (2-3): 197-207
39. Nyce JW, Metzger WJ. DNA antisense therapy for asthma in an animal model. Nature 1997; 385 (6618): 721-5
40. Finotto S, Buerke M, Lingnau K, et al. Local administration of antisense phosphorothioate oligonucleotides to the c-kit ligand, stem cell factor, suppresses airway inflammation and IL-4 production in a murine model of asthma. J Allergy Clin Immunol 2001; 107 (2): 279-86
41. Finotto S, De Sanctis GT, Lehr HA, et al. Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. J Exp Med 2001; 193 (11): 1247-60
42. Allakhverdi Z, Allam M, Renzi PM. Inhibition of antigen-induced eosinophilia and airway hyperresponsiveness by antisense oligonucleotides directed against the common beta chain of IL-3, IL-5, GM-CSF receptors in a rat model of allergic asthma. Am J Respir Crit Care Med 2002; 165 (7): 1015-21
43. Massaro D, Massaro GD, Clerch LB. Noninvasive delivery of small inhibitory RNA and other reagents to pulmonary alveoli in mice. Am J Physiol Lung Cell Mol Physiol 2004; 287 (5): L1066-70

44. Levin AA. A review of the issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides. Biochim Biophys Acta 1999; 1489 (1): 69-84
45. Crooke ST. Progress in antisense technology. Annu Rev Med 2004; 55: 61-95
46. Tanaka M, Nyce JW. Respirable antisense oligonucleotides: a new drug class for respiratory disease. Respir Res 2001; 2 (1): 5-9
47. Stenton GR, Ulanova M, Dery RE, et al. Inhibition of allergic inflammation in the airways using aerosolized antisense to Syk kinase. J Immunol 2002; 169 (2): 1028-36
48. Stein CA. Is irrelevant cleavage the price of antisense efficacy? Pharmacol Ther 2000; 85 (3): 231-6
49. Guvakova MA, Yakubov LA, Vlodavsky I, et al. Phosphorothioate oligodeoxynucleotides bind to basic fibroblast growth factor, inhibit its binding to cell surface receptors, and remove it from low affinity binding sites on extracellular matrix. J Biol Chem 1995; 270 (6): 2620-7
50. Fennewald SM, Rando RF. Inhibition of high affinity basic fibroblast growth factor binding by oligonucleotides. J Biol Chem 1995; 270 (37): 21718-21
51. Cotter FE. Antisense oligonucleotides for haematological malignancies. Haematologica 1999; 84 Suppl EHA-4: 19-22
52. Sereni D, Tubiana R, Lascoux C, et al. Pharmacokinetics and tolerability of intravenous trecovirsen (GEM 91), an antisense phosphorothioate oligonucleotide, in HIV-positive subjects. J Clin Pharmacol 1999; 39 (1): 47-54
53. Klinman DM. Immunotherapeutic uses of CpG oligodeoxynucleotides. Nat Rev Immunol 2004; 4 (4): 249-58
54. Agrawal S, Kandimalla ER. Role of Toll-like receptors in antisense and siRNA. Nat Biotechnol 2004; 22 (12): 1533-7
55. Bjorck T, Gustafsson LE, Dahlen SE. Isolated bronchi from asthmatics are hyperresponsive to adenosine, which apparently acts indirectly by liberation of leukotrienes and histamine. Am Rev Respir Dis 1992; 145 (5): 1087-91
56. Ali S, Mustafa SJ, Metzger WJ. Adenosine-induced bronchoconstriction and contraction of airway smooth muscle from allergic rabbits with late-phase airway obstruction: evidence for an inducible adenosine A1 receptor. J Pharmacol Exp Ther 1994; 268 (3): 1328-34
57. Maus U, Rosseau S, Mandrakas N, et al. Cationic lipids employed for antisense oligodeoxynucleotide transport may inhibit vascular cell adhesion molecule-1 expression in human endothelial cells: a word of caution. Antisense Nucleic Acid Drug Dev 1999; 1: 71-80
58. Lysik MA, Wu-Pong S. Innovations in oligonucleotide drug delivery. J Pharm Sci 2003; 92 (8): 1559-73
59. Darby C, Geahlen RL, Schreiber AD. Stimulation of macrophage Fc gamma RIIIA activates the receptor- associated protein tyrosine kinase Syk and induces phosphorylation of multiple proteins including p95Vav and p62/GAP- associated protein. J Immunol 1994; 152 (11): 5429-37
60. Costello PS, Turner M, Walters AE, et al. Critical role for the tyrosine kinase Syk in signalling through the high affinity IgE receptor of mast cells. Oncogene 1996; 13 (12): 2595-605
61. Kiefer F, Brumell J, Al-Alawi N, et al. The Syk protein tyrosine kinase is essential for Fc gamma receptor signaling in macrophages and neutrophils. Mol Cell Biol 1998; 18 (7): 4209-20
62. Matsuda M, Park JG, Wang DC, et al. Abrogation of the Fc gamma receptor IIA-mediated phagocytic signal by stem-loop Syk antisense oligonucleotides. Mol Biol Cell 1996; 7 (7): 1095-106
63. Stenton GR, Kim MK, Nohara O, et al. Aerosolized Syk antisense suppresses Syk expression, mediator release from macrophages, and pulmonary inflammation. J Immunol 2000; 164 (7): 3790-7
64. Coopman PJ, Do MT, Barth M, et al. The Syk tyrosine kinase suppresses malignant growth of human breast cancer cells. Nature 2000; 406 (6797): 742-7
65. Moroni M, Soldatenkov V, Zhang L, et al. Progressive loss of Syk and abnormal proliferation in breast cancer cells. Cancer Res 2004; 64 (20): 7346-54
66. Wang S, Ding YB, Chen GY, et al. Hypermethylation of Syk gene in promoter region associated with oncogenesis and metastasis of gastric carcinoma. World J Gastroenterol 2004; 10 (12): 1815-8
67. Ulanova M, Puttagunta L, Marcet-Palacios M, et al. Syk tyrosine kinase participates in β1 integrin signaling and inflammatory responses in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2005; 288 (3): L297-507
68. Lowell CA. Src-family kinases: rheostats of immune cell signaling. Mol Immunol 2004; 41 (6-7): 631-43
69. Jouvin MH, Adamczewski M, Numerof R, et al. Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. J Biol Chem 1994; 269 (8): 5918-25
70. Lin S, Cicala C, Scharenberg AM, et al. The FcεRI β subunit functions as an amplifier of FcεRI γ-mediated cell activation signals. Cell 1996; 85 (7): 985-95
71. Stafford S, Lowell C, Sur S, et al. Lyn tyrosine kinase is important for IL-5-stimulated eosinophil differentiation. J Immunol 2002; 168 (4): 1978-83
72. Duan W, Chan JH, McKay K, et al. Inhaled p38α mitogen-activated protein kinase antisense oligonucleotide attenuates asthma in mice. Am J Respir Crit Care Med 2004; 171 (6): 571-8
73. Wulczyn FG, Krappmann D, Scheidereit C. The NF-kappa B/Rel and I kappa B gene families: mediators of immune response and inflammation. J Mol Med 1996; 74 (12): 749-69
74. Choi IW, Kim DK, Ko HM, et al. Administration of antisense phosphorothioate oligonucleotide to the p65 subunit of NF-kappaB inhibits established asthmatic reaction in mice. Int Immunopharmacol 2004; 4 (14): 1817-28
75. Karras JG, McGraw K, McKay RA, et al. Inhibition of antigen-induced eosinophilia and late phase airway hyperresponsiveness by an IL-5 antisense oligonucleotide in mouse models of asthma. J Immunol 2000; 164 (10): 5409-15
76. Zsebo KM, Williams DA, Geissler EN, et al. Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell 1990; 63 (1): 213-24
77. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 1997; 89 (4): 587-96
78. Quarcoo D, Weixler S, Groneberg D, et al. Inhibition of signal transducer and activator of transcription 1 attenuates allergen-induced airway inflammation and hyperreactivity. J Allergy Clin Immunol 2004; 114 (2): 288-95
79. Popescu FD. New asthma drugs acting on gene expression. J Cell Mol Med 2003; 7 (4): 475-86
80. Komiya T, Tanigawa Y, Hirohashi S. Cloning and identification of the gene gob-5, which is expressed in intestinal goblet cells in mice. Biochem Biophys Res Commun 1999; 255 (2): 347-51
81. Nakanishi A, Morita S, Iwashita H, et al. Role of gob-5 in mucus overproduction and airway hyperresponsiveness in asthma. Proc Natl Acad Sci U S A 2001; 98 (9): 5175-80
82. Sime PJ, Xing Z, Graham FL, et al. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. J Clin Invest 1997; 100 (4): 768-76
83. Xing Z, Braciak T, Ohkawara Y, et al. Gene transfer for cytokine functional studies in the lung: the multifunctional role of GM-CSF in pulmonary inflammation. J Leukoc Biol 1996; 59 (4): 481-8
84. Knowles MR, Hohneker KW, Zhou Z, et al. A controlled study of adenoviral-vector-mediated gene transfer in the nasal epithelium of patients with cystic fibrosis. N Engl J Med 1995; 333 (13): 823-31
85. Dreyfus DH, Matczuk A, Fuleihan R. An RNA external guide sequence ribozyme targeting human interleukin-4 receptor alpha mRNA. Int Immunopharmacol 2004; 4 (8): 1015-27
86. Plehn-Dujowich D, Altman S. Effective inhibition of influenza virus production in cultured cells by external guide sequences and ribonuclease P. Proc Natl Acad Sci U S A 1998; 95 (13): 7327-32
87. Rippmann JF, Schnapp A, Weith A, et al. Gene silencing with STAT6 specific siRNAs blocks eotaxin release in IL-4/TNFalpha stimulated human epithelial cells. FEBS Lett 2005; 579 (1): 173-8
88. Allen M, Heinzmann A, Noguchi E, et al. Positional cloning of a novel gene influencing asthma from chromosome 2q14. Nat Genet 2003; 35 (3): 258-63
89. Van Eerdewegh P, Little RD, Dupuis J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. Nature 2002; 418 (6896): 426-30

90. Laitinen T, Polvi A, Rydman P, et al. Characterization of a common susceptibility locus for asthma-related traits. Science 2004; 304 (5668): 300-4

91. Stahel RA, Zangemeister-Wittke U. Antisense oligonucleotides for cancer therapy: an overview. Lung Cancer 2003; 41 Suppl. 1: S81-8

92. Ziegler A, Luedke GH, Fabbro D, et al. Induction of apoptosis in small-cell lung cancer cells by an antisense oligodeoxynucleotide targeting the Bcl-2 coding sequence. J Natl Cancer Inst 1997; 89 (14): 1027-36

93. Zangemeister-Wittke U, Leech SH, Olie RA, et al. A novel bispecific antisense oligonucleotide inhibiting both bcl-2 and bcl-xL expression efficiently induces apoptosis in tumor cells. Clin Cancer Res 2000; 6 (6): 2547-55

94. Kim R, Emi M, Tanabe K, et al. Therapeutic potential of antisense Bcl-2 as a chemosensitizer for cancer therapy. Cancer 2004; 101 (11): 2491-502

95. Herbst RS, Frankel SR. Oblimersen sodium (Genasense bcl-2 antisense oligonucleotide): a rational therapeutic to enhance apoptosis in therapy of lung cancer. Clin Cancer Res 2004; 10 (12 Pt 2): 4245s-8s

96. Villalona-Calero MA, Ritch P, Figueroa JA, et al. A phase I/II study of LY900003, an antisense inhibitor of protein kinase C-alpha, in combination with cisplatin and gemcitabine in patients with advanced non-small cell lung cancer. Clin Cancer Res 2004; 10 (18 Pt 1): 6086-93

97. Wang H, Cai Q, Zeng X, et al. Antitumor activity and pharmacokinetics of a mixed-backbone antisense oligonucleotide targeted to the RI alpha subunit of protein kinase A after oral administration. Proc Natl Acad Sci U S A 1999; 96 (24): 13989-94

98. Coudert B, Anthoney A, Fiedler W, et al. Phase II trial with ISIS 5132 in patients with small-cell (SCLC) and non-small cell (NSCLC) lung cancer: a European Organization for Research and Treatment of Cancer (EORTC) Early Clinical Studies Group report. Eur J Cancer 2001; 37 (17): 2194-8

99. Jansen B, Zangemeister-Wittke U. Antisense therapy for cancer: the time of truth. Lancet Oncol 2002; 3 (11): 672-83

100. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997; 3 (8): 917-21

101. Olie RA, Simoes-Wust AP, Baumann B, et al. A novel antisense oligonucleotide targeting survivin expression induces apoptosis and sensitizes lung cancer cells to chemotherapy. Cancer Res 2000; 60 (11): 2805-9

102. Suzuki C, Daigo Y, Kikuchi T, et al. Identification of COX17 as a therapeutic target for non-small cell lung cancer. Cancer Res 2003; 63 (21): 7038-41

103. Sak A, Wurm R, Elo B, et al. Increased radiation-induced apoptosis and altered cell cycle progression of human lung cancer cell lines by antisense oligodeoxynucleotides targeting p53 and p21 (WAF1/CIP1). Cancer Gene Ther 2003; 10 (12): 926-34

104. Raben D, Helfrich B. Angiogenesis inhibitors: a rational strategy for radiosensitization in the treatment of non-small-cell lung cancer? Clin Lung Cancer 2004; 6 (1): 48-57

105. Pold M, Krysan K, Pold A, et al. Cyclooxygenase-2 modulates the insulin-like growth factor axis in non-small-cell lung cancer. Cancer Res 2004; 64 (18): 6549-55

106. Kuhn H, Kopff C, Konrad J, et al. Influence of basic fibroblast growth factor on the proliferation of non-small cell lung cancer cell lines. Lung Cancer 2004; 44 (2): 167-74

107. Stabile LP, Lyker JS, Huang L, et al. Inhibition of human non-small cell lung tumors by a c-Met antisense/U6 expression plasmid strategy. Gene Ther 2004; 11 (3): 325-35

108. Hopkins-Donaldson S, Cathomas R, Simoes-Wust AP, et al. Induction of apoptosis and chemosensitization of mesothelioma cells by Bcl-2 and Bcl-xL antisense treatment. Int J Cancer 2003; 106 (2): 160-6

109. Yin JQ, Gao J, Shao R, et al. siRNA agents inhibit oncogene expression and attenuate human tumor cell growth. J Exp Ther Oncol 2003; 3 (4): 194-204

110. Sumimoto H, Yamagata S, Shimizu A, et al. Gene therapy for human small-cell lung carcinoma by inactivation of Skp-2 with virally mediated RNA interference. Gene Ther 2005; 12 (1): 95-100

111. Spankuch-Schmitt B, Bereiter-Hahn J, Kaufmann M, et al. Effect of RNA silencing of polo-like kinase-1 (PLK1) on apoptosis and spindle formation in human cancer cells. J Natl Cancer Inst 2002; 94 (24): 1863-77

112. Brummelkamp TR, Bernards R, Agami R. A system for stable expression of short interfering RNAs in mammalian cells. Science 2002; 296 (5567): 550-3

113. Martinez LA, Naguibneva I, Lehrmann H, et al. Synthetic small inhibiting RNAs: efficient tools to inactivate oncogenic mutations and restore p53 pathways. Proc Natl Acad Sci U S A 2002; 99 (23): 14849-54

114. Nieth C, Priebsch A, Stege A, et al. Modulation of the classical multidrug resistance (MDR) phenotype by RNA interference (RNAi). FEBS Lett 2003; 545 (2-3): 144-50

115. Maggon K, Barik S. New drugs and treatment for respiratory syncytial virus. Rev Med Virol 2004; 14 (3): 149-68

116. Xu Z, Kuang M, Okicki JR, et al. Potent inhibition of respiratory syncytial virus by combination treatment with 2-5A antisense and ribavirin. Antiviral Res 2004; 61 (3): 195-206

117. Wu CJ, Huang HW, Liu CY, et al. Inhibition of SARS-CoV replication by siRNA. Antiviral Res 2005; 65 (1): 45-8

Correspondence and offprints: Dr *A. Dean Befus*, Department of Medicine, Pulmonary Research Group, University of Alberta, Room 550A HMRC, Edmonton, AB, T6G 2S2, Canada.
E-mail: dean.befus@ualberta.ca