The Future of Antisense Oligonucleotides in the Treatment of Respiratory Diseases

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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.

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.

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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 penetration 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.

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 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
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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. 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. 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. 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.

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. 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. 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.

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. Despite enhanced delivery to airway epithelial cells, PEI has toxicity and can be detrimental to lung function. A new strategy using chitosan-DNA nanospheres for intranasal delivery of DNA recently showed advantages over lipid cationic carriers. These nanospheres can protect DNA from nuclease degradation, and multiple compounds can be incorporated into nanospheres to achieve additional effects. 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. Several studies on local ASO application to the airways have relied on surfactant rather than using artificial carrier systems. In a recent study, a single instillation of siRNA mixed with surfactant and elastase decreased ex-
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
pression of a targeted protein in mouse lungs by 50–70% during 7
days of examination.[43]

1.6 ASO Pharmacokinetics

The pharmacokinetics and toxicity 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 oligo-
nucleotides bind to plasma proteins, ensuring their prolonged
effect.[44] Various ASO doses for systemic (intravenous or subcu-
taneous) 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 stud-
ied 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 com-
pared with intravenous therapy of lung diseases. In a study of
phosphorothioate ASO to the type 1 adenosine receptor
(ADOR1), 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

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oligonucleotide degradation product.\cite{46} It can activate adenosine receptors that induce bronchoconstriction. Adenosine receptors are up-regulated in certain clinical conditions, particularly asthma,\cite{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,\cite{29,30} or cationic liposomes may affect cellular functions directly, e.g. by inhibiting TNF-induced endothelial vascular cell adhesion molecule-1 expression.\cite{57} Cytotoxicity of cationic liposomes is dose-dependent and requires careful evaluation when liposomes are used.\cite{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 (FceRI) on mast cells and basophils.\cite{59-61} A 60 bp ASO directed against the SYK gene was constructed as a stem-loop structure, containing three phosphorothioate modifications,\cite{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.\cite{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.\cite{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.\cite{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 | Binding to heparin-binding proteins | Thrombocytopenia |
| | Binding of various proteins | Complement activation |
| | 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 |

\textbf{ASO} = antisense oligonucleotides; \textbf{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\(_h\)2) 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. Intrasulcal 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\(_h\)2 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\(_h\)2 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, a disintegrin and metalloprotease domain 33 (ADAM33) and G protein-coupled receptor for asthma susceptibility (GPRA), which may be new targets for antisense therapy. Although biochemical mechanisms linking many of these candidate genes to asthma pathogenesis are poorly understood, 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 for review). In particular, the anti-apoptotic protein BCL-2 is a promising target for ASO therapy in non-small cell lung cancer. ASO directed against the BCL2 gene induces apoptosis of cancer cells and potentiates effects of chemotherapy.

Other molecular targets for ASO therapy of lung cancer include signal transduction molecules: protein kinase C-α (PRKCA), the regulatory subunit R1-α of protein kinase A (PRKAR1A), RAF kinase (RAF1), and the protein encoded by the HRAS oncogene. 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; COX17, several growth factor receptors and receptor tyrosine kinases, as well as transcription factors. 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 bi-specific activity against BCL-2 and BCL-xL, another anti-apoptotic BCL protein, was developed and showed enhanced anti-tumor activity in cancer cells.

Since human cancer cell lines preserve their RNAi machinery, use of siRNA to silence oncogenes involved in cancer pathogenesis has been suggested. 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. Although there are several in vitro studies using siRNA to various target molecules potentially important in tumorigenesis, 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. In some clinical trials, ASO-based therapeutics were combined with chemotherapeutic agents, 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. Application of ASO with 2′-5′ linked tetra-adenylates was shown to recruit the cellular nucleas RNAse L and successfully inhibit RSV replication. Importantly, a combination of subtherapeutic doses of ASO with the antiviral drug ribavirin demonstrated a potent inhibitory effect. 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. Inhibition of both RSV and PIV following intranasal instillation of siRNA in the mouse was also demonstrated. 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). This is the beginning of a potentially important research area, with opportunities to develop innovative ASO therapies for infectious diseases of the lung.

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<sub>h</sub>2 cytokines                                | 42         |
| IL-4 receptor α chain                                | IL4RA     | Asthma        | Inhibition of development of T<sub>h</sub>2 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<sub>h</sub>2 responses                                 | 41         |
| Transcription factor STAT-1                          | STAT1     | Asthma        | Down-regulation of CD40 expression, important in T<sub>h</sub>2 responses             | 78         |
| Transcription factor STAT-6                          | STAT6     | Asthma        | Down-regulation of T<sub>h</sub>2 responses                                           | 87         |
| Type 1 adenosine receptor                            | ADORA1    | Asthma        | Regulation of bronchial smooth muscle contraction and inhibition of inflammation       | 39, 79     |
| Ca<sup>2+</sup>-dependent chloride channel Gob-5      | CLCA1     | Asthma        | Down-regulation of airway hyper-responsiveness and mucus production                   | 81         |
| Anti-apoptotic proteins: BCL-2, BCL2L1, 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<sub>h</sub>2** = 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.

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