A Review of Antisense Therapeutic Interventions for Molecular Biological Targets in Various Diseases

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Abstract: The principle of antisense technologies is based on the specific inhibition of unwanted gene expression by blocking mRNA activity. It has long appeared to be an ideal strategy to leverage new genomic knowledge for drug discovery and development. During the past 20 years the technology associated with the development of antisense has improved dramatically and emerging chemistries have made antisense oligonucleotides into powerful and versatile tools to study the function of proteins in living cells. In recent years, antisense technologies have been widely used as potent and promising tools for this purpose. There is a rapid increase in the number of antisense molecules progressing in clinical trials. Antisense technologies provide a simple and efficient approach for drug discovery and development and are expected to become a reality in the near future.

Key words: Cancer, drug discovery, oligonucleotides, RNA interference, ribozymes

INTRODUCTION

Antisense agents are medicines that interfere with the communication process that tells a cell to produce an unwanted protein. Proteins play a central role in almost every aspect of human metabolism. Almost all human diseases are the result of inappropriate protein production or disordered protein performance (Chan et al., 2006). Traditional drugs are designed to interact with protein molecules throughout the body that support or cause diseases but antisense drugs are designed to inhibit the production of disease-causing proteins by binding to the blueprint of a protein and specifically preventing its conversion into a pathogenic factor for example that causes an uncontrolled tumor growth (Aboul-Fadl, 2006). They can be designed to treat a wide range of diseases including infectious, inflammatory, cancer, cardiovascular diseases and have the potential to be more selective, as a result, more effective and less toxic than traditional drugs. In contrast to gene therapy antisense drugs do not alter human genes or have any effect on human genetic information. Therapeutic applications of antisense techniques are currently under investigation in many different fields (Galderisi et al., 1999). The first antisense drug Fomiviren, for the treatment of cytomegalovirus retinitis in AIDS patients, was approved in 1998 (Calvez et al., 2004; Van Aerschot, 2006). The use of antisense agents as therapeutic agents has generated considerable enthusiasm in the research and medical community. The last few years have seen a rapid increase in the number of antisense molecules progressing phase I, II and III clinical trials (Aboul-Fadl, 2005). ISIS Pharmaceuticals is the leader with 11 phase I, 7 phase II and 3 phase III trials. Genta is active with Genasense, an antisense to Bel 2 for antitumor cell treatment is in phase III. AVI Biopharm has a third generation antisense oligonucleotide platform and around this it is testing 4 phase I, 5 phase II and 2 phase III trials. Hybrion has conducted 2 phase I and has two phase 2 trials planned (Phillips, 2004). This review outlines the basic concept of the antisense technology, its development and recent potential therapeutic applications.

MOLECULAR MECHANISM OF ANTISENSE AGENTS

Mostly antisense agents are synthetic, single-stranded short sequences of DNA bases designed to hybridize to specific sequences of mRNA forming a duplex. This DNA-RNA coupling attracts an endogenous nuclease, RNase H which destroys the bound RNA and frees the DNA antisense to rehybridize with another copy of mRNA (Crooke, 1998). In this way, the effect is not only highly specific but prolonged because of the recycling of
the antisense DNA sequence. The general mechanism for inhibition of synthesis of protein molecule is given in Fig. 1.

The reduction in mRNA reduces the total amount of protein specified by mRNA. It is also theorized that hybridization sterically prevents ribosomes from translating the message of the mRNA into protein. Therefore, there are at least two ways in which antisense can work effectively to reduce the amount of protein being elaborated: RNase H based degradation of RNA (e.g., phosphodiester, phosphorothioate) and hindering of ribosomal assembly and translation (e.g., peptide nucleic acids, morpholino oligonucleotides) (Bennett and Swayne, 2009; Dryselius et al., 2003; Maguire, 2009).

**ANTISENSE AGENTS**

There are some major classes of antisense agents: antisense sequences, commonly called Antisense Oligonucleotides (ASOs); ribozymes and RNA Interference (RNAi).

**Antisense oligonucleotides**: Antisense oligonucleotides as therapeutic agents were proposed by Zamecnik and Stephenson, 1978. Nonetheless, it has taken almost a quarter of a century for this potential to be realized. A minimum length for antisense oligonucleotides in order to get specific binding is 11 bases but most being tested are in the 15-25 base range. Synthetic oligonucleotides are foreign to the cells into which they are introduced and they immediately become prey for endogenous nucleases. If synthetic oligonucleotides were to attain the level of persistence in the cell that would be needed for them to accomplish their tasks, they would have to be protected from those endogenous nucleases. In order to meet all these requirements it is necessary for normal oligonucleotides (Fig. 2a) to be chemically modified in a suitable manner. There are three possible sites on a nucleotide where protective modifications could be introduced (Kurreck, 2003). In both DNA and RNA nucleotides the base can be altered or changes can be effected in the phosphate backbone. In RNA nucleotides the 2’ hydroxyl group which is missing in DNA nucleotides, can also be modified. The “trick” involved in protective modifications of nucleotides is to introduce an alteration that is protective against nuclease degradation that does not, at the same time, eliminate the desired effect of the oligonucleotide sequence by blocking complementary hybridization or harming the cell. According to their generations they have been categorized into three types.

![Fig. 1: Sites of action of antisense agents](image)

**First generation antisense-oligonucleotides**: The first generation of antisense agents contains backbone modifications such as replacement of oxygen atom of the phosphate linkage by sulfur (phosphorothioates) (Fig. 2b), methyl group (methylphosphonates) (Fig. 2c) or amines (phosphoramidates) (Fig. 2d). Of these, the phosphorothioates have been the most successful and used for gene silencing because of their sufficient resistance to nucleases and ability to induce RNase H functions (Campbell et al., 1990; Zon, 1995). Phosphorothioate oligonucleotides were first synthesized in the 1960s by Eckstein and colleagues and were first used as Antisense-oligonucleotides (ASOs) for the inhibition of HIV replication by Matsukura and coworkers (Matsukura et al., 1987). However, their profiles of
binding affinity to the target sequences, specificity and cellular uptake are less satisfactory (Chen et al., 2005).

**Second generation antisense-oligonucleotides:** The problems associated with phosphorothioate oligodeoxynucleotides are to some degree solved in second generation oligonucleotides containing nucleotides with alkyl modifications at the 2' position of the ribose. 2'-O-methyl and 2'-O-methoxyethyl RNA are the most important members of this class. 2'-O-methyl and 2'-O-methoxyethyl derivatives can be further combined with phosphorothioate linkage (Fig. 3) (Manoharan, 1999). Antisense oligonucleotides made of these building blocks are less toxic than phosphorothioate ASONs and have a slightly enhanced affinity towards their complementary RNAs. Questions regarding its efficiency to induce RNase H cleavage of the target RNA are the matter to concern regarding this second generation oligonucleotides. Since RNase H cleavage is the most desirable mechanism for antisense effect and since 2'-O-alkyl modifications are desirable for nuclease resistance, a hybrid oligonucleotide construct incorporating both characteristics has appeared in the form of the “gapmer” antisense oligonucleotide. A gapmer contains a central block of deoxynucleotides sufficient to induce Rnase H cleavage flanked by blocks of 2'-O-methyl modified ribonucleotides that protect the internal block from nuclease degradation (Monia et al., 1993).

**Third generation antisense-oligonucleotides:** A variety of nucleic acid analogs have been developed that display increased thermal stabilities when hybridized to with complementary DNAs or RNAs as compared to unmodified DNA:DNA and DNA:RNA duplexes. These are the third generation antisense oligonucleotide modifications. The third generation contains structural elements, such as zwitterionic oligonucleotides (possessing both positive and negative charges in the molecule), Peptide Nucleic Acids (PNAs) (with a pseudopeptide backbone) (Fig. 4a), Locked Nucleic Acids (LNAs)/Bridged Nucleic Acids (BNAs) (Fig. 4b), Hexitol Nucleic Acids (HNA) (Fig. 4c) and Morpholino oligonucleotides (Fig. 4d) (Hyrup and Nielsen, 1996; Elayadi and Corey, 2001; Doelker et al., 2002). PNAs are dramatic alterations in which the sugar phosphate backbone is replaced completely by polyamide linkages. While these constructs afford increased stability and favorable hybridization kinetics, they suffer from being unavailable to the RNase H cleavage mechanism, problematic solubilities and delivery difficulties (Persato et al., 2007). The newest and most promising third generation modification is the Locked Nucleic Acids (LNAs). LNAs nucleotides are a class of nucleic acid analogues in which the ribose ring is “locked” by a methylene bridge connecting the 2'-O atom and the 4'-C atom. LNAs were immediately seen to display remarkably increased thermodynamic stability and enhanced nucleic acid recognition. LNAs has been proven to be a powerful tool in many molecular biological applications in which standard DNA oligonucleotides or RNA riboprobes

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**Fig. 3:** General structures of second generation Antisense oligonucleotides (2'-alkyl substituted phosphorothioate oligonucleotides)

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**Fig. 4:** General structures of third generation Antisense oligonucleotides
do not show sufficient affinity or specificity (Veechu and Wengel, 2009; Stein et al., 2010). In the Hexitol Nucleic Acids (HNAs) the furanose sugar moiety of an ASON is replaced by a six-membered hexitol component (De Bouwer et al., 1997). The replacement of the conformationally flexible deoxyribose by the restricted anhydrohexitol ring results in a structural reorganization of HNA to form A-type helices. They showed significant increase in binding affinity towards complementary RNA (approximately 3°C per modification). In addition, the HNAs were found to be stable against enzymatic degradation (Kolb et al., 2005; D’Alconzo et al., 2009). However, antisense effect of these modifications can be attributed only to the steric blocking of target mRNA, as they do not activate RNase H (Hendrix et al., 1997). In the Morpholino oligonucleotides (MFs) the ribose is replaced by a morpholino moiety and phosphorodiimidate linkages are used (Abramova et al., 2009). Similar to the PNAs, these modifications do not activate RNase H and can be used only as steric blockers to inhibit gene expression in biological system. They are stable against nucleases and have similar target affinity to that of the isosequential unmodified ASNs. Because backbone in MFs is uncharged they are unlikely to have unwanted interactions with proteins but on the other hand it affects their cellular uptake (Amantana and Iversen, 2005; Iversen et al., 2009).

**RIBOZYMES**

Ribozymes are RNA enzymes that were first described in Tetrahymena thermophila by Cech et al. (1981). They are catalytic RNAs which cleave covalent bonds in a target RNA. The catalytic site is the result of the conformation adopted by the RNA-RNA complex in the presence of divalent cations. Ribozymes are true catalysts and can carry out RNA slicing by transesterification (splicesome) and peptidyl transfer (in ribosomes) (Doudna and Lorsch, 2005). These molecules, even greater potential advantages than antisense oligodeoxynucleotides, are able to bind specifically and cleave an mRNA substrate. There are advantages of using ribozymes instead of antisense oligodeoxynucleotides. Ribozymes can inactivate the target RNA without relying on the host cell’s machinery and they have the capacity to cleave more than one copy of the target RNA by dissociating from the cleavage products and binding to another target molecule. There are multiple types of ribozymes; the two most commonly used for research and therapeutic purposes are the hammerhead ribozyme and the hairpin ribozyme (Fedor and Westhof, 2002; Bevilacqua and Yajima, 2006). Most of the studies performed to date have described the use of ribozymes as therapeutic agents for viral and cancer diseases (Khan, 2006; Spizzo et al., 2009). However, some dominant genetic disorders may also benefit from this approach. This is the case for some connective tissue disorders such as osteogenesis imperfecta, marfan syndrome and the caramosynostotic syndromes (Wood et al., 2007). A greater understanding of how ribozymes work and the methods to optimize their function will enhance their attractiveness as potential therapeutic agents.

**RNA INTERFERENCE**

Only recently, research in the antisense field increased in impact by the discovery of RNA interference (RNAi) (Downward, 2004). This naturally occurring phenomenon as a potent sequence specific mechanism for post-transcriptional gene silencing was first described for the nematode worm Caenorhabditis elegans (Fire et al., 1998). RNA interference is initiated by long double-stranded RNA molecules (dsRNA) which are processed into 21-23 nucleotides long RNAs called short interfering RNA (siRNA) by the Dicer enzyme (Bernstein et al., 2001; Hammond, 2005). These Small Interfering RNAs (siRNAs) are then incorporated into the RNA Induced Silencing Complex (RISC), a protein RNA complex and guide a nuclease which degrades the target RNA (Chen et al., 2005). So it is thought to provide a significantly higher potency compared to traditional antisense approaches. In principle, RNA interference might be used to treat any disease that is linked to elevated expression of an identified gene. This might make it suitable for combating viral diseases, cancers and inflammatory diseases. Indeed, in tissue culture models, impressive results have been achieved against various cancer cells by using RNA interference to target oncogenes and against HIV, influenza and polio viruses by targeting viral genes (Damm-Welk et al., 2003). siRNAs act against a variety of viruses, HIV, Hepatitis C Virus (HCV), hepatitis B virus, papillomavirus, herpesvirus, rotavirus and influenza virus have been tested in cell cultures and displayed high efficiency in inhibiting viral infection and replication (Jaque et al., 2002; Yokota et al., 2003; Hamasaki et al., 2003; Hall and Alexander, 2003; Jia and Sun, 2003; Dector et al., 2002; Ge et al., 2003; Zamore and Aronin, 2003).

**ADVANTAGES OF ANTISENSE THERAPY OVER TRADITIONAL DRUG THERAPY**

There are several aspects of antisense therapy using oligonucleotides that are potentially advantageous over traditional drug mechanisms (Askari and
Oligonucleotides can be manufactured quickly, some within one week; the sequence of the miRNA is all that is needed.

- The target is often one-dimensional (in contrast to multiple dimensional domains often targeted with proteins); sensitivity can be measured through database scanning for known genes or northern/southern blotting for unknown genes.

- Inhibition of miRNA expression will produce quicker and longer lasting clinical response than inhibition of protein formed by the ribosome, targeted by conventional drug therapy.

- Hydrogen bonding between oligonucleotide and miRNA target exceeds by several orders of magnitude as compared to Van der Waals and other forces needed to bind protein targets.

- ASONs accumulate in specific organs and tissues like liver, spleen, kidneys, bone marrow, fat cells. They can be administered by several routes i.e., oral, rectal, subcutaneous, intravenous, intrathecal, intravitreal, aerosol and topical.

**THERAPEUTIC APPLICATIONS OF ANTISENSE AGENTS**

The potential applications for antisense oligonucleotides are limited only by the genetic information available. Antisense oligonucleotides can be developed against any target in which the inhibition of protein production or the inhibition of RNA processing yields the therapeutic result. Currently, clinical trials are underway using antisense oligonucleotides to treat rheumatoid arthritis, psoriasis, renal transplant rejection and inflammatory bowel disease (Crohn’s disease) (Fig. 5) (Fichou and Ferec, 2006).

However, the primary targets remain refractory viral diseases and cancers for which the necessary target genetic information is typically available. The following are some of the major antisense agents in clinical trials for various diseases (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Company</th>
<th>Target</th>
<th>Phase</th>
<th>Indication</th>
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<td>CMV miRNA</td>
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<td>Rettinitis</td>
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<td>Genzyme With Isis Pharmaceuticals</td>
<td>Apob-100</td>
<td>III</td>
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<td>ICAM-1</td>
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<td>I</td>
<td>Advanced cancer</td>
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<td>IRS-1/Translation HCV</td>
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<td>HCV</td>
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<td>Isis Pharmaceuticals</td>
<td>PKC-β</td>
<td>III</td>
<td>Solid Tumors</td>
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<td>Genasense (Augmensoen, Genta)</td>
<td>(<a href="http://www.genta.com">http://www.genta.com</a>)</td>
<td>Bel-2</td>
<td>III</td>
<td>Malignant melanoma, NHL, CLL, MM, NSCLC</td>
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<td>II</td>
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<td>Preclinical</td>
<td>Colon cancer, breast cancer and brain cancer</td>
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<td>Prostate Cancer</td>
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<td>HSP 27</td>
<td>Preclinical</td>
<td>Prostate Cancer</td>
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<td>OGGX-011 ISIS 112989</td>
<td>Onco-GeneX Technology (<a href="http://www.oncogenx.com">http://www.oncogenx.com</a>)</td>
<td>ChPPP-1</td>
<td>III</td>
<td>Prostate, breast and lung cancers</td>
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<td>Growth Hormone Receptor – I</td>
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<td>AVI BioPharma</td>
<td>C-my c inhibitor</td>
<td>II</td>
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<td>AVI 4126</td>
<td>AVI BioPharma</td>
<td>C-myc mRNA</td>
<td>I</td>
<td>Restenosis, cancer and polycystic kidney disease</td>
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<td>Monarsen (EN101)</td>
<td>Ester Neurosciences (<a href="http://www.esterneuro.com">www.esterneuro.com</a>)</td>
<td>AChE</td>
<td>I</td>
<td>Myasthenia Gravis</td>
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Table 1: Continued

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<th>Indication</th>
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<td>Preclinical</td>
<td>Lung Carcinoma, Prostate Carcinoma</td>
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<td>I/II</td>
<td>Malignant glioma</td>
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Fig. 5: Fields of applications of antisense therapy

ANTISENSE AGENTS IN CANCER THERAPY

Emerging novel strategies of cancer treatment are based on the selective down-regulation of specific molecular targets involved in the process of neoplastic development and progression (Elsayed and Sausville, 2001). As the aim of cancer therapeutics is becoming streamlined to target more specific biological pathways, genetic components and/or cellular proteins, the role of antisense therapy utilizing oligonucleotides is evolving as a potential treatment strategy in the fight against cancer. A number of antisense oligonucleotides against different genes that code for important cellular protein involved in cancer cell signaling, proliferation and survival, including protein kinase A, protein kinase C, c-raf, bel-2 etc., have been tested in phase I/II clinical trials (Cho-Chung, 1999; Tamm et al., 2001; Gewirtz, 2000). Although no antisense agents have been approved for the treatment of cancer, their movement toward potential use in future clinical oncology settings is apparent. The various protein targets and antisense agents in clinical trials in cancer treatment are as follows:

Bel-2: Regulation of cell death (apoptosis) is frequently affected in the development of malignant diseases and all molecular steps from extracellular signalling receptors through intracellular pathways, cell death rheostats and cell death executioners may be involved. Bel-2 is an anti-apoptotic member of a family of anti-and pro-apoptotic proteins that is upregulated in a variety of cancers and specifically overexpressed through chromosomal translocation in some Non-Hodgkin Lymphomas (Gross et al., 1999; Kang and Reynolds, 2009). One of the most promising antisense oligonucleotides in clinical development is an 18-mer Phosphorothioate (PS)-oligonucleotide targeting the human bel-2 mRNA is G3139, Genasense (oblimersen sodium). G3139 has been evaluated in a number of phase II/III studies in different malignancies, including melanoma, Non-Hodgkin’s Lymphoma (NHL) and prostate cancer, in which antitumour activity has been demonstrated (Jansen et al., 2000; Gjertsen et al., 2007; Tarhini and Kirkwood, 2007; Shah et al., 2009). Genasense has demonstrated synergy with several chemotherapeutic agents, radiation therapy and immunotherapy and is often administered a few days prior to standard therapies. Furthermore, Genasense has been evaluated or is being evaluated in combination with the following agents: Gleevac, Rituxan, Paclitaxel, Camptosar, Fludara, Cyclophosphamide, Taxotere, Mylotarg, Dexamethasone and Cytosine arabinoside (Charan-Khan et al., 2004; Benimetskaya et al., 2005; Knox et al., 2008).
c-raf: Raf kinases are serine/threonine kinases that regulate mitotic signaling pathways, most notably the mitogen-activated protein kinase pathway (MAPK) that transmits signals from ras (Skolnik et al., 1993). c-raf has been reported to bind to bel-2 and to be involved in the regulation of apoptosis (Wang et al., 1996). The Raf/MAPK extracellular-signal-regulated kinase pathway has profound effects on proliferative, apoptotic and differentiation pathways as well as the sensitivity and resistance to chemotherapeutic drugs (McCubrey et al., 2009; Kaur et al., 2010). Isis has developed a 20mer Phosphoro-oligonucleotide, ISIS 5132, that targets the 3'-Untranslated Region (UTR) of c-raf-1 (Monia et al., 1996). In vitro studies of ISIS 5132 using A549 lung cells showed inhibition of c-raf-1 mRNA. Results of other phase I clinical trials of ISIS 5132, conducted in patients with a variety of advanced solid tumours showed that the drug is generally well tolerated with only mild side effects which were generally the same as those attributed to phosphorothioate ASON treatment (Rudin et al., 2001). ISIS 5132 can be safely combined with standard doses of carboplatin and paclitaxel in treatment of non small cell lung cancer (Fidias et al., 2009). Another raf-1 ASON, LEraFAON (Neo-Pharm) is a 15-mer antisense oligodeoxynucleotide (ASON) directed to the translation initiation site of c-raf-1 mRNA has also entered clinical trials. LEraFAON is also the first ASON containing liposomal drug tested in humans. The PS modification of this ASON is limited only to the terminal base at the 3' and 5' end. LEraFAON has been encapsulated in a cationic liposome in order to protect the ASONs from degradation and to improve their serum half life. Preclinical studies of this drug have shown inhibition of tumour growth, more than 50% inhibition of raf-1 expression in tumour xenografts and increased sensitization of tumour cells to radiation and to chemotherapeutic agents. Phase I study of LEraFAON has shown that the i.v. delivery of drug is well tolerated along with palliative radiotherapy (Pei et al., 2004; Dritschilo et al., 2006).

Protein Kinase C-α: Protein Kinase C (PKC) is a family of serine/threonine kinases that is involved in the transduction of signals for cell proliferation and differentiation. The important role of PKC in processes relevant to neoplastic transformation, carcinogenesis and tumour cell invasion renders it a potentially suitable target for anticancer therapy (Mackay and Twelves, 2003). Isis/El Lilly have developed a 20mer Phosphoro-oligonucleotide ISIS 3521 (also referred to as LY900003 or Affinitak or Aprinocarsen) that targets the 3'-UTR of PKC-α (Roychowdhury and Lahn, 2003). The phase I trials of ISIS 3521 were carried out successfully in patients with treatment resistant solid tumours. In cell culture studies, Affinitak was shown to inhibit mRNA and protein expression in A549 lung and T24 bladder carcinoma cells with resultant inhibition of proliferation at 100-200 nM concentrations (Cripps et al., 2002; Holmlund, 2003). Phase I/II studies, evaluating the combination therapy of ISIS 3521 and chemotherapeutic agents, were initiated in patients with stage III B or IV Non-small Cell Lung Carcinoma (NSCLC) (Villalona-Calero et al., 2004; Ritch et al., 2006; Luis et al., 2006).

H-ras: Abnormal expression of Ras proteins is frequently found with oncogenic transformation, making ras a promising therapeutic target (Saxena et al., 2008, Kaur et al., 2010). A 20-base phosphorothioate ASON (ISIS 2503) that binds to the translation initiation region of human H-ras mRNA selectively reduced the expression of H-ras mRNA and protein in cell culture (Cunningham et al., 2001). Expression of other family members including N-ras, Ki-rasA and Ki-rasB was not affected by ISIS 2503 in vitro. In a phase I trial, ISIS 2503 caused no dose-limiting toxicity at doses up to 10 mg/kg/d by 14-d continuous iv. infusion every 3 wk. ISIS 2503 in combination with chemotherapy is now in phase II clinical trials for the treatment of metastatic breast cancer, pancreatic cancer and NSCLC (Adjei et al., 2003; Alberts et al., 2004).

DNA Methyltransferase: Hypermethylation by the enzyme DNA methyltransferase has been postulated to inactivate tumor suppressor genes, resulting in neoplastic transformation and tumorigenesis (Reid et al., 2002; Gore, 2009; Issa and Kantarjian, 2009). Agents that prevent or reverse DNA methylation might therefore restore normal growth control to cancer cells (Ibrahim, 2010). MG 98 is a phosphorothioate ASON that is a highly specific inhibitor of translation of the mRNA for human DNA methyltransferase with IC50 values of 50-70 nM in cell lines (Amato, 2007). Tumor growth delay and regression were observed with MG 98 in human lung and colon cancer xenografts. Phase II trials are currently being conducted in patients with head and neck as well as metastatic renal cancer (Davis et al., 2003; Winquist et al., 2006; Plummer et al., 2009).

Clusterin: Clusterin is a glycoprotein with a nearly ubiquitous tissue expression and an apparent involvement in various biological processes. Clusterin acts as a cell-survival protein that is overexpressed in response to tumor-killing strategies, such as chemotherapy, hormone ablation and radiation therapy.
Overexpression of clusterin prolongs cell survival and leads to enhanced metastatic potential of cancer cells in vitro (Wei et al., 2009). OX-011 (OncoGeneX Technologies Inc.) is a second-generation ASON complementary to the translation-initiation site of human Clusterin mRNA. OX-011 incorporates a phosphorothioate backbone with 2-Methoxyethyl (MOE) modifications to the four bases on either end of the 21-mer molecule (Chi et al., 2008a). Such gapmer modifications maintain the improved tissue pharmacokinetic profile of the second generation chemistry but preserve the high affinity for target mRNA and the recruitment of RNase H necessary for activity. Phase II trials of combined OX-011 and chemotherapy are ongoing in patients with prostate, breast and lung cancers (Chi et al., 2008b; Chia et al., 2009).

**Transforming Growth Factor-β2 (TGF-β2):** Overexpression of the cytokine transforming growth factor-beta 2 (TGF-β2) is a hallmark of various malignant tumors including pancreatic carcinoma, malignant glioma, metastasizing melanoma and metastatic colorectal carcinoma. This is due to the pivotal role of TGF-β2 as it regulates key mechanisms of tumor development, namely, immunosuppression, metastasis, angiogenesis and proliferation (Flanders and Burmester, 2003; BonaFoux and Lee, 2009). The most advanced ASONs for the therapy of high-grade gliomas is a phosphorothioate-modified ASON, AP 12009 (tracedeser) which targets mRNA encoding TGF-β2. AP 12009 is administered intratumorally using convection-enhanced delivery. A series of phase I and II clinical trials have evaluated the toxicity profile and optimal dose of the substance (Hau et al., 2007; Hau et al., 2009; Schlingensiepen et al., 2008).

**Survivin:** Survivin is one of the most cancer-specific proteins identified to date, being upregulated in almost all human tumors. Biologically, survivin has been shown to inhibit apoptosis, enhance proliferation and promote angiogenesis. Because of its upregulation in malignancy and its key role in apoptosis, proliferation and angiogenesis, survivin is currently attracting considerable attention as a new target for anti-cancer therapies. In several animal model systems, downregulation of survivin or inactivation of its function has been shown to inhibit tumor growth (Ambrosini et al., 1997; Mita et al., 2008). Survivin is highly expressed in a wide variety of human cancer types, including lung, colon, pancreas, prostate, breast and gastric tumors. Interestingly, survivin is expressed in a cell cycle-dependent manner with highest levels in G2/M and rapid down regulation following cell-cycle arrest. At the beginning of mitosis, survivin associates with the mitotic spindle and disruption of this interaction results in a loss of its antiapoptotic function. However, LY2181308/ISIS 23722 is a second-generation 2-MOE ASON that potently and specifically down regulates survivin expression in a broad range of human cancer cells including lung, colon, pancreas, breast and prostate (Pennati et al., 2007; Ryan et al., 2009).

**XIAP:** The X-linked mammalian inhibitor of apoptosis protein (XIAP) was the first IAP identified and has been shown to bind several partners. XIAP is highly expressed in Acute Myeloid Leukemia (AML), glioblastoma, prostate, pancreatic, gastric and colorectal tumors (Salvesen and Duckett, 2002; Sasaki et al., 2000). AEG35156/GEM640 (Aegera Therapeutics Inc.) is a 19-mer ASON targeted to human XIAP mRNA that incorporates 2′-O-methyl chemistry with a phosphorothioate backbone. In vitro and in vivo preclinical proof-of-concept studies have demonstrated that inhibition of XIAP protein expression by AEG35156/GEM640 enhances the antitumor activity of chemotherapy in several xenograft models (Hu et al., 2003). A phase I dose-escalation tolerability study of AEG35156 as a single agent is currently underway in the United Kingdom as a 7-day continuous i.v., infusion in patients with advanced tumors and phase I trials evaluating shorter infusion schedules in combination with docetaxel or reinduction chemotherapy for AML (Acute myeloid leukemia) (Lacassie et al., 2005; Cummings et al., 2005; Lacassie et al., 2006).

**Thymidylate Synthase (TS):** Thymidylate Synthase (TS) is a key enzyme in the synthesis of DNA and a target for cancer chemotherapeutic agents (Rose et al., 2002; Bertino, 2009). Oligodeoxynucleotides (ODNs) target different regions of TS mRNA, inhibit human tumor cell proliferation as single agents and enhance cytotoxicity of clinically useful TS protein-targeting drugs. ODN 491, a novel 20mer AS ODN complementary to a previously untargeted portion of the TS mRNA coding region. AS ODN 491 decreased TS mRNA levels to different degrees in a panel of human tumor-derived cell lines and induced different physiological effects in a tumor cell line-dependent manner. ODN 491 (like ODN 83, previously shown to be effective) decreased TS protein levels in HeLa cells with a concomitant increase in sensitivity to TS-targeting chemotherapeutics (Flynn et al., 2006; Jason et al., 2008).

**Ribonucleotide reductase (RNR):** Ribonucleotide reductase (RNR) is an important enzyme for cell division and tumor growth that is required for the reductive conversion of ribonucleotides into deoxyribonucleotides which is a
crucial step in the synthesis and repair of DNA (Fan et al., 1998). Mammalian RNR has a dimeric structure composed of two dissimilar subunits, R1 and R2, encoded on different chromosomes and each inactive on its own. Both subunits consist of a nucleotide binding site (M1) and a metal binding site (M2). M1-affected RNR inhibitors are nucleoside analogs, for example, gemcitabine. M2 contains nonheme iron and a tyrosine-free radical which are required for the enzymatic reduction of ribonucleotides. Inhibitors of M2 act by destroying the free radical. Hydroxyurea is a clinically approved RNR inhibitor acting at the iron free radical site but the inhibition is reversible due to the ease in regenerating the tyrosine-free radical by mammalian cells (Cerqueira et al., 2007). The R1 subunit protein levels are constant during cell cycle, however, the expression of the R2 subunit increases in late G1/early S phase of the cell cycle when DNA replication occurs. The R2 subunit was also shown to be overexpressed in tumor tissues and appears to influence transformation and malignant potential of some oncogenes. GTI-2501 and GTI-2040 (Lorus Therapeutics Inc.) are first-generation phosphorothioate antisense molecules that target and inhibit expression of the R1 and R2 subunit of RNR, respectively (Desai et al., 2005). A phase I trial of GTI-2040 has been reported and dose-limiting toxicity of hepatic enzyme elevation was observed (Yoon et al., 2006). The recommended phase II dose was determined to be 185 mg m²d⁻¹ given as a 21-day continuous i.v., infusion. Phase I trials of GTI-2501 and GTI-2040 in combination with docetaxel have been completed and phase II trials of these combination regimens are underway (Leighl et al., 2009).

**AGENTS FOR VIRAL INFECTIONS**

Oligonucleotides hold considerable promise for treating viral infections. Although much recent attention has focused on small interfering RNAs, the majority of oligonucleotides that have been studied as antiviral agents to date are modified Oligodeoxynucleotides (ODNs) designed to work via an antisense mechanism.

**Cytomegalovirus:** Cytomegalovirus (CMV) belongs to the Herpes-viridae family of viruses which are DNA viruses that exhibit the biological properties of latency and reactivation. In the developed countries, up to 80% of individuals develop sub-clinical CMV infections (Mocarski, 1988). CMV retinitis is one of the most common opportunistic infections in patients with Acquired Immunodeficiency Syndrome (AIDS). AIDS patients infected with CMV retinitis can develop either intolerance or resistance to commonly used anti-CMV treatment regimens, necessitating the development of alternative treatment options (Hoffman and Skiest, 2000). Much of the research on inhibition of CMV replication by ASONs has mainly focused on inhibition of CMV Immediate-early (IE) gene products. ISIS 2922 (Fomiviren also called Vitraveryne, ISIS Pharmaceuticals), a PS-ASON with a 21 nucleotide sequence complementary to RNA of IE2 showed at least 30 fold more potent antiviral activity as compared to nucleoside analog ganciclovir. The results showed that ISIS 2922 inhibits viral production in a specific and dose dependent manner (Azad et al., 1993). Till date, only one compound, fomiviren, the first gene-based therapeutic agent has received FDA approval. The approval for fomiviren was important to antisense technology as a whole because it demonstrated that antisense drugs can be used effectively in the treatment of a local disease. The results of phase I, II and III trials showed intravitreal injections of ISIS 2922 to be safe and effective. The drug halted the progression of both acute and chronic CMV retinitis in AIDS patients. Moreover, the local treatment with the drug reduced the incidence of systemic side effects. Most commonly reported side effects were increase in intraocular pressure and mild to moderate intraocular inflammation, both of which were transient or treatable with topical steroid treatment (Schreiber et al., 2009, Andrei et al., 2009). Following the successful clinical trials, in 1998, ISIS 2922 was approved by FDA, for the treatment of CMV-induced retinitis in patients with AIDS and became the first ASON drug to be marketed commercially (Haasnoot and Berkhout, 2009). A second-generation ASON with the identical sequence as fomiviren, ISIS 13312, is a 2'-MOE ASON that has been shown to have antiviral activity comparable to fomiviren in fibroblasts and retinal pigment epithelial cells (Mansoor and Melendez, 2008; Detrick et al., 2001). Both fomiviren and ISIS 13312 demonstrated comparable and consistent antiviral activity with the IC₅₀ between 0.1 and 1.0 μM (Henry et al., 2001). Other viruses that manifest as an ocular condition in the anterior segment include Herpes Simplex (HSV), Varicella-Zoster (VZV), Epstein-Barr (EBV) and adenovirus. Among these, HSV-1 is commonly associated with ocular infections and is the leading cause of corneal blindness. Targeting HSV-1 is yet another opportunity for ASONs as therapeutic agents that have not been fully developed for ophthalmology.

**Hepatitis C virus:** Hepatitis C infection is an inflammation of the liver caused by the hepatitis C virus. According to the World Health Organization, approximately 170 million people worldwide are chronically infected with HCV (Kamal, 2008). It is the most
common chronic blood-borne infection in the developed world and the leading cause of liver transplants in the United States. However, currently available anti-HCV compounds are not broadly effective, especially against the chronic HCV infection. Therefore, continuous efforts are being made to develop newer and better therapeutic strategies (Tan et al., 2002; O’Leary and Davis, 2010). The cytosolic viral single stranded RNA (ssRNA) is a vulnerable potential target for therapeutic antisense oligonucleotides. The Internal Ribosomal Entry Site (IRES) at the 5' end of the viral RNA is that highly conserved target. IRES are the landing pad that directs the positive strand HCV-RNA to the Endoplasmic Reticulum (ER) for protein translation. Thus, inhibition of attachment of IRES to both cellular and viral proteins by oligonucleotides could effectively inhibit HCV replication. ISIS 14803 is a 20-unit 5'-methylecytidine antisense phosphorothioate oligodeoxynucleotide that binds to (HCV) RNA at the translation initiation region of the internal ribosome entry site (IRES) and inhibits protein expression in cell culture and mouse models (McHutchison et al., 2006). This Phase II, open-label, dose-escalation trial of ISIS 14803 was performed in chronic HCV patients (Gordon et al., 2002).

Human Immunodeficiency Virus (HIV): HIV is a single stranded RNA virus that uses Reverse Transcriptase (RT) to create a DNA copy of its RNA genome. The viral DNA then gets integrated into the DNA of the host and is subsequently transcribed and translated by the host cell. HIV infection is spreading worldwide at an alarming rate, representing the difficulties in controlling viral replication. Many regions of the HIV genome have been targeted with ASOs including the rev, tat, gag, pol and env genes, 5' untranslated region and psi sequences. Various ASOs have been shown to be effective in acute and chronic viral infections (Suzuki et al., 2002). GEM 91 (Hybriden), a 25-mer PS-ASO that binds to the translation initiation site of the HIV gag mRNA, was shown to effectively reduce HIV replication in vitro (Yamaguchi et al., 1997). Although the initial reports of GEM 91 clinical trials showed it to be well tolerated (Sereni et al., 1999), its use was later discontinued because of dose-limiting thrombocytopenia and elevated serum transaminase levels. GEM 92 is an orally administered second generation ASO synthesized on a truncated GEM 91 sequence (Zheng, 1999). GEM 92 is currently in phase I clinical trials, with preclinical studies showing an improved stability and safety profile (Pandey et al., 2009).

AGENTS USED IN ASTHMA

Asthma is now one of the world's most common long-term conditions, affecting as many as 300 million people worldwide. This number could increase by a further 150 million by 2025, according to the World Health Organisation (WHO). Moreover, current therapies fail to restore the immunological imbalance, frequently do not produce an optimal control of asthma symptoms and sometimes are associated with adverse effects (Humbert et al., 2007). Despite significant advances that have been made in recent years, there is still an urgent need for novel, more effective and safer asthma drugs. An important objective in molecular pharmacology is the manipulation of gene expression with new drug molecules. RNA-based gene silencing strategies have been proposed not only as research tools but also as potential therapeutic interventions in allergic asthma (Mahato et al., 2005; Popescu, 2005; Pan and Clawson, 2006). In the treatment of allergic asthma, ASO can be used for silencing of gene expression, at post-transcriptional level, for many molecular targets i.e., cell membrane receptors (G-protein coupled receptors, cytokine and chemokine receptors), membrane proteins, ion channels, cytokines and related factors signaling non-receptor protein kinases (tyrosine kinases, such as Syk and serine/threonine kinases, such as p38 MAP kinase) and regulators of transcription belonging to Cys4 zinc finger of nuclear receptor type (GATA-3) or beta-scaffold factors with minor groove contacts (p65, STAT-6) classes/superclasses of transcription factors (Popescu and Popescu, 2007; Adcock et al., 2008). Respiratory diseases, including asthma, are well suited for inhaled therapies and present an attractive opportunity for topical antisense strategies. Some important targets for antisense agents in asthma are given below.

Adenosine A1 receptor: The adenosine A1 receptor is involved in inflammation, bronchoconstriction and surfactant depletion observed in asthma and certain other respiratory diseases (Spieuzza et al., 2006). Respirable ASOs (RASOs), as compared to systemically administered ASOs, offer the potential to selectively downregulate the AR-1 expression in the lung at much smaller doses and also minimize the risk of systemic side effects and toxicity. EPI 2010 is a first-generation RASO designed to target the initiation codon of the human A1 receptor mRNA is a 21-mer phosphorothioate shown to inhibit each of these aspects of asthma and to be effective in multiple models of human asthma including the primate (Zhang, 2002). Results of 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 (Ball et al., 2004). Further clinical trials are ongoing to determine its clinical efficacy.

Mitogen-activated Protein Kinases (MAPK): Among the various MAPK families p38 is mostly implicated in cytokine biosynthesis as well as in recruitment of inflammatory cells (Pelaia et al., 2005). p38 MAPK is a
non-receptor proline-directed serine/threonine kinase with a pivotal role in the activation of inflammatory cells (Schiindler et al., 2007). In particular, p38 MAPK significantly contributes to neutrophil recruitment by up-regulating the vascular expression of intercellular adhesion molecule-1 (ICAM-1) and the release of TNF-α into airspaces. p38 MAPK inhibitors appear to have a preferential inhibitory effect on synthesis of Th2 compared to Th1 cytokines, indicating their potential application in the treatment of atopic diseases. p38 alpha MAPK ASON (ISIS 101757) is a 2’ MOB-ASON delivered as aerosol for inhalation or nose-only exposure, in Ovalbumin (OVA) sensitized mice with asthma. It inhibited p38 alpha mRNA and protein expression in Bronchoalveolar Lavage (BAL) fluid cells and peribronchial lymph node cells, reduced mucus hypersecretion, suppressed Th2 cytokine production (IL-4, IL-5 and IL-13 levels in BAL fluid) and inhibited airway eosinophilia Airway Hyperresponsiveness (AHR) (Duan et al., 2005). Safety issues remain a concern for long-term use, although delivery to the airways by aerosol may reduce side-effects. Interestingly, the corticosteroid insensitivity seen in peripheral blood cells and macrophages obtained from BAL fluid in patients with severe asthma can be overcome by the combination of a p38 MAPK inhibitor and dexamethasone (Adcock et al., 2008).

Interleukin receptor and C-C chemokine receptor type 3: The IL-4 receptor alpha chain plays special roles in allergy, being a common subunit of the IL-4 and IL-13 receptor complex. Signaling through it by IL-4 is important in Th2 differentiation and the blocking of its production inhibits the activity of IL-4 and IL-13, regulating allergic inflammation, mucus overproduction and airway hyperresponsiveness (AHR) (Isidoro-Garcia et al., 2005). The IL-4 receptor alpha chain was studied as a target for new inhaled second ASON. The drug, ISIS 369645, is a second-generation antiinflammatory alpha inhibitor of the alpha subunit of the interleukin 4 receptor (IL4R-alpha). Inhibiting the production of the IL4R-alpha inhibits the activity of two important cytokines in asthma, IL-4 and IL-13 that regulate inflammation, mucus overproduction and airway hyperresponsiveness (Oh et al., 2010). ISIS 369645 is currently being evaluated in phase 1 studies. Topigen Pharmaceuticals (Montreal, Quebec, Canada) recently demonstrated the safety, tolerability and pharmacological activity of TPI-ASMS, a first generation antisense drug that combines two oligonucleotides into a single inhaled product, in patients with mild allergic asthma. TPI-ASMS is a combination of two ASONs targets two distinct cellular pathways involved in allergic airway inflammation by inhibiting the recruitment of allergic inflammatory cells, via an effect on the cysteine-cysteine CCR3 receptor and by reducing the persistence of allergic inflammatory cells via interference with the common beta sub-unit for the receptors of interleukin 3, interleukin 5 and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) (Gauvreau et al., 2008). This pioneering multi-targeted approach of blocking the synthesis of specific receptors with RNA-silencing technology is expected to have advantages over current therapeutics by providing broader but specific, pharmacological activity. TPI ASM8 is delivered in a convenient, cost-effective inhaled formulation and systemic exposure is minimal resulting in a favorable safety profile in clinical trials to date. Now TPI ASM8 is in phase II clinical development for the management of moderate to severe asthma (Séguin and Ferrari, 2009).

AGENTS USED IN OTHER INFLAMMATORY DISEASES

Antisense oligonucleotides have been explored for their therapeutic benefits in various inflammatory diseases e.g., Rheumatoid arthritis, Crohn’s Disease (CD), Ulcerative Colitis (UC), Psoriasis etc. Some of the important targets of ASON therapy for these disease models are discussed below.

Tumor necrosis factor α: Tumor necrosis factor α (TNF-α) also known as cachectin is an important cytokine that plays a role in host defense. The cytokine is produced primarily in macrophages and monocytes in response to infection, invasion, injury or inflammation. TNF-α interacts with two different receptors, TNF receptor I (TNFR1) and TNF receptor II (TNFRII), in order to transduce its effects, the net result of which is altered gene expression. Cellular factors induced by TNF-α include interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interferon-γ (IFN-γ), Platelet Derived Growth Factor (PDGF) and Epidermal Growth Factor (EGF) and endothelial cell adhesion molecules including endothelial leukocyte adhesion molecule 1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Wajant et al., 2003). Despite the protective effects of the cytokine, overexpression of TNF-α often results in disease states, particularly in infectious, inflammatory and autoimmune diseases. This process may involve the apoptotic pathways (O’Shea et al., 2002). High levels of plasma TNF-α have been found in infectious diseases such as sepsis syndrome, bacterial meningitis, cerebral malaria,
AIDS and autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease (including Crohn's disease), sarcoidosis, multiple sclerosis, Kawasaki syndrome, graft-versus-host disease and transplant (allograft) rejection; and organ failure conditions such as adult respiratory distress syndrome, congestive heart failure, acute liver failure and myocardial infarction (Lin et al., 2008). ISIS 104838 (ISIS Pharmaceuticals), a 20 nucleotide gapmer targeting the mRNA of human TNF-α is the first ASON belonging to the second generation to be tried in clinical trials (Kennawell, 2003). The preclinical studies with ISIS 104838 showed it to be effective for the treatment of collagen induced arthritis with activity comparable to that by anti-TNF-α antibody and that it successfully knocks down TNF-α expression by 85% in stimulated keratinocytes. In phase I of clinical trials, ISIS 104838 was administered either by i.v., or s.c routes (Sewell et al., 2002). ISIS 104838 is currently in phase II of clinical trials for the treatment of Rheumatoid Arthritis (RA) and Crohn Disease (CD) (De Boer et al., 2007).

**Intercellular adhesion molecule-1 (ICAM-1):** Intercellular Adhesion Molecules (ICAMs) are structurally related members of the immunoglobulin supergene family and are ligands for the beta2 integrin molecules present on leukocytes. Of the five ICAMs identified, ICAM-1 is the most extensively studied. Although ICAM-1 is expressed constitutively at low levels on endothelial cells and on some lymphocytes and monocytes, its expression can be significantly increased in the presence of cytokines (TNF-α, IL-1, IFN-γ) and reactive oxygen species. Depending upon cell type, ICAM-1 participates in trafficking of inflammatory cells, in cell-cell interactions during antigen presentation, in microbial pathogenesis and in signal transduction through signaling events. Again, depending upon cell type examined, ICAM-1 engagement has been documented to activate specific kinases through phosphorylation, resulting in transcription factor activation and increased cytokine production, increased cell membrane protein expression, reactive oxygen species production and cell proliferation (Hubbard and Rothlein, 2000; Philpott and Minner, 2008). ISIS 2302 (Alicaforsen) is a 20 base phosphorothioate oligonucleotide targeting intercellular adhesion molecule-1 (ICAM-1), hybridizes to a sequence in the 3' untranslated region of human ICAM-1 mRNA. Alicaforsen offers the potential for broad effectiveness in the treatment of immune-mediated and inflammatory diseases (Barish, 2005; Baumgart and Sandborn, 2007).

**Insulin-like growth factor type 1 receptor (IGF-IR):** The Insulin-like Growth Factor 1 (IGF-1) Receptor is a transmembrane receptor that is activated by IGF-1 and by the related growth factor IGF-2. It belongs to the large class of tyrosine kinase receptors. This receptor mediates the effects of IGF-1 which is a polypeptide protein hormone similar in molecular structure to insulin (LeRoith et al., 1995; Rodon et al., 2008). The type 1 Insulin-like Growth Factor Receptor (IGFIR) regulates multiple aspects of malignancy and is the target of several drugs currently in clinical trials (Saxena et al., 2007). Whereas topical application and subsequent penetration of large oligonucleotides into normal skin is problematic, the impaired barrier function of psoriatic lesions permits the uptake of antisense drugs. ATL1101 is a second-generation antisense drug designed to block the synthesis of the IGF-1 receptor, a protein involved in the regulation of cell overgrowth in psoriasis. ATL 1101 is based on Isis Pharmaceuticals' proprietary second-generation antisense chemistry called 2'-O-methoxyethyl and is being developed as a cream for the topical treatment of mild to moderate cases of psoriasis (Sachdev et al., 2010; Ma et al., 2009; White et al., 2004).

**AGENTS USED IN CARDIOVASCULAR DISEASE**

**Hypercholesterolemia:** Apolipoprotein B is an important structural protein on the surface of atherogenic lipoproteins such as remnant very-low-density lipoprotein and low-density lipoprotein and facilitates the clearance of these particles from the circulation by binding to the low-density lipoprotein receptor. Overproduction of apolipoprotein B or reduced receptor-mediated clearance of lipoproteins leads to elevated serum cholesterol levels and premature atherosclerosis (Krause, 2004; Chapman and Caslake, 2004). Mipomersen (ISIS301012) is the nonadecasodium salt of a 20-base phosphorothioate oligonucleotide, a second-generation antisense drug developed by Isis Pharmaceuticals that inhibits apolipoprotein B production by binding directly to and reducing the expression of apolipoprotein B messenger RNA (Kastelein et al., 2006; Fatima et al., 2007; Matthew, 2007). In a clinical trial, ISIS 301012 50-400 mg administered weekly via subcutaneous injection for 4 weeks reduced apolipoprotein B by 14.3-47.4% and low-density lipoprotein cholesterol by 5.9-40% at 55 days. The most frequent adverse event was injection-site erythema that resolved spontaneously. Studies are ongoing to further define the safety, efficacy and pharmacokinetics of ISIS 301012 as add-on therapy in patients with heterozygous and homozygous familial hypercholesterolemia (Kastelein et al., 2007; Athyros et al., 2008). No pharmacokinetic interactions have been demonstrated with ezetimibe and simvastatin for the treatment of hypercholesterolemia based on its ability to inhibit apolipoprotein B-100 (apoB-100)
(Yu et al., 2009). Early results from phase II clinical trials of ISIS-301012 in patients with high cholesterol demonstrated that it produced rapid, dose-dependent and prolonged reductions in apoB-100, LDL cholesterol, Very-Low-density Lipoprotein (VLDL) cholesterol, total cholesterol and triglyceride levels and was safe and well tolerated. Phase III clinical trials of ISIS-301012 in patients with hypercholesterolemia are ongoing. Mipomersen has been assessed in several phase 2 trials in a variety of patient phenotypes initially as monotherapy, followed by combinations with lower-dose statins and then high-dose statins and other lipid-lowering agents (Yoshitaka et al., 2008).

**Restenosis:** Restenosis literally means the recurrence of stenosis, a narrowing of a blood vessel, leading to restricted blood flow. Restenosis usually pertains to an artery or other large blood vessel that has become narrowed, received treatment to clear the blockage and subsequently become renarrowed (Dangas and Kuepper, 2002). Inhibition of c-myc would also interfere with expression of downstream genes such as those associated with cellular adhesion, the cell cycle and connective tissue matrix remodeling (De Marisa et al., 2006). Specific to the development of obstructive vascular disease, c-Myc is quickly induced in Vascular Smooth Muscle Cell (VSMC) after arterial injury and activated by proliferative signals, including a number of mediators of vascular Endothelial Cell (EC) biology, such as LDL, thrombin, endothelin and angiotensin II (Liang et al., 2007). Inhibition of c-Myc has been shown to inhibit smooth muscle cell proliferation in vitro and in several animal models. Recently introduced, AVI-4126 belongs to a family of molecules known as the phosphorodiamidate morpholino oligomers (PMOs) with sequence complementary to the translation initiation start site of the c-myc mRNA. The mechanism of action of AVI-4126 involves interference with ribosomal assembly, thus preventing translation of c-myc and interference with intron 1-exon 2 splicing of the c-myc pre-mRNA, preventing appropriate translation of the c-myc mRNA (Hudziak et al., 2000). The IC50 for inhibition of c-myc by AVI-4126 is 0.3 μM in cell culture. The cellular response to AVI-4126 is diminished cell growth associated with arrest of cells in the G0/G1 phase of the cell cycle (Stephens, 2004; Kipshidze et al., 2005). c-Myc amplification and overexpression has also been correlated with progression and chemotherapy resistance in lung cancer. AVI-4126, a neutral antisense Phosphorodiamidate Morpholino Oligomer (PMO) has been identified to specifically inhibit c-MYC expression in multiple disease models and identified in phase I clinical studies to be safe and bioavailable in solid tumors (Sekhon et al., 2008).

**AGENTS USED IN NEUROLOGICAL DISEASE**

**Multiple sclerosis:** Multiple Sclerosis (MS), also known as disseminated sclerosis or encephalomyelitis disseminata is an autoimmune condition in which the immune system attacks the Central Nervous System (CNS), leading to demyelination. It may cause numerous physical and mental symptoms and often progresses to physical and cognitive disability (Sospendra and Martin, 2005; Taragh and Ilahi, 2010). Antagonism of T-cell trafficking has been investigated as a therapeutic approach to MS using antibody and antisense technologies (Sorbera et al., 2005). Clinical evidence of the central role of very late antigen-4 (VLA-4) (α4β1 integrin) in lymphocyte transmigration into the CNS has recently been confirmed based on the efficacy of the humanized mAb to α4 integrin, natalizumab (Antegren, Tysabri, Elan Pharmaceuticals and Biogen/Idec) in patients with relapsing MS. Long-term administration of natalizumab provided significant benefits in clinical trials, including a marked reduction in the risk of new lesions and a significant reduction in the risk of exacerbations within 2 months of the initiation of therapy (Comi, 2009). In early 2005, Biogen Idec and Elan Corporation voluntarily suspended marketing of Tysabri in the United States based on two reported cases of Progressive Multifocal Leuкоencephalopathy (PML) (Aksamit, 2006; Linda et al., 2009). VLA-4 is the α4β1 integrin localized on many inflammatory cells that participates in cell adhesion, trafficking and activation, through binding to VCAM-1 and fibronectin. A second-generation antisense inhibitor of VLA-4 (ATL1102, Antisense Therapeutics Limited) is currently being investigated as a subcutaneous therapy for relapsing-remitting MS. The pharmacokinetics and safety of ATL1102 was investigated in a double blind, placebo-controlled study in healthy subjects. This product is directed to inhibiting the expression of CD49d (an alpha integrin), a subunit of the VLA-4 integrin complex which is considered to be responsible for the progression of MS (Lothhouse et al., 2005; Tilley, 2008).

**Myasthenia gravis:** Myasthenia Gravis (MG) is a chronic and debilitating disease which affects about 100,000 people worldwide, characterized by muscle weakness especially inability to open one’s eyes, hand and leg muscle problems. The body’s immune system attacks acetylcholine receptors at the neuromuscular junction (NMJ), interfering with normal muscular function (Conti-Fine et al., 2006). In severe cases the disease can involve the respiratory muscles, causing potentially life-threatening respiratory failure. The current management of MG includes the use of anticholinesterase drugs for temporary improvement of neuromuscular
transmission, removal of Anti-acetylcholine Receptor (AChR) Antibodies (Abs) by plasma exchange or specific immunoadsorption procedures, use of nonspecific immunosuppressants or immunomodulators to curb the anti-AChR response and thymectomy (Keesey, 2004; Rasool et al., 2008). A novel approach to long-term therapeutic inhibition of AChE activity in MG patients is based on the observation that in the NMJ of both MG patients and Experimental Autoimmune MG (EAMG) animals, there is enhanced transcription and altered splicing of AChE pre-mRNA, with accumulation of a normally rare readthrough AChE-R variant (Brenner et al., 2003). The commonly occurring synaptic AChE-S variant forms membrane multimers. In contrast, AChE-R exists as soluble monomers that lack the carboxyterminal cysteine needed for membrane attachment. Thus, AChE-R permeates the synaptic space and degrades ACh before it reaches the postsynaptic membrane, thereby compromising AChR activation. These observations prompted the design and use of EN101 (Monarsen), synthetic 20-base antisense oligonucleotide that suppresses the expression of AChE-R. EN101 normalizes neuromuscular transmission in Experimental Autoimmune MG (EAMG) by modulating the synthesis of AChE variants, thereby affecting the rate of ACh hydrolysis and the efficacy of AChR activation (Argov et al., 2007; Sussman et al., 2008). The U.S. Food and Drug Administration have granted Orphan Drug Designation status for Monarsen. EN101 is undergoing human trials. It is modified to achieve stability for oral administration. Monarsen is now being investigated in a phase II study (Katzberg and Bril, 2009).

**DIABETES MELLITUS**

Diabetes mellitus defines a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. It is one of the most common metabolic syndromes, since there are 200 million diabetic individuals in the world; this creates a need to understand the etiology of the disease and the factors influencing its onset (Malecki and Klupa, 2005; Al-Zubairi and Eid 2010). Many researchers and companies are engaged in enhancing bioavailability of insulin in natural physiological way (Singh et al., 2011). The molecular biology approach based on Protein Tyrosine Phosphatase (PTP)-1B that antagonizes insulin signaling is a potential therapeutic target for insulin resistance associated with obesity and type 2 diabetes. To date, studies of PTP-1B have been limited by the availability of specific antagonists; however, treatment of rodents with Antisense Oligonucleotides (ASOs) directed against PTP-1B improves insulin sensitivity, inhibits lipogenic gene expression and reduces triglyceride accumulation in liver and adipose tissue. ISIS 113715 is a 20-mer phosphorothioate Antisense Oligonucleotide (ASO) that is complementary to the Protein Tyrosine Phosphatase 1B (PTP-1B) messenger RNA and subsequently reduces translation of the PTP-1B protein, a negative regulator of insulin receptor (Montalibet and Kennedy, 2005; Geary et al., 2006). ISIS 113715 is currently being studied in early phase II clinical studies to determine its ability to improve or restore insulin receptor sensitivity in patients with type 2 diabetes mellitus. Future work will investigate the combination of ISIS 113715 with antidiabetic compounds (Swarbrick et al., 2009).

**CONCLUSION**

Drugs based on antisense are potentially valuable options for new drug treatments. Antisense-based compounds may offer advantages for carefully selected targets and conditions, such as those of multiple sclerosis, psoriasis, lung cancer, colorectal carcinoma, pancreatic carcinoma, malignant glioma and malignant melanoma, diabetes and diseases such as asthma and arthritis with an inflammatory component. The ability to use antisense compounds to inhibit the expression of proteins already known to be drug targets is a valuable strategy and should increase the probability of developing clinically useful compounds. The rapid development of antisense technology offers almost unlimited scope for the development of new and highly specific therapeutics.

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