Journal of Medical Genetics and Genomics Vol. 3(5), pp. 77 - 83, May 2011
Available online http://www.academicjournals.org/jmgg
ISSN 2141-2278 ©2011 Academic Journals

Review

Antisense therapy, a magic bullet for the treatment of various diseases: Present and future prospects

Jyotsna A. Saonere

Department of Pharmacy, Government Polytechnic, Amravati 444603, M. S. India. E-mail: saonereja@gmail.com.

Accepted 9 February, 2011

Antisense oligonucleotides (ASO) are becoming more widely accepted as potential therapeutics for various diseases. Antisense therapy has emerged as an exciting and promising strategy for the treatment of various diseases. Antisense are different from conventional drugs that are designed to interact with protein molecule. Antisense drugs have more selective action and they have the potential to be more effective and less toxic than conventional drugs. Recent clinical trials confirm the ability of antisense to significantly suppress target-gene expression. Antisense oligonucleotides are usually highly selective and thus produce fewer adverse effects than conventional therapeutics. Antisense technology might be a gateway to the treatment of diseases by targeting the expression of genes rather than permanently altering them. Antisense oligonucleotides can alter target gene expression by binding to RNA. Once bound, the ASO either disables or induces the degradation of the target RNA. This technology may be used to treat various conditions including cancer, diabetes, and hypertension, as well as autoimmune and cardiovascular diseases. ASOs are potentially potent, selective and well-tolerated drugs. The current status and future direction of several antisense drugs that have potential clinical use in treatment of various diseases are reviewed here.

Key words: Antisense, oligonucleotide, gene, autoimmune disease, degradation.

INTRODUCTION

Antisense drugs are a new generation of gene-silencing therapeutic agents with potential for targeted causal treatment of as yet incurable diseases. Antisense therapy is a form of treatment for genetic disorders or infections. When the genetic sequence of a particular gene is known to be causative of a particular disease, it is possible to synthesize a strand of nucleic acid (DNA, RNA or a chemical analogue) that will bind to the messenger RNA (mRNA) produced by that gene and inactivate it, effectively turning that gene "off". This is because mRNA has to be single stranded for it to be translated. Alternatively, the strand might be targeted to bind a splicing site on pre-mRNA and modify the exon content of an mRNA

This synthesized nucleic acid is termed an "anti-sense" oligonucleotide because its base sequence is complementary to the gene's messenger RNA (mRNA), which is called the "sense" sequence (so that a sense segment of mRNA " 5'-AAGGUC-3' " would be blocked by the anti-sense mRNA segment " 3'-UUCCAG-5' ").

Antisense drugs are being researched to treat cancers including lung cancer, colorectal carcinoma, pancreatic carcinoma, malignant glioma and malignant melanoma, diabetes, Duchenne muscular dystrophy and diseases such as asthma and arthritis with an inflammatory component (Agrawal et al., 1995; 2000). Some of the examples of antisense drug like fomivirsen (marketed as Vitravene), has been approved by the US Food and Drug Administration (FDA) as a treatment for cytomegalovirus retinitis. Mipomersen (ISIS 301012) inhibits human apolipoprotein (apo) B-100 synthesis and lowers circulating apoB and low-density lipoprotein cholesterol levels.

ANTISENSE TECHNOLOGY

Antisense refers to short DNA or RNA sequences, termed oligonucleotides, which are designed to be complementary to a specific gene sequence. The goal is

Abbreviations: ASO, Antisense oligonucleotides; **CFTR,** cystic fibrosis transmembrane conductance regulator; **ODN,** antisense oligodesoxynucleoties.

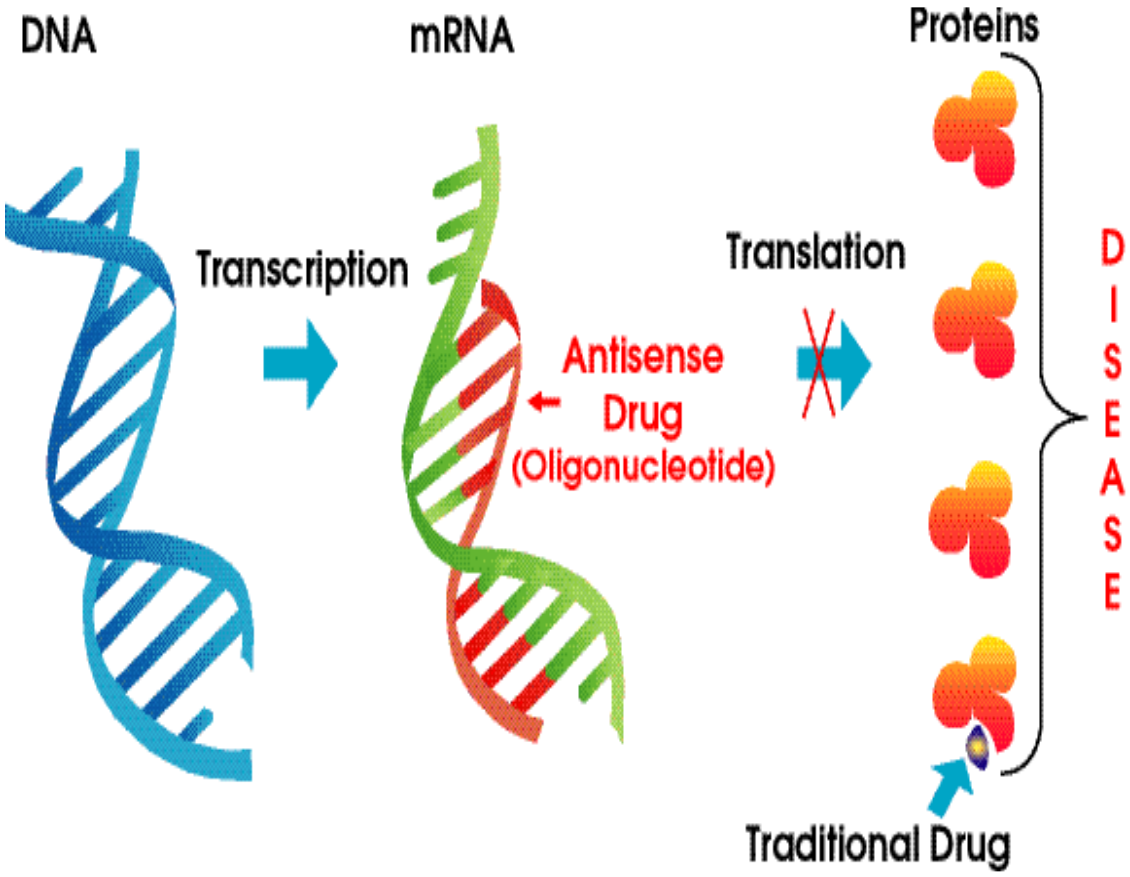

Figure 1. Antisense technology.

to alter specific gene expression resulting from the binding of the antisense oligonucleotide to a unique gene sequence (Crooke et al., 1992, 1995, 1996, 2000) (Figure 1).

Antisense technology was first effectively used in plants to alter the levels of various degradative enzymes or plant pigments. The technology was rapidly applied to mammalian cells and in 1992 Science named antisense its runner-up in the molecule of the year award.

In principle, antisense technology is supposed to prevent protein production from a targeted gene. The exact mechanism by which this occurs remains uncertain. Proposed mechanisms include triplex formation, blocking RNA splicing, preventing transport of the mRNA antisense complex into the cytoplasm, increasing RNA degradation, or blocking the initiation of translation.

Potential advantages

There are several aspects of antisense therapy utilizing oligonucleotides that are potentially advantageous over traditional drug mechanisms.

1. Oligonucleotides may be manufactured quickly, some within one week, and the sequence of a gene is all that is needed.
2. Potential sensitivity to therapy may be easily measured, as the target is often one-dimensional versus multiple-dimensional domains often targeted within proteins. Sensitivity can be measured through database scanning or Northern/Southern blotting for unknown genes.
3. Potential to produce longer lasting responses, as clonal expansion may require more time to produce clinical disease once mRNA is inhibited, versus just inhibition of protein typical with conventional therapies.
4. Potential for enhanced binding affinity to target, as hydrogen bonding between oligonucleotide and target appears to exceed, by several orders of magnitude, Van der Waals and other forces used by standard agents to bind to protein targets.

How antisense works

Antisense compounds are designed to have the right nucleotide sequence to bind specifically to and interfere with its associated mRNA, the instructions for the production of a particular protein (Leonetti et al., 1993).

To create antisense drugs, special chemically stabilized nucleotides are synthetically linked together in short chains of about 12 to 30 nucleotides (called oligonucleotides). Each antisense drug is designed with the right complementary genetic code to bind to a specific sequence of nucleotides in its mRNA target to form a short area of double strands.

ANTISENSE USED IN TREATMENT OF VARIOUS DISEASES

Hemorrhagic fever viruses

In early 2006, scientists studying the Ebola hemorrhagic fever virus at USAMRIID announced a 75% recovery rate after infecting four rhesus monkeys and then treating them with an antisense Morpholino drug developed by AVI BioPharma, a U. S. biotechnology firm. The usual mortality rate for monkeys infected with Ebola virus is 100%. In late 2008, AVI BioPharma successfully filed Investigational New Drug (IND) applications with the FDA for its two lead products for Marburg and Ebola viruses. These drugs, AVI-6002 AVI-6003 are novel analogs based on AVI's PMO antisense chemistry in which anti-viral potency is enhanced by the addition of positively-charged components to the morpholino oligomer chain. Preclinical results of AVI-6002 and AVI-6003 demonstrated reproducible and high rates of survival in non-human primates challenged with a lethal infection of the Ebola and Marburg viruses, respectively.

Cancer

Several pre-mRNAs derived from cancer-associated genes can produce splice variants with antagonistic effects. The ratio of the splice variants changes in a number of cancers and cancer cell lines with the splice variant which stops cell death predominating and promoting cancer cell proliferation (Neckers et al., 1992). Being able to reverse this change in production of the different splice variants to increase the apoptotic (promoting cell death) version in cancerous cells would therefore offer an approach to cancer treatment and a form of therapy.

In the case of the Bcl-x gene, a member of the family of apoptosis regulators, AONs have been demonstrated to be effective in altering the ratio of alternatively spliced variants Ho PT, Parkinson DR (1997). The Bcl-x gene has two 5’ splice sites in exon 3. The use of both splice sites results in the generation of two different splice variants: Bcl-xL and Bcl-xS; the former giving rise to a protein with strong anti-apoptotic functions. This protein has been found in over 60% of invasive human breast carcinomas and is thought to play a major role in the pathogenesis of various lymphomas.

Transfection of cancer cell lines with the antisense oligonucleotides specific to one of the alternative 5' splice sites caused a shift of splicing from the Bcl-xL to the Bcl-xS variant. As a consequence, the cells exhibited increased apoptosis and also increased sensitivity towards apoptosis-inducing agents (Mani et al., 1999). These results suggest that antisense oligonucleotides might be used for modification of the splicing pattern of genes involved in cancer as a therapeutic tool.

Also in 2006, German physicians reported on a dose-escalation study for the compound AP 12009 (a phosphorothioate antisense oligodeoxynucleotide specific for the mRNA of human transforming growth factor TGF-beta2) in patients with high grade gliomas. At the time of the report, the median overall survival had not been obtained and the authors hinted at a potential cure.

HIV/AIDS

Starting in 2004, researchers in the US have been conducting research on using antisense technology to combat HIV. In February 2010, researchers reported success in reducing HIV viral load using patient T-cells which had been harvested, modified with an RNA antisense strand to the HIV viral envelope protein, and re-infused into the patient during a planned lapse in retroviral drug therapy.

Cardiovascular and renal diseases

Antisense oligodesoxynucleoties (ODN) provide a novel strategy to inhibit RNA transcription and thereby the synthesis of the gene product. Because antisense ODN hybridize with the mRNA strand, they are highly specific. Their backbone structure has been modified to phosphorothioates or phosphoamidates so that they can better withstand degradation after delivery (Martens et al., 1998). We have shown that antisense ODN are a useful research tool to elucidate intracellular processes. The example we provide involves the inhibition of PKC signaling. Furthermore, we have shown the potential clinical utility of antisense treatment. We successfully inhibited the expression of the surface adhesion molecule ICAM-1 with antisense ODN in a model of reperfusion injury. This model is highly applicable to the problem of delayed graft function in humans. However, "getting there" is a major problem and clearly less than half the fun. Cationic substances such as lipofectin have worked sufficiently well in the experimental setting. Viral gene transfer offers a possibility; however, viruses produce an additional series of problems. Liposomes may not provide sufficient transfer efficiency. Coating liposomes with viral fusion proteins may offer an ideal way with which to deliver the goods into the cytoplasm of the target cell. Antisense approach to acute renal failure and reperfusion

injury could have great clinical utility. The production of angiotensin II by cells feeds back on those cells resulting in cell growth and other changes. In our view, this tissue (cellular renin angiotensin system) could therefore potentially play a role in a wide variety of cardiovascular disorders, including atherosclerosis and vascular The introduction of angiotensin II to the cells restored this growth. Thus, through the application of antisense technology, we were able to demonstrate the biological principle that cells can make their own angiotensin II with growth promoting effects.

β-thalassemia and cystic fibrosis

Antisense oligonucleotides were first used to restore normal splicing of the β-globin gene in β-thalassemia patients. In this context, the antisense sequences were targeted to block the use of cryptic splice sites in β-thalassemia patients. Splicing of the cystic fibrosis transmembrane conductance regulator (CFTR), a protein which is spliced incorrectly in some cystic fibrosis patients, could likewise be re-established via AONs. Both approaches do offer therapeutic potential by the redirection of normal splicing in patients.

Duchenne muscular dystrophy

The dystrophin gene contains more that 20 exons which encode a repeated region in the dystrophin protein. DMD patients carry mutations in the dystrophin gene, which change the reading frame of the mRNA leading to the formation of premature terminated protein products which cannot carry out the function of dystrophin.

One current application of AONs is to induce the specific skipping of exons in order to restore the correct open reading frame of a mutated transcript. Transcripts can then be translated into a partially or even fully functional protein and ameliorate symptoms of muscular dystrophy. Some mutations even require the deletion of two exons in order to gain the correct reading frame. Translation can be performed subsequently. In fact, antisense-mediated exon skipping is one of the most promising therapeutic approaches for Duchenne muscular dystrophy (DMD).

A first human trial using optimised antisense oligonucleotides in order to induce exon skipping was completed recently in the Netherlands; a second trial is about to start in the United Kingdom. Local intramuscular injection of optimised AON sequences resulted in dystrophin levels of up to 20% compared to wild type dystrophin. This observation is accompanied by improvement in muscle histology and function.

The exon skipping approach would be suitable for up to 90% of all DMD patients. However, a disadvantage of the therapy is that different mutations require skipping of different exons to restore the reading frame and currently the focus is concentrated on two hotspots where the deletions take place. The results of the human trials mean that antisense-mediated exon skipping could become a standard method in the clinical treatment of DMD in the near future.

Spinal muscular atrophy

Another field of application for antisense oligonucleotides is the treatment of spinal muscular atrophy (SMA) patients. SMA is caused by a deletion or inactivation of the SMN1 gene. The nearby SMN2 gene carries the same genetic information but a single nucleotide exchange impairs inclusion of one exon. Therefore the product is only an incomplete substitute for SMN. In an analogous approach to AONs, the U7 small RNA which is usually involved in production of other RNAs, namely the histone mRNAs, can be converted to induce exon skipping in several - also including medically important - genes and therefore in the case of SMN as well.

The research group of Daniel Schümperli, a EURASNET member in Bern, Switzerland has pioneered the use of modified U7 RNAs as a delivery vehicle to modify splicing. The U7 RNA constructs carry a sequence allowing them to bind to exon 7 of SMN2 and a splicing enhancer sequence that will improve the recognition of the targeted exon. The strategy could be optimised in a way that nearly all SMN2 mRNAs become spliced correctly. In SMA patient fibroblasts, this technique led to the induction of a prolonged restoration of SMN protein and its correct localisation to dot-like nuclear structures called gems. This approach is being tested in animal models, to examine whether the method can be used in nerve cells whose death causes SMA.

Antisense in diabetes mellitus and obesity

Mipomersen, which is a first-in-class antisense drug, was designed to reduce LDL (low density lipoprotein)-cholesterol. Also, it is an antisense drug used to inhibit PTP-1B, and it is an insulin sensitizer for the treatment of type 2 diabetes. In addition to lowering glucose, ISIS 113715 has anti-obesity and lipid-lowering potential. We reported positive top-line Phase 2 data on ISIS 113715 in 2009 (Figure 2).

Asthma

In allergic asthma experimental models, antisense oligonucleotides (ASO) are administered by inhalation or systemically. ASO can be used for a large number of molecular targets: Cell membrane receptors (G-protein coupled receptors, cytokine and chemokine receptors),

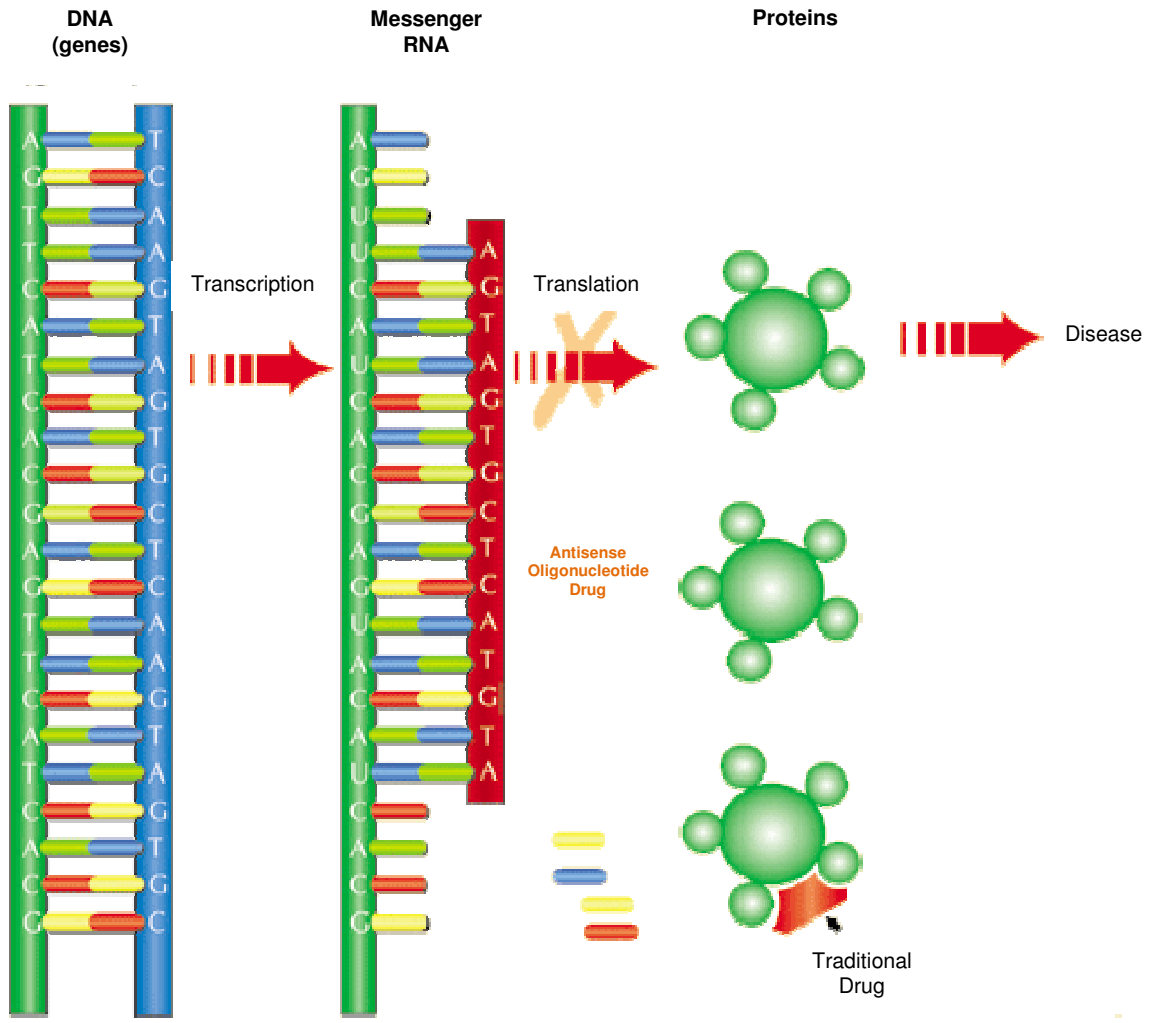

Figure 2. Mechanism of action of antisense.

membrane proteins, ion channels, cytokines and related factors, signaling non-receptor protein kinases (tyrosine kinases, and serine/threonine kinases) and regulators of transcription belonging to Cys4 zinc finger of nuclear receptor type or beta-scaffold factors with minor groove contacts classes/superclasses of transcription factors. A respirable ASO against the adenosine A1 receptor was investigated in human trials. RNase P-associated external guide sequence (EGS) delivered into pulmonary tissues represents a potentially new therapeutic approach in asthma as well as ribozyme strategies (Tanaka et al., 2001). Small interfering RNA (siRNA) targeting key molecules involved in the patho-physiology of allergic asthma are expected to be of benefit as RNAi immunotherapy. Antagomirs, synthetic analogs of microRNA (miRNA), have important roles in regulation of gene expression in asthma. RNA interference (RNAi) technologies offer higher efficiency in suppressing the expression of specific genes, compared with traditional antisense approaches.

MARKETED ANTISENSE PREPARATION

Perspective

The use of antisense therapy in treatment of various diseases hold promissible effect, particularly as research progresses in refining its drug delivery methods, increasing its affinity and improving its specificity. Currently, research effort in antisense strategies are aimed at understanding the degree of involvement of single genes or combinations of genes, in the course of diseases. The purpose of this review to make more attention to the area of research is to isolate the most critical targets and identify the feasibility of using genes as targets for therapeutic purposes. Thus antisense technology has progressed rapidly over recent years and continues to do so. Furthermore, the possibility that antisense approaches will play an important role in future to advance our current understanding of the molecular events underlying human disease and to provide a novel

Table 1. Antisense agents and their targets.

S. no.	Agent	Target
01	Genasense (oblimersen)	Bcl-2
02	Affinitak (ISIS 3521)	PKC-alpha
03	ISIS 112989 (OGX 011)	Secretory protein clusterin
04	ISIS 23722	Survivin
05	AP 12009	TGF-Beta2
06	GEM 231	Protein kinase A
07	GEM 240	MDM2
08	IGF-1R/AS ODN	Insulin-like growth factor
09	MG98	DNA methyltransferase
10	LErafAON	C-raf-1
11	Ki-67 antisense oligonucleotide	Ki-67
12	GTI-2040	Ribonucleotide reductase
13	ISIS 2503	H-ras
14	AP11014	TGF-Beta1
15	Mipomersen	apoB-100
16	ISIS-CRP_{Rx}	CRP
17	BMS-$PCSK9_{Rx}$	CRP
18	BMS-$PCSK9_{Rx}$	PCSK9
19	ISIS-FXI_{Rx}	Factor XI
20	ISIS-$APOCIII_{Rx}$	ApoCIII
21	ISIS 113715	PTP-1B
22	ISIS-$GCGR_{Rx}$	GCGR
23	OGX-011	clusterin
24	ISIS-$SGLT2_{Rx}$	SGLT2
25	OGX-011	Clusterin
26	LY2181308	Survivin
27	ISIS-$EIF4E_{Rx}$	eIF-4E
28	Alicaforsen	ICAM-1
29	ATL1102	VLA-4
30	AIR645	IL-4Ra
31	Vitravene	CMV
32	ACHN-490	Aminoglycoside
33	iCo-007	C-raf kinase
34	ATL1103	GHr
35	EXC 001	Fibrosis

antisense therapy is very encouraging. Antisense technology is a formidable tool for investigating physiologic and pathologic processes. In addition, it is soon likely to become a mainstay of therapy, particularly in infectious diseases, with wider applications in the future as gene therapy techniques are developed further. Antisense pharmaceuticals will soon be available for the routine care of patients and are expected to prove to be effective, specific agents with favorable therapeutic profiles (Table 1).

CONCLUSION

Antisense drugs have the potential therapeutic efficacy in the treatment of various diseases than the conventional drug. The rapid development of antisense technology offers almost unlimited scope for the development of new and highly specific therapeutics. Antisense therapeutics therefore is well positioned to play a significant role in the progression of antisense technology for drug development in human diseases. More research attention should be given on these area that can be effective in treatment of various diseases like cardiovascular diseases, autoimmune diseases and other such diseases.

REFERENCES

Agrawal S, Temsamani J, Galbraith W, Tang JY (1995).

Pharmacokinetics of antisense oligonucleotides. Clin. Pharmacokinet., 28: 7-16.12
Crooke ST (1996) Progress in antisense therapeutics. Med. Res. Rev., 16: 319-344
Crooke ST (2000) Progress in antisense technology: the end of the beginning. Methods Enzymol., 313: 3-45.
Crooke ST, Jansen B, Wacheek V, Heere Resss E (1992) Therapeutic applications of oligonucleotides. Annu. Rev. Pharmacol. Toxicol., 32: 329-376.
Crooke ST, Lemonidis KM, Neilson L, Griffey R, Lesnik EA, Monia BP (1995). Kinetic characteristics of Escherichia coli RNase H1: cleavage of various antisense oligonucleotide-RNA duplexes. Biochem. J., 312: 599-608.
Leonetti JP Degols G, Clarenc JP, Mechti N, Lebleu B (1993). Cell delivery and mechanisms of action of antisense oligonucleotides. Prog. Nucleic Acid Res. Mol. Biol., 44: 143-166.
Mani S, Gu Y, Wadler S (1999). Antisense therapeutics in oncology: points to consider in their clinical evaluation. Antisense Nucleic Acid Drug Dev., 9: 543-547.
Neckers L, Whitesell L, Rosolen A, Geselowitz DA (1992). Antisense inhibition of oncogene expression. Crit. Rev. Oncog., 3(1-2): 175-231.
Tanaka M, Nyce J, Ulanova M, Duta F, Puttagunta L (2001). Respirable antisense oligonucleotides: a new drug class for respiratory disease. Respir. Res., 2: 5-9.