



Epigenetics - Gene Silencing

RD Lele*

Abstract

The discovery of the mechanism of RNA interference by ds RNA by Prof. Andrew Fire and Prof. Craig Mello in 1998, gave them the Nobel Prize in 2006. This discovery revealed a new mechanism for gene regulation through “gene silencing” at the transcriptional level (TGS) or at the post-transcriptional level (PTGS), which play a key role in many essential cellular processes. Today dsRNA is used as a powerful tool to experimentally elucidate the function of essentially any gene in a cell. The immense impact of the discovery of RNA interference (RNAi) on biomedical research and its novel medical applications in the future are reviewed in this article, with particular stress on therapeutic applications of radio-labeled antisense oligonucleotides (RASONS) for diagnosis and treatment of various cancers and neurodegenerative diseases by “gene silencing”. Antisense oligonucleotides (ASONS) can also modulate alternative splicing which 74% of all human genes undergo. The most effective targeting strategy employs simultaneous blocking SnRNP binding sites and splice junctions. Correction of splicing by ASONS can be used to silence mutations causing aberrant splicing as in thalassemia, Duchenne muscular dystrophy and cystic fibrosis. ©

INTRODUCTION

This article intends to elaborate the new concept of gene silencing and its mechanisms and its potential therapeutic applications, which will enhance the reader’s appreciation of the role of epigenetics and gene silencing in health and disease.

RNA INTERFERENCE

The Nobel Prize in Physiology and Medicine in 2006 was shared by Professor Andrew Z. Fire at Stanford University California and Profesor Craig C. Mello at University of Massachusetts Medical school in USA, for their discovery that double stranded RNA triggers suppression of gene activity in a homology-dependent manner, a process named RNA interference (RNAi). This discovery revealed a new mechanism for gene regulation and the biochemical machinery involved plays a key role in many essential cellular processes. This control system for gene expression has proven to be important for both the development of an organism and the physiological functions of cells and tissues. Today double-stranded RNA is used as a powerful tool to experimentally elucidate the function of essentially

any gene in a cell. The immense impact of the discovery of RNAi on biomedical research and its novel medical applications in the future, will be reviewed in this article.

Prof. Bertil Daneholt, Professor of Molecular genetics, Karolinska Institute Stockholm, Chairman of the Nobel Assembly has given an excellent review of RNA interference, which is a major source of information in this article.¹

The evolution of our knowledge about gene expression is studded with many Nobel prizes. The double helix nature of DNA revealed by Francis Crick, James Watson and Maurice Wilkins (Nobel Prize 1962); transcription of a gene into a specific RNA species, messenger RNA (mRNA) envisioned by Francois Jacob and Jaques Monod (Nobel Prize 1965); triplets of nucleotides as the genetic code for twenty aminoacids, revealed by Marshall Nirenberg and Hargobind Khorana (Nobel Prize 1968); long RNA molecules (pre-mRNA consisting of expressed mRNA sequences – exons, and intervening sequences-introns which are degraded, revealed by Philip Sharp and Richard Roberts in 1977 (Nobel Prize 1993). The discontinuous arrangement of mRNA sequences, and alternative splicing to generate different mRNAs and coding more than one protein from the primary transcript totally changed the earlier hypothesis – “one gene, one mRNA, one ribosome, one protein”. An estimated 74 percent of 30,000 human genes undergo alternative splicing. Spliced variants have been linked to a variety of cancers and genetic diseases such as thalassemia and cystic fibrosis.

The discovery that RNA can act as a Catalyst by Sydney Altman and Thomas Cech (Nobel Prize 1989) and the revelation that RNA is able to catalyze its own replication

*Hon. Chief Physician and Director, Nuclear Medicine Department Jaslok Hospital and Research Centre, Mumbai. Hon. Director, Nuclear Medicine and RIA Department Lilavati Hospital and Research Centre, Mumbai. Emeritus Professor of Medicine (for life) and Ex-Dean, Grant Medical College and Sir JJ Hospitals, Mumbai. Dean (Academic), All India Institute of Diabetes, Mumbai. Emeritus Professor of the National Academy of Medical Sciences, India.

and the synthesis of other RNA molecules (the ribozyme concept) led to the idea that RNA was the first genetic material on earth – an “RNA World” existed before DNA took over as the key genetic material. The discovery of catalytic RNA has evolutionary implications, and suggests that RNA plays a more active role in gene expression than earlier realized. It is now well established that ribosomal RNA catalyses peptide bond formation during translation.

A large number of small RNA molecules work in conjunction with proteins in ribonucleoprotein (RNP) complexes. There are non-coding RNA molecules that affect transcription (e.g. human 7SK snRNA bound to elongation factors), translation (e.g. SRPRNA in the signal recognition particle), replication (e.g. telomerase RNA) and chromosome structure (e.g. XIST RNA causing X chromosome inactivation). Others regulate RNA processing (e.g. miRNA in RNase P, snRNAs and snoRNAs) and RNA editing (guide RNAs). The specific roles of these various RNA particles in the cell are being extensively investigated.

Small RNA molecules (about 100 nucleotides in length) which can bind to a complementary sequence in mRNA and inhibit its translation, were discovered in *E. Coli* in the early 1980's. Today about 25 cases of regulatory trans-acting antisense RNAs are known in *E. Coli*. Similar regulation of translation by antisense RNA was demonstrated in 1993 in the worm *C. elegans*. In 2001 a large number of small RNA molecules called micro RNAs (miRNAs) were revealed, causing post-transcriptional inhibition (RNA silencing). In plant experiments around 1990, homology-dependent gene silencing could be induced at the transcriptional level (TGS-transcriptional gene silencing) or at the post-transcriptional level (PTGS – post transcriptional gene silencing). A PTGS like process called quelling was also established in the fungus *Neurospora Crassa*. The phenomenon of RNA causing gene silencing remained enigmatic until the discovery of the mechanism of RNA interference in 1998 when Andrew Fire and Craig Mello published their break-through study in *Nature*.² Since both antisense and sense RNA could cause silencing, Mello argued that the mechanism could not just be a pairing of antisense RNA to mRNA. He coined the term RNA interference for the unknown mechanism. They showed that injection of double-strand RNA (dsRNA) led to an efficient loss of the target mRNA. The dsRNA has to correspond to the mature mRNA sequence; neither intron nor promoter sequences triggered a response, indicating a post-transcriptional, presumably cytoplasmic mechanism, which degraded the targeted mRNA (which disappeared). Only a few dsRNA molecules per cell were sufficient to accomplish full silencing. The dsRNA effect could spread between tissues and even to the progeny, suggesting transmission of the effect between cells. Fire and Mello speculated that “dsRNA could be used by the organism for physiological gene silencing”, at the post-transcriptional level (RNAi). Within a year, the presence of RNAi had been documented in many other organisms including fruit flies, trypanosomes, plants, planaria, hydra and zebrafish. A short

21 nucleotide long dsRNA can achieve efficient targeted silencing in eukaryotes. A remarkable exception is the budding yeast, *saccharomyces cervisiae*.

The molecular machinery involved in RNAi has been subsequently revealed.³⁻⁵ A large complex called RISC (RNA – induced silencing complex) is targeted to the mRNA via a short antisense RNA; the mRNA is cleaved and subsequently degraded. A ribonuclease III like nuclease called Dicer is responsible for the processing of dsRNA to short dsRNA (small interfering RNA – siRNA). An RNA dependent RNA polymerase (RdRP) plays an important role in generating and/or amplifying siRNA.⁶

SIGNIFICANCE OF RNAi

1. RNAi protects against viral infections, in plants, worms and flies. Its relevance, for vertebrates including humans is to be clarified.
2. RNAi secures genome stability by keeping mobile elements silent. If RNAi is not efficient the transposons are not kept under control and can start to jump and cause deleterious effects in the genome.
RNAi represents an “immune defense” of the genome. Close to 50% of our genome consists of viral and transposon elements that have invaded the genome in the course of evolution. The RNAi machinery can recognize invading ds viral RNA and degrade it.⁷
3. RNAi represses protein synthesis and regulates the development of the organism. Small RNA molecules called microRNA (miRNA) are processed from larger hair pin – like precursors by an RNAi like machinery. Today it is estimated that about 500 miRNAs in mammalian cells control gene expression. About 30% of all genes are regulated by miRNAs during development in plants, *C. elegans* and mammals. The role of mi RNA dependent control of gene expression in humans is yet to be elicited.
4. RNAi keeps chromatin condensed and suppress transcription, and regulates the activity of genes in the immediate vicinity of the condensed blocks of chromatin, through histone modification, binding of specific chromatin condensing proteins (HPI) and DNA methylation. This action on chromatin is most important for proper functioning of the genome and for maintenance of genome integrity.
5. Targeted gene silencing by RNAi can be used to study the function of individual genes, in cultured cells as well as in transgenic organisms.
6. RNAi via specifically designed oligonucleotides might be a useful approach in future gene therapy. Promising results have been reported in several animal models and in clinical trials, as described in the subsequent sections in this article.⁸⁻¹¹

In a recent experiment in monkeys a single injection of a drug to induce RNAi against mRNA of PCSKg lowers cholesterol by about 60%, an effect lasting for 3 weeks.¹²

Currently there are at least six oligonucleotide drugs inducing RNAi for illness including cancer.

Prof. John Mattick, Professor of Molecular Biology at the University Queensland in Australia has commented that RNAi is the biggest conceptual change since the discovery of the Double Helix."

Therapeutic Antisense Oligonucleotides

The idea of designing specific oligonucleotides for inhibition of specific mRNA has been implemented since 1977, at a time when it was not known, as we know today, that Nature has been using this strategy since antiquity ! The first oligomer of nucleotides was made by Hargobind Khorana in 1967. The radiolabeling of specific anti-sense oligonucleotides with I-125, Tc-99m and In-118 for imaging was pioneered by Mrinal Deewanjee in 1992. The c-myc oncogene mRNA in nude mouse implanted with human breast-tumour (MCF7) Xenograft has been imaged with In-111 labeled antisense oligonucleotide probe (RASON) by Deewanjee.¹³

With RASONS one could detect cancer in-situ by external imaging while the lesions are too small to be detected by other means, as well as treat them. Only 10 µg of RASON are needed for specific mRNA imaging in Yorkshire pigs (20-30 kg).^{14,15}

Theory of Antisense Technology

During gene transcription, the double strand of DNA becomes partially uncoiled and the two complementary strands – "sense" and "anti-sense" separate. The antisense strand of DNA is utilized as a template to generate mRNA. This cytoplasmic mRNA is in the "sense" orientation and a sequence of as short as 18 bases of an "antisense" oligonucleotide can sterically block the translation. Thus, by selecting appropriate antisense nucleotide sequences, it is possible to inhibit the expression of virtually any gene product in a cell without directly affecting other cellular components (Fig. 1).

One important hurdle is the ubiquitous presence of nucleases in living tissues and serum which degrade the single stranded oligonucleotide chains. To overcome this initial impediment, a sulfur atom is substituted for the non-binding oxygen in the nucleotide linkages of the synthesized chain (Fig. 2), which makes it resistant to nucleases, whereas normal phosphoesterase-linked nucleotides are immediately degraded by nucleases. Phosphorothioate-linked nucleotides have the advantage (because of their charge – to mass ratio) to cross cell membrane but still retain the capacity to activate RNase-H enzyme, which mediates the degradation of mRNA.

Radiolabeled antisense oligonucleotides (RASONS) have been designed for specific mRNAs (e.g. C myc, Erb B2, telomerase) expressed in breast cancer cells. Such sequences of 15 - 25 nucleotides can traverse the membrane of living cells and bind to specific mRNAs (in vitro hybridization) and provide an image of the cancer in vivo. The specificity of the "Molecular Velcro" for target cells

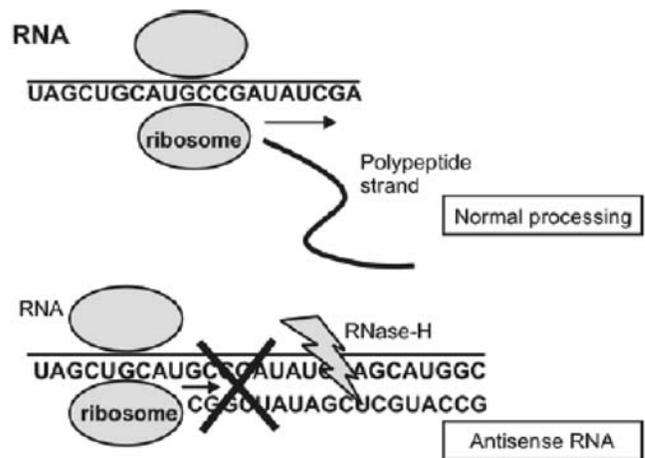


Fig. 1 : The schematic representation of antisense oligonucleotides that inhibit transcription of mRNA

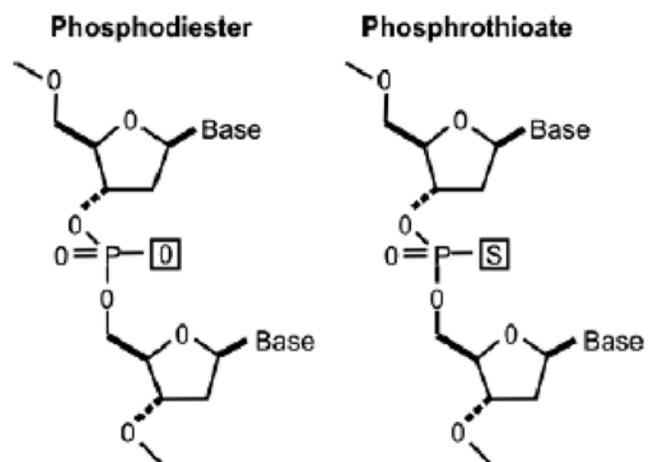


Fig. 2 : Creation of phosphorothioate backbone

is excellent and provides therapy to kill the cells by using α emitters or Auger electrons - "Molecular Surgery". Thus by selecting appropriate antisense oligonucleotide sequence it is possible to inhibit the expression of virtually any gene in cells without attacking any other cellular components. Although the word "gene silencing" was not used for this activity it was precisely that, with the, potential for therapy – prevention of production of harmful proteins by virus – infected cells, bacteria – infected cells, tumor cells or genetically transformed cells.¹⁶⁻¹⁸ The oligonucleotide drug Vitravene, the first of such drugs to be approved by FDA in USA, is used to treat cytomegalovirus retinitis in AIDS patients. Antisense oligonucleotides have been developed to block the c-myc proto-oncogene mRNA which codes for substances that cause atheroma in blood vessels. Table I lists the currently tested antisense agents and their mRNA targets.

POTENTIAL ADVANTAGES

There are several aspects of antisense therapy using oligonucleotides that are potentially advantageous over traditional drug mechanisms. (J. Maxon 2005).¹⁹

1. Oligonucleotides can be manufactured quickly, some

within one week; the sequence of the mRNA is all that is needed.

2. 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 (Tanaka and Nyce 2001).²⁰
3. Inhibition of mRNA expression will produce quicker and longer lasting clinical response than inhibition of protein formed by the ribosome, targeted by conventional drug therapy.
4. Hydrogen bonding between oligonucleotide and mRNA target exceeds by several orders of magnitude Van der Waals and other forces needed to bind protein targets.

ASONS accumulate in specific organs and tissues- liver, spleen, kidneys, bone marrow, fat cells. They can be administered by several routes- oral, rectal, subcutaneous, intravenous, intrathecal, intravitreal, aerosol and topical. Their potential applications include cancer, metabolic and cardiovascular, ocular, viral and neurodegenerative disorders.

At the Mary Crowley Medical Research Centre at Baylor University, Texas, phase 1 clinical trials have been completed involving antisense molecules for PKCa, C raf, H ras and BCL-2. Tumor cell growth is often discontinuous so that at any given time a large proportion of tumor cells are quiescent. Therefore the mRNA binding agents have to be administered over prolonged periods of time.

BCL-2 is an inhibitor of apoptosis in cancer cells. G3139 an ASON targeting BCL-2 mRNA leads to increased apoptosis in both in vitro cell lines of malignant cells as well as in animal models of cancers.

PKC is responsible for transformation and proliferation of variety of tumor cells including breast cancer. ASON for mRNA of PKC suppresses the growth. ISIS 3521 at doses ranging from 0.15 – 6 µg/Kg/day for three days per week for three weeks showed complete response in 2 patients and stabilization in 10 patients of NHL.

Inhibition of H ras mRNA by ISIS 2503 in 4 patients had resulted in stabilization lasting longer than 2 months although no patient achieved a complete or partial response. ISIS 5132, which specifically binds to mRNA of C raf kinase caused stabilization of disease for 9 to 10 months in renal and pancreatic cancers. A patient with ovarian Ca had 97% decreases in Ca 125 levels in response to antisense infusion.

RASON therapy for neurodegenerative disease

Neurotoxicity from accumulation of misfolded/mutant proteins is causative of neurodegenerative disease. A therapeutic strategy is to decrease the levels of these proteins. Antisense oligonucleotides to superoxide dismutase 1(SOD1) reduces both SOD1 protein and mRNA levels in the brain and spinal cord. In an animal model

of amyotrophic lateral sclerosis (ALS), treatment with ASON initiated near onset significantly slowed disease progression.²¹

Short synthetic 15-25 base oligonucleotides is a substrate for degradation by endogenous RNase H. the ASON is directly pumped into the CSF for 14 days. Significant ASON concentrations (3.7-7 µM) are achieved in the brain, brainstem and spinal cord. These micromolar concentrations are comparable to the 106 micromolar concentrations commonly achieved in CNS active drugs such as anti-epileptic drugs. (M. Tanaka and JW Nyce).²⁰

RASONS for respiratory diseases

The lung offers an exceptional target for RASON delivery by inhalation. The lung surfactant containing cationic lipids enhances the uptake and distributions of oligonucleotides throughout the respiratory tract as shown by autoradiography. Hence the effective dose is several orders of magnitude less than parenteral administration.

EPI2010 is a 21-mer phosphorothioate RASON targeting adenosine A1 mRNA, which is involved in bronchoconstriction, inflammation and surfactant depletion. It is effective as a single delivered inhaled dose of 50 µg/kg. The half-life of EPI2010 in the lungs is less than 24 hours; the duration of its effect is 6 days (range 4-11 days) (Tanaka and Nyce 2001).²⁰

Alternative splicing of pre mRNA

Alternative splicing is the major source of proteome diversity in humans and this is highly relevant to understanding disease and its therapy. For example the target of the analgesic and antipyretic drug acetaminophen is a neural-specific alternatively spliced isoform of cyclooxygenase-1 (Cox-1). Several important diseases have been linked to mutations or variations in either cis-acting elements or trans-active factors that lead to aberrant splicing and abnormal protein production. Correction of erroneous splicing is an important goal of molecular therapies (Garcia-Blanco MA et al 2004).²²

Genome-wide survey of human alternative pre mRNA splicing with exon-junction microarrays has revealed that 74 percent of all 30,000 human gene transcription undergo alternative splicing of pre mRNA. Close to 50 percent of human genetic disorders are caused by mutations that cause defects in pre-mRNA splicing (Johnson JM et al 2003).²³

A powerful technique for studying functions of individual transcripts is to use steric-blocking antisense oligos to block RNA processing events, splicing events in particular, and thereby force the expression of altered transcripts. Morpholino oligos can be used as tools to characterize functions of alternatively spliced transcripts, or generate "Loss of function" (knockdowns) by means of exon deletion. Morpholino oligos directly alter splicing of events by blocking small nuclear Ribonucleoprotein (sn RNP) binding sites and splice junctions. Morpholino oligos can block other sequences involved in the control of RNA processing

including mi RNA, intronic splicing silencers (ISSs), exonic splicing enhancers (ESEs) and ribozyme catalytic sites. Targeting a normal gene and generating an exon deletion produces a phenotype equivalent to a loss of function mutation. (Morcos 2007).²⁴

Alternative RNA splicing and its modulations

Techniques that trick the splicing machinery to alter the splicing pathways can be of high therapeutic value. Morpholino-antisense oligos can specifically block splicing events. Alteration of splicing patterns result from the binding of the oligonucleotide to the nuclear pre-mRNA. Efficient delivery of morpholino oligos in cytoplasm of cells is now possible. The etiology of many human genetic disorders involve splicing errors. The morpholino-oligo approach has been used for hundreds of successful splice-blocking experiments in the tissue culture and in developmental model systems including zebrafish and frog embryo. Targeting internal exon-intron or intron-exon boundary results in exon deletion, and targeting the first exon-intron insertion. The therapeutic potential of antisense oligonucleotides as modulators of alternative RNA splicing has been discussed by P. Sazani and R. Kole (2003).²⁵ This is a promising approach to RNA repair based on ASON, and production of therapeutic gene products.

Modification of natural alternative splicing or induction of aberrant splicing can produce clinically relevant benefits. For example Bcl X codes for two splice variants with opposing functions that are derived by the use of two alternative 5' splice sites. The long form Bcl X L has antiapoptotic properties, while the short form BclxS has proapoptotic properties. Both forms are required for normal cellular function. Many cancers over-express bclxL. An antisense 2'O methyl oligonucleotide targeted to the xL splice site induced apoptosis in PC3 prostate cancer cells. The level of expression of bclC gene determines the success of bcl oligonucleotide treatment c-myc, a powerful oncogene is over expressed or mutated in several types of cancer. Application of an ASON 28 mer morpholino oligomer directed toward the AUG codon was unexpectedly shown to cause aberrant splicing. In addition to AUG, the oligonucleotide blocked the intron-exon junction of exon 2 activated a cryptic 3' splice site further upstream with the exon. Thus the region of exon 2 containing the start codon was omitted from the spliced message, leading to translation initiation from a down-stream AUG and generating a truncated nonfunctional cMYC protein.

Prostate-specific membrane antigen (PSMA) has a long and short splicing, isoform (PSM and PSM/) generated by the use of alternative 3' splice sites in exon 3. The PSM/PSM ratio is 150 times higher in prostate cancer cells relative to normal tissue. Oligonucleotide-induced exon skipping and restoration of the correct PSM/PSM ratio is an attractive proposition.

Correction of splicing by ASONS:

βThalassemia is a serious genetic blood disorder in which

the production of the β globin subunit of Hb is partially or totally ablated by mutation in the gene. Mutations at position 654, 705 or 745 or intron 2 (IVS2) human β globin pre mRNA lead to creation of an aberrant 5' splice site and activation of a cryptic 3' splice site within the intron. The result is the inclusion of part of IVS2 (creating an inframe stop codon) into the supplied mRNA. AN ASON (resistant to RNase-H) targeted to the aberrant splice site blocks its use by the spliceosome, restoring the use of the proper splice sites and thus normal β globin expression (Sezani and Kole 2003).

Cystic fibrosis

A mutation in intron 19 of the CFTR gene (3849 + 10 kb C to T) creates an aberrant – 5' splice site and activates a cryptic 3' splice site resulting in decreased CFTR protein expression. This can be corrected by ASON targeted to either 9' or 3' aberrant splice sites. Interestingly, this double delivery effect, is not seen with β globin.

Dystrophin

Mutations that create stop codons in the dystrophin gene are the main cause of Duchenne muscular dystrophy (DMD). This premature stop codon can be eliminated by 2' O. Methyl oligonucleotides targeted to the 5' splice site of the exon. This treatment causes exclusion of exon 23 from the spliced mdx message, thus reducing its length by 213 nucleotides and eliminating the premature stop codon. The ASON – drivers shift in splicing is a particularly attractive approach to treat DMD since the dystrophin gene is very large (2400 kb) and therefore difficult to deliver by current gene therapy protocols.

Manipulation of alternative splicing

Sanazi and Kole have discussed the clinically relevant potential targets for antisense oligonucleotides that shift splicing.

Human Insulin Receptor gene is expressed as two different tissue-specific variants, HIR-A and HIR B that exclude or include exon 11 respectively. The levels of inclusion of exon 11 in tissues might contribute to T2DM. HIR-A binds insulin with higher affinity than HIR-B but has lower signaling capacity than the latter.

GABA_Aγ₂ gene is alternatively spliced to generate a short (γ_{2s}) and long (γ_{2L}) form. The ratio of γ_{2s} to γ_{2L} is severely reduced in the prefrontal cortex in schizophrenia and in animal models of alcoholism. The alternatively spliced exon only 24 bp allowing for complete targeting by ASON that would promote skipping and improve the ratio of γ_{2s} to γ_{2L}.

Fibronectin is an extracellular matrix protein critical for the maintenance of normal skin. Different forms of fibronectin can arise by alternative splicing of three domains EDA, EDB and III CS. An increase a ratio of EDA*Fn to EDA' Fn was found in epidermis of psoriasis, an immune hyperproliferative disorder of the skin. This represents a clinically relevant alternative splicing event. ASON- induced skipping of the EDA exon might ameliorate the disease.

Table 1 : Therapeutic Antisense oligonucleotide drugs and their targets

Infection/ Inflammation	- Fomivirsen (vitravene)	CMV retinitis in AIDS
	- ISIS 353512	C-reactive protein (CRP)
	- Alicaforsen	ICAM-1 (ISIS 2302)
	- ATL/TV1102	CD49d, sub unit of very late Antigen (VLA-4) active in several Inflamm inflammatory disorder and multiple Sclerosis
	- TPI ASM8	CCR3 and βc (common beta chain of IL-5 and G-M CSF receptors
Metabolic	- Mipomersen (ISIS 301012)	apo B 100 – lowering LDL cholesterol levels
	- BMS – PCSK9	Proprotein convertase subtilisin Kexin 9 a protease interacts with LDL receptor – lowering LDL cholesterol.
Diabetes	- ISIS 113715	Protein tyrosine phosphatase (PTP-Ib) enhancing availability of insulin
	- ISIS325568	Glucagon receptor GCGR lowering hepatic glucose production
	- ISIS377131	Glucocorticoid receptor GCCR in liver and adipose tissue.
	- ISIS388626	Sodium dependent glucose Transporter (SGLT2) in kidney.
	- Ico-007	C-raf kinase-diabetic retinopathy
Cancer	- OGX-011	Clusterin (survival protein in Cancer cell in response to therapy)
	- LY2181308	Survivin over expressed in cancer cell only
	- LY2275796	Eukaryote initiation factor 4E (eIF4E) critical switch for cancer progression
	- O6X427	Heat shock protein HSP 27 cell Survival protein.
	- Wickstrom's ASON	C-myc in Burkitt's lymphoma
	- Genasense (G3139)	BCL2 over expressed in all cancer cells.
	- Affinitak ISIS13521	Protein kinase C (PKC γ)
	- AP12009	TGF β 2
	- AP11014	TGF β 1
	- Le raf AON (ISIS 5132)	C-raf 1
	- ISIS2503	H-ras
	- GEM 231	Protein Kinase A (PKA)
	- GEM 240	MDM2
	- IGF-IR ASON	Insulin- like growth factor (IGF)
	- ALT 1103	DNA methyl transferase
- MG 98	Ribonucleotide reductase	
- GTI 2040	Ki-67	
- Ki 67 AON		
Neurodegen- erative	- ISIS333611	Cu/Zn Superoxide Dismutase SO D1 Amyotrophic Lateral Sclerosis (ALS)

Gene function rendered ineffective by the splicing mutations can be restored by ASONs to correct splicing. This is important since close to 50% of genetic disorders are caused by mutations that cause defect in pre-mRNA splicing. This approach has therapeutic potentials (Sazani and Kole 2003). Exogenous control of gene expression through modulation of RNA self-cleavage is possible (L. Yen et al 2004).

I expect a resurgence of this approach in the light of the new awareness about the therapeutic potential of “gene silencing”. The ability of antisense technology to provide ease of product synthesis, targeting of a single intended gene for silencing and quick, reproducible laboratory results, will accelerate the maturation of functional genomics and thus oncology therapeutics. ISIS has built a state-of-the-art commercial scale manufacturing facility since 2003. The facility has the capability of producing hundreds of kilograms per year of antisense drugs, I urge the Indian Biotechnology community to be actively involved in this biotechnology revolution and “the pharmacology of the future” (Table 1).

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Announcement

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