

# **Nucleic acid based therapeutic molecules**

**Shiv Charan Prasad and Ipsita Roy\***

Department of Biotechnology  
National Institute of Pharmaceutical Education and Research  
Sector 67, S.A.S. Nagar, Punjab, India-160062  
\*Corresponding Author: e-mail: ipsita@niper.ac.in

---

Oligonucleotides (and deoxyoligonucleotides) have proved to be valuable tools in the modulation of gene expression in a highly specific manner. In fact, a large number of proteins of widely varied character have been successfully downregulated or upregulated using an assortment of oligonucleotide-based approaches. They thus play important roles in functional genomics, target validation and therapeutic strategies. This article briefly discusses the different nucleic acid moieties, viz. antisense nucleotides, short inhibitory sequences, ribozymes, aptamers, etc., their mechanism of action inside the cell, chemical modifications undertaken to improve their stability and their use in various disease conditions like cancer, AIDS, neurodegenerative diseases, etc.

---

Cells are small membrane-enclosed compartments that contain a host of structures and biochemicals made up of proteins and other biomolecules. The genetic blueprint for the manufacture and control of these structures and biochemicals is found in the nucleus of the cell, where chromosomes contain ~25,000 genes, collectively known as the human genome. The gene is responsible for the synthesis of proteins, and these proteins, once synthesized, are responsible for carrying out various biological processes and also for causing devastating diseases. These 25,000 genes in the human genome can be transcribed into about 85,000 different mRNAs, and each can be used as a template by the cell to synthesize one or more different proteins. The majority of pharmaceutical drugs available today comprise of small organic molecules, peptides or proteins (e.g. hormones), and antibodies which typically bind to target proteins directly to inhibit or modulate the functions of proteins. So, for the last decade or so, researchers have tried to create tools that allow precise control over a specific gene to inhibit or silence the functions of lethal genes. In the early 1980s, a breakthrough technology known as antisense technology was developed. This technology involves base complement based recognition of the nucleic acid sequence, which helps to block the transfer of the message for protein synthesis, thereby preventing the synthesis of particular proteins that may be responsible for a disease.<sup>1</sup> Subsequently, explosion of genetic information provided by the completion of Human Genome Project provided a rich source of information about the target genes for the design of antisense and other nucleic acid-based drugs.

## **Why nucleic acids?**

The dream of modern drug research is to discover a biologically active molecule or a class of biologically active molecules, that are totally specific and able to act efficiently

only on the target responsible for the disease. The majority of available drugs today work by interacting with proteins which have been identified as critical for the dysfunction of certain cells or tissues. The problem with these conventional therapies is that they act on the target proteins in the body but also often bind to non-target proteins or exert an effect through unknown interactions. For example, thiazolidinediones (TZDs), a major class of drugs used to improve insulin sensitivity in the treatment of type 2 diabetes, are proposed to act by binding to Peroxisome Proliferator-Activator Receptor- $\gamma$  (PPAR $\gamma$ ), a key adipogenic transcription factor in white adipose tissue and activating genes involved in glucose and lipid metabolism like *CD36* and *adiponectin*.<sup>2</sup> The adverse effect of administration of TZDs, including renal and liver toxicity are believed to be due the indiscriminate and non-specific activation of (PPAR $\gamma$ ) systemically, including in the kidney and liver tissues.<sup>3,4</sup> Suppose an enzyme has five domains all with the same shape and size and we would like to inactivate only one, it may not be possible to do so without introducing additional complications. Knock-out technologies may achieve truncation of a particular domain but that may alter the three dimensional structure of the multidomain protein. Fortunately, progress in genetics and functional genomics has enabled definitive studies on some of the fundamental molecular mechanisms that regulate the expression of genes, as well as the dysfunction of these mechanisms. This has led to drugs that may turn off genes by targeting the RNA that codes for the protein instead of the protein product itself. RNA is an ideal target for drug discovery because it is an important mediator of gene expression. The most straightforward way in which inhibition can be brought about is by synthesizing complementary sequences that recognize single stranded RNA or DNA sequences and bind to them via base pairing, thus interfering with their function. This principle, and its many variations, is responsible for the various nucleic acid inhibitors, viz.

# Review Article

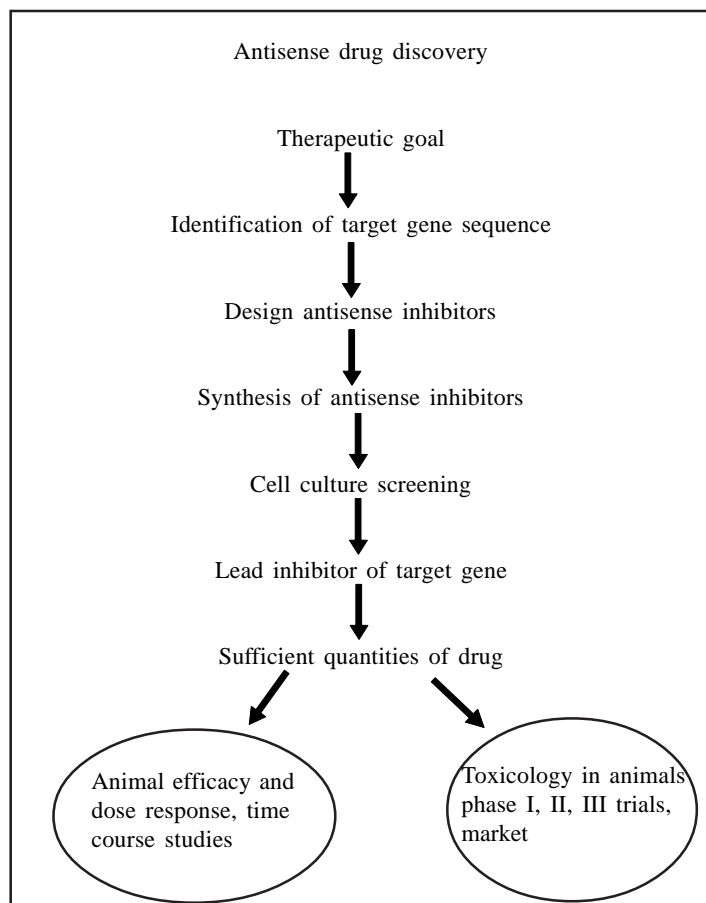
antisense sequences, short inhibitors sequences, ribozymes, aptamers, etc. Some of these are discussed below.

## ANTISENSE OLIGONUCLEOTIDES

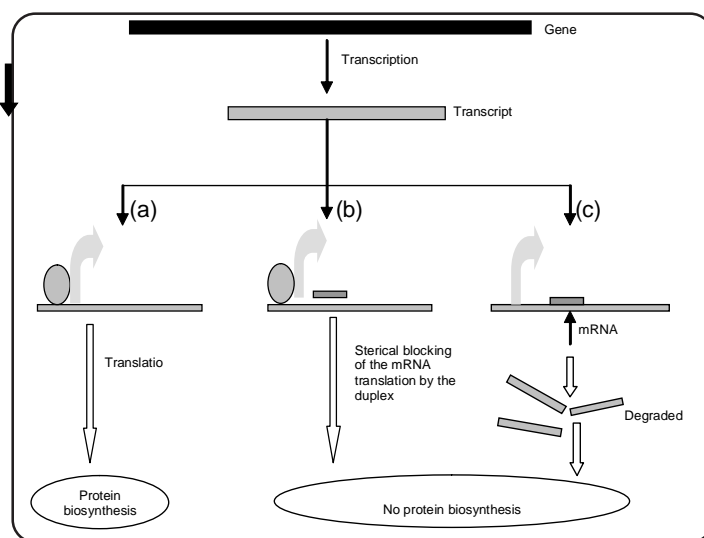
The potential of oligodeoxynucleotides to act as antisense agents that inhibit viral replication in cell culture was discovered by Zamecnik and Stephenson.<sup>5</sup> Antisense oligonucleotides consist of ~15-20 nucleotides that are complementary to their target mRNA. This strategy can serve as a helpful tool in the area of drug discovery (Figure 1). Antisense technology works by two mechanisms (Figure 2): (a) Most antisense oligonucleotides are designed to activate RNAase H which cleaves the RNA moiety of a DNA-RNA heteroduplex and leads to the degradation of the target, (b) They do not induce RNAase H mediated cleavage but can be used to inhibit translation by blocking the binding of either the polymerase or the ribosome to the 5'-terminus of the target sequence, thus preventing the assembly of the target machinery.

Antisense oligonucleotides show poor stability *in vivo* because unmodified oligonucleotides are rapidly degraded in biological fluids by nucleases.<sup>6</sup> So a variety of heterocyclic modifications have been carried out, in antisense oligonucleotides to

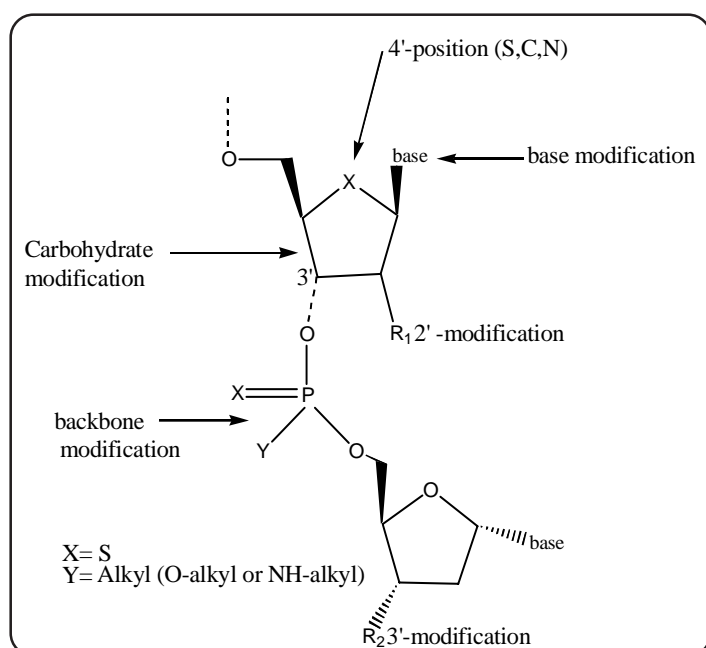
promote resistance to nuclease degradation and also to stabilize the duplex between antisense oligonucleotides and the target mRNA. The various possible modifications are shown in (Figure 3). Phosphorothioate (PS) oligonucleotides were first synthesized by Eckstein and colleagues in the early 1960s in which the nonbridging oxygen atom in the phosphodiester bond is replaced by sulfur.<sup>7</sup> The modified antisense oligonucleotide was first used for the inhibition of HIV replication by Matsukura and coworkers.<sup>8</sup> Because of this modification, phosphorothioate nucleic acids form regular Watson Crick base pairing with the target mRNA, activate RNase H, carry negative charge for delivery to cells and exhibit attractive pharmacokinetic properties. But the problem with phosphorothioate oligonucleotides is that they show some non-specific binding to certain proteins. This problem has been solved in second generation oligonucleotides which contain alkyl modification at the 2' positions of the ribose, i.e 2'-O-methyl and 2'-O-methyl-ethyl RNA. This has slightly enhanced affinity towards the complementary mRNA. A variety of modified oligonucleotides have been developed to improve target affinity, nuclease resistance and pharmacokinetic properties in third generation antisense oligonucleotides like peptide nucleic acid (PNAs), 2-deoxy-2'-fluoro- $\alpha$ -D-arabino nucleic acid (FANA) and locked nucleic acid (LNA). As result



**Figure 1** Steps involved in the generation of antisense sequences as drug candidates.



**Figure 2** Overview of inhibition of specific gene expression using nucleic acids. A gene is transcribed into a mRNA (transcript). (a) In normal conditions, the transcript is subsequently translated into a functional protein. (b) An antisense oligonucleotide (AS ODN) or the antisense strand of a small interfering RNA (siRNA), with sequences that are complementary to the transcript, specifically binds to mRNA sequence. This duplex sterically blocks the access of the translation machinery to the translation initiation signal. (c) An AS ODN, a hammerhead ribozyme or the antisense strand of a siRNA specifically interacts with its target sequence creating an heteroduplex, which induces the cleavage of the transcript by RNase H degradation, or the activated RNA-induced silencing complex (RISC), respectively. As a result of gene silencing, in (b) and (c), the protein is not synthesized.



**Figure 3 Possibilities of structural modifications of oligonucleotides to increase their stability against various agents.**

Out of these modifications, the nuclease resistance increases with increasing half-life (approximately 9-10 h compared to 1-2 h for unmodified oligonucleotides).<sup>9</sup> To enhance the cellular uptake, nuclease resistance and half-life of these

nucleotides, several modifications have been carried out in second and third generations. Pharmacokinetic studies showed that phosphorothioate oligodeoxynucleotides are well absorbed from parenteral sites and are distributed broadly to organs and peripheral tissue (they do not cross blood-brain barrier in the absence of special delivery systems). After the success of Vitravene™ (ISIS Pharmaceuticals) (which targets IE2 region of cytomegalovirus (CMV) and inhibits CMV-induced retinitis, thus preventing blindness in acquired immunodeficiency syndrome (AIDS) patients), several biotechnology companies have bet their future on this technology and are moving ahead to develop antisense therapeutics. Some of the potential antisense drug candidates are listed in Table 1.

## RNA INTERFERENCE

RNA interference (RNAi) is a primitive mechanism of gene regulation, seen in eukaryotes as diverse as yeast and mammals and probably plays a central role in controlling gene expression in all eukaryotes. The use of antisense RNA to interfere with a gene's activity in *Caenorhabditis elegans* was first used by Guo and Kemphues to study *par-1*; however, it was reported that the control sense RNA also produced a *par-1* mutant phenotype.<sup>10</sup> Subsequently, it was discovered by Fire *et al.* that it is the presence of dsRNA, formed from the annealing of sense and antisense strands

**Table 1 Antisense oligonucleotides approved for use or in clinical trials**

Company	Product	Target	Disease	Chemistry	Status
ISIS Pharmaceuticals	Vitravene	CMV IE2	CMV retinitis	PS DNA	Approved
ISIS Pharmaceuticals	Affinitac	PKC-a	Cancer	PS DNA	Phase III
Genta	Genasense	Bc12	Cancer	PS DNA	Phase III
ISIS Pharmaceuticals	Alicaforsen	ICAM-I	Psoriasis's disease	PS DNA	Phase II/III
Alnylam Pharmaceuticals in partnership with Medtronic, Inc	Huntingtin gene antisense	Huntingtin protein	Huntington Disease	-	Preclinical
ISIS Pharmaceuticals	iCO-007	c-Raf kinase	Diabetic retinopathy	-	Phase I
Epigenesis pharmaceuticals	EPI-2010	Adenosine A 1 receptor	Asthma	PS DNA	Phase II
Lorus Therapeutics	GTI 2040	Ribonucleotide (reductase R2)	Cancer	PS DNA	Phase II
AVI Biopharma	Avi 4126	c-myc	Restenosis, Cancer, Polycystic Kidney disease	3 <sup>rd</sup> generation	Phase I/II
Hybridon	Gem 92	HIV gag	AIDS	2 <sup>nd</sup> generation	Phase I
Merck in partnership with Isis Pharmaceuticals	Merck drug	Hepatitis C virus genome	Hepatitis C	-	Phase II

CMV: cytomegalovirus; PS-DNA: Phosphorothioate DNA; ICAM: Inter Cellular Adhesion Molecule; PKC: Protein Kinase C

# Review Article

present in the *in vitro* RNA preparation, that is responsible for producing the interfering activity.<sup>11</sup> Introduction of dsRNA into an adult worm results in the loss of the targeted endogenous mRNA from both the adult and its progeny. This phenomenon has been effectively harnessed to study an ever increasing number of maternal and zygotic genes in *elegans*.<sup>11</sup> RNA interference (RNAi) is the process of using specific sequence of double stranded RNA (dsRNA) to knockdown the expression level of target genes either for basic scientific studies or for therapeutic purposes. After its discovery and within less than a decade, it has become one of the most powerful and indispensable tools in the molecular biologist's tool kit. It uses a short dsRNA molecule (short hairpin RNA, shRNA), which is perfectly complementary to the target mRNA. After uptake by cells, this dsRNA molecule is cleaved into segments approximately 21-22 nucleotides long, known as short interfering RNA (siRNA) (see Figure 4). The complex of siRNA and target mRNA formed by base pairing then binds to the multiprotein complex known as RNA Induced Silencing Complex (RISC) that contains the "splicing" proteins Argonaute-2 that cleave the target mRNA molecules at defined positions (between bases 10 and 11 relative to 5' end of antisense siRNA strand) followed by its degradation by cellular RNase. This leads to highly efficient and specific knockdown of this particular gene expression.

## Advantages of RNAi in therapeutics

One of the potential advantages of sequence-based gene suppression technologies is the ability to design precisely targeted therapeutics for almost any gene, regardless of the

**Table 3 RNA interference trials in rodent models of neurodegenerative diseases**

Disease	Target	Delivery vehicle
Huntington's disease	HD-N171-82Q	rAAV1 <sup>25</sup> Liposome <sup>26</sup>
Spinocerebellar ataxia type 1	Ataxin-1/Q82	rAAV1 <sup>27</sup>
Familial amyotrophic lateral sclerosis	SOD1(G93A)	Lentivirus <sup>28</sup>
Alzheimer's disease	Endogenous BACE1	Lentivirus <sup>29</sup>
Parkinson's disease	$\alpha$ -Synuclein	Lentivirus <sup>30</sup>

APP: Amyloid precursor protein; BACE1: Beta-site of APP-cleaving enzyme 1; rAAV: recombinant adeno-associated virus; SOD1: Superoxide dismutase 1

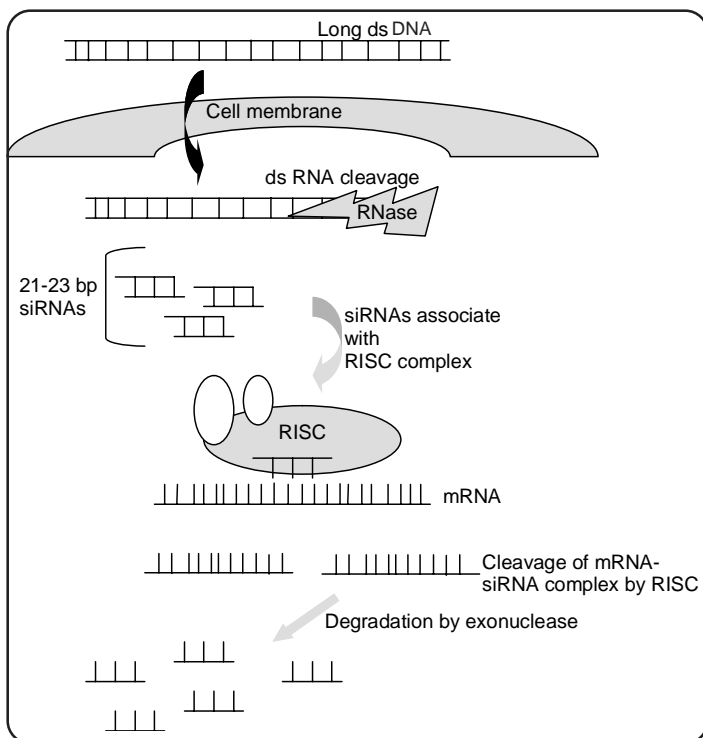
function of the gene product, whether that function is clearly defined, and in the absence of any protein structure information. This offers the opportunity to selectively inhibit expression of only the defective gene, particularly in the fields of oncology and genetic neurological disorders where the disease is often caused by a dominant mutation in a single allele. Some of these areas are listed in Table 2.

While it is difficult to directly compare the efficiency of gene suppression technologies due to differences in the rules for optimal design and target sequence selection, several studies have supported the conclusion that RNAi-mediated inhibition is more potent than that achieved with antisense oligonucleotides even in cases where site selection has been optimized for antisense effectiveness.<sup>12,13</sup> This is also empirically evident by the remarkable speed at which RNAi techniques have been implemented in scientific research, and the number of successful RNAi-based experiments already published. It is speculated that this advantage is derived from the fact that RNAi is an innate biological response, and hence represents a more natural strategy for manipulating gene expression. In any event, it means that physiologically, beneficial silencing can be achieved with lower concentrations of effector molecules. Many difficult-to-

**Table 2 Ongoing clinical trials for siRNA-mediated therapeutic approaches**

Company	siRNA product	Disease	Status
Acuity Pharmaceuticals	Cand5	AMD, diabetic retinopathy	Phase II
Alnylam Pharmaceuticals, Inc.	ALN-RSV01	RSV lung infection	Phase II
	ALN-VSP01	Liver cancer	Preclinical
Silence therapeutics	RTP-801i	AMD	Phase I
	AKIi-5	Acute renal injury	Preclinical
	Atu027	Gastrointestinal cancers	Preclinical
Sirna	Sirna-027	AMD	Phase I
Therapeutics/Merck	Sirna-034	HCV	Preclinical
Calando Pharmaceuticals	siRNA targetting	Cancer, solid tumors	Phase I
	M2 subunit of ribonuclease reductase		

AMD: Age-related Macular Degeneration; HCV: Hepatitis C Virus



**Figure 4 Mechanism of RNAi**

treat maladies, like neurodegenerative diseases, have been targeted using siRNA technology (Table 3).

## RIBOZYMES

Ribozymes and deoxyribozymes are nucleic acid enzymes and can be designed to cleave substrate mRNA in a highly sequence specific manner, resulting in selective inhibition of the expression of deleterious genes. A variety of ribozymes, catalyzing intermolecular splicing or cleavage reaction, have been found in lower eukaryotes, viruses and some bacteria. These RNA enzymes were first discovered in the protozoan *Tetrahymena thermophila*.<sup>14</sup> Ribozymes, with advantageous properties like accessibility of new target sites,<sup>15</sup> high activity under physiological  $Mg^{2+}$  concentrations<sup>16</sup> and enhanced biostability, can be generated by *in vitro* selection techniques using combinatorial libraries. A ribozyme or deoxyribozyme binds specifically to its intracellular substrate mRNA strand and cleaves it, preventing its translation. After cleavage, the enzyme is released and is ready for binding and destroying another mRNA molecule, leading to highly specific 'knockdown' of the encoded protein(s). A highly active ribozyme against a K-ras target sequence could be selected in the presence of 2'-fluoro and 2'-amino modified ribonucleotides.<sup>6</sup> The optimized ribozyme, named ZINZYME<sup>TM</sup> (Ribozyme Pharmaceuticals), has relatively high catalytic activity at 1 mM  $Mg^{2+}$  and cleaves a new Y-G-H target sequence (where Y is C or U and H is A, C or U). ANGIOZYME<sup>TM</sup> (Ribozyme Pharmaceuticals) is a hammerhead ribozyme that is targeted against the vascular

endothelial growth factor (VEGF) receptor. It is used to reduce tumor growth by inhibition of the formation of new blood vessels. HERZYME<sup>TM</sup> is a ZINZYME<sup>TM</sup> that is targeted against the human epidermal growth factor-2 (HER2), which is over expressed in certain breast and ovarian cancers.<sup>6</sup> In a phase I trial, it was found to be well tolerated with no significant systemic adverse events, as reported by the company. RNA can fold into a diverse set of structural conformation, because it is single-stranded and even fold into unique structures that carry out chemical reactions. According to RNA world hypothesis, the first enzyme in evolution was not proteins but small RNA molecules that formed unique structures. Using gene therapy, the gene encoding the ribozyme is cloned downstream of a suitable promoter into a vector, either a plasmid or a retroviral vector. After transfection or transduction, the gene becomes stably integrated in the host DNA, and its transcription provides a continuous intracellular supply of ribozyme. Retroviral vectors have been used to introduce ribozymes directed against sequences in the HIV-1 genome infecting CD4<sup>+</sup> lymphocytes.<sup>17</sup>

Deoxyribozymes are not reported to exist in nature, but several such molecules have been prepared in the laboratory, by a combinational technique know as *in vitro* selection. By *in vitro* selection, a number of deoxyribozymes with RNA cleaving activity have been isolated and characterized.<sup>18,19</sup> An *in vivo* study with DNAzymes directed against the early growth response factor-1 (Egr-1) was shown to inhibit neointima formation following balloon injury to the carotid artery wall of rats.<sup>20</sup> Further successful *in vivo* application of DNAzymes raised against TNF- $\alpha$  reported improved hemodynamic performance in rats with postinfarction heart failure.<sup>21,22</sup>

## APTAMERS

In 1980s, research on viruses (e.g. HIV and adenovirus) led to the discovery of a new class of small RNAs called aptamers. These molecules were first described in HIV-1: a virus-encoded transcript, the trans-activation response (TAR) aptamer, was found to bind the viral Tat protein. It was shown that formation of this complex inhibits Tat function and regulates both viral and cellular gene expression as well as replication of the virus.<sup>23</sup> Aptamers are short, single stranded oligonucleotides (RNA or DNA) that recognize their targets on the basis of shape complementarity.<sup>24</sup> The binding to the target is highly specific and affinity for the target is of the same order as monoclonal antibodies. In this manner, aptamers are different from other oligonucleotides because most of the conventional oligonucleotide therapies target the translational machinery of the cell by destroying the mRNA. On the other hand, aptamers target proteins directly and bind with them, altering their function. A problem with oligonucleotide therapeutics is that they block the protein

# Review Article

**Table 4 Diagnostic and therapeutic uses of aptamers**

Title	Title/Example
Pathway elucidation	Aptamers can be used as specific inhibitors to dissect intracellular signalling and transport pathways. An example of this kind is an RNA aptamer acting as a nanomolar inhibitor of the transcription factor NF- $\kappa$ B that works by blocking the interaction with the DNA recognition site. Inhibition of NF- $\kappa$ B-dependent gene activation could be a valuable strategy for enhancing the activities of antiviral and anticancer agents. <sup>31</sup>
Tools for study of disease	A high affinity single stranded DNA aptamer population against the kinetoplastid membrane protein-11 (KMP-11) of <i>Leishmania infantum</i> has been selected by a SELEX methodology using colloidal gold. This protein is an integral part of the cell membrane of the parasites that is involved in mobility or in some other aspects of the flagellar structure. The aptamer population has been characterized in a series of <i>in vitro</i> experiments, suggesting that it may be used as a powerful tool to further investigate the role of KMP-11 during <i>Leishmania</i> development and as a diagnostic tool for the infection. <sup>32</sup>
Tools for purification	The affinity and specificity of aptamers is sufficient for use as affinity chromatography media. Two DNA aptamers were covalently linked to fused silica capillary columns to serve as stationary-phase reagents in capillary electro chromatography. Separations of binary mixtures of amino acids (D-trp and D-tyr), enantiomers (D-trp and L-trp), and polycyclic aromatic hydrocarbons (naphthalene and benzo[a]pyrene) were achieved. <sup>33</sup>
Target validation	The creation of transgenic <i>Drosophila melanogaster</i> species coexpressing B52 protein and anti-B52 aptamer. <sup>34</sup> The experiment helped to validate the role of B52 in the development of various morphological features and in screening a number of drug molecules against B52 protein.
Cancer	The vascular endothelial growth factor (VEGF) a factor involved in tumour growth, that stimulates the growth of vessels into the tumours. Another example is the VEGF stimulation of growth of new vessels in the eye which cause age-related sight defects. <sup>35</sup> RNA aptamers, which specifically bind and inactivate VEGF <i>in vitro</i> , have been isolated. A clinical study on humans with injection of anti-VEGF-aptamers in the eye showed that 80 % of the patients retained or improved the eye sight and they had no side-effects. <sup>36</sup>
AIDS	One of the most urgent research areas concern anti-HIV drugs, for treatment for AIDS. A series of aptamers, which interfere with virus multiplication in human cells, have been developed. The aptamers interact with various components involved in virus propagation, i.e. HIV reverse transcriptase (RT), <sup>37</sup> integrase, <sup>38</sup>

synthesis that creates an unnatural cellular environment. In biological systems, all the pathways, mainly in the case of cell signaling have multiple mechanisms of action or activate cascades where one protein is responsible for the function of more than one protein. If the synthesis of a target protein is blocked by stable transfection of antisense or siRNA oligonucleotides, the protein is totally absent from the cellular milieu, and its deficiency could have more than one effect. Also, if only one domain of the protein is responsible for a diseased function, knocking down the whole protein may cause more harm than good. Using aptamer technology, it is possible to block the function of one domain of the protein which retaining its complete three-dimensional structure. It can also help decide druggability of a target. So, an aptamer can be a drug of choice. The other areas of application are listed in Table 4. Aptamers are usually selected following iterative rounds of binding and elution in the presence of

the target. The starting library is steadily enriched for the specific binder molecules. Since the molecules are synthesized chemically, the diversity is very high and aptamers possessing very high affinities for the target molecules can be isolated.

## Attractive properties of aptamers

(a) *Ability to disrupt interactions between proteins:* The large surface area of interaction between aptamers and their protein targets makes aptamers well-suited to block interactions between proteins. Because abnormal interactions between proteins are involved in many disease processes, the use of aptamers to inhibit these interactions may have meaningful clinical significance. Furthermore, since aptamers interact with proteins found on the surface of and outside cells, they do not have to cross the cell membrane, which may make it easier to deliver an

effective quantity of aptamer to the target.

(b) *High affinity binding and specificity*: Aptamers have well-defined, three-dimensional shapes, which allow them to interact with a folded, three-dimensional protein target, like a key in a lock. The complementary shapes of an aptamer and its target allow aptamers to bind to their targets with high affinity and specificity. The only marketed aptamer drug till date, Macugen<sup>®</sup> (Eyetechn/Pfizer) for example, recognizes only one isoform of VEGF (VEGF<sub>165</sub>) since it binds to the heparin-binding domain of the growth factor. This isoform is responsible for pathological ocular neovascularization in age-related macular degeneration (AMD).

(c) *Negligible immunogenicity*: Because nucleic acids are not typically recognized by the human immune system as foreign agents, aptamers do not generally trigger an immunogenic response.

(d) *Versatility*: Interaction of aptamer is due to shape complementary, so it can be used for a variety of targets, e.g. proteins, carbohydrates, nucleic acids, small molecules, whole cells and even organisms.

## CONCLUSION

Unlike small molecules, use of oligonucleotides as therapeutic aids gives rise to specific action against the drug target. Chances of side effects are minimized. The delivery of these oligonucleotides to the target, cells and tissues is an area needs to be worked on. Using them, hitherto untouched diseases like cancer, AIDS, diabetes, etc. can be targeted.

## REFERENCES

- Alama, A.; Barbieri, F.; Cagnoli, M.; Schettini, G. *Pharmacol Res*, 1997, 36, 171-8.
- Tontonoz, P.; Hu, E.; Spiegelman, B. M. *Cell*, 1994, 79, 1147-56.
- Guan, Y.; Hao, C.; Cha, D. R.; Rao, R.; Lu, W.; Kohan, D. E.; Magnuson, M. A.; Redha, R.; Zhang, Y.; Breyer, M. D. *Nat Med*, 2005, 11, 861-6.
- Zhang, H.; Zhang, A.; Kohan, D. E.; Nelson, R. D.; Gonzalez, F. J.; Yang, T. *Proc Natl Acad Sci U S A*, 2005, 102, 9406-11.
- Stephenson, M. L.; Zamecnik, P. C. *Proc Natl Acad Sci U S A*, 1978, 75, 285-8.
- Kurreck, J. *Eur J Biochem*, 2003, 270, 1628-44.
- De Clercq, E.; Eckstein, E.; Merigan, T. C. *Science*, 1969, 165, 1137-9.
- Matsukura, M.; Shinozuka, K.; Zon, G.; Mitsuya, H.; Reitz, M.; Cohen, J. S.; Broder, S. *Proc Natl Acad Sci U S A*, 1987, 84, 7706-10.
- Phillips, M. I.; Zhang, Y. C. *Methods Enzymol*, 2000, 313, 46-56.
- Guo, S.; Kempfues, K. J. *Cell*, 1995, 81, 611-20.
- Fire, A.; Xu, S.; Montgomery, M. K.; Kostas, S. A.; Driver, S. E.; Mello, C. C. *Nature*, 1998, 391, 806-11.
- Uprichard, S. L. *FEBS Lett*, 2005, 579, 5996-6007.
- Achenbach, T. V.; Brunner, B.; Heermeier, K. *Chembiochem*, 2003, 4, 928-35.
- Cech, T. R.; Zaugg, A. J.; Grabowski, P. J. *Cell*, 1981, 27, 487-96.
- Vaish, N. K.; Heaton, P. A.; Fedorova, O.; Eckstein, F. *Proc Natl Acad Sci USA*, 1998, 95, 2158-62.
- Peracchi, A. *Rev Med Virol*, 2004, 14, 47-64.
- Weerasinghe, M.; Liem, S. E.; Asad, S.; Read, S. E.; Joshi, S. J. *Virol*, 1991, 65, 5531-4.
- Breaker, R. R. *Nat Biotechnol*, 1997, 15, 427-31.
- Sen, D.; Geyer, C. R. *Curr Opin Chem Biol*, 1998, 2, 680-7.
- Santiago, F. S.; Lowe, H. C.; Kavurma, M. M.; Chesterman, C. N.; Baker, A.; Atkins, D. G.; Khachigian, L. M. *Nat Med*, 1999, 5, 1264-9.
- Schubert, S.; Kurreck, J. *Curr Drug Targets*, 2004, 5, 667-81.
- Iversen, P. O.; Nicolaysen, G.; Sioud, M. *Am J Physiol Heart Circ Physiol*, 2001, 281, H2211-7.
- Fichou, Y.; Ferec, C. *Trends Biotechnol*, 2006, 24, 563-70.
- Kaur, G.; Roy, I. *Expert Opin Investig Drugs*, 2008, 17, 43-60.
- Harper, S. Q.; Staber, P. D.; He, X.; Eliason, S. L.; Martins, I. H.; Mao, Q.; Yang, L.; Kotin, R. M.; Paulson, H. L.; Davidson, B. L. *Proc Natl Acad Sci USA*, 2005, 102, 5820-5.
- Rodriguez-Lebron, E.; Denovan-Wright, E. M.; Nash, K.; Lewin, A. S.; Mandel, R. J. *Mol Ther*, 2005, 12, 618-33.
- Xia, H.; Mao, Q.; Eliason, S. L.; Harper, S. Q.; Martins, I. H.; Orr, H. T.; Paulson, H. L.; Yang, L.; Kotin, R. M.; Davidson, B. L. *Nat Med*, 2004, 10, 816-20.
- Ralph, G. S.; Radcliffe, P. A.; Day, D. M.; Carthy, J. M.; Leroux, M. A.; Lee, D. C.; Wong, L. F.; Bilsland, L. G.; Greensmith, L.; Kingsman, S. M.; Mitrophanous, K. A.; Mazarakis, N. D.; Azzouz, M. *Nat Med*, 2005, 11, 429-33.
- Singer, O.; Marr, R. A.; Rockenstein, E.; Crews, L.; Coufal, N. G.; Gage, F. H.; Verma, I. M.; Masliah, E. *Nat Neurosci*, 2005, 8, 1343-9.
- Sapru, M. K.; Yates, J. W.; Hogan, S.; Jiang, L.; Halter, J.; Bohn, M. C. *Exp Neurol*, 2006, 198, 382-90.
- Lebruska, L. L.; Maher, L. J., 3rd. *Biochemistry*, 1999, 38, 3168-74.
- Moreno, M.; Rincon, E.; Pineiro, D.; Fernandez, G.; Domingo, A.; Jimenez-Ruiz, A.; Salinas, M.; Gonzalez, V. M. *Biochem Biophys Res Commun*, 2003, 308, 214-8.
- Romig, T. S.; Bell, C.; Drolet, D. W. *J Chromatogr B Biomed Sci Appl*, 1999, 731, 275-84.
- Shi, H.; Hoffman, B. E.; Lis, J. T. *Proc Natl Acad Sci U S A*, 1999, 96, 10033-8.
- Drolet, D. W.; Nelson, J.; Tucker, C. E.; Zack, P. M.; Nixon, K.; Bolin, R.; Judkins, M. B.; Farmer, J. A.; Wolf, J. L.; Gill, S. C.; Bendele, R. A. *Pharm Res*, 2000, 17, 1503-10.
- The Eyetechn Study Group. *Retina*, 2002, 22, 143-52.
- Joshi, P.; Prasad, V. R. *J Virol*, 2002, 76, 6545-57.
- de Soultrait, V. R.; Lozach, P. Y.; Altmeyer, R.; Tarrago-Litvak, L.; Litvak, S.; Andreola, M. L. *J Mol Biol*, 2002, 324, 195-203.
- Bai, J.; Banda, N.; Lee, N. S.; Rossi, J.; Akkina, R. *Mol Ther*, 2002, 6, 770-82.
- Kim, S. J.; Kim, M. Y.; Lee, J. H.; You, J. C.; Jeong, S. *Biochem Biophys Res Commun*, 2002, 291, 925-31.