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Effacing diseases with Antisense Oligonucleotides

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Abstract

Anti-sense oligonucleotides are short, modified single stranded DNA or RNA that hybridize with target mRNA and inhibit synthesis of encoded peptide. This inhibition is achieved by either degradation of target mRNA by RNase enzyme or by blocking translation. Novel therapeutics are the need of the hour to combat newer diseases. High specificity and lower costs are core issues in the development of novel therapeutic agents. Antisense oligonucleotides meet all these criteria and are also effective in targeting a number of diseases. The presence of formivirsen in the market has provided an impetus for development of new antisense oligonucleotides and several others are in the pipeline. This review focuses on various facets of antisense oligonucliotides.

Keywords: Antisense, Oligonucleotides, RNase-H, mRNA.

INTRODUCTION

Disease states do not exhibit a consistent pattern from generation to generation and newer diseases are on the rise. New drug development should receive greater focus in order to handle a barrage of symptoms induced by novel diseases. Targeting unwanted gene expression could be regarded as one of the most widely accepted approaches in circumventing the development and progression of diseases [1]. Antisense oligonucleotides are being developed as therapeutic tools for the control of gene expression. Specific hybridization of antisense oligonucleotide to its cognate gene product could impinge the process of gene expression [2, 3 and 4]. The first piece of concrete work on oligonucleotide was undertaken by Belikova and co-workers in 1967 who proposed that RNA sequences serve as endogenous inhibitors of gene expression in prokaryotes. The use of complementary sequence can inhibit the expression of a specific mRNA, inducing a blockade in the transfer of genetic information from DNA to protein [5]. Later on, Paterson and co-workers elucidated the role of exogenous, single-stranded nucleic acids in inhibiting RNA translation in cell-free systems [6]. Zamecnik and Stephenson in 1978 showed the potential of oligodeoxynucleotides as antisense agents by inhibiting viral replication in cell cultures [7].

From this humble beginning, the journey of developing novel antisense therapeutics has continued unabated. Fomivirsen was the first antisense drug to be approved by the US FDA as a treatment for Cytomegalovirus retinitis. Drug development have traditionally focused on modifying active sites of proteins by directly blocking interactions with natural substrates, however the possibility of using drugs that interact with nucleic acids are on the rise [8].

Rationale and Nature of Antisense Technology

Natural oligonucleotides have been extensively used for gene construction, selection, determination of DNA sequences (hybridization probes or affinity purification) and site-directed mutagenesis [9]. The process of gene transcription involves unwinding of duplex DNA into the "sense" and "antisense" strands. The template for the generation of mRNA is derived from the antisense strand of DNA and the "sense" conformation is borne by the cytoplasmic mRNA [10]. Complementary base pair binding of antisense oligonucleotides (As-ODNs) to cytoplasmic mRNA which is in sense orientation prevents the translation either by steric blocking or by destruction of bound mRNA via RNase-H [11].

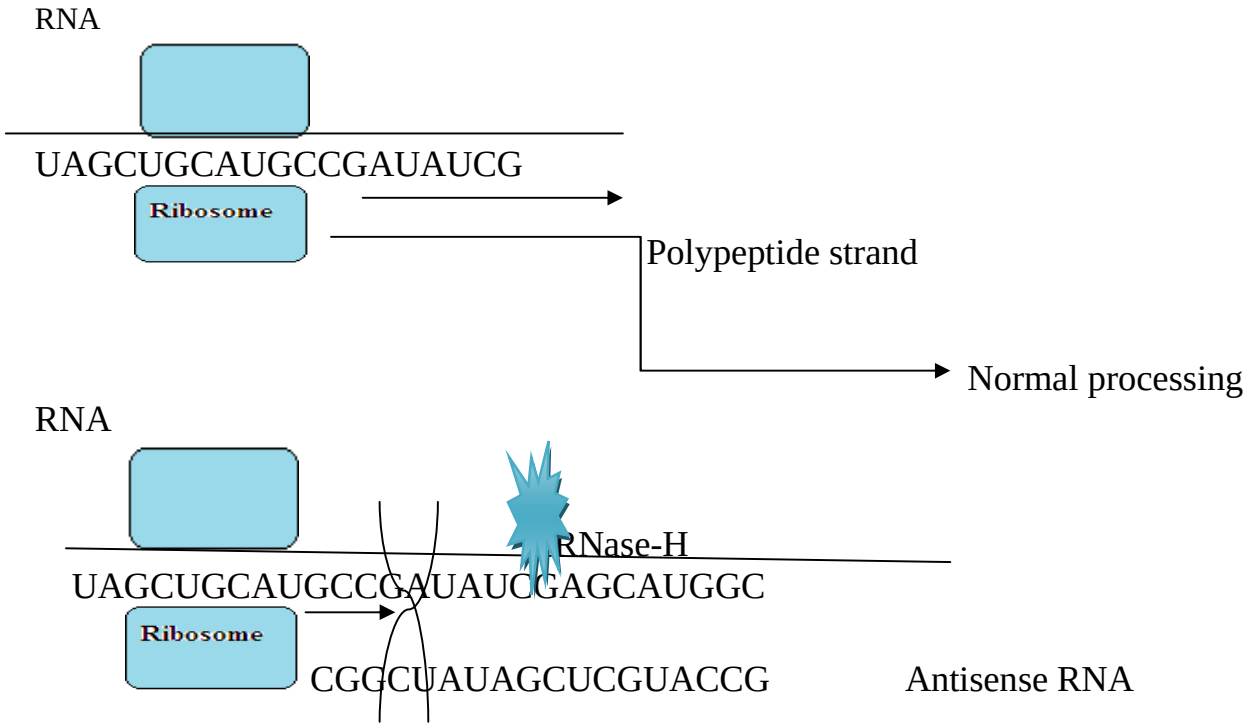


Fig-1: Antisense oligonucleotide transcription of mRNA

Oligonucleotides are referred as miniature stretches of DNA or RNA sequences. Antisense oligonucleotides contain 13-25 nucleotides, single stranded, synthetically prepared strands of deoxynucleotide sequences or analogs which are complementary to the specific regions of cellular mRNA or DNA sequence of the target gene. Antisense molecules composed of single stranded DNA are termed antisense oligodeoxynucleotides (As-ODNs) [12, 13]. Interference with the process of gene expression, blockade of translation or degradation by attracting RNase-H activity are the measures utilized by antisense oligodeoxynucleotides. RNA interference

(RNAi) mechanisms for sequence-specific gene silencing are new horizons in the application of antisense technology [14].

Approximately 15 percent of genetic diseases are caused by defects in splicing. Although correct splice sites exist, mutant splice sites may also be present, resulting in aberrantly spliced mRNA. Antisense oligonucleotides (As-ODNs) can be used to alter transcript splicing patterns. They can direct the splicing machinery to use the wild-type splice donor and acceptor sites, thus resulting in correctly spliced mRNA and functional protein. The most dramatic example of this type of antisense therapy concerns the ex vivo correction of splicing defect that leads to a form of ß-thalassemia. Specifically, elytroid cells from thalassemic patients treated with antisense oligonucleotides targeted aberrant splice sites, thereby correcting the nature of hemoglobin. A wide variety of other genetic diseases occurs due to aberrant splicing which could be corrected with antisense-based therapy [14]. When the genetic sequence of a particular gene responsible for a specific disease is known, it becomes easier 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 by effectively turning that gene "off". Alternatively, the strand might be targeted to bind a splicing site on pre-mRNA and modify the exon content of an mRNA [15].

Design for novelty in antisense oligonucleotides

One of the hurdles with As-ODNs is their rapid degradation in blood and in cells by exonucleases and endonucleases. To remedy this, modified backbones were introduced that resisted nuclease degradation. These modifications included subtle as well as not too subtle changes to the phosphate or the sugar portion of the oligonucleotide. A simple and straightforward modification was the replacement of non-bridging oxygen on the phosphate backbone with sulfur, producing a phosphorothioate linkage [16, 17]. This modification has the distinct ability of retarding nuclease degradation of oligonucleotides. Most of the oligonucleotide constructs in clinical trials are generally phosphorothioates without any modification. The bear a lower cost coupled with possession of desired properties such as antisense drug - nuclease resistance and retention of RNase-H activity [18].

Oligonucleotide modification involves replacement of the hydrogen at the 2_ position of ribose by an *O*-alkyl group, such as methyl moiety. These oligonucleotides form high melting heteroduplexes with targeted mRNA and induce an antisense effect by a non RNase-H dependent mechanism. Stable oligonucleotides lacking the natural phosphate-ribose backbone have also been designed. They are capable of producing stable duplexes or triplexes with single or doublestrand DNA or RNA. Phosphorodiamidate morpholino oligomers have several properties considered desirable for antisense purposes. The deoxyribose moiety is replaced by a morpholine ring, and the charged phosphodiester intersubunit linkage is replaced by an uncharged phosphorodiamidate linkage. These oligonucleotides are very stable in biological systems and exhibit efficient antisense activity. An example of a second-generation oligonucleotide is the N3_3P5_PN, which result from the replacement of oxygen at the 3_position of ribose by an amine group [18].

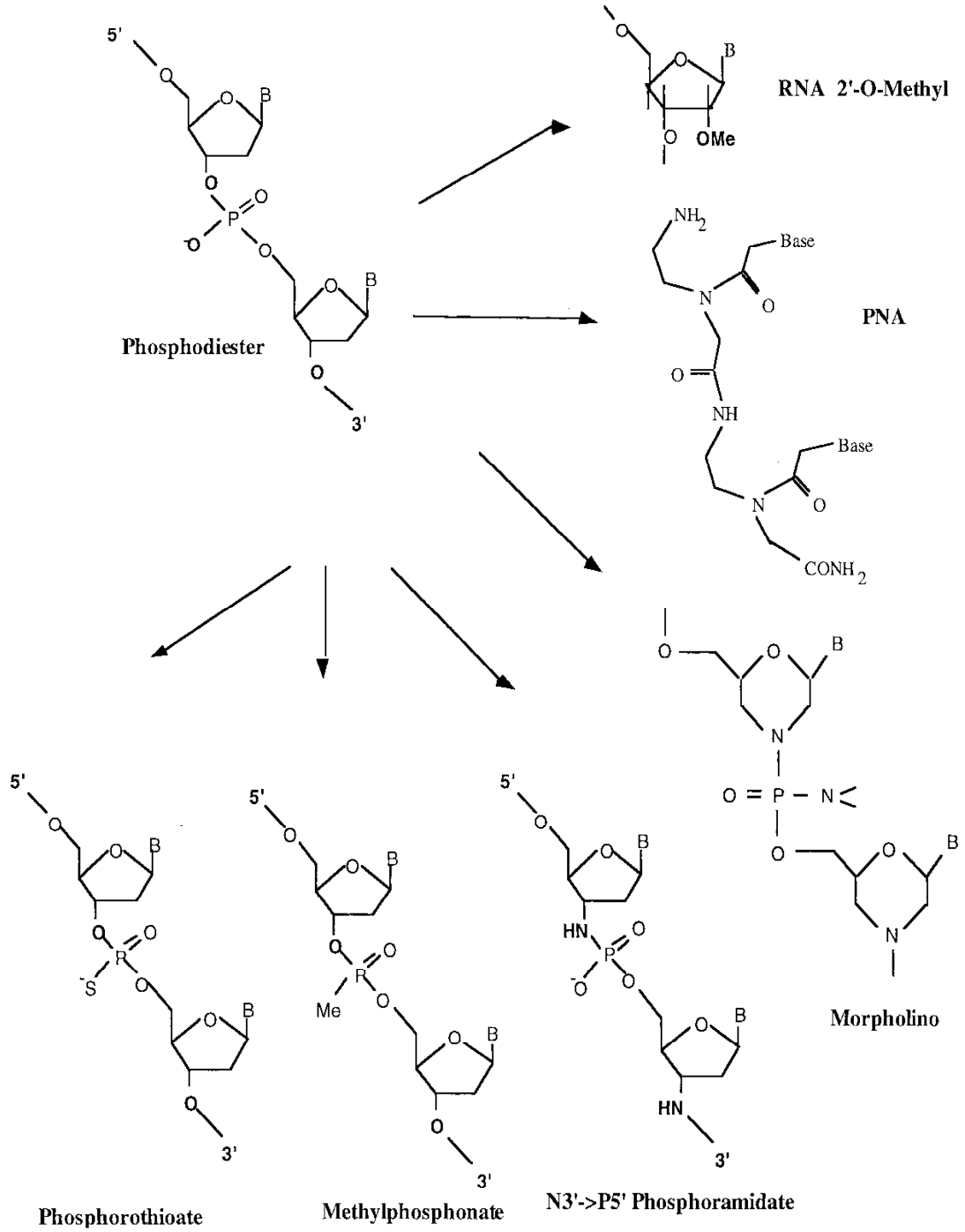


Fig-2: Chemical structures of oligonucleotides, peptide nucleic acid (PNA)

Family tree of Antisense Oligonucleotides

First-generation antisense oligonucleotides:

Phosphorothioate DNA (PS)

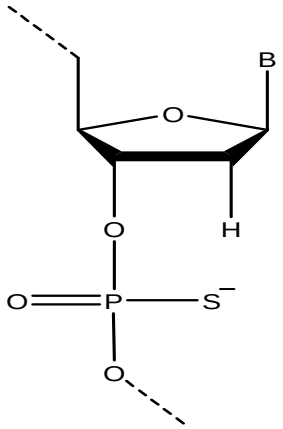
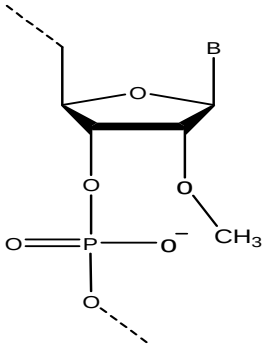


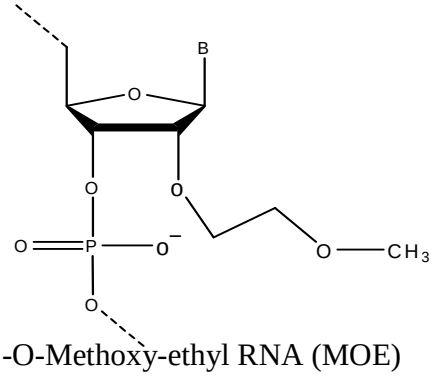
Fig-3: B denotes one of the bases adenine, guanine, cytosine or thymine.

Phosphorothioate oligodeoxynucleotides (PS-ODNs) represent the first generation DNA analogs. Replacement of one of the non-bridging oxygen atom in the phosphate group with either sulfur group (phosphorothioates), methyl group (methyl phosphonates) or amines (phosphoramidates) can result in the conception of As-ODNs. The first generation ODNs shows more resistance to nucleases and longer plasma half life as compared with phosphodiester oligonucleotides. Due to their nuclease resistance, PS-ODNs form regular Watson–Crick base pairs, activate RNase-H, carry negative charges for cell delivery and display attractive pharmacokinetic properties. The disadvantages of the first generation As-ODNs are their side effects such as immune stimulation and complement activation due to interaction with poly anions such as heparin binding proteins [19].

Second-generation antisense oligonucleotides:



2’-O-Methyl RNA (OMe)



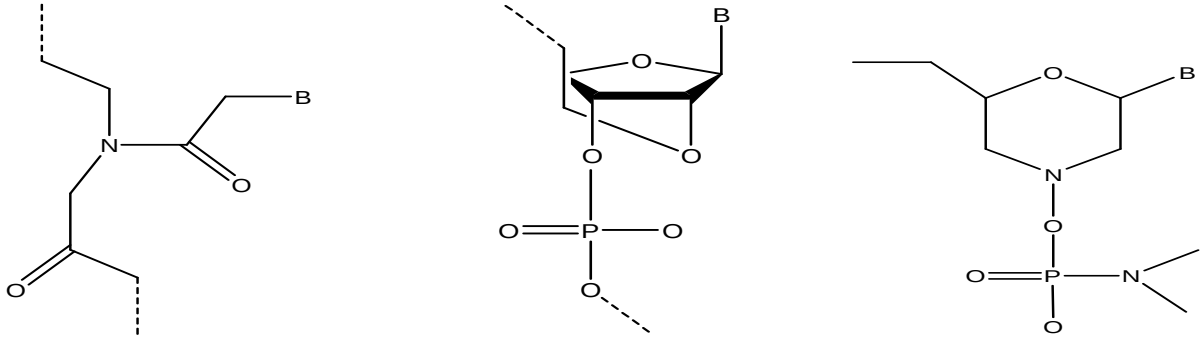
2’-O-Methoxy-ethyl RNA (MOE)

Fig-4: B denotes one of the base adenine, guanine, cytosine or thymine

Poor binding affinity, hybridization stability with target mRNA and poor nuclease resistance are the major deterrents in the use of first generation As-ODNs. In order to circumvent these

problems, second generation As-ODNs with alkyl modifications at the 2' position of ribose, were developed. Commonly used second generation As-ODNs are 2'-O- Methyl (2'-OME) and 2'-O-Methoxyethyl (2'-MOE) ODNs. 2'-OME and 2'-MOE have the ability to activate RNAse-H, an endonuclease, whose involvement is vital for the activity of As-ODNs. RNAse-H activation is induced by developing chimeric As-ODNs, surrounded by a central gap region consisting of a phosphorothioate deoxyribose core, with nuclease resistant arms such as 2'-OME or 2'-MOE. The "gapmer" allows RNAse-H to occupy the central gap facilitating activation of target specific mRNA degradation. Due to alkyl modification of arms, degradation of As-ODNs by nucleases can be prevented. Second generation As-ODNs have a higher affinity for mRNA, better tissue uptake, increased resistance to nucleases, longer in vivo half life and lesser toxicity as compared to first generation As-ODNs. GEM 231 and GEM 92 (Hybridon) are examples of second generation As-ODNs currently being tested in clinical trials [20].

Third-generation antisense oligonucleotides



Peptide nucleic acid (PNA) Locked nucleic acid (LNA) Morpholino phosphoroamidates (MF)

Fig-5: B represents one of the bases adenine, guanine, cytosine or thymine

A spurt in the development of third generation chemically modified As-ODNs began with modification in the furanose ring, phosphate linkages; ribose as well as nucleotides has led to the generation of newer ODNs. Locked nucleic acid (LNA), peptide nucleic acid (PNA) and morpholinophosphoroamidates (MF) are the three most commonly used third generation As-ODNs. Enhancement in target affinity, greater hybridization affinity with mRNA, nuclease, peptidases stability and alteration in pharmacokinetic profiles are some of the measures incorporated in the development of third generation As-ODNs. In addition, PNAs have the ability to recognize double stranded DNA and are capable of modulating gene expression or inducing mutation by strand invasion of chromosomal duplex DNA. However, third generation As-ODNs do not activate RNAse-H and are most likely to produce their biological effects by causing steric hindrance of ribosomal machinery resulting in translational arrest. Presence of uncharged molecules does not permit them to interact with serum proteins. This reduces non-specific interactions and hastens clearance from the body. However, electrostatically neutral backbones of As-ODNs make their solubility and uptake difficult [21].

Design and mechanism of action of antisense oligonucleotides

The main principle involved in antisense oligonucleotide technologies is specific inhibition of unwanted gene expression by blocking mRNA activity. Antisense oligonucleotides are recognized to be very efficient tools for the inhibition of gene expression in a sequence-specific manner. They are designed to target specific mRNA sequences via Waston-Crick base pairing, there by inhibiting the translation process [22]. ODNs are usually agonists or antagonists of receptors, or they inhibit or stimulate enzymes or protein protein interactions. They have a distinct ability of down regulating gene expression thus enabling them to progress into rational forms of drug therapy. Ribozymes, aptamers, spiegelmers and immunostimulatory cytidine-phospho-guanosine (CpG) ODNs are some of the other therapies working on an identical concept. The essential steps in rational drug design are the identification of an appropriate target responsible for certain disease and the development of a drug with a specific affinity to that target. One of the most general approaches of drug targeting is the specific manipulation of gene expression at the DNA or RNA stage of protein synthesis [23]. Testing as many oligonucleotides as possible improves the chance of identifying one that is particularly potent. This can be expedited by monitoring gene expression using RT-PCR in a 96- well format or utilizing other high-throughput procedure such as micro-array technology. A significant advantage of As-ODNs to traditional small-molecule-based drug development is the ability to target any gene, irrespective of the protein structure. Therefore, this biotechnological revolution could serve as a primary focus for novel drug development [24]. The success of As-ODNs relies on several factors such as cell permeability, nuclease resistance and target binding affinity. Efficacies of As-ODNs can be enhanced by recruiting RNase-H to cleave only the RNA strand of the As-ODN: RNA hybrid duplex [25].

Antisense ODNs exerts its mechanisms of action by blockage of translation, RNA transport and splicing. Antisense ODNs hybridize with target mRNA, thereby providing a steric block for the translation machinery [26] or an effective target for RNase-H resulting in mRNA degradation [27, 28]. The RNA-DNA hybrid can be recognized and cleaved by RNase-H, yielding an additional mechanism of repression of gene expression (**Figure 1**). ODNs consisting of regions of DNA (e.g., phosphodiester, phosphorothioate and chimeric) can act by recruiting endogenous ribonuclease H (RNase H), an enzyme that recognizes RNA–DNA duplexes and cleaves the RNA strand. Modified antisense oligomers that do not recruit RNase-H can inhibit protein synthesis by hybridizing in the 5' untranslated region or near the AUG start codon, which blocks ribosome assembly. Such oligomers can also inhibit splicing when hybridized to intron–exon junctions. As-ODNs cause direct inhibition of the target gene at the RNA or protein level. Alternatively, antisense RNA generated by expression cassettes or viral vectors can block target gene expression through activation of double-stranded RNase-H [29]. Target specificity and functional consequences would be high in order to down regulate protein expression [30-33].

mRNA molecules have a distinct property of forming complex secondary and tertiary structures in vivo and are capable of interacting with a number of proteins. Selection of accessible target sequence for As-ODNs becomes difficult and different approaches have been proposed to overcome this obstacle. Software like RNAFOLD provides proposals to predict mRNA structure. Ionic strength, temperature variations, interaction with proteins is some of the intricate events taking place within a cell which can strongly affect mRNA structures. Sophisticated methods relying on DNA microarrays or on the screening of libraries of ODNs in presence of the RNase-

H have been proposed. Testing As-ODNs in cell cultures could be a successful prospect as might be masked due to folding of mRNA in the presence of cytoplasmic protein [34].

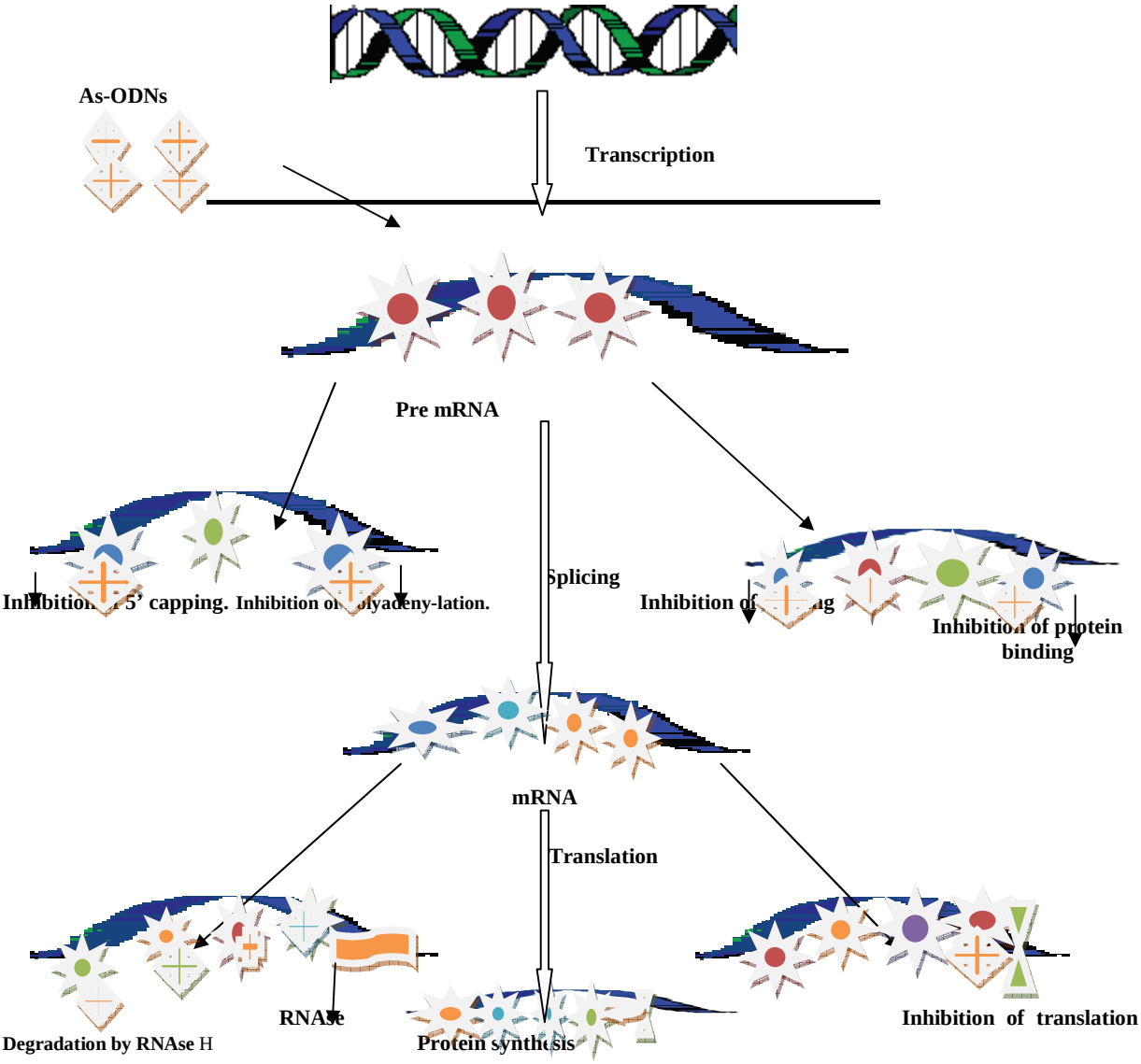


Fig-6: Mechanism of action of antisense oligonucleotides. As-ODNs interacts with the target mRNA by sequence-specific base pairing with in the cell. As-ODN-mRNA duplex then prevents protein synthesis by interfering with various steps of mRNA synthesis

Potential clinical applications of antisense therapy

Antisense technology developed for the treatment of different human diseases would play a major role in future healthcare.

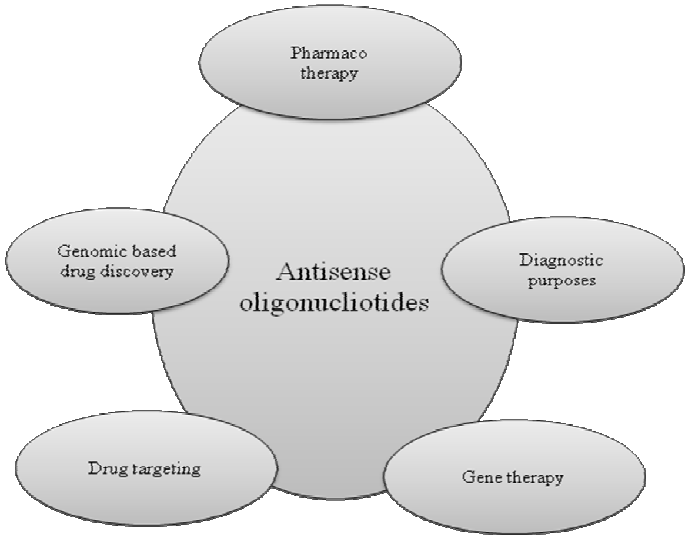


Fig-7: Utilization of As-ODNs

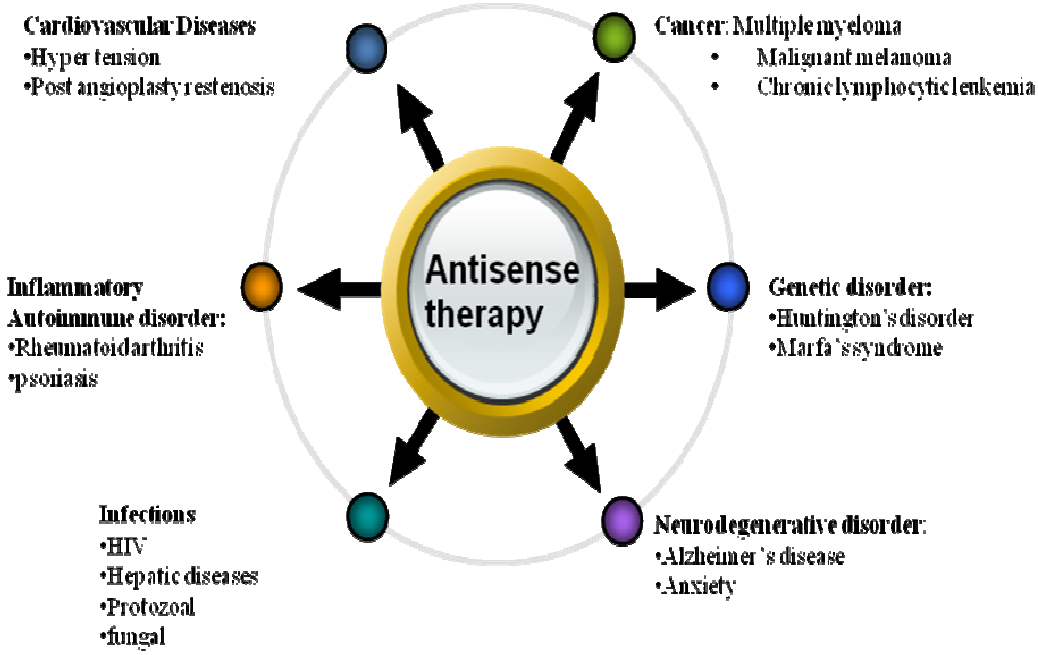


Fig-8: Clinical uses of As-ODNs

ß$_1$antisense oligonucleotides in cardiovascular diseases:

ß$_1$-As-ONDs are specifically designed to act against the ß$_1$-adrenceptors (ARs) through down regulation mechanisms by reducing cardiac output and ventricular contractility. ß$_1$-As-ODNs could regulate heart rate [35]. As-ODNs directly act on ß$_1$-ARs mRNA and block the expression of ß$_1$-AR in the ischemic myocardium [36]. ß$_1$-As-ODNs developed as cationic liposomes are capable of achieving sustained hypotension. The advantages of the ß-As-ODNs over ß-blockers are their ability to reduce blood pressure without affecting heart rate [37, 38].

Antisense Therapy in Cancer

Antisense therapy in oncology is beneficial as the speed of their production is very rapid, generally utilizing less than a week. A more consistent response is expected with ODNs they are capable of inhibiting mRNA by delaying clonal expansion. The affinity of ODNs for the cancer target is generally of higher magnitude unlike conventional therapy which utilizes van der walls forces to bind protein targets. Antisense therapy in oncology mainly utilizes specific 20 nucleotides synthesized to be complementary to the specific "sense" (5' to 3'orientation) mRNA sequence responsible for coding of targeted protein. The important targets of As-ODNs in cancer therapeutics are proteins such as B-cell lymphoma-2 (Bcl-2), Harvey Rat Sarcoma Viral Oncogene (V-Ha-Ras) and Protein Kinase C-Alpha (PKC-Alpha.) These proteins exert an effect on the development, growth and maintenance of several types of cancer [39]. Other antisense oncogenes belong to c myb, bcr/abl, and K-ras family. In oncogenesis, growth factors and their receptors also play major role. TGF-beta, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF) are some important targets for anti-tumor gene therapies. In neoplastic differentiation, insulin-like growth factor (IGF-I) and its receptor (IGF-I-R) plays a significant role. Therefore IGF-I antisense and IGF-I-R antisense gene therapies are used to treat different malignant tumors [40].

Bcl-2

Bcl-2 is a mitochondrial-membrane protein which blocks apoptosis. Development of tumors and resistance to chemotherapy can ensure due to over expression of Bcl-2. G3139 an antisense oligonucleotide is used to treat wide variety of cancers, such as multiple myeloma, malignant melanoma, chronic lymphocytic leukemia (CLL), non- Hodgkin's lymphoma (NHL), breast cancer and small cell lung cancer (SCLC). G3139 and dacarbazine combination produce favorable results in cancer treatment [41, 42].

Table1: Antisense Agents and their Targets

AGENT	TARGET
Geneses (oblimersen)	Bcl-2
Affinitak (ISIS 3521)	PKC-alpha
ISIS 112989 (OGX 011)	Secretory Protein Clusterin
ISIS 23722	Survivin
AP 12009	TGF-Beta2
GEM 231	Protein kinase A
GEM 240	MDM2
IGF-1R/AS ODN	Insulin-like growth factor
MG98	DNA methyltransferase
LErafAON	C-raf-1
Ki-67 AS-ODN	Ki-67
GTI-2040	Ribonucleotide reductase
ISIS 2503	H-ras
AP11014	GFBeta1

Protein kinase Cα (PKC)

Protein kinase Cα is a cytoplasmic serine-threonine protein kinase family member. It controls cell proliferation by myriad signal transduction pathways. Currently 13 iso forms of PKC

inhibitors are available which are unable to differentiate between the various isoforms there by reducing specificity. As-ODNs are underway which can challenge a specific isoform and interfere in the growth of tumour [43].

H-ras

Ras family components are mainly involved in cell-signaling pathways, control of cellular proliferation, differentiation and cell death. Abnormal cell growth and malignant transformation is also caused by mutation in genes encoding the ras family of proteins [44]. A 20 mer PS-ODN, ISIS 2503 (ISIS Pharmaceuticals) targets translation, affects initiation region of H-ras mRNA there by interfering with the expression of H-ras protein invitro [45].

Brain tumors and antisense approach

Manifestation of neuroblastoma is largely due to dysregulation of C-myb gene producing faulty gene expression leading to the dysregulation of hMYCN protein. IMR-32, a type of human neuroblastoma cell exhibits elevated levels of hMYCN gene which is treated with implanted micro-osmotic pumps for subcutaneous delivery of hMYCN ODNs. Differentiation of normal and abnormal cells depends up on supply of growth factors (IGF-I-R, IGF-I). As-ODNs targeting IGF-I-R and IGF-I gene are beneficial in containing growth of malignant tumor. In glioma invasion, uPAR and p16 tumor suppressor gene plays a significant role. It was observed that in tumor model, the growth of glioblastoma cell lines into tumor is mainly due to uPAR and p16 gene over expression which can be substantially inhibited by As-ODNs. Antisense oligonucleotides were found to produce an ample reduction in tumor incidence [46].

AS-ODNs role on TGF growth factor in scaring and fibrosis

Transforming growth factor beta 1 (TGF-ß1) plays a critical role in connective tissue remodeling, scarring and fibrosis. TGF-ß2 also plays a pivotal role in conjunctival scarring. Second generation phosphorothiated oligonucleotides are effective against TGF-ß1 and TGF-ß2. There by serving as powerful tools utilized to minimize conjunctival scarring and glaucoma. TGF-ß1 and Type-1 procollagen induces subcutaneous tissue injury, leading to an elevation in the levels of collagen. Double stranded oligodeoxynucleotide decoys decrease collagen production by indirectly inhibiting TGF- ß1 [47].

AS-ODNs therapy in Neurodegenerative disorders

Delivery of phosphorothioate As-ODNs encapsulated with a cationic lipid was capable of interfering with specific region of mRNA of 5HT1A receptor. Experimental evidence revealed efficacy of As-ODNs in down regulation of 5HT1A auto receptor expressed in RN46A cells, SN48 cells and LLP-K1 cells. Down regulation was due to effective impinging with expression of 5HT1A mRNA resulting in reduction in 5HT1A receptors [48].

AS-ONDs -Targeting viral nucleic acids

Viruses are particularly suitable targets for As-ODNs because they carry genetic information that is distinct from the host cells. As-ODNs are capable of specifically interfering with HIV genes. Inhibition of HIV replication is a complex process occurring by inhibition of virus adsorption to the host cell, inhibition of transcription via anti-sense or as the result of triple helix formation and inhibition of viral encoded enzymes such as reverse transcriptase and integrase [49, 50].

Antisense HIV oligonucletides have lower toxicity which is an advantage over existing medication. As-ODNs can act on the genome of a negative strand RNA virus by inactivation of RNase L, there by inhibiting viral replication. Viruses on which As-ODNs act are Influenza A, hepatitis B virus (HBV), hepatitis C virus (HCV), Cytomegalovirus (CMV), Human papillomavirus (HPV), Respiratory syncytial virus (RSV), Herpes simplex virus (HSV), Epstein - Barr virus (EBV) and HIV [51].

Human immunodeficiency virus (HIV)

As-ODNs that target HIV genome are being evaluated in clinical trials. The potential therapeutic effect of synthetic oligonucleotides was first published in 1978 and showed the inhibition of Rous sarcoma viral RNA translation and viral replication in cells. Production of human immunodeficiency virus (HIV) in both acutely and chronically infected human cells can be inhibited by unmodified and modified antisense oligonucleotides. Recently, expression of human papilloma viral gene was inhibited by a 20-residue phosphorothioate oligomer [52].

Cytomegalovirus (CMV)

These are DNA viruses exhibiting biological properties of latency and reactivation. CMV infections are the major causes of morbidity and mortality in most immune compromised patients. Antisense oligonucleotides are being used for the inhibition of CMV replication. ISIS 2922 is used for the treatment of CMV induced retinitis in AIDS patients [53].

Antisense oligonucleotide therapy in Hepatic diseases:

Liver injury and inflammation can cause chronic liver disease and hepatic fibrosis. Fibrosis of liver may be produced in hepatitis B (HB) and hepatitis C viral (HCV) infection. Tissue inhibitors of metalloproteinase gene-1 (TIMP-1) and tissue inhibitors of metalloproteinase gene-2 (TIMP-2) play a major role in development of liver fibrosis. Inhibiting the expression of TIMP-1 by antisense oligonucleotides can prevent liver fibrosis by decreasing the deposition of collagen 1 and 111 [54].

Anti-Inflammatory Action

Intercellular adhesion molecule 1 (ICAM-1) play an important role in the trafficking and activation of leukocytes and is up-regulated in inflamed mucosa in Crohn's disease. ISIS 2302, As-ODNs inhibits ICAM-1 expression. Trials with the ICAM-1 antisense oligonucleotide, ISIS-2302, showed that the drug is well-tolerated and provides a promising therapy for Crohn's disease and ulcerative colitis [55].

Antisense Therapy for Genetic Disorders

Approximately 15 percent of genetic diseases are caused by defects in splicing. Antisense oligonucleotides (As-ODNs) can be used to alter transcript splicing patterns. Genetic disorders like Huntington’s disease and Marfa’s syndrome could be treated with antisense therapy. Synthesis of huntingtin in a human terato carcinoma cell line can be inhibited by phosphorothioate oligodeoxynucleotides [56]. Marfan’s syndrome which is mainly due to negative effect of mutant fibrillin can be down regulated by antisense oligonucleotides [57]. Huntington’s disease and myotonic dystrophy occurs due to mutations in trinucleotide repeats. Successful targeting and trans splicing of such genes showing trinucleotide repeats with ribozymes has been achieved [58, 59].

Oligonucleotide Uptake

- **Microinjection:** Microinjecting oligonucleotides into cells results in rapid accumulation of the oligonucleotide in the nucleus ensures site directed release and minimizes toxicity [60].
- **Receptor-mediated endocytosis**: Oligonucleotide enters into living cells through receptor-mediated endocytosis. Small oligonucleotides are taken up more rapidly than long oligonucleotides. This uptake can be competitively inhibited by other small oligonucleotides, especially if they contain a 5' phosphate. Uptake of ODNs is temperature-dependent, occurring more rapidly at 37^{o}C than at 4^{o}C. Modifying the oligonucleotide can change its uptake efficiency [61].
- **Lipids:** The entry of wide array of compounds including As-ODNs into cells can be achieved by using cationic lipids. Initially, the DNA cation lipid complex is internalized into the endosome. Anionic lipid which is present on cytoplasmic face of endosome fuses with cationic transfect ion agent, displacing the oligonucleotides into the cytoplasm [62, 63].
- **Encapsulation:** Encapsulation of concentrated oligonucleotides in lipid vesicles by minimum volume entrapment method enhanced their uptake 20 times and retained their stability in serum. Oligonucleotides formulated as liposome can be detected in the nucleus for up to two weeks after administration by intravenous, subcutaneous, or intraperitoneal injections [64].

Other methods of facilitated entry

Group of cells pretreated with streptolysin O exhibited better oligonucleotides permeation with minimal cellular toxicity [65]. Antennapedia homeodomain protein is translocated through cell membranes and targeted to nuclear localization. A 16–amino acid peptide fragment from the third helix has shown to confer this property to the protein [66]. This agent protected the oligonucleotide from nucleolytic degradation, enabling the use of phosphodiester DNA as an antisense agent [67]. This peptide is commercially available with a sulfhydryl-modifier enabling its attachment to a SH-modified oligonucleotide. Glycoprotein tagging has also been used to modify oligonucleotide to target them for uptake into specific cell types such as hepatocytes [68].

Targeted Delivery of Oligonucleotides

As-ONDs enter into the endosomal or lysosomal compartments through pinocytosis, endocytosis and caveolar potocytosis. As-ONDs of large size and ionic charges have difficulty in diffusing across the plasma membrane. In order to achieve the specific target delivery, phosphorothioate or phosphodiester oligonucleotides are conjugated to an OX-26-Streptavidin vector to improve penetration across the blood-brain barrier [69].

CONCLUSION

Very few As-ODNs were capable of passing the rigors of clinical trials with only a handful of them being subjected to testing in 1996. The past few years has seen explosive growth in the number of antisense related clinical trials. Formivirsen is the first As-ODNs that have been approved by the FDA for local administration to treat CMV retinitis. This has paved the way for an array of antisense therapeutics to undergo testing. This will be a boon for the management of intractable diseases.

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