Quest Journals
Journal of Research in Pharmaceutical Science
Volume 2 ~ Issue 3 (2014) pp: 01-13
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Antisense Oligonucleotide: Basic Concept and its Therapeutic Application

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Abstract:- Antisense oligonucleotides are synthetic genetic materials that interact with natural genetic material and modulate them in a systematic way. Antisense oligonucleotides as a form of molecular medicine to modulate gene function was first acknowledged in the late 1970s. This therapy involves blocking translation, thereby inhibiting protein formation. Recently, antisense technology has been resurrected and has generated considerable enthusiasm in the research. Antisense oligonucleotides have proven to be valuable in gene functionalization and target validation and also represent a novel therapeutic strategy for wide range of diseases such as genetic disorders, cancers, and infectious diseases. Thus, in the present review an attempt is made to help the apprentice understand the basic concept of the antisense technology and its therapeutic applications.

Keywords:- Antisense oligonucleotide, antisense technology, cancer, genetic disorders, Infections

I. INTRODUCTION

An antisense oligonucleotide [ASO]refers to a short synthetic strand of deoxyribonucleotide analogue that hybridizes with the complementary mRNA via Watson–Crick base pairing.ThemRNA in RNA-DNA duplexis a substrate for cellular Ribonuclease H [RNase H],an enzyme that destroys the RNA. RNase H cleaves the RNA-DNA duplex region of the mRNA thus induce a blockade in thetransfer of genetic information from DNA to protein. [1]

Antisense oligonucleotides have been used to modify the expression of specific genes. [2]They are not only usefulin the study of loss-of-gene function and target validation, but also act as a novel therapeutic strategy to treat any disease that is linked to dysregulated gene expression [Table-1].Antisense oligonucleotides can also manipulate alternative splicing, thus can be usedto modulate the ratio of different splice variants or correct splicing defects[3].

II. MECHANISM OF ACTION

ASO is taken up by cellular endocytosis, hybridize with the target mRNA resulting in the formation of ASO-mRNA heteroduplex leading in majority of times to: either activation of RNAse H or sterichindrance of ribosomal subunit binding.Both these mechanisms result in selective degradation of bound mRNA and ultimately target protein knockout.

RNase H-dependent oligonucleotides can induce the degradation of mRNA when targeted to any region of the mRNA. However, the steric-blocker oligonucleotides physically avert the progression of splicing only when targeted to the 5'or AUG initiation codon region. [4]

Other mechanisms by which ASO can act is by entering the nucleus directly and altering maturation of mRNA,splicing activation, 5'-cap formation inhibition, arrest of translation and double strand RNAse activation. [5]

III. OLIGONUCLEOTIDE ALTERATIONS

Oligonucleotides with natural phosphodiester bonds have short stability and are highly susceptible to rapid degradation by intracellular endonucleases and exonucleases. Thus chemical modificationshave been developed to enhance nuclease resistance,cellular uptake, distribution, prolong tissuehalf-life, increase affinity and potency. [1] The modifications can be made to the nucleobases, sugar moiety [especially at the 2' position of the ribose] or phosphate backbone. [6] Oligonucleotides with modified sugar moieties and phosphate backbones are divided into three generations.

3.1 First-generation ASOs- Phosphorothioate [Fig 1]

First generation ASOs are those in which one of the non-bridging oxygen atoms in the phosphodiester bond is replaced by a sulphur atom which introduces chirality at phosphorus The phosphorothioates are the most widely studied oligonucleotides. The advantages of phosphorothioate oligonucleotide includes the relative ease of synthesis and higher bioavailability by conferring higher resistance to the ASO against nuclease degradation, and the. They are highly soluble and are also capable of activating RNase H. [4] However, the stability of a phosphorothioate oligonucleotide has been shown to vary with each sequence and the cell line examined. [7]

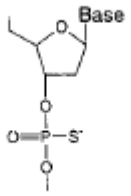


Fig 1. Phosphorothioate DNA

The disadvantages of this modification are slight reduction in the affinity of the ASO for its mRNA target because of decrease in the melting temperature of the ASO–mRNA heteroduplex approximately by 0.5 degree C per nucleotide[8] and production of non-specific effects by interactions with cell surface and intracellular proteins. [9, 10]

After a single application to tissue culture cells, the antisense effects of the phosphorothioates can be observed for over 48 hours. [11] Pharmacokinetics study of phosphorothioates in mice have demonstrated that following intravenous or intraperitoneal administration, it is distributed in most of the tissues, is degraded mostly by exonucleases and that up to 30% is excreted in urine in 24 hour and an additional 10% in 24-48 hours. [12]

Phosphorothioate oligonucleotide can be further modified by the addition of C-5 propyne pyrimidines to increase their relative binding affinities and compensate for the decrease in melting temperature. Propyne-modified oligonucleotide allow for a decrease in the length such that oligonucleotide as short as 11 bases can have potent antisense effects. [13]

3.2 Second generation ASOs [Fig 2]

Second-generation ASOs with 2'-O-alkyl modifications were developed to further enhance nuclease resistance and increase binding affinity for target mRNA. 2'-O-Methyl (2'-O-Me) and 2'-O-Methoxyethyl (2'-O-MOE) modifications ofPhosphorothioate -modified ASOs are the two most widely studied second-generation ASOs. [14] Other substitutions that can be made at the ribose 2' position includes 2'-fluoro, 2'-O-propyl, and 2'-O-pentyl which can alter an oligonucleotide nuclease stability and binding properties. [15]These second generation ASOs are less toxic than phosphorothioate oligonucleotide and have slightly greater affinity towards their complementary RNAs.[15]

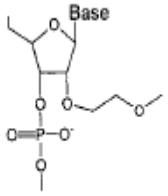
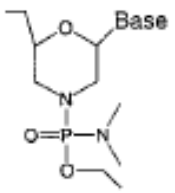


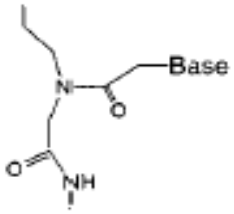
Fig 2. 2'-methoxyethyl RNA

Antisense effects of these second generation ASOs may be attributable to the steric block of translation.Methyl and ethyl substitutions and 2' modified analogs were shown not to support RNase H-mediated cleavage of target mRNA. [16]

However, the most desirable mechanism for antisense effect is the cleavage of target RNA by RNase H. Gapmer technology is used to circumvent this shortcoming which consists of chimeric ASO with central 'gap' region consisting of phosphorothioate oligonucleotide [sufficient to induce RNase H cleavage] is flanked on both sides (5' and 3' directions) by nucleotide 'wings' composed of 2'-OMethyl or 2'-Methoxyethyl modified nucleotides. [6]

3.3 Third-generation ASOs [Fig 3-5]





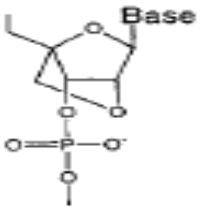


Fig 3. Peptide Nucleic Acid (PNA) **Fig 4 Locked Nucleic Acid (LNA)** **Fig 5. Morpholino oligonucleotide**

The third generations ASOs were developed to further enhance target affinity, nuclease resistance, biostability and pharmacokinetics. [14]Peptide nucleic acid ([PNA) [Fig 3], locked nucleic acid (LNA) [Fig 4] and phosphoroamidate morpholino oligomer [PMO] [Fig 5] are the three most studied third-generation ASOs.

In PNAs, the sugar-phosphate backbone is replaced with a pseudo-peptide polymer. [17] They are electrostatically neutral molecules, with high biological stability and favorable hybridization properties. [17] PNAs do not contain any (pentose) sugar moieties or phosphate groups and exhibit little or no binding to serum proteins. [18] PNA which is not a substrate for RNase H act by forming a sequence-specific duplex with mRNA, which then causes steric hindrance of translational machinery leading to protein knockdown.[19]PNAs are electrostatically neutral molecules with high target affinity, specificity and stability.[18] Experimentally and therapeutically, PNA could not be explored as a regulator of gene expression because of its poor cellular uptake. However it has been demonstrated that intracellular delivery can be improved bymicroinjection, electroporation, co-transfection with DNA or pairing of PNAs with negatively charged oligomers, lipids, or peptides. [20]

PNAs have also demonstrated its usefulness in cytogenetics for the rapid in situ identification of human chromosomes and the detection of aneuploidies.PNAs could become a powerful tool for in situ chromosomal investigation.[21]

LNA is a ribonucleotide comprising of a methylene bridge that connects the 2'-oxygen of the ribose with the 4'-carbon. This structure increases the binding affinity for complementary sequences and offers a new chemical approach for the control of gene expression and optimization of microarrays. [22]Oligos containing locked nucleic acids [LNAs] possess enhanced affinity towards target mRNA and DNA, are resistant to nuclease degradation and are not a substrate for RNase H.[23]

LNA monomer can be freely incorporated into DNA to form chimeric oligonucleotides (DNA/LNA co-polymers) resulting in stable, non-toxic and potent antisense oligonucleotide that are able to recruit RNase H. [24]LNA oligonucleotide has been demonstrated as a most promising molecule for the development of oligonucleotide-based therapeutics for gene silencing, [25] suppression of tumor growth, [23] modulation of RNA splicing [26, 27] and RNA interference. [28] Suppression of Tat-dependent transcription and telomerase activity has been efficiently achieved by LNA oligomers. Cleavage of highly structured RNA has also been achieved using LNA-modified DNAzymes. Furthermore, application of LNA to nucleic acid diagnostics has also been reported. [29]

Phosphorodiamidate morpholino oligomers (PMOs) are nonionic ASO in which the ribose sugar is replaced by morpholino ring and the phosphodiester bond is replaced by a phosphoroamidate linkage. PMOs interfere with target gene expression either by binding and sterically blocking the assembly of translation machinery resulting in inhibition of translation, or by altering splicing of pre-mRNA. They do not act by RNAase H mechanism. They possess favorable hybridization, nuclease stability, and toxicity profiles. [30] Both animal and human studies have demonstrated the efficacy of Phosphoroamidate morpholino oligomer. [31-33]

IV. DELIVERY OF OLIGONUCLEOTIDE

ASOs penetrate into the targeted cells through active transport [adsorptive endocytosis and fluid phase pinocytosis], which in turn depends on temperature and concentration and structure of the oligonucleotide.[34-36]Oligonucleotides endocytosis has shown to be mediated by the nucleic acid specific receptors.[35] Naked oligonucleotides are internalized poorly by cells. [37] Numerous delivery strategies have been developed to improve cellular uptake of the ASO.

Cationic liposomes, such as Lipofectin and Transfectam have been used to protect ASO and to ease their entry into the cell. [38] These liposomes have high affinity for negatively charged cell membranes and are delivered by endosomal pathway into cells. It has been documented that helper molecules such as

dioleoylphosphatidylethanolamine, when added into the liposomes allow the oligonucleotides to escape from the endosomes.[39, 40] Liposomes or immunoliposomes have been demonstrated to increase the delivery of synthetic antisense oligonucleotides into human myeloid and lymphoid leukaemia cells. [41]

Dendrimer are spherical and highly branched polymers with cationic polyamidoamine moieties proficient of forming a covalent complex with the ASO. The dendrimer–ASO complex offers advantage over the liposomal formulation by being stable and active even in the presence of serum. It enhances the delivery of ASO into the cytosol and nucleus and also increases the retention time of ASO in the cells. [1] Starburst polyamidoamine[PAMAM]dendrimers are a new type of cationic polymers with a molecular architecture characterized by regular, dendric branching with radial symmetry and modest toxicity. [42,43]

ASO can also be conjugated to cell-penetrating peptides [CPP] that promote the cellular uptake of the ASO. CPP are relatively short (9–30 amino acids)polycationic peptides rich in arginine and lysine, with net positive charge. [44]Commonly used cell penetrating peptides include HIV-1 Tat protein, Transportan, Antennapedia protein of Drosophila, synthetic Pep-1 peptide. [45] Some of the internalization mechanisms proposed for the cellular uptake of CPPs include endocytosis and direct translocation or cell penetration. [46] CPP-based systems appear to be very versatile and efficient.

Recently, various newer staragies have been used to improve delivery of ASO to its target like use of monnosylated chitosan nanoparticles and conjugation with histidine rich peptide.[47, 48]

IV. BIOLOGICAL BARRIERS TO IN VIVO DELIVERY OF THE ASO

A major biological barrier between oligonucleotide and its ultimate site of action (cytosol or nucleus) is the rapid excretion via the kidney. Other major barriers include vascular endothelial wall, degradation by serum and tissue nucleases, uptake by the phagocytes of the reticuloendothelial system, slow diffusion through and binding in extracellular matrix and inefficient release from endosomes. [44]

V. TOXICOLOGY OF ASO

In general, ASO drugs have shown to produce dose-dependent, transient and mild-to-moderate toxicities manifested in rodents, primates and humans. Toxicity study of phosphorothioateoligodeoxynucleotide and its analogues done in animals have shown to cause thrombocytopenia, dose-dependent elevation of liver transaminases, reduction of the levels of alkaline phosphatase, albumin, and total protein. Splenomegaly, lymphoid hyperplasia, diffused multi-organ mixed mononuclear cell infiltrates, lymph nodes necrosis, cytological alterations and necrosis in hepatocytes and renal tubule regeneration were also observed. [49]

Clinical observations in monkeys included transient lethargy, periocular oedema, susceptibility to bruising, transient decreases in peripheral total leucocytes and neutrophil counts, hemoconcentration, and a brief increase followed by a prolonged decrease in arterial blood pressure, acute mortality. [50- 52]

Phosphorothioatedeoxynucleotides have also shown to bind to proteins like factor H, leading to activation of the alternative complement pathway and a transient self-limited prolongation of activated partial thromboplastin time (Aptt). [51]

Justin S Waters et al evaluated antisense oligonucleotide targeting bcl-2 in patients with non-Hodgkin's lymphoma and found no significant systemic toxicity. The patients developed skin inflammation at the subcutaneous infusion site. Other dose-limiting toxicities observed were thrombocytopenia, hypotension, fever, and asthenia. [53]

Stuart A. Grossman et al study results showed that the toxicities attributed to aprinocarsen an antisense oligonucleotide directed against protein kinase C-α were mild, reversible, and infrequent which included thrombocytopenia, elevations of ALT and AST, nausea, vomiting, and fatigue.[54]

VI. ASO AS THERAPEUTIC AGENT IN VARIOUS DISEASES

6.1 Muscular dystrophy

Duchenne muscular dystrophy [DMD] is a X-linked progressive muscle-wasting disease caused by frameshift mutations in the human DMD gene such that it disrupt the open reading frame, leading to aberrant translation and absence of the essential muscle protein dystrophin. The allelic disease i.e., Becker muscular dystrophy [BMD], is a much milder phenotype, caused by mutations maintaining the open reading frame, resulting in the production of a partially deleted but functional dystrophin.[55]

Treatment using ASOs for DMD aims to remove the mutated exon alone or together with additional exons to restore the reading frame and consequently induce the expression of "BMD-like" partially functional dystrophin protein. Pramono et al and Dunckley et al demonstrated for the first time the principle of the exon-skipping therapy for DMD in lymphoblastoid cells and cultured mouse cells respectively. [56, 57]

Lu Ql and co-workers tested the therapeutic benefits of ASOs in vivo in the mdx dystrophic mouse (carrying a mutation in exon 23 of the dystrophin gene) by combining a potent transfection protocol with a 2-O-

methylated phosphorothioated antisense oligoribonucleotide(2OMeAO) designed to promote skipping of the mutated exon. The treated mice showed persistent production of dystrophin at normal levels in large numbers of muscle fibres and functional improvement of the treated muscle was also seen. Repeated administration has shown to enhance dystrophin expression without eliciting immune responses. [58] In another study, functional and hybridisation array screens have been used to select optimised splicomers directed to exon 23 of dystrophin mRNA which carries a nonsense mutation in the mdx mouse. Splicomers were transfected into cultured primary muscle cells, and dystrophin mRNA assessed for exon exclusion. Splicomers were also administered to the muscles of mdx mice. The results of the study suggested that in cases for DMD correctly designed splicomers may have direct therapeutic value in vivo, [59]Use of the phosphorodiamidatemorpholino oligomers (PMOs) and peptide-linked PMOs [PMO-Pep] applied to DMD canine model, has shown to induce high and sustained levels of exon skipping and highest level of dystrophin expression with no apparent adverse effects upon in-vitro cells.[60]

The molecular therapy for Duchenne muscular dystrophy (DMD) by the process of exon recognition and intron removal during gene transcript splicing that converts dystrophin mRNA from out-of-frame to in-frame transcripts with antisense oligonucleotides is now approaching clinical application. Intramuscular injection of antisense oligonucleotide PRO051 had shown to induced dystrophin synthesis in four patients with Duchenne's muscular dystrophy who had suitable mutations. [61].Geomans and others administered weekly abdominal subcutaneous injections of antisense oligonucleotide PRO051 for 5 weeks intotal of 12 patients, with each of four possible doses (0.5, 2.0, 4.0, and 6.0 mg per kilogram of body weight), followed by a 12-week open-label extension phase, during which they all received PRO051 at a dose of 6.0 mg per kilogram per week. PRO051 induced detectable, specific exon-51 skipping at doses of 2.0 mg or more per kilogram and also showed dose-dependent increase in novel dystrophin expression in patients with Duchenne's muscular dystrophy, with only modest improvement in the 6-minute walk test after 12 weeks of extended treatment. [62] Another breakthrough study from UK reported that systemic administration of AVI-4658, a PMO based antisense, induced restoration of dystrophin expression in skeletal muscle of patients with Duchenne muscular dystrophy. This open label, phase II, dose-escalation study (0·5, 1·0, 2·0, 4·0, 10·0, and 20·0 mg/kg bodyweight) study showed good tolerability and safety profile with no serious drug related events with single doses of up to 900 mg and cumulative exposure exceeding 10 000 mg during 12 weeks of study period and thus consolidates the potential of AVI-4658 to become a disease-modifying drug for Duchenne muscular dystrophy. [63]

6.2 Cancer

The high specificity of binding of antisense oligodeoxynucleotide to their target mRNA make these compounds useful as therapeutic agents against human cancer. Antisense nucleic acids can be used to modulate the expression of selected genes, and to suppress malignant behaviour in cancer cells. Promising targets for antisense cancer therapy that have been extensively studied include proteases and protease receptors, telomerase, fusion genes, the Bcl family of proteins and various protein kinases. Extreme specificity of their expression allows them to target cancerous cells with minimal impact to healthy tissue.[64] The mechanism by which ASOs act is induction of apoptosis and sensitisation of cancerous cells to the cytostatic effect of anticancer drugs. Huo X & co-workers have documented slowing of the growth of the ovarian cancer cell line SKOV3 and reduction in its activity after being transfected by Wilmstumortumor suppressor gene 1 (WT1) antisense oligonucleotide, with the inhibition rate of 49.48%. WT1 antisense phosphorothioate oligonucleotides have shown to inhibit cell proliferation, arrest cell cycle at G0-G1 phase and induce apoptosis in SKOV3 ovarian carcinoma cells. Down regulation of WT1 mRNA and protein expression was also observed, which further contributed to the apoptosis. [65]Cyclin D1 antisense oligonucleotide was shown to inhibit cell growth stimulated by epidermal growth factor and induce apoptosis of gastric cancer cells.[66] Another study in ZL34 mesothelioma cell lines showed that treatment with antisense oligonucleotides 4259 or 4625 of Bcl-xL or of Bcl-xL and Bcl-2 both, respectively, induced apoptosis and also sensitized ZL34 cells to the cytostatic effect of cisplatin and gemcitabine. [67] Zhang et al (2012) tested a nanoscale-based tumour-targeted system containing two anticancer drugs, two antisense oligonucleotides and a ligand specific to receptors overexpressed in cancer cells in a mouse metastatic xenograft model. This approach demonstrated decrease in the primary tumour as well as prevention of intraperitoneal metastases along with limited side effects thus having potential for the management of ovarian carcinoma and its metastases with better patient compliance. [68] Overexpression of oncogenes such as HER-2, bcl-2/bcl-xL, protein kinase A, and transferrin receptor gene, V integrin gene etc have shown to affect the prognosisof breast cancer patients. Antisense therapy directed to inhibit specially the expression of these target genes have showed encouraging therapeutic effects in vitro and in vivo on breast cancer cell lines or xenografts.[53, 69-73] Combinations of antisense oligonucleotides with cytotoxic agents offer important advantages in cancer therapy. Studies have also demonstrated the synergistic antitumor effect when these antisense therapies are combined along with normal chemotherapeutic agents.[70, 74] Preclinical

data support the clinical evaluation of ASOs in many tumours including ovarian, nasopharyngeal, gastric, bladder, prostrate and many more.[66, 68, 75-78]

6.3 Thalassemia

El-BeshlawyA et al studied the effect of antisense oligonucleotides against the 3' aberrant splice site in beta-thalassemia Egyptian patients with IVSI-110 mutation. Peripheral blood mononuclear cells of ten thalassemia patients with IVS1-110 mutation were obtained duplicated and treated with 20 µmol/mlmorpholino ASO. Their study showed correction with ASON treatment in 50% of the cases, of which 2 cases showed the appearance of corrected mRNA band and 3 cases showed an increased ratio of the corrected to the aberrant mRNA band. These results suggest the applicability of ASOs for the treatment of thalassemia. [79]Sierakowska et al studied the use of antisense oligonucleotides to repair thalassemia human β-globin mRNA in mammalian cells. Antisense 2'-O-methylribooligonucleotides were targeted against specific sequence elements in mutated human beta-globin pre-mRNAs to restore correct splicing of these RNAs in vitro. The authors concluded that mammalian cells expressing the IVS2-654 human β-globin gene after treatment with antisense oligonucleotides, were able to restore the correct splicing in a dose-dependent fashion, generating correct human b-globin mRNA and polypeptide.[80]Study done by Lacerra G and co-workersin thalassemia patients treated with morpholino ASO directed against aberrant splice sites in mutant beta-globin precursor mRNAs, showed restoration of haemoglobin A synthesis in erythroid cells.[81]

6.4 Arthritis

Morita Y et al evaluated the feasibility of antisense oligonucleotides as therapeutic agents to inhibit synovial cell growth in rheumatoid arthritis(RA). Fibroblast-like cells obtained from RA synovium were stimulated with interleukin-1beta and treated with antisense or sense oligonucleotides targeting proliferating cell nuclear antigen (PCNA) messenger RNA. The authors concluded that antisense strategies designed to suppress PCNA expression inhibited IL-1-stimulated fibroblast proliferation and thus have potential use as therapeutic agents for RA. [82]Spleen-specific suppression of TNF-alpha by cationic hydrogel-delivered antisense nucleotides for the prevention of arthritis in animal model was studied by Dong L et al. The therapeutic efficacies of ASO-Gel was evaluated in three types of animal models, including the adjuvant-induced arthritis (AA), carrageen/lipopolysaccharide (LPS)-induced arthritis (CLA) and collagen-induced arthritis (CIA) models. The effects of ASO-c-agarose in alleviating inflammation and tissue destruction were evidenced in more than 90% of the testing animals, with decrease of main inflammatory cytokines, lightening of joint swelling and tissue damage, as well as increase in their body weights. [83]Kai M. Hildner& others showed that targeting of the Transcription Factor STAT4 by Antisense Phosphorothioate Oligonucleotides suppresses Collagen-Induced Arthritis in mice and may be a potential target to therapy of chronic arthritis.[84]

6.5 Diabetes

Antisense oligonucleotides have demonstrated a decrease of c-Raf kinase expression improvement in the neovascularization severity score, and good ocular tolerability in a pig model of venous-occlusion retinal neovascularization. [85] iCo-007, an antisense drug currently in trial, that targets c-Raf kinase may offer a significant advantage in the treatment of diabetic retinopathy by down regulating the signal pathways of multiple growth factors that seem to play a critical role in the process of ocular angiogenesis and vascular leakage. [86]

6.6 Asthma

ASOs directed against chemokine receptor 3 (CCR3) and the common beta chain of IL-3, IL-5, and granulocyte-macrophage colony stimulating factor (GM-CSF)receptors have been shown to attenuate antigen induced eosinophil efflux to the airways in animals.[87-90] TPI ASM 8, combination of two phosphorothioate antisense oligonucleotides, designed to inhibit allergic inflammation by down-regulating human CCR3 and the common beta chain has shown attenuation of allergen-induced increase in target gene mRNA and airway responses in subjects with mild asthma. [91]Antisense oligonucleotide targeting the adenosine A1 receptor represents a potentially new therapeutic approach in asthma. Nyce JW and Metzger WJ administered aerosolised phosphorothioate antisense oligonucleotide targeting the adenosine A1 receptor to the dust mite-conditioned allergic rabbit model of human asthma. Their study showed that the antisense therapy desensitized the animals to subsequent challenge with either adenosine or dust-mite allergen. [92] EPI 2010 an antisense drug targeting adenosine A1 receptor has shown to significantly improve allergen-induced airway obstruction and bronchial hyper-responsiveness in animal models of human asthma. [93] In clinical studies also EPI 2010 has demonstrated efficacy in patients with mild asthma. [94, 95]

6.7Amyloidosis

Antisense oligonucleotides specific for human transthyretin(TTR) has shown to inhibit hepatic synthesis of transthyretin mRNA levels and serum transthyretin levels by as much as 80% in transgenic mouse model carrying the human TTR Ile84Ser mutation and thus may offer a medical means of treating systemic transthyretin amyloidosis.[96] ISIS-TTR Rx, an antisense drug in development with Isis Pharmaceuticals and UK pharma giant GlaxoSmithKline for the treatment of TTR amyloidosis, is currently under evaluation in a Phase 1.[97] Recently US Food and Drug administration has granted ISIS-TTRRx fast track designation for the treatment of familial amyloid polyneuropathy.[98] As a result, Isis Pharmaceuticals in collaboration with GlaxoSmithKline started a multicentric phase III trial to assess the efficacy and safety of ISIS TTR Rx in patients with familial amyloid polyneuropathy. [99]

6.8 Antisense oligonucleotides in clinical trials[Table-2]

There has been steady progress in discovery and development of Antisense oligonucleotides based therapeutics over the past several years. Approximately 30 odd antisense oligonucleotides are being evaluated in humans. Many of them are still in phase 1 or 2 of clinical trials. Fomivirsen is the only approved drug in this category for the last 14 years. Marketed as Vitravene, it has been approved for treatment of cytomegalovirus induced retinitis. [100] Recently, in Jan 2013 FDAapproved second ASO based drug Mipomersen. ISIS pharmaceutical with Genzyme developed KYNAMRO^{TM}(mipomersen sodium) and received its approval as lipid lowering drug in patients with homozygous familial hypercholestremia. Mipomersen is a novel second-generation antisense drug which inhibits synthesis of apolipoprotein-B (Apo-B). Apo-B provides the structural core for atherogenic lipids, including Low density lipoprotein – cholesterol (LDL-C), which carries cholesterol through the bloodstream. Mipomersen reduces LDL-C and other key atherogenic lipids linked to cardiovascular disease by preventing their formation. Results of recently published Phase III trial of Mipomersen showed 36% reduction in LDL-C levels in patients of familial hypercholestremia.[101]Mipomersen is currently being studied in patients who are at high risk for cardiovascular disease [CVD] and are intolerant of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) inhibitors (statins). [102]Further research is required involving clinical outcome of cardiovascular disease end points in patients receiving Mipomersen.

Custirsen, PRO051 and Alicaforsen are other ASO based drugs which are currently under phase III evaluation and thus these ASOs hold high hopes for reaching phase 4 of clinical trials. Custirsen targets clusterin, stress-induced protein which improves cell-survival and is over-expressed in response to anti-cancer agents. Custirsen had shown good results in phase II trials in advanced castration-resistant prostate cancer. [103] Two phase III trials, SYNERGY and SATURN, are currently going on to confirm the effect of custirsen on tumour progression and survival in combination with first-line and second-line docetaxel for progressive metastatic prostate cancer. [104]

Alicaforsen is an antisense drug that blocks intercellular adhesion molecule 1 (ICAM-1) by disabling target RNA molecules and blocking the translation of protein. ICAM-1 is upregulated in the presence of inflammation. Alicaforsen has been evaluated in both Crohn's disease and ulcerative colitis. Results of Phase II/III trial in Crohn's disease were disappointing and hence its further development was discontinued. [105] However phase II trials showed convincing results in Ulcerative colitis as enema formulation and is currently planned to undergo phase III evaluations for this condition.[106]

PRO051 induces skipping of exon 51 of the dystrophin gene by binding to a sequence within the dystrophin pre-mRNA and restore the translational reading frame to dystrophin transcripts in patients with Duchenne's muscular dystrophy. Phase II study showed promising results with no major adverse effects. PRO051 is currently undergoing phase III clinical trials.[107]

VII. CONCLUSION

The dearth of a method to deliver ASO-based drugs to cells and the immunostimulatory side effects have emerged as a major challenge to its clinical utility in the past years, however, recently ASO as therapeutic agents have emerged as a valid approach to selectively modulate gene expression which has prompted a great deal of interest. The immense potential of this technology is still to be fully comprehended. Buoyantly, new research will shed light on ways to increase the therapeutic efficacy of this novel technology.

Table - 1. Various potential gene targets for ASO

Potential Target Gene	Therapeutic area
HER-2, PKA, PKAI, PKAII, TfR gene, Bcl-2/Bcl-xL, V integrin gene, VEGF	Breast Cancer
insulin-like growth factor receptor (IGF-1R)	prostate cancer

c-myb	Colon cancer and leukemia
PKA-I	breast, lung and prostate cancers
L-Grb-2	CML, ML, CLL
(PCSK9)Proprotein convertase subtilisin/kexin-9	hypercholestremia
apoC-III	hypercholestremia
Lp(a)	hypercholestremia
c-Raf kinase	Diabetic retinopathy
APP	AD
Mycolyl transferases	antitubercular therapy
VLA-4, GATA-3, tyrosine kinase Syk	ASTHMA
IL-1 receptor	Skin inflammation
IL-4, IL-5	Lung inflammation
TNF-a	Lung inflammation, Rheumatoid Arthritis
NF-kB p50	Lupus
NF-kB p65	Colitis
CD47	reconstructive surgery, organ transplantation, angioplasty, and cancer.
cyclin D1 (CD1)	Mesothelioma, non-small cell lung cancer, cancers over expressing it

Table - 2. List of ASOs in clinical drug development

Drug	Target	Disease condition	clinical trial phase
Custirsen [OGX-011]	clusterin	metastatic castration-resistant prostate cancer Metastatic Breast Cancer NSCLC	Phase 3 Phase 2 Phase 1
Mipomersen	apolipoprotein B	familial hypercholesterolaemia	Phase3
Alicaforsen	intercellular adhesion molecule1 (ICAM 1)	ulcerative colitis and pouchitis, Crohn's Disease	Phase 2 Phase 3
ATL1102	VLA-4	multiple sclerosis	Phase 2

LY2181308	survivin mRNA	advanced solid tumors	Phase 1
EXC 001	connective tissue growth factor	fibrotic diseases	Phase 2
iCo-007	c-Raf kinase	diabetic macular edema and diabetic retinopathy	Phase 2
XEN701	hepcidin-hemojuvelin pathway	anemia of inflammation	Phase 1/2
ISIS-EIF4E$_{Rx}$	eukaryotic initiation factor-4e (eIF-4E)	NSCLC and prostate cancer	Phase 2
OGX-427	heat shock protein 27 (Hsp27)	Advanced solid tumors	Phase 2
ISIS-STAT3$_{Rx}$	signal transducer and activator of transcription 3 (STAT 3)	Advanced cancers	Phase 2
ISIS-FGFR4$_{Rx}$	FGFR4	Obesity	Phase 1
ISIS-GCCR$_{Rx}$	glucocorticoid receptor (GCCR)	Diabetes	
ISIS-GCGR$_{Rx}$	glucagon receptor (GCGR)	Diabetes mellitus with sever hyperglycaemia	Phase 1
ISIS-PTP1B$_{Rx}$ (ISIS 113715)	PTP-1B	Type 2 diabetes	Phase 1
ISIS-CRP$_{Rx}$	CRP	Rheumatoid arthritis	Phase 2
ISIS-APOCIII$_{Rx}$	apoC-III	Hypertriglyceridemia	Phase 2
ISIS-FXI$_{Rx}$	factor XI	heart attack and stroke	Phase 1
ISIS 104838	tumor necrosis factor	Rheumatoid Arthritis	Phase 1
AVI-4658	exon 51 of the dystrophin gene	Duchenne Muscular Dystrophy	Phase 1
LErafAON-ETU	Raf-1	advanced cancers	Phase 1/2
EZN-2968	HIF-1 alpha	advanced solid tumors and advanced lymphomas	Phase 1
Oblimerson sodium, G3139 (Genasense)	bcl-2	subcutaneous solid tumors, small cell lung carcinoma, Bcell lymphoma, Breast Cancer, advanced solid tumors, AML metastatic renal cell carcinoma, relapsed or refractory CLL, advanced Malignant melanoma, Multiple Myeloma,	Phase 1 Phase 2 Phase 3
GTI-2040	R2 subunit of ribonucleotide reductase	Renal Cell	Phase 1/2
ASM8	CCR3 receptor	Asthma	Phase 1/2

AEG35156	X-linked inhibitor of apoptosis protein (XIAP)	Pancreatic carcinoma, breast carcinoma and Non-Small-Cell Lung carcinoma and Advanced Cancers, Refractory acute myeloid leukemia	Phase 1/2
GED0301	smad7	Crohn's disease	Phase 1
LY2275796	eukaryotic initiation factor 4E (eIF-4E)	Advanced cancers	Phase 1
Cenersen	p53	acute myeloid leukemia chronic lymphocytic leukaemia	Phase 2
PRO051	exon 51 of the dystrophin gene	Duchenne's muscular dystrophy	Phase 3
ATL1103	Growth Hormone receptor (GHr)	Acromegaly, diabetic retinopathy	Phase 1/2
Aganirsen	Insulin Receptor Substrate-1 (IRS-1)	Corneal graft rejection, Retinopathy of prematurity, Neovascular Glaucoma, diabetic retinopathy, Age related macular degeneration	Phase 2/3
Trabedersen	transforming growth factor-beta 2 (TGF-β2	high grade glioma malignant melanoma pancreatic cancer	Phase 2

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