International Standard Serial Number (ISSN): 2249-6807

*International Journal of Institutional Pharmacy and Life Sciences 5(4): July-August 2015*

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

**Pharmaceutical Sciences**

**Review Article……!!!**

Received: 17-04-2015; Revised: 21-07-2015; Accepted: 22-07-2015

# OLIGONUCLEOTIDES – A NEW GENERATION DRUGS

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**Keywords:**

Oligonucleotides, mRNA, therapeutic, clinical trials, ethylphosphonates

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## ABSTRACT

The principle of antisense oligonucleotides (AS-OD) technologies is based on the specific inhibition of unwanted gene expression by blocking mRNA activity. Antisense oligonucleotides show great potential as a molecular biological tool and therapeutic agent but there are some difficulties while using them such as toxicity, non-specific side effects, and low intracellular uptake. The first chemically synthesized modified oligonucleotides were the ethylphosphonates. Although these oligonucleotides have excellent stability in biological systems the absence of charge reduces their solubility and also reduces their cellular uptake. In human clinical trials, phosphorothioate oligonucleotides, the first generation antisense oligonucleotides show various hematologic toxicities mainly due to non-specific effects. Due to their charge and polarity, uptake by targeted cell is not efficient. Antisense therapy is an active field of drug development, and currently there are around 30 different oligonucleotides tested in about 40 different clinical trials, mostly in Phase II.

## 1.INTRODUCTION

Antisense therapy has been investigated extensively over the past two decades, either experimentally for gene functional research or clinically as therapeutic agents owing to the conceptual simplicity, ease of design and low cost[1]. The concept of this therapeutic approach is promising because short antisense oligonucleotides (ASOs) can be delivered into target cells for specific hybridisation with target mRNA, resulting in the inhibition of the expression of pathogenic genes. Because of their excellent targeting capacities and easiness of synthesis in high diversity, oligonucleotides are extensively used in vitro as ligands for nucleic acids (antisense oligonucleotides), proteins and small molecules (aptamer oligonucleotides[1,2,3]. Although the seductive idea to use oligonucleotides in vivo for therapy, diagnostic, imaging, etc appeared more than 30 years ago, these applications are still in their infancy and it seems that tremendous efforts are still necessary to develop these compounds as pharmaceuticals.

## 2. MECHANISM

Based on the formation of a Watson-Crick hybrid between an oligonucleotide and an RNA, the antisense technology provides a simple and elegant approach to inhibit the expression of a target gene. An antisense is a short oligonucleotide whose sequence is complementary and can bind to that of its target RNA (viral RNA or mRNA), thereby inhibiting its translation (Fig 1). The mechanism of inhibition is either through a steric blockage of the pre-mRNA splicing or of the initiation of translation, or through ribonuclease H mediated recognition of the mRNA-oligonucleotide duplex and ablation of the mRNA. Antisense oligonucleotides complementary to a target region of a candidate mRNA have been successfully used to inhibit protein synthesis in a number of biological systems[4,5].

This method of gene regulation, based on the hybridisation of two nucleic acids strands through Watson-Crick base pair formation, is extremely simple to design and has many potential therapeutic application: in cancer, viral infections and in inflammatory disorders[4,5,6,7,8,9].

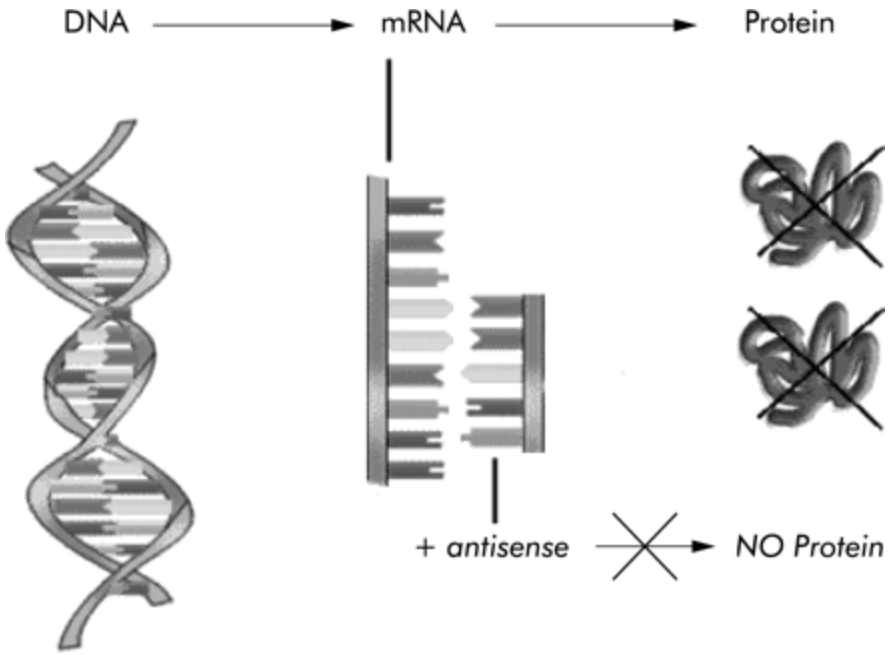


**Fig 1: Mechanism of action of Oligonucleotides**

## 3. DESIRED PROPERTIES OF OLIGONUCLEOTIDE DRUGS

Similar to all drugs, Oligonucleotide drugs should have the following properties: 1)high affinity and specificity for the target receptor, 2) ability to effect the desired pharmacology(activate RNase H cleavage of mRNA ,3) favourable pharmacokinetics 4) a good therapeutic index and economical.

## 4. SYNTHETIC OLIGONUCLEOTIDES

The first chemically synthesized modified oligonucleotides were the ethyl phosphonates. Ethylphosphonates oligonucleotides are non charged oligomers, in which a non bridging oxygen atom is replaced by a methyl group at each phosphorus in the oligonucleotide chain. Although these oligonucleotides have excellent stability in biological systems[10] the absence of charge reduces their solubility. Absence of charge also reduces their cellular uptake[11,12] which appears to occur predominately via the process of adsorptive endocytosis[13] and not by diffusion through membranes[14].

The phosphorothioates are the most widely studied oligonucleotides, because of their nuclease stability (although they are by no means nuclease proof) and relative ease of synthesis. However, the replacement of one of the non bridging oxygen's by sulphur at each phosphorus in the oligonucleotide chain introduces chirality at phosphorus. These molecules can activate the

complement casacade[15] and cause hematologic changes such as reduced heart rate, blood pressure and cardiac output. There is also a transient inhibition of the clotting times shown by an increased activated partial thromboplastin time (aPTT) [16].

Delivery vectors can take care of both toxicity and drug delivery problems by mediating the entry and their delivery to the target, not just of oligonucleotides but of other drugs. [17]

Antisense for therapy is an active field of drug development, and currently there are around 30 different oligonucleotides tested in about 40 different clinical trials, mostly in Phase II. So far, only one has been approved by the FDA, Vitravene for cytomegalovirus retinitis. About one half of the oligonucleotides in clinical trials are built with the phosphorothioate chemistry, 50% target cancer related genes, 25% target viral infections including hepatitis C, and three target chronic inflammatory diseases such as Crohn's disease and haemorrhagic rectocolitis[18,19]. Many of these candidate antisense drugs target haematological disorders such as chronic myeloid leukaemia[20] by targeting specific proto-oncogenes involved in cell proliferation and neoplasic transformation: Bcr/ab144 c-myb, c-myc or the tumour suppresser gene[21]. Other antisense strategies are based on the chemo sensitisation of tumour cells by depressing anti-apoptotic genes such as Bcl-2 expression[22]. The antisense drug Genasense (Genta, Inc) is an anti Bcl2 antisense now in Phase 3 clinical trials in lymphoma[23] and is also assayed as a chemosensitiser for dacarbazine treatment of human melanoma.[23,24]. In another approach, glioma cells collected at surgery are treated ex vivo with an antisense oligonucleotide against the type I insulin-like growth factor receptor and re-implanted into the patient, inducing apoptosis and a host response[25].

Although there are now a number of reports of antisense inhibition of human tumours, it should be emphasised that only for a very small number of patients has complete remission been observed. Many antisense oligonucleotide have been found to induce a variety of biological effects not related to their specific hybridisation to the target mRNA, including immune stimulation and other activities by oligonucleotide containing CpG motifs, release of pharmacologically active concentration of deoxy ribonucleosides, or aptameric binding to proteins. Clinical efficacy of antisense on tumour growth and development is difficult to evaluate and could certainly benefit from in vivo imaging evaluation methods[25].

## 5. DELIVERY OF OLIGONUCLEOTIDES TO CELLS

In order for an antisense oligonucleotide to down-regulate gene expression, it must penetrate into the targeted cells. To date, the precise mechanisms involved in oligonucleotide penetration are not clear. Uptake occurs through active transport, which in turn depends on temperature, the structure and the concentration of the oligonucleotide and the cell line[26]. At the present time, it is believed that adsorptive endocytosis and fluid phase pinocytosis are the major mechanisms of oligonucleotide internalization, with the relative proportions of internalized material depending on oligonucleotide concentration. At relatively low oligonucleotide concentration, it is likely that internalization occurs via interaction with a membrane-bound receptor [27,28].

Helen L. Lightfoot and Jonathan Hall identified 26 oligonucleotide-target combinations which are, or were until recently, in phase 2 clinical status or above (Table 1) according to Thomson Reuters Integrity database. In the following examples suppression of the target mRNA and/or protein levels strongly implies that the drugs are being delivered to appropriate disease tissues.

**Table 1: Oligonucleotides which are in the phase 2 clinical trails**

| Sl.No | Action | Drug | Reference |
|---|---|---|---|
| 1. | Dystrophin(Mutation induce a frame shift or non-sense residue and produces dysfunctional protein | Eteplirsen (phase 2) | 29 |
| 2 | | Drisapersen(phase 3:DMD) administered sc causes exon skipping and restores dystrophin levels in muscle biopsies of patients | 30 |
| 3 | B cell lymphoma-2(inhibitor of cancer cell apoptosis associated with chemotherapy or radiotheraphy resistance | Oblimersen(phase 3:cancer) | 31,32,33 |
| 4 | Surviving (inhibitor of cancer cell apoptosis highly expressed in tumors associated with chemotherapy . | ISIS-23722(Phase 2: cancer) | 34 |
| 5 | Clusterin(CLU) Secreted stress induced cytoprotective protein associated with chemotherapy | Clustirsen(phase 3:cancers) | 35,36,37 |
| 6 | Heat shock(27kDa protein 1) | OGX-427 | 38 |
| 7 | Eukaryotic translation initiation | ISIS-EIF4Erx(phase 2:cancer_ | 39 |
| 8 | Ribonucleoside-diphosphate reductase M2 chain | GTI-2040(Phase 2: cancer) Phosphorothioate oligodeoxynucleotide ASO administered Ivwith cytarabine in AML PATIENTS | 40,41 |

## CONCLUSION

A number of other antisense oligonucleotides are currently in clinical trials, including those for treating malignancies and for targeting various diseases. The antisense approach could reduce the cost of drug discovery by expediting the identification of lead targets for pharmaceutical intervention. In spite of the number of antisense oligonucleotides currently in Phase I, II and III clinical trials, there are still significant hurdles to be overcome. The main barrier is achieving systemic delivery of the oligonucleotides to the correct target, and in the desired time frame, to achieve functional down regulation of the target gene. These issues are at present being actively addressed and will hopefully continue to shed light on ways to increase therapeutic efficacy and specificity.

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