ANTISENSE THE RAPY AND ITS UNIQUE APPLICATION TO TREAT GENETIC DISORDERS-A REVIEW

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ABSTRACT:

A genetic disorder is a genetic problem caused by one or more abnormalities formed in the genome. Most genetic disorders are quite rare and affect one person in every several thousands or millions. Genetic disorders may be hereditary, meaning that they are passed down from the parents' genes. In other genetic disorders, defects may be caused by new mutations or changes to the DNA. The principle behind the technology is that an antisense nucleic acid sequence base pairs with its complementary sense RNA strand and prevents it from being translated into a protein or the inhibition of RNA processing yields the therapeutic result.The Oligonucleotide are used to block coded massage carried by mRNA this known as antisense therapy the primary sequence of mRNA called oligonucleotide contains nucleic acid bases complimentry to mRNA molecule. An oligonucleotide has a complimentry base sequence called antisense oligonucleotide, when mixed with mRNA antisense oligonucleotide Recognize mRNA and forms duplex and act as barrier to translation process and block protein synthesis. Antisense drugs have the potential therapeutic efficacy in the treatment of various diseases than the conventional drug. The rapid development of antisense technology offers almost unlimited scope for the development of new and highly specific therapeutics. Antisense therapeutics therefore is well positioned to play a significant role in the progression of antisense technology for drug development in human diseases.

Keyword: Antisense therapy, genetic disorder, oligonucleotide, protein, Nucleic acid.

INTRODUCTION:

A genetic disorder is a genetic problem caused by one or more abnormalities formed in the genome. Most genetic disorders are quite rare and affect one person in every several thousands or millions. Genetic disorders may be hereditary, meaning that they are passed down from the parents' genes. In other genetic disorders, defects may be caused by new mutations or changes to the DNA. In such cases, the defect will only be passed down if it occurs in the germ line. Some types of recessive gene disorders confer an advantage in certain environments when only one copy of the gene is present. Genetic disorders may also be complex, multifactorial, or polygenic, meaning they are likely associated with the effects of multiple genes in combination with lifestyles and environmental factors. Multifactorial disorders include heart disease and diabetes. Although complex disorders often cluster in families, they do not have a clear-cut pattern of inheritance. This makes it difficult to determine a person’s risk of inheriting or passing on these disorders.[1]

Antisense therapy is a form of treatment for genetic disorders or infections. When the genetic sequence of a particular gene is known to be causative of a particular disease, it is possible 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, effectively turning that gene "off". This is because mRNA has to be single stranded for it to be translated. When the genetic sequence of a particular gene is known to be causative of a particular disease, it is possible to synthesize a strand. A Sense strand is a 5‘ to 3‘ mRNA molecule or DNA molecule. The complementary strands or mirror strand to the sense is called an antisense. Antisense technology is the process in which the antisense strand hydrogen bonds with the targeted sense strand. When an antisense strand binds to a mRNA sense strand, a cell will recognize the double helix as foreign to the cell and proceed to degrade the faculty mRNA molecule thus preventing the production of undesired protein.Also Oligos which bind to complementary RNA sequences are commonly called _antisense‘ oligos because they are typically used to bind the _sense‘ sequence of a messenger RNA. Antisense oligos are used

for identifying the function and studying the control of genes, as well as for validating prospective protein targets in drug development programmes. Of the several hundred oligo structural typesdeveloped over the past three decades, less than half a dozen have gained acceptance for research and biopharmaceutical development.[2]

Antisense compounds are designed to have the right nucleotide sequence to bind specifically to and interfere with its associated mRNA, the instructions for the production of a particular protein To create antisense drugs, special chemically stabilized nucleotides are synthetically linked together in short chains of about 12 to 30 nucleotides (called oligonucleotides). Each antisense drug is designed with the right complementary genetic code to bind to a specific sequence of nucleotides in its mRNA target to form a short area of double strands.[3]

MECHANISM OF ANTISENSE TECHNOLOGY:

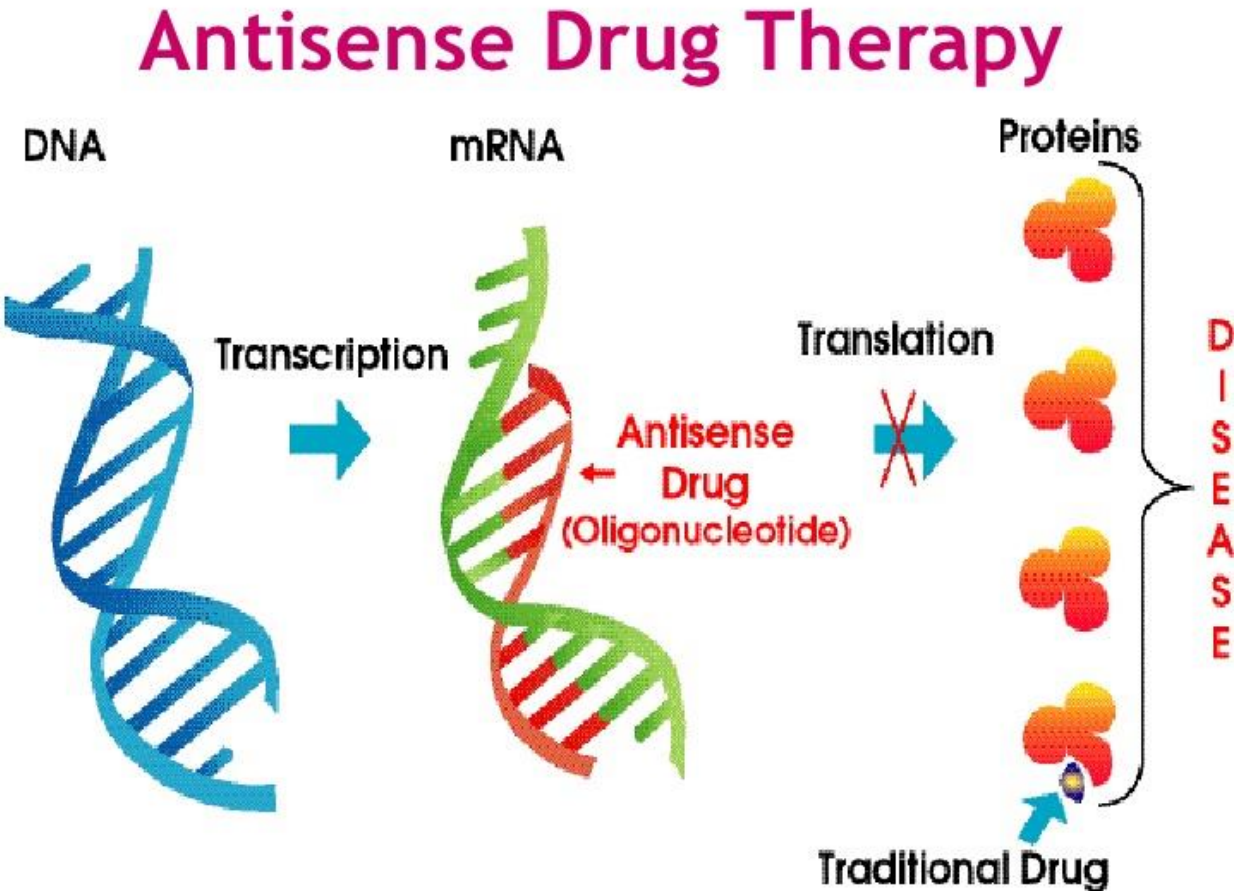

Fig 1: Mechanism of antisense therapy

Antisense compounds are designed to have the right nucleotide sequence to bind specifically to and interfere with its associated mRNA, Oligonucleotide are used to block coded massage carried by **mRNA** this known as antisense therapy the primary sequence of **mRNA** called oligonucleotide contains nucleic acid bases complimentry to **mRNA** molecule. An oligonucleotide has a complimentry base sequence called antisense oligonucleotide, when mixed with **mRNA** antisense oligonucleotide Recognize **mRNA** and forms duplex and act as barrier to **translation** process and block protein synthesis. ***figure 1***

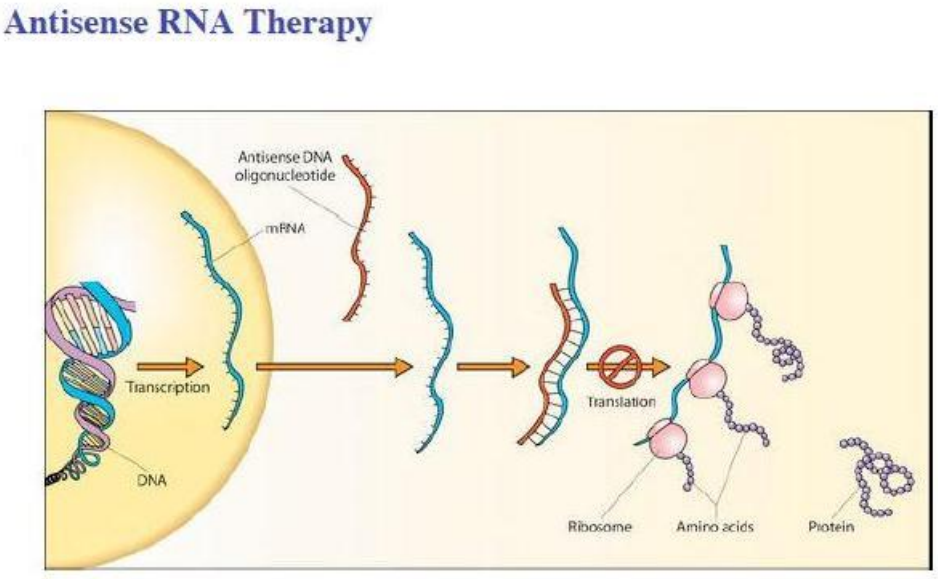

Fig 2: Antisense RNA therapy

THERAPEUTIC APPLICATIONS OF ANTISENSE AGENTS:

information available. Antisense oligonucleotides can be developed against any target in which the inhibition of protein production or the inhibition of RNA processing yields the therapeutic result. Currently, clinical trials are underway using antisense oligonucleotides to The potential applications for antisense oligonucleotides are limited only by the genetic treat rheumatoid arthritis, psoriasis, renal transplant rejection and inflammatory bowel disease (crohn's disease)

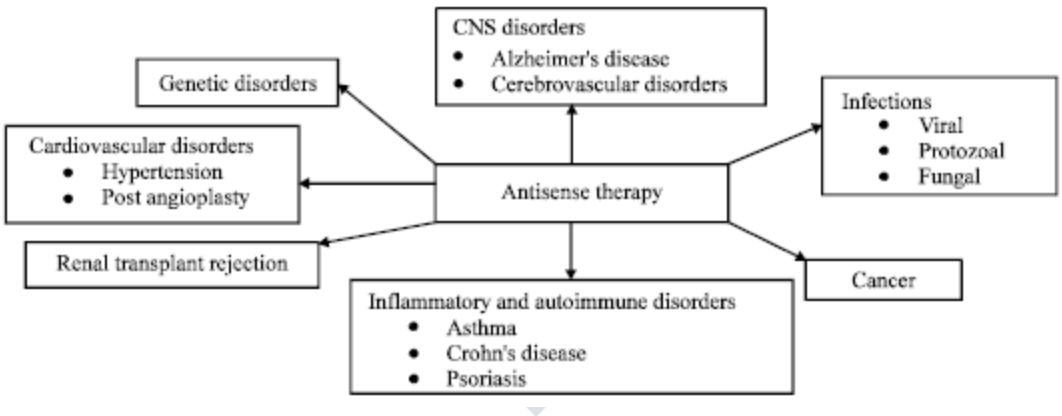

Fig 3: Field of application of antisense therapy

a) Antisense Agents In Cancer Therapy:

Emerging novel strategies of cancer treatment are based on the selective down-regulation of specific molecular targets involved in the process of neoplastic development and progression (Elsayed and Sausville, 2001). As the aim of cancer therapeutics is becoming streamlined to target more specific biological pathways, genetic components and/or cellular proteins, the role of antisense therapy utilizing oligonucleotides is evolving as a potential treatment strategy in the fight against cancer.
The various protein targets and antisense agents in clinical trials in cancer treatment are as follows:

Bcl-2: Regulation of cell death (apoptosis) is frequently affected in the development of malignant diseases and all molecular steps from extracellular signalling receptors through intracellular pathways, cell death rheostats and cell death executioners may be involved. Bcl-2 is an anti-apoptotic member of a family of anti-and pro-apoptotic proteins that is upregulated in a variety of cancers and specifically overexpressed through chromosomal translocation in some Non-Hodgkin Lymphomas (Gross *et al.*, 1999; Kang and Reynolds, 2009).

Protein Kinase C-α: Protein Kinase C (PKC) is a family of serine/threonine kinases that is involved in the transduction of signals for cell proliferation and differentiation. The important role of PKC in processes relevant to neoplastic transformation, carcinogenesis and tumour cell invasion renders it a potentially suitable target for anticancer therapy (Mackay and Twelves, 2003). Isis/Eli Lilly have developed a 20mer Phosphorooligonucleotide ISIS 3521(also referred to as LY900003 or Affinitak or Aprinocarsen) that targets the 3'-UTR of PKC-α (Roychowdhury and Lahn, 2003).

Clusterin: Clusterin is a glycoprotein with a nearly ubiquitous tissue expression and an apparent involvement in various biological processes. Clusterin acts as a cell-survival protein that is overexpressed in response to tumor-killing strategies, such as chemotherapy, hormone ablation and radiation therapy. Overexpression of clusterin prolongs cell survival and leads to enhanced metastatic potential of cancer cells ***in vitro*** (Wei *et al*., 2009).

Survivin: Survivin is one of the most cancer-specific proteins identified to date, being upregulated in almost all human tumors. Biologically, survivin has been shown to inhibit apoptosis, enhance proliferation and promote angiogenesis. Because of its upregulation in malignancy and its key role in apoptosis, proliferation and angiogenesis, survivin is currently attracting considerable attention as a new target for anti-cancer therapies. In several animal model systems, downregulation of survivin or inactivation of its function has been shown to inhibit tumor growth (Ambrosini *et al*., 1997; Mita *et al*., 2008).

a) **Tubulointerstitial injury**-Midkine, a heparin-binding growth factor, is involved in the migration of inflammatory cells. The inflammatory cell migration to the tubulointerstitium of the kidney after ischemia/reperfusion (I/R) injury is attenuated in midkine gene–deficient mice. Midkine antisense phosphorothioate oligodeoxyribonucleotide (ODN) at a dose of 1 mg/kg in saline was intravenously administered to mice 1 day before or after I/R. These results indicated that intravenousinjection of midkine antisense ODNis a candidate for a novel therapeutic strategy against acute tubulointerstitial injury induced by I/R injury.

b) **Cytomegalovirus:** Cytomegalovirus (CMV) belongs to the Herpes-viridae family of viruses which are DNA viruses that exhibit the biological properties of latency and reactivation. In the developed countries, up to 80% of individuals develop sub-clinical CMV infections (Mocarski, 1988). CMV retinitis is one of the most common opportunistic infections in patients with Acquired Immunodeficiency Syndrome (AIDS). AIDS patients infected with CMV retinitis can develop either intolerance or resistance to commonly used anti-CMV treatment regimens, necessitating the development of alternative treatment options (Hoffman and Skiest, 2000). Much of the research on inhibition of CMV replication by ASONs has mainly focused on inhibition of CMV Immediate-early (IE) gene products. ISIS 2922 (Fomivirsen also called Vitravene; ISIS Pharmaceuticals), a PS-ASON with a 21 nucleotide sequence complementary to RNA of IE2 showed at least 30 fold more potent antiviral activity as compared to nucleoside analog gangciclovir. The results showed that ISIS 2922 inhibits viral production in a specific and dose dependent manner (Azad *et al*., 1993).

c) **Acute injury**- Closed head injury (CHI) is an important cause of death among young adults and a prominent risk factor for nonfamilial Alzheimer‘s disease. A single intracerebroventricular injection of 500 ng 2¢-O-methyl RNA-capped antisense oligonucleotide (AS-ODN) against acetylcholinesterase (AChE) mRNA blocks overexpression of the stress-related read through AChE (AChE-R) mRNA splicing variant in head-injured mice. These findings demonstrate the potential of antisense therapeutics in treating acute injury, and suggest antisense prevention of AChE-R overproduction to mitigate the detrimental consequences of various traumatic brain insults.

d) **Duchenne muscular dystrophy (DMD):** The dystrophin gene contains more that 20 exons which encode a repeated region in the dystrophin protein. DMD patients carry mutations in the dystrophin gene, which change the reading frame of the mRNA leading to the formation of premature terminated protein products which cannot carry out the function of dystrophin. One current application of antisense is to induce the specific skipping of exons in order to restore the correct open reading frame of a mutated transcript. Local intramuscular injection of optimised AON sequences resulted in dystrophin levels of up to 20% compared to wild type dystrophin. This observation is accompanied by improvement in muscle histology and function.

e) **Spinal Muscular Atrophy (SMA)**- SMA is caused by a loss of, or defect in, the survival motor neuron 1 (SMN1) gene.The SMN1 gene produces most of the SMN protein, which is critical to the health and survival of the nerve cells in the spinal cord responsible for muscle function.The severity of SMA is correlated with the amount of SMN protein in the cell. Antisense therapy could be used to change the functioning of the SMN2 gene by utilizing alternative RNA splicing. Alternative splicing is a normal mechanism that the cell uses to produce different, but closely related proteins from a single gene.

f) **Cardiovascular diseases**- Few studies revealed an inhibition of expression of the surface adhesion molecule ICAM-1 with antisense oligodesoxynucleoties (ODN) in a model of reperfusion injury. Antisense approach to acute renal failure and reperfusion injury could have great clinical utility. The production of angiotensin II by cells feeds back on those cells, resulting in cell growth and other changes.

g) **Antisense antivirals**- Despite the navailability of antiviral chemotherapies, human pathogenic viral infections remain a global health problem, causing formidable morbidity and mortality worldwide. Of particular concern are those virus types developing (multi) drug-resistant, eg. Herpes viruses, hepatitis C virus (HCV), and human immunodeficiency virus (HIV), that fail almost all the available antiviral drugs in clinical practice. Novel antiviral strategy of using single-stranded antisense oligo (deoxy) nucleotides (ASOs) as gene silencers has been a prospective area of anti-infective study, which has shown greatadvantages in providing fast-respond postexposure therapeutics for emerging viruses.

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i) **Multiple Sclerosis**- Subcutaneously administered injection of ATL1102 (IInd generation antisense therapeutics) has found a significant success in the treatment of multiple sclerosis.

j) **Diabetic retinopathy**- It is neovascularisation of the retina leading to blindness. Presently there are no approved drug treatments for the advanced (proliferative) form of diabetic retinopathy. Antisense (ATL1103) can be a good choice.

k) **Diabetic nephropathy**- It is one of the complications of diabetes and may treated by the help of antisense technology e.g. ATL1103

l) **Acromegaly**- It is caused due the overproduction of growth hormone by the anterior pituitary. ATL1103 was be found effective in most of the cases.

m) **HIV/AIDS-** Starting in 2004, researchers in the US have been conducting research on using antisense technology to combat HIV. In February 2010, researchers reported success in reducing HIV viral load using patient T-cells which had been harvested, modified with an RNA antisense strand to the HIV viral envelope protein, and re-infused into the patient during a planned lapse in retroviral drug therapy.

Table 1: Some antisense therapeutics currently under phases of clinical trials

S. No.	ANTISENSE THERAPEUTICS	TARGET/PURPOSE	TRIALS / UNDER PHASE
1	EGFR antisense DNA	Squamous Cell Carcinoma of the Head and Neck	Phase 1
2.	AEG35156 antisense	Advanced Hepatocellular Carcinoma	Phase 2
3.	Insulin-like growth factor receptor-1 antisense oligodeoxynucleotide	Malignant Glioma	Phase 1
4	XIAP antisense	Acute Myelomonocytic Leukemia	Phase 2
5.	TGF-β2 antisense oligonucleotide	Primary Open Angle Glaucoma	Phase I
6	AVI-4658 (PMO)	Duchenne Muscular Dystrophy (DMD)	Phase 3
7	ISIS 3521	Pulmonary Neoplasms	Phase 2

CONCLUSION

Antisense drugs have the potential therapeutic efficacy in the treatment of various diseases than the conventional drug. The rapid development of antisense technology offers almost unlimited scope for the development of new and highly specific therapeutics. Antisense therapeutics therefore is well positioned to play a significant role in the progression of antisense technology for drug development in human diseases. More research attention should be given on these area that can be effective in treatment of various diseases like cardiovascular diseases, autoimmune diseases and other such diseases.

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