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Review Article

A Review On Analytical And Clinical Applications Of Anti Sense Oligonucleotides

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ABSTRACT

Antisense drugs are drugs that seek to block DNA transcription or RNA translation in order to moderate many disease processes. Antisense drugs consist of nucleotides linked together in short DNA or RNA sequences known as oligonucleotides. Some of these drugs have been designed to target viral disease and cancer cells in the body. Vitravene (ISIS Pharmaceuticals), an oligonucleotide targeted to cytomegalovirus, was the first antisense oligonucleotide drug approved by the US Food and Drug Administration (FDA). Presently, numerous antisense drug candidates are in clinical trials to treat cardiovascular, metabolic, endocrine, neurological, neuromuscular, inflammatory, and infectious diseases. Antisense therapy has been useful in the treatment of cardiovascular disorders such as restenosis after angioplasty, vascular bypass graft occlusion, and transplant coronary vasculopathy. Purity checking is the main challenge so many analytical techniques were used . Some researches were used high performance liquid chromatography (HPLC) Or PAGE to purify the synthesized antisense oligonucleotides to achieve the desired purity . The present review focused on analytical techniques and clinical applications of Anti sense oligonucleotides used to treat various diseases and disorders.

INTRODUCTION

Antisense drugs consist of nucleotides linked together in short DNA or RNA sequences known as oligonucleotides. Oligonucleotides are designed knowing the sequence of target DNA/RNA (e.g. messenger RNA) to block transcription or translation of that targeted protein. An

oligonucleotide that binds complementary ("sense") mRNA sequences and blocks translation is referred to as antisense [1]’). ASOs are short, single stranded chemically modified nucleotides, usually 12-24 bases in length that bind to their RNA targets via Watson –Crick base pairing. Once bound to the target RNA sequence, ASOs regulate

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the function of target RNA through broad set of mechanisms;

- a) Bind to RNA and hindered its function without facilitating RNA degradation such as translation inhibition or modulation of RNA processing
- b) Promote degradation of the RNA via endogenous enzymes, such as RNase H or argonaute-2[RNA interference (RNAi)]

ASOs can increase protein production by binding to sequences in up stream of primary ORF or by antagonizing miRNAs, which normally diminish protein production. To further stabilize the drug, many chemical modifications have been made to the oligonucleotide structure. The most common modification used involves substitution of non bridging Oxygen in the phosphate backbone with sulphur resulting in a phosphorothioate-derived antisense oligonucleotide. Some of these drugs have been designed to target viral disease and cancer cells in the body. Vitravene an oligonucleotide targeted to cytomegalovirus, the cost of a second oligonucleotide drug, Macugen has made the treatment prohibitive given the availability of cheaper, equally effective drugs. Both drugs act locally (in the eye) but several other antisense drugs administered intravenously have also been approved such as Alicaforsen and Mipomirsen[1]). Unlike small molecule-based protein targeting, antisense drugs exhibit their effect by Watson–Crick base pairing rules with target RNA sequence. This principle of Watson–Crick molecular recognition provides the antisense field more flexibility in RNA-based drug design and expedites its development, which is imperative for targeting a myriad of rare and genetic diseases [2]). The amalgamation of chemical structure modifications of oligonucleotides and diverse delivery platforms provides an additional boost to the antisense field. Recently United States Food and Drug Administration (FDA) approval of several nucleic

acid-based drugs has further spurred interest in the antisense research[3]).

Synthesis:

- (i) To make antisense drugs, chemically stabilized nucleotides are linked together in short chain[4]). The methyl phosphonate backbone yields molecules that are non-ionic and form a stable duplex with mRNA. The duplex formed is degraded by nucleases. The first successful backbone chemistry that modified the phosphodiester is phosphorothioate linkage[5]). The resulting compound is the broadest range of activities in which the oxygen atom is replaced by sulphur in the phosphonate group. The obtained molecule has chiral chiral, negative charged, nuclease resistance and form a stable duplex with mRNA. The major drawback of phosphorothioates is a low concentration of drug in plasma, less attraction for their target mRNA and side effects such as clotting abnormalities and immune stimulation[6]). The second-generation molecules (2'-O-methoxyethyl or 2-MOE) are composed of both DNA- and RNA-like nucleotides. These compounds provide therapeutic effects at lower doses. They significantly slow down the degradation of the drugs by protecting the drug from destructive nucleases[7]). Third-generation drugs were developed by chemically modifying the furanose ring of the antisense oligonucleotides, along with modifications of phosphate linkages or of riboses, as well as of nucleotides[8]). They were made to improve the nuclease stability, target affinity and pharmacokinetic profiles of the antisense oligonucleotide. Locked nucleic acid (LNA), peptide nucleic acid (PNA) and morpholino phosphoroamidates (MF) are the three most commonly used third-generation antisense oligonucleotides[9]).
- (ii) Antisense oligonucleotides are synthetic polymers in which some or all of the natural nucleotide monomers of the oligonucleotide are



chemically modified deoxynucleotides (in DNA) or ribo nucleotides (in RNA). Normally, antisense oligonucleotides contain 15 to 25 monomers. In antisense technology single-stranded DNA or RNA molecules are used to target a specific sense mRNA. Antisense compounds have become effective tools for basic molecular biology, genomics, and proteomics research which is often used for drug discovery, targeted screening, and validation. For example antisense oligonucleotides can act by blocking the upstream message for receptor substrates, proteins over expressed in pathological versus physiological states[10]'').

Importance :

Antisense therapy has been useful in the treatment of cardiovascular disorders such as restenosis after angioplasty, vascular bypass graft occlusion, and transplant coronary vasculopathy. Antisense oligonucleotides also have shown promise as anti viral[11]''). Depending on the sequence and modifications, antisense oligonucleotides can alter RNA function through several distinct mechanisms, making them a diverse tool. They can be used to restore protein expression, reduce expression of a toxic protein, or modify mutant proteins to reduce their toxicity[12]'').

ANALYTICAL TECHNIQUES / METHODS:

□ Evaluation of ultrahigh-performance liquid chromatography columns for the analysis of unmodified and antisense oligonucleotides:

Sylwia Studzińska et al. has been used for the separation and analysis of unmodified and modified antisense oligonucleotides. For this reason, authors tested various columns of low particle sizes in the analysis of unmodified and phosphorothioate oligonucleotides. The influence of both the type and concentration of ion-pair reagent on the retention of the studied biomolecules was tested. The developed methods were used for separation of unmodified

oligonucleotides and to determine antisense oligonucleotides in human serum samples. The results proved that octa decyl and phenyl columns are the most selective in the resolution of oligonucleotides which differ in the position of single nucleotides in the sequence. The phenyl column was selected and applied for the analysis of phosphorothioate oligonucleotides in serum samples . The results obtained as shown in the figure -1 [13]'').

□ Analysis of Antisense Oligonucleotides and Their Metabolites with the Use of Ion Pair Reversed-Phase Liquid Chromatography Coupled with Mass Spectrometry

Kaczmarkiewicz A et al. have been widely investigated as a potential drugs because of their ability to bind with the target DNA or RNA strands, which may lead to inhibition of translational processes. Special attention was paid to the stationary phases selection for the separation of OGNs and the impact of different compositions of mobile phases on retention and signal intensity in mass spectrometry (MS). Moreover the application of ion pair liquid chromatography coupled with MS for the separation and determination of metabolites of ASOs was described[14]'').

□ Analysis of antisense oligonucleotides with the use of ionic liquids as mobile phase modifiers

Sylwia Studzińska et al. have investigated the impact of several ionic liquids, commonly used as free silanol suppressors, on the retention and separation of phosphorothioate oligonucleotides. Three various stationary phases (octadecyl, octadecyl with embedded polar groups and penta fluorophenyl) as well as ionic liquids with the concentration range of 0.1–7 mM were used for this purpose. The results obtained during the study showed that the increase in concentration of ionic liquids results increasing retention of the oligonucleotides. Such an effect was observed

regardless of the stationary phase used. Moreover, elongation of the alkyl chain in the structure of ionic liquids caused an increase of antisense oligonucleotide retention factors. The results obtained during retention studies confirmed that addition of ionic liquids to the mobile phase influences antisense oligonucleotide retention in a way similar to the case of commonly used ion pair reagents such as amines. A method of oligonucleotide separation was also developed. The best selectivity was obtained for the octadecyl stationary phase since separation of mixtures of antisense oligonucleotides and their metabolites differing in sequence length was successful. It has to be pointed out that ionic liquids were used for the first time as mobile phase additives for oligonucleotide analysis[15]”).

□ **Pharmacokinetic Studies of Antisense Oligonucleotides Using MALDI-TOF Mass Spectrometry**

Markus Herkt et al. In the study MALDI-TOF mass spectrometry (MS) was utilized to establish a robust and fast method to sensitively detect and accurately quantify anti-microRNA (antimiR) oligonucleotides in blood plasma. An anti miR oligonucleotide isolation protocol containing an ethanol precipitation step with glycogen as oligonucleotide carrier as well as a robust and reproducible MS- analysis procedure has been established. Proteinase K treatment was crucial for releasing anti miR oligonucleotides from plasma as well as cellular proteins and reducing background derived from biological matrices. Anti miR oligonucleotide detection was achieved from samples of studies in different animal models such as mouse and pig where locked nucleic acids(LNA)modified anti miR oligonucleotides have been used to generate pharmacokinetic data[16]”).

□ **Investigation of antisense oligonucleotides with the use of mass spectrometry:**

Sylwia Studzińska et al. have been investigated as potential drugs for years. They inhibit target gene or protein expression. The application of mass spectrometry was described with regard to the ionization type used for the determination of these potential therapeutics. [17]”).

□ **Characterization of antisense oligonucleotide and guide ribonucleic acid diastereomers by hydrophilic interaction liquid chromatography coupled to mass spectrometry**

Alexandre Goyon et al. describe as oligonucleotides have become an essential modality for a variety of therapeutic approaches, including cell and gene therapies. Rapid progress in the field has attracted significant research in designing novel oligonucleotide chemistries and structures. Beyond their polar nature, the length of large RNAs and presence of numerous diastereomers for phosphorothioate (PS)modified RNAs pose heightened challenges for their characterization. In the study the stereochemistry of a fully modified antisense oligonucleotide (ASO) and partially modified guide RNAs (gRNAs) was investigated using HILIC and orthogonal techniques. The profiles of three lots of a fully modified ASO with PS linkages were compared using ion-pairing RPLC (IPRP) and HILIC. Interestingly three isomer peaks were partially resolved by HILIC for two lots while only one peak was observed on the IPRP profile. Model oligonucleotides having the same sequence of the five nucleotides incorporated to the 3-end of the gRNA but differing in their number and position of PS linkages were investigated by HILIC, IPRP, ion mobility spectrometry–mass spectrometry (IM-MS) and nuclear magnetic resonance (NMR). An strategy was ultimately designed to aid in the characterization of gRNA stereochemistry. Ribonuclease (RNase) T1 digestion enabled the characterization of gRNA diastereomers by reducing their number from 32 at

the gRNA intact level to 4 or 8 at the fragment level[18]”).

□ **Ultra-High-Performance Reversed-Phase Liquid Chromatography Hyphenated with ESI-Q-TOF-MS for the Analysis of Unmodified and Antisense Oligonucleotides**

Sylwia Studzińska et al. The reversed-phase ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry method was developed during the study and successfully applied to separation and analysis of unmodified and antisense oligonucleotides. It was shown that oligonucleotide separation strongly depends on the stationary phase type. The type pH and salt content in the mobile phase influence also their chromatographic behavior and mass spectrometry sensitivity. Increasing the apparent pH causes retention decrease and increase of sensitivity, while increasing the salt concentration produces greater oligonucleotide retention and lower sensitivity. Utilization of octadecyl stationary phase with aryl rings in reversed-phase mode allows successful separation of various modified and unmodified oligonucleotides, differing in length or sequence[19]”).

□ **Clinical Applications :**

Antisense drugs are being researched to treat haemorrhagic fever, HIV/AIDs, amyotrophic lateral sclerosis, cardiovascular disease, diabetes, obesity, renal disease, asthma, inflammation, arthritis, spinal muscular atrophy, Duchenne muscular dystrophy, cystic fibrosis and cancer diseases. These diseases are currently being addressed by antisense oligonucleotides and will hope to target the new potential therapeutic compounds for many diseases. However, the minimum use of ASOs in the treatment of disease requires effective design and specificity.

□ **Antiviral Agents:**

Burrer and co-workers reported the antiviral activity of novel phosphorodi amidate antisense morpholino oligomers (P-PMOs) in mouse models

infected with murine hepatitis virus (MHV). Authors utilized different strains of virus in cell culture and evaluated the effect of P-PMOs in tested models in vivo. There are ten P-PMOs engaged against different target mRNA genomes that were tested in culture. The result reveals that one of these molecules called 5TERM PMO perfectly complementary to the virus genomic RNA. Therefore, the mentioned PMO was effective against 6 different strains of murine hepatitis virus. In addition to that, the authors performed various arginine-rich peptides conjugated to the 5TERM PMO sequence to calculate potency and toxicity, in that way authors selected appropriate PMO for in vivo testing. selected compound inhibits viral titres in the organs of mice and saved against the different tissue damages. Chadwick et al. narrated that antisense RNA has been recognized as a powerful inhibitor of gene expression and prevents retroviral replication at different phases in virus life cycle. The novel antisense RNA drug was complementary to 3 target regions in the 5' leader of HIV-1. They are TAR region, the splice donor packaging signal region, the primer binding site and were confirmed by RT-PCR. The result unveils that the packaging signal (ψ) of HIV-1 is an attractive target for ASOs therapy. Kobayashi-Ishihara et al. found that latently infected T lymphocytes are a vital barrier towards reducing persistent HIV. The authors describe that an HIV-based recombinant fluorescent lentivirus (rfl-HIV) enables to notice antisense and sense transcription of the virus using fluorescence genes. Hence, rfl-HIV transcripts can display the core suppressor activity and be able to lock an incorporated provirus into a non-functional condition. Therefore, it was a significant step to eliminate HIV from infected individuals.

□ **Antidiabetic Agents:**

Liang et al. carried out a novel handling of type 2 diabetes by using gene therapy.



The study illustrates the effects of a specific antisense oligonucleotide (GR-ASO) in the mice model. These drugs inhibit glucagon receptor mRNA expression and were confirmed by a quantitative real-time RT-PCR test. The antisense drug was administered through intraperitoneal (IP) route at a dose of 25mg/kg two times a week in mice for 21 days resulted in reduced GR mRNA expression in the liver, extensively reduced blood glucose, free fatty acids, triglyceride and reduced glucagon stimulated cAMP intervention in hepatocytes isolated from tested animals were absorbed. In addition to that, the antisense drug also enhanced glucose tolerance and reduced hyperglycaemic response to glucagon challenge. In another report, the author evaluated that non obese diabetic mouse dendritic cell (NOD) bone marrow-derived DCs intending to avoid diabetes in syngeneic recipients by Machen et al. The low surface CD40, 80 and 86 cells were exclusively regulated by treating NOD DCs ex vivo with a combination of anti-sense oligonucleotides. Engineered DC encouraged an increased occurrence of CD25 and CD4 T cells in NOD recipients at all age groups studied, and diabetes-free recipients exhibited greater numbers of CD25 and CD4 T cells compared with untreated NOD mice. Therefore, the occurrence of diabetes was considerably postponed by a single-dose administration of the engineered NOD DCs into syngeneic recipients. Oshitari et al. reported the outcome of combined antisense oligonucleotides against elevated sugar levels, diabetes-induced over expression and increased vascular permeability was identified in rat microvascular endothelial cells (RMECs). The result shows the combined AS-oligonucleotide approach is useful in simultaneously dropping laminin overexpression, fibronectin, collagen IV expression and decreasing vascular leakage in the retinal capillaries in the tested rats. The decision strongly recommended that the unusual level of

ECM components may give vascular out flow in the diabetic retina. Sloop et al. found that setback of diabetes by glucagon receptor antisense oligonucleotide (GCGR ASOs). The rodents were utilized to determine the level of blocking effect of glucagon receptor (GCGR). Here, the experimental animals were treated with a 2-methoxyethyl- modified phosphorothioate ASO. The effect of GCGR ASOs reduced GCGR expression, regulated blood glucose, preserved insulin secretion and improved glucose tolerances, notably reducing the expression of cAMP-regulated genes and avoiding glucose production in the liver. Moreover, GCGR reserve improved blood concentrations of active glucagon-like peptide-1 and insulin levels in pancreatic islets.

□ **Anti-Arthritic Agent:**

Akhavain and coworkers examined the effect of micro-encapsulated antisense oligonucleotides as a potent healing agent employed to selectively reduce nuclear factor- kappa B (NF-kB), which acts as a vital transcription factor in the inflammatory condition. The study suggested that there is a considerable inhibition of NF-kB after treating with microencapsulated antisense oligonucleotides. Therefore, it can be suggested as a potential treatment in the pathogenesis of inflammation. A novel method of treatment of ASOs for a reserve of ADAMTS in co-delivered and resident joint cells in osteoarthritis by Garcia et al. The ADAMTS enzyme is responsible for the loss of proteoglycans during cartilage deterioration in osteoarthritis. Locked ASOs released from biomaterial scaffolds for specific and prolonged ADAMTS inhibition in co-delivered and resident chondrocytes. Inclusion of the gapmer is a fibrin-hyaluronic acid hydrogel that displayed a delayed-release profile for up to 2 weeks. The effective knockdown of ADAMTS5 was exhibited up to 2 weeks in gapmer loaded and gapmer-free hydrogel. Makalish et al. discussed the effect of antisense oligonucleotide Cytos-11



inhibiting TNF- α gene expression in a rat model. The Cytos-1ASOs has been shown a potent suppression of TNF- α expression for joint inflammation, peripheral blood concentrations and reduce pan-nus development. The obtained results were compared to adalimumab. Morita et al. were reported the inhibition effect of rheumatoid synovial fibroblast growth by ASOs. The fibroblast cells secreted interleukin-1 β responsible for rheumatoid arthritis (RA). Interleukin-1 β has been treated with ASOs by targeting messenger RNA. Both mRNA and protein levels of proliferating cell nuclear antigen were concealed in the cell treated with antisense oligonucleotides, demonstrating that the anti proliferative effect was attained through a novel method of treatment.

Hildner et al. demonstrated that the transcription factor STAT4 induced arthritis which was treated effectively by novel antisense oligonucleotides (ASPOs). The STAT4 factor is responsible for creating signals to different pro inflammatory cytokines such as IL-12, IL-23 and IL-15 that commence and stabilize the production of 1 cytokine. A specific ASPOs focused on the translation site and reduced STAT4 levels and signs of CIA even when applied during the onset of disease symptoms. In vitro and in vivo studies of antisense oligonucleotide were performed for targeted delivery to tissues and cells by Nakamura et al. ASOs are able to bind specific gene regions and control protein translation, they are helpful to avoid abnormal endogenous processes connected with a particular illness such as dyslipidaemia and hepatitis. The author targeted potential road blocks in the particular clinical translation through ASO-based therapies for the management of osteoarthritis. The stage is set with the ongoing surge in human genomic and proteomic data that will enable the detection of promising RNA targets for ASOs.

□ **Anti-Inflammatory Agent:**

A multi-target antisense technique has been used against PDE7 and PDE4 decline smoke- induced respiratory inflammation in mice by Fortin et al. The study revealed that the effect of 2'-deoxy-2-Fluoro- β -D- arabinonucleic acid (FANA)-containing ASON targeting the messenger RNA of PDE4B, of PDE 4D and PDE 7A subtypes of pulmonary inflammatory markers. When used in combination ASONs, drastically abrogated the cytokine-induced discharge to near baseline levels. Therefore, the experimental animals treated with combined AONs and exposed to cigarette smoke, considerable inhibition of target mRNA in cells from lung lavages. Karras et al. found the anti-inflammatory effect of inhaled IL-4 receptor- α antisense oligonucleotide (IL-4RA) in mice models. The Th2 cytokines called IL4 and IL13 cause allergic lung inflammation as well as airways hyperreactivity (AHR) in asthma. The mentioned receptor-lacking mice are opposed to allergen-induced asthma, which stress the healing effect of selective inhibitors. The authors designed a chemically modified IL-4RA that specifically reduces IL-4R α protein expression in lung eosinophils, dendritic cells, macrophages and airway epithelium following inhalation in allergen challenged mice. The experimental results support the possible utility of IL-4RA in asthma/allergy. Ramelli et al. found a novel drug delivery of LNA oligonucleotides that curbs airway inflammation in a HDM model. During the literature study, an ASO preventing mmu-miR-145a-5p was estimated in a mouse model for mild-moderate asthma. ASOs in the form of nanoparticles dispersed to the majority of cells in the lung but were not there in the smooth muscle of the upper respiratory tract. Therefore authors reduced obstructive airway remodelling, mucosal metaplasia, eosinophilia and CD68 immuno reactivity. The result reveals that the nanoparticles were delivered in the lungs and treated pulmonary inflammation due to the normalization of interferon pathways.



Donner et al. described that a new method of treatment for doxorubicin-induced nephropathy and renal inflammation through antisense oligonucleotides. The data obtained from preclinical and clinical suggested the activation of CD40 gives to nephropathy, renal injury and inflammation. The inhibition of renal CD40 expression is a novel way to treat injury and unilateral ureter obstruction in mouse models. Experimental animals were administered with 2.5 CD40 ASO inhibiting CD40 mRNA levels between 75 and 90% in the renal organ. Therefore, the study recommends strongly CD40 ASO as an effective therapy in doxorubicin-induced nephropathy, renal inflammation and injury. Zorzi et al. investigated a new method of treatment for inflammatory bowel disease using Smad7 antisense oligonucleotide (ASOs). Transforming growth factor- β 1 (TGF- β 1) is a potent director of numerous mucosal inflammation in the gut and other parts of the body. Smad7 antisense oligonucleotide inhibiting TGF- β 1 has been documented in inflammatory bowel disease (IBD). These findings demonstrate that Smad7 ASOs is safe and well tolerated in patients with Crohn's disease.

□ **Anticancer Agents:**

Vanderborght et al. described the effect of antisense oligonucleotides (HIF-1 α and HIF-2 α) on tumorigenesis, fibrosis and inflammation in a mouse model. Hepatocellular carcinoma (HCC) is naturally associated with the hypoxia-inducible factor (HIF) that acts a vital role in HCC growth and development. Hence, the author examined the therapeutic action of isoform-specific HIF-1 α and HIF-2 α ASOs on the tumorigenesis, fibrotic and inflammatory components of the tumour micro environment. The results discourage the use of both iso forms of HIF-1 α and HIF-2 α . ASOs as targets for the treatment of hepatocellular carcinoma .

Abaza et al. reported c-myc antisense oligonucleotides used to treat human colon tumour and carcinoma. Authors learned the process of making the sensitivity of human colorectal cancer cells using c-myc antisense phosphorothioate oligonucleotides ([S]ODNs) with chemotherapeutic drugs such as 5-fluorouracil(5FU), taxol, vinblastine and doxorubicin either alone or in combination. After administering of c-myc AS[S]ODNs alone, the development of tumour cells was inhibited considerably ($p < 0.006$) and levels of protein and c-myc mRNA were significantly reduced. The combinations of chemotherapy exhibited time and dose-dependent additive and/or synergistic anti tumour properties. Here, cells treated with either c-myc AS[S]ODNs alone or in the combination with cytotoxic drugs were arrested the cell growth in G2/M and S Phase. The combination of treatments also showed a noticeable apoptotic effect compared to a single treatment. A recently published article reported that an antisense oligonucleotide drug (ASOs) targeting miR-21 induces H1650 apoptosis by Ge et al. MicroRNAs have been considered as a vital role in the progression of many tumours. Here, five molecules of oligonucleotides were designed, synthesized and identified for anti tumour activity. The suggested antisense oligonucleotides target microRNAs by gene silencing. Among these five antisense oligonucleotides, phosphorothioate oligonucleotide 4 inhibited the proliferation of H1650 cells due to the stimulation of apoptosis by triggering the caspase 8-apoptotic pathway. Ciardiello et al. found the reserve of bcl-2 as tumour therapy. Antisense oligonucleotides bind to particular-lar mRNAs and control the endogenous expression of genes. Therefore, tumour development and progression could slow down the tumour cell. Here, ANOs bind to the target messenger RNA by Watson-crick base-pairing resulting in the reserve of mRNA translation into protein. A recently published



article reported that a phase I/II clinical study of antisense oligonucleotides (LY900003) against cancer treatment by Villalona-Calero et al. It inhibits protein kinase C- α , with a combination of gemcitabine and cisplatin in patients with advanced non-small cell lung cancer (NSCLC). The protein kinase C- α is involved in malignant transformation and development. The mentioned ASOs with the combination of existing chemotherapeutic agent ensure the safety and requisite pharmacokinetic interactions were evaluated in phase I clinical trials. There are no significant adverse effects seen in any of the patients. In phase II, the combination of dose with gemcitabine and cisplatin was given and assessed anticancer activity in patients with advanced NSCLC. Finally, the author concluded that LY90003 can be administered safely along with the existing chemotherapeutic agents and has shown excellent action against cancer cells [20]’).

6. General Applications:

- Anti-viral agents
- Obesity agents
- Anti diabetic
- Cardiovascular diseases
- Amyotrophic lateral sclerosis
- HIV/AIDS
- Haemorrhagic fever
- Spinal muscular atrophy
- Arthritis
- Inflammation
- Asthma
- Renal disease
- Cancer

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