NEW HORIZONS IN ONCOLOGY- THE YEARS TO COME...

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Over the last fifty years the field of oncology has gone through a spectrum of changes showing major paradigm shifts from single cell cycle theory to cellular biology and genetics in cancer. During the initial developmental phase, cancer was considered as rapidly dividing cells, which had lost their normal growth control and divided continually and uncontrollably. It was identified as immunologically foreign and related to cancer specific antigens. Hence stress came on drugs acting on the cell cycle and hence disturbed the kinetic of cancer cell cycle. Use of BCG and C Parvum in cancer for non-specific stimulation of immune system had shown regression of primary tumours and also restriction of secondary spread. Use of cancer vaccines, interferons, interleukins, TIL, LAK cells and monoclonal antibodies marked the basis of immunological modulation in cancer medicines till twenty years back.

In the last two decades the shift in limelight came on treating the molecular defect and focus came to be on oncogenes, tumour suppression genes, antiangiogenesis and apoptosis. The modern concept of cancer management is focussing on mechanism based, rational anticancer drugs and the treatment of disseminated disease. Inhibitors of oncogene function, check point repair processes, angiogenesis and cancer cell immunization will form the fundamentals of cancer therapy in next years to come.

The major areas where the next decade will see changes in cancer management are

I) Radiation and chemotherapy protectors.

II) Use of IMRT and Stereotactic RT

III) Immunotoxins and cancer vaccines

IV) Antiangiogenesis

V) Antisense inhibition of genes and gene therapy.

I) RADIATION AND CHEMOTHERAPY PROTECTORS

Administration of a protective agent before chemotherapy or radiation or following therapy, to rescue normal cells over malignant cells has gained prominence because cancer therapy is associated with adverse reactions which limit the intensity of treatment delivered, significantly impact the quality of life and can be life threatening at times.

Amifostine, a novel compound, which is administered before radiotherapy or chemotherapy, came from a clan of compounds investigated by US Army for a classified nuclear warfare project. It protects cell by scavenging free radicals. It has the ability to selectively protect broad range of normal, but not neoplastic tissue from the cytotoxic effects of radiotherapy and chemotherapy. Amifostine (WR - 2721) being a prodrug in its active form 'WR - 1065' protects cells by scavenging oxygen free radicals and hydrogen donation to repair damaged target molecules. Ability of normal organs to take up higher concentration of free thiol than a tumour, along with hypovascularity and lower interstitial pH as compared to a tumour results in low rates of producing activation in tumour. Due to its very short half-life no protection is conferred in those cases where cytotoxic drugs have prolonged half-life or drugs that require a prolonged infusion time. From many pre-clinical and clinical studies it has been established that amifostine can protect normal tissues like jejunum, colon, lung, bone marrow and kidney from the side effects of radiation and chemotherapeutic agents. It can be given in a dose of 340 mg/m2 on alternate days or 200 mg/m2/d before Radiotherapy. Amifostine is a well tolerated drug with transient side effect including nausea, vomiting, sneezing, flushes, somnolence and occasional allergic reactions. The most clinically significant toxicity is hypotension.

Another new drug dexrazoxane, a cyclized analogue of EDTA, is used to rescue many patients who receive anthracycline, from it's cardiotoxic actions. Anthracycline induces cardiotoxicity, irreversible diffuse myocardial injury leading to cardiomyopathy. Dexrazoxane, the only cardio protective agent, acts through chelation of iron. After penetrating the cell membrane it is converted to a opening chelating agent that interferes with iron mediated free radical generation, thereby reducing the amount of iron available and preventing the formation of

oxygen radical producing metal iron-doxorubicin complex. Many preclinical and clinical studies have clearly shown its beneficial effects with only mild side effects like transient leukopenia, thrombocytopenia, decrease in haemoglobin level, alopecia, anorexia, nausea, vomiting and dose dependent elevation of bilirubin, SGOT, SGPT.

Haematopoietic growth factors and cytokines are being evaluated as agents which diminish haematopoitic toxicity and mucositis due to chemotherapy. The pathogenesis of epithelial injury is multifactorial. Mechanism of protection involves direct binding on cytokine receptors of epithelial cells or secondary cytokine release. G - CSF and GM - CSF along with cytokines like IL - 1,2,11, EGF and TGF31 are under study to evaluate whether they truly decrease the toxicity of Chemotherapy.

Leucovorin is used as a rescue agent in patients receiving high dose methotrexate as in lymphoma, leukemia and osteosarcoma. MTX inhibits dihydrofolate reductase and depletes intra-cellular folate pool, thereby impair synthesis of purine pyrimidine thus preventing the toxic effects of MTX like myelosuppression and GI side effects.

Cyclophosphamide and Ifosphamide produce haemorragic cystitis by producing acrolein, a metabolite. Mesna, a sulfhydryl compound, acts in urinary tract to detoxify acrolein.

ACTH analogues like alfa-MSH, metanocortin, ORG - 2766 and neurotropins are under active evaluation.

II) INTENSITY MODULATION OF THE RADIATION BEAM AND STEREOTACTIC RADIATION

Routine radiation techniques cannot always fully control localized human tumour because of variation in clonogen radiation sensitivity, inaccuracies in tumour target coverage and inability to apply maximum tumour dose levels because of proximity of normal tissues. In theory, three-dimensional conformal radiotherapy could overcome these problems by conforming the spatial distribution of the prescribed radiation dose to the precise 3D configuration of the tumour and minimizes dose to surrounding normal tissue. But in actual practice, conventional 3D - CRT failed to pinpoint tumour tissue accurately with resultant spillage.

To improve the conformity of dose distribution came IMRT, a new approach of 3D treatment planning. Optimized intensity modulated beams produce non uniform dose distribution for each of a number of beams with the requirement that all the dose distributions added together result in a desired pattern of optimum dose distribution conforming much closer to the shape of the target volume. Intensity modulation involves the optimum assignment of non-uniform intensities (i.e. weights) to tiny sub-division of beams - 'beamlets' or rays. The ability to manipulate the intensities of individual rays within each beam permits greatly increased control over radiation fluency, with can be used to custom design optimum dose distribution. Multi leaf Collimator (MLC), Computer Controlled Radiotherapy (CCRT) and use of MLC in dynamic mode made this possible.

Designing intensity modulated treatments requires determining optimum intensity distributions within the fields of a number of radiation beams incident on the tumour volume. Planning of intensity modulated treatments is similar to that of standard 3D - CRT conformal treatment which begins with delineation of outlines or colours of clinical target volume (CTV). PTV is used by treatment planners to design treatment. Then determination of the direction of beam is carried out followed by optimum intensity distribution by Computer aided optimization method. It is imperative to define qualitative criteria to evaluate and rank treatment plans in a clinically relevant manner. Criteria based on dose limits have been used. In general, however the criteria should incorporate biologic indices. A commonly used method for optimization is inverse radiotherapy technique where the criteria of effectiveness of a treatment plan is specified in terms of the desired dose to the target volume and the desired upper limits to the surrounding normal tissue. The intensity distributions within each of a set of beams are determined by the mathematical inversion of a desired dose distribution. Application of inverse technique is limited to the optimization of plans for which the criteria are based only on dose limits, but it would be advantageous to specify the criteria of optimization to include biologic indices like Tumour Control Probability (TCP) and Normal Tissue Complication Probabilities (NTCP). In addition to the clinically relevant definition of the optimization criteria a number of other factors affect the optimality of the treatment plans. Proper accounting of scattered

radiation can lead to sharper beam boundaries, steeper gradient at the interfaces of overlapping or adjacent normal structures and reduced margin, thus providing greater protection of normal tissue and more homogeneous target dose. Another significant factor is the number of beams and their angles. But IMRT is less sensitive to the above-mentioned factor. Minimum number of beams should be used. Beams are normally placed at equiangular steps around the target volume. Optimization of angles of intensity modulated beams is a highly computer resource demanding process.

Multi leaf collimator has dynamic mode leaves, which can be used to modulate intensities to produce 2-D non-uniform profiles of arbitrary shape. Since intensity modulated treatments are delivered remotely or automatically under computer control, extra safety measures are required to ensure collision free trajectories of the components of the treatment machine. These include immobilization of the patient and a " Virtual Treatment " before the actual treatment is delivered. The treatment machine computer automatically sets up the various components of the machine and switches on the radiation beam. It moves leaves during irradiation as specified in the leaf motion data set.

For simplicity, beams are often constrained to be in the same transverse plane. However, non-coplanar beams may provide additional gain in the quality of treatment. It may also be apparent that when optimized intensity modulated beams are employed the quality of the resulting plan is relatively insensitive to the orientation of beams. But as dynamic MLCs now can deliver multi field intensity modulated conformal treatments in short times and automated treatment planning particularly computer aided optimization, reduces the time and effort required even when a large number of intensity modulated beams are used, beams numbering in the range of 6-15 may be quite acceptable. Intensity modulated treatments delivered with a dynamic MLC require more precise quality control. Though the treatment is complex, automation is possible in planning and delivery and is likely to result in cost effective world class solutions.

Stereotactic radiotherapy (SRT) is another specialized branch of radiotherapy where high single dose of radiation is given to small intracranial targets by stereotactic target localization technique. In

larger targets (fractional External Radiotherapy) is given. Stereotactic radiotherapy combines two major approaches, stereotactic target dose localization along with biologic advantage of dose fractionation.

Now with the advent of CT and MRI we can localize and define intracranial tumours more accurately for RT treatment planning. The therapeutic ratio within the relative probability of tumour control and normal tissue complication can be improved by reducing the volume of irradiated normal tissue and avoiding large single doses of radiation. Optimal treatment planning for RT would provide conformity of the treated volume to the target volume in 3 dimensions which is best accomplished by the use of multiple treatment portal shaped to conform to the projection of the tumour volume along the beam axis (beam's eye view). Small tumours can be treated by linac based SRT, but in larger lesions it may create significant complication. Relocatable stereotactic frames with dedicated stereotactic linacs have made it possible to deliver fractionated Radiotherapy using stereotactic treatment planning technology, with the same precision in target localization, patient immobilization and focal dose distribution as in SRT with the added advantage of dose fractionation. Larger tumours and those near to or involving critical structures can be treated effectively.

Patients' positioning is done by patient specific dental appliance. Once the frame fitting is accomplished, a stereotactic CT Scan at 3 to 5 mm intervals is obtained. MRI is required when CT does not provide adequate imaging of target lesion or critical normal structure (Optic chiasma), but MRI does not have spatial resolution of CT and is subject to distortion artifact. Spatial resolution of CT and target definition of MRI can be merged. For SRT, MRI is done a few days before treatment. MRI - CT fusion is most useful in cases of low-grade, non-enhancing astrocytoma, acoustic neuroma and base of skull meningioma, which are poorly visualized on CT.

The treatment planning process must consider all possible beam approaches and select only those that cover the target volume projection in the beam's eye view and exclude critical normal structures. An iso center is then established and different diameter collimators are assessed for target coverage. It follows dose computation to make a non-spherical dose distribution. The treatment

plan is evaluated by analyzing the dose distribution delivered by beam configuration. For SRT, this evaluation requires 3D dose computation and dose reporting. The treatment dose is normally prescribed to the 95% isodose line.

In high-grade astocytomas, SRT can be used as a boost to conventional Radiotherapy (or for tumour recurrence). Altered fractionation scheme with stereostatic technique appears to be a promising approach for patients with recurrent intracranial tumours and prior exposure to Radiotherapy.

For small recurrent, post surgery residual or inoperable meningiomas, SRT has been used with mixed results. It is conferred that small lesions not located near or within any critical structures can be effectively treated by SRT. Tumour volume (4.1 cm^3) and location (sites involving cranial nerves or its nuclei) were found to be important predictors of complications.

Basics of conformal treatment are precise patient's immobilization, target delineation and field shaping. Stereotactic methods offer a means of delivering precise treatment to intracranial targets while minimizing dose to uninvolved normal tissue. Increasing clinical experience in stereotactic technique in Radiotherapy will allow assessment of SRT's role in improving the therapeutic ratio and long term survival of patients.

III) IMMUNOTOXINS AND CANCER VACCINES

Immunotoxins are conjugates of monoclonal antibody and a toxin. It is a new class of biologic anticancer agents designed to specifically bind and kill cancer cells that express specific target antigen. First generation toxins were made up of MAb (Monoclonal antibodies) and native plants or bacterial toxins. By recombinant DNA technology, DNA elements encoding the Fv fragment of an antibody can be fused to a toxin gene. Fv fragments are generally fused to toxins by recombinant DNA methods to form single chain immunotoxins and when growth factors or cytokines are linked to a toxin, it is termed recombinant toxin or oncotoxin.

Though first generation immunotoxins showed tumour regression in some patients suffering from lymphoid malignancies, severe toxicity due to non-specificity limits their use. So ligands should be specific

and should target a molecule that is abundant and uniformly present on cell surface. Bacterial or plant toxins are proteins with enzymatic activity that cause cell death either by inhibiting protein synthesis or inducing apoptosis. Riew, gelonin, sporin, poke weed antiviral protein (pap) are all plant toxins. Ricin is a glycoprotein synthesized from castor bean (ricinus communis). It is composed of A and B subunits where A subunit kills the cell by catalytically inactivating ribosomes and B subunit is responsible for cell binding. The mode of action of other plant toxins is similar to that of ricin, and is relatively nontoxic to cells and is used for immunotoxin production.

Bacterial toxins include pseudomonas exotoxin (PE) and diphtheria toxin (DT). X-ray crystallography and mutational studies have shown that PE is composed of 3 major functional domains: domain Ia - cell binding domain, domain Ib - no known function; domain II - translocation domain and domain III- catalyzes ADP - ribosylation and inactivation of elongation factor, thereby inhibiting protein synthesis leading to cell death. PE binds to cell - surface glycoprotein, which is the receptor for alpha2 macroglobulin and low-density lipoprotein. Then it is internalized by endocytosin and after enzymatic breakdown inactivates elongation factor (EF-2) leading to cell death. DT, produced by corynebacterium diphtheriae is made up of 3 domains. Fragment a, the catalytic domain, arrest protein synthesis and kills cell. Fragment b helps fragment a to enter into cells.

Targeting haematologic malignancies with ricin immunotoxins have shown some encouraging results, the treatment of metastatic epithelial tumours has been less successful. One of the major obstacle in developing immunotoxins for epithelial tumours has been the difficulty in finding antibodies that do not cross react with essential normal tissue. Several solid tumours including melanoma, cancers of colon, breast, ovary, bladder, and SCLC were treated with ricin immunotoxins. Life threatening toxicities were encountered but unfortunately response was very minimum. Immunotoxin from a conjugate of saperin, a plant toxin and anti CD30, a member of TNF receptors has been clinically evaluated in a advanced refractory Hodgkin's disease with good results.

The first PE containing immunotoxin was formulated in 1985.

Due to severe toxicity like encephatopathy these first generation PE toxins were modified. MAb B3 is an antibody that reacts with many cancers of colon, oesophagus, gastric, lung, breast, mucinous carcinoma of ovary, transitional cell carcinoma bladder and advanced Prostate carcinoma. For the first time in the history of immunotoxins against solid tumours a very encouraging response was noted. The difference in blood flow within tumours and elevated interstitial pressure greatly inhibit the penetration of macromolecular weight immunotoxins into the interior of tumour and this causes an unequal distribution of drugs, though these problems do not arise in haematologic malignancies and small tumours

The second-generation immunotoxins are genetically engineered small single chain immunotoxins made up of variable region of antibody coupled to toxin with overcome the tumour distribution problem. The most important property of these single chain immunotoxins is that they are more active.

Several problems that limit the clinical efficacy of immunotoxins are:

1. Using chemicals more specific for tumour cells can circumvent toxicity due to antigens present on normal cells.
2. Non specific toxicity due to uptake by liver or kidney.
3. Non specific dose limiting toxicity.
4. Immunogenicity
5. Size and stability of protein

Vascular leak syndrome is a major dose limiting toxicity of ricin, saporin, PAP and PE. Damage to endothelial cells results in reducing serum albumin and accumulation of fluid in interstitial spaces leading to weight gain and edema and in severe cases pulmonary edema.

The concept of immunotoxins is now more than 20 years old. Although the initial clinical trials were disappointing, recent trials show good antitumour activity in human mainly in haemopoietic tumours because tumour cells are readily accessible and antigens are better defined. Progress in the treatment of epithelial tumours has been slower, because tumour specific antibodies have been more difficult to identify

and difficulty in penetrating these tumours. The recent findings show that LMB-1 has antitumour activity against colon and breast cancers that can cause regression of tumours. Smaller more active and more stable immunotoxins may produce even more dramatic responses, making them useful agents for treating solid tumours.

Vaccines are generally used to induce passive immunity against infectious diseases; recently antigens are being used as vaccines to modulate immune system. Anticancer vaccines increase the immune recognition of cancer cells by variety of means like gamma- irradiation of tumour cells and admixture with BCG, virus and by gene modified tumour cells with Cytokines (IC-2, IC-4), GMCSF and MHC molecule. Most important influence on cancer vaccine development has been the recognition of the critical role that T-Lymphocytes play in antitumour immune response. In response to stimulation with autologous tumour cells, T cells lyse tumour cells. The antitumour response of T cells created new vaccines which induce antigen specific T-lymphocyte anti tumour immune response. Tumour antigens have an ability to clone and manipulate these antigens and lead to generation of defined antigen vaccine. Any intra cellular proteins, specifically expressed in tumour or unexpressed in composition to normal tissue can be a target of T-cell based immunotherapy.

The earliest type of vaccines are based on non-specific immunostimulation of specific antitumour reactivity by injecting tumours in vivo with bacteria, virus or other foreign antigens. Autologous or allogenic cancer cell preparation are generally modified exvivo to increase their immunogenicity by gamma irradiation or admixing immunologic adjuvants like alum, detox, BCG etc. Lately employing gene therapy, genes encoding immunomodulation (like IL, costimulatory molecules) were inserted into tumour cells. Tumour cell derived heat shock protein and dendritic cells pulsed with tumour cell extract also have gained importance. Recombinant and synthetic anti cancer vaccines are prepared by cloning of tumour associated antigen (TAA) which are recognized by T lymphocytes.

Autologous tumour cell vaccines are prepared from surgical resection or biopsy specimen. Autologous cells are usually killed, attenuated or irradiated before re injection. Autologous cell vaccine

are advantageous as tumours contain many unique and potentially immunogenic mutations that cannot feasibly be characterized and produced individually for each patient which allow immunization to the diverse antigens.

In allogenic tumour cell vaccine tumour cells from several patients are run in cell cultures and then whole cell line lysates are used as vaccines. Now it is used as a source of shared potential T Cell antigen for vaccination of patients with melanoma and in other types of tumour.

Allogenic cells may be infected with certain viruses to increase their immunogenicity. Another approach is to use shed tumour antigens in combination with adjuvants like alum or DETOX. As it is made of multiple cell lines there will be low probability that any individual tumour cell may escape immune detection. It is more consistent thanthe autologous one. Allogenic cells can be more easily transfected with cytokines to increase their immunogenicity.

Modern vaccines are composed by encoding of tumour associated antigens whose proteins and DNA sequences are known. Vaccines of these type can induce only cellular or humoral immune response or both .TAA can be produced synthetically or by recombinant DNA technology or the gene for TAA can be encoded by plasmid DNA or can be incorporated in certain viruses. These vaccines are more potent as it allows greater control over quantity and kinetics of immune stimulation. Antigenic sequences can be fused with IL-2 or co-stimulating molecules like B7- 1. As the tumour cells have heterogenicity due to mutation, they may escape immune recognition of a defined antigen.

Historically the most common approach to enhancing immunogenicity of an antigen was to mix it with non-specific immune adjuvants like BCG, C Parvum, alum etc. A similar strategy involves the xenogenization of tumour defined as introduction of highly immunogenic foreign antigens into or onto the tumour. Increased immunogenicity can also be achieved by gene modification of tumour cells which then express cytokines, T cell costimulatory molecules etc., Cytokines can be used systemicaly or locally to increase immunogenicity. Many helper peptides can activate helper T-cells

which can facilitate the generation of cytotoxic T- Cell response. Adhesion molecules or costimulatory molecules may be transfected to tumour cells to increase their immunogenicity by interaction between APC's and T-cells leading to T-cells activation. As CD 8 + T cells are critical for producing tumour regression antigens are delivered by viral vectors like adenovirus, retrovirus which can infect APC's to induce CD 7 + T cells activation. In DNA based immunization process, DNA propagated as a bacterial plasmid can be injected intramuscularly or deposited in the epidermis through 'gene gun'. Strategies using naked DNA can be enhanced by the provision of cytokines involved in T-cell differentiation and growth. Antigen genes contained in bacteria like Listeria, oral BCG and APCs (dendritic cells) can be used to immunize patients.

The vast majority of cancer vaccine trials have been conducted in patients with melanoma both in metastatic and in surgical adjuvant setting. Now focus is on defined antigen vaccines to immunize against CEA. Antiidiotype vaccines are being currently tested in clinical trials. In patients of renal cell carcinoma, lymphoma, myeloma and leukemia vaccines showed good objective response rates and survival benefit.

The specificity of expression of prostate specific antigen (PSA) in normal prostate and prostate adenocarcinoma makes it a potential target antigen for vaccine immunotherapy. Human papilloma virus is linked to the development of cervical cancer. Vaccines against cervical cancer have been developed with E6 and E7 proteins of HPV. Development of vaccines consisting of the viral capsid proteins is being considered for prevention of HPV infections. Breast and ovarian tumour are similar in their expression of several molecules like MAGE genes, p53 mutations, her - 2 - neu and MUC - 1 that are targets for vaccines immunization. Clinical trials are beginning to address these issues. Mutation occurs in the ras oncogene protein in about 30 % of all malignancies. Fusion proteins characterize several malignancies, for example bcr-abl oncogene in CML. These proteins may be the targets of vaccines in near future.

Difficulties in successful immunotherapy are faced in antigen specificity and generalized immune deficit in patients, insufficient magnitude of immune response to activate a mechanism that can

produce tumour regression and tumour heterogenicity. Tumour cells can escape immune recognition by a number of mechanisms and can produce immuno - suppressive mediators. High tumour pressure or inadequate vascularization may prevent the entry of T cells or other effector cells. Perhaps in the future more potent immunization methods may minimize these problems and bring in major breakthrough in immunotherapy.

IV) ANTI-ANGIOGENIC THERAPY

Angiogenesis means growth of new microvessels. This process depends mainly on locomotion, proliferation and tube formation by capillary endothelial cells. During angiogenesis endothelial cells emerge from their quiescent state and proliferate rapidly. Pathologic angiogenesis while still a focal process persists for months or years. The fundamental goal of antiangiogenesis therapy is to return the foci of proliferating microvessels to their normal resting state and to prevent regrowth.

The rationale for antiangiogenic therapy is that progressive tumour growth is angiogenesis dependent. In the complete absence of neovascularization human tumours spontaneously are restricted to very small sizes which range from microscopic insitu carcinoma to micrometastases. Angiogenic activity can occur before or after the onset of neoplasia. For example, in carcinoma of cervix angiogenic activity first appears in the pre noeplastic stage of dysplasia, whereas in many other tumours (breast, prostate), the angiogenic phenotype appears after the neoplastic change. The positive regulators of angiogenesis include at least 14 angiogenic proteins. Basic fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) are found in majority of different types of human tumours. Other evidences suggest that certain tumour suppressor genes may normally code for proteins, which inhibit angiogenesis, and that angiogenic factors may be under the control of specific oncogenes.

More than 40 angiogenesis inhibitors have been reported till date. Majority of inhibitors are still in preclinical study phase. A group of endogenous proteins, which inhibit endothelial growth, may play a physiologic role in maintaining the normally low replication rate of vascular endothelial cells. Some of these endogenous endothelial

inhibitors can be demonstrated to inhibit angiogenesis in vivo. They include platelet factor 4, thrombospondin, and interferon alfa -2a, interleukin - 12 and angiostatin.

Angiostatin is a potent antitumour agent. It is a 38 kd protein where sequence is identical with the first four triple loops structures of plasminogen. Angiostatin is a specific inhibitor of endothelial proliferation and has no effect on tumour cell growth or the growth of non-neoplastic cells. It is the first angiogenesis inhibitor that can regress human carcinoma in mice to a microscopic dormant state, in which all neovacularization has been seen to be blocked without toxicity. Antiangiogenic therapy began in humans in 1988 with the first use of interferon alpha - 2a for life threatening hemangiomas in children. Interferon alfa - 2a is less toxic in infants and childrens than in adults and its toxic effects are usually reversible. The active compound fumagilin, secreted by a fungus (Aspergillus fumigatus) was discovered, showed to inhibit growth of capillary endothelial cells in culture. A synthetic analogue of fumagillin, angiogenesis modulator 1470 (AGM - 1470) was more potent angiogenesis inhibitor than the parent compound. It is more selective agent for endothelial cells. Other angiogenesis inhibitors have entered clinical trials for patients with advanced cancers are carboxyamino triazole, Tecogalen, Thalidomide. Antiangiogenesis inhibitors are slowly showing results in clinical trials and in near future will come in successful clinical use.

V) ANTISENSE INHIBITION OF GENE EXPRESSION AND GENE THERAPY

The antisense technology came into being from the specificity of the Watson - Crick base pair interaction between m RNA and an exogenous complementary oligo deoxy nucleotide. The specificity resides in sequence of the oligos in RNA strand, which is the sense strand and the oligo forms the antisense strand. The antisense oligo, forms m RNA - DNA duplex, specifically prevents the translation of the m RNA into protein.

Antisense oligos have recently entered clinical therapeutic trials. First generation antisense oligos were manually synthesized but recent methods of synthesis creates oligos of virtually any length. Automation quickly followed, leading to several generations of so called gene

machines. A major problem with the use of phospho di ester oligos is the fact that intra and extra cellular nucleasedigestthem. Antisense oligos (phospho di ester and phosphorothionate) successfully inhibits a whole lot of cancer related targets like MYB, BCR-ABL, MYC, BCL 2, JUN, EGF, IL-1, g-protein etc. Oligos may also be useful in targeting individual cellular genes at the DNA level, termed antigene therapy.

For oligonuleotides to be effective antisense agents, they must first enter cells and achieve the appropriate concentration in the correct intra cellular compartment. Charged oligo cannot passively diffuse through cell membrane. They are taken up actively by cells. Metabolic inhibitors slow the process of internalization. The rate of oligo internalization is temperature dependant.Oligo internalization in cells depends predominantly on two processes of adsorptive endocytosis and fluid phase endocytosis. The most successful strategy to increase the intracytoplasmic and intranuclear concentrations of antisense oligo deoxynucleotide has been with the use of cationic lipids. Microinjection of specialized oligos into the cytoplasm is an effective method of producing antisense inhibition of target translation.

The bcl-2 gene, involved in the t (14:18) translocation in most follicular NHL, plays a role in the survival and growth of normal cells mainly by suppressing programmed cell death (apoptosis). The over production of bcl - 2 protein can increase the resistance of cells to a wide variety of chemotherapeutic agents like adriamicin, oncovin, CDDP, MTX making it an ideal target for antisense therapy. Many investigations using different tissue culture systems showed reduction of bcl - 2 protein along with sharp drop in cellular viability, which lead to increased sensitivity of cells to chemotherapeutic agents. These results suggest that a therapeutic strategy involving marrow purging of malignant cells by a combination phosphorothioate antisense oligos and standard chemotherapy is worth considering.

The BCR-ABL fusion gene present in haemotologic malignancies like CML, is the target of oligos in many trials. These trials showed decrease in those cells, which harbour bcr-abl fusion gene with virtual disappearances of bcr- abl mRNA transcript. Further research showed that antisense oligos alone were not sufficient to entirely eliminate the bcr-abl mRNA transcript. Thus at present the role of antisense oligos

targeted to the bcr-abl mRNA for the treatment of CML is not clear.

Basic fibroblast growth factor (BFGF) in brain directly promote glial cell tumour growth or indirectly, through stimulation of angiogenesis. Many studies have used oligos targeted to BFGF, mRNA to reduce the growth rate of human glioma cells. NF-KB is a heterodimeric transcriptional regulatory factor, which participates in regulation of a large number of cellular and viral genes. It appears to have particular relevance to the malignant phenotype.

Several studies showed, on blocking of myc protein production cells were blocked at G1/S interface. Inhibition of myc-protein levels leads to decrease in cellular proliferation. This method has been used in Burkitt's lymphoma lines and human leukemia lines by different investigators with equivocal results.

The major challenges in development of antisense therapeutics include the following:

- Developing appropriate mRNA and cellular targets based on pharmacological principles.
- Delivery of the oligo to cells and to an appropriate intra-cellular compartment.
- Understanding the extent of nonsequence specificity and developing ways to minimize the interaction of oligos with the cell membrane.
- Optimizing pharmacokinetic properties for maximum oligo efficacy.
- Designing new oligo backbones optimized for efficacy and toxicity.

Gene therapy is a technique by which a functioning gene is inserted into a cell to correct its metabolic abnormality or to introduce a new function. As cancer occurs due to mutation or loss of genetic material within cells, genes can be introduced into the cells to correct the abnormality. Broadly there are two methods of gene transfer: viral and non-viral. The choice depends on the biologic requirements of the specific therapeutic strategy e.g. to protect haemopoietic stem cells from the toxic effects of chemotherapy, and to immunize patients against cancer only transient expression of antigen genes are required. Virus is used extensively to introduce genetic material because virus can

deliver genes to cell with higher efficiencies compared with non-viral methods. Many viruses have been used for gene transfer e.g. Retrovirus, adenovirus, adenoma associated virus (AAV), vaccinia virus and fowl poxvirus.

Retroviruses are RNA viruses that are capable of stably integrating DNA within the host cell genome. Retroviral vectors for gene transfer have been constructed by substituting the genes of interest in place of the viral protein coding regions, thus making these vectors replication incompetent, so these viruses can infect target cells but cannot replicate due to the absence of the protein coding region. The most common method of gene transfer is by the retroviral vector. Their advantage includes the ability to stably integrate into the host genome in absence of viral protein expression. Disadvantages include low level of gene transfer efficiency, low activity in vivo resulting from complement mediated viral inactivation and safety hazards like probability of generating replication competent retrovirus and potential of inducing insertional mutagenesis. Initially it was reported that replication competent amphotropic retroviruses were not pathogenic they established they a chronic retroviremia in a severely immuno compromised host. Now modified form of retroviral vectors are produced which greatly decreases the likelihood of recombination to generate replication competent recombinant retroviruses (RCRS).

Adenovirus can be used for gene transfer after making them replication deficient by removing the E1 gene region, which is responsible for transcriptionally activating other genes. The recombinant adenovirus is produced by cloning the gene of interest into the deleted E1 region. Adenoviral gene transfer can be performed in both dividing and non-dividing cells with higher efficiency and can result into high levels of gene expression. Adenoviral vectors can be produced in high titres and are capable of infecting some tissues directly in vivo, such as pulmonary epithelial cells.

Expression of transgene is transient and is lost as the infected cell divides and In vivo administration of current adenoviral vectors trigger host immune response both humoral and cellular are the major disadvantages.

Adenoma associated virus (AAV) can only replicate in the presence of a helper virus, such as adenovirus. So in the absence of helper virus it cannot replicate but instead integrates into host genome, which makes it a vehicle for gene transfer.

Several properties of pox virus, a family of DNA virus makes them an attractive vector for the introduction of foreign genes, because of the large size of the pox virus genome, large amounts of foreign DNA can be incorporated without adversely affecting viral infectivity. Multiple genes can be inserted each at a different site in the virus. Gene expression is not dependent on potential regulatory mechanisms of the host cell because it is entirely cytoplasmic and does not rely on host cell transcriptional machinery, hence a large quantity of gene expression has been observed in a wide variety of cells.

The earliest non-viral method of gene transfer used to introduce DNA into mammalian cells was calcium phosphate transfection. As the percentage of stable transfectants is exceedingly low, it is not useful for gene transfer for human clinical studies. Similarly electroporation of cells which reversibly disabilizes or permeabilizes cell membrane in order to introduce DNA, results in low percentage of cells that stably express the foreign gene. Another method is to inject the DNA directly into the cell by micro injection technique. As very few cells can be injected manually hence this method is not practical for clinical purposes. Gene gun propels gold beads coated with DNA into cells, although it results in low transfection efficiencies, gene transfer to epithetial cells can be performed in vivo, giving it a potential role in immunization strategies. The direct in vivo injection of DNA, termed naked DNA into selected tissues, such as muscle and thyroid has been utilized to immunize against the products of the encoded genes.

Non viral methods of gene delivery are more convenient and have safety advantages but result in transient gene expression with the added disadvantages of lower gene transfer efficiency.

The ability to label stem cells with unique integrated DNA sequences in haematopoietic stem cell marking studies has enabled to track stem cells and their differentiated progeny. This has helped to assess the sources of disease recurrence following ABMT therapy

in AML. Gene transfer itself is being investigated as a purging techniques, using antisense oligo deoxy nucleotides against activated oncogenes as a means to eliminate tumour cells within the autologous marrow.

There are several approaches in cancer gene therapy technique:

i) Genetic modification of the immune response

ii) Modification of tumours with genes that have direct antitumour effects

iii) Introduction of genes into haematopoietic stem cells to decrease toxicity from chemotherapy.

Genetic modification of the immune response can be done by cytokine genes TNF, IL-1 ,4,6,7,8 , GCSF etc. In human studies both ex vivo and in vivo gene transfer with both viral and non-viral vectors using numerous cytokines genes, HLA-b-7 and costimulatory molecule b7 has been done. This has been used in malignancies of breast, kidney, colon, prostate, GI tract, ovary, head and neck cancer, neuroblastoma, glioblastoma and melanoma. No significant therapeutic benefit has been reported so far.

Other method of genetic modification of the immune response is by immunization with genes encoding tumour antigens. This can be done by recombinant vaccines and by dendritic cells. In recombinant vaccines whole tumour cells have been used as immunogen. Recently cloning of several melanoma antigens recognized by T-cells has opened new possibilities for active immunization strategy for cancers. Antigens expressed at high levels in recombinant viral vectors can induce a significant antitumour immune response. Significant antitumour effect is also seen in immunization studies using naked DNA or by gene gun technique. Thus the mainstay of gene therapy is genetic modification of immune effector cells by

i) Enhancing survival of immune cells

ii) Increasing tumour recognition by using novel receptor genes,

iii) Increasing antitumour efficacy of immune cells.

For T cells to be functional against a tumour, they must survive in vivo, recognize the target and then execute an adequate antitumour

effector mechanism, either by direct cell lysis or by release of cytokines that may attract and stimulate other immune cells. There are several ways to potentially enhance these steps: systemic administration of IL-2, inserting the gene for IL-2 in T - lymphocytes. A variety of monoclonal antibodies have been developed that bind to specific cancers. Cancer therapy with antibodies has been largely disappointing, partially because of the lack of an adequate effector mechanism to destroy tumour cells on binding. TNF has potent antitumour activity and can result in dramatic tumour regression in some cancers but TNF has dose limiting adverse effects like severe hypotension.

‘ Suicide gene ‘ is a unique technique of gene therapy in cancer patients. Retroviral vectors integrate preferentially into dividing cells, so replicating tumour cells may be target of retroviral vectors compared to normal cells. This treatment approach is limited to those cancers whose primary morbidity is a result of local unresectable disease. Current trials are evaluating this approach in patients with brain tumours, ovarian cancer and malignant mesothelioma. These trials utilize retroviral and adenoviral /gene transfer technique to deliver the herpes simplex virus (HSV) -TK or cytosine de aminase suicide genes, followed by systemic therapy with ganciclovir (for HSV-TK) or 5-fluorocytosine (for cytosine deaminase).

Cancer gene therapy approaches also involve introduction of genes into haematopoietic stem cells to decrease toxicity from chemotherapy. Expression of the multidrug resistance (MDR) gene reduces the toxicity of taxols, actinomycin D, adriamycin and VCR by encoding for a transmembrane molecule that actively pumps these cytotoxic agents out of the cell. Expression of the MDR gene in tumour cells is commonly associated with tumour resistance to these cytotoxic agents. Clinical trials are now underway that attempt to genetically modify bone marrow cells with the MDR gene, followed by reinfusion into the patients in an attempt to decrease haematopoietic toxicity from subsequent chemotherapy

Gene expression is variable, depending on the target cell types and cannot be dependably regulated by current vectors. Progress in gene therapy is limited by the restriction imposed by the present

techniques of gene transfer and gene expression and hence improved vectors need to be developed.

IN INDIAN PERSPECTIVE

Radiation and chemotherapy protectors are being successfully used in Indian scenario, especially in radiotherapy for head and neck cancers, pelvic cancers, magna field irradiations and with chemotherapuetic agents. Many prospective trials are underway, results of, which may be available in few years.

IMRT and SRT are being clinically used with success in the major cancer hospitals in India. Since the technology is quite new which needs state of the art machines as well as trained personnel the pace of popularization is slow. Within half a decade atleast all Regional Cancer Centers will be in a position to utilize these technologies in clinical setting.

India has to go a long way in field of researches in cancer vaccines, immunotoxins, antiangiogenesis and gene therapy. Though few premiere research centres are doing commendable work in these following fields, but lot needs to be done specially in phase II and phase III trials. We might be optimistic in bringing out a positive outcome in these researches and further convert them into clinical practice by the end of this decade.