Current status of gene therapy in gastroenterology

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INTRODUCTION

The potential role of genetic intervention extends from diseases caused by single gene defects, through severe viral infections, to polygenic disorders, such as diabetes mellitus and arteriosclerosis. However, gene therapy can be defined as the introduction and expression of an exogenous gene in human cells for therapeutic benefit, and is conventionally restricted to human diseases associated with single gene defects. There are wider opportunities for genetic intervention and these include strategies to reduce or block gene expression as well as the introduction of nonmammalian genes. The rapid progress in our understanding of some of the molecular mechanisms involved in the pathogenesis of cancer and metabolic disorders, coupled with the development of gene delivery vector technology, has urged us to consider novel genetic approaches to digestive diseases.

There is no shortage of ideas and applications for genetic intervention in human diseases, but there are great limitations not only with the efficiency and targeting of the present generation of gene transfer vectors but also with our incomplete understanding of transcription control.

GENE TRANSFER TECHNOLOGY

The gene delivery technology is advancing rapidly and there have been specific developments that could be translated into gene based therapies for gastroenterological diseases. For example, *ex vivo* transfer methods are being studied extensively using hepatocytes obtained through liver biopsy, partial hepatectomy, and from specimens harvested for liver transplantation. Adult liver cells transiently undergo active proliferation permitting *in vitro* gene transfer even with vectors that require active cell division for entry and expression. Gene transfer may then be facilitated through a number of methods, including viruses, liposome, calcium phosphate coprecipitation, particle bombardment, naked DNA injection, and electroporation. The transfected cells are reintroduced into the host by using, for example, a microcarrier system into the peritoneum, gel beads, hepatocyte coated cell support matrix implanted next to liver tissue, or into

the spleen or portal circulation through direct injection.

The spectrum of delivery systems for *ex vivo* gene transfer is broadly applied also to the *in vivo* model. Although the transfer efficiency of liposomes is low, these lipids can be made comparatively easily to high chemical purity and have low immunogenicity, which may permit repeated administrations. They have been used successfully in an *in vivo* model, by topical administration to epithelial cells both in the airways and the intestinal tract and also by the intravascular route. A recent study showed high efficiency transfer of the APC tumor suppresser gene in liposome complexes delivered to normal mouse colonic epithelium by rectal catheter infusion. Almost 100% of epithelial cells expressed the gene for up to four days, which is consistent with the known rate of turnover of this tissue[1]. Intravenous injection of a rat insulin gene expression vector in liposome complexes results in uptake primarily by the liver and spleen. Improvement in hepatocyte uptake can be achieved by incorporating lactosyl ceramide into the phospholipid bilayer; this galactosyl terminal asialoganglioside is specifically recognized by a receptor highly selective for hepatocytes. Many different lipid agents are now being explored for efficacy of DNA transfer and it seems likely that the composition of the complex will have to be optimized for different targets and different routes of administration.

Of the available methods of gene delivery, viruses have been proved the most efficient so far. Achieving viral gene transfer to specific organs for clinical application will be difficult, however, particularly as viral titres 10 to 1000 times higher than those usually attained (typically 10^6 infectious units per milliliter) will be necessary for *in vivo* strategies. There is now extensive experience with retroviruses whose main advantages include their small size and easy manipulation, and with stable colinear integration with host genome. They are comparatively non-toxic and are efficient for gene transfer. Retroviruses persist in up to 5% of hepatocytes three months after injection of an infected hepatocyte cell suspension into the portal vein after partial hepatectomy. The small intestinal epithelium is an attractive target for gene therapy because of its large surface area, easy accessibility, and the presence of stem cells with known locations. Although few studies have yet targeted the intestinal system *in vivo*, marker genes have been transferred to the epithelial surface with retroviral vectors in animal models. Clearly, unless the therapeutic or marker gene is transferred to the stem cells, the rapid turn-over of this specialized

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epithelium would seriously limit potential benefits of delivered genes. Retroviruses have a number of disadvantages, notably the requirement for cells that are actively dividing to permit viral DNA integration, the ability to carry only small DNA sequences, and a small but finite risk of causing insertional mutagenesis as a result of random integration.

Currently alternative viral vectors with potential advantages over retroviruses in specific applications are under development. Adenoviruses can infect non-dividing cells, can be concentrated to high titres, and are comparatively highly efficient vectors. Adeno-associated viruses are ubiquitous and non-pathogenic in humans and can also infect non-replicating cells, but, like retroviruses and adenoviruses, are limited in the size of the foreign gene that can be inserted. This last problem may be overcome by the use of herpes simplex group viruses and possibly even vectors based on hepatitis B virus, which has potential additional advantages of hepatotropism and an ability to integrate with host genome *in vivo*.

GENE THERAPY IN GASTROENTEROLOGY

Strategies for genetic intervention can be divided into five main aspects: replacement or augmentation of gene expression; reduction of expression of genes by antisense or ribozyme technology; genetic prodrug activation; augmentation of immune responses; and polynucleotide vaccination.

Some strategies can be achieved by *ex vivo* gene transfer into isolated human cells, which can then be reimplanted into the host, while others require delivery and expression of genes to target cells *in vivo*—a major challenge with current vector technology. In this review we consider important clinical applications within these categories and outline the directions of study that should lead to clinical trials in the near future.

Cystic fibrosis

Replacement strategies for disorders resulting from a single gene defect are attractive candidates for gene therapy. Inheritance of two mutated copies of the cystic fibrosis transmembrane conductance regulator (CFRT) gene on chromosome 7q22 causes this common autosomal recessive disorder[2]. The CFRT functions primarily as a cyclic adenosine monophosphate activated chloride channel in epithelial cell. The CFRT gene has a complex mechanism of regulation and is expressed mainly in particular classes of epithelial cell. In humans these sites include certain cells of the lung, crypt cell in the ileum, duodenum and colon, the pancreatic ducts, and gall bladder. There is a recent evidence of endogenous CFRT gene expression in intrahepatic biliary epithelial cells, which is consistent with clinical findings of cystic fibrosis induced biliary damage.

Despite recent molecular genetic information regarding the CFRT gene and its product, the exact cause of the mucosal abnormalities in cystic fibrosis is unclear. The changes are not fully explained simply on the basis of loss or poor functioning of a small cAMP activated chloride conductance in the apical membranes of certain specialized epithelial cells. It is not clear how different mutations in the CFRT gene actually cause specific phenotypic presentations of cystic fibrosis.

Despite this problem, substantial progress has been made with gene therapy strategies for cystic fibrosis. The four transgenic mouse models available for study have severe intestinal disease with relative sparing of lung and pancreatic epithelia (unlike those in humans). Correction of the lethal intestinal defect has been shown by transfection of human CFRT by liposomal delivery in a vector under the control of rat intestinal fatty acid binding protein gene promoter. Treated mice survived for up to seven months, well beyond the expected four weeks of the control group, and also showed functional correction of ileal goblet cell and crypt cell hyperplasia and cAMP-chloride secretion. Gene therapy aimed at correcting lung abnormalities in human subjects has entailed the direct introduction of non-mutant CFRT cDNA into the epithelial cells of the respiratory tract in the hope that this will result in sufficient CFRT protein being made in these cells to correct the disease process. Vectors used so far include liposomes and adenoviruses, each has its own drawbacks: the liposomal route is proving relatively inefficient while immunological responses to adenovirus may limitits efficacy. Nevertheless initial data from two phase I clinical trials of cationic liposomal mediated CFRT delivery of a plasmid containing the CFRT cDNA to the nasal epithelium has resulted in over 20% correction of conductance abnormalities in nasal mucosal biopsies and the overall treatment was well tolerated. Attempts have also been made to transfer the CFRT gene into the biliary epithelium *in vivo* using adenoviruses. In one study, injection of the vector directly into the common bile duct during laparotomy resulted in gene expression in nearly all intrahepatic bile duct epithelial cells. Gene expression remained stable after 21 days in epithelial cells of small ducts. These results are encouraging particularly because gene delivery might be achieved by endoscopic retrograde cholangiopancreatography in the future[2].

α₁ antitrypsin deficiency

Approximately 1% of the British population carry the PiZ defect caused by a point mutation in the α_1 antitrypsin gene in chromosome 14, leading to low serum concentrations of α_1 antitrypsin and predisposition to emphysema and liver cirrhosis. Savransky et al[3] have proposed one approach to correcting this gene defect in human PiZ GM2522 fibroblasts using the technique of targeted homologous recombination to the gene locus, replacing exon V of the abnormal gene with the exon V counterpart of a normal complementary DNA. Other groups have shown a similar effect in hepatoma cell lines and hepatocytes in rat liver *in vivo* using soluble carrier systems, which utilize the asialoglycoprotein receptors on hepatocytes. It is conceivable that in due course harvested human fetal

hepatocytes identified as possessing the PiZ defect could be subjected to targeted homologous recombination *in vitro* and then reinfused into the portal vein.

Low density lipoprotein (LDL) receptor deficiency

Homozygous LDL receptor deficiency leads to familial hypercholesterolaemia, in which patients have six-to eight-fold increases in plasma LDL-cholesterol values associated with premature atherosclerotic disease and early death. This is a common disease resulting from a mutation in the genitics of the LDL receptors affecting one in 500 of the population. LDL receptors are found in most tissues but it is hepatic expression of the receptor that is most crucial in cholesterol homeostasis. This condition may respond to drug treatment in many cases but transfer of LDL receptor genes to the liver may be a treatment option for a few subjects with intractable hypercholesterolaemia for whom the only treatment option is liver transplantation. Animal studies using the model for homozygous familial hypercholesterolaemia, the Watanabe heritable hyperlipidemic rabbit, have shown successful *ex vivo* retroviral transfer of the receptor gene to 20% of cultured hepatocytes. Transduced cells then expressed receptor levels four or five times higher than normal hepatocyte controls, and after reinjection into the host liver, resulted in lowering total cholesterol value by 50%-70% of its pretreatment value, an effect that lasted over four months. Furthermore there are indications that clinical benefit may result from even partial correction of the total receptor defect, and clinical study has already been underway in the United States using retroviral *ex vivo* gene transfer.

Antisense DNA oligomer treatment: suppressing HBV expression

Using asialo-orosmucoid coupled with poly-L-lysine, both single and double stranded DNA has been delivered specifically to hepatocytes by targeting their asialoglycoprotein receptors. An antisense oligodeoxynucleotide complementary to the polyadenylation signal for human hepatitis B virus was introduced into HepG2 hepatoma cell line by Wu *et al*[4]. This cell line has been transfected with a complete human hepatitis B virus genome and secreted infectious viral into the culture medium. At 24 hours and seven days after exposure to the antisense sequence, HBV DNA values were 80% and 95% lower than controls respectively. Furthermore, by using similar carrier systems, two separate antisense sequences injected into Beijing ducks (an animal model for HBV infection) resulted in suppression of viral replication by over 90% compared with controls.

Replacing defective tumor suppressor genes

In cell culture, malignant properties can often be reversed by inserting normaltumor suppresser genes. The difficulty for corrective strategies lies in delivering actively expressed vectors to each single tumor cell of an established and possibly disseminated cancer *in vivo*. The same argument may not apply for prophylactic therapy to replace gene function in cells of patients with inherited abnormalities of tumor suppresser genes, where it may not be necessary to correct the constitution of every cell to significantly reduce the risk of cancer development within the lifetime of a person.

Normal (wild type) *p53* is involved in the control of cell cycle progression as well as in arresting replication to permit repair in DNA-damaged cells. It may also be involved in restricting precursor po-pulations by mediating apoptosis or programmed cell death. Abnormal or mutant *p53* permits the accumulation of gene mutations and chromosomal rearrangements and has been associated with virtually every sporadic malignancy including gastrointestinal tumors and hepatocellular carcinoma. There is experimental evidence showing the benefits of correcting *p53* abnormalities. Replacement of wild type *p53* using retroviral expression vectors in both human lung cancer cell lines with mouse model of orthotopic human lung cancer resulted in suppression of the malignant phenotype. Furthermore, there was also evidence that the combination of restoration of *p53* function and sequential administration of the cytotoxic drug cisplatin was synergistic in reducing the malignant behavior of these cell lines[5]; this is clearly an important finding that may influence approaches to adjuvant treatment for cancer.

There could also be a role of replacement therapy for *p53* in patients with Barrett esophagus, a condition that may respond poorly to medical treatment. Adenocarcinoma arising in Barrett esophagus is often preceded by mucosal dysplasia. Several researchers have found an association between *p53* mutation and adenocarcinoma related to Barrett esophagus[6]. In addition, recent reports suggest that *p53* dysfunction may participate in the progression from dysplasia to carcinoma, and that there is a correlation between presence of mutant *p53* and increasing dysplastic features[7]. For those patients with histological evidence of progressive dysplasia who are unfit for surgery and refractory to treatment with acid suppression, correction by insertion of wild type *p53* using submucosal endoscopic injection of retroviral or adenoviral suspensions may be an alternative treatment. Clearly such a strategy will require considerable improvement in gene delivery systems and targeting mechanisms for the preneoplastic cells, as well as more sophisticated understanding of transcription control to permit appropriate expression.

The identification of the APC gene participating in familial adenomatous polyposis affecting the large and small intestine, and the DNA mismatch repair gene families (hMSH2, hMLH1, hPMS1, and hPMS2) involved in hereditary non-polyposis colorectal cancer syndrome will stimulate further interest in the replacement of tumor suppresser gene function in stem cells in organs at risk of cancer development. However, the lack of knowledge concerning the temporal and special control of expression of these genes will

delay the application of such prophylactic gene therapy.

Antisense DNA oligomer treatment: suppressing oncogene expression

Antisense oligodeoxynucleotides are short (10 - 15 bases) synthetic nucleotide sequences formulated to be complementary to specific DNA or RNA sequences. By the binding of these nucleotides to their targets, the transcription or translation of a single gene can be selectively inhibited by triggering RNase H degradation of the target RNA and interfering with the processing of pre-mRNA. Examples of antisense oligomers with significant *in vitro* antiproliferative activity include those against *c-myb* in colorectal cell lines, *c-myc* in lymphoma lines, and *bcr-abl* in chronic myeloid leukemia blast cells.

The *ras* oncogenes are obvious potential targets for antisense therapeutics as they are implicated in many solid tumors including more than 75% of pancreatic cancers and colorectal cancers. Inhibition of the *ras* signaling pathway modulates critical aspects of *ras* oncogene mediated transformation in whole cells. The resulting phenotypes include reduced anchorage dependent and anchorage independent growth and morphological reversion of the cells. Recently, reduced biological aggressiveness and loss of anchorage independent growth were reported in experiments using homologous recombination to target *k-ras*. Using antisense oligonucleotides to target regions of *k-ras* mRNA there are some antiproliferative effects in human pancreatic cancer cell lines, but there is wide variability of response rates in different cell lines and little evidence of sequence specificity[8]. Moreover, there is no correlation of antiproliferative effect with reduction in amounts of *k-ras* protein expressed and these agents theoretically directing against cellular genes act instead through unpredictable sequence independent mechanisms. Similar findings have been reported for oligonucleotides designed against *c-myc* and *c-myb* sequences[9]. The amplification of *c-myc*, *c-k-ras*, and *c-Ha-ras* oncogenes in a series of 124 gastric carcinomas did not reveal any new independent prognostic factor. On the contrary, amplification of *c-erb B-2* had a significant negative impact on overall survival. An examination of the tumors involving the thymidine labeling index highlighted the need to identify the most suitable biologic material for estimating proliferative activity in gastric carcinoma[10].

Inserting drug activating 'suicide' genes

There are many examples of genes preferentially expressed in tumors compared with normal tissues. Genetically directed enzyme prodreatment therapy (GDEPT) exploits the differences in gene expression between different cell types to increase the specificity of cell destruction by coupling the promoters of differently expressed genes to prodrug activating enzymes. Examples of useful promoters include carcinoembryonic antigen in colorectal cancer, *erbB2* in breast and pancreatic cancer[11], and prostate specific antigen in prostate; the most widely used prodrug activating enzymes are cytosine deaminase (which converts 5-fluorocytosine to the cytotoxic 5-fluorouracil) and herpes simplex virus thymidine kinase (which converts ganciclovir to toxic phosphorylated derivatives). The system is designed so that significant transcription of the enzyme gene is activated only in tumor cells by linking the enzyme gene to transcription control elements (a 'molecular switch') selective for a particular tumor or tissue type. Significant antitumor effects from conversion of 5-FC to 5-FU have been seen in colorectal cancer cell lines transduced with the cytosine deaminase gene under a constitutive promoter[12]. There was also significant regression of tumor volume even when as little as 2% of the tumor mass contained cytosine-deaminase expressing cells. The last effect was also seen when a CEA-HSVTK construct was used to transduce CEA expressing pancreatic cancer cells, which were then engrafted into several combined immune deficient mice. When ganciclovir was given, only 10% of the cells expressed HSVDK[13]. This important feature of the GDEPT system is probably due to a so-called 'bystander effect'⁻attributed to transfer of the activated drug from a cell in which it is produced to others in the vicinity lacking the enzyme. There has been some success in targeting tissue such as the gastrointestinal epithelium and pancreas using the carcinoembryonic antigen gene promoter[12], and also targeting tumor specific transcriptional activation using the α-fetoprotein promoter in hepatocellular carcinoma.

Augmentation of the immune response and cancer vaccines

The promise shown by systemic high dose immunotherapy to treat cancer in murine studies has not been fulfilled in human trials except in a few cases of renal cell carcinoma and melanoma. Indeed the initial dose regimens were extremely toxic. It may be more important to deliver a small dose of cytokine at a specific site rather than high doses systematically, which has led to two approaches to cytokine based gene therapy that augment naturally produced immune response to malignancies. The first approach entails the insertion of cytokine genes into cultured tumor infiltrating lymphocytes *ex vivo*. This subset of T cells is critical for the prevention and elimination of tumors, and their antitumor efficacy improves with the transfer of the genes for tumor necrosis factor and interleukin 2. 'Cancer vaccines'⁻ constitute the second approach and entail the induction of cytokine expression in the tumor cells so that T cell recognition of tumor antigens is enhanced. Immunostimulatory cytokine genes are transduced into tumor cells *ex vivo*, the tumor cells are irradiated to eliminate malignant activity and reintroduced into the host. Cytotoxic T cells recognize tumor specific antigens presentred on the surface of these cells. They are induced by the local secretion of the transferred cytokine gene product to expand, target, and destroy cancer cells.

In addition to cytokines, a number of other genes are also capable of inducing an antitumor response including allogeneic HLA (human leucocyte antigen) genes and costimulatory molecules such as the B7 family, B7.1 and B7.2. B7 is expressed mainly on antigen presenting cells and serves as costimulatory signals for T cells, by interacting with its ligands CD28 or CTLA-4.

Cytotoxic T cells depend on antigen present in the context of self class I MHC molecules, whereas T helper lymphocytes require activation by antigen present in the context of self class II MHC molecules. Aberrant expression of MHC is a common feature of gastrointestinal cancers. Class I antigens are frequently lost, while class II expression is often unregulated. Polynucleotide vaccination (in contrast with conventional vaccines consisting of peptides, whole tumor cell lysates) has great therapeutic potential in that delivery of genes that express unique oncoproteins such as *k-ras* or *p53* endogenously within a cell and may result in an MHC class I CD8 + response and proliferative activation of cytotoxic T cells, rather than a less effective class II CD4 + response. This may be a further means of breaking down immunotolerance to tumors, which could lead to the generation of tumor specific responses. Mutated forms of the ras protooncogene, in particular, contain potentially antigenic T cell epitomes specific for the malignant phenotype. Certain k-ras mutations that produce new peptides including consensus binding motifs could cause increased immunogenicity as a direct result of differential MHC class I binding. Carcinoembryonic antigen has been utilized for active immunotherapy.

The finding that individual tumor cells often express much higher levels of this antigen suggested that carcinoembryonic antigen may be a target for immunotherapy, initially using DNA vaccines. Although humoral immune responses were seen, there was no evidence of a more powerful cell-mediated cytotoxicity. Secondly, the injected gene was of human sequence, which will be recognized by the mouse host as a foreign antigen, while the product of a gene from the homologous species may be less immunogenic. Thirdly, the challenge with a small inoculum of tumor cells at the end of a course of vaccination is clearly different from the treatment of a patient with extensive metastatic colorectal cancer. Patients with nm23-H1 allelic deletions are 3 times as likely to develop distant metastases as patients without nm23-H1 deletions (relative risk, 3.89; 95% confidence interval, 1.39, 10.89; $P = 0.01$).

PROSPECT IN THE FUTURE

These fields are under intense investigation and real advances are likely in the next decade. In the short-term investigation, further useful improvements are possible from manipulation of the RNA and DNA viruses with which we are already familiar. Development of high efficiency viral packaging systems and refinement of the purification and concentration processes can be expected to improve the titres of viral vectors to values that could permit gene transfer by systemic administration. Targeting of delivery should be possible by incorporating single chain antibodies to cell surface antigens or ligands for transmembrane receptors into the envelope or penton coat proteins of retroviruses and adenoviruses respectively, and there are encouraging signs that this approach can be successful. In the intermediate term, we expect the arrival of 'designer vector' incorporating the most useful elements from both viral and synthetic systems and these can be varied depending on the particular application. For instance, the inverted terminal repeats of adeno-associated virus that mediate stable chromosomal integration can be combined with a backbone of another vector with a large insert capacity such as herpes virus or a bacteriophage. Integration may be enhanced by packaging a functional recombinase enzyme with the gene expression construct in liposomal complexes targeted to a particular class of cells with an antibody. In the long term, basic research will be made into the structure and organization of mammalian chromosomes, which can carry whole clusters of genes with their natural control elements into cells.

The control of gene transcription is extremely complicated and, even for the most intensely investigated systems such as the globin genes, our understanding is still fragmental. While most protocols presently use strong viral promoters to drive expression of recombinant cDNA copies of therapeutic genes, future work must be directed to defining the genomic elements that enable temporal and spatial control of expression through a lifetime. The identification of locus control regions that can insulate gene clusters from interference by surrounding genetic influences has been an important step, and many investigators are now working to understand how the promoter and enhancer/silencer elements of a gene interact with structures within the nucleus. Advances in this area will require parallel deve-lopments in the sophistication of vector design before they can be transferred into practice.

REFERENCES

1   Westbrook C, Chimera S, Arenas R, Kim S, Otto G. Human APC gen expression in rodent colonic epithelium using *in vivo* liposomal delivery. *Hum Mol Genet*, 1994;3(9):2005 - 2010
2   Caplen N, Alton EW, Middleton P, Dorin J, Stevenson B, Gao X. Liposomal-mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Nature Med*, 1995;1(1): 39 - 46
3   Savransky E, Hytroglou P, Harpaz N, Thung SU, Johnson EM. Correcting the PiZ defect in the alpha-1 antitrypsin gene of human cells by targeted homologous recombination. *Lab Invest*, 1994;70(2):676 - 683
4   Wu GY, Wu CH. Specific inhibition of hepatitis B viral gene expression in vitro by targeted antisense oligodeoxynucleotide. *J Biol Chem*, 1992;267(10):12436 - 12439
5   Roth J, Mukhopadhyay T, Zhang WW, Fujiwara T, Georges R. Gene replacement strategies for the prevention and therapy of cancer. *Eu J Cancer*, 1994;30(9):2032 - 2037
6   Symmons PJ, Linehan JM, Brito MJ, Filipe M. p53 expression in Barrett's esophagus, dysplasia and adenocarcinoma using antibody DO-7. *J Pathol*, 1994;173(1):221 - 226
7   Hardwick RH, Shephard NA, Mooghen M. Adenocarcinoma arising in Barrett's oesophagus: evidence for the participation of p53 dysfunction in the dysplasia/carcinoma sequence. *Gut*, 1994;35(2):764 - 768
8   Carter G, Gilbert C, Lemoine N. Effect of antisense oligodeoxynucleotide targeting KRAS expression in pancreatic cell lines. *Int J Cancer*, 1995;6(4):1105 - 1112
9   Burgess TL, Fisher EF, Ross SL, Bready J, Qian Y, Bayewitch L et al. The antiproliferative activity of c-myb and c-myc antisense oligonuleotides in smooth muscle cells caused by a nonantisense mechanism. *Proc Natl Acad Sci USA*,1995;92(10):4051 - 4055
10   Amadort D, Maltoni M, Volpi A, Nanni O, Sxanrpi E, Renault B *et al*. Gene amplificative kinetics in relation to prognosis of patients with gastric carcinoma. *Cancer*,1997;79(2):226-232
11   Harris J, Guttierez A, Hurst HC, Lemoine NR. Gene therapy for cancer using tumour specific prodrug activation. *Gene Ther*, 1994;1(1):174 - 179
12   Huber BE, Austin E, Richards CA, Davis ST, Good SS. Metabolism of 5-fluorocytosine to 5-fluorouracil in human colorectal tumour cells transduced with the cytosine deaminase gene. *Proc Natl Acad Sci USA*, 1994;91(20):8302 - 8306