Pergamon

European Journal of Cancer Vol. 31A, No. 9, pp. 1531–1537, 1995

0959–8049/95 $9.50+0.00

0959-8049(95)00295-2

Educational Report

European School of Oncology Position Paper. Gene Therapy for the Medical Oncologist

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INTRODUCTION

FOR MORE than a decade, it has been possible to take a gene, introduce it into a vector (usually viral), have the vector bind to a target cell receptor and integrate its recombinant DNA into the host cell genome, where it will remain for the duration of the cell's life. It was initially expected that this technique would find its greatest use in attempts to combat the hereditary single gene defects that cause such diseases as cystic fibrosis. However, the bulk of the 90 clinical gene therapy protocols which have now been approved concern patients with cancer. This enthusiasm reflects the real prospect that gene modification may be used both to optimise existing treatments, such as chemotherapy, and as the basis for entirely new management options.

Among the possible uses of gene therapy are the modification of tumour cells to suppress their malignant phenotype or to induce them to become more immunogenic; the modification of healthy cells (particularly haematopoietic cells) to enhance resistance to cytotoxic agents, so allowing more intensive or more prolonged treatment; and the modification of immune effector cells so that they better recognise tumour or express different and hopefully more effective cytokines. Attempts are also being made to introduce into tumour cells genes coding for enzymes which might prime those cells for selective killing by a prodrug.

There are undoubtedly limitations in current attempts to exploit gene therapy in cancer. These arise mainly because available vectors are inadequate for the task of delivering genes to malignant cells distributed over a wide anatomical area, while also being selective in their targeting. Other constraints, however, are manmade. Strict governmental controls, justified by the need to minimise risk from the generation of replication competent viruses and the risk of germline transmission, regulate both the production and use of vectors and the genetic manipulation of cells. Further, concern to safeguard intellectual property rights hampers the co-operation between commercial and academic centres.

Despite these problems, considerable progress is being made. This paper reviews the range of gene therapy approaches outlined above, but begins by considering the gene marking of cells as a means of monitoring the efficacy of treatment. Such studies have already contributed substantially to the understanding of how to improve cancer treatment.

GENE MARKING OF BONE MARROW

Bone marrow is an attractive target for gene therapy since its cells are easy to obtain, to manipulate *ex vivo* and to return to the patient. Further, it is possible in principle—through the modification of a single stem cell—to repopulate the entire patient with haematopoietic and lymphoid cells expressing a therapeutic gene and to have that effect last a lifetime. However, the efficiency of gene transfer to bone marrow cells using current vectors is extremely low. Only retroviral vectors are able stably to integrate cDNA into the host cell genome; and such vectors infect only cells in cycle, which excludes the great majority of haematopoietic stem cells. Nevertheless, while transfer efficiencies are too low for current gene therapy to be of direct clinical benefit, they are sufficient to allow gene marking studies to expand our understanding of the biology of bone marrow. Such studies have been conducted by Professor Malcolm Brenner and colleagues at the St Jude Children's Research Hospital, Memphis, Tennessee, U.S.A.

Identifying the source of relapse

Relapse following autologous marrow transplantation (ABMT) is common and the main reason for treatment failure. However, there has been uncertainty as to whether relapse is due to residual disease in the patient or to occult tumour cells present in bone marrow obtained in remission and then re-infused.

To examine this question, a retroviral vector was used to mark bone marrow harvested from AML and neuroblastoma patients with the neomycin resistance (*Neo-R*) gene. Bone marrow was

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Received 8 Mar. 1995; accepted 12 Apr. 1995.

then reinfused. An initial point of interest is that the insertion of cDNA had no effect on time to engraftment, suggesting that transduced stem cells are not made more immunogenic by marking. More importantly, however, the study provided clear evidence that harvested bone marrow contains tumorigenic cells, and that these cells can contribute to relapse at both medullary and extramedullary sites. Of the 12 AML patients studied, 4 relapsed. The *Neo-R* marker was evident in bone marrow clones from 3 of them. Of 9 neuroblastoma patients, 5 relapsed, and *Neo-R*-containing cells were found in 4. In one of these patients, relapse took the form of a liver metastasis. Cells taken from this deposit were *Neo-R*-positive.

This evidence that tumour-specific markers can and do appear in the gene-marked bone marrow cells supports the view that improved outcome following ABMT will depend—at least in part—on bone marrow purging. Given the likely importance of purging, gene marking can also help determine the relative efficiency of alternative techniques in eradicating clonogenic cells with individual patients.

We took bone marrow from 10 patients. One sample from each was transduced using the LNL-6 viral vector and the other with G1N. These vectors are closely related, but can be distinguished by PCR amplification. Within each patient, one aliquot was randomised to either immunological purging using IL-2 or to pharmacological purging with 4-hydroperoxycyclophosphamide (4HC), the other aliquot being purged using the alternative technique. Both samples of bone marrow were then reinfused. Subsequent relapses will be related to the presence of the different viral markers, allowing the efficacy of the two purging techniques to be compared.

Nine patients so far have been treated using this protocol. No one has yet relapsed. However it is already clear that the impact of the two purging techniques on normal progenitor cells is different: results to date suggest that IL-2 is far less supportive of stem cell maintenance than 4HC. Our ability to identify and quantify the presence of different viral markers in the same patient will also enable us to assess the effect of the *ex vivo* use of growth factors on early engraftment and on long-term stem cell viability. The protocol for such a study has been approved.

Adoptive transfer of T cell immunity

In addition to identifying cells that induce relapse, gene marking techniques may also have a role in identifying cells that combat malignancy. Although allogeneic BMT carries with it the risk of graft versus host disease, it is recognised that the graft versus tumour effect may decrease the rate of relapse. This protective effect is mediated by T lymphocytes and may result in part from their recognition of tumour-specific antigens. An alloreactive effect is almost certainly of greater importance, i.e. the malignancy is of host origin and, therefore, a target for donor-derived alloreactive T cells. In addition, many tumours contain fusion proteins, mutant oncogenes and viral antigens which, if processed and presented by the malignant cells, could potentially be recognised by incoming effectors. Attention at St Jude Children's Research Hospital has focused on particular antigens expressed by Epstein-Barr Virus (EBV) infected B lymphocytes. EBV lymphoproliferative disease is common in allogeneic BMT recipients who receive T cell depleted HLA mismatched or unrelated donor transplants.

EBV-specific cytotoxic T cell lines (CTL) from bone marrow donors were, therefore, established and marked with the *Neo-R* gene before infusion into patients. Studies show that these CTLs survive long-term in the recipient and expand in the peripheral circulation: signal can be detected at least 10 months after infusion when the CTLs are restimulated by exposure to EBV-infected lymphoblastoid cells.

Infused at a dose of 10^6 donor cells/kg body weight, these CTLs have both antiviral and antiproliferative properties. 3 patients have been treated at the stage when a rise in EBV DNA in blood heralded the onset of lymphoproliferative disease. Within 2–3 weeks, levels of EBV DNA had returned to normal. In a further patient with florid EBV lymphoma, use of the anti-EBV CTLs resolved fever and lymphadenopathy. Hence, given the right target antigen and the right effector cells, the immune system can eradicate even bulky disease. The challenge now is to determine whether such findings can be repeated in other diseases in which tumour-specific antigens are putatively expressed.

BIOLOGICAL RESPONSE MODIFIERS

Over the past 20 years, despite great expenditure of resources, the incidence of cancer and its associated mortality have in fact increased. Traditional approaches, therefore, allow considerable scope for improvement through the development of novel strategies.

Tumours that escape destruction by the immune system do so for several reasons. First, tumour cells are poor antigen presenters. Secondly, they may have the ability to induce anergy in the effector cells. The task of immunotherapy is, therefore, not only to stimulate the immune response, but also to reverse immunosuppression. Many of the gene therapy protocols now being implemented involve the attempt to increase the antigenicity of tumours by inserting genes that express biological response modifiers. Dr Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, described progress to date.

At least four cell types—natural killer cells, lymphokine activated killer cells, macrophages and tumour-infiltrating lymphocytes—are capable of killing tumour cells *in vitro*. Gene therapy will mainly be used when the tumour is not localised. The T cell is, therefore, the most obvious candidate for manipulation since this cell alone has both memory and specificity, giving it the ability to seek out target cells anywhere in the body. The precise mechanisms of T cell killing are not clear, but the cells possess granules containing pore-forming perforins and granzymes which are released when the T cell receptor engages a major histompatibility complex (MHC) molecule presenting an immunogenic peptide.

Cytokine expression by tumours

Lymphocytes known to kill tumour cells *in vitro* require interleukin-2 (IL-2) for their activation and proliferation. An obvious idea is, therefore, to introduce the *IL-2* gene into tumour cells in the hope that lymphocytes will be activated, expanded in numbers, and transformed into killer cells. This approach has met with some success in murine tumour models. In mice carrying an established tumour, immunotherapy with irradiated tumour cells secreting cytokines such as IL-2, IL-4, IL-6 and GM-CSF has been successful in activating effector cells to the extent that tumour is eradicated.

However, there is little consistency between the animal models. Thus, the same vector containing the same IL-2 cDNA and inducing the same amount of IL-2 expression induces regression of established bladder carcinoma, for example, but is ineffective against established CMS5 fibrosarcoma. Many questions are unanswered. The optimum dose of genetically modified tumour

cells remains to be established, as does the best cytokine gene to be introduced. GM-CSF is advocated by certain groups, on the basis that it activates local antigen presenting cells. However, there is no consensus that this cytokine has greatest potential benefit. Indeed, there are human tumours which already secrete GM-CSF, such as many renal carcinomas, but despite this form tumours in humans. It is unlikely that any one form of gene therapy will be active against all tumour types, which show great heterogeneity in inherent immunogenicity. For example, the introduction of the co-stimulatory molecule *B7* gene has an effect in relatively immunogenic tumours and much less effect in non-immunogenic tumours.

The effector cell

Mechanisms of tumour-induced immunosuppression, which are likely to be of great importance in the clinical context, are actively under study. One line of investigation concerns signal transduction in T cells, which plays a key role in inducing the expression of genes that transform them into killer cells. Recent work shows that signal transduction from receptor to nucleus is abnormal in T cells from tumour-bearing animals [1].

T cells from naive, healthy mice have been compared with T cells from mice carrying a growing parental tumour and T cells from mice with a tumour of the same size that secretes IL-2. T cells from mice with the IL-2-secreting tumour were identical to those from healthy mice. However, T cells from those with the growing, non-cytokine secreting tumour produced significantly less zeta chain protein, which is important in the process of signal transduction from receptor to nucleus. The same phenomenon was evident when the tyrosine kinases p56 lck and p59 fyn (which are also involved in signal tranduction) were assayed. The amount of p56 lck and p59 fyn protein in the T cells from the mice with IL-2 secreting tumours was identical to that found in healthy animals. However, p56 lck and p59 fyn expression had clearly been significantly downregulated in animals with the tumour that did not secrete cytokine [2]. Such work demonstrates the importance of considering the effector cells and their compromised ability to respond, as well as the tumour itself, when seeking to induce host rejection of tumour.

Limitation of animal models

The literature contains spectacular examples of the efficacy of cytokine gene therapy in certain animal models of cancer [3]. However, even if the technique can be made to work well in such models, there is no guarantee of similar efficacy against human tumours. First, several human tumours, such as renal carcinoma, grow well in cancer patients despite the fact that they secrete cytokines. Secondly, the natural history of spontaneously arising humun tumours, which develop for years, is totally different from that of tumours induced in mice, which are rapidly fatal. Thirdly, the well documented ability of human tumour cells to secrete immunosuppressive factors, such as the TGF-beta produced by glioma, will have a major impact on the ability of cytokine gene therapy to induce their rejection. MHC expression, which is crucial to the stimulation of T cells, is also very heterogeneous among human tumours.

Clinical protocols

There is evidence that tumour-specific CTLs are present in cancer patients, although in relatively small numbers. In melanoma patients whose tumour expresses MAGE1, the frequency of specific CTL precursors ranges from 1 in 900 to 1 in 33 000 mononuclear cells [4]. The task of gene therapy is, therefore, not to induce an immune response *de novo* but to expand the available pool of CTLs and to restimulate them. Clinical gene therapy protocols aimed at achieving this goal are now being put into effect, mostly in patients with solid tumours.

A common approach is to use irradiated tumour cells, whether allogeneic or autologous, as antigen presenting cells. Tumour cells are clearly not ideally suited for this role. Even when they are induced to express IL-2, for example, work on the CMS5 system demonstrates that tumour cells are far inferior to professional antigen presenting cells in activating CTLs and in stimulating their proliferation. However, animal work suggests that the additional expression of the co-stimulatory signal B7 may have a synergistic effect in inducing tumour rejection.

At Memorial Sloan-Kettering, patients are now being recruited into two gene therapy protocols. 12 melanoma patients and 11 patients with renal carcinoma have been treated with an HLA-matched allogeneic tumour cell vaccine [5]. The use of HLA-matched allogeneic tumour cells is based on evidence that HLA-A2 molecules can present shared immunogenic peptides to CTLs and that alloantigens may serve as an adjuvant, provoking a stronger antitumour response. Data are only preliminary. However, it is interesting to note that of the 11 renal carcinoma patients treated, 5 had stable disease over the protocol period of approximately 3 months.

Continued work with animals is essential to determine the optimum antigen presenting cell to use in gene therapy and the optimum cytokine, and to establish means of minimising the effect of tumour cells on the host lymphocyte. Nevertheless, carefully controlled trials of immunostimulatory gene therapy are now appropriate in certain patients, and the first results are awaited with great interest.

DRUG RESISTANCE GENE TRANSFER

In contrast to the uses of gene therapy already described, the transfer of drug resistance genes to a patient's haematopoietic stem cells is a hybrid approach. Rather than being a novel therapy in its own right, the technique is designed to improve the efficacy of an existing treatment, in this case chemotherapy, by reducing its toxicity. Chemotherapy has a narrow therapeutic index, its use being limited by adverse effects on normal tissues, and in particular by myelosuppression. It is intellectually appealing to take advantage of mechanisms used by tumour cells to escape death to improve the tolerability of standard cancer treatment, and possibly to intensify it.

There is a second potential use in inserting drug resistance genes into haematopoietic cells. Gene transfer to stem cells remains relatively inefficient, even with the use of new techniques such as haematopoietic cytokines and autologous stromal layers. It may be possible to overcome this limitation if genetically modified cells containing an introduced drug resistance can be expanded *in vivo* by post-transplant drug treatment. Exposure of the cells to a cytotoxic drug will then give the modified cells a survival advantage, and result in their selective amplification. This technique might have particular application in the gene therapy of non-malignant disease. Both aspects of drug resistance gene transfer were considered by Dr Brian Sorrentino, St Jude Children's Research Hospital, Memphis, Tennessee.

MDR1

The human *MDR1* gene is the best characterised of the drug resistance genes regarding its potential as a gene therapy strategy. Its transmembrance glycoprotein product promotes the energy-dependent efflux of a wide range of cytotoxic compounds from

the cell interior. Studies have demonstrated that the transduction of mouse haematopoietic stem cells with human *MDR1* cDNA protected against drug-induced haematotoxicity. Six months after infusion of the modified haematopoietic cells, animals were treated with paclitaxel, a drug whose dose-limiting toxicity is myelosuppression. On this first exposure, mice with the MDR-containing cells experienced neutropenia that was as profound as that seen in controls. However, when these animals were allowed to recover and subsequently rechallenged with paclitaxel, the situation was quite different. Animals with MDR-transduced cells showed little neutropenia. It was subsequently shown that the initial myelosuppressive pressure resulting from first exposure to a cytotoxic drug confers survival advantage on MDR-modified cells. This was evident on quantitative PCR which showed a substantial increase in the proportion of gene-modified cells in the peripheral circulation. For the whole group of animals, the mean proviral copy number in total white blood cell count, which reflects the number of modified haematopoietic cells, increased from 0.19 initially to 0.62 in the period immediately before rechallenge with paclitaxel. This amplication was not seen in a control group of animals transfected with retrovirus containing the *Neo-R* gene.

Further studies showed that selection for drug resistance occurs not at the level of precursor cells, which have limited self-renewal capacity, but at the level of the more primitive stem cells. Two animals which had shown substantial and stable increases in proviral copy number after paclitaxel were sacrificed and their bone marrow infused into other animals. 6 months following this secondary transplant, the recipient animals also demonstrated a stable increase in proviral copy number.

It is also possible to transfer the *MDR1* gene to human CD34+ cells. Early attempts were limited by the fact that around 50% of cDNA integrations resulted in expression of a non-functional short form of MDR protein. This was due to an aberrant splice donor and splice acceptor site within the *MDR1* cDNA. However, modification of the retroviral vector has now overcome this problem.

In conjunction with the National Cancer Institute, researchers at the St Jude Children's Research Hospital have developed a clinical protocol for transfer of the *MDR1* drug resistance gene. Patients with metastatic breast cancer and no evidence of bone marrow involvement who are about to undergo autologous BMT will be recruited. One-third of their bone marrow cells will be transduced with the *MDR* gene before retransfusion. The study aims to establish whether it is possible to transfer and express the *MDR1* gene in a clinical context. It should also be possible to determine whether modified cells can be selected for by administering paclitaxel, as in the mouse model. If so, a low initial transduction efficiency can perhaps be overcome by multiple rounds of paclitaxel exposure. Finally, if a sufficient proportion of modified cells can be achieved, it should be possible to treat patients with paclitaxel at relapse and determine whether drug-induced myelosuppression has been reduced.

Other drug resistance genes

Broadly speaking, drug resistance can be achieved by three mechanisms: cells may increase expression of structurally normal gene products (as is the case with human *MDR1*); they can express a mutated, structurally altered gene (as is the case with dihydrofolate reductase (DHFR)); or they can decrease expression of gene products essential for cytotoxity. This latter mechanism is not at present being studied in the context of potential gene therapy. Although the *MDR1* system has been intensively investigated, there is no *a priori* reason to suppose that it is the best drug resistance gene for gene therapy applications. Other drug resistance genes are, therefore, being tested.

DHFR is inhibited by methotrexate, resulting in reduced DNA synthesis. Several methotrexate resistant enzymes have been identified and variant enzymes that function in the presence of antifolate drugs are being investigated at St Jude Children's Research Hospital. Using genes identified by Dr Ray Blakley's laboratory, it has proved possible to develop viral vectors able to confer high levels of methotrexate resistance to haematopoietic cells in mouse models.

Researchers at St Jude are also working on resistance to cyclophosphamide, a drug widely used and effective in many malignancies. Cyclophosphamide is converted to aldophosphamide after passage through the liver. Once in this form, the drug enters cells, where it spontaneously degrades to the alkylating agent phosphoamide mustard. A cytoplasmic enzyme aldehyde dehydrogenase (ALDH) converts aldophosphamide into an inert compound, thereby attenuating cytotoxicity. Retroviruses containing the *ALDH* gene have been developed, and studies have shown that enforced overexpression of the enzyme in transduced murine leukaemia cell lines significantly reduces mafosfamide toxicity. To date, it has been possible to increase ALDH expression to 2–3 times that in non-transduced cells. This vector also caused increased mafosfamide resistance in transduced primary murine bone marrow cells. It is hoped that new vector designs will further increase ALDH expression and mafosfamide resistance.

There are thus a number of systems which may protect haematopoeitic cells from the effects of cytotoxic drugs, so broadening the therapeutic index of commonly used chemotherapeutic agents. A potential pitfall with resistance gene transfer is the risk of transferring drug resistance to contaminating tumour cells in the bone marrow graft. Trials should, therefore, be designed to minimise this possibility and provide reasonable alternative therapy even if this should occur, such as salvage therapy with non-crossresistant drugs. Currently, it is possible to transfer potentially protective drug resistance genes to patients only at the time of bone marrow transplant, which typically occurs late in the course of disease. However, it may become possible to transfer genes to haematopoietic cells at an earlier stage, potentially without preceding myeloablation. Another potential limitation is that non-haematological toxicities may prevent dose escalation even if myelosuppression can be significantly reduced. The murine transplant model will be useful in identifying drug resistance systems, whereby non-haematopoietic toxicities start to become limiting at drug doses substantially higher than those causing dose-limiting myelosuppression in control animals. The utility of this approach will be ultimately determined in appropriately designed clinical trials.

SUICIDE GENES

If one can imagine a situation in which exogenous genes can effectively be delivered to every tumour cell in the body, a range of therapeutic options emerges. Tumour suppressor genes could be inserted into tumours *in situ*, a variety of antisense molecules could be used to regulate the expression of activated oncogenes and growth factors, programmed cell death could be induced specifically in tumour cells, exogenous toxins under the control of tumour-specific promoters could be administered, and the metabolism of tumour cells could be fundamentally altered to make them more susceptible to conventional forms of therapy. In disseminated disease, the possibility of targeted delivery of

genes to every tumour cell must be considered remote. However, even at this early stage, there are aspects of this comprehensive programme that can usefully be explored.

One such element is the idea of introducing 'suicide genes' into localised tumour deposits. The term refers to genes that remain latent until activated, for example by a prodrug. Dr Michael Blaese and colleagues at the National Institutes of Health, Bethesda, Maryland, have been working on two such systems. The first employs the herpes simplex thymidine kinase (*TK*) gene, which phosphorylates ganciclovir into its active form. Ganciclovir phosphate is a chain terminator and inhibitor of DNA polymerase which leads to the death of dividing cells. A second system involves the cytosine deaminase gene from fungi which deaminates 5'-fluorocytosine, thereby generating 5'-fluorouracil. In both systems, the presence of the inserted gene, which is not present in healthy host cells, induces the tumour to subject itself to hopefully fatal chemotherapy when the appropriate prodrug is administered.

Bystander cell killing

Early work in mice demonstrated the potency of the approach. A tumour nodule containing cells modified earlier *in vitro* to express the *TK* gene was eliminated after treatment with ganciclovir while a nodule on the same animal which contained only wild-type cells was unaffected. The direct delivery of a suicide gene to tumour deposits *in vivo* poses more formidable problems. No delivery system available is likely to reach more than a small fraction of the tumour cells. However, in this context, the *TK* system demonstrates a particularly useful feature. Animal work shows that a tumour nodule containing as few as 10% modified cells (and 90% wild-type cells) can still be eradicated: cells adjacent to those that have been transduced with the foreign gene also die when ganciclovir is administered. The implication of this strong bystander effect—probably due to the transmission of the nucleotide toxin through gap junctions shared by cells within the tumour mass—is that it may not be necessary to transfect every cell in a malignant deposit to achieve a substantial therapeutic effect. A corollary, however, is that such an effect would only be seen in tumours which express gap junctions, and not, for example, in CNS lymphomas.

Clinically, the tumours that lend themselves to the suicide gene approach are localised cancers that are devastating by virtue of their position, that cannot be surgically removed, and that have not responded to conventional therapy. Techniques have been developed allowing such tumours, in this case in the brain, to be stereotactically injected with a vector carrying the *TK* gene. In practice, injection of retroviral vectors is unsuitable for this approach. First, they are unstable at 37°C and disappear with a half life of around 6 h. Secondly, retroviral vectors will only integrate into cells actively synthesising DNA. Even in active brain tumours, the proportion of cells in mitosis at any one time is small. One means of overcoming this limitation would be continually to infuse vector into the tumour deposit. However, an alternative and more practical approach has been used. This involves injection into the tumour bed of mouse fibroblasts (of the PA317 packaging line) genetically engineered to produce retroviral vector particles. Thus, whenever a tumour cell goes into cycle, there is vector close by ready to integrate the *TK* gene.

All host cells have receptors for the retrovirus, which might pose a difficulty if the vector were to escape the tumour. However, in this instance, the selectivity of the vector for dividing cells is to our advantage. Since integration of the *TK* gene occurs only in dividing cells, the enzyme will not be expressed in healthy brain tissue.

Animal and early clinical data

The strategy has been validated in a rat model in which a gliosarcoma was injected into the brain, followed a week later by injection of retroviral vector-producing fibroblasts using the same steroatactic coordinates. In control animals treated with saline, extensive outgrowth of tumour was seen. However, in littermates which received ganciclovir, there was complete elimination of tumour both macroscopically and microscopically. In 60% of the ganciclovir-treated animals, use of the suicide *TK* gene resulted in long-term survival of up to 1 year.

The difference between treating a small tumour in an animal model and a large cancer in a patient is substantial, even given the advantages of the bystander effect. Nevertheless, early results were encouraging enough to justify a phase I trial of the therapeutic strategy, which began 2 years ago at the National Institutes of Health. The study included 15 patients with stage IV glioma or brain metastases unresponsive to other treatment. Following magnetic resonance imaging, vector-producing fibroblasts were injected throughout the tumour bed along parallel needle tracks roughly 5 mm apart. One week later, ganciclovir was infused. 8 patients have shown substantial reductions in tumour volume, and four responses have lasted between 4 and 22 months.

The fact that localised gene delivery has been accomplished can be demonstrated. *In situ* hybridisation from human biopsies shows that mRNA from the herpes *TK* gene is expressed by tumour cells. However, such studies also show the limitation of the current approach. Gene delivery is confined to an area within 5–15 cell diameters of the fibroblast injection track. The retroviral vectors used are relatively large and do not diffuse well through solid tissue. Certain patients with glioma might have been fortunate enough to have tumours that expressed sufficient gap junctions for the nucleotide toxin to spread far enough to have a good therapeutic effect. However, this bystander phenomenon cannot be relied on. The real challenge is now to find ways of spreading the suicide gene further through the tumour deposit.

Various approaches to the wider delivery of suicide genes are being pursued. One idea is to use an adenovirus vector which can be grown to titres of 10^{11} viral particles per ml, far higher than the level of 10^6 particles per ml which is typically found with retroviral vectors. At least in animal models, use of an adenovirus vector is effective in inserting multiple copies of the therapeutic gene into tumour cells. However, the ideal means of delivering the suicide gene would be to have a replication competent viral vector that would infect its way throughout the tumour and yet stop at its margins. Early studies with replication competent viruses that are restricted in their replication to certain types of tumour cell suggest this may be possible.

REGULATORY ISSUES

Safety issues are of considerable concern in all applications of gene therapy. During transduction, the recombinant virus could in principle recapture deleted structural genes and so revert to a replication competent form. It is important to demonstrate that there is no replication competent helper virus after transduction and irradiation of transduced cells. The presence of such a virus can be excluded by procedures such as the co-culture of the transduced tumour cells for 6 weeks with murine fibroblasts.

A second issue relates to insertional mutagenesis. Retroviruses

integrate into the host genome at any number of possible locations, some of which are almost certainly involved in the control of cell division. The use of gene therapy, therefore, has the potential to be oncogenic. However, the risk is low; and in 65 patient years' follow up at the St Jude Children's Research Hospital, for example, there have been no cases attributable to the gene marking studies being conducted. Nevertheless, such concerns provide the background for the discussion on the regulation of gene therapy. The current position was presented by Dr Odile Cohen-Haguenauer, Hôpital Saint Louis, Paris.

The deliberate alteration of the genetic material of living cells, and the therapeutic use of somatic cells manipulated *ex vivo* to change their biological characteristics, raise concerns that are scientific, ethical and social. These issues need to be considered at various levels, relating to the individual patient, the family and carers, and the wider environment.

Public health concerns centre on the possible spread of recombinant DNA beyond the patient. Whatever the context, the non-propagation and non-transmission of systems used to achieve gene transfer is essential.

To ensure the safety, efficacy and ethical nature of proposed therapy, clinical protocols should be designed with care and subjected to review by specialised scientific and ethical bodies. The review should pay attention to the appropriateness of trial design and to the balance of potential benefit and risk for patients involved. This must take into account the possible availability of alternative treatment strategies, such as targeted drug delivery, in the individual patient.

Regulating the safety and efficacy of the gene therapy products themselves (whether vectors or modified cells) is more properly the province of medicine control agencies. This applied to any level of manufacture and production, however large or small in scale. In the U.S.A., such regulation is the province of the Division of Cellular and Gene Therapy of the FDA. A section of the European Medicines Evaluation Agency, operative from January 1995, will have to perform a similar function in ensuring strict adherence to good manufacturing and laboratory practice. Among the elements in such practice are the control of biological sources, the adequacy of production facilities and the testing of output to maintain reproducibly high standards of quality in the final product. We are familiar with such regulation in the area of biotechnology in general and the engineering of vaccines in particular.

In the European Community, this level of regulation will be covered by the centralised procedure for marketing authorisation within the forthcoming European Medicines Evaluation Agency; the CPMP (Committee for Proprietary Medicinal Products) has recently adopted guidance to support marketing authorisation of gene therapy products [6, 7]. In the U.S.A., the FDA is the relevant authority and a notice (Federal Register, 14 October 1993) has been released dealing with the application of current statutory authorities to human somatic cell therapy products and gene therapy products [8].

Many of the principles laid down in existing Community Guidelines on Biotechnology Products, such as the requirements for cell banks, genetic stability and safety testing, as well as the principles of good clinical practice for clinical trials, will be applicable to the products designed for the gene therapy [9]. Additional new guidance is being developed to focus on the key areas of manufacture and control which need to be taken into account for these products. This guideline on the quality and safety of gene therapy products [6] was released for consultation of interested parties until August 1994. The CPMP adopted a final updated version in December 1994. The note for guidance is currently available.

There are already European Community Directives on Genetically Modified Organisms [10–13]. They include regulations on the containment of such organisms, their deliberate release into the environment, and the protection of persons at work. In the U.S.A., stringent containment does not apply to the facilities where patients are being treated. This should receive much attention as the requirement for contained treatment facilities in Europe contributes to the current limitation of protocols which could otherwise be initiated; and will involve significant financial investment. The absolute requirements should carefully be assessed according to the gene therapy product, the nature and function of the genetic sequence to be transferred and to the disease concerned. More specifically linked to gene therapy products, are provisions that relate to the amplification of nucleic acid sequences, uses of virus vectors as delivery systems, and genetically modified somatic cell products. Dealing with direct *in vivo* gene transfer, the risk of genetic modification of germline cells has to be assessed. In this setting, the long-term follow up of patients is, therefore, essential to determine the survival of genetically modified somatic cells and their biodistribution, as well as to assess their therapeutic benefit, toxicity and potential adverse effects.

In the United States, early phases in the clinical investigation and development of biological products have tended to be less stringently regulated than later phases, and their status is that of an Investigational New Drug. Application to the FDA includes the molecular biology of vector inserts, the production and testing of producer cell banks and safety and activity testing in animals [14]. The FDA has been primarily concerned with large scale production and the granting of product licences, while the Recombinant DNA Advisory Committee (RAC) of the NIH has overseen investigational gene therapy protocols. RAC has, in the past, encouraged broad public discussion as well as scientific evaluation. Procedures are now being streamlined so that the role of RAC will probably be confined to substantially new applications of gene therapy. In the consolidated procedure, the FDA alone will receive protocol submissions. The Cellular and Gene Therapy Division will forward a copy of each submission to the RAC. The two organisations will be jointly responsible only for review of gene transfer protocols that involve novel approaches, new diseases, unique applications, and other issues requiring public review. Slight modifications to existing or new protocols following a route already accepted by RAC would not have to be resubmitted to them [15, 17].

Currently in Europe a multiplicity of individual local and national agencies governs the safety, efficacy and ethics of gene therapy trials and the manufacture of gene therapy products. With regard to gene therapy protocols, the RAC's 'Points to Consider' has been used as a guide. However, this is now itself being reviewed. European countries should take advantage of the creation of the European Medicines Evaluation Agency to coordinate and centralise regulation, and thus anticipate the evolution towards regulation by a single body that is now taking place in the U.S.A. With regard to environmental safeguards, those EC directives on bitotechnology which do exist, and which were established in 1990, have so far been fully implemented only in three member states (France, the U.K. and the Netherlands) and partially in two (Germany and Belgium). The establishment of guidelines is, therefore, not the same as their application.

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Acknowledgements——The European School of Oncology gratefully acknowledges an educational grant from Rhône-Poulenc Rorer which made the meeting possible.