

IMPLICATIONS OF GENE THERAPY FOR HOSPITAL PHARMACISTS

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In this article, the authors highlight the potential changes hospital pharmaceutical services may require to undergo in order to meet the demands of the increasing number of gene therapy products entering clinical trials

Advances in genetic engineering and recombinant DNA technology have led to an increase in the number of biotechnology products reaching clinical trials. The development of genetically modified viruses, advances in cloning and sequencing of the human genome are beginning to offer the opportunity to treat a wide variety of diseases using gene therapy.

The first gene therapy trial was initiated in a human patient over a decade ago. Although clinical success has been slow, gene therapy will be used for some diseases without a conventional treatment. Gene therapy clinical trials are currently being undertaken in cystic fibrosis, cancer, cardiac disease and HIV.^{1,2} Such new therapies provide pharmacists with the opportunity and responsibility to develop new skills and expand pharmacy services. Gene therapy clinical trials, however, are quite different from those for standard small drug molecules. The aim of this article is to discuss the extra considerations required to set up a gene therapy clinical trial in a hospital pharmacy and the challenges met by two teaching hospitals in the UK. We hope to highlight the potential changes hospital pharmaceutical services may require to meet the demands of the increasing numbers of gene therapy products entering clinical trials.

GENE THERAPY

Gene therapy can be divided into two main categories: gene replacement and gene addition. Gene replacement tends to be used for monogenic diseases, where a single "faulty" gene, for example, an abnormal cystic fibrosis transmembrane conductance regulator (CFTR) gene, can be replaced with a normal gene. Currently, most gene therapy clinical trials are against cancer and involve gene addition, whereby a gene or genes may be "added" to a cell to provide a new function.^{1,2} Cancer gene therapy strategies include:

- Introduction of tumour suppressor genes
- Inhibition of the expression of dominant oncogenes
- Selective delivery of a toxin gene to cancer cells
- Chemoprotection by introduction of a multidrug resistance gene to normal tissues during high dose chemotherapy

- Introduction of genes encoding enzymes for prodrug activation
- Production of anti-angiogenesis proteins or cytokines
- Genetic immunopotentialiation by genetic modification of irradiated tumour cells (*in vitro* transduction)
- Immunisation with virus encoding tumour-associated antigens (*in vivo* transduction)

For gene therapy to be successful a therapeutic gene must be delivered to the nucleus of a target cell where it can be expressed as a therapeutic protein. Genes are delivered to target cells by vectors in a process called "gene transfer". The greatest challenge to gene therapy is finding a vector that can transfer therapeutic genes to target cells specifically and efficiently.

Gene transfer vectors can be broadly divided into non-viral and viral systems. Non-viral vectors, such as liposomes, have so far shown limited efficiency. Genetically modified (GM) viruses have proved to be the most efficient way of delivering genes. Viruses are merely genetic information protected by a protein coat. They have a unique ability to enter (infect) a cell, deliver viral genes to the cell nucleus and use the host cell machinery to express those viral genes. A variety of viruses have been used as vectors, including retroviruses, herpes simplex viruses and adenoviruses.

Many viral vectors used are "replication-deficient" or "replication-defective". They have been genetically modified by removal of the viral genes required to form new viral particles or revert back to a pathogenic virus. The deleted genes are replaced with a therapeutic gene, thus allowing the delivery and expression of the therapeutic gene without subsequent spread of the virus to surrounding cells.²

REGULATORY APPROVAL

In the United Kingdom, gene therapy clinical trials using viral gene transfer vectors are different from trials for conventional drugs.³ Viral vectors are genetically modified organisms (GMOs) and as such are subject to additional controls complicating the set up, handling and management of the trial. As with all clinical trials in the UK, a gene therapy trial must gain an appropriate licence from the Medicines and Healthcare products Regulatory Authority (MHRA) and the approval of the local research ethics

committee (LREC).³ For gene therapy, however, two additional steps are required before a protocol can be put into practice (Figure 1). First, the protocol has to be approved by a government body called the Gene Therapy Advisory Committee.⁴ Secondly, gene therapy clinical trials involving GMOs must comply with regulations for "contained use" and "deliberate release" of GMOs as set out by the Health and Safety Executive.^{3,5}

Gene Therapy Advisory Committee The 1992 report of the Committee on the Ethics of Gene Therapy (the Clothier committee) recommended that gene therapy (genetic engineering in humans) should be limited to life-threatening diseases or disorders. In 1993 the Gene Therapy Advisory Committee was established by the Government to advise on the ethical acceptability of proposals for gene therapy research on humans. All UK gene therapy clinical trial protocols in humans are subject to review by the advisory committee and take place under the strict rules they set out.⁴

Health and Safety Executive The aim of the Health and Safety Executive (HSE) is to minimise exposure of people and the environment to GMOs, ie, to "contain" GMOs as far as is feasibly possible. The use of GM bacteria, viruses and cells, but not DNA alone, is covered by the Genetically Modified Organisms (Contained Use) Regulations 2000.⁵ Aimed at laboratories, these regulations list a number of requirements for institutions involved in handling GMOs. Considerable interpretation is required to adapt these requirements for use in the context and practice of clinical trials.

Before any work involving GMOs can start, a biological safety officer must be appointed and a local genetic modification safety committee established. Under the guidance of this safety committee, it is the responsibility of the biological safety officer to ensure that all activities involving GMOs are assessed for risk to human health and

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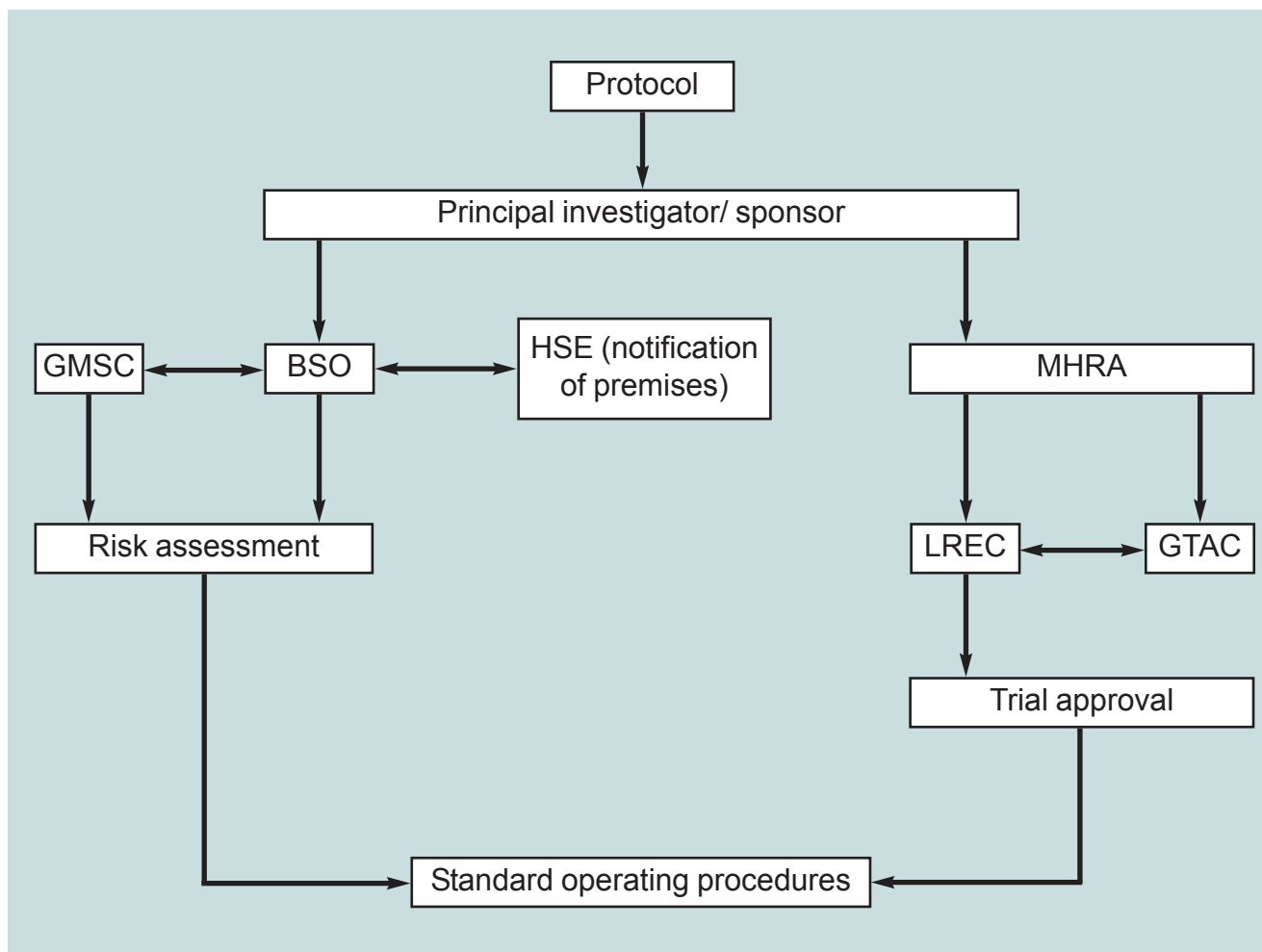


Figure 1: The process of a clinical trial involving a genetically modified organism (GMO) through the different regulatory bodies before study initiation. Key: GMSC, genetic modification safety committee; BSO, biological safety officer; HSE, Health and Safety Executive; MHRA, Medicines and Healthcare products Regulatory Authority; LREC, local research ethics committee; GTAC, Gene Therapy Advisory Committee

safety and to the environment. Records of this assessment must be maintained. Risk assessments classify activities involving GMOs into one of four classes (1, 2, 3 and 4). This classification determines the level of containment required to control the degree of risk involved; hence there are also four corresponding containment levels (1, 2, 3 and 4). Class 1 activities involve the least and class 4 the highest risk. Class 1 activities are unlikely to result in harm to humans or the environment and require containment level 1. Likewise, class 4 activities — for example, those involving the ebola virus — require containment level 4. Work with any agent able to cause human disease is categorised as class 2 or higher. A replication-deficient adenovirus (or “cold virus”) vector encoding a harmless bacterial enzyme requires class 1 containment but if such a vector were to encode a human growth factor, the increased risk to those handling the virus might change the containment level required to that of class 2. The exact requirements of the four containment levels are set out in the HSE “contained use” regulations and include the essential information to be included in a risk assessment with respect to genetic modification.⁵ At present, there are no examples in the UK of clinical trials for gene therapy involving GMOs that require level 3 or 4 containment.⁴

The HSE requires notification before any activities involving GMOs are undertaken. Information regarding the work, containment level, genetic modification safety committee and waste inactivation are provided in this notification. For work of risk class 2 or higher, the HSE must agree to the work before it commences. In all cases once the GMO has been classified, all the containment measures from the corresponding containment level must be applied to the work, unless specific permission (a “derogation”) has been obtained from the HSE to waive certain requirements.⁶

SETTING UP A GENE THERAPY TRIAL

In the rest of this article we will attempt to address some of the issues raised in two major hospitals carrying out gene therapy trials in the UK.

The appointment of a biological safety officer is the first obstacle to setting up a gene therapy trial. It could have a major cost implication for trusts, especially if the required expertise and knowledge are not readily available within the hospital. To overcome this problem, at University Hospital Birmingham NHS Trust the role is performed by a university academic staff member working in close liaison with the trust’s health and safety adviser. At the

Oxford Radcliffe Hospital, the consultant virologist initially performed the role. Owing to the large workload, however, the Radcliffe has now appointed a part-time biological safety officer who works closely with her Oxford University counterpart.

A working party or task force with representatives of all those directly and indirectly involved in gene therapy is a good way to approach setting up a gene therapy trial. Members of the working party can be physicians, nurses, porters and domestic staff, and there may be representation from pharmacy, health and safety, occupational health, mortuary, microbiology, virology, ethics and research and development departments. Working party viewpoints can be brought together to circumvent expected problems. Often, however, this role is instead undertaken by the genetic modification safety committee.

Genetic modification safety committee

There are no set requirements concerning who should be a member of the genetic modification safety committee (GMSC). It should include the biological safety officer, managers and employees, and should provide adequate representation for all those that may “handle” the GMO. For example, at the University Hospital Birmingham and the Oxford Radcliffe, the GMSC includes

representatives from pharmacy, occupational health, health and safety, research and development, microbiology and virology departments as well as nurses and clinicians. A member of the committee reports to the infection control committee at Birmingham and the risk management committee at Oxford.

As well as considering proposals for any new GMO work within the trust, any incidents involving gene therapy, eg, needlestick injuries and spillage, are reported to the GMSC in addition to the standard trust reporting procedures. Pharmacy has an important presence on the GMSC. The pharmacy department needs to be aware of proposed new trials for gene therapy. Pharmacy representatives can advise on the practicalities of dispensing gene therapy, the facilities needed, the procedures to be implemented and the implications for standard pharmacy services. It is useful if the pharmacy representative has some gene therapy knowledge and experience.

The GMSC performs the final step in approving a gene therapy clinical trial protocol and ensures that all other relevant authorities (see Figure 1) have given approval before a trial can go ahead. Because gene therapy clinical trials are new, at Birmingham and Oxford every aspect is documented as a series of standard operating procedures. In Birmingham, these have been incorporated into the cancer centre quality assurance system providing an audit trail within a system accredited to ISO 9001. Every gene therapy trial requires specific instructions which are dependent on the protocol and the risk assessment. In both Oxford and Birmingham, we have found that general procedures, such as the approval requirements for a gene therapy trial, are needed in conjunction with specific work instructions.

PHARMACY AND GENE THERAPY

For pharmacy, standard operating procedures specific to gene therapy include instructions for storage, cleaning, dispensing, spillage, protective clothing, transport and waste disposal. Also included are standard prescriptions and worksheets specific to the virus being dispensed and the dose required. Dispensing worksheets record patient details, batch numbers, dilution volumes, expiry data, operators and labelling. All these documents are intended to help pharmacy personnel safely dilute a concentrated gene therapy viral product into a dose suitable for administration in a way that can be fully audited.

There are no guidelines for dispensing gene transfer agents and consequently no definitive answers in the interpretation of the regulations. Each trial will have its own specific instructions, which need to be documented and followed. Safe handling of gene therapy is intimately related to the technical aspects of drug preparation, dispensing and administration. The HSE "contained use" regulations, occupational health and safety and Committee on Substances Hazardous to Health guidelines have to be interpreted and adapted to the hospital environment

while incorporating the principles of good aseptic practice.

In the pharmacy, the appropriate equipment, supplies, protective clothing and waste disposal systems must be available. In addition, operators must be adequately trained. They must be familiar with the safe use of these products, understand the calculations required for reconstitution, and they must be capable of using the equipment and preparation techniques involved.

Handling Pharmacists must not assume that they will automatically be given the responsibility for dispensing gene therapy products. Many DNA and virus products may not be considered "drugs" in the traditional sense and other health care specialties may assume responsibility for their dispensing and clinical use. Pharmacists have training in preparation and dispensing of drugs and therefore the expertise to store and dispense gene therapy products. Pharmacy can provide the use of validated biological safety cabinets or isolators to protect the product, operator, and environment.

In Birmingham, pharmacy staff use a negative pressure isolator to dispense gene therapy viruses. In Oxford, medical research staff use a class II biological safety cabinet in the laboratory adjacent to the research ward. Other UK hospitals have facilities for handling gene therapy viruses in negative pressure suites either adjacent to existing aseptic facilities or adjacent to en-suite patient rooms. There is currently no consensus on whether central or satellite facilities are more appropriate.

Storage Most hospital pharmacies do not possess the ultra-low temperature freezers required for gene therapy products, most which have to be stored below -70°C . Provision of the correct storage facilities can be costly and adequate space with controlled access can be a problem. If possible, the storage facility should be positioned near the dispensing area to minimise risk of spillage and contamination of other areas. Validation and monitoring of normal pharmacy refrigerators and freezers is standard practice but proves difficult and expensive with ultra-low temperature freezers. Pharmacy departments may be able to negotiate the purchase of storage facilities, service contracts, and monitoring systems with investigators and trial sponsors.

Cleaning Minimal exposure of GMOs to humans and the environment is required by the HSE, yet there is no method readily available to hospital pharmacy departments for the detection of viruses. At Birmingham and Oxford the action of virucidal detergents has been validated against all the gene therapy vectors used. In Birmingham, isolators are cleaned before and after use with these virucidal agents and also with standard 70 per cent industrial methylated spirits. In Oxford, the virucidal agent is washed off with sterile water to minimise corrosion damage to the cabinet. Decontamination and disinfection procedures need to be appropriate to each viral product.

Dispensing Often gene therapy products are of small volume and some require multiple dilutions to reconstitute them. The dilution process can be complex and calculations should be recorded and checked. Viral products are often quantified in units by the number of viral particles that can be found in 1ml. For gene therapy trials doses in the region of 1×10^{12} viral particles are not uncommon. With large numbers of particles in small volumes of liquid, the measurement of volumes for dilution is critical to the dose the patient will receive. For accuracy and also to reduce the risk of needlestick injuries, micropipettes may be considered instead of needles and syringes during dispensing. The volume of liquid remaining in syringes and needles after administration, the dead volume, is surprisingly large. This means that for some trials involving the injection of small volumes, compensation for dead volume is required in calculations and dispensing.

The strength of gene therapy products can be described in a number of different ways. Generally, doses of viral vectors are prescribed in viral particle units (PUs) regardless of whether the viral particles are active or not. The strength of a gene therapy vector can also be described in terms of the number of active viral particles, known as plaque forming units (PFUs). These two measurements can be different and need to be distinguished: for example, an adenovirus vector of 1×10^{11} PU/ml may only contain 1×10^{10} PFU/ml.

Labelling All gene therapy clinical trial products are classed as investigational medicinal products and as such should be labelled according to the instructions found in annex 13 of the "Orange guide".⁷ Problems may be encountered with pharmacy computer systems that do not have the facility for labelling with exponential figures or unusual units of measurement (PU/ml or PFU/ml). There is no pharmacy requirement to label the product as genetically modified, but for on-site transport of the product to the patient it is good practice to label transport containers with a biological hazard warning sign.

Operators For most of the hospital staff involved it will be their first encounter with GMOs. Education should overcome initial fears that may result from the negative media coverage of genetic modification of food and crops. It is essential that all staff involved in handling gene therapy vectors are trained in the procedures specific to handling gene therapy. Importantly, there is a requirement that staff are not pregnant, breastfeeding or immunosuppressed. Employees need to be aware of any risk associated with these therapies and have an option not to be involved in the dispensing process. Staff training should be documented, recorded and regularly updated. In Oxford, this information is held centrally on the computer system.

Policies need to be implemented for employee exposure to such agents. In Oxford an electronic database is kept for all staff who

have been involved in handling GMOs, even class 1 agents. In Birmingham, the occupational health and safety department keeps records of employees working directly with GMOs. This is an HSE requirement for containment level 2 agents and above.

Transport Transport of GMOs within the trust is subject to risk assessment. It should, however, be noted that transport of GMOs to other sites might be subject to the Carriage of Dangerous Goods by Road and Rail Regulations 1994, a requirement of which is that those transporting GMOs must be authorised to do so.

Waste disposal Aseptic technique and decontamination procedures are used as specified by standard operating procedures and all contaminated materials inactivated and then disposed of as clinical waste.⁵ Consideration has to be made of the relevant biohazard labelling. According to the "contained use" regulations waste materials contaminated with GMOs must be inactivated before disposal. Standard disposal of clinical waste at Birmingham and Oxford is performed by a contractor not authorised to handle GMOs. All contaminated materials must, therefore, be inactivated on-site before collection by contractors. Currently, at Birmingham, all materials potentially contaminated are autoclaved on trust premises by the microbiology department. In Oxford, waste is either autoclaved on trust premises by microbiology, or a separate authorised contractor is used to collect potentially contaminated waste. Sealed Linbins labelled as GMO waste are used prevent any spillage and are stored separate from other waste while awaiting collection.

Spillage In the event of a spill, every conceivable circumstance needs to be addressed, from contamination of operators and clothing to contamination of surfaces and equipment. Manufacturers will specify the detergents able to inactivate their products. This can mean, however, that different spillage procedures are needed for each product. Procedures must contain any spilt gene therapy product and allow for its subsequent inactivation before leaving the hospital site. The risk assessment and class of the GMO will determine the contents of spillage kits. For example, a spillage kit may contain gloves, masks, aprons, goggles, disposable shoe covers, virucidal detergent, absorbent material, disposable forceps and a biohazard incineration bag. It is essential that spillage kits are available in areas where gene therapy is handled.

CONCLUSION

At Birmingham and Oxford we have tried to interpret the level of containment required for GMOs under the "contained use" regulations and integrate them with the principles of aseptic technique. The lack of guidelines does not make this an easy task. With GMOs, as with cytotoxics, we want to minimise the exposure of the operator and the environment to these agents. At Bir-

mingham, the satellite hospital pharmacy aseptic suite easily meets the requirements for level 1 containment. The facility is used daily for conventional cytotoxic reconstitution which must be stopped for GMO related cleaning to allow for GMO dispensing. Standard microbiological monitoring used in hospital pharmacy aseptic units will not detect the presence of viruses. There is no easy way to determine viral contamination of dispensing equipment. In an effort to compensate for these factors vigorous clean down procedures are performed before and after dispensing using validated virucidal agents and generous purge times before conventional chemotherapy dispensing can take place. Problems arise with class 2 GMOs where a greater level of containment is required. An alternative may be to dispense in an isolator at the patient's bedside. Birmingham is fortunate enough to have a research block (Wellcome Trust clinical research facility) equipped with two isolation rooms, specially designed for gene therapy patients with interchangeable positive and negative air pressures adequate to contain GMOs.

At Oxford, a biological safety cabinet is used to protect the operator handling gene therapy. It is not in an aseptic pharmacy unit but aseptic technique is used to protect the product. Since the biological safety cabinet is also used to handle body fluid samples, there are also vigorous cleaning procedures before and after dispensing. With the prospect of high containment level gene therapy vectors, the option of a specific aseptic facility is being investigated in both centres.

The trend for gene therapy is towards non-viral vectors, DNA vaccines and antisense oligonucleotides. Class 2 viral vectors are already in clinical trial, so hospitals need to plan for containment level 2 facilities in the future. In the UK, no gene therapy products have yet received regulatory approval for marketing. Most clinical trials for gene therapy are still at phase I/II for safety and dose determination, although of the current gene therapy trials registered in the UK, two have now reached phase III.⁴ Clearly, gene therapy has entered the med-

ical environment and pharmacy must adapt to meet the challenge. If pharmacists recognise the unique requirements that result from the use of these products, they can develop pharmacy services to meet those needs. Hospital pharmacy departments are ideally placed for ensuring the development of policies and procedures for the safe and effective acquisition, storage, distribution and handling of these products. Plans need to be put into place to ensure that appropriate facilities will be available in the future for handling gene therapy clinical trials.

There are currently no national guidelines on the pharmacy facilities required for handling gene therapy. In November 2002, the National Quality Assurance Pharmacists Group approved a working document on the plans that trusts should incorporate into future hospital pharmacy facilities. Recommendations are made that future aseptic suites include a separate dedicated unit with a dedicated vented negative pressure isolator and separate air handling plant. In the meantime, dedicated negative pressure isolators or type II biological safety cabinets, which are extracted externally, should be used to prepare viral gene therapy vectors. Preferably this would be in a dedicated room, but if this is not possible then other activities must be stopped during gene therapy dispensing and a suitable validated clean down must be allowed in the room after dispensing.⁸ The HSE has also drafted some guidelines on handling gene therapy in the clinical setting, which are yet to go out for consultation.

Genetic modification and its application to the clinical setting is a novel and exciting therapy for many diseases including cancer. It is essential as treatments develop and progress that the pharmacy profession is aware of the requirements for the handling and preparation of gene therapy products.

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REFERENCES

1. Gomez-Navarro J, Curiel DT, Douglas JT. Gene therapy for cancer. *Eur J Cancer* 1999;35:2039-57.
2. Brooks G. Gene therapy — the use of DNA as a drug. London: Pharmaceutical Press; 2002.
3. Cohen-Haguener O, Rosenthal F, Gansbacher B, Bolhuis R, Dorsch-Hasler K, Eshhar Z et al. Opinion paper on the current status of the regulation of gene therapy in Europe. *Human Gene Ther* 2002;13:2085-110.
4. Gene Therapy Advisory Committee. Available at: www.doh.gov.uk/genetics/gtac/index.htm (accessed 10 June 2003).
5. Health and Safety Executive. A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000. Norwich: HM Stationery Office; 2000.
6. Searle PF, Spiers I, Simpson J, James ND. Cancer gene therapy: from science to clinical trials. *Drug Delivery Syst Sci* 2002;2:5-13.
7. Medicines Control Agency. Rules and guidance for pharmaceutical manufacturers and distributors 2002. London: Stationery Office; 2002.
8. National Quality Assurance Pharmacists Group. Guidance on handling of monoclonal antibody and gene therapy products. Available at www.qainfozone.nhs.uk/publications.html#NHSQACCommittee (accessed 22 July 2003).