From vector toxicity to successful clinical evaluation: highlights of the 2nd annual meeting of the British Society for Gene Therapy

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Manchester was the setting for the second annual meeting of the British Society for Gene Therapy (BSGT). With an increase in BSGT membership, there was also a welcome increase in the number of delegates attending the conference, from 208 to 222, compared with the previous year. Recent advances in the field of gene therapy were presented and these spanned the development, use and refinement of a wide range of gene-delivery approaches using nonviral vectors and many types of viral vectors, including adenovirus, adeno-associated virus (AAV), lentivirus, Semliki Forest virus (SFV) and herpes simplex virus (HSV). This year, there was a particular focus on advances in cancer gene therapy and in-depth discussion on vector toxicity and safety, with particular regard to retroviral vectors. In addition, there were also sessions that focused on gene therapy for genetic disease, methods to improve gene delivery and expression as well as discussions on the use of stem cells in cellular therapy. One particular aim of the conference was to reinforce the need for high-quality, basic research in gene therapy to facilitate a successful translation into clinical studies. Evidence of this was provided at the conference with many encouraging results from clinical trials, principally in oncolytic gene therapy and in the use of gene therapy for the treatment of genetic disease. This report describes some of the highlights of the data presented.

Vector toxicity & safety

Recent adverse effects reported in clinical trials following viral-mediated gene transfer have resulted in the need for thorough safety evaluation of these types of vectors [1–3]. On the first full day of the conference, Mike Themis (Imperial College, London, UK) presented data on an *in vivo* fetal model to test the safety of lentivirus gene-therapy vectors that highlighted the potential oncogenic risks of lentiviral vectors. The fetal model is an attractive proposition: potentially it could provide early treatment for genetic disease, may allow stem-cell transfer for permanent correction, has immune tolerance for long-term gene expression and allows for the easy identification of any adverse effects of gene delivery such as developmental problems. Themis and colleagues have previously shown that *in utero* administration of several lentiviral vectors to day-16 mouse fetuses resulted in efficient vector spread and long-term gene expression. However, this talk focused on the high frequency of liver tumors observed after injection of third-generation lentiviral vectors based on equine infectious anemia virus (EIAV). In mice treated with these EIAV lentiviral vectors, originating from Oxford BioMedica Ltd (UK), tumors were detected, in some cases in eight out of ten mice, depending on the vector type. Multiple tumors were presented in some animals. It was concluded that the fetal model may now present itself as a useful model to determine the effects of vector insertion and to study genomic insertion sites that induce oncogenesis in more detail.

The next speaker, Susan Kingsman (Oxford BioMedica Ltd, Oxford, UK) urged caution in the interpretation of the results presented by Themis, as experiments are ongoing to verify whether tumor generation was due to the vector. Alternative mechanisms were suggested, including the possibility that the fetal liver is well known to develop tumors after chemical or surgical intervention. Hence, the procedure itself may be oncogenic and tumor generation completely unrelated to the EIAV vector. Kingsman went on to suggest that the more likely explanation was the presence of the woodchuck hepatitis post-transcriptional regulatory element (WPRE) in these vectors, which may provide the potential to express an X protein-derived fragment that may act as a weak cofactor for oncogenesis [4]. The UK Gene Therapy Advisory Committee have also released advice on this safety issue in 2004 and further details can be found on the Department of Health Gene Therapy Advisory Committee website [101]. It was highlighted that Oxford BioMedica have a number of lentiviral vectors in the pipeline for clinical applications to treat such diseases as Parkinson's, motor neuron disease and age-related blindness. However, these vectors contain a modified WPRE that do not express any X protein fragments, which should help to attenuate any oncogenesis concerns.

The leukemic side effects of retroviral gene transfer were also discussed and it was highlighted that they originate from insertional mutagenesis. Several strategies were presented that could be used to overcome the oncogenic issues associated with these types of vectors. Christopher

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Baum (Hannover Medical School, Germany), for example, described how an increase in the safety profile of these vectors was achieved by careful control of the amount of virus used. Mice transplanted with repopulating hemopoetic cells transduced at low multiplicity of infection (MOI) remained free of leukemic complications for more than 5 months compared with those transduced at high MOI, which revealed many leukemic events, often with multiple vector insertions. In addition, careful vector design may also reduce complications due to insertion into proto-oncogenes. Data were presented on the use of retroviral pseudotransduction as a novel form of gene delivery. The usual route of retroviral infection is via receptor-mediated uptake, followed by reverse transcription of plus-stranded genomic mRNA into double-stranded proviral DNA, which then integrates into the host genome. The vectors used by Baum and colleagues were mutated in the primer binding site, thus blocking reverse transcription of the virion mRNA leading to at least a 1000-fold attenuation in their capacity for integrative gene transfer. However, this retroviral mRNA, whilst not undergoing reverse transcription, served as an immediate translation template, resulting in efficient transient transgene expression.

An alternative strategy to improve the biosafety of lentiviruses was described by Rafael Yanez (Queen Mary University of London, UK) using integration-deficient lentiviral vectors. Efficient expression was mediated by the formation of double-stranded episomal circles that were highly stable in postmitotic cells, although transient expression was also seen in proliferating cells. High levels of lentiviral transduction and transgene expression, in the absence of vector integration, was described during the correction of RPE65 defects in Leber congenital amaurosis (LCA), a clinically severe retinal degenerative condition (which has been treated successfully by Robin Ali, UCL, London, UK, that will be discussed later).

Gene therapy in the treatment of genetic disease

There were several presentations on the use of gene therapy to treat genetic diseases, and the conference profiled several successful treatments, including therapies for haemophilia B (Amit Nathwani, UCL UK) and Hunter syndrome (Ilaria Bellantuono, Royal Manchester Children's Hospital, UK). Some of the most promising results were described by Kathrina Quinn (Trinity College Dublin, Ireland) and Robin Ali (UCL, UK).

Kathrina Quinn gave a short presentation describing the intranasal delivery of interferon (IFN)-β to mice using SFV particle vectors to treat experimental encephalomyelitis, which is an animal model of multiple sclerosis. Increased expression of IFN-β was thought to promote a T-helper (Th)2 response leading to downregulation of the Th1–IFN-γ disease-promoting response. Interestingly, the efficacy of treatment was highly dependent on both the number and timing of treatments, with fewer treatments during the disease-effector stage, therefore suggesting that these factors should be carefully considered when delivering therapies.

Robin Ali gave a particularly encouraging talk on ocular gene therapy for retinal disease. The eye appears to be an ideal organ for this type of therapy, as together with its obvious accessibility, it allows for localized delivery so that very small amounts of reagent are required compared with other organs and, importantly, the eye contains a large population of postmitotic cells so it is less susceptible to oncogenic events. In addition, only a few 100 functional photoreceptor cells may be sufficient for light perception; therefore, even modest therapy success can provide a real therapeutic benefit. Experiments were described using AAV (mainly based on AAV-2 but often pseudotyped with AAV-4 or -5 capsid proteins) and lentiviral vectors to deliver transgenes to correct those mutated in inherited retinal degenerative diseases and in combination with delivery of neurotrophins, in a variety of animal models. This work is in preparation for imminent human clinical trials and a Department of Health grant has been awarded to test therapy for RPE65 defects in LCA – one of the most clinically severe retinal degenerations. LCA results in near total blindness with early onset in childhood and patients becoming totally blind from their late teens. RPE65 is a highly conserved 61 kDa protein present in smooth endoplasmic reticulum of the retinal pigment epithelium and is essential for retinoid metabolism. In mice, the lack of RPE65 results in the accumulation of all transretinyl esters, lack of rhodopsin, rod photoreceptor dysfunction, retinal pigment epithelial inclusions and subsequent retinal degeneration. Promising results were shown using an AAV-2 vector expressing RPE65, with restoration of normal rod function in animal models, including murine and canine models. With such promising results in animal models, it is hoped that this will lead to successful treatment in the clinic, not only for LCA but also for the treatment of many other gene defects associated with early onset severe retinal dystrophy.

Cancer gene therapy

New treatments, or the refinement of existing treatments, are required to successfully combat many types of cancer. Oncolytic gene therapy is one of the most promising approaches based on exploiting the ability of viruses to specifically infect, replicate and subsequently lyse tumor cells, while sparing normal cells. Strategies were highlighted at this conference to target infection and replication to tumor cells. One such approach involved utilising specific cellular receptors expressed exclusively, or at high levels, by cancer cells. Andre Lieber (University of Washington, DC, USA) described the development of chimeric adenovirus vectors that incorporated fibers derived from Ad group B serotype 35 (Ad5/35). Ad35 utilises CD46 as a cellular receptor and can efficiently target malignant cells where CD46 is upregulated. An alternative strategy to target cancer cells by selective viral replication was described by Nick Lemoine (Barts

and the London School of Dentistry, UK) with the use of replication-selective oncolytic adenovirus. Deletions in the *virus-associated I (VAI)* gene, required for translation of viral RNAs, resulted in viral replication and cytolytic cell death only in EBV-associated tumor cells, where the EBV-encoded small RNA 1 can complement *VAI* deletion mutants. In tumor xenografts, *VAI*-deleted adenovirus produced an increase in antitumoral efficacy, compared with wild-type virus. These results are most encouraging for the treatment of virus-associated malignancies.

Success using this type of targeting and oncolytic anticancer therapy was also highlighted in clinical studies. Moira Brown (Crusade Laboratories Ltd, Glasgow, UK) described the use of a selectively replication-competent HSV vector, termed HSV1716, to target cancer cells. HSV1716 is deleted in the *RL1* gene encoding the virulence factor ICP34.5 and subsequently cannot replicate in nondividing cells. However, in tumor cells there is a significant increase in the expression of proliferating cell nuclear antigen (PCNA), which compensates for the lack of ICP34.5 expression, allowing HSV1716 to replicate and resulting in tumor cell death. Previous work has shown that HSV1716 delivery results in tumor regression and an associated mean increase in survival times in most cancer patients. For example, several patients with glioma that were treated with HSV1716 had survival rates significantly longer than the average 1-year expectancy. In metastatic melanoma patients, flattening of previously palpable tumor nodules was observed after intratumoral injection of HSV1716. In particular, selective tumor killing has been achieved in clinical trials with cancers such as the brain tumor glioblastoma multiforme (GBM), malignant melanoma and squamous cell carcinoma of the head and neck.

Brown also described how HSV1716 is now being used as a component in combination therapies. For example, HSV1716 used in combination with small interfering (si)RNA technology to deliver specific siRNA molecules down-regulates a squamous cell cancer oncogene, which significantly delays tumor growth compared with HSV1716 alone. Alternatively, HSV1716 used in combination with conventional therapies, such as chemo- or radiation therapy, showed enhanced antitumor effects.

A combinatorial approach was also described by Richard Iggo (Swiss Institute for Experimental Cancer Research) for targeting colon cancer cells using adenoviral vectors with Tcf binding sites inserted into early promoters. In these cells, there is constitutive activation of transcription from promoters containing binding sites for Tcf/LEF transcription factors and so viral replication can be restricted to colon tumors. Tumor cell infection is further enhanced by insertion of an integrin-targeting peptide into the virus fiber gene. Furthermore, the oncolytic effectiveness of these targeted viruses is greatly enhanced by combining them with RAD001, a mammalian target of rapamycin (mTOR) kinase inhibitor that directly inhibits tumor growth and also has antiangiogenic and immunosuppressive effects.

Rachel Cowen (University of Manchester, Manchester, UK) presented data on a different combinatorial gene therapy/drug treatment anticancer approach based on the hypoxic nature of most solid tumors. These tumor cells are hypoxic as a consequence of the rapid tumor growth outstripping the blood supply and poor organization of the tumor vasculature. Thus, the specific physiologic conditions of tumor cells can be exploited for therapeutic strategies. In particular, a novel prodrug, 629 (5-aziridinyl-3-hydroxymethyl-1-methylindole-4,7-dione), was described that is converted into a cytotoxic compound in the hypoxic environment of tumor cells. The efficacy of 629 is greatly increased when used in combination with non-replicating adenoviral vectors expressing P450 reductase from a hypoxia responsive promoter. This elevation in reductase levels increases the sensitivity of hypoxic cells to the 629 prodrug and was shown to be successful in controlling the hypoxic fraction of breast and fibrosarcoma xenografts.

Altogether, several combinatorial anticancer approaches were highlighted at the meeting including the combination of viral oncolysis with conventional chemo- and radiation therapies or novel drugs. These strategies appear promising for future clinical evaluations.

Nonviral vectors

Nonviral technology has often been dismissed as an ineffective gene-delivery tool. However, more recently, and especially with the current safety concerns associated with viral vectors, there has been a re-energising of the nonviral field. Much progress has been made in the development of more effective vectors, such as the use of synthetic methods to manufacture novel lipids and polymers to enhance plasmid delivery to the nucleus, as well as the incorporation of elements from viral vectors to increase levels and longevity of transgene expression [5]. On the final day of the conference, several presentations described recent developments in the design of nonviral vectors that improve their efficiency. Briefly, highlights included the use of nonviral episomal DNA vectors containing a nuclear scaffold/matrix attachment region enabling long-term maintenance in mammalian cells (Dean Jackson, University of Manchester), inclusion of nuclear localization signals in plasmid DNA to increase the nuclear import of gene delivery vectors (Vaysse Laurence, Imperial College), and the use of antisense oligonucleotides to create a shortened, mature dystrophin transcript that has the mutation-bearing region excluded for the treatment of Duchenne muscular dystrophy (DMD) (George Dickson, Royal Holloway, University of London, London, UK). A clinical trial to utilise this technology is now being planned in DMD patients.

Expert commentary & outlook

The 2005 Second Annual British Society for Gene Therapy conference spanned only two and a half days but contained 39 oral presentations and 87 posters covering a wide range of

gene-therapy strategies. In particular, encouraging progress in the development, use and refinement of many different types of gene delivery vectors, both viral and nonviral was described, highlighting the numerous modifications being made to these vectors in order to improve their delivery efficiency, transgene expression, cell targeting and safety. Many successful proof-of-principle experiments, right through to preclinical and clinical-trial data were also highlighted, including trials such as those described by Robin Ali for retinal degeneration and Moira Brown in cancer therapy. It is also encouraging to see that the field is starting to address important issues, such as vector toxicity, in more detail. In particular, the use of fetal *in vivo* models will likely become a sensitive model to test the oncogenic potential of certain vectors such as lentiviral vectors. This will help to facilitate rapid progress towards vectors with minimum toxicity profiles. Indeed, the gene therapy community now eagerly awaits further updates of the precise mechanism of how lentiviral vectors cause tumorogenesis in this model.

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Website

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