

Review

Tripartite Meeting in Gene and Cell Therapy, 2008: Irish Society for Gene and Cell Therapy, British Society for Gene Therapy, and International Society for Cell and Gene Therapy of Cancer

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Abstract

The second annual meeting of the Irish Society for Gene and Cell Therapy was held in Cork, Ireland on May 15 and 16, 2008 (<http://crr.ucc.ie/isgct/>). The meeting was jointly organized with the British Society for Gene Therapy and the International Society for Cell and Gene Therapy of Cancer. Because of the location of the conference and the co-organization of this meeting with the British and International Gene Therapy societies, the meeting enjoyed a range of talks from some of the major leaders in the field. Particularly notable were the talented molecular and cell biologists from Ireland who have contributed cutting edge science to the field of gene therapy. Topics including cardiovascular disease, repair of single-gene disorders, and cancer gene therapy were discussed with presentations ranging from basic research to translation into the clinic. Here we describe some of the most exciting presentations and their potential impact on imminent clinical gene therapy trials.

Introduction

THE MEETING WAS introduced by Noriyuki Kasahara (president-elect of the International Society for Cell and Gene Therapy of Cancer [ISCGT]; and David Geffen School of Medicine [University of California Los Angeles, Los Angeles, CA]). He overviewed the renewed commitment of industry to gene therapy (GT) and described some of the notable advances in cancer GT since the 1980s. He said that important barriers to effective GT have been removed and, of particular note in the laboratory, that more efficient gene transfer into target cells is now being achieved. Dr. Kasahara stated that we are gaining a greater understanding of the interaction between GT and the immune system, so that not only can we circumvent its protective effects; we can also use the immune system to enhance the efficacy of GT. Strategies are now being devised to maximize GT efficiency (e.g., transfection of maximal cell numbers and optimized cancer cell killing) and we are on the frontiers of a new era in reparative medicine using stem cells. It seemed appropriate that

Dr. Kasahara quoted the Irish playwright James Joyce: "A man's errors are his portals of discovery." We may not yet have achieved mainstream clinical application, but we are certainly moving in that direction. Last, Dr. Kasahara described the increasing realization of the need to assess risk versus benefit when considering cancer GT. We review this productive meeting and discuss future directions in GT.

Treatments for Genetic Diseases

Simon Waddington (Royal Free Hospital, University of London, London, UK) delivered a thorough review of *in utero* GT, highlighting the factors that characterize this treatment as an attractive alternative to adult GT, while also identifying some of the other diseases that may be targetable by this therapy. The capacity for more efficient transduction is possible *in utero* because there are fewer physical and biological barriers to vector passage. There is greater access to stem cell and progenitor compartments at a significantly greater vector-to-cell ratio than would be possible in the

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adult model (Waddington *et al.*, 2005). The absence or limited development of immune mechanisms at the time of *in utero* GT can serve to avoid immune elimination (normally seen in the adult environment) and can lead to the development of immune tolerance toward the transgenic protein (Waddington *et al.*, 2007). This concept has been supported by Dr. Waddington in studies into the expression of human factor IX (hFIX) in a murine model of hemophilia B. hFIX protein tolerance observed after vector-mediated gene transfer was shown to be due to the presence of a regulatory T cell (Treg) population in the *in utero*-treated animals. A number of autosomal recessive disorders, because of a current absence of conventional treatment options and their association with fatality during infancy, are all possible targets for fetal GT. These include neural ceroid lipofuscinosis, surfactant B deficiency, and ornithine transcarbamylase deficiency. Early-intervention GT for cystic fibrosis (CF) lung disease was assessed with a view to developing a potentially permanent correction due to the equal distribution of transduced epithelial cells throughout the airways after fetal administration of a gp64/HIV-green fluorescent protein (GFP) lentivirus (LV). Transduced cells often appear in a clustered formation, which may suggest that infection of progenitor cells and clonal expansion has occurred (Buckley *et al.*, 2008). The choice between nonintegrating (adenovirus [Ad], adeno-associated virus, integration-defective LV) and integrating (integration-proficient gammaretrovirus [RV] and LV) vectors and their associated benefits represents an additional consideration for the development of further treatment strategies. A cautionary note was given in relation to the need for future studies in larger animal models, which would better reflect the potential of any *in utero* GT to treat the chosen condition encountered in a human setting.

Richard Wade-Martins (University of Oxford, Oxford, UK) presented current research into the area of high-capacity episomal DNA delivery vectors, using both viral and nonviral approaches. Three important features make this approach an attractive means of complementation GT: the nonintegrating, extrachromosomal nature of these vectors, the high transgene capacity, and advances in both viral and nonviral vector delivery technology. Work on long-term extrachromosomal persistence has focused on three systems of episomal vector retention; Epstein-Barr virus-based plasmids, S/MAR (scaffold/matrix attachment region)-based vectors, and human artificial chromosomes. In each case the high transgene capacity (152 kb) of the herpes simplex virus type 1 (HSV-1) amplicon vector is used. Such capacity is sufficient to accommodate large genomic loci containing the gene of interest together with both upstream/downstream sequences encompassing native promoters, noncoding regulatory flanking sequences, and introns allowing for the production of alternative splice variants, prolonged expression, and physiological regulation. The HSV-1-based infectious bacterial artificial chromosome (iBAC) system was first demonstrated in the delivery of the complete human hypoxanthine phosphoribosyltransferase (*HPRT*) locus to human and mouse cell lines (Wade-Martins *et al.*, 2001). Further evidence of the merits of the iBAC system can be observed in restoration of expression of the low-density lipoprotein receptor (LDLR) gene, mutated in familial hypercholesterolemia, at broadly physiological levels while retaining normal expression regulation by sterol levels (Wade-Martins *et al.*, 2003). This approach offers significant improvement compared

with the use of vectors under strong, constitutive promoters driving cDNA expression, which are likely to lead to unregulated transgene expression. This is equally a concern in the regulation of the protein encoded by the frataxin gene, *FRDA*, associated with Friedreich's ataxia. Unregulated accumulation of the frataxin protein has been proven to be toxic to the cell, whereas iBAC-*FRDA* was expressed at physiological levels and corrected the underlying biochemical deficit in Friedreich's ataxia patient cells (Gomez-Sebastian *et al.*, 2007). The S/MAR-based iBAC system combines the high transgene capacity of HSV-1 with the episomal retention properties of the S/MAR. The inclusion of these elements can help increase the level of expression and avoid silencing of the transgene. Similar levels of transgene expression (compared with those detailed above) with physiological regulation was observed in the functional recovery of LDLR activity (Lufino *et al.*, 2007). A nonviral approach to the delivery of BAC constructs has also been examined and involves hydrodynamic tail vein injection, leading to high-efficiency transduction of hepatocytes. The technique is well tolerated in small animals and long-term expression (4 months) of the *LDLR* gene with physiological regulation has been demonstrated (Hibbitt *et al.*, 2007). The delivery of large aliphoid DNA sequences, using HSV-1 vectors, to form human artificial chromosomes represents another application for the delivery of large genomic payloads to cells by HSV-1 vectors resulting in long-term expression profiles (Moralli *et al.*, 2006).

Ciaran Lee (Department of Physiology and BioSciences Institute, University College Cork [UCC], Cork, Ireland) presented on the capacity of zinc finger nucleases (ZFNs) to effect gene repair by homologous recombination in the context of CF. Most CF patients have the $\Delta F508$ mutation in both alleles of the *cftr* gene, which disrupts an apical membrane chloride channel. A pair of ZFNs can induce a double-stranded break at a unique genomic site and enable a donor repair sequence to effect homology-directed repair of a target allele in up to 20% of treated cells (Urnov *et al.*, 2005). Repair of one allele of the *cftr* gene in just 8% of lung epithelial cells could be sufficient to restore wild-type levels of chloride flux; hence the interest in this approach as a potential therapeutic approach to CF. As a first step toward *cftr* gene correction, this group described the design and synthesis of a pair of ZFNs to recognize two 9-bp sequences, separated by a 4-bp spacer, corresponding to a 22-bp region of intron 9 in the *cftr* gene, close to the $\Delta F508$ site. Using an *in vivo* plasmid DNA cleavage assay, they were able to show that both ZFNs could recognize their respective 9-bp target sequences with high specificity. Moreover, the ZFNs could heterodimerize to recognize and cleave the 22-bp target region in the same assay, indicating that they could be used with a suitable donor sequence to effect homologous recombination of the *cftr* gene. However, before evaluation in an *in vivo* repair assay of the *cftr* gene, the nuclease domain will be modified to prevent nonspecific homodimerization, in order to further increase specificity when the ZFNs are expressed in a genomic context.

Gene and Stem Cell Treatments for Cardiovascular Disease

Timothy O'Brien (president of the Irish Society for Gene and Cell Therapy [ISGCT]; Regenerative Medicine Institute

[REMEDII], National University of Ireland [NUI], Galway, Ireland) described his work in a talk entitled "Endothelial Progenitor Cell (EPC) Dysfunction in Diabetes Mellitus: Implications for Autologous Cell Therapy." The use of stem/progenitor cells in regenerative medicine is an area of major importance in cardiovascular disease, where accumulating evidence suggests that bone marrow (BM)-derived endothelial, hematopoietic stem, and progenitor cells can contribute to tissue vascularization. Professor O'Brien gave an informative overview of the study of EPCs and the challenges facing the scientific community in identifying the nature of the cells used for EPC transfer. He addressed one of the fundamental challenges in EPC biology, namely, the need for a unifying approach to characterizing these cells when isolated and grown under culture conditions. Commonly used markers are CD34, CD133, and KDR, and early and late outgrowth cells are distinguished on the basis of their proliferative capacity and their ability to form *de novo* blood vessels. Correct identification of EPCs is a fundamental requirement in order to realize their full clinical potential for providing an endogenous repair mechanism to (1) counteract endothelial injury, (2) replace dysfunctional endothelium, and (3) form new vessels in ischemic areas.

Reduced levels of circulating EPCs have been found in patients with multiple risk factors for cardiovascular disease and have been found to predict disease progression, thus supporting an important role for endogenous vascular repair to modulate the clinical course of coronary artery disease. With this in mind, Professor O'Brien discussed the advantages and disadvantages of autologous or allogeneic transplantation, with the former avoiding any problems with immunogenicity; however, allogeneic transplantation may be necessary as a patient's EPCs could be dysfunctional in their homing or reparative function. Some of the potentially therapeutic molecules that the O'Brien laboratory is investigating to improve EPC function are the phosphoglycoprotein osteopontin (OPN) and an enzyme essential for neovascularization, endothelial nitric oxide synthase (eNOS). Professor O'Brien presented data generated from EPCs isolated from diabetic versus nondiabetic rabbits and demonstrated a possible link with EPC dysfunction and reduced OPN levels in these animals. One of the functions of OPN has been suggested to involve cell migration and survival, and it is thought to influence angiogenesis via integrin signaling because OPN contains an Arg-Gly-Asp (RGD) binding site and has high affinity for the integrin $\alpha_v\beta_3$ receptor. Investigating angiogenesis via a Matrigel tube formation assay, Professor O'Brien and colleagues have shown that EPCs from diabetic rabbits were unable to form tubes, and that adding OPN to the medium could rescue the tubule formation function of the EPCs. This conclusion was strengthened by demonstrating a role for OPN in EPC function, using a hind-limb ischemia model and the OPN knockout mouse presented in part in a poster by Erin Vaughan from the group.

Professor O'Brien also described eNOS as a potential therapeutic enhancer of EPC function. Impaired wound healing is a major problem in the diabetic clinic and the O'Brien laboratory has been investigating this problem using the diabetic rabbit ear ulcer model. They measured wound size 7 days after transplantation of EPCs with or without transduction with eNOS and showed that wound healing was enhanced in rabbits receiving EPCs transfected with and over-

expressing eNOS, confirming the idea that EPCs may be dysfunctional in the disease state and therefore have the potential for manipulation *ex vivo* to yield enhanced therapeutic capacity. A poster from his group, presented by Erin Vaughan, showed the role of OPN in angiogenesis.

Frank Barry (REMEDII, NUI) presented "Cell-Based Strategies in Tissue Repair," using three biological models of stem cell engraftment to investigate stem cell-host interactions, and discussed whether the major player was modification of the host or the transplanted stem cell phenotype. Professor Barry presented pioneering work from his laboratory, demonstrating reversal of the diseased articular cartilage in patients with osteoarthritis (OA). Professor Barry explained how multipotent adult mesenchymal stem cells (MSCs) isolated from bone can be used to differentiate into cells of the chondrogenic lineage, opening the potential to develop new therapies that would stimulate repair of damaged cartilage in OA. OA is a disease of gradual degeneration with loss of the cartilage that serves as the cushion between bones within a joint. Research from the Barry laboratory, including posters presented by Udo Greiser, Garry Duffy, Claire Kavanagh, and Catherine Malone, showed that the major benefit of this approach may be that the MSCs will act as trophic producers of bioactive factors to initiate endogenous regenerative activities in the OA joint. Current results show there is limited cell engraftment in the site of the transplanted cells, providing the impetus for further research into how the stem and host cells interact. Future work may also focus on the use of cell-seeded scaffolds in the damaged areas in order to release stimulating factors for repair.

The next model described by Professor Barry was that of a mouse myocardial infarct (MI), where stem cells were injected into the damaged left ventricular wall of the mouse heart. The group investigated how the stem cell phenotype may change in response to the injured environment and their work suggested that transplanted cells retained their stem cell characteristics, adding support for the conclusion that a trophic signaling network was at play in the repair response. Finally, a breast cancer xenograft mouse model was used, in which MSCs were labeled with fluorescent dye PKH26 and delivered systemically. It was found that the cells engrafted into breast tumor tissue, suggesting chemotactic activity of the tumor. Claire Kavanagh from the Barry group presented a poster in which they had used a chemiarray to identify active chemokines post-MI. This identified several proteins of interest including MCP-1. Migration assays with various concentrations of MCP-1 showed a dose-dependent migration of MSCs toward MCP-1. The data suggested that MSC-targeted homing could be associated with locally released cytokines, which are upregulated after an MI. These data may influence the design of clinical therapies responsible for homing MSCs to MI-damaged cardiac tissue. The group has used a chicken immune phage display to generate antibodies to human MSCs, based on the finding that chickens have a high immune response to human MSCs. Undoubtedly, these innovative molecular tools will be invaluable in future. Professor Barry finished with the take-home messages that (1) MSCs are associated with limited engraftment capacity; (2) the differentiation capacity of the transplanted MSCs is uncertain; however, they may contribute to tissue repair through chemokine effects without the ability and/or need to differentiate into the target tissue; and (3) they may also be metabolically flexible, showing resistance to apoptosis.

On a philosophical note, he added that there is a limited amount of information on the characteristics of MSCs in the human database, that the long-term effect of stem cell transplantation is unknown territory, and that the manufacturing technology for large-scale preparation of stem cells is not developed, leaving us with a lot of exciting data and challenges for the future.

Annette Enright (REMEDI, NUI) talked about the regulation and role of microRNAs (miRNAs) in human MSC behavior. miRNAs are 22-nucleotide-long endogenous non-coding regulatory RNAs that interfere with the translation of target genes by binding to complementary sequences on the 3' untranslated regions of their target miRNAs. They play important regulatory roles in stem cell differentiation. Ms. Enright described her work in generating an LV-mediated inducible knockdown of Droscha, a protein involved in synthesizing miRNAs. She used this system to show enhanced adipogenesis of MSCs among cells with reduced levels of Droscha and hence reduced synthesis of new miRNAs when compared with controls. She and her colleagues from the Barry and Howard laboratories went on to use MiChip and GeneSpring analysis of MSCs, isolated from four healthy human volunteers and induced along an adipogenic pathway, to establish that certain miRNAs were up- and downregulated during adipogenic differentiation of these cells. The roles of these modulated miRNAs in adipogenesis are now the focus of current work in the laboratory. Ronan Murphy (Dublin City University, Dublin, Ireland) presented a poster in which miRNA expression signatures were investigated by *in vivo* modeling of human endothelial cells exposed to various levels (physiological and pathological) of hemodynamic stimuli. They screened for and identified various mechano-responsive miRNAs, via VE-cadherin and integrin signaling, using the novel TaqMan low density array (TLDA) technology platform from Applied Biosystems (Foster City, CA). They discovered that various important mechano-responsive proteins within ECs, for example, the tight junction protein zonula occludens-1 (ZO-1), were not regulated at the transcriptional level but by the modulation of translation by hemodynamically sensitive miRNAs. Deciphering the miRNAs involved in vascular cell function has potential applications in vascular tissue engineering, may enable the design of novel therapies for vascular disease, and may prove to be accurate molecular diagnostic markers of vascular competence.

Jeffrey Schmeckpeper from Noel Caplice's laboratory in the Centre for Research in Vascular Biology (UCC) spoke of their work in designing LV vectors containing lineage-specific promoters to track the differentiation of BM progenitor cells *in vivo* and *in vivo*. VE-cadherin and smoothelin-B promoters were cloned into self-inactivating LV vectors to drive eGFP expression specifically in endothelial and smooth muscle cells, respectively. Using these vector systems, BM progenitor cell differentiation into endothelial or smooth muscle cells was monitored over time in an *in vivo* colony-forming assay and in an *in vivo* Matrigel angiogenesis model. These studies demonstrated the feasibility of LV vector-driven fluorescent tags as a valuable molecular tool for analysis and lineage tracking of BM progenitor cell differentiation.

Noel Caplice (Centre for Research in Vascular Biology, UCC) presented an overview of the field of cardiac stem cell

therapy, including his group's work, in a talk entitled "Repair Therapy for the Myocardium after Acute MI." The presentation began by detailing the origins of data supporting BM stem cells and cardiac regeneration, which demonstrated the ability of BM cells to repair MIs in mice, with 70% of infarcted ventricle being regenerated (Orlic *et al.*, 2001). However, 2 years later, it was indicated that findings were not as significant as first believed, with the level of integration at about 1%, again in mice (Murry *et al.*, 2004). After the publication of these murine studies, Professor Caplice's group undertook human studies involving chimeric patients who had undergone BM transplantation and identified BM-derived cardiomyocytes in the hearts of patients at autopsy at a frequency of only 0.02% (Deb *et al.*, 2003). However, in the same patient subsets, when vascular chimerism was examined, the level of BM-derived cell chimerism was 100-fold greater than for cardiomyocytes (Caplice *et al.*, 2003; Simper *et al.*, 2003). Professor Caplice explained that the high frequency of BM differentiation into vascular cells correlates with the embryonic differentiation pathways followed by these cells, and it was plausible that the cell types would retain similar genetic dispositions in adults, whereas BM hematopoietic cells would not be expected to differentiate to cardiomyocytes. Professor Caplice's studies address two mechanistic questions: (1) do cells traffic to and remain at the site of action after MI, and (2) do these cells secrete factors that have repair effects, with the possibility of using these factors in themselves as a therapy, thereby overcoming patient-specific immunological requirements? The Centre for Research in Vascular Biology uses a porcine acute MI model and radiolabeled EPC and positron emission tomography scanning to monitor trafficking. Data showed the rapid clearing of labeled autologous cells within 2 hr of balloon occlusion intracoronary administration. Retention of cells was dramatically improved by using continuous bolus injection (Doyle *et al.*, 2007). Given the data indicating that the therapeutic effects are unlikely to be mediated by the transdifferentiation of cells, the Caplice group showed the likely involvement of paracrine factors. These studies demonstrated a significant reduction in infarct size as a result of administration of cell-derived conditioned medium alone. *In vivo* hypertrophy assays with rat cardiomyocytes substantiated these findings. Professor Caplice also overviewed some core findings from clinical trials involving autologous BM cells. There are now approximately 25 such trials involving more than 1000 patients (Assmus *et al.*, 2002; Strauer *et al.*, 2002; Janssens *et al.*, 2006). It is becoming evident that the severity of the initial disease is paramount to therapeutic efficacy in these trials, in that patients with milder infarction do not demonstrate a therapeutic response. The use of new imaging technology such as cardiac magnetic resonance imaging in clinical trials has exposed the importance of microvascular obstruction in cardiac stem cell therapy, with 50% of patients displaying evidence of this phenomenon (Janssens *et al.*, 2006). Work in Professor Caplice's laboratory demonstrated that microvascular obstruction correlated with reduced cell access to the treatment zone and reduction in therapeutic effect in this porcine acute MI model.

Yvonne Alexander (University of Manchester, Manchester, UK) gave an insightful presentation on vascular calcification. Dr. Alexander discussed the problem of calcification in the clinic and its limited clinical management, providing

the impetus for the development of novel treatment strategies for intervention or treatment. The current thinking on calcification is that it is a dynamic remodeling event, sharing many similarities to osteogenesis. A number of laboratories, including Dr. Alexander's, are engaged in identifying the cells and molecules involved in this process. There is increasing evidence for the existence of vascular progenitor cells in the vessel wall with the capacity to upregulate bone-related proteins and thus differentiate into osteogenic cells. Transplantation studies have shown that marrow-derived stem cells enter the circulation and engraft to areas of tissue damage, although whether these cells act to release signaling molecules that trigger osteogenic differentiation remains to be determined. Cytokines are important in the mobilization and differentiation of progenitor cells, an example of which is hepatocyte growth factor (HGF). HGF is produced by osteoblasts and is a key player in bone metastasis. Dr. Alexander showed the occurrence of elevated levels of circulating HGF in the serum of patients with cardiovascular disease, as well as elevated localized HGF expression within calcified atherosclerotic lesions. Adenoviral technology was used to demonstrate that HGF has the ability to induce the osteoblastic differentiation of explanted smooth muscle cells, a finding that correlates with Notch3 activation, and upregulation of the notch target gene *HES-1*. In addition, treatment with AdNK4, an inhibitor of the HGF receptor c-Met, attenuates the progression of human smooth muscle cell mineralization, suggesting that the inhibition of HGF/c-Met signaling, or blockade of the downstream Notch3 pathway, may be a novel therapeutic target for the inhibition of vascular calcification in atherosclerosis. This hypothesis will be tested in future *in vivo* studies. Dr. Alexander suggested that a better understanding of the mechanisms underlying excess vascular mineralization could have important implications for the development of promising new therapies of this clinically important, multifaceted vasculopathy.

David Morrissey (Cork Cancer Research Centre [CCRC], Cork, Ireland) presented his work on "Variables Affecting Duration of Plasmid Transgene Transcription *in Vivo*." Dr. Morrissey spoke about the common use of promoters of viral origin in GT approaches, and presented data from the CCRC on silencing of these promoters by tumors *in vivo*. Luciferase reporter gene expression resulting from promoters of viral origin, cytomegalovirus (CMV) and simian virus 40 (SV40), was compared with that of the native mammalian ubiquitin B promoter both *in vitro* and *in vivo* within tumor, liver, and muscle when delivered by plasmids. Dr. Morrissey showed that a single administration of plasmid to muscle produced high-level expression for more than 1 year regardless of the promoter. In contrast, luciferase expression associated with viral promoters was shown to be silenced in tumor and liver tissue within 1 week of administration, unlike that of ubiquitin-mediated transcription. This silencing was also evident, but delayed, when the CMV luciferase cassette was integrated *in vivo*, mediated by a Sleeping Beauty transposon vector. When a Tet-inducible promoter cassette was examined, uninduced plasmids did not express on later addition of doxycycline 7 days postdelivery, indicating that nontranscribing DNA was silenced. This suggested that promoter DNA is responsible for the observed silencing, and ruled out the involvement of protein-mediated immune stimulation. Dr. Morrissey proposed that traditional "con-

stitutive" promoter-mediated expression is dependent on target tissue, and is an important variable to be addressed in achieving appropriate transgene expression for DNA therapeutic strategies, singling out the significance of silencing of viral promoters by tumor.

Delivering Gene Therapies

Dr. Matthew Wood (Department of Physiology, Anatomy, and Genetics, University of Oxford) described the use of nucleic acid therapies for neuromuscular diseases such as Duchenne Muscular Dystrophy (DMD) and spinocerebellar ataxia type 7. His group has focused on the use of nucleic acid-based gene silencing and gene modification, and nucleic acid-based delivery to the neuromuscular system. Dr. Wood spoke about his group's work on treatments for DMD, a degenerative disorder of the muscle. Workers have studied the potential of single-stranded antisense oligonucleotides for exon skipping and splice correction of mutations in the dystrophin gene. This resulted in restoration of dystrophin expression in the *mdx* mouse, with dystrophin-positive fibers still evident 8 and 12 weeks after a single intramuscular delivery. These data led to the formation of a UK consortium running clinical trials for muscular dystrophy, called the Muscular Dystrophy EXon-skipping (MDEX) consortium, to establish a phase 1 clinical trial for the use of antisense oligonucleotides for DMD in 2007.

Dr. Wood also outlined proof-of-principle studies carried out within his group for the treatment of spinocerebellar ataxia type 7, using mRNA silencing. Spinocerebellar ataxia type 7, a disorder leading to cerebellar and retinal degeneration, has been linked to a single nucleotide polymorphism, a G:U mismatch, and his group has developed a short hairpin RNA with a mismatch at position 16 that specifically silences the mutant *ataxin-7* gene. *In vivo* studies have shown that this short hairpin RNA restores the normal cellular phenotype with the elimination of the mutant protein aggregates and that wild-type protein was restored to wide cellular distribution. They have also demonstrated wild-type protein restoration in the retina *in vivo*.

Another aspect of Dr. Wood's work is in developing efficient and specific delivery mechanisms for these nucleic acid therapies. He outlined the developments that have taken place in the chemistries of conjugation to nucleic acids, focusing on advances with peptide nucleic acids (PNAs). PNAs have a number of advantages: they are nuclease and protease resistant, and they can be modified and conjugated easily. He outlined a number of proof-of-concept experiments carried out by his group, in which they have demonstrated that a single intramuscular injection of the antisense oligonucleotide-conjugated PNAs into 2-month-old dystrophin-deficient mutant (*mdx*) mice resulted in restoration of dystrophin expression and persistent expression at 20 weeks posttreatment. His group has also studied the use of arginine-rich cell-penetrating peptides such as R9F2, (RXR)₄, and derivatives of (RXR)₄ in combination with morpholino chemistry to ensure more efficient delivery. After *in vitro* and *in vivo* screens they have now identified a number of active candidates for intravenous administration-based, systemic delivery studies. Studies carried out with the peptide-conjugated antisense have shown systemic muscle and cardiac

correction in DMD after a single intravenous injection at a dose of 25 mg/kg in 2-month-old *mdx* mice. Striking amounts of correction were observed, with higher than normal levels of dystrophin produced in some tissues. To ensure tissue-specific delivery Dr. Wood discussed their work with a number of tissue-specific homing peptides, both known and novel. Dr. Wood also mentioned a novel formulation that facilitates antisense oligonucleotide uptake resulting in higher numbers of dystrophin-positive fibers.

Andrew Baker (BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK) discussed his group's studies on mechanisms that define liver gene transfer by adenovirus *in vitro* and *in vivo*. Despite the advantages of using adenovirus for GT strategies there still remains one main disadvantage: the fact that systemic administration of the vector results in high transduction of the liver because of native vector tropism. One of the main aims of his group has been to retarget adenoviral vectors for systemic delivery in order to prevent liver transduction. Previous studies have used small peptides or antibodies to retarget the adenovirus to specific cell types, such as endothelial cells. Although successes were reported *in vitro*, liver transduction was still not evident *in vivo*. Professor Baker's group has focused on the use of fibers other than Ad5, or on the use of different adenoviral serotypes. They have shown that pseudotyping the fibers from Ad16, which has poor hepatic tropism, resulted in transduction of human vascular endothelial and smooth muscle cells with efficiencies equal to or greater than that of non-modified Ad5. In addition, there was also less accumulation of the vector and less transgene expression detected in the liver. The next stage in their vector development was to target this adenovirus to the renal system, using peptides (HTT and HIT) identified by *in vivo* phage display (Denby *et al.*, 2007). Intravenous delivery of each peptide-modified virus resulted in selective renal targeting; however, the vast majority of the vector genomes were still detected in the liver. The work of others into Ad5 infectivity in the liver has identified a novel pathway for adenoviral infection. These researchers demonstrated that blood factors play a major role in targeting adenoviral vectors to hepatic cells, with coagulation FIX and complement component C4-binding protein implicated in adenoviral binding. The binding of these factors to the adenoviral fiber knob domain provides a bridge for virus uptake through cell surface heparan sulfate proteoglycans and low-density lipoprotein receptor-related protein. An adenoviral vector containing mutations in the fiber knob domain removed blood factor binding and demonstrated reduced transduction of liver cells *in vivo*.

The Baker group have performed studies on the role of coagulation factors on adenoviral infectivity. They have shown that FIX is not the main factor involved in adenoviral infectivity, as transduction of hFIX null mice with the mutated vector and wild-type Ad5 resulted in the same levels of gene transfer in the liver. Instead, their studies have demonstrated that other blood factors such as factor X (FX), protein C, and factor VII (FVII) enhance hepatocyte transduction *in vivo*. FX in particular demonstrated a good effect on infectivity, with high levels of gene transfer mediated by direct virus-factor binding. They have also shown that human FX substantially enhanced hepatocyte transduction by wild-type and mutated viruses in an *ex vivo* liver perfusion model and that systemic downregulation of these factors by warfarin signifi-

cantly reduced liver uptake of coxsackievirus-adenovirus receptor (CAR)-deleted adenoviruses *in vivo*. Adenoviral infectivity of hepatocytes could, however, be rescued by infusion of human FX alone. Further studies demonstrated that FX binds to the Ad5 hexon, and not the fiber as previously thought. Adenovirus has been demonstrated to bind the hexon via an interaction between the FX Gla domain and hypervariable regions of the hexon surface. Liver infection by the FX-Ad5 complex is mediated through a heparin-binding site in the FX serine protease domain. Intravenous delivery of Ad5 with hypervariable regions from Ad48, which does not bind FX, showed reduced uptake and expression in the liver in comparison with Ad5. FX binding *in vivo* can be blocked with an FX Gla domain-binding protein (X-bp) from the hundred-pace snake. Preinjection of this X-bp before adenoviral administration blocked liver gene transfer.

Leonard Seymour (president of the British Society for Gene Therapy [BSGT]; and Department of Clinical Pharmacology, University of Oxford) spoke about his group's efforts to overcome the issue of inadequate delivery in GT strategies, specifically in relation to adenoviral vectors (Ad5). Intravenous delivery of viral vectors is hampered by the harsh environment of the blood system. The group has demonstrated that adenoviral clearing from the blood stream is not entirely due to the presence of neutralizing antibodies but is also cell mediated. In an *ex vivo* model more than 90% of the Ad5 is bound to human but not murine erythrocytes. Further studies showed that in phosphate-buffered saline (PBS) this binding is mediated by the Ad5 fiber and can be displaced with free fiber but not with hexon. They also saw the same increase in adenoviral infectivity on some cells *in vivo* by FX as previously demonstrated by Professor Baker's group. But this effect could be significantly inhibited by human erythrocytes. His group observed that Ad5 also binds to human erythrocytes in plasma, subject to different conditions, but not in the presence of EDTA or when the plasma was heat-treated. This brought into question whether the binding was mediated by complement as both EDTA and heat treatment of the plasma inhibited complement.

In nonobese diabetic/severely compromised immunodeficient mice transduced with human washed red blood cells, a challenge with Ad5 resulted in Ad5 being bound entirely by the erythrocytes. The presence of these erythrocytes resulted in a 50% knockdown of tumor-targeted Ad5 entering tumor cells. Ad11 has proven better for targeting tumors in animals that have human erythrocytes.

The Seymour group has also studied the use of a polymer coat to protect the virus from unwanted interaction(s) in the blood. Vector tropism can be altered by attaching specific ligands to the surface of the polymer coat, for example, herceptin targeting shows good systemic circulation and kinetics. Another style of polymer is currently being studied as the initial polymers investigated did not offer complete protection from neutralizing antibodies. They have also introduced cleavable bonds onto the side chains of the polymer. This material can be retargeted effectively and is resistant to neutralization by antibodies. In terms of red blood cell binding, in PBS, polymer-coated adenovirus targeted with epidermal growth factor no longer binds to human red blood cells and is completely free in the plasma; the same is seen in neutralizing plasma. Professor Seymour also illustrated the importance of focusing on clinical translation and the use

of human samples as Ad5 binds strongly to human erythrocytes but not to mouse erythrocytes, making the use of murine models potentially misleading.

Cancer Gene Therapy

Noriyuki Kasahara stated that one of the main problems with applying GT in the clinic is the low efficiency of gene delivery to the majority of cells in solid tumors. Human clinical trials of glioblastoma show <1% and often <0.1% transfection efficiency despite prior debulking and tattooing of the resected cavity (Lang *et al.*, 2003). The group had showed that p53 staining was limited to around the needle track. They have now started using replication-competent virus for cancer therapeutics, so that each tumor cell becomes a source of more virus. The development of cancer therapy has included adenovirus, HSV, influenza virus, measles virus, Newcastle disease virus, parvovirus, polio virus, reovirus, vaccinia virus, and vesicular stomatitis virus (VSV), and all of these are being tested in current clinical trials demonstrating the return of oncolytic virotherapy, which exploits naturally cytolytic virus. Many large complex viruses are difficult to manipulate, and the basis for attenuations are not well understood. Many such viral strains are used in vaccinations and, due to the availability of U.S. Food and Drug Administration (FDA)-pertinent data, they were advanced to clinical trial quickly. However, such vaccine strains generated a significant immune response, leading to rapid clearing of the virus, and because of a lack of viral persistence there is a reoccurrence of the tumor.

Murine leukemia virus (MLV) is a stable integrating RV, a simple virus that is well understood. It fails to infect unless the cell is proliferating, providing a level of selectivity, and it is not intrinsically cytolytic. It behaves as a benign parasite, being less immunogenic than many of the other oncolytic viruses noted previously, making it a less destructive virus. It can be modified to carry prodrug-activating "suicide" genes, enabling a strategy involving seeding of tumor with MLV, allowing it to spread in daughter cells around the tumor before administering prodrug to induce cell death. ACE-GFP is a replication-competent retroviral (RCR) vector. It allows precise insertion at the *env*-U3 boundary (Logg *et al.*, 2001) and GFP shows the spread of this virus in a metastatic colon cancer model, a multifocal CT26 liver tumor, after locoregional delivery. Dr. Kasahara's data showed the clear demarcation of where the virus was expressed, in tumor tissue but not normal cells, by staining and polymerase chain reaction (PCR). RCR vectors can also be used to deliver intracellular chemotherapy using yeast cytosine. Applications to various cancer types can be made and have included glioblastoma, prostate cancer, and brain cancer. When the tumor develops, virus is applied and then a single cycle of prodrug (5-fluorocytosine [5-FC]). Again there is a sharp demarcation between the absence of expression in non-dividing normal brain parenchyma and expression in tumor cells. Multiple cycles of prodrug improve survival and the data suggest that persistence of RCR-CD (CD, cytosine deaminase) in metastatic intracranial glioma cells does occur (Tai *et al.*, 2005). It should also be noted that 5-FU acts only on actively dividing cells, making this even more tumor cell specific. The persistence of RCR neoantigen in metastatic glioma cells offers a unique opportunity for immunotherapy.

It may be possible to target viral antigens that are persistently expressed in metastatic tissue through the use of anti-MLV antibodies. Persistent expression of viral proteins may act as a stimulus, leading to a response against a tumor neoantigen target. ACE-hIL-2 (hIL-2, human interleukin-2) leads to the complete eradication of tumor mass compared with controls, whereas with ACE-hIL-2 but after pretreatment with the anti-retroviral agent azidothymidine (AZT) which suppresses replication of the virus, the tumor grows and quickly, catches up with the size of the tumor in the untreated control group, demonstrating that replication of the virus is imperative for this treatment to work. The FDA stipulates that to progress to pre-IND (investigational new drug application) status, the Kasahara group does not need to perform large animal studies but that the group needs to repeat these experiments with clinical-grade vectors, therapeutic efficiency, and perform biodistribution studies. So the clinical trial will include 3 × 3 dose escalation studies. There is an international consortium for the development of RCR vectors, including groups in California, Minnesota, New York, London, Denmark, Vienna, and Paris. We are all aware of the differences between mice and men, making mice a poor model for GT treatments, and so the Kasahara group is moving toward heterotypic models to better mimic the true human disease. They are putting primary human brain tumor samples into immunodeficient mice in collaboration with Dr. Mitch Burger's group, an effort that will be described at future meetings.

Stephen Russell (Mayo Clinic, Rochester, MN) described a novel approach to target the replication cycle of an oncolytic virus, by modifying its genomic sequence to make it vulnerable to cellular microRNAs. Coxsackievirus A21 (CVA21) is a potent oncolytic agent, but also causes fatal myositis. When miRNA target sequences corresponding to muscle-specific miRNAs were engineered into its genome, the virus retained its oncolytic activity in mouse models of melanoma and multiple myeloma, but no longer killed the mice. Dr. Russell suggested that this novel targeting approach might be applicable to control unwanted toxicities and off-target tropisms of other oncolytic viruses.

Gerald O'Sullivan (CCRC, UCC) presented aspects of his group's work with nonviral GT of solid cancers and developments for application to foregut cancer. Minimal residual disease (MRD) is the undetectable residual cancer that remains after complete ablation of locoregional disease by surgery. MRD exists as individual cells or multicellular aggregates of less than 2 mm that lack an autonomous blood supply, and are normally resistant to chemotherapy. It has been shown by others that metastases start early in disease and metastatic niches in distal tissues precede malignant cells. In a clinical study by Prof. O'Sullivan's group, micrometastases were examined in 50 consecutive patients selected for cure with respective surgery (Murphy *et al.*, 2000). Frequent micrometastases were found in the BM and 90% of these patients had MRD after surgery, independent of nodal status, tumor size, or spread. This study demonstrated that the degree of immune reaction to the primary tumor correlates with survival of patients, because of immune targeting of micrometastases sharing the same antigen spectrum as the primary tumor cells. This led to the hypothesis that immune therapy of the primary tumor might establish a systemic immune response to effectively control MRD.

One of the treatment modalities being examined involves *in vivo* electroporation of plasmids or drugs. In electrochemotherapy, the tumor mass is injected with chemotherapeutic drug (bleomycin) followed by electroporation. In murine models complete ablation of various tumor types was achieved by this treatment. Electrochemotherapy has been used successfully by this group in clinical trials, with 85% treated subcutaneous metastatic tumor nodules (previously recalcitrant to multimodal treatment) regressing in patients (Larkin *et al.*, 2007). Professor O'Sullivan displayed results on the use of the same electroporation technology to deliver plasmid-based GT into solid tumors of mice. When murine fibrosarcomas growing in BALB/c mice were administered, by electroporation, a plasmid encoding granulocyte-macrophage colony-stimulating factor (GM-CSF) and the B7-1 costimulatory immune molecule, complete tumor regression occurred in more than 60% of treated animals (Collins *et al.*, 2006). This response was systemic, durable, and tumor specific, with all responding animals resistant to repeat tumor challenge. Using a liver metastatic model, the development of metastases was prevented after treatment of the primary subcutaneous tumor. Thus immunogene therapy of the primary tumor was also effective against systemic disease at a cellular or pretumor phase. Survival was increased to 100% when treatment was applied as a neoadjuvant to surgical removal of the primary tumor 4 days after gene delivery (including nonresponding primary tumors), and indicated that elimination or control of the primary tumor by immunogene therapy was not a prerequisite for containment of systemic disease (Cashman *et al.*, 2008). The CCRC has developed endoscopic electroporation technology to target internal gastrointestinal tumors. Immunogene therapy alone proved ineffective in larger tumors, which may be due to increased local Tregs at the time of treatment, hypothesized Professor O'Sullivan, showing data demonstrating correlation between tumor size and Treg infiltration.

Maria Whelan from the O'Sullivan group described, in a poster presentation, their work on Treg depletion to induce effective antitumor responses. BALB/c mice with subcutaneously injected JBS (fibrosarcoma), C26 (colon cancer) or 4T1 (metastasizing mammary) tumors were depleted of Tregs systemically via intraperitoneal administration of anti-CD25 antibody at the time of or after tumor induction. The group showed that CD25 depletion at the time of JBS tumor induction led to 100% tumor rejection, whereas the growth of both C26 and 4T1 tumors was significantly retarded with an overall increase in survival. Systemic Treg depletion of fibrosarcoma-bearing mice significantly improved the efficacy of *in situ* electroporation GT with B7-1 and GM-CSF when administered in combination, increasing survival from 60% (immunogene therapy alone) to 90% (combined Treg depletion and immunogene therapy). In summary, Professor O'Sullivan stated that effective targeting or elimination of tumors and micrometastases required an optimal effector cell-to-target ratio that is achievable by a combination of local immunogene therapy, Treg depletion, and inhibition of tumor cell metabolism or mitosis (chemotherapy or biotherapy).

This group has also examined electroporation as a physical method of gene delivery in other contexts. A poster was presented by Timothy Doody (CCRC), describing his research investigating whether compacting DNA could improve DNA delivery in electrogene transfer (Walsh *et al.*,

2006). They found that transfection with both PEGylated poly-L-lysine and modified cyclodextrin could significantly improve transfection rates in combination with electroporation, with no significant change in cell viability.

Farzin Farzaneh (president of the ISCGT; and King's College London, UK) described the genetically modified autologous cell vaccine they have been developing for relapsed acute myeloid leukemia (AML) (Chan *et al.*, 2006). In 1992 Schwartz described the need for professional antigen-presenting cells (APCs) to have CD28, which is also known as CD80, on their surface to stimulate T cells. Once stimulated in the presence of CD28, the APCs could destroy target cells without the need for CD28. AML blasts express both MHC class I and II; they express a range of tumor antigens such as WT1, PRAME, and G250; and they share a common lineage with APCs. They express many markers that are present on DCs, but not CD80. B7-2, also known as CD86, is expressed on 80% of AML patient samples, so by adding back CD80 they will look like professional APCs. Leukemogenicity was examined with the mouse 32p210 leukemia cell line, which was modified to express B7-1 and/or IL-2. Modification of the cell line with either improved survival, but modification with both led to 100% survival rates. In a therapy model, mice were inoculated intravenously with 10^5 cells and 1 week later were given three weekly subcutaneous vaccinations with 10^6 irradiated cells that expressed B7-1 and IL-2. This led to the eradication of a previously initiated leukemia and 80% of mice became leukemia free. The group has made efforts to titer and concentrate LV (Chan *et al.*, 2005). To concentrate RVs they biotinylated RVs, using avidin paramagnetic particles. A bridge was used to target RV vectors, and this was achieved through the direct chemical modification of the cell surface with biotin. A biotin acceptor peptide (LNGFR) was used to facilitate concentration of the vector by 4200-fold while reducing the volume 125-fold. Of note, recovery was more than 100%. The infectivity of the virus was increased, probably because of the removal of inhibitory factors. This has also been done with LV vectors, amphotrophic vectors, and vesicular stomatitis virus glycoprotein G (VSV-G) protein (Nesbeth *et al.*, 2006). The group has now been able to achieve the efficient transduction of primary AML blasts. At a multiplicity of infection (MOI) of 1 more than 40% of blasts were transduced and 43 ng of p24 per 10^6 cells per 24 hr was achieved. At an MOI of 5 a total of 200 ng of p24 was produced, and greater than 95% transduction efficiency was achieved. Using CBA beads they were able to show the profile of cytokine secretion by autologous *in vitro*-stimulated T cells, indicating that the T cells have a helper T cell type 1 phenotype. There was an increase in the number of functionally competent T cell generated in *in vitro* culture when incubated with AML cells that expressed B7-1 and IL-2. The Farzaneh group then investigated autologous CTL activity and showed that the target cells (AML blasts) could be lysed by autologous T cells that had been stimulated with AML blasts transduced with B7-1/IL-2 and that there was a specificity to the recognition. The group showed they were also expanding and activating natural killer cells in coculture. There was a substantially larger number of CD56⁺CD3⁻ cells in cultures where AML blasts transduced with B7-1 and IL-2 were used, and this was less notable if the blasts were transduced with either B7-1 or IL-2. The group is aware of the concern regarding the expansion of

Tregs in cocultures, particularly because of the IL-2. By examining the frequency of CD4⁺CD25^{high}FoxP3⁺CD27⁺ cells they could show an increase in the numbers of Tregs in the *in vitro* cultures compared with unstimulated T cells; however, the increase in Treg numbers was not significant. Clinical trials are now being initiated and patient recruitment has begun.

Barbara Guinn (King's College London) described her work identifying tumor antigens as targets for the immunotherapy of AML. Cancer-testis (CT) antigens have tissue-restricted expression; they are expressed in normal tissues that lack MHC class I expression, referred to as immunologically protected sites, such as the testes or placenta, while also being expressed in cancer cells (Scanlan *et al.*, 2004). To identify CT antigens that may act as targets for the immunotherapy of AML the Guinn laboratory investigated the expression of 10 known CT antigens (*MAGE-A1*, *-A3*, *-A6*, and *-A12*; *BAGE*; *GAGE*; *HAGE*; *LAGE-1*; *NY-ESO-1*; and *RAGE-1*) in 26 presentation AML patients and 20 normal donor samples (Adams *et al.*, 2002). They found that 23% of AML patient samples expressed *HAGE*, and the frequency of expression of any of the other antigens (*MAGE-A1*, *-A3*, *-A6*, or *-A12*, or *NY-ESO-1*) was 8% or less. The group used cDNA microarrays to investigate the expression patterns of 82 known CT and tumor-associated antigens in 124 presentation AML patients and 8 normal donor mobilized peripheral blood stem cell samples (Guinn *et al.*, 2005a). Eleven antigens were found to have present calls in patient samples but not normal donors. Of these, nine were known CT antigens (*RAGE-1*, *MGEA6*, *SYCP1*, *SAGE*, *GAGED2*, *GAGE5*, *MAGE-C1*, *GAGE3/5*, and *CTP11*), with the highest frequency of expression for *RAGE-1* (21%) and *MGEA6* (12%). Analysis of an extended cohort of 195 presentation AML patients showed that the frequency of antigen expression remained consistent (Guinn *et al.*, 2006). In addition, this extended group allowed the demonstration of an increased frequency of present calls in patients with the less differentiated forms of AML (M0/M1/M2) as compared with patients in the lineage-restricted or recurrent translocations groups (M4–M7). *MGEA6* was also found to have an increased frequency of expression in AML patients with cytogenetic abnormalities associated with poor versus standard versus good prognosis. To identify novel CT antigens that may be expressed in presentation AML the Guinn group used the SEREX technique to immunoscreen a commercially available normal donor testis library with sera from five newly diagnosed AML patients (M4 and M5 subtype) with no cytogenetic abnormalities (Guinn *et al.*, 2005b). They identified 44 independent antigens recognized by presentation AML patient sera, which we immunoscreened with sera from a further 16 AML, 19 chronic myeloid leukemia, and 22 normal donors. They found eight antigens that were recognized solely by patient sera and eight that were preferentially recognized by patient sera compared with normal donor sera. Three antigens (*GRINL1A*, *PASD1*, and *SSX2IP*) were found to be expressed in presentation AML patient samples but not normal donor hematopoietic samples (including CD34⁺ cells) by RT-PCR and in the case of *PASD1*, the sole CT antigen identified by this SEREX study, RT-PCR data were confirmed by real-time PCR. The group is now examining the capacity of epitopes within *PASD1* to induce T cell responses in normal donors and AML patients.

Frank Roche (Trinity College Dublin, Ireland) talked about his group's work with a Semliki Forest virus (SFV)-like particle system. This is a nonreplicating expression system that leads to high-level, transient expression of the encoded transgene. The project is focused on the treatment of glioblastoma multiforme. There are three cytokines, IL-12, interferon (IFN)- γ , and IFN- β , being examined in this project. All cytokines are involved in immune stimulation and lead to the downstream inhibition of angiogenesis. SFV is considered level 2 in Europe but is level 3 in the United States, which makes it difficult to work with in the United States according to Stephen Russell. This was due to a death that was reported from SFV in Germany in the 1970s; now the notes are missing regarding the incident and the facts of what happened are in dispute.

Sarfraz Ahmad (CCRC, UCC) discussed his work on prostate cancer. Prostate cancer is the most common noncutaneous male malignancy in the Western world. According to the Irish National Cancer Registry approximately 1900 new cases are diagnosed every year and there is expected to be a 275% increase in prostate cancer patients between 2000 and 2020. Prostate cancer is associated with various antigens, including prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA). These antigens are largely restricted to the prostatic epithelial cell and are strongly upregulated in prostate cancer. DNA vaccinations can induce effective antitumor immune responses. A DNA vaccine given by the intramuscular route has the potential to activate both humoral and cellular immunity. The TRAMPC1 cell line is derived from transgenic adenocarcinoma mouse prostate model. These transgenic mice develop both local and metastatic prostate cancer that bear resemblance to human prostate cancer. Ahmad's group used intramuscular administration of plasmid (expressing one of the prostate cancer-associated antigens) with electroporation to transfect muscle. Successful muscle transfection was demonstrated by reporter gene and also by RT-PCR, showing muscle transfection with the gene of interest. Four doses (50 μ g of DNA vaccine per dose) were given at weekly intervals. One week after the last vaccination each mouse was challenged subcutaneously with TRAMPC1 (5×10^6 cells per mouse). Four of 11 mice were tumor free after intramuscular vaccination with mPSCA compared with mice treated with empty vector or untreated mice (no survivors). Tumor-bearing mice in the mPSCA vaccination group had a statistically significant lower tumor burden. The vaccinated animals also lived significantly longer. Adoptive transfer of the lymphocytes was shown by the Winn assay. Significantly higher levels of IFN- γ were produced by the immunized mice compared with control groups. The study also showed long-term protection against tumor growth by this immunization technique. In tumor rechallenge experiments, using four doses of vaccine followed by rechallenge of same tumorigenic dose of TRAMPC1 (5×10^6), only 16% of mice receiving mPSCA vaccines developed tumors, compared with 100% of untreated mice. In addition, mice were injected with a metastatic melanoma cell line, B16F10, which had been stably transfected with mPSCA. When the mice were culled, metastases were observed in the lung. Mice treated with the mPSCA vaccines had few to no metastases compared with untreated mice. The group also reported that the effectiveness of the DNA

vaccine could be enhanced by coadministration of synthetic CpG oligodeoxynucleotides with vaccine. Synthetic CpG oligodeoxynucleotides have immunological effects similar to those seen with bacterial DNA and represent a promising vaccine adjuvant. The suppression of *in vivo* growth of tumor was augmented by coadministration of the CpG oligodeoxynucleotides. Dr. Ahmad summarized that immune activation could be achieved against prostate cancer-associated antigens. This vaccination strategy could be effective in providing tumor protection and indicates that DNA vaccination has the potential to be used as a neoadjuvant or adjuvant therapy in clinical trials.

Targeted killing of autoreactive CD4⁺ effector cells reveals their importance for the induction and maintenance of acute autoimmune central nervous system disease as described by Mikhail Nosov (REMEDI, NUI and Max Planck Institute of Neurobiology, Martinsried, Germany). Dr. Nosov has examined the efficiency of the suicide system red blood cell-thymidine kinase (TK) system to target antigen-experienced GFP-producing CD4⁺ effector T cells in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. Myelin basic protein (MBP)-specific T cells were retrovirally transduced to express HSV-TK and eGFP, to track and specifically target autoaggressive effector T cells during adoptive transfer of EAE in rats. Subsequent treatment with the prodrug ganciclovir (GCV) induced cell death specifically in HSV-TK⁺ cells *in vitro* by apoptosis 24 hr after GCV treatment (with up to 80% cell killing). A similar efficiency regarding cell killing was observed in the *in vivo* setting. Sequential administrations of GCV led to a gradual decrease in numbers of the autoaggressive effector T cells in peripheral immune organs and the central nervous system. However, although elimination of effector T cells before the onset of clinical disease significantly ameliorated EAE, GCV treatment after the onset of clinical symptoms did not significantly shorten disease duration or reduce disease severity.

Kornelia Kis (REMEDI, NUI) presented her work on Tregs in the context of transplant rejection. In a presentation entitled "IL-2 Does Not Increase TGF- β -Dependent FoxP3 Expression in Alloantigen-Specific Rat Lymphocytes," Dr. Kis explained that it has been shown that exogenously added IL-2 increases transforming growth factor (TGF)- β -dependent FoxP3 expression after polyclonal stimulation of mouse CD4⁺ lymphocytes. The additive effect of retinoic acid and TGF- β increasing FoxP3 expression on TCR-transgenic CD4⁺ T cells has been also described; however, it is not clear whether IL-2 and retinoic acid treatment facilitate the generation of alloantigen-specific Tregs as well. To generate the highest proportion of alloantigen-specific Tregs, Dr. Kis investigated the effect of IL-2 on FoxP3 expression of rat T cells treated with TGF- β and/or retinoic acid during alloantigen-specific activation. Dr. Kis confirmed that retinoic acid or TGF- β alone could not mediate this effect but that, together with retinoic acid, they increased FoxP3 expression in treated compared with control cells. Addition of IL-2 slightly, but not significantly, decreased FoxP3 expression in control, retinoic acid- and TGF- β -treated cells. These data indicate that IL-2 has a different effect on TGF- β -dependent Treg formation in the case of allogeneic stimulation when compared with polyclonal stimulation and indicates a complex role of IL-2 in Treg development. The alloantigen-specific TGF- β

and TGF- β - plus retinoic acid-treated cells used in her work, showed a regulatory phenotype in an *in vitro* suppression assay. These results suggest that alloantigen-specific Tregs generated *ex vivo* can inhibit allogeneic activation of T cells and might be promising tools for the inhibition of transplant rejection.

Moving Science into Business

Kevin Scanlon (CEO, International BioScience, Altadena, CA) defined the complex process of transforming novel concepts into medical products. He explained the commitment that has been made by governments worldwide, including Ireland, to commercialize the technology from universities, and in particular biotechnology. By detailing the established route for development and commercialization of medical products, and providing a historical perspective on examples of successful biotechnology strategies, Dr. Scanlon clearly demonstrated the need for better synergy between science and business. He explained the German government's promotion of the petrochemical industry in the 1880s, which he said succeeded because of the strong interrelationship between government, academia, and business, while explaining the importance of the culture of scientists, business people, and risk taking in the evolution of the biotech industry in California in the 1980s, citing the creation of genetic insulin as an example. He noted that academics today are not particularly entrepreneurial and that there are two fundamental commercialization problems facing academics and the business communities: (1) the ability of the academic community to change the culture of the scientists to commercialize their technology and (2) the ability of the business community to communicate successfully with the scientists. Universities across the world are now realizing the importance of the technologies being produced and the value that these can add to the community, and so a concerted effort is being made to commercialize this, through the university technology transfer office. The need to improve the success rates for more biotechnology products (currently less than 10%) was made clear, and the main reasons for failure were outlined. The academic-entrepreneur must become more knowledgeable about the commercialization of ideas. The design of a medical product coming out of an academic environment will be dependent on mentoring, funding, the involvement of a business incubator, and the investment community. At the later stages of development, support for selling to the customer and expanding the market will require the help of the pharmaceutical industry. The "funding gap" between research grant and pharmaceutical company requires the building of business communities, including Angel Investor groups, that can work with universities to build products and bring them to market. Dr. Scanlon described gene and cell therapy positively as having the potential to be a disruptive technology, changing the way patients are treated. However, the primary limitation of GT in clinical trials has been the inability to achieve a sufficiently high dose in the target tissue to have a therapeutic impact. Delivery, selectivity, and lack of toxicity are therefore the prime issues to be overcome. Dr. Scanlon finished his presentation by noting some of Ireland's advantages with respect to research commercialization: for example, European Union membership, a tertiary level-educated population, an

English-speaking, opportunistic culture, and the global economy are all in Ireland and Britain's favor.

Summary

This meeting, because of the novel conjunction of three societies in the planning committee, provided a rare opportunity to include a range of topics and speakers. The ISGCT, BSGT, and ISCGT seamlessly brought together a range of talks that were heard over the 2-day meeting and spanned topics from basic science through to marketable clinical medicines. The current problems faced by GT were highlighted as getting the transgene to the target tissue, infecting a significant portion of the target cells, overcoming the restraints of an effective immune system and the need to stimulate a damaged antitumor immune response. Assistance from the Computer Resources for Research group in UCC provided a novel opportunity for talks to be filmed and published on the ISGCT web site (www.isgct.ie), where all abstracts are also available. The next ISGCT meeting will be in Dublin in 2009 (www.isgct.ie), details of the next BSGT meeting can be found at www.bsgt.org, and the next ISCGT meeting will be held in conjunction with the Fifth Chinese Conference on Oncology (CCO) in Shijiazhuang, People's Republic of China (www.iscgt2008.com).

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