Cancer Gene Therapy (2013) 20, 141–149

www.nature.com/cgt

REVIEW

The innovative evolution of cancer gene and cellular therapies

P Lam[1], G Khan[2], R Stripecke[3], KM Hui[1,4,5], N Kasahara[6], K-W Peng[7] and B-A Guinn[2,8,9]

We provide an overview of the latest developments in cancer gene therapy—from the bench to early-stage clinical trials. We describe the most recent work of worldwide teams including experienced scientists and clinicians, reflecting the recent emergence of gene therapy from the 'Valley of Death'. The treatment efficacy of clinical gene therapy has now been shown in a number of diseases including cancer and we are observing a renewed interest by big pharmaceutical and biotechnology companies most obviously demonstrated by Amgen's acquisition of Biovex for up to USD$1 billion. There is an opportunity to be cautiously hopeful regarding the future of gene therapy in the clinic and we review here some of the most recent progress in the field.

Cancer Gene Therapy (2013) **20,** 141–149; doi:10.1038/cgt.2012.93; published online 1 February 2013

Keywords: stem cells; immunotherapy; cancer research

INTRODUCTION

This review is based on presentations given at the Annual Meeting of the International Society for the Cell and Gene Therapy of Cancer, which was held from 4 to 6th October 2012 in Singapore. It provided an opportunity for a small group of clinicians and scientists, all passionate about cancer gene therapy, to meet and exchange their most recent data. Even in the short time since our last meeting in 2009,[1] a lot has changed, reflecting a recent definition of perseverance, 'the courage to ignore the obvious wisdom of turning back' (www.despair.com). There was a significant increase in the number of studies that have now transitioned into Phase I/II clinical trials. A number of talks focused on improved gene therapy practices, as we learn from patient data how to improve vectors, their targeting and their administration. The cutting edge identification of patients who will respond to treatment and the utilization of personalized therapies have enabled researchers to focus time and money on those patients for whom an appropriate treatment is still lacking. Our small but international society continues to evolve, collaborate and achieve. Some of the highlights are described here.

VIRAL-BASED THERAPIES

Adenoviral studies

The synergistic effects of adenovirus (Ad)-p53 infection when used in conjunction with platinum chemotherapy were described by Robert Sobol (p53, Houston, TX, USA). These effects were further enhanced through the use of combination radiotherapy, chemotherapy and Ad-p53 treatment in mouse models. The treatment was shown to be effective even against cells that were refractory to chemotherapy. Mda7 is equivalent to interleukin (IL)-24, a secretory protein. Intratumoral injection is an effective mode of transfer. There was demonstration of broad expression, reflecting good distribution following a single intratumoral injection. Ad-p53 efficacy has been evidenced in a range of tumors including Li–Fraumeni syndrome.[2] P53 activates p21, which induces a cascade of caspases including caspase 3, which leads to apoptosis. After treatment with Ad-p53, there is an induction of both p21 and caspase 3.[3] Complete remission (CR) rates were 19% with radiation alone; however, these increased to 64% when a combination of Gendicine and radiation were given to hepatocellular carcinoma (HCC) patients. Transarterial chemoembolization plus Ad-p53 led to 6-month survival rates versus 4 months when chemotherapy (transarterial chemoembolization) alone was used.

Masatoshi Tagawa (Chiba Cancer Center Research Institute, Chiba, Japan) reported about their work on mesothelioma. More than 80% of mesothelioma patients have been exposed to asbestos and this form of lung cancer has an average latent period of 38 years. There is no useful diagnostic marker for mesothelioma, although loss of functions of a BRCA1 family gene have been recently identified in US family cases. Asbestos has not yet been banned in Asia and the consumption in emerging countries is increasing, with China and India being leading asbestos users. Asbestos imported into the emerging countries is less toxic, but there still exists a potential risk to individuals. The mean survival period after the diagnosis is 8.2 months and there is no preventative measure, which can be taken after asbestos exposure. The current first-line chemotherapy is a combination of cisplatin and premetrexed, but the mean survival period with the treatment is only 12 months. Mesothelioma lacks expressions of the $p14^{ARF}$ and the $p16^{INK4A}$ genes at the INK4A/ARF region in chromosome 9p21, and this is caused by a deletion or methylation of the region. The loss of expression leads to deficient p53 functions and phosphorylated retinoblastoma hyperphosphorylation, but the activities of both tumor suppressors can be restored by re-expressed p53. The group of Masatoshi Tagawa has shown that the treatment of patients with mesothelioma with Ad-p53

[1]Division of Cellular and Molecular Research, National Cancer Centre, Singapore, Singapore; [2]Department of Life Sciences, University of Bedfordshire, Luton, UK; [3]Lymphatic Cell Therapy Program, Excellence Cluster for Regenerative Biology (REBIRTH), Hannover Medical School, Hannover, Germany; [4]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; [5]Program in Cancer and Stem Cell Biology, Duke-NUS Graduate Medicinal School, Singapore, Singapore; [6]Department of Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA; [7]Department of Molecular Medicine, Mayo Clinic, Rochester, MN, USA; [8]Cancer Sciences Unit, Somers Cancer Sciences Building, Southampton University Hospitals, University of Southampton, Southampton, UK and [9]Department of Haematological Medicine, King's College London School of Medicine, London, UK. Correspondence: Dr BA Guinn, Department of Life Sciences, University of Bedfordshire, Park Square, Room B303, Luton LU1 3JU, UK.
E-mail: barbara.guinn@beds.ac.uk
Received 3 November 2012; revised 3 December 2012; accepted 10 December 2012; published online 1 February 2013

induced not only a cleavage of caspases 3, 8 and 9, leading to apoptosis, but also dephosphorylated phosphorylated retinoblastoma through induced p21, inhibiting cell cycle progression. Combination of Ad-p53 and the first-line chemotherapy produced synergistic effects in an orthotopic animal model. Ad defective of E1B-55 kDa (ONYX-015) have similar effects, except that it induced hyperploidy with an enlarged nucleus. Neutlin-3a, used to inhibit the p53–MDM2 interaction, produced cytotoxicity greater to *wild-type p53*-bearing mesothelioma than to those with the mutation, demonstrating that upregulation of p53 was important for mesothelioma treatment. Zoledronic acid (ZOL), a third generation of biophosphonates, upregulated p53 expression levels and was cytotoxic to mesothelioma. The ZOL-mediated apoptosis was, however, independent of p53 activations. Nevertheless, combination of ZOL and cisplatin achieved synergistic antitumor effects. A Phase I clinical trial with ZOL for mesothelioma is now being initiated in collaboration with Chiba University.

The group of Albert Deisseroth, who is now based at the US Food and Drug Administration (FDA), has developed a vaccine that is based on the attachment of the tumor-associated antigen (TAA) to the extracellular domain (ecd) of CD40L, a potent immunostimulatory signal. The attachment of the TAA to the ecdCD40L promotes uptake of the TAA into dendritic cells (DCs), the activation of DCs, major histocompatability (MHC) class I presentation of the associated TAA on DCs and expansion of antigen-specific B and T cells. The Deisseroth group has shown that the TAA/ecdCD40L vaccine strategy overcomes the anergy, which exists to Mucin-1 (MUC-1) and Her2Neu in MUC-1 and Her2Neu transgenic mice. Original studies used a first-generation non-replicating Ad-sig-TAA/ecdCD40L vector and showed increased presentation of TAA on MHC class I of DCs. The treatment induced a memory response that persisted for >1 year[4] and overcame the defective immune response that is seen in subjects of advanced chronological age due to decreased levels of CD40L on CD4 T helper cells, which is necessary for responsiveness to vaccination in both humans and mice. Vaccines can consist of a DNA or viral expression vector encoding the TAA/ecdCD40L protein, the TAA/ecdCD40L protein or a mix. Vaccination with the Ad-sig-TAA/ecdCD40L vector followed by two boosting vaccinations with the TAA/ecdCD40L protein enhances the immune response for TAAs as compared with what can be generated by the Ad-sig-TAA/ecdCD40L vector alone in the 18-month-'old' mice. Regulatory T cells decrease as a secondary consequence (perhaps) following multiple protein boosts.

MUC-1, which was chosen as a target (TAA) for the vaccine, is overexpressed in 90% of epithelial cancers and is a predictor of adverse prognosis. In normal tissues, MUC-1 is heavily glycosylated and immunologically silent. In cancer, MUC-1 is hypomethylated, which leads to the exposure of amino-acid epitopes that are not exposed on MUC-1 in normal epithelial cells. One vector injection followed by two protein boosts (VPP) is the preferred schedule. A Phase I trial, which is designed to study the efficacy and toxicity of the Ad-sig-MUC-1/ecdCD40L vaccine, is now being undertaken at the National Cancer Center (Singapore, Singapore) led by Han Chong Toh. The trial involves the administration of Ad-sig-MUC-1/ecdCD40L, and 12 patients will be given vector alone, in four cohorts of 1×10^9, 1×10^{10}, 5×10^{10} and 1×10^{11} viral particles (VP) per dose. Patients will have relapsed breast, lung, prostate, ovary and colon cancer. Their clinical end points, which are related to tumor response and immunological response, will be measured in the peripheral blood mononuclear cells collected before and after vaccination by John Connolly of the Immunology Network in Singapore.

Ad-p53 has excellent safety profile in the approximately 600 patients tested.[3] Ad-Mda7 works well with chemotherapy agents.[5] Robert Sobol (p53) described the potential of VirRx 007, which enhances oncolytic activity by increasing the expression of the adenoviral death protein. Effective at low doses now to build up to the dose level usually given approximately 10^{10} viral particle units (vpu).

The development of personalized ovarian cancer therapy has benefitted from the pioneering use of transcriptionally and/or transductionally targeted Ad5 vectors by David Curiel's group based at Washington University School of Medicine (St Louis, MO, USA). These conditionally replicating adenoviral vectors are being used for personalized therapies. In addition, the group is exploiting the increasingly affordable methods of microarray analysis and next-generation sequencing, to aid the identification of an optimal tumor-specific promoter for each tumor and plans to build a customized transcriptionally targeted Ad5 vector for each patient. As with all personalized therapies, David Curiel acknowledged that this approach, although desirable, might be expensive.

The use of PEGylated Ad[6] provides an opportunity to increase plasma retention and reduce immunogenicity. However, PEGylation has been shown to decrease the accessibility of virus particles to target cells, which has been overcome to some extent by Chae-Ok Yun's group, based at Hanyang University (Seoul, Korea), through the conjugation of PEGylated Ad to herceptin to treat Her2/neu-positive cells *in vitro* and *in vivo*. The treatment resulted in higher plasma retention along with lower neutralizing antibody (Ab) and IL-6 production than naked Ad alone. Further modification produced a Her2/neu-targeted, PEGylated oncolytic Ad called DWP418-PEG-HER. This modified Ad virus specifically killed Her2/neu-positive cells and performed better than naked Ad *in vivo*. Immunohistochemical staining confirmed accumulation of Ad E1A in tumors, suggesting that DWP418-PEG-HER could be used to treat metastatic cancer in the future.

Herpes simplex and measles virus

Cell cycle-dependent regulation of genes in viral vectors has been achieved through the transcriptional targeting of phylogenetically conserved sequences by Paula Lam's group at the National Cancer Center. The transcriptional targeting of suicide gene therapy has been demonstrated in a patient-derived human HCC mouse model. The group used the cell cycle-dependent element (CDE) and cell cycle genes homology regions sites located within many of the cell cycle gene promoters such as cyclin A promoter to achieve cell cycle regulation. In proliferating HCC cells, transactivating chimeric protein, Gal4/NF-YA, under the regulation of hybrid promoter (apolipoprotein E enhancer elements/human α-antitrypsin promoter) binds to Gal4 binding sites upstream of the minimal cyclin A2 promoter. Thus, selective killing is achieved through conversion of prodrug 5-fluorocytosine to its toxic 5-fluorouracil derivatives. Intratumoral delivery of these vectors effectively suppressed the growth of patient-derived HCC xenograft model. In addition, the group also reported on the generation of an oncolytic herpes simplex virus type-1 vector where the cell cycle-regulatory transgene cassette is inserted into the *ICP6* gene of the F-strain. Enhanced transgene expression could be demonstrated in proliferating tumor regions, but significantly less when the same amount of viruses was introduced into the contralateral normal brain of the same animal.

Lack of tumor tropism and the presence of pre-existing anti-measles Abs in vaccinated individuals can pose a major obstacle during systemic measles virotherapy. Using mesenchymal stem cells (MSCs) that reportedly express low MHC class I antigen, and no CD40, CD80 and CD86, together with its tumor-homing properties, measles virus (MV) infection was successfully delivered to orthotopic patient-derived HCC tumor in severe combined immunodeficiency mice as demonstrated by Tina Ong Hooi Tin (National Cancer Center). Tumor growth in both measles-naïve and passively immunized mice receiving MSC-associated MV was significantly suppressed. In contrast, tumor growth delay was only

evident in measles-naïve mice and not in passively immunized mice when given naked virus.

Replication-incompetent viruses

The group of Yasufumi Kaneda (Division of Gene Therapy Science, Graduate School of Medicine, Osaka University, Osaka, Japan) has been able to demonstrate that ultraviolet irradiation could render the envelope of replication-incompetent hemagglutinating virus of Japan (HVJ; Sendai virus) able to mediate oncolytic activity without producing any toxic effects. The suppressive effect induced by replication-incompetent envelope HVJ-E on prostate cancer cells is similar to those induced by the live virus particles, suggesting that the mechanism for the oncolysis induced by HVJ-E does not involve viral genome or viral protein synthesis.[7] Furthermore, the transfer of HVJ-E RNA fragments via lipofection could induce selective prostate cancer cell death in a dose-dependent manner, but this was not observed in non-malignant prostate epithelial cell lines. Using small interfering RNA (siRNA) knockdown experiments, the group subsequently showed that the suppression effect was mediated through retinoic acid-inducible gene I/mitochondrial anti-viral signaling protein. Intact HVJ genome is recognized by the cytoplasmic RNA receptor retinoic acid-inducible gene I. Retinoic acid-inducible gene I is known to activate nuclear factor-κB and interferon (IFN) regulatory factors through the adaptor protein mitochondrial anti-viral signaling protein, which is essential for anti-viral innate immunity. When retinoic acid-inducible gene I/mitochondrial anti-viral signaling protein signaling pathway is activated, tumor necrosis factor-related apoptosis-inducing ligand and NOXA were found to be effector molecules responsible for the selective prostate cancer cell death induced by HVJ-E. A single injection of HVJ-E (5×10^{10}) particles has eradiated 70% tumors in an orthotopic prostate cancer model in immunodeficient non-obese diabetic-severe combined immunodeficiency mice.

NON-VIRAL DELIVERY SYSTEMS

Won Jong Kim (Pohang University of Science and Technology, Pohang, Korea) described his multifunctional non-viral gene delivery for cancer therapy. A total of 74% of vectors used in clinical trials are viral. The benefits of non-viral vectors are that they are non-immunogenic and there is no limit to DNA size. However, they have an associated low transfection efficiency.

Analog peptides have been identified in one of the most recently described cancer-testis antigens, PASD1, by Barbara Guinn's group at the University of Bedfordshire (Bedfordshire, UK).[8] The group identified a number of HLA-A*0201-binding wild-type sequences and modified them at a single anchor residue to enhance T-cell stimulation. They have shown that one such analog peptide, named Pa14, was to be able to induce normal donor and leukemia patient T cells to produce IFNγ. DNA vaccines incorporating the first domain of tetanus toxin[9] and Pa14 were used to show that Pa14 can induce epitope-specific T-cell expansion, IFNγ secretion and killing of both analog and wild-type epitope-loaded antigen-presenting cells (APCs). The group has shown that Pa14-specific T cells from a colon cancer patient could be expanded to 13.6% of the $CD8^+$ population after 4 weeks of *in vitro* culture.[8] In addition, the Guinn group has been developing pMHC arrays[10,11] to detect antigen-specific T cells in colon cancer and to prioritize PASD1 epitopes in collaboration with colleagues at the University of Oxford (Oxford, UK). Through the incubation of $0.8–1.4 \times 10^6$ negatively isolated, 'untouched' $CD8^+$ T cells with up to 40 pMHC tetramers on the array, they could show that cancer antigen-specific T cells were detectable in 25% of the colon cancer patients analyzed.

HCC is the fifth most common cancer and the third leading cause of cancer-related deaths worldwide. Celastrol is an anticancer, antioxidant and anti-inflammatory compound isolated in China from the 'Thunder of God' vine.[12] The problem is its low water solubility and toxicity. Hong Ping Xia of the National Cancer Center in Singapore described their use of nanotechnology to deliver HCC therapy. Hong Ping Xia described the development of multifunctional nanoparticles that were modified with targeting peptide ligands for targeted human HCC therapy. Among the various compounds screened, the multifunctional nanoparticle conjugated with celastrol produced specific inhibitory effects against HCC cells compared with normal hepatocytes, and suppressed the growth of orthotopic HCC xenograft tumors with much reduced toxicity in athymic nu.nu mice. These promising preclinical results suggest the potential of targeted multifunctional nanoparticles as anticancer drug delivery systems for cancer therapy.

It has been known that bacteria can grow in tumors for the past 200 years, and this property has been exploited as a potential means of specifying therapeutic activity to malignant tissue in a number of clinical trials over the years. The benefit of using bacteria for gene delivery is that they have a huge payload capacity for DNA, RNA and protein, and scientists can also exploit the large bacterial genome. However, so far the clinical results have not been impressive. Mark Tangney of the Cork Cancer Research Center, University College Cork (Cork, UK) explained that this may be due to (i) insufficient understanding of the precise nature of bacterial growth within tumors, and (ii) the use of pathogen-derived species in patients, where the maximum tolerable dose was less than the therapeutic dose. Mark Tangney's group has developed a strategy to use non-invasive, non-pathogenic strains often found in food such as probiotic yoghurts. The interplay between bacteria and tumors is now believed to be multifactorial, involving the disorganized and leaky vasculature within tumors and necrosis featuring regions of immune suppression and hypoxia, and nutrients such as purines. This bacterial growth within tumors is tumor-type independent and occurs with all bacteria.[13] He explained his group's strategies to improving our understanding of what is occurring inside bacterially colonized tumors. For example, his team has been developing *in vivo* imaging tools to elucidate the precise locations of bacteria within the tumor over time, to date utilizing luminescence imaging, but moving to more clinically applicable versions.[13] They are currently developing variants of non-invasive bacteria with improved tumor growth, for use with their BDEPT (Bacterial Directed Enzyme Prodrug Therapy) strategy.

SMALL-MOLECULE THERAPIES

The dysregulation of sphingolipid metabolism leads to therapeutic resistance to radiation, chemotherapy and gene therapy. The ceramide-sphingosine-sphingosine 1-phosphate biostat represents a control pathway where ceramide elevation leads to cell death and elevation of sphingosine 1-phosphate leads to cell survival. Most cancer therapy agents including radiation cause elevation of ceramide as part of their mechanism of action and this has been demonstrated by the group of James Norris of the Medical University of South Carolina (Charleston, SC, USA). In normal cells, increases in ceramide leads to cell cycle arrest, but in cancer it frequently leads to induction of apoptosis. However, during radiation or chemotherapy the ceramide catabolic enzyme acid ceramidase (AC) that deacylates ceramide is upregulated shifting the biostat to the right with increased production of sphingosine 1-phosphate. Over 50% of prostate cancer patients are treated with radiotherapy and 21–44% of patients relapse. The group analyzed the tissues before radiotherapy and after relapse and showed that AC levels were even higher ($N = 3$) in patients who relapse. It is now understood that AC protects against

chemotherapy and radiotherapy, and that it is activated by radiation, and quite quickly over time is counterproductive for the success of the therapy. This effect is not as pronounced in normal epithelium and stroma. The synthetic C6 ceramide also induces AC, suggesting that ceramide is the signal that induces AC expression. To understand this better, studies were performed and the transcription factor AP1 was shown to be responsible for AC upregulation. This was proven using TAM67 (dominant-negative c-Jun) and siRNA to AC. To address this therapeutically, Sphingogene (Mt Pleasant, SC, USA) developed a small-molecule inhibitor called SPG105 (LCL521). LCL521 inhibits AC activity and is safe up to 290 mg kg^{-1}. Tumors in mice treated with LCL521 have decreased AC levels, and when mice are treated with LCL521 plus radiation, this leads to a cure in 100% of animals in a prostate cancer model. The next step is to move this drug into clinical trials for prostate cancer in the near future. As all major cancers that receive radiation therapy also upregulate AC, it is likely that the therapeutic benefit of SPG105 will extend to these cancers as well.

CELLULAR AND IMMUNOTHERAPIES

DC therapies

Lentivirus (LV) vectors expressing combinations of cytokines and antigens direct the autonomous differentiation of DC precursors in mice and man into activated and antigen-loaded DCs. Renata Stripecke (Hannover Medical School, Hannover, Germany) detailed her group's development of LV-induced DCs.[15,16] Experimental syngeneic and humanized mouse models demonstrated that LV-induced DCs are highly viable (>3 weeks *in vivo*) and effectively migrate to lymph nodes in immune-competent mice or promote the development of lymphatic structures in immune-deficient mice. Smart DC-TRP2 (coexpressing granulocyte–macrophage colony-stimulating factor, IL-4 and the TRP2 melanoma antigen) stimulate autologous cytotoxic T lymphocytes (CTLs) from melanoma patients and have shown no biosafety concerns in syngeneic or humanized mouse systems. Good manufacturing practice-compatible methods for the production of SmartDC-TRP2 have been developed while also achieving high recovery and high viability of the cells. The pilot batch of LV vector for clinical development of SmartDC-TRP2 has been produced in Farzin Farzanehs' facility at King's College London (London, UK). LV-induced DCs are also being developed for immunotherapy after SCT to enhance the graft-versus-leukemia and graft-versus virus (cytomegalovirus). The use of integrase-defective LV vectors for DC reprogramming has been demonstrated and increases the biosafety of the gene transfer by lowering the risk of insertional mutagenesis.

The group of Yajun Guo (PLA General Hospital Cancer Center, Shanghai, China; SMMU Cancer Institute, Shanghai, China) has shown that DCs genetically modified with scFV-CD40 were able to migrate towards HER2-positive tumor cells, were activated after HER2 ligation, homed to draining lymph node and induced tumor-specific CTL. The Guo group also showed that they could target DCs to specific tumor antigens, *in vivo*, using TAA-scFV vectors specific for CD11c.

T-cell therapies

T cells are highly efficient at recognizing internally processed antigens if appropriately presented and are self-amplifying with a good biodistribution. They are directed to tumor through native T-cell receptor and can invade bulky refractory disease. Recent developments of chimeric antigen receptors (CARs) expressed in T cells has led to promising data from Carl June's group,[17] which showed that CARs composed of Ab-binding domains connected to domains that activate T cells could overcome tolerance by allowing T cells to respond to cell surface antigens. Unlike many previous studies, these engineered T cells were shown to expand >1000-fold *in vivo* and express functional CARs for >6 months. A CD19-specific immune response was demonstrated in the blood and bone marrow, accompanied by CR, in two of three patients. Malcolm Brenner of the Baylor College of Medicine (Houston, TX, USA) is a pioneer of cellular immunotherapy who has taken his work into several clinical trials with a focus on T-lymphocyte therapy for cancer. His own studies have also showed tumor responses (albeit anecdotally) when they used a CD30 CAR for refractory Hodgkin's disease.[18] They have extended beyond hematological malignancies to solid tumors. A GD2-directed CAR expressed on Epstein–Barr virus (EBV) CTL was given to 11 patients with relapsed neuroblastoma and induced CR in 3 (27%), and a tumor response was observed in more than 50% of patients.

Tumor-directed T cells may crossreact with normal tissue antigens or release lethal quantities of cytokines such that fatal consequences may result.[19] As T cells can also expand over time and produce ever worsening adverse effects, for example, graft-versus-host disease (GvHD) after allogeneic hemopoietic stem cell transplantation, it has been essential to develop an exit strategy, and so the use of a suicide gene is also required. Because of the limitations of current systems, such as immunogenicity, the requirement for a potentially therapeutic activating drug, and slow activity primarily in dividing cells, Malcolm Brenner and his co-workers tested a new inducible caspase 9 human-derived (so less immunogenic) suicide gene, which is activated by an otherwise bioinert chemical inducer of caspase 9 dimerization. They tested the approach in recipients of T-cell-depleted transplants who received an addback of T cells containing the *inducible caspase 9* gene.[20] If GvHD developed as indicated by increased bilirubin levels (indicates liver damage), skin rash or diarrhea, they gave a single dose of caspase 9 dimerization drug, which lead to 90% depletion of the infused inducible caspase 9 T cells within 30 min, and resolution of GvHD within 24 h. The residual T cells re-expanded and repopulated patients without recurrence of GvHD because the inducible caspase 9 dimerization/activation selectively eliminated the most allo-reactive cells while sparing other T cells, including virus-specific T cells. The recovering T cells remained polyclonal. There was also a broad caspase 9 dimerization dose–response curve, so a small dose (1/100th of the full dose) could, for example, be used to eliminate half the cells so that it may be possible to use the drug as a rheostat to control the intensity and toxicity of the T-cell activity.

Yajun Guo described his group's work on Ab-mediated cellular immunotherapy. scFV-CD28-zeta chimeric Ag receptors had been devised for adoptive T-cell therapy. The group showed that a memory response develops in animals cured through treatment with HER2scFV-CD28 CAR-adoptive T-cell therapy. In addition, there was evidence of protection against rechallenge with HER2-positive and -negative cancer cells.

An unintentional induction of regulatory T cells by IL-2 during DC-based cancer immunotherapy was described by David Klatzmann (Pierre & Marie Curie University, Paris, France). Five patients had no clinical response because of major regulatory T cells induction.[21] This should question the use of IL-2 in cancer immunotherapy protocols. In contrast, in a clinical trial evaluating regulatory T cells' depletion in donor lymphocyte infusions after leukemia relapse post allogeneic hematopoietic stem cell transplantation, 4/4 patients responded to treatment.[22]

Ab therapies

Yajun Guo's group has developed nanoparticle-conjugated Abs, anti-epidermal growth factor receptor (EGFR)/HER2 bi-specific Abs and have determined the crystal structure of Rituximab (anti-CD20 type I Ab) so they can develop variants that take advantage of the characteristics of type I and type II Abs.

Stem cell therapy

Human placental chorionic-plate-derived MSCs from healthy donor mothers exhibit many markers common to MSC, such as CD73, CD90, CD105, CD44 as well as HLA-G surface markers, and are capable of differentiating into the mesodermal linage and can be propagated in the absence of serum. Using a paraquat-induced lung injury mouse model, Thai-Yen Ling (National Taiwan University, Taipei City, Taiwan) has shown that the administration of placental chorionic-plate-derived MSCs can restore the biological function of lung tissues. Suppressed cytokines that are known to associate with neutrophil recruitment and activation (for example, granulocyte–macrophage colony-stimulating factor; IL-6; monocyte chemoattractant protein-1/CCL2; keratinocyte-derived chemokines (KC/GRO-α)) and improved survival were all demonstrated.

Novel adjuvants

Farzin Farzaneh described a potent adjuvant called CASAC,[23] which has been shown to enhance antitumor effects in aged mice. The adjuvant contains two or more TLR agonists, anti-CD40, IFNγ and surfactant has been shown to drive unprecedented levels of CD8 response to peptide or protein Ag and highly polarized T helper 1 CD4 responses. Studies by Farzin Farzaneh and his co-workers have shown that CASAC can boost responses to tumor antigens, including SIINFEKL, WT-1 (important for acute myeloid leukemia) and glypican-3 (important for HCC), TRP2 (important in malignant melanoma) as well as hepatitis B in an HBsAg transgenic mouse model of chronic virus infection.

siRNA and saRNA

Inhibition of vascular endothelial growth factor with siRNA has been used by Won Jong Kim's group to block angiogenesis. They developed an siRNA carrier—an arginine-rich peptide, an oligoarginine called R9.[24] For targeting tumors actively, the Kim group has also developed a bioreducible gene carrier by conjugating NGR peptide,[25] which is used to actively gene target CD13 on angiogenic endothelial cells leading to an accumulation of gene moiety into the tumor site. The blood–brain barrier can be targeted by bioreducible gene carrier using RVG peptide, which has high affinity to neuronal cells.[26,27] *In vivo* experiments using a magnet on one side of the tumor shows localization of treatment to tumor on that side by imaging.[25] The technology can be used to target plasmids to the site of tumor, but it is not clear if it will be expressed.

Patients with viral hepatitis, a history of alcohol abuse and/or obesity have less than a 10% survival rate at 5 years. Associated low serum albumin levels cause ascites, as well as leg and pulmonary edema so there is an urgent need to improve quality of life. Nagy Habib (Imperial College London, London, UK) described for the first time his work using small activating RNA (saRNA) to change gene expression profiles in liver cancer cells. HepG2 liver cancer cell lines express some albumin, while saRNA was shown to boost albumin expression. Nagy Habib considered 'if the cancer cell is so busy making albumin how does it make enough energy to go into mitosis' and decided to develop a 'metabolic diversion'. By adding saRNA his group could increase albumin production by the tumor and decrease cell proliferation *in vivo veritas* in an animal model. *In vivo* 9-day Wistar rats were injected three times with saRNA and there was hardly any impairment of the liver function, but the burden of tumor was significantly reduced. This has led to worldwide collaborations, as it appears 'you can do anything with saRNA'. This has included IFNγ saRNA oligonucleotides and IL-2 saRNA for DCs and Nagy Habib's group has treated 50 different genes with saRNA and it has worked every time. SaRNA is cheap compared with other biologics, it is a double-stranded RNA that takes the place of repressor on the promoter, its effects last for 30 days and are reversible.

BIOMARKERS AND PERSONALIZED MEDICINE

Biomarkers for the efficacy of treatment[28] have been used to indicate whether p53 gene therapy will be beneficial in patients with head and neck cancers by Robert Sobol (p53). They have shown that the more wild-type p53 monomers present in each tetrameric complex of p53, the higher the number of functional p53 tetramers in the tumors. Patients with entirely wild-type p53 are classed as having a favorable profile having a median survival of 7.2 versus 2.7 months if they had an unfavorable profile (predominantly mutant p53 harboring tetramers). If patients with a favorable level of wild-type p53 treatment are treated with methotrexate, there is little improvement compared with those patients with a poor profile. Mda7 has apoptotic effects in cells, its levels increase as IL-10 decreases, suggesting that IL-10 may be a potential biomarker for Mda7. In all, 75% of tumors such as lung cancer have low levels of IL-10.

HCC is not one of the top three cancers in the world (with regards to frequency), but with regards to death caused by cancer, it is number three owing to the high associated mortality. Multistage HCC is very well defined. The group of Kam M Hui (National Cancer Center) have identified diagnostic biomarkers, which can be used to detect HCC. The group used U133 Plus 2.0 array chips to assess the human genome. Each chip contains 47 000 transcribed and 21 000 well-characterized human genes. In addition to analyzing 270 HCC patients samples the group also analyzed adjacent normal tissue and for markers of early recurrence they analyzed samples from patients who relapsed less than 24 months after initial disease occurrence ($n = 34$) and non-responders to treatment ($n = 36$). One of the first issues was to look at the quality of the database. The group was able to show that distinct genes had increased or decreased expression.[29] Rac GTPase-activating protein 1 is particularly associated with the early recurrence of HCC.[30] Using siRNA to determine why Rac GTPase-activating protein 1 is important they found that knockdown of this gene led to decreased tumorigenicity and prevents cytokinesis. The earlier the detection of cancer, the greater the chance of survival. Two tests exist for HCC screening—α-fetal protein, which has 63% sensitivity and 87% specificity, and ultrasound, which is 60% sensitive and 95% specific but is very operator dependent. The 'Gold' standard is biopsy but the need for a non-invasive screening method is apparent. Kam Man Hui's group started to look at peripheral blood mononuclear cell from patients who are indicative of when tumor cells go into circulation. In all, 13 genes segregated with HCC, gastric and pancreatic cancers. Using a binary logistic regression model, the group used 53 HCC and 16 healthy volunteer peripheral blood mononuclear cell as a training set for receiver operating characteristic curve. They showed that the assay was very specific and sensitive (area under the curve = 0.986). HCC differs greatly among regions and whether patients are hepatitis B virus or HCV carriers (deep in Asia or Europe). Investigating 250 samples from patients who either did or did not have hepatitis B virus infection and HCC on the binary logistic regression model, they found the area under the curve from the training set was 0.937,[31] indicating that a high degree of sensitivity and precision could be attained with this binary logistic regression model.

MAGE-A, a cancer-testis antigen, with expression restricted to testis and placenta in normal tissues was first identified as the gene that directed the expression of antigen MZ2-E on a human melanoma cell line.[32] Most people look for cancer-testis antigen expression using techniques such as reverse transcription-polymerase chain reaction and microarray. There are two Abs for Mage: αMage-A1 6C1, which recognizes MAGE-A2, -A3, -A4, -A6, -A10 and -A12, and αMage-A3 57B, which recognizes MAGE-A1, -A4, -A6 and -A12. Baoen Shan (Cancer Research Center at the Fourth Hospital of Hebei Medical University, Shijiazhuang, China) described his group's interest in MAGE-A4 expression in normal

and breast cancer. There is an interaction between MAGE-A4 and p53, with MAGE-A4 being shown to enhance the transcriptional activity of p53. MAGE-A11 affects the expression of estrogen receptor-induced breast cancer. Most Mage, including MAGE-A4, -A9, -A10, -A11 and -A12, are commonly expressed in various tissues. MAGE-A9 and -A11 may be related to endocrine therapy and poor prognosis in breast cancer. MAGE-A9 and -A11 increases lead to the proliferation of monocyte chemoattractant factor-7 cells and MAGE-A4 suppresses it.

MicroRNA markers are being used to predict response to therapy in head and neck cancer by Mahvash Tavassoli's group at Kings College London (London, UK). Fifty percent of patients do not respond to radiochemotherapy and tumors in general have complex genetic aberrations.[33] Radiation treatment is associated with lifelong disabilities, while promising therapies include radiotherapy plus cetuximab.[34] The future vision for treatment includes targeted therapies to avoid the cost burden and side effects by treating unresponsive patients. Apotosis is one of the six most important hallmarks of cancer[35] and one of the focuses for targeted therapy include restarting apoptosis. *In vitro* studies have shown efficacy in killing head and neck cancer cells with either SMAC mimetics or recombinant TRAIL, a combination of both resulted in killing all 15 cell lines tested. They found an increased level of TNFα in cell lines sensitive to SMAC mimetics. A microRNA signature has been obtained, which may predict response of head and neck cancers to SMAC mimetic and/or TRAIL. MicroRNAs have multiple targets and are more versatile than gene expression.

Personalized medicine has the potential to greatly progress cancer treatment. It has allowed substantial progress to be made, for example, with regards to the use of herceptin in breast cancer and the targeting of the EGFR in colon cancer cells harboring wild-type KRAS with cetuximab or panitumumab. A prerequisite of personalized medicine is the capability to predict pretherapeutically the response of individual tumors to certain (targeted) drugs. For this, we need reliable and reproducible biomarker assays. 'Prediction is difficult especially about the future' Niels Bohr (1885–1962). Manfred Dietel (Institute for Pathology, Charité Universitätsmedizin, Berlin, Germany) has described how we should not be too pessimistic, especially when we consider the progress we have made. For example, we now have predictive tissue-based biomarkers for targeted FDA/European Medicines Agency (EMA)-approved drugs such as trastuzumab, which requires the overexpression of Her-2 to be effective. Eligibility tests are requisite to ensure unnecessary side effects and costs are minimized. Currently, 35% of all tumors get predictive tests in the Institute for Pathology-Charite Berlin. It is now an EMA/FDA mandatory prerequisite that there is an eligibility test if a so-called targeted drug is considered for therapy. Some examples: If a metastasized colon cancer carries a mutated *KRAS* gene, the therapeutical Abs panitumumab or cetuximab would not work. This is the case for 40% of patients and there is no point in giving the drug in this situation—not only due to financial cost but also due to the unnecessary side effects for patients receiving the treatment. This means, subtype analysis is essential to predict response. Prahallad *et al.*[36] demonstrated that in BRAF(V600E) mutant colon cancers (approximately 8–10% of all colon cancers), the BRAF inhibitor PLX4032 (a small-molecule drug also known as vemurafenib) would not be effective alone since an compensatory upregulation of the EGFR was observed. As a consequence, a combination therapy consisting of BRAF and EGFR inhibitors might be beneficial.

In 1999, non-small-cell lung cancer was subtyped predominantly as adenocarcinoma, squamous cell carcinoma and large-cell carcinoma. Progress to 2012 means that the same groups of cancers are subtyped by no mutations, KRAS (22%), EGFR (17%), EML4-ALK (7%), double mutants (3%) and BRAF (2%) for example. Routine tests for EGR, KRAS and ALK indicate whether tyrosine kinase inhibitors are suitable treatments to interfere with tyrosine kinase molecules when they are activated. Therefore, kinase inhibitors are only approved if used in conjunction with a diagnostic eligibility test. These drugs are Gefitinib (Iressa; Astra Zeneca, Wedal, Germany) and Erlotinib (Tarceva; Roche, Penzberg, Germany) for EGFR-mutated non-small-cell lung cancer. Crizotinib, an ALK inhibitor (Xalkori; Pfizer, Berlin, Germany), is very effective when there is an EML4-Alk inversion in non-small-cell lung cancer tumors. Ou *et al.*[37] showed that this treatment could be even more effective when combined with other drugs. Proving the inversion is presently essential. The gold standard currently is fluorescence *in situ* hybrization; in the future, immunohistochemistry may be used as additional screening method.

Fifty percent of all malignant melanomas have a mutated BRAF kinase. These patients show a dramatic response to vemurafenib, a BRAF inhibitor. Although patients will still die of malignant melanoma, there is a significant clinical and psychological impact in the patient knowing that the tumor is shrinking. Flaherty *et al.*[38] showed that a combination of BRAF and MEKi doubles life expectancy/overall survival (OS) from 5.8 to 9.9 months. This is another option for a combined targeted therapy.

With regards to breast cancer, it is well known that the estrogen receptor (ER)-positive and HER2-negative breast cancer cases can be divided into a low-risk group to be treated with tamoxifen alone and a high-risk group to be treated with tamoxifen plus chemotherapy. However, this stratification is difficult regarding the individual case. To reliably stratify the individual patient, the group developed the multigene EndoPredict assay (Sividon Diagnostics, Cologne, Germany).[39,40] Following a combined morphological and molecular assessment of the tumor tissue to identify low-risk patients, it was shown that 96% of the patients who qualify for the low-risk group do not have metastasis within 10 years. This allows the physician to come to a scientifically based decision.

Manfred Dietel explained that pathology reports of the future will all be based on paraffin-embedded tissues. The primary step will continue to be the classical histology with hematoxylin and eosin stain, McMannus' *Periodic acid Schiff's* and other stainings. However, to provide more information on prognosis and therapy response, additional methods have to be included, such as immunohistochemistry, fluorescence and bright-field *in situ* hybridization, and in particular new molecular techniques. Most of them are based on tissue-adapted PCR/PCR variants and tissue-based sequencing/mutation analyses. Even new-generation sequencing is applicable on formaldehyde-fixed paraffin-embedded tissue; this will give us deeper insights in the pathogenesis of many malignant tumors. The combined morphological/molecular pathology reports will provide the bases of reliable personalized medicine.

PHASE I/II CLINICAL TRIALS

Acute myeloid leukemia cells are viewed as being good APCs because they express MHC class I and II, tumor antigens and share a common (myeloid) lineage with APC. They also express many of the surface molecules found on DCs including the costimulatory molecule CD86 (B7-2), although not CD80 (B7-1).[41,42] A Phase I clinical trial for relapsed acute myeloid leukemia following allohemopoietic stem cell transplantation has now started recruiting under the leadership of Farzin Farzaneh. The trial involved two rounds of vaccination and to date no patients have evidenced an immune response at that stage. However, one patient received all of the required doses ($n = 3$) and the last dose induced a pronounced delayed-type hypersensitivity response. One year out and there has been no evidence of molecular or clinical disease.

A tale of two viruses was the description given by Kah-Whye Peng (Mayo Clinic, Rochester, MN, USA) for 'the blob' (measles) and 'the bullet' (vesicular stomatitis virus). Of particular note was that 'the blob' (MV) is now in several Phase I clinical trials, including ovarian cancer, myeloma, mesothelioma, glioma and

head and neck cancer. Virally encoded reporter genes (for example, one which secretes carcinoembryonic antigen or thyroidal sodium iodide symporter (NIS) to show the location of infected tumors) are used to monitor virotherapy.[43] Phase I clinical trials for ovarian cancer involve six cycles of measles-carcinoembryonic antigen therapy. Dose escalation of this first-in-man clinical trial started with 10^3 vpu, increasing to 10^9. The main aim was to look at safety, toxicity and maximum-tolerated dose.[44] Overall median survival is 12.2 months, with disease stabilization in patients given higher doses of virus.

Vesicular stomatitis virus, 'the bullet' is in Phase I clinical testing at Mayo Clinic (Phoenix, AZ, USA). This virus is ideal for bulky disease, which can be debulked with virus, with delivery of the virus killing the majority of infected cells, and the host immune system for clearing residual disease.[45] Vesicular stomatitis virus-mIFNβ-NIS is a fast replicating virus, significantly extended survival of myeloma bearing immunocompromised mice at doses of 10^5–10^8 vpu. In immune-competent mice with myeloma, tumor cures are correlated with viral dose given.

T cells specific for the viral latent and lytic cycle antigens have been used as prophylaxis and treatment of post-transplant lymphoproliferative disease occurring after SCT. Cliona Rooney from Baylor College of Medicine described their clinical trials using T cells for EBV. In the prophylaxis group, no patients developed post-transplant lymphoproliferative disease compared with 12% of patients who did not receive EBV-specific CTLs. Among 13 patients with active disease at the time of CTL treatment, 11 attained CR. These T cells persisted for up to 10 years as demonstrated by gene marking using a retroviral vector, which also demonstrated T-cell homing to tumor tissues. When used to EBV-positive Hodgkin's disease, EBV-specific-CTL again homed to tumor sites, persisted for up to 12 months and produced two CRs among patients with disease. To improve upon this therapy, a manufacturing strategy was developed to target specifically LMP1 and LMP2, two of the three viral antigens expressed in Hodgkin's disease, and the therapy was extended to patients with EBV-positive Hodgkin's disease and non-Hodgkin's lymphoma. LMP-CTLs produced 11 CRs among 21 patients with relapsed or refractory disease; of these, one patient also received rituximab while one received multiple doses of T cells. There were no severe toxicities related to these T-cell therapies, in contrast to the severe short- and long-term toxicities associated with standard chemo-radiotherapies, highlighting the need for targeted immuno-therapies to be made more widely available. Therefore, to enable pivotal late-phase studies, the time and complexity of T-cell manufacture has been decreased by using overlapping peptide libraries as a source of antigen and eliminating the autologous EBV-transformed B lymphoblastoid cell line as a source of APCs.

Nasopharyngeal cancer (NPC) is endemic in South East Asia and Southern China. By stage 4 of disease, patients frequently have <2 years median survival and treatment is palliative and not curative. Small-molecule therapies have a limited role in NPC. Han Chong Toh has led first-in-man clinical trials using DC vaccines to treat NPC. NPC cells, however, express a type 2 latency response expressing LMP1 and LMP2 viral oncoproteins, offering antigenic targets for immune targeting. Mini-allogeneic bone marrow transplant has been previously evaluated for metastatic NPC, but although responses are observed, the treatment is associated with high toxicity.[46] Using mini-allo-SCTs Han Chong Toh has treated 19 patients and achieved mixed chimerism in 16 patients, while the three remaining patients had late responses. Chronic GvHD is associated with better survival than patients with non-chronic GvHD. One patient 1 year post allo-SCT developed a late Response Evaluation Criteria In Solid Tumors (RECIST) response, indicating that graft-versus-tumor responses may take time to develop. A Phase II clinical trial exploring a strategy of four cycles of gemcitabine–carboplatin chemotherapy followed by six cycles of adoptively transfered EBV-specific T cells has been completed.[47] Preliminary data indicate a 2-year OS is 62.9% with 12 patients still alive after a median follow-up of 29.9 months. Five patients have not required further systemic chemotherapy as the initiation of CTL therapy, for a median duration of approximately 36 months. Exploratory analysis showed that LMP2-specific CTLs in the CTL product infusate was associated with a statistically significant OS benefit. The median and 3-year OS in this trial is high when compared with historical NPC studies.[48]

Noriyuki Kasahara (University of California, Los Angeles, CA, USA) has been developing tumor-selectively replication-competent retroviruses expressing prodrug activator ('suicide') genes for cancer therapy. One such vector, Toca 511, is a retroviral replicating vector, which is currently in clinical development by Tocagen (San Diego, CA, USA). Toca 511 spreads with high efficiency throughout solid tumors in a non-lytic manner, resulting in widespread integration of the yeast cytosine deaminase prodrug activator gene into the tumor cells' own genome. Yeast cytosine deaminase expressed from the retroviral replicating vector then converts the anti-fungal prodrug 5-fluorocytosine into the chemotherapy drug 5-fluorouracil directly within the infected tumor cells.[49] Using two different brain tumor models in immunocompetent hosts, it has recently been reported that Toca 511 intratumoral injection followed by several cycles of 5-fluorocytosine eventually leads to tumor eradication and long-term survival even when prodrug administration is subsequently discontinued, with cured animals maintained alive for 1 year without any adverse effects, and evidence indicating that antitumor immunological responses had been activated.[50] In 2009, Tocagen received FDA approval of its Investigational New Drug application, and in late 2010 initiated a Phase I dose escalation clinical trial involving stereotactic intratumoral injection of Toca 511 into patients with recurrent high-grade glioma. By mid-2011, FDA reviewed the safety data from the first cohort of three patients and concluded that safety was acceptable, and in 2012, approved a second clinical trial of Toca 511 gene therapy administered into the post-resection tumor bed, also in recurrent high-grade glioma patients. To date, multiple neuro-oncology centers in the United States have treated more than 30 patients with Toca 511, with dose escalation up to four dose cohorts. All patients have tolerated the treatment well, without dose-limiting toxicity, and promising signs of therapeutic efficacy have been observed, including symptomatic improvement, radiographic evidence of tumor stabilization or shrinkage and progression-free survival. In future studies, Noriyuki Kasahara envisaged combining retroviral replicating vector-mediated gene therapy with adoptive immunotherapy and/or tumor vaccine therapy, incorporating imaging capabilities into the virus to enable real-time monitoring of viral spread in the tumor before timely application of the prodrug for maximal killing of the tumor cells, and the use of tumor-homing cellular delivery vehicles for more efficient vector dissemination to multiple tumor foci.

The preclinical assessment of the potency, safety and selectivity of the group B oncolytic Ad ColoAd1 has been performed by Len Seymour (University of Oxford) and his group. ColoAd1 is a chimeric Ad11p/Ad3 replication-competent Ad isolated from a colon cell line on which a viral pool of Ads from groups B to F were passaged. ColoAd1 uses CD46 as it entry receptor. Len Seymour presented data on the impressive stability of ColoAd1 in the serum and blood. In contrast to herpes simplex virus, vaccinia or Ad5, coincubation of ColoAd1 with whole human blood did not significantly reduce viral titers, suggesting minimal interaction with blood components or cells. This is an important feature as it may improve systemic delivery of the virus to tumors. ColoAd1 is highly selective and potent against a range of carcinomas and is currently in Phase I testing.

A limited Phase II clinical trial that involved the angiographic implantation of an encapsulated HEK293 producer cell line overexpressing the cytochrome *P*450 subenzyme 2B1 into the

vasculature leading to the tumor has been performed by John A Dangerfield (COO; Austrianova Singapore Pte Ltd, Singapore, Singapore). The cells are protected from the patient's immune system because they are microencapsulated in bioinert polymers of cellulose sulfate (Cell-in-a-Box Technology). In the presence of ifosfamide, the *CYP2B1* gene converts the prodrug into its active cytotoxic compounds, phosphoramide mustard and acrolein, which renders DNA of dividing cells and proteins dysfunctional via alkylation. This forms the basis for its selectivity in restricting the cytotoxicity to fast-growing tumors and at the site where the capsules are implanted, which significantly reduces systemic toxicity and provides more effective local therapeutic concentrations. John Dangerfield reported that the latest clinical trial with 13 patients exhibited no capsule or cell-related adverse effects, which support the findings of the original Phase I/II with 14 patients. The median life expectancy of the patient collective was almost doubled when compared with the data available for gemcitabine (Gemzar), the current standard treatment for advanced pancreatic cancer. There was also a twofold improvement in 1-year survival.

More than 160 patients with solid tumors have now received the oncolytic vaccinia virus JX-594 intratumorally or intravenously[51] as described by Erica Sommermann from Jennerex Biotherapeutics (San Francisco, CA, USA). The virus was well tolerated and patients showed transient flu-like symptoms. The lead indication is HCC.[52] It was reported that HCC patients given high-dose intratumoral JX-594 have better OS (14.1 months) compared with patients given low-dose virus (6.7 months, $P = 0.02$). Importantly, objective tumor responses have been seen in some of the patients treated with JX-594. Furthermore, immunohistochemical staining of liver tumor biopsies from patients treated by intravenous infusion alone showed tumor-specific presence of vaccinia virus even in the presence of baseline neutralizing Abs for vaccinia. A randomized Phase IIb trial (TRAVERSE) is underway in multiple countries and is designed to enroll 120 patients with advanced liver cancer who have failed sorafenib therapy.

SUMMARY

In the brief period since our last meeting, we have seen significant developments in gene therapy by this dedicated team of clinicians and scientists, which merits the description 'an innovative evolution'. Field leaders whose work have been described in this review have continued to change the face of gene therapy demonstrating successful treatment strategies, which translate to the clinic. Malcolm Brenner describes a suicide gene therapy, which is dose dependent and can control the potentially fatal GvHD often induced by successful hemopoietic stem cell transplantation. Paula Lam described the use of cell cycle-dependent promoters to regulate gene expression in viral vectors, while Nagy Habib described their use of saRNA to upregulate gene expression profiles in liver cancer cells and cause their death by exhaustion.

Outcome data from a number of Phase I and II clinical trials was not only presented but was also seen to inform research and future treatment strategies. Vesicular stomatitis virus from Kah-Whye Peng's lab is being used to debulk late-stage cancer allowing the host immune system to remove residual disease. Indeed, we enjoyed Erica Sommermann's descriptions of Phase II clinical trials involving vaccinia virus-based therapies for patients with advanced liver disease who have failed Ab therapy. Manfred Dietel described the near future of pathology in which detailed pathology reports on formaldehyde-fixed paraffin-embedded tissue indicate which gene therapies will be effective—saving money and unnecessary side effects for patients who do not have the genetic makeup in their tumor needed for effective treatment.

Gene therapy has established itself as able to impact on survival rates for patients with cancer and we look forward to its continued success.

ABBREVIATIONS

AC, acid ceramidase; Ad, adenovirus; AML, acute myeloid leukaemia; APC, antigen presenting cell; CAR, chimeric antigen receptor; CID, Caspase 9 dimerization; CR, complete remission; CTA, cancer-testis antigen; CTL, cytotoxic T lymphocyte; DC, dendritic cell; Ecd, extracellular domain; EBV, Epstein Barr virus; FDA, Food and Drug Administration; GM-CSF, granulocyte–macrophage colony-stimulating factor; GvHD, graft versus host disease; HCC, hepatocellular carcinoma; HBV, Hepatitus B virus; HCV, Hepatitus C virus; HD, Hodgkin's disease; HSCT, hemopoietic stem cell transplantation; iC9, inducible caspase 9; LN, lymph node; LV, Lentivirus; MAVS, mitochondrial anti-viral signaling protein; MSC, mesenchymal stem cell; MHC, Major Histocompatability; MUC-1, Mucin-1; MV, measles virus; NIS, sodium iodide symporter; NPC, nasopharyngeal cancer; NSCLC, non-small cell lung cancer; OS, overall survival; PBMC, Peripheral blood mononuclear cells; pRb, phosphorylated Retinoblastoma; PTLD, post-transplant lymphoproliferative disease; RACGAP1, Rac GTPase-activating protein 1; RIG-I, retinoic acid-inducible gene-I; saran, small activating RNA; siRNA, small interfering RNA; TAA, tumor associated antigen; Treg, regulatory T cell; Vpu, viral particle units; VSV, Vesicular stomatitis virus; ZOL, Zoledronic acid

CONFLICT OF INTEREST

Dr Kasahara is the lead inventor of Tocagen's core technology, and is a co-founder and consultant to the company.

ACKNOWLEDGEMENTS

The 2012 ISCGT Singapore meeting was sponsored by National Cancer Center in Singapore, Beckman Coulter, CellGenix, ion torrent and Research Instruments. The next ISCGT meeting will be held in conjunction with the 9th Hebei Conference on Oncology (CCO) in Shijiazhuang, People's Republic of China in 2013. For more details see www.iscgt.net

REFERENCES

1 Guinn B, Casey G, Möller MG, Kasahara N, O'Sullivan GC, Peng KW *et al.* International Society for Cell and Gene Therapy of Cancer 2009 Annual Meeting held in Cork, Ireland. *Hum Gene Ther* 2010; **21**: 9–26.
2 Senzer N, Nemunaitis J, Nemunaitis M, Lamont J, Gore M, Gabra H *et al.* P53 therapy in a patient with Li–Fraumeni syndrome. *Mol Cancer Ther* 2007; **6**: 1478–1482.
3 Peng Z. Current status of gendicine in China: recombinant human Ad-p53 agent for treatment of cancers. *Hum Gene Ther* 2005; **16**: 1016–1027.
4 Zhang L, Tang Y, Akbulut H, Zelterman D, Linton PJ, Deisseroth AB. An adenoviral vector cancer vaccine that delivers a tumor-associated antigen/CD40-ligand fusion protein to dendritic cells. *Proc Natl Acad Sci USA* 2003; **100**: 15101–15106.
5 Pataer A, Hu W, Xiaolin L, Chada S, Roth JA, Hunt KK *et al.* Adenoviral endoplasmic reticulum-targeted mda-7/interleukin-24 vector enhances human cancer cell killing. *Mol Cancer Ther* 2008; **7**: 2528–2535.
6 Kim PH, Sohn JH, Choi JW, Jung Y, Kim SW, Haam S *et al.* Active targeting and safety profile of PEG-modified adenovirus conjugated with herceptin. *Biomaterials* 2011; **32**: 2314–2326.
7 Matsushima-Miyagi T, Hatano K, Nomura M, Li-Wen L, Nishikawa T, Saga K *et al.* TRAIL and Noxa are selectively upregulated in prostate cancer cells downstream of the RIG-I/MAVS signaling pathway by nonreplicating Sendai virus particles. *Clin Cancer Res* 2012; **18**: 6271–6283.
8 Hardwick N, Buchan S, Ingram W, Pulford K, Mufti GJ, Stevenson FK *et al.* An analogue peptide from the cancer-testis antigen, PASD1, induces CD8+ T cell responses against naturally processed peptide. Submitted.
9 Rice J, Buchan S, Stevenson FK. Critical components of a DNA fusion vaccine able to induce protective cytotoxic T cells against a single epitope of a tumor antigen. *J Immunol* 2002; **169**: 3908–3913.
10 Soen Y, Chen DS, Kraft DL, Davis MM, Brown PO. Detection and characterization of cellular immune responses using peptide-MHC microarrays. *PLoS Biol* 2003; **1**: E65.

11 Chen DS, Soen Y, Stuge TB, Lee PP, Weber JS, Brown PO *et al.* Marked differences in human melanoma antigen-specific T cell responsiveness after vaccination using a functional microarray. *PLoS Med* 2005; **2**: e265.
12 Kannaiyan R, Shanmugam MK, Sethi G. Molecular targets of celastrol derived from Thunder of God Vine: potential role in the treatment of inflammatory disorders and cancer. *Cancer Lett* 2011; **303**: 9–20.
13 Cronin M, Stanton RM, Francis KP, Tangney M. Bacterial vectors for imaging and cancer gene therapy: a review. *Cancer Gene Ther* 2012; **19**: 731–740.
14 Cronin M, Akin AR, Collins SA, Meganck J, Kim JB, Baban CK *et al.* High resolution *in vivo* bioluminescent imaging for the study of bacterial tumour targeting. *PLoS One* 2012; **7**: e30940.
15 Koya RC, Kimura T, Ribas A, Rozengurt N, Lawson GW, Faure-Kumar E *et al.* Lentiviral vector-mediated autonomous differentiation of mouse bone marrow cells into immunologically potent dendritic cell vaccines. *Mol Ther* 2007; **15**: 971–980.
16 Pincha M, Salguero G, Wedekind D, Sundarasetty BS, Lin A, Kasahara N *et al.* Lentiviral vectors for induction of self-differentiation and conditional ablation of dendritic cells. *Gene Therapy* 2011; **18**: 750–764.
17 Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A *et al.* T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med* 2011; **3**: 95ra73.
18 Savoldo B, Rooney CM, Di Stasi A, Abken H, Hombach A, Foster AE *et al.* Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30zeta artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. *Blood* 2007; **110**: 2620–2630.
19 Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010; **18**: 843–851.
20 Tey SK, Dotti G, Rooney CM, Heslop HE, Brenner MK. Inducible caspase 9 suicide gene to improve the safety of allodepleted T cells after haploidentical stem cell transplantation. *Biol Blood Marrow Transplant* 2007; **13**: 913–924.
21 Mesel-Lemoine M, Cherai M, Le Gouvello S, Guillot M, Leclercq V, Klatzmann D *et al.* Initial depletion of regulatory T cells: the missing solution to preserve the immune functions of T lymphocytes designed for cell therapy. *Blood* 2006; **107**: 381–388.
22 Maury S, Lemoine FM, Hicheri Y, Rosenzwajg M, Badoual C, Cherai M *et al.* CD4 + CD25 + regulatory T cell depletion improves the graft-versus-tumor effect of donor lymphocytes after allogeneic hematopoietic stem cell transplantation. *Sci Transl Med* 2010; **2**: 41ra52.
23 Wells JW, Cowled CJ, Farzaneh F, Noble A. Combined triggering of dendritic cell receptors results in synergistic activation and potent cytotoxic immunity. *J Immunol* 2008; **181**: 3422–3431.
24 Kim WJ, Christensen LV, Jo S, Yockman JW, Jeong JH, Kim YH *et al.* Cholesteryl oligoarginine delivering vascular endothelial growth factor siRNA effectively inhibits tumor growth in colon adenocarcinoma. *Mol Ther* 2006; **14**: 343–350.
25 Son S, Namgung R, Kim J, Singha K, Kim WJ. Bioreducible polymers for gene silencing and delivery. *Acc Chem Res* 2012; **45**: 1100–1112.
26 Son S, Singha K, Kim WJ. Bioreducible BPEI-SS-PEG-cNGR polymer as a tumor targeted nonviral gene carrier. *Biomaterials* 2010; **31**: 6344–6354.
27 Namgung R, Singha K, Yu MK, Jon S, Kim YS, Ahn Y *et al.* Hybrid superparamagnetic iron oxide nanoparticle-branched polyethylenimine magnetoplexes for gene transfection of vascular endothelial cells. *Biomaterials* 2010; **31**: 4204–4213.
28 Nemunaitis J, Clayman G, Agarwala SS, Hrushesky W, Wells JR, Moore C *et al.* Biomarkers predict p53 gene therapy efficacy in recurrent squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2009; **15**: 7719–7725.
29 Wang SM, Ooi LL, Hui KM. Identification and validation of a novel gene signature associated with the recurrence of human hepatocellular carcinoma. *Clin Cancer Res* 2007; **13**: 6275–6283.
30 Wang SM, Ooi LL, Hui KM. Upregulation of Rac GTPase-activating protein 1 is significantly associated with the early recurrence of human hepatocellular carcinoma. *Clin Cancer Res* 2011; **17**: 6040–6051.
31 Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. American Association for the Study of Liver Diseases. *Hepatology* 2011; **53**: 1020–1022.
32 van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B *et al.* A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991; **254**: 1643–1647.
33 Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. *Lancet* 2008; **371**: 1695–1709.
34 Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB *et al.* Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006; **354**: 567–578.
35 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646–674.
36 Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D *et al.* Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012; **483**: 100–103.
37 Ou SH, Bazhenova L, Camidge DR, Solomon BJ, Herman J, Kain T *et al.* Rapid and dramatic radiographic and clinical response to an ALK inhibitor (crizotinib, PF02341066) in an ALK translocation-positive patient with non-small cell lung cancer. *J Thorac Oncol* 2010; **5**: 2044–2046.
38 Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M *et al.* Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med* 2012; **367**: 107–114.
39 Filipits M, Rudas M, Jakesz R, Dubsky P, Fitzal F, Singer CF *et al.* A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res* 2011; **17**: 6012–6020.
40 Denkert C, Kronenwett R, Schlake W, Bohmann K, Penzel R, Weber KE *et al.* Decentral gene expression analysis for ER + /Her2 − breast cancer: results of a proficiency testing program for the EndoPredict assay. *Virchows Arch* 2012; **460**: 251–259.
41 Hardwick N, Chan L, Ingram W, Mufti G, Farzaneh F. Lytic activity against primary AML cells is stimulated *in vitro* by an autologous whole cell vaccine expressing IL-2 and CD80. *Cancer Immunol Immunother* 2010; **59**: 379–388.
42 Ingram W, Chan L, Guven H, Darling D, Kordasti S, Hardwick N *et al.* Human CD80/IL2 lentivirus-transduced acute myeloid leukaemia (AML) cells promote natural killer (NK) cell activation and cytolytic activity: implications for a phase I clinical study. *Br J Haematol* 2009; **145**: 749–760.
43 Hasegawa K, Pham L, O'Connor MK, Federspiel MJ, Russell SJ, Peng KW. Dual therapy of ovarian cancer using measles viruses expressing carcinoembryonic antigen and sodium iodide symporter. *Clin Cancer Res* 2006; **12**: 1868–1875.
44 Galanis E, Hartmann LC, Cliby WA, Long HJ, Peethambaram PP, Barrette BA *et al.* Phase I trial of intraperitoneal administration of an oncolytic measles virus strain engineered to express carcinoembryonic antigen for recurrent ovarian cancer. *Cancer Res* 2010; **70**: 875–882.
45 Naik S, Nace R, Federspiel MJ, Barber GN, Peng KW, Russell SJ. Curative one-shot systemic virotherapy in murine myeloma. *Leukemia* 2012; **26**: 1870–1878.
46 Childs R, Chernoff A, Contentin N, Bahceci E, Schrump D, Leitman S *et al.* Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 2000; **343**: 750–758.
47 Straathof KC, Bollard CM, Popat U, Huls MH, Lopez T, Morriss MC *et al.* Treatment of nasopharyngeal carcinoma with Epstein–Barr virus—specific T lymphocytes. *Blood* 2005; **105**: 1898–1904.
48 Leong SS, Wee J, Rajan S, Toh CK, Lim WT, Hee SW *et al.* Triplet combination of gemcitabine, paclitaxel, and carboplatin followed by maintenance 5-fluorouracil and folinic acid in patients with metastatic nasopharyngeal carcinoma. *Cancer* 2008; **113**: 1332–1337.
49 Logg CR, Robbins JM, Jolly DJ, Gruber HE, Kasahara N. Retroviral replicating vectors in cancer. *Methods Enzymol* 2012; **507**: 199–228.
50 Ostertag D, Amundson KK, Lopez Espinoza F, Martin B, Buckley T, Galvao da Silva AP *et al.* Brain tumor eradication and prolonged survival from intratumoral conversion of 5-fluorocytosine to 5-fluorouracil using a nonlytic retroviral replicating vector. *Neuro-Oncology* 2012; **14**: 145–159.
51 Breitbach CJ, Burke J, Jonker D, Stephenson J, Haas AR, Chow LQ *et al.* Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. *Nature* 2011; **477**: 99–102.
52 Heo J, Breitbach CJ, Moon A, Kim CW, Patt R, Kim MK *et al.* Sequential therapy with JX-594, a targeted oncolytic poxvirus, followed by sorafenib in hepatocellular carcinoma: preclinical and clinical demonstration of combination efficacy. *Mol Ther* 2011; **19**: 1170–1179.