

Gene Therapy: Right Here, Right Now. Highlights from the Society for Medicines Research Meeting

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Summary

The concept, application and enormous potential of gene therapy to repair the direct cause of monogenic, genetically driven diseases ‘at source’ has tantalized and fascinated the scientific community for many years; the development of suitable treatments using this technology has encountered many challenges and disappointments. Only few gene therapy trials reported clear clinical benefits and in some, severe adverse events including treatment-related lethality were observed. Overall, concern and scepticism rose over the further deployment of these strategies. However, these attitudes are changing with the introduction of new more targeted and specific

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delivery approaches and gene engineering and improved adenoviral and retroviral delivery vector technologies. Several trials in inherited genetic diseases, as well as cancers, have now clearly demonstrated evidence of efficacy and safety for gene therapy medicines. Additional precision gene editing technologies, in particular CRISPR/Cas9, are now nearing the clinic, further increasing the possibilities of specifically altering the human genome to treat serious diseases. Practical gene therapy challenges many ingrained standard approaches to medicines discovery, not least in the safety, production and regulatory stages. This meeting brought together experts from academia and industry to discuss the promise, challenges and reality of gene therapy as a therapeutic approach.

Key words: Gene therapy – Monogenic blood diseases – Hemophilia B – Cystic fibrosis – Short activating RNA

Advancements and Challenges in Gene Therapies from an Industrial Perspective

Johan Hyllner, of the UK Cell and Gene therapy catapult, spoke on ‘Advances and challenges in gene therapies from an industrial perspective,’ providing a historical overview and introduction to the field with a particular focus on the translational medicine aspects, and although this novel therapeutic class can potentially act on many disease areas and through correction of defects in many diverse cell types, there is relatively little case experience to guide routine application of gene therapy approaches, and each new therapeutic program is an active area of research. The progression of such therapies brings a whole raft of scientific, bio-processing, analytical, regulatory and commercialization issues to be solved, for which the expertise and experience does not exist in house, even for the largest of pharmaceutical companies. It was therefore clear that collaborations between academic institutions, industry and other stakeholders underpin any progress in the field. This is especially true for innovative academic labs, where the barrier to entry

into translational medicine studies using gene therapy is too high and subsequently significantly stifles innovation.

In a United Kingdom setting, the major barriers for the seeding, and subsequent growth, of the fledgling gene and cell therapy industry were identified as regulatory, business, manufacturing and supply-chain related. To overcome some of these hurdles, Innovate UK, a national governmental agency, founded the Cell and Gene Therapy Catapult, a nonprofit organization aimed at providing the assembly and networking of a critical mass of expertise to 'bridge the translational gap' from bench to bedside.

Understanding AAV and its Potential for Gene Therapy

Els Henkaerts, of King's College London School of Medicine, Guy's Hospital, London, presented, 'Understanding AAV and its potential for gene therapy'. Their lab's research focuses on adeno-associated virus (AAV), a parvovirus which displays features that are unique among currently known DNA viruses—specifically the low pathogenicity, immunogenicity and the ability to infect both dividing and quiescent cells and persist in an extrachromosomal state without integrating into the genome of the host cell. Consequently, engineered AAV vectors have become frontrunners for gene therapy in humans, leading to positive results from several human clinical trials. Although AAV is a widely used vector in the field, there are limitations due to its restricted biological activity and the challenge with respect to consistent commercial manufacturing. Owing to its complex lifecycle of AAV and dependence on helper viruses, molecular insights into viral replication have become essential for the development of vectors with increased bioactivity and production schemes that promise maximal, broad therapeutic application.

Their lab has most recently focused on studies on the complex interactions of AAV with the host cell and helper virus, leading to the discovery of a cellular protein that interacts with the AAV genome and causes transcriptional silencing, a phenomenon against which both helper virus and AAV have evolved unique countermeasures. These findings point to the potential to anticipate how, and then modulate, cellular proteins influence vector production and bioactivity of recombinant AAV vectors.

Gene Editing for the Therapy of Monogenic Blood Diseases

Professor Fulvio Mavilio, Scientific Director at Génethon (Evry, France) and Professor of Molecular Biology at the University of Modena, provided an overview about the advancement of gene therapy and gene editing technology to treat monogenic blood disorders. The first clinical study to utilize genetically modified cells as a treatment for monogenic blood disease was conducted in the early 1990s on a child suffering

from ADA-SCID, a rare genetic disorder. The disease is caused by adenosine deaminase (ADA) deficiency and characterized by loss of T, B and NK immune cells and metabolic defects. It is lethal in the first years of life, unless patients are kept in complete isolation. Fully matched allogeneic bone marrow transplantation is only possible for less than 25% of patients. There was therefore a strong rationale to attempt gene therapy as an effective way to re-express ADA in patients' cells and create a potential cure. A first patient received bone marrow cells and PBMCs that were retrovirally transduced *ex vivo* with an ADA minigene. This pioneer treatment showed that long-lasting T cells derived exclusively from the bone marrow cells, opening the way to transplantation of genetically corrected stem cells as a treatment for this disease. Subsequently, 14 ADA-SCID patients were treated with gene therapy from 2000 to 2009, achieving immunological reconstitution in all patients, and correction of the systemic metabolic defect as well as restoration of normal growth in the majority of patients. Eventually the ADA gene therapy was further developed in a partnership between GlaxoSmithKline (GSK), the San Raffaele Telethon Institute for Gene Therapy and MolMed. In 2016 it received approval in Europe as the first *ex vivo* stem cell gene therapy and is now marketed under the name Strimvelis (formerly known as GSK-2696273).

As a second example of gene therapy in blood disorders Dr. Mavilio reported on the approaches to cure SCID-X1, an X-linked trait that is caused by mutations in the *IL2RG* gene. This rare genetic disorder is characterized by loss of T, B and NK immune cells and is lethal in the first years of life, unless patients are kept in complete isolation. As in the case of ADA deficiency, only approximately 25% of patients can be treated with a matched allogeneic bone marrow transplant, leaving many of them with no option for a cure. *Ex vivo* retrovirus-mediated transfer of *IL2RG* was initially successful at correcting immune dysfunction in 8 of the 9 patients. However, acute leukemia developed in 4 patients, and 1 died. Studies designed to understand the pro-oncogenic property of this therapy showed that the MLV-based vector systems used have a preference to insert into regulatory DNA sequences, including enhancers that drive the expression of oncogenes and induce neoplastic progression. More recent gene therapy approaches therefore utilize lentivirus-based vectors that preferably insert away from regulatory regions and are devoid of viral enhancer elements, reducing the risk of insertional oncogenesis. Such a HIV-based vector was successfully used in the therapy of Wiskott-Aldrich Syndrome, an X-linked, rare primary immunodeficiency. Treatment with this vector led to rapid and sustained immune reconstitution, substantial clinical improvement without treatment-related severe adverse events. Currently, over 20 trials assessing gene therapies for inherited blood disorders are ongoing, illustrating the progress in the field.

In the final part of his presentation Dr. Mavilio expanded from gene therapy to the topic of gene editing. Technologies

like CRISPR/Cas9 or Zn-Finger nucleases have been developed to directly alter gene sequences by introducing deletions, mutations or insertions. CRISPR in particular, due to the apparent ease of the technology and the ability to design highly specific target sequences, has led to significant activity in the field. However, the effectiveness of this technology varies dependent on the kind of DNA manipulation attempted, and treatment strategies need to take this into account. Dr. Mavilio illustrated this by outlining different possible approaches to treat sickle cell anemia or beta-thalassemia, diseases caused by mutations in the beta hemoglobin (HbB) gene. CRISPR could either be used to correct the mutated *HBB* gene or to delete sequences that prevent the expression of fetal Hb, a protein that can compensate for the dysfunction of *HBB*. While targeting *HBB* directly would correct the root cause of disease, the chances of effective therapy are moderate due to the low effectiveness of correcting DNA by CRISPR in stem cells. Also, different therapeutics with mutation-specific target sequences would need to be developed. The alternative approach, a downregulation of a gene that prevents fetal Hb expression (*Bcl11A*) or disruption of its target sequences on the *HBB* locus, however could be generic for all sickle cell anemia and beta-thalassemia patients and may be highly effective. Dr. Mavilio closed with remarks that while gene editing is an exciting technology, there are still a number of questions that need to be addressed to understand the risks and true scope of this approach.

Nonviral *CFTR* Gene Therapy in Cystic Fibrosis Patients

The presentation by Stephen Hyde, from the Radcliffe Department of Medicine at the University of Oxford, covered work conducted as part of the UK Cystic Fibrosis Gene Therapy Consortium (UK CFGTC). Cystic fibrosis (CF) is the most common lethal genetic disease in Caucasian populations, affecting one in ca. 3,000 births. The autosomal-recessive disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which encodes a chloride/bicarbonate channel responsible for controlling ion transport across the cells lining of the lung, pancreas and other organs. *CFTR* dysfunction leads to the build-up of dehydrated and sticky mucus, which eventually clogs airways and may affect other organs. Progressive lung disease is therefore the key cause of death in these patients. The current median age of death is approximately 28 years, while effective treatments have been approved for some CF subgroups, predicted median survival of a child born today is still approximately 40 years. Despite the development and approval of small molecules that target the function of *CFTR*, *CFTR* remains an attractive target for gene therapy, as in vitro studies indicated that even minor restoration of *CFTR* activity can lead to correction of ion flux and mucus production in epithelial sheets. Perceived ease of local delivery and dosing to the lungs was also seen as a distinct

opportunity for gene therapy in CF. However, several early gene therapy trials in CF using aerosols had only very limited success due to modest gene transfer, merely transient correction of *CFTR* defects and an emerging immune response (flu-like symptoms) against some of the vectors used. In 2001, the UK's three leading CF gene therapy groups (Edinburgh, Oxford, London) joined together as one organization, the UK CFGTC, to share expertise and funding. As key goals the initiative aimed to initially identify the best currently available gene transfer agent, extend duration of *CFTR* expression in patients and reduce immunogenicity (Wave 1 program). Also, novel clinical assays were required to evaluate clinical efficacy. In a second stage (Wave 2), a best-in-class gene transfer agent was to be developed to successfully advance the CF gene therapy efforts.

For the Wave-1 program it was decided to utilize a nonviral gene transfer approach. Preclinical studies indicated that, while correction of *CFTR* activity was possible, inflammation occurred in response to plasmid administration. A strategy to remove CG nucleotides from the *CFTR* plasmid DNA and to use a cationic lipid mixture resulted in pGM169/GL67A, a formulation that did not cause an inflammatory response in vivo and achieved extended duration of *CFTR* expression after aerosol administration. In early clinical studies, single dosing of pGM169/GL67A led to a noticeable increase in chloride secretion over time that waned after approximately 9 weeks, in line with the approximate turnover of lung epithelial cells. These encouraging data led to a double-blind, randomized phase II multidose study where monthly doses of pGM169/GL67A caused a statistically significant increase in lung function (FEV₁ being the primary endpoint). Discussions are currently ongoing with potential partners about progression towards phase III trials. The presentation was concluded by presenting the progress of the Wave 2 program, which follows the aim of developing a vector system that can improve substantially over the promising activity seen with pGM169/GL67A. An approach was used to combine features of Sendai virus (efficient infection of lung cells) and HIV/SIV (long-lived expression), which resulted in rSIV.F/HN, a vector with long-lived expression and high airway tropism. Preclinical studies in mice, sheep and human lung tissue models demonstrated highly efficient gene transfer into lung tissue, including primary human epithelium from CF patients. rSIV.F/HN appeared safe in mouse studies and can be delivered in a range of lung delivery devices. Manufacturing to scale has also been achieved and a single escalating dose first-in-human trial is planned this year while discussions are ongoing with potential partners to conduct a phase IIb lung study.

Strimvelis: The First Ex Vivo Stem Cell Gene Therapy to Treat Patients with ADA-SCID

Jonathan Appleby, of the GSK Rare Diseases Gene Therapy group, detailed the development of 'Strimvelis: the first

ex vivo stem cell gene therapy to treat ultra-rare disease patients with ADA-SCID.⁷ ADA deficiency is a rare, inherited, autosomal-recessive monogenic disorder of purine metabolism which results in accumulation of the toxic metabolites deoxyadenosine and deoxyadenosine triphosphate (dAXP). Left untreated, patients with minimal or no residual enzyme will develop severe combined immunodeficiency (ADA-SCID), characterized by impaired differentiation and function of T, B and NK cells, profound lymphopenia, cognitive impairment and other systemic issues. ADA-SCID is very rare with an annual incidence of between 1 in 200,000 and 1 in 1,000,000 of live births and accounts for approximately 10-15% of all cases of SCID. Without an effective treatment, patients with ADA-SCID rarely survive for more than 2 years.

In describing the history surrounding the development of Strimvelis (GSK-2696273), Dr. Jonathan Appleby (Rare Diseases Gene Therapy Unit, GSK, Stevenage, UK) commented that this had been a 20-year product concept in the making. Established in 1995, the treatment was initially developed at San Raffaele Telethon Institute for Gene Therapy (SR-Tiget). In 2010 the collaboration between GSK and Fondazione Telethon and Ospedale San Raffaele was established and following review and a positive recommendation by the European Medicines Agency in April 2016, Strimvelis was approved by the European Union marketing commission in May 2016.

Prior to the availability of Strimvelis, treatment options included hematopoietic stem cell transplantation (HSCT) or enzyme replacement therapy (ERT). HSCT requires an allogenic human leukocyte (HLA)-compatible sibling donor and is the treatment of choice, although options are limited since < 25% of infants have access to a suitable HLA-matched related donor. ERT with polyethylene-glycol-modified bovine ADA (PEG-ADA) improves immune function, decreases incidence of severe infections and supports growth with survival rates up to 78% over a 20-year period. However, treatment is expensive and not available in many countries.

Strimvelis contains autologous genetically modified CD34⁺ cells. The product is manufactured from the patient's own CD34⁺ cells isolated from their bone marrow and these cells are then transduced with a retroviral vector that encodes for the human ADA cDNA sequence. The finished product is presented as dispersion for infusion containing 1-10 million cells mL⁻¹ of autologous CD34⁺ enriched cell fraction that contains CD34⁺ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence. The cells are formulated in 0.9% sodium chloride and are then re-infused into the patient. Manufacturing is limited to one single site, MolMed in Milan, situated in close proximity with the treatment center.

Results have shown that ADA gene modified cells have a survival advantage, Dr. Appleby commented, highlighting

key survival data with bone marrow CD34⁺ and peripheral blood CD15⁺ cells and evidence that gene-modified stem cells give rise to proportionally higher numbers of gene-modified CD3⁺ T cells that approach almost normal levels. In addition, ADA enzyme activity in lymphocytes increased sufficiently in peripheral blood to prevent dAXP accumulation in red blood cells to $\leq 100 \mu\text{M}$, levels that are equal to those found in successfully allogenic transplanted patients. Severe infections decreased from around 1.1 to 0.2 infections/person-year in the 12 patients monitored over an 8-year period. Both height and weight in males and females were sustained and in the integrated patient population as a whole (18 patients over a 7-year period) survival rate is currently 100% of which 82% are intervention free. Though very encouraging, this is still early days for Strimvelis, Dr. Appleby reminded the meeting. Since the long-term effects are unknown it is recommended that patients are monitored with annual visits (as a minimum) for the first 11 years and then at 13 and 15 years post Strimvelis treatment. Nevertheless, as the first approved ex vivo stem cell gene therapy for a life-threatening ultra-rare disease, Strimvelis represents a potential launch-pad for a gene therapy platform that tackles more prevalent conditions.

A Gene Therapy Approach in the Treatment of Hemophilia B

Prof. Michael Linden's (Genetic Medicine Institute, Pfizer Rare Diseases, UK) outlined the potential for gene therapy to overcome many of the shortcomings of factor replacement, the current standard of care. Hemophilia A and hemophilia B are caused by genetic deficiencies in the intrinsic pathway of coagulation of factor VIII (FVIII) or factor IX (FIX), respectively. Hemophilia A is five times more prevalent compared to hemophilia B and the minimal functional gene for FVIII is three times larger than the gene for FIX. FVIII and FIX form a single enzymatic complex generating factor Xa and thrombin and ultimately the fibrin clot. FVIII and FIX are normally secreted from liver and the site of action is plasma. Hemophilia severity is defined by the factor level in the plasma; < 1% of normal is severe, 2-5% is moderate, ≥ 5 to $\leq 50\%$ is mild and $\geq 50\%$ is considered normal.

In hemophilia B patients with $\leq 2\%$ of circulating FIX activity levels, recurrent bleeding results in painful hemarthroses, disabling hemophilic arthropathy and other sequelae. Although prophylactic therapy of frequent intravenous infusions with FIX protein products to maintain protective levels (above 2% of normal) has provided significant reduction in spontaneous bleedings, the adherence to the demanding frequency of intravenous infusions is the major burden to patients. Gene therapy in hemophilia is not new, Prof. Linden commented and over the past two decades, AAV-mediated gene transfer trials for hemophilia B have

demonstrated a good safety profile and the potential of a transformational cure.

To reach the protective FIX activity levels, Spark Therapeutics and Pfizer have developed a novel gene transfer product (SPK-9001), using the codon-optimized naturally occurring human FIX variant (hFIX-Padua) delivered by AAV-Spark100 novel capsid for the treatment of hemophilia B. The hFIX-Padua variant has a higher specific activity than the wild-type protein. Presenting the interim results from the ongoing phase I/II multicenter, open-label, dose-escalation gene transfer study of SPK-9001 (AAV-hFIX-Padua) in hemophilia B adults with $\leq 2\%$ FIX activity, Prof. Linden reported that at the starting dose of 5×10^{11} vg/kg of SPK-9001, the mean steady-state FIX activity was $31.9\% \pm 7.4\%$ at 12 to 52 weeks follow-up. In the 9 patients dosed with a cumulative follow-up period of 238 weeks there was a 99% reduction in factor use in these subjects at an estimated saving of > 1.1 million IU FIX and an estimated cost saving of > USD 2.1 million. To date, SPK-9001 appears to be well tolerated with no major safety concerns and to Prof. Linden's knowledge, the starting dose of SPK-9001 is the lowest dose thus far to achieve detectable FIX levels > 5% of normal in AAV-mediated gene transfer trials for hemophilia B.

Much work though still remains for gene therapy medicines, Prof. Linden concluded. In both historical trials and the current study, patient-to-patient variability in expression and immune response to vector is observed, the source of which is unknown and not predicted by current in vivo animal models. The lifetime of expression and mechanism for persistence is still yet to be fully understood and the manufacturing of gene therapy products at a commercial scale remains a challenge.

Gene Activation Using saRNA—An Emerging Therapeutic Approach with Broad Potential

David Blakey, of MiNA Therapeutics Ltd., spoke on 'Gene activation using short activating RNA (saRNA)—an emerging therapeutic approach with broad potential.' Small activating RNAs (saRNAs) are short, double-stranded oligonucleotides that are designed to selectively increase gene transcription. Previous work had shown that a designed saRNA could upregulate the transcription factor CCATT/enhancer-binding protein α (C/EBP- α). Rapidly, this saRNA oligonucleotide was further optimized and developed into a clinical candidate. A 'nucleotide walk', performed around hotspots for saRNA activity in the *CEBPA* gene, identified by bioinformatics analysis and subsequent design, led to the selection of CEBPA-51—this saRNA upregulates CEBPA mRNA 2.5-fold and C/EBP- α target gene serum albumin by 2.3-fold in human hepatocellular carcinoma HepG2 cells. A nuclear run-on assay confirmed this is a transcriptionally driven process. Mechanistic experiments demonstrated that argonaute-2 (Ago2) is required for saRNA activity, with

the guide strand of the saRNA duplex shown to be associated with Ago2 and localized at the CEBPA genomic locus using RNA ChIP assays. Mutations in the seed sequence of CEBPA-51 caused a complete loss of activation, supporting a sequence-specific on-target saRNA activity of CEBPA-51. The CEBPA-51 saRNA has been formulated in SMARTICLES nanoparticles (MTL-CEBPA) for liver delivery. When administered at 4 mg/kg over 2 weeks, it leads to 90% inhibition of tumor growth in a diethylnitrosamine-induced cirrhotic liver cancer model and improvement in liver function parameters such as bilirubin, AST and ALT. MTL-CEBPA dosed at 1-3 mg/kg in a CC14-induced rat model of fibrosis and liver failure demonstrated reversal of fibrosis, improved liver function and improvement in survival. MTL-CEBPA is currently in a phase I clinical trial for patients with liver cancer, and is the first human study of a saRNA class therapeutic. Given the positive efficacy observed in the rat fibrosis model, the data also supports additional trials of MTL-CEBPA for liver cirrhosis.

Gene-modified Immune Cells: Next-generation Cancer Therapy

Emma Morris, of the UCL Institute of Immunity and Transplantation, Royal Free Hospital, London, closed the meeting with a tour de force presentation covering 'Gene-modified immune cells as next generation cancer therapy.' Immunotherapies, applied against cancer, infection and immune disorders, are now being tested widely in early phase clinical trials across Europe and the U.S. These approaches rely on engineered genetic modification of T cells, leading to altered specificity or function (or both).

Altering the specificity of T cells can be achieved through the introduction of chimeric antigen receptors (CARs) or T-cell receptors (TCRs) using either retro- or lentiviral vectors. CARs are modified single-chain antibody fragments that recognize cell-surface tumor antigen and are not limited by MHC restriction. However, CARs are unable to recognize tumor-associated intracellular proteins, which are presented on the cell surface in the context of MHC presentation; for these, TCRs are required to target T cells to these intracellular-derived antigens. Morris' research has focused on modifying the function, persistence, homing, cytotoxicity, cytokine secretion profile and differentiation status of T cells, providing a platform for the generation of next-generation 'designer' immune cell-based therapies.

Conclusions

In summary, the Society for Medicines Research (SMR) meeting on gene therapy provided a current and broad overview of gene therapy discovery and development, using an increasing number of therapeutic modalities.

A key unifying feature of these is the possible exquisite targeting, and more excitingly, in the context of an industry that has typically been focused on discovery and development of small-molecule therapeutics, the ability to restore function to a broken molecular process. Although many hurdles still exist, it is truly an exciting time for molecular medicine.

Disclosures

P. Weber, M. Konneh, P. Jeffrey and J. Overington are in paid employment of their respective organizations. All authors are SMR Committee members for which no remuneration is paid.

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