Anexos

ASGCT LATIN AMERICAN SYMPOSIUM

Symposium Title: *Successes in Clinical Gene Therapy: from cancer to genetic diseases.*

Katherine Ponder, MD

Presentation Title: Vectors for Gene Therapy

Presentation Summary:

Gene therapy involves the transfer of genetic material that encodes a therapeutic gene into cells of the body. *Ex vivo* gene therapy involves removal of cells from the body, genetic modification in tissue culture, and re-infusion into the patient; this has been used to modify hematopoietic stem cells. *In vivo* gene therapy involves injection of a gene into a blood vessel or locally into a specific organ or tissue. Intravenous injection is efficient at modifying liver cells because of their excellent blood supply and the direct contact between blood and hepatocytes, while direct injection has modified cells in the eye and brain. All gene therapy approaches require a vector, which is a piece of genetic material that produces a messenger RNA that encodes a therapeutic protein, or encodes an siRNA that can reduce expression of a gene. Although some vectors are simple circles of DNA that can be produced in bacteria, naked DNA has generally not been efficient at transferring genetic material into cells. In contrast, viruses have evolved effective mechanisms to transfer genetic information into cells. Thus, most gene therapy trials have used so-called viral vectors which are able to transfer genetic information into a cell, but have been crippled to prevent them from replicating in the body. A simple example of a gene therapy vector is the retroviral vector. A wild-type retrovirus has elements that are necessary for packaging into a viral particle and integration into the chromosome, as well as sequences that code for proteins that are necessary for producing a viral particle such as the *GAG*, *POL*, and *ENV* genes. A retroviral vector can be generated by deleting the genes that encode the viral proteins and replacing them with a therapeutic gene. Although this vector cannot replicate on its own, viral particles can be produced in cells that also contain the genes that encode the viral proteins. Cloning of a therapeutic gene into a retroviral vector is simple, and a transfection into mammalian cells that already contain the viral protein genes is straightforward. Large scale culture can produce viral particles, which can then be frozen for later use. These vectors bind to a cell surface receptor, which results in transfer of the genetic information into the cell, after which the RNA that is the genome of retroviral vector is copied into DNA via reverse transcriptase, and integrates into the chromosome and is maintained for the life of the cell. There are many other types of viral vectors that use the same principle of deleting genes that encode viral proteins, replacing them with the therapeutic gene, and producing them in cells that also express the genes for viral proteins. Other vectors that are used for gene therapy include lentiviral vectors (a subset of retroviral vectors), adenovirus-associated virus (AAV) vectors, and adenoviral vectors. The advantages and disadvantages of different vectors will be discussed.

Katherine Ponder, MD

Presentation Title: Gene Therapy for Lysosomal Storage Diseases

Presentation Summary:

Lysosomal storage diseases (LSD) are due to deficiency of enzymes that degrade a variety of compounds in the lysosome, and result in the accumulation of storage material and clinical symptoms that vary according to the specific disease. Hematologists can encounter patients with lysosomal storage diseases such as Gaucher disease or mucopolysaccharidosis (MPS) that are treated with enzyme replacement therapy (ERT), or patients with MPS, Krabbe disease, or other LSD that receive hematopoietic stem cell transplantation. Three approaches have been taken to achieve gene therapy for LSD in humans and/or animal models. Ex vivo hematopoietic stem cell-directed gene therapy has been used to treat adrenoleukodystrophy, and has slowed, but not eliminated neurological deterioration , while direct injection into the brain has been used to treat MPS in animal models (2). Finally, intravenous injection of vectors has been used to treat MPS in animal models, and the clinical effect evaluated for up to a decade. This talk will focus on the effect of neonatal intravenous injection of a retroviral vector in the canine model of MPS VII, which is due to b-glucuronidase (GUSB) deficiency and results in accumulation of the glycosaminoglycans heparan, dermatan, and chondroitin sulfate. Newborn MPS VII dogs were injected intravenously with a retroviral vector expressing canine GUSB, which resulted in transduction of liver cells that secreted GUSB that was modified with mannose 6-phosphate, which is necessary for uptake of enzyme by cells. This resulted in serum GUSB activity that varied considerably from near-normal to 100-fold normal, and was stable for the lifetime of individual dogs. Treated MPS VII dogs had a median survival of 6 years, which was 20-fold that of untreated dogs at 0.3 years. One treated dog survived 11 years, while untreated dogs are invariably dead by 2 years. Treated dogs could all walk throughout their lives due to improved bone and joint disease, while untreated dogs could not stand after 6 months of age. Treated dogs also had improvements in their cardiovascular system. The clinical effect was similar in all animals, suggesting that near-normal levels of enzyme in serum were sufficient to exert a beneficial effect. However, one of 50 treated dogs developed a benign hemangioma in the spleen that expressed retroviral vector sequences at 8 years of age, and it is likely that insertional mutagenesis played a role in the generation of the tumor. We conclude that neonatal intravenous injection of a retroviral vector expressing GUSB resulted in stable expression for up to 11 years (the duration of evaluation) and marked clinical improvement in MPS VII dogs, but the development of a benign tumor expressing retroviral vector sequences raises safety concerns. Current studies are attempting to identify a vector with similar efficacy that does not have the ability to activate expression of oncogenes located near the vector integration site. It is likely that systemic gene therapy will be successful for several LSD in the near future, although no clinical trials have yet been initiated.

Adrian Thrasher, MD, PhD

Presentation Title: Gene Therapy Approaches for Primary Immunodeficiencies

Presentation Summary:

At the start of the 1990s, the first clinical trials of gene therapy were attempted for an inherited severe combined immunodeficiency (SCID) caused by deficiency of the intracellular enzyme adenosine deaminase. In the absence of definitive treatment, SCID of any molecular type is usually fatal within the first year of life, although patients with ADA deficiency can be supported by administration of exogenous enzyme replacement. Even so, this is often only partially effective, and is extremely expensive. The rationale for the development of gene therapy for SCID therefore derives from the severity of the illness, the inadequacy of conventional therapy, and the considerable morbidity and mortality associated with stem-cell transplantation, particularly from a mismatched donor. Efficacy in these early studies was limited, but a decade further on, gene transfer technology and cell handling protocols had been refined sufficiently to produce real clinical benefit. Four recent studies have demonstrated highly effective gene therapy for the X-linked form of SCID (SCID-X1) and ADA deficiency, using retroviruses to deliver the therapeutic genes into haematopoietic stem cells *ex vivo* (two examples from our own centre referenced below). Similar 'proof of principle' studies have been conducted in patients with Chronic Granulomatopus Disease and Wiskott-Aldrich Syndrome. Bearing in mind the outcome and adverse effects of conventional therapy, these are remarkable results and the first clear indication that gene therapy can offer a cure for some human

diseases. In a few patients the treatment has failed, indicating that there is more to learn about the effective dose of corrected cells and the potential for host factors to influence immune cell development. Many different types of vector have been tested in laboratory experiments to deliver therapeutic genes, and their effectiveness is largely determined by the host and tissue type. For stable gene transfer to dividing cells, such as haematopoietic cells, the new genetic material has to be retained through cell division and passed on to daughter cells. Although retroviruses are highly effective for this, their dependence on chromosomal integration brings with it the risk of inadvertent gene activation or inactivation. Having initially achieved successful immunological reconstitution, several patients with SCID-X1 (out of a total of 19 treated worldwide) developed T cell lymphoproliferative disease up to 6 years after the gene therapy procedure. In four of these patients, the enhancer sequences in the retroviral vector, which are responsible for effective transgene expression, had activated the *LMO-2* proto-oncogene. There are likely to be other factors that contributed to cell transformation, but they have not yet been defined. It is therefore unclear whether all patients are at significant risk, or whether this is more pronounced in a few with SCID-X1. However, reports of similar adverse events in other applications, once again in the context of early generation gammaretroviral vectors, indicates that this technology requires considerable refinement. Fortunately, it is likely that much can be done to improve efficiency and safety of current protocols, and these developments have recently entered clinical testing. The design of vectors used for gene delivery is clearly important, and modifications are possible that limit the risks of mutagenesis, such as incorporation of DNA and RNA insulator sequences in integrating vectors; use of self-inactivating vectors in which the powerful viral enhancer sequences are deleted; or targeting of safe regions in the genome. Ultimately, the development of homologous recombination or gene repair to accurately correct genetic mutations, or the construction of mitotically stable extrachromosomal vectors, would obviate many of these problems, even though current technologies are inefficient.

Michael Milone, MD, PhD

Presentation Title: Building supernatural immunity to cancer using chimeric antigen receptors

Presentation Summary:

The immune system possesses significant cytotoxic potential. Stimulating natural immunity through vaccination has shown promising effects in some cancers; however, a number of barriers limit the efficacy of the natural immune system including tolerance to self-antigens and immunodeficiency associated with cancer and associated chemotherapy. Adoptive immunotherapy using T-cells that are genetically modified to express an artificial receptor that combines the antigen specificity of an antibody with the signal transduction machinery of the T-cell receptor in a single chimeric antigen receptor (CAR) hold significant promise for overcoming many of the barriers to anti-cancer immunity. This talk will describe the design and production of T-cells bearing CARs that target CD19, a molecule expressed by normal B-cells and cells in a range of B-cell malignancies including acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). The use of lentiviral vectors for the efficient generation of anti-CD19 CAR-modified T cells (CART19) will be reviewed. The remainder of the talk with focus on the application of CAR-modified T cells in the treatment of B-cell malignancy. Results from phase I clinical trials at the speaker's institution using CTL019 (ClinicalTrials.gov Identifiers: NCT01626495, NCT01747486) and trials at other institutions will be presented. Toxicity related to CART19 cell therapy that includes a frequent cytokine release syndrome and long term B-cell aplasia will also be discussed.

Valder Arruda, MD, PhD

Presentation Title: Gene therapy for hemophilia: progress and challenges

Presentation Summary:

Over the last decade a longstanding goal of gene therapy for hemophilia was envisioned by the characterization of the FVIII and FIX genes, and by the availability of large animal models of the disease for critical preclinical studies. Optimization of recombinant viral vector expression systems and vector manufacture allow for the initiation of clinical trials. The use of adeno-associated viral (AAV) vectors for expression of FIX resulted in the expression of therapeutic levels of the transgene. Recent advances in the field led to the successful long-term sustained expression of therapeutic levels of FIX with consequent amelioration of severe to moderate/mild phenotypes of hemophilia B subjects. These findings raise the possibility that a similar approach for hemophilia A could be envisioned with ongoing development of promising novel strategies for FVIII expression

Jean Bennett, MD, PhD

Presentation Title: The retina as a gene therapy target: Progress and Prospects

Presentation Summary:

Jean Bennett MD, PhD

Gene therapy has restored sight in patients with Leber's Congenital Amaurosis (LCA), an inherited blindness that begins at birth. This presentation will describe the safety and efficacy results after gene therapy in 12 subjects, born with LCA. The subjects were treated at The Children's Hospital of Philadelphia in one eye and all eligible subjects have had re-administration to the contralateral eye. The presentation will also describe how this data has been used to initiate a Phase 3 study aimed at obtaining US Food and Drug Administration approval of this intervention. While LCA is a rare blinding disease, the results from these studies could pave the way for development of other gene -based treatments for more common forms of blindness and for other inherited diseases.