Gene Therapy in Hemophilia

Prof. Bulent Antmen, MD
KEY SUBJECTS:

- Gene therapy
- Vectors and non-viral vectors
- Risks and regulations
- Hemophilia and gene therapy
- Clinical trials and problems last decade
- The first successful gene therapy in Hemophilia B
- Potential Complications
- Conclusion and near future
Gene Therapy

- Developed in the 1970’s as a product of molecular biology
- **Gene Therapy** - technique that uses genes as a means of treatment and/or prevention for particular diseases and/or disorders caused by genetic defects
- **Includes**: Replacing a mutated gene with a healthy copy, Inactivating a mutated gene, and Introducing a new gene into the body
- In the 1980’s researchers developed a method to insert a gene into the cell
Vectors

- **Vector** – carrier molecule used to deliver the healthy gene to the target cell; most common vectors are viruses, which have been genetically modified to carry normal human DNA
- **Viral Vectors** – Retrovirus, Adenovirus, and Adeno-associated virus
- **Non-Viral Vectors** – Sleeping Beauty Transposon ™ System (SBTS) and Phi C integrase
- In 1990, researchers at the National Institute of Health first used gene therapy as treatment for a patient with severe combined immune deficiency (SCID).
Non-Viral Vectors

- Over the last ten years, two non-viral methods of gene delivery have been developed,
- **Transposon** - segment of DNA, which can be integrated at many different sites along a chromosome
- **Sleeping Beauty Transposon™ System** - utilizes a unique gene found in fish, can be used to insert FVIII of FIX, once inserted it becomes permanently saved; random insertion, however, significantly less likely to cause adverse reactions compared the use of viral vectors;
- **Phi C integrase** - similar to the SBTS, however insertion is not random; DNA is only inserted in limited number of locations; even safer than the SBTS

Researchers are optimistic about both the SBTS and the Phi C integrase delivery methods; however, because these non-viral delivery methods have been discovered only within the last ten years much remains to be unknown and researchers need to learn more before these methods can be safely tested in humans.
Associated Risks

• **Immune Response** - an individual’s immune system may view the newly introduced gene as a virus and attack

• **Viral Spread** - viral vectors may infect other cells in addition to the target cells that they were intended

• **Reversion of the Original Virus** - the virus may recover its original ability to cause disease

• **Tumor** – a tumor may form if the gene is inserted into the wrong location in the genome

• **Reproductive Damage** - reproductive cells may also be affected due to the new DNA that has been introduced
Regulation & Safety

- The **National Institute of Health** establishes guidelines for researchers and institutions to adhere by when conducting gene therapy clinical trials.
- The **U.S. Food and Drug Administration** regulates all clinical trials to help protect the individuals who participate.
- FDA also has the authority to intervene, deny, or discontinue any research that may be unsafe for those involved.
Hemophilia is an attractive disease for gene therapy for many reasons.

It is a relatively prevalent disorder compared with many other monogenetic disorders and the functions of the missing proteins are well studied.

For over 25 years, it has been suggested that hemophilia is a particularly favorable condition for correction via gene therapy. Clinical trials for hemophilia A conducted over a decade ago used a variety of gene delivery strategies including FVIII-transformed autologous fibroblasts, and FVIII-expressing retrovirus or gutted adenovirus.

No persistent FVIII activity was achieved in these trials.
Table 1. Currently ongoing product development.

<table>
<thead>
<tr>
<th>Technique used</th>
<th>Product</th>
<th>Published clinical results hemophilia indication</th>
<th>Clinical studies</th>
<th>ClinicalTrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEGylation</td>
<td>FVIII</td>
<td>No</td>
<td>2 Phase 1/II compl., Phase III open</td>
<td>NCT01184820, NCT01205724, NCT01480180</td>
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<td></td>
<td>FIX</td>
<td>Yes [Negrier et al. 2011]</td>
<td>Phase 1/II compl., Phase III open</td>
<td>NCT00956345, NCT01333111, NCT00922792, NCT00951405</td>
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<tr>
<td></td>
<td>FVIIa</td>
<td>Yes [Moss et al. 2011]</td>
<td>Phase 1 compl., Phase II compl.</td>
<td></td>
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<tr>
<td>PEGylated liposomes</td>
<td>FVIII</td>
<td>Yes [Spira et al. 2006, 2008, 2010]</td>
<td>Phase III and project closed due to lack of efficacy</td>
<td>NCT00245297, NCT00629837, NCT00623727</td>
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<tr>
<td>Polysialic acid</td>
<td>FVIII</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Albumin fusion</td>
<td>FVIIa</td>
<td>No</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>FIX</td>
<td>No</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Fc fusion</td>
<td>FVIII</td>
<td>No</td>
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<td></td>
<td>FIX</td>
<td>Yes [Shapiro et al. 2011]</td>
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<td>NCT01027377, NCT00716716</td>
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<td>Increased activity</td>
<td>FVIIa</td>
<td>Yes [Moss et al. 2009]</td>
<td>Phase I and II compl.</td>
<td>NCT00486278, NCT01392547</td>
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<tr>
<td>Technique used</td>
<td>Product</td>
<td>Published clinical results hemophilia indication</td>
<td>Clinical studies</td>
<td>ClinicalTrials.gov identifier</td>
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<tr>
<td>Gene therapy</td>
<td>FVIII, FIX</td>
<td>Yes, reviewed by Pierce et al. [2007]</td>
<td>Two phase I trials ongoing</td>
<td>NCT00979238, NCT00515710</td>
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<tr>
<td>Anti-TFPI</td>
<td>antibody aptamer</td>
<td>No</td>
<td>Phase I ongoing</td>
<td>NCT01228669</td>
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<td></td>
<td></td>
<td>No</td>
<td>Phase I registered</td>
<td>NCT01191372</td>
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<tr>
<td>Read-through of premature stop codons</td>
<td>Hemophilia A or B</td>
<td>No</td>
<td>Phase II study suspended</td>
<td>NCT00947193</td>
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<td>Transgenic animals</td>
<td>FVIII, FIX</td>
<td>No</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Transgenic tobacco plants</td>
<td>FIX</td>
<td>No</td>
<td>–</td>
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TFPI, tissue factor pathway inhibitor
Hemophilia A; Clinical Trials–Retroviral Vector

- **Mice** - Retroviral vectors with high levels of human FVIII were injected intravenously into thirteen newborn, FVIII deficient mice
- 6/13 displayed high levels of functional FVIII; 5/6 mice began to produce FVIII
- 6/7 mice that had been injected with retroviral vectors, **not** displaying functional FVIII, developed inhibitors
Hemophilia B; Clinical Trials—Adenoviral Vector

- **Canines** - Hemophilia B canines displayed increased FIX levels after being injected with an adenoviral vector; however, the levels rapidly decreased to 1% of the normal level three weeks post vector injection and 0-1% two months post injection.

- **Mice** - Factor IX deficient mice were given a comparable dose of the same vector. The mice displayed 20% of the normal FIX level for four months.

- **Non-Human Primate** - Eighty percent FIX levels were achieved at the highest dose, followed by a dramatic decrease in human FIX levels undetectable three weeks post injection.

- *It was concluded that larger animal subjects which had been injected with the adenoviral vectors produced FVIII or FIX for shorter periods compared to smaller animals.*
Hemophilia B; Clinical Trials– AAV Vector

- The most promising viral vectors being studied currently are those derived from adeno-associated viruses (AAV)
- Canines have been permanently cured using AAV vectors
- **Human 1** - 80-90 intramuscular leg injections, no side effects and no immune responses; however, only very low levels of FIX were displayed
- **Human 2** - another AAV vector was injected into the liver. The results were similar, low vector dose produced no side effects and the patient displayed very low FIX levels
- **Human 3** - a higher dose of the AAV vector was administered. He showed significant levels of FIX in his blood; however, he developed minor side effects, which indicated an immune response and liver toxicity

*It was concluded that countless injections would be required to cure hemophilia in humans*
Gene Therapy

- In the last 3–5 years,
- Several pioneering applications in gene therapy have resulted in measurable and sustained phenotypic corrections in human clinical trials.

- In particular, the use of the adeno-associated virus (AAV) for the correction of monogenic disorders has directed lasting clinical improvement in conditions as various as a retinopathy, a neurodegenerative disorder, dyslipidemia, and in hemophilia B.
• There are two phase I/II gene therapy trials in hemophilia B registered [ClinicalTrials.gov identifiers: NCT00979238 and NCT00515710].

• The publication of a successful gene therapy approach in adenosine deaminase deficient severe combined immuno-deficiency (SCID) patients and the recent publication of gene transfer in hemophilia B by Nathwani et al. provides hope that gene therapy is a possibility.
Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

• The first and still the only human gene therapy trial for any bleeding disorder to achieve consistently measurable and persisting clotting factor expression.

• This trial, which used a self-complementary AAV serotype 8 codon-optimized FIX vector, posed ongoing safety concerns at the highest dose, defining the challenge for attempts to advance the AAV factor IX approach.
Table 2. Typical inclusion criteria in AAV gene therapy trials, current and proposed

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Note</th>
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<tbody>
<tr>
<td>Severe hemophilia B: FIX $\leq$ 1%</td>
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<td>Age $\geq$ 18 y</td>
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<tr>
<td>HCV RNA viral load-negative*</td>
<td></td>
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<tr>
<td>HIV-negative†</td>
<td></td>
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<tr>
<td>No previous history of FIX inhibitor</td>
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<tr>
<td>At least 20 exposure days to FIX concentrate</td>
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<tr>
<td>Anti–AAV-neutralizing antibody titer $\leq$ 1:5</td>
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*Earlier trial included subjects who were HCV RNA viral load-positive, but they are now excluded because of potential need for course of steroids.
†Some trials may include HIV-positive persons who are stable with adequate CD4 counts on highly active antiretroviral therapy.
METHODS

We infused a single dose of a serotype-8–pseudotyped, self-complementary adeno-virus-associated virus (AAV) vector expressing a codon-optimized human factor IX (FIX) transgene (scAAV2/8-LP1-hFIXco) in a peripheral vein in six patients with severe hemophilia B (FIX activity, <1% of normal values). Study participants were enrolled sequentially in one of three cohorts (given a high, intermediate, or low dose of vector), with two participants in each group. Vector was administered without immunosuppressive therapy, and participants were followed for 6 to 16 months.
RESULTS

AAV-mediated expression of FIX at 2 to 11% of normal levels was observed in all participants. Four of the six discontinued FIX prophylaxis and remained free of spontaneous hemorrhage; in the other two, the interval between prophylactic injections was increased. Of the two participants who received the high dose of vector, one had a transient, asymptomatic elevation of serum aminotransferase levels, which was associated with the detection of AAV8-capsid–specific T cells in the peripheral blood;
RESULTS

had a slight increase in liver-enzyme levels, the cause of which was less clear. Each of these two participants received a short course of glucocorticoid therapy, which rapidly normalized aminotransferase levels and maintained FIX levels in the range of 3 to 11% of normal values.
CONCLUSIONS

Peripheral-vein infusion of scAAV2/8-LP1-hFIXco resulted in FIX transgene expression at levels sufficient to improve the bleeding phenotype, with few side effects. Although immune-mediated clearance of AAV-transduced hepatocytes remains a concern, this process may be controlled with a short course of glucocorticoids without loss of transgene expression. (Funded by the Medical Research Council and others; ClinicalTrials.gov number, NCT00979238.)
Potencial Complications of Gene Therapy in Hemophilia

<table>
<thead>
<tr>
<th>General</th>
<th>AAV-FIX specific*</th>
<th>Potential solutions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene silencing</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Genotoxicity</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
| Immunotoxicity          | Neutralizing antibodies that block delivery | Current; exclude subjects with neutralizing antibodies  
Plasmapheresis?  
Pharmacologic reduction of antibodies?  
Prednisolone | 23, 24, 25 |
| Phenotoxicity           | T-cell response to capsid     | —                                                                                    | 2          |
| Vertical transmission   | Risk of germline transmission | Barrier birth control until semen is negative by PCR                                  | 14         |
| Horizontal transmission | —                             | —                                                                                    | —          |

— indicates not applicable.
*Based on observations in clinical trials.
Potential applicability to hemophilia A
The use of an AAV vector to direct expression of FVIII in mice was first reported by Burton et al in 1999. To circumvent the limitations imposed by the limited genome packaging capacity of AAV vectors (∼ 5 kb is the maximum size of a single-stranded DNA fragment that can be accommodated within the AAV capsid), they used 2 vectors: one encoding the heavy chain and the other the light chain. Subsequently Chao et al reported the use of a single vector expressing B domain-deleted FVIII to correct the bleeding diathesis in hemophilia A mice. The published data in large animal models, which have been a better guide to dosing in humans than have mouse data, required doses on the order of $0.6-4 \times 10^{13}$ vg/kg to achieve circulating levels of canine FVIII in the range of 1%-7.8%. These doses are at least a log higher than doses
Conclusion

• Gene therapy is a promising treatment option for a number of genetic diseases and/or disorders

• “Safe, long-term expression of clotting factors has been successfully achieved in hemophilia using multiple gene transfer strategies

• There is still a vast amount that researchers and/or scientists must fully understand to ensure safety

• Currently, adeno-associated viral vectors are being pursued as a means of achieving long-term production of both FVIII and FIX
Conclusion

- Although there was evidence of gene transfer and therapeutic effects in some of these trials, stable expression of therapeutic FVIII or FIX levels has not yet been obtained long time or life long.

- Further improvements of the vectors and a better understanding of the immune consequences of gene transfer are warranted and ongoing.

- A review of the approach to achieving clearly measurable and persistent correction of hemophilia using a self-complementary AAV serotype 8 vector to deliver an optimized factor IX cDNA to the liver and a review of the path to this milestone highlights the ongoing experimental and translational challenges.
Future;

• Two international multicenter gene transfer phase I clinical trials in human up 12 years will be conducted using direct in vivo gene delivery with AAV-8 vectors in Hemophilia B and A.
In Turkey and Gene Therapy

• Kavakli and et al
• Ege University, Faculty of Medicine, Dept. Of Pediatric Hematology
Thank you for your attention...