PROGRESS IN GENE THERAPY FOR HEMOPHILIA A AND B

Glenn Pierce MD PhD
Vice President, Medical
World Federation of Hemophilia
La Jolla, California, USA
I currently have, or I have had in the past two years, an affiliation or financial interest with business corporation(s):

(1) Consulting fees:
   BioMarin, Genentech/
   Roche, Shire, Pfizer, St Jude

(2) Research fundings:
   None

(3) Boards, Employment:
   Voyager Therapeutics, Third
   Rock Ventures, Ambys Medicines
Role of Technology Advances in Evolving Hemophilia Care

Brief History of Hemophilia Treatments

- **Plasma-Derived Clotting Factors (1969)**: Widespread viral contamination
- **Recombinant Clotting Factors (1990s)**: Eliminated potential for transmission of blood-borne pathogens
- **EHL Clotting Factors (2014 - )**: Biosimilars, Humanized
- **Novel Platform Therapies (2015 - )**: Gene therapies, Bi-specific monoclonal antibodies, siRNAs

24/7 coverage
More prophylaxis
Some prophylaxis
On-demand

THE SPREAD OF HCV AND HIV THROUGH THE BLOOD SUPPLY AND ITS LASTING IMPACT
1% TROUGHS ARE A HISTORICAL ARTIFACT

Treatment Goal: Prevent and Treat Bleeding

Non-factor therapies include 5 in clinical testing: Emicizumab (marketed), Fitusiran, and 3 anti-TFPIs; EHL, extended half life

Mazepa et al Blood 2016
WHY GENE THERAPY FOR HEMOPHILIA?

Scientists
• “Perfect” disease to study with this novel biotechnology
  single gene defect with directly correlated phenotype
• Wide target range
  5-150% factor activity levels
  no tight regulation needed
• Model for progress

Patients
• High burden of replacement therapy
• Continued risk of Morbidity and Mortality despite prophylaxis due to
  • Target 1% factor troughs
  • Variable compliance with regimen
• 70% of world - no access to clotting factor, no hope in foreseeable future

Almost every genetic and acquired disease has been studied in animal models. >200 human trials using AAV – based gene therapy have been conducted in the past 30 years.

Image: National Human Genome Research Institute's Talking glossary
ADENO-ASSOCIATED VIRUS: ADVANTAGES AND DISADVANTAGES

Advantages

• Wild type not pathogenic
• Many serotypes evolved with different species and tissue specificities
• Minor, transient toxicity as vector administration
• Only a minor amount of integration
• It works

Disadvantages

• Difficult to manufacture
• Small payload capacity
• Variably high levels of immunity to many serotypes
• Antibody response precludes 2nd administration
• Variable toxicity upon administration
• Minor amount of integration
• Duration of effect unclear
• Variable responses
• Many steps from manufacture to protein expression in vivo not understood
DISRUPTIVE TECHNOLOGIES: BALANCING BENEFITS AND RISKS

BENEFITS of Gene Therapy
• Elimination of factor troughs
• Phenotypic “cures”
• Benefit/risk differs in developing world

RISKS of Gene Therapy
• May not be immediately obvious
• Known knowns
• Known Unknowns
• Unknown unknowns*


## Gene Therapy for Hemophilia: Early Clinical Trials Late 1990s-2005

**Clinical studies for gene and cell therapy of hemophilia A and B.**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Vector</th>
<th>Route of administration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A</td>
<td>Transient transfection of hFVIII into autologous dermal fibroblasts</td>
<td>Transplantation of fibroblasts into omentum</td>
<td>Transient elevation of FVIII levels in three out of six patients</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>Retroviral vector expressing B-domain deleted hFVIII</td>
<td>Intravenous: peripheral vein</td>
<td>Transient elevation of FVIII levels in nine out of 13 patients</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>Gutless adenoviral vector expressing full-length hFVIII</td>
<td>Intravenous</td>
<td>Toxic reaction in one patient, resulting in discontinuation of treatment</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>rAAV2 vector expressing hFIX</td>
<td>Intramuscular: skeletal muscle</td>
<td>Effective gene transfer into muscle tissue. Therapeutic levels of FIX were not seen</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>rAAV2 vector expressing hFIX</td>
<td>Right hepatic artery</td>
<td>Therapeutic levels of FIX attained for 2–4 weeks at the highest dose of administered vector. This dose was also associated with an immune response against transduced hepatocytes, abrogating clinical benefits</td>
</tr>
</tbody>
</table>

“Transient elevations” – maybe?

In the high-dose group, a consistent increase in the factor IX level to a mean (±SD) of 5.1±1.7% was observed in all 6 patients, which resulted in a reduction of more than 90% in both bleeding episodes and the use of prophylactic factor IX concentrate.

Prednisone was enabling 10 patients treated over >5 years Mean levels ~5% and stable
THE NEXT STEPS: THERAPEUTIC FACTOR LEVELS ACHIEVED

December 2017

Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant

Savita Rengarajan, M.B. B.S., Liron Walsh, M.D., Will Lester, M.D., Ch.B., Ph.D., Christien Vettermann, Ph.D., Glenn F. Pierce, M.D., Ph.D., Bella Madan, M.D., Michael Laffan, D.M., Hua Yu, Ph.D., Michael Y. Wong, M.D., and K. John Pasi, M.B., Ch.B., Ph.D.

AAV5-Factor VIII Gene Transfer in Severe Hemophilia A

John E. J. Rasko, M.B. B.S., Ph.D., Benjamin J. Samelson-Jones, M.D., Lindsey A. George, M.D., Spencer K. Sullivan, M.D., Adam Giermaszewski, M.D., Lie, Adam Cuker, M.D., Calleigh O. M. Majumdar, M.D., Jerome Teitel, M.D., Cathie Y. Altern, M.D., tankin K. Dan, M.D., Margarit V. Ragnhi, M.D., Alvin Y. Luk, Ph.D., Katie W. Wright, Ph.D., Yifeng Chen, M.D., Yuni Liu, Ph.D., Valder R. Vinters, M.P.H., Xavier M. A. H. Winters, M.P.H., Stefano Tiefenbacher, Ph.D., Dani, Olga Zeleznaya, Ph.D., Ph.D., Katherine A. High, M.D., Marcus E. Carr, M.D., Ph.D., Linda B. Couto, Ph.D., Ph.D.


A Cure for Hemophilia within Reach

H. Marijke van den Berg, M.D., Ph.D.


WORLD FEDERATION OF HEMOPHILIA
FEDERATION MONDIALE DE L'HÉMOPHILIE
FEDERACIÓN MUNDIAL DE HEMOFILIA
AAV-Factor IX Studies
Pfizer has initiated a Phase 3 noninterventional study to be followed by delivery of AAV-FIX Padua.

Padua mutant = R338L, 6-8x increased specific activity FIX

Spark data on file, Jan 2019, from K High
UNIQUE PHASE 1/2 RESULTS

Factor IX cassette identical to Nathwani AAV5 vector
Same FIX results as Nathwani
Steroid responsive LFT elevation; no loss of FIX

AMT-061: SINGLE AMINO ACID CHANGE TO AMT-060 TRANSGENE (UNIQURE)

Leucine instead of Arginine at position 338 (R338L)

Enabling mutation: 6-8x increase specific activity

Chang...Stafford, Changing Residue 338 in Human Factor IX from Arginine to Alanine Causes an Increase in Catalytic Activity. JBC 1998; 273, 12089–94
Effect of Padua (R338L) mutant on FIX levels at same 2E13/kg dose as Ph 1/2 (8% FIX)

Efficacy: FIX activity up to 16 weeks post-treatment

Mean FIX activity at 12 weeks: 38.0%

Von Drygalski et al, EAHAD 2019
First gene editing with AAV-Fix

DeKelver et al. Enhancing ZFN Expression Construct and Nuclease Activity Leads to Improvement of In Vivo Genome Editing Platform. ASGCT, 2018
# AAV-FIX Interventional Clinical Studies

<table>
<thead>
<tr>
<th>Drug (sponsor)</th>
<th>Number of patients at highest vector dose (vg per kg)</th>
<th>Mean steady-state factor activity (range of % normal)</th>
<th>Clinical stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor IX</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPK-9001 (Spark Therapeutics)</td>
<td>10 at 5e11</td>
<td>33.3 (14–79)</td>
<td>III, Pfizer</td>
</tr>
<tr>
<td>AMT-060 (uniQure)</td>
<td>5 at 5e12</td>
<td>4.6 (1.6–7.6)</td>
<td>Phase I/II</td>
</tr>
<tr>
<td></td>
<td>5 at 2e13</td>
<td>7.1 (3.2–11.1)</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>AMT-061 (uniQure)</td>
<td>3 at 2e13</td>
<td>~30</td>
<td>Entering phase III</td>
</tr>
<tr>
<td>scAAV2/8-LP1-hFIXco (UCL and SJCRH)</td>
<td>6 at 2e12</td>
<td>5.1 (2.9–7.2)</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>FLT180a (Freeline Therapeutics)</td>
<td>18</td>
<td>NR</td>
<td>Phase I</td>
</tr>
<tr>
<td>SB-FIX (Sangamo) [ZFN]</td>
<td>NR</td>
<td>NR</td>
<td>Phase I/II</td>
</tr>
</tbody>
</table>

2 studies stopped enrollment
- Baxter/Baxalta/Shire/Takeda AAV8-FIX Padua (BAX326): transaminases and steroid unresponsive loss of expression of FIX: high CpGs
- Dimension/Ultragenyx AAVrh10-FIX: high transaminases, low expression native FIX

AAV-Factor VIII Studies
Preliminary *SPK-8011* Phase 1/2 data in hemophilia A: Persistent, stable FVIII activity seen in first two cohort participants out >1 year

5e11 vg/kg dose cohort exhibiting persistent and stable FVIII activity out over 1.5 years at last read

1e12 vg/kg dose cohort exhibiting persistent and stable FVIII activity out to ~1 year at last read

Note: *SPK-8011* data as of November 2, 2018. Data points represent 4-week interval average FVIII activity as measured by the study central laboratory.

*Due to non-adherence with study protocol, most recent reads for Participant 3 are not available in the study database. However, the participant’s home hemophilia treatment center reports a FVIII level drawn in October 2018 (~15 months post-vector infusion) remains stable vs. last reported read in study database.*
FACTOR VIII LEVELS 1-2 YEARS POST-AAV5-FVIII GENE THERAPY

BioMarin Phase 1/2 study, Valoctocogene Roxaparvovec (Valrox)
SUBSTANTIAL REDUCTION IN BLEEDING AND FVIII USAGE POST VALROX

**6E13 Dose Through Week 104**
97% Reduction in Mean ABR

<table>
<thead>
<tr>
<th>ABR (episodes/year)</th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16.5</td>
<td>16.3</td>
</tr>
</tbody>
</table>

All patients off prophylaxis
100% resolution in target joints

**% Patients Bleed Free**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Patients Bleed Free</td>
<td>15%</td>
<td>71%</td>
<td>86%</td>
</tr>
</tbody>
</table>

**4E13 Dose Through Week 52**
92% Reduction in Mean ABR

<table>
<thead>
<tr>
<th>ABR (episodes/year)</th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>12.2</td>
</tr>
</tbody>
</table>

All patients off prophylaxis

**% Patients Bleed Free**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Year 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Patients Bleed Free</td>
<td>17%</td>
<td>83%</td>
</tr>
</tbody>
</table>

**6E13 vg/kg Dose Through Week 104**
96% Reduction in Mean FVIII Usage

<table>
<thead>
<tr>
<th>Annualized FVIII Usage (infusions/year)</th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>139</td>
<td>137</td>
</tr>
</tbody>
</table>

0 5.3

**4E13 vg/kg Dose Through Week 52**
98% Reduction in Mean FVIII Usage

<table>
<thead>
<tr>
<th>Annualized FVIII Usage (infusions/year)</th>
<th>Pre-infusion</th>
<th>Post-infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>156</td>
<td>147</td>
</tr>
</tbody>
</table>

0 2.5
SUMMARY OF SAFETY FOR VALROX PHASE 1/2

• BMN 270 was well tolerated across all doses

• No subject developed inhibitors to FVIII

• No subject withdrew

• Most common AEs across all dose cohorts: ALT elevation (10 subjects, 67%), arthralgia (7 subjects, 47%) and back pain, fatigue, headache (5 subjects each, 33%)

• 2 Subjects reported SAE’s during the study:
  • Grade 2 pyrexia with myalgia and headache at time of infusion; observed in hospital overnight and resolved without sequelae
  • Planned total knee replacement for chronic arthropathy
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<th>Clinical stage</th>
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</thead>
<tbody>
<tr>
<td><strong>Factor VIII</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valoctocogene roxaparvovec (BMN-270; BioMarin)</td>
<td>7 at 6e13</td>
<td>146 (20–218)</td>
<td>Phase III</td>
</tr>
<tr>
<td></td>
<td>6 at 4e13</td>
<td>51 (48–54)</td>
<td>Phase III</td>
</tr>
<tr>
<td>SPK-8011 (Spark Therapeutics)</td>
<td>2 at 5e11</td>
<td>10^b</td>
<td>Phase I/IIa</td>
</tr>
<tr>
<td></td>
<td>3 at 2e12</td>
<td>~13</td>
<td>Phase I/IIa</td>
</tr>
<tr>
<td>SB-525 (Sangamo)</td>
<td>NR</td>
<td>NR</td>
<td>Phase I/IIa</td>
</tr>
<tr>
<td>SHP654 (Shire)</td>
<td>NR</td>
<td>NR</td>
<td>Phase I/IIa</td>
</tr>
<tr>
<td>AAVhu37(BAY2599023) (Bayer/Ultradeutics)</td>
<td>NR</td>
<td>NR</td>
<td>Phase I/IIa</td>
</tr>
<tr>
<td>AAV2/8-HLP-FVIII-V3 (St Jude/UCL) (GO-8)</td>
<td>18@NR</td>
<td>NR</td>
<td>Phase I/IIa</td>
</tr>
<tr>
<td>AAV LKO3-FVIII (Spark/Roche)</td>
<td>NR</td>
<td>NR</td>
<td>Entering Ph III</td>
</tr>
<tr>
<td>Valrox (BioMarin GENEr8-1)</td>
<td>40@6e13</td>
<td>NR</td>
<td>Phase III</td>
</tr>
<tr>
<td>Valrox (BioMarin GENEr8-2)</td>
<td>40@4e13</td>
<td>NR</td>
<td>Phase III</td>
</tr>
</tbody>
</table>

1 study is evaluating efficacy with pre-existing antibodies
• BioMarin AAV5-FVIII: enrolling patients with anti-AAV5 antibodies

1 study is planning on a clinical trial in an inhibitor population
• Spark, undisclosed vector

AAV Vector Biology and Conundrums
Challenges to Successful Gene Therapy


WHAT ARE CAUSES OF LIVER TRANSAMINASE ELEVATIONS?

Variable but small elevations in liver transaminases in multiple AAV clinical studies in hemophilia and other diseases

- First noted in Manno et al, w loss transgene expression
  - Cytotoxic T-cell response to AAV capsid peptides displayed in w HLA class 1
- Nathwani et al administered steroids when LFTs rose, saved some FIX expression
- Rangarajan et al: inc LFTs w/o loss of FVIII, unclear steroid benefit; no evidence of cytotoxic T cell response
- George et al: LFTs up w loss of FIX, FVIII, steroid responsive
- UniQure study, LFTs up, steroid responsive w no loss of FIX

2 independent causes: Immune and intracellular toxicities?

- FVIII induces ER stress and an unfolded protein response
- Long term implications?

CAPSID DOSE AND AAV IMMUNE RESPONSES

F Mingozzi, KA High Blood 2013;122:23-36
# AAV Mediated Gene Therapy for Hemophilia: Conclusions and Outstanding Issues

## Known Knowns

<table>
<thead>
<tr>
<th>Category</th>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAFETY</strong></td>
<td>Integration, Pre-existing immunity, Variable acute subclinical liver toxicity</td>
</tr>
<tr>
<td><strong>DOSING</strong></td>
<td>“High” doses needed</td>
</tr>
<tr>
<td><strong>MOA</strong></td>
<td>Heterogeneous transduction, Variability in cellular protein production</td>
</tr>
<tr>
<td><strong>MANUFACTURING</strong></td>
<td>Commercial scaling; purification and QC assays not well standardized</td>
</tr>
</tbody>
</table>

## Known Unknowns

<table>
<thead>
<tr>
<th>Category</th>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAFETY</strong></td>
<td>Pre-existing immunity, Integration, Mild acute liver toxicity</td>
</tr>
<tr>
<td><strong>EFFICACY</strong></td>
<td>Pediatrics?, Inhibitors?</td>
</tr>
<tr>
<td><strong>DURABILITY</strong></td>
<td></td>
</tr>
<tr>
<td><strong>MOA</strong></td>
<td>Opportunities to enhance transduction efficiency</td>
</tr>
</tbody>
</table>

## Conclusions

- Multiple ongoing Phase 3 clinical trials for both FVIII and FIX
- Benefit/risk appears ok, huge benefit in developing world
- Improvements needed from vector construction to capsid selection to in vivo delivery
- Paradigm shift in treatment is coming