Gene Therapy…

...where are we today... and how did we get here??

Tim MacLachlan
NESOT Fall meeting
Shrewsbury, MA
October 24, 2008
Gene therapy… the rogue biologic

- Gene therapy… what is it exactly?

- Early days of gene therapy
  - Integration of Neo into TIL
  - ADA-SCID

- Safety events… high profile and others
  - Cancer induction
  - Uncontrolled inflammation

- Where is the field now… what can we expect in the future?
Gene therapy... what is it exactly?

<table>
<thead>
<tr>
<th>Type</th>
<th>Approximate insert-size capacity</th>
<th>Integration</th>
<th>Duration of persistence</th>
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</thead>
<tbody>
<tr>
<td><strong>Nucleic-acid-based</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Oligonucleotides: decoys, antisense, ribozymes, siRNA</td>
<td>10–100 bp</td>
<td>No</td>
<td>Hours–days</td>
</tr>
<tr>
<td>Expression plasmids</td>
<td>2–10 kb</td>
<td>Extremely rare</td>
<td>Days</td>
</tr>
<tr>
<td>Transposons</td>
<td>2–10 kb</td>
<td>Efficient, relatively random</td>
<td>Stable</td>
</tr>
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<td>Bacteriophage integrase</td>
<td>2–10 kb</td>
<td>Efficient, site-restricted</td>
<td>Stable</td>
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<tr>
<td>Artificial chromosome</td>
<td>50–300 kb</td>
<td>Episomal</td>
<td>+/- Stable</td>
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<td><strong>Virus-based</strong></td>
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<td>Retroviral</td>
<td>2–6 kb</td>
<td>Efficient, relatively random</td>
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<td>Lentiviral</td>
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<td>Stable</td>
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<td>Adeno-associated virus</td>
<td>2–5 kb</td>
<td>Rare</td>
<td>Months–years</td>
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<tr>
<td>Adenoviral</td>
<td>2–30 kb</td>
<td>No</td>
<td>Weeks–months</td>
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<tr>
<td>Herpesvirus</td>
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<td>Episomal</td>
<td>Months–years</td>
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<tr>
<td>Epstein–Barr virus</td>
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<td>Episomal</td>
<td>Months–years</td>
</tr>
<tr>
<td>SV40</td>
<td>1–5 kb</td>
<td>Moderate</td>
<td>Unproven</td>
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</table>

Bp, base pair; kb, kilobase.
Gene transfer into humans—immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction

SA Rosenberg, P Asberson, K Cornetta, A Kasid, RA Morgan, RM Moen, EM Karson, MT Lotze, JC Yang, SL Topalian, and et al.

- Took tumor infiltrating lymphocytes from melanoma
- Used retrovirus to introduce NEO gene...
- Amplified in neomycin culture...
- Reintroduced into patients...
- Found to be safe
Firsts in gene therapy…

- Defect in adenosine deaminase
  - No nucleotides, no active replication, no immune system
  - Replace ADA with retrovirus into T cells extracted from patient
  - Infuse cells back into patients… long term responses…

T Lymphocyte–Directed Gene Therapy for ADA− SCID: Initial Trial Results After 4 Years


In 1990, a clinical trial was started using retroviral-mediated transfer of the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency (ADA− SCID). The number of blood T cells normalized as did many cellular and humoral immune responses. Gene treatment ended after 2 years, but integrated vector and ADA gene expression in T cells persisted. Although many components remain to be perfected, it is concluded here that gene therapy can be a safe and effective addition to treatment for some patients with this severe immunodeficiency disease.
Classic targets addressed

- X-linked SCID

Gene Therapy of Human Severe Combined Immunodeficiency (SCID)–X1 Disease

Marina Cavazzana-Calvo,*1,2,3 Salima Hacein-Bey,⁎1,2,3 Genèviève de Saint Basile,1 Fabian Gross,4 Eric Yvon,3 Patrick Nusbaum,2 Françoise Selz,1 Christophe Hue,1,2 Stéphanie Certain,1 Jean-Laurent Casanova,1,4 Philippe Bousso,5 Françoise Le Deist,1 Alain Fischer1,2,4†

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Nature Reviews | Cancer
Safety events part I... oncogenesis

Chemotherapy successful in \( \frac{3}{4} \) patients...

BMI1 and CCND2 as well...
Safety events part I... oncogenensis?

- Treatment of MPS VII (lacking b-glucoronidase)
- MPSVII mice were dosed IV **neonatally**
Safety events part II… uncontrolled inflammation

Molecular Genetics and Metabolism 80 (2003) 148–158
Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer

Steven E. Raper, a Narendra Chirmule, b Frank S. Lee, c Nelson A. Wivel, b Adam Bagg, c Guang-ping Gao, b James M. Wilson, b and Mark L. Batshaw d,*

- Jesse Gelsinger case
- Adenovirus serotype 5, E1 and E4 deleted expressing OTC
- Clinical trial had already infused 17 patients at doses ranging from 2e9 to 6e11 particles/kg
  - Previous adverse events were transient elevations in LFTs, thrombocytopenia
- Massive systemic inflammatory response, DIC, multi-organ failure after hepatic artery dose of 6e11 particles/kg
Safety events part II…
uncontrolled inflammation

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Steven E. Raper,a Narendra Chirmule,b Frank S. Lee,c Nelson A. Wivel,b Adam Bagg,c Guang-ping Gao,b James M. Wilson,b and Mark L. Batshawd,*

- Did animal studies predict? A question of debate…
  - An early study investigating administration of Ad-LacZ to rhesus, similar to the therapy except with E4 intact, found a dose of 5e12 safe, but 1e13 acutely hepatotoxic and pursuant DIC
  - Safety study investigating the therapy at doses of 2e9 and 6e11 into baboons found mild and reversible portal inflammation.
  - Using a different vector and transgene, 2 animals died at ~1e13
    - Using the same vector but different transgene, 1 animal became ill
Inflammation continues… CTLs against AAV

Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response

Catherine S Manno1,2,15, Glenn F Pierce3,15, Valder R Arruda1,2,15, Bertil Glader4,15, Margaret Ragni5, John J E Rasko6, Margaret C Ozelo7, Keith Hoots8, Philip Blatt9, Barbara Konkle10, Michael Dake4, Robin Kaye1,2, Mahmood Razavi4, Albert Zajko10, James Zehnder4, Pradip K Rustagi11, Hiroyuki Nakai4, Amy Chew13, Debra Leonard3,12, J Fraser Wright3, Ruth R Lessard3, Jürg M Sommer3, Michael Tigges3, Denise Sabatino1, Alvin Luk3, Haiyan Jiang3, Federico Mingozi3, Linda Couto3, Hildegund C Ertl1,13, Katherine A High1,2,14 & Mark A Kay4

- AAV-FIX for hemophilia B
- Dosed at a range of 8e10 to 2e12 vg/kg through hepatic artery
- Preclinical work…
  - Rhesus at 4e12 vg/kg… no AEs, stable expression for 1 yr
  - Multiple studies in hemophilia B dogs at doses up to 1e12 – stable expression, no AEs
  - All published preclinical work done in different labs with different material
  - Clinical trial injecting vector intramuscularly resulted in no AEs, but little systemic expression
Inflammation continues… CTLs against AAV

- Four patients dosed via hepatic artery at doses of 8e10-4e11… no AEs, but no expression either
- Third cohort (fifth) patient dosed at 2e12…

- Sixth patient dosed at 2e12… no AEs, very low expression
- Seventh patient… backed off to a dose of 4e11
  - No expression
  - Mild transaminitis
  - Cytotoxic T-lymphocytes against AAV capsid identified by 2 weeks post infusion (earliest timepoint)
Inflammation continues… CTLs against AAV

- Conclusion – AAV capsid induces CTLs, could induce toxicity by targeting AAV infected hepatocytes
- Issues with hypothesis
  - Toxicity occurred 4 weeks after infusion… AAV capsid proteins still presented on hepatocytes at this time??
  - CTLs formed 2 weeks after infusion… why the two week delay in hepatocyte targeting??
  - Animals efficiently form AAV-CTLs in preclinical work… yet express transgene long-term with no toxicity

Pre-existing AAV Capsid-specific CD8$^{+}$ T Cells are Unable to Eliminate AAV-transduced Hepatocytes

Hua Li$^{1,4}$, Samuel L Murphy$^{2,4}$, Wynetta Giles-Davis$^{1}$, Shyrie Edmonson$^{2,3}$, Zhiquan Xiang$^{1}$, Yan Li$^{1}$, Marcio O Lasaro$^{1}$, Katherine A High$^{2,3}$ and Hildegund CJ Ertl$^{1}$

$^{1}$Wistar Institute, Philadelphia, Pennsylvania, USA; $^{2}$The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; $^{3}$Howard Hughes Medical Institute, Philadelphia, Pennsylvania, USA

- Result – Sponsors now required by CBER to conduct thorough CTL examination in preclinical safety studies
Onto the future... the bright spots

- New direction... compartmentalized gene therapy
  - Limit spread of therapy, possible immune reactions
  - Concentrate therapy to maximize effect
  - One candidate... the eye
  - Disease candidate - macular degeneration
Onto the future… the bright spots

- Macular degeneration gene therapies…
  - GenVec Ad-PEDF
    - Phase II
    - Preclinical work shows inflammation
  - Genzyme AAV-sFLT01
    - Phase I
    - Preclinical work shows long term efficacy
  - …and mild but persistent inflammation
  - CTLs and humoral response also examined
    - No effect on inflammation
Onto the future... the bright spots

- Retinal genetic diseases... Leber's Congenital Amarosis
  - Defect in RPE65 gene

> therapy restores vision in a canine model of childhood blindness

M. Acland³, Gustavo D. Aguirre, Pearce-Kelling¹, Vibha Arora, John H. Fisher² & Jean Bennett²
### Effect of Gene Therapy on Visual Function in Leber’s Congenital Amaurosis


- Increase in sight

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Mutation</th>
<th>Visual Acuity</th>
<th>ETDRE§</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>F</td>
<td>Homozygote p.Glu102Lys c.304G→A</td>
<td>Hand motion</td>
<td>20/1050</td>
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<tr>
<td>2</td>
<td>26</td>
<td>M</td>
<td>Homozygote p.Glu102Lys c.304G→A</td>
<td>Hand motion</td>
<td>20/710</td>
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<tr>
<td>3</td>
<td>19</td>
<td>F</td>
<td>Homozygote p.R234X c.700C→T</td>
<td></td>
<td>20/640</td>
</tr>
</tbody>
</table>

### Safety and Efficacy of Gene Transfer for Leber’s Congenital Amaurosis

Albert M. Maguire, M.D., Francesca Simonelli, M.D., Eric A. Pierce, M.D., Ph.D., Edward N. Pugh, Jr., Ph.D., Federico Mingozzi, Ph.D., Jeannette Bennicelli, Ph.D., Sandro Banfi, M.D., Kathleen A. Marshall, C.T., Francesco Testa, M.D., Enrico M. Surace, D.V.M., Settimio Rossii, M.D., Arkady Lyubarsky, Ph.D., Valder R. Arruda, M.D., Barbara Konkle, M.D., Edwin Stone, M.D., Ph.D., Junwei Sun, M.S., Jonathan Jacobs, Ph.D., Lou Dell’Osso, Ph.D., Richard Hertle, M.D., Jian-xing Ma, M.D., Ph.D., T. Michael Redmond, Ph.D., Xiaosong Zhu, M.D., Bernd Hauck, Ph.D., Olga Zelenaia, Ph.D., and Raser Wright, Ph.D.,  to Auricchio, M.D., Ph.D.
Where have we been, where are we going?

- Early promise in gene therapy not met with lots of success
- Very high profile safety events stopped progress
- Recent initiatives suggest limiting spread of therapy may be best way to go