Review of Selected ASGT Annual Meeting Sessions related to Retroviral Vectors

- Hematopoietic Marking Studies Session
- Ethical and Policy Dilemmas in Clinical Gene Therapy Studies: Balancing Real Benefits and Real Risks
- SCID-X1 Clinical Trial: Current State

- Marina Cavazzano-Calvo, Hôpital Necker-Enfants Malades in Paris - **Haploidentical Hematopoietic Stem Cell Transplantation vs. Gene Transfer for X-SCID and SCID-X1 Clinical Trial: Current State**
- Adrian Thrasher, Great Ormond Street, London - **Gene Transfer for X-SCID Using a GALV-Pseudotyped Retroviral Vector**
- Phil Noguchi, FDA - **FDA actions**
- Ted Friedmann, UCSD - **RAC actions**

Ethical and Policy Dilemmas in Clinical Gene Therapy Studies: Balancing Real Benefits and Real Risks

- **Haploidentical Hematopoietic Stem Cell Transplantation vs. Gene Transfer for X-SCID and SCID-X1 Clinical Trial: Current State**
  - **Marina Cavazzana-Calvo, M.D., Ph.D.**
- **Gene Transfer for X-SCID Using a GALV-Pseudotyped Retroviral Vector**
  - **Adrian Thrasher, M.D., Ph.D.**

Haploidentical Hematopoietic Stem Cell Transplantation vs. Gene Transfer for X-SCID

- **Current Status of subjects in X-SCID gene transfer trials:**
  - **All subjects reconstituted full TCR repertoire**
    - **15 to 100 integration sites per subject**
    - **TREC normal; thymopoiesis restored at 3 month and maintained to date**
  - **All subjects reconstituted B cell function**
    - **Stopped IVIG at 6 months and maintained Immunoglobulin production**

Haploidentical Hematopoietic Stem Cell Transplantation vs. Gene Transfer for X-SCID

- **Current Status of subjects in X-SCID gene transfer trial:**
  - **P4 and P5 developed T-ALL about 30-34 months post administration**
    - **Both successfully completed chemotherapy**
    - **P4 received an unrelated donor BMT**
      - **CD3, CD5, CD7, CD28, CD45RO positive**
    - **P5 awaiting BMT**
  - **No other subject has developed T-ALL to date (P1 and P2 > 4 years post administration)**

Haploidentical Hematopoietic Stem Cell Transplantation vs. Gene Transfer for X-SCID

- **Current Status of subjects in X-SCID gene transfer trial:**
  - **P4 and P5 had integration in region of LMO-2 oncogene with cis-transcriptional activation**
  - **No "overexpression" of γc transgene compared to expression in developing T cells**
  - **No mutation of transgene**
  - **No abnormal Jak-3/STAT5 signaling/activation**
  - **Normal blast apoptosis**

Haploidentical Hematopoietic Stem Cell Transplantation vs. Gene Transfer for X-SCID

- **Current Status of subjects in Paris X-SCID gene transfer trial: Molecular Analysis**
  - **P4**
    - **Single γδTCR T cell clones**
  - **P5**
    - **Three αβTCR T cell clones**
      - **Single integration site in all clones**
      - **15% Vβ1; 64% Vβ2; 17% Vβ3)**
        - **Vβ1 11% at 6 months**
        - **Vβ1 52% at 17 months (normal total T cell count)**

Proposed Changes to the Gene Transfer for X-SCID Protocol - Paris

- **Enrollment on hold until analysis of current subjects finished more preclinical studies performed**
  - **Complete integration analysis**
  - **Wait another year to allow f/u on most recent subjects**
  - **Develop appropriate, predicable animal model**
  - **Modify vector (SIN, insulator, ?)**
  - **Case by case enrollment going forward**
  - **Only subjects > 3 months of age enrolled**
  - **Between 10^6 and 10^7 transduced $CD34^+$ cells/kg administered**

Great Ormond Street X-SCID Protocol

- Vector strategy similar to French study
- PG13 producer cell
  - (GALV pseudotype; transduction of very early cells)
- 40 hour pre-activation of $CD34^+$
  - *Ex vivo* serum free
  - scf + flt + tpo + IL-3
  - Transduction 3x over 72 hours
  - No conditioning at time of infusion
    - No analysis done to identify the cell types during preactivation and transduction

Great Ormond Street X-SCID Protocol

	Age	Total infused (10^6)
P1	10 mo	180
P2	10 mo	180
P3	4 mo	78
P4	20 yr*	150
P5	3 yr	115

60-100% transduction efficiency; only 30% will be $CD34^+$

* Failed haploBMT; no reconstitution

Great Ormond Street X-SCID Protocol

P1:

- At one year
  - T cell reconstitution to normal level
  - TCR repertoire normal by TCR Vβ usage and normal TCR spectrotype
  - B cell reconstitution
    - IgG level just below normal
    - IgM and IgA normal level
    - Class switch normal
    - Ig spectrotype normal
  - Exposed to VZV
    - Normal clinical course with “normal” anti-VZV T cells (elispot analysis)

Great Ormond Street X-SCID Protocol

P1:

- Severe gastrointestinal complication at 12 weeks with surgery
  - Delayed immune reconstitution

P3/P4/P5:

- Too early post administration
- All continue to receive IVIG

- **Integration analyses ongoing**
  - All show > 100 integration sites

Great Ormond Street X-SCID Protocol

Monitoring of current subjects:

- TCR and Ig spectratype
- Integration analysis
- LMO-2 expression
- VL30 detection (murine vector sequence)
- "Gene Chip" analysis
  - 300,000 genes analyzed
  - 20,000 genes show expression differences compared to "normal" T cells

Great Ormond Street X-SCID Protocol

Future enrollment:

- **Only if can't find matched BMT donor (even unrelated)**
- **Continue no conditioning prior to administration**
- **> 3 months of age**

RAC recommendation

- “pending further data or extenuating circumstances reviewed on case-by-case basis, retroviral gene transfer studies for X-SCID should be limited to patients who have failed identical or haploidentical stem-cell transplantation or for whom no suitable stem cell donor can be identified”

RAC recommendations (cont'd)

- "there are not sufficient data or reports of adverse events directly attributable to retroviral vectors…to warrant cessation of other retroviral human gene transfer studies, including studies of non-X-linked SCID. Such studies *may be justified* contingent upon appropriate risk/benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans".

Haploidentical Hematopoietic Stem Cell Transplantation vs. Gene Transfer for X-SCID

- **European HSCT results:**
  - **T^-B^- SCID: ≈ 50% 1 year survival**
  - **T^-B^+ SCID: ≈ 80% to 90% 1 year survival**

Poor T cell function in a significant number

Haploidentical Hematopoietic Stem Cell Transplantation vs. Gene Transfer for X-SCID

- 6 subjects received haploidentical BMT at the time the γc gene transfer subjects were enrolled
  - 3 died (GVHD, VOD, EBV lymphoma)
  - 2 "cured" with full immune reconstitution
    (1 year of hospitalization required for 1 case)
    Required 6 months for immune reconstitution
  - 1 still in hospital with Grade III/IV GVHD four months post BMT

- Noguchi
  - 27 retrovirus/hematopoietic stem cell studies previously on “clinical hold” being re-evaluated (PI responses to issues of patient monitoring and informed consent process)
  - 13 acceptable PI responses

- Germany
  - clinical hold lifted on all retrovirus vector studies
- Italy
  - immune reconstitution 3 patients - no adverse events
- Japan
  - clinical hold on SCID (1 X-SCID, 1 ADA-SCID)

New emphasis in RAC review

- number of independent integrations, number of independently transduced cells
- selective or proliferative advantage?
- vector design - conditional expression, suicide elements, SIN vectors, silencers, etc.
- long-term studies in large animal models?
- functions of transgene product - transgenic or knockout studies?
- monitor for clonally expanding cells
- re-evaluation of consent processes

Hematopoietic Marking Studies

- **Claudio Bordignon- ADA-SCID**
  - **12 subjects enrolled in 3 temporal trials**
  - **Non-myeloablative conditioning (Busulfan)**
  - **13% to 40% $CD34^+$ cell transduction efficiency (CFU-C)**
  - **0.9 to 9 x 10^6 $CD34^+$ per kg infused**

Claudio Bordignon- ADA-SCID

- **Results to date:**
  - **Restoration of immune function at 20 months**
    - **Normal B and T cell and near normal NK cell counts**
    - **Granulocytes restored at 10% of normal level**
  - **Correction of metabolic defect**
  - **Multi-lineage stable engraftment**
- **Superior to mismatched BMT**
- **Equal to matched BMT**

Claudio Bordignon- ADA-SCID

- **Results to date:**
  - **Inverse PCR to analyze integration sites**
    - **In P1- smear; no definitive bands in population analysis**
    - **Cloned T cells from P1- sequence analysis of integration sites**
      - **Integration within and outside gene coding sequences**
      - **Saw integration within *evi*-1, but no pathology to date**
    - **P1, P3 & P4- highly polyclonal integration in T cells**
    - **P2- oligoclonal integration (discrete bands)**

No clinical pathology seen to date

Report from the ASGT *Ad Hoc* Committee on Retroviral-mediated Gene Transfer to Hematopoietic Stem Cells

http://www.asgt.org/reports/042003/

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Table of Contents