Recognizing and treating
Severe Combined Immune Deficiency in 2011

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Case # 1

Baby girl, born at term, normal gestation
Normal weight and length, no dysmorphisms, normal P.E.

7 wks: thrush, otitis media, “bronchitis”, fails to gain weight
progressive weight loss, pneumonia

4 mo: respiratory distress, anemia
unirradiated blood transfusion -> rash, fever
extreme lymphopenia noticed

5 mo: exitus

Post mortem examination: severe depletion of thymus,
spleen, lymph nodes
Case # 2

Baby boy, 38 wga, BW 3380 g, length 52 cm
Discharged at DOL +4

4 wk: intervention for pyloric stenosis

4 mo: dyspnea, cough. CXR: left lobe pneumonia
In spite of AB treatment, worsening of symptoms
Protracted diarrhea, FTT

5 mo: Chest CT: bilateral interstitial pneumonia

6 mo: BW 5000 g (<3rd centile)
PE: severe dystrophy, dry skin, hypotonia, polypnea
WBC 2.7 (N65%, L5%, M26%, E 4)
IgG <66 mg/dL; IgA <2; IgM <5 mg/dL
CD3: <1%, CD19: <1%; CD16: 89%
Case # 3

Baby boy (2\textsuperscript{nd} in birth order)
Born at term (39 wga), normal gestation
BW 3289 g, l 50 cm, no dysmorphisms
Normal P.E. except bilateral clubfeet

Day 1: 2 episodes of mild desaturation that self-resolved
(thought to be due to maternal use of SSRI)

Initiated enteral feeding in the first 24 hours

Discharged on day 4 with BW 3207 g, minimal jaundice
and no distress
Three presentations, one diagnosis

<table>
<thead>
<tr>
<th>Case #</th>
<th>Year</th>
<th>Diagnosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1948</td>
<td>SCID</td>
<td>Glanzmann and Riniker, 1950</td>
</tr>
<tr>
<td>2</td>
<td>2004</td>
<td>SCID</td>
<td>Grunebaum et al, 2006</td>
</tr>
<tr>
<td>3</td>
<td>2010</td>
<td>SCID</td>
<td>Hale et al., 2010</td>
</tr>
</tbody>
</table>
pre-B cell → B lymphocyte → plasma cell

Thymus

SCID

helper T cell

hematopoietic stem cell

T lymphocyte

cytokines

cytotoxic T-cell precursor

cytotoxic T-cell

dashed arrows indicate cytokines

viruses, fungi

antibodies

bacteria

phagocytes
SEVERE COMBINED IMMUNE DEFICIENCY (SCID): A MEDICAL EMERGENCY

• 1/40,000 – 1/100,000 live borns
• early onset (first months of life)
• severe infections
  - interstitial pneumonia
  - chronic diarrhea
  - candidiasis
  - opportunistic pathogens often involved
• failure to thrive
• lymphopenia
• presence of maternal T cells is common and may cause GvHD
• lethal within 2 years of age

but......

can be cured with hematopoietic stem cell transplantation
Typical opportunistic pathogens in SCID

- Candida spp.
- Pneumocystis jiroveci
- Cytomegalovirus
- Adenovirus
- Parainfluenzae virus 3

Disseminated BCGosis in recipients of BCG vaccine
Severe diarrhea in recipients of rotavirus vaccine
Essentielle Lymphocytophthise.

Ein neues Krankheitsbild aus der Säuglingspathologie.

Von EDUARD GLANZMANN und PAUL RINIKER (Prosektor).

I. Klinischer Teil, von E. Glanzmann.

Lymphocytopenie und Lymphocytophthise.

Aus der Kinderklinik (Direktor: Prof. G. Fanconi) und dem Pathologisch-anatomischen Institut (Direktor: Prof. E. Uehlinger) der Universität Zürich

Agammaglobulinämie und Alyphocytose
mit Schwund des lymphatischen Gewebes

Von W. H. Hitzig, Z. Biró, H. Bosch und H.J. Huser

Eingegangen am 20. Oktober 1958
Lymphopenia is a hallmark of SCID

(Buckley, J Pediatr 1997)
CHALLENGES IN DIAGNOSIS OF SCID: DISEASE HETEROGENEITY

- Immunological heterogeneity
- Genetic heterogeneity
- Phenotypic heterogeneity
Variability of B and NK cell numbers in SCID

Lymphocytes/µL

0 2000 4000 6000 8000 10000

γc  JAK3  IL7R  RAG  Artemis  ADA  Unkn.  Normal Range
N=15  N=15  N=4  N=6  N=7  N=4  N=13

T  B  NK
Immunological heterogeneity of SCID

1) T- B+ NK- SCID
2) T- B+ NK+ SCID
3) T- B- NK- SCID
4) T- B- NK+ SCID

Each of these may be due to various gene defects
cell survival

ADA, PNP

γc, IL7R, JAK3

cytokine-mediated proliferation

NKp

T/NKp

DN

DP

CD4

CD8

CD45

CD3δ,ε,ζ

RAG1/2, Artemis, DNA-PKcs

Lig4, Cernunnos

FOXN1

thymus organogenesis

expression of pre-TCR

myeloid progenitor

HSC

CLP

Bp

NK

B

CD4
Different distribution of SCID genotypes in different countries

Brescia, Italy (n=109)

Duke Univ., USA (n=166)

- IL2RG
- JAK3
- IL7R
- ADA
- RAG1
- RAG2
- Artemis
- AK2
- FOXN1
- RMRP
- SCID + MIA
- T-B+ unknown
- T- B- unknown

- IL2RG
- JAK3
- IL7R
- ADA
- RAG1
- RAG2
- Artemis
- CD3 chains
- CD45
- RMRP
IMMUNOLOGICAL RECONSTITUTION OF
SEX-LINKED LYMPHOPENIC
IMMUNOLOGICAL DEFICIENCY

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From the Department of Pediatrics and the Pediatric Research
Laboratories, University of Minnesota, Minneapolis, Minnesota
55455
The Perfect SCID Transplant

- FAST
- Cheap
- No side effects
- 100% effective
- Lasts 90 years
Hematopoietic cell transplantation for SCID

Factors influencing outcome:

• type of donor and HLA-matching
• nature of the cell defect
• age and clinical status at transplantation
• year of transplantation
• transplant-related toxicity
• acute and chronic Graft versus Host disease
• lineage-specific chimerism
• quality of immune reconstitution
Sources of long-term repopulating cells

HLA-identical sibling donor
- unfractionated bone marrow

Haploidentical related donor
- T-cell depleted (Soybean lectin + E-rosetting)
- T-cell depleted (mAb)
- positive selection (CD34)

Adult unrelated donor
- unfractionated bone marrow
- T-cell-depleted bone marrow
- positively selected peripheral blood CD34+ cells

Related or unrelated cord blood (unfractionated)
Initial recovery of T cells after MRD-HCT for SCID is extrathymic and is immediate.

1-4 months

4-12 months

HSC

T cells

host cells
donor cells

naïve CD4 T

memory CD4 T
Recovery of T cells after T-cell depleted haplo-identical HCT for SCID depends on intra-thymic differentiation and may take 4-6 months.
Probability of survival after HCT for SCID according to donor-recipient compatibility

n = 681
10 years survival rate

Geno : 84%
Pheno : 64%
MUD : 66%
mmRel : 54%
p<0.0001

(Gennery, JACI 2010)
Early recognition of SCID is key to optimize survival and quality of life after HSCT
Comparison of outcome in proband and sibling cohort

Probands: n=48
- Death before HSCT: n=17, 35% mortality
- Progress to HSCT: n=31
  - Deaths after HSCT: n=12, 39%

Siblings: n=60
- Death before HSCT: n=1, 1.8% mortality
- Progress to HSCT/GT: n=59
  - Deaths after HSCT/GT: n=5, 8.5%

Overall mortality/survival:
- Proband: 29/48 (60%) (40%)
- Sibling: 6/60 (10%) (90%)

(Brown, BLOOD 2011)
HCT for SCID gives optimal survival if performed early in life.

(Buckley, 2008)
Why diagnose SCID early

• Establish diagnosis, institute treatment

• Avoid inefficient, costly, dangerous “diagnostic Odyssey”

• Provide families with genetic diagnosis and counseling

• Learn true incidence and spectrum of SCID

• Educate providers and public about SCID

• Collaborate to determine optimal treatment
Thymus Blood

BM  THYMUS  BLOOD

$V_\alpha$  $V_\delta$  $\delta$Rec  $D_\delta J_\delta C_\delta$  $\psi J_\alpha$  $J_\alpha$  $C_\alpha$

HSC  $\rightarrow$  Tpro  $\rightarrow$  TREC

- sjTREC generated at DP stage
- dilute with subsequent divisions in periphery

$\bullet$  = signal joint TREC

CD4+
CD8+
Identification of Severe Combined Immunodeficiency by T-Cell Receptor Excision Circles Quantification Using Neonatal Guthrie Cards

Yoichi Morinishi, MD, PhD, Kohsuke Imai, MD, PhD, Noriko Nakagawa, MD, Hiroki Sato, MHSc, Katsuyuki Horiuchi, MD, PhD, Yoshitoshi Ohtsuka, MD, PhD, Yumi Kaneda, MD, Takashi Taga, MD, PhD, Hiroaki Hisakawa, MD, PhD, Ryosuke Miyaji, MD,
### SCID meets criteria for newborn screening

<table>
<thead>
<tr>
<th>Screening criteria</th>
<th>Why SCID meets criteria</th>
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<tbody>
<tr>
<td>Disease is serious</td>
<td>Fatal unless definitive treatment</td>
</tr>
<tr>
<td>Disease is not detected by PE</td>
<td>SCID babies look normal at birth</td>
</tr>
<tr>
<td>Incidence supports screening</td>
<td>Presumed incidence at least 1/100,000</td>
</tr>
<tr>
<td>Well established confirmatory test</td>
<td>Flow cytometry/functional assays</td>
</tr>
<tr>
<td>Efficient treatment exists</td>
<td>HCT (enzyme replacement therapy, gene therapy)</td>
</tr>
<tr>
<td>Earlier treatment is better</td>
<td>Survival 95% if HCT at &lt;3.5 months of life</td>
</tr>
<tr>
<td>Diagnosis and treatment available</td>
<td>Both available at many centers</td>
</tr>
</tbody>
</table>
Screening for SCID using dried blood spots at birth in United States

screening now performed in 5 states and 1 territory

$\delta$Rec-$\varphi$J$\alpha$ TREC detected by quantitative PCR in DBS

Sept 2007: SCID nominated for addition to universal panel
Jan 2008: WI begins screening
Feb 2009: MA begins screening
Jan 2010: SCID nominated again for addition to universal panel
May 2010: Secretary of Health and Human Services recommends addition
Screening for SCID using dried blood spots at birth in United States

incidence (at least 1:100,000)
- fatal without treatment
- early treatment improves outcome
- robust feasible test
- reasonable “false positive” rate

Figure 5. Map of Newborn Screening for SCID Implementation Status
# Summary of newborn screening for SCID to date in US

(publicly available data through April 2011)

Secretary’s Advisory Committee on Heritable Diseases in Newborns and Children

<table>
<thead>
<tr>
<th></th>
<th>Date started</th>
<th>Births/year</th>
<th># screened</th>
<th># SCID</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wisconsin</td>
<td>Jan 2008</td>
<td>69,322</td>
<td>243,707</td>
<td>4</td>
<td>~1:61,000</td>
</tr>
<tr>
<td>Massachusetts*</td>
<td>Feb 2009</td>
<td>77,022</td>
<td>194,056</td>
<td>4</td>
<td>~1:48,000</td>
</tr>
<tr>
<td>California*</td>
<td>Aug 2010</td>
<td>510,000</td>
<td>500,000</td>
<td>7</td>
<td>~1:71,000</td>
</tr>
<tr>
<td>New York*</td>
<td>Sept 2010</td>
<td>236,656</td>
<td>239,454</td>
<td>4</td>
<td>~1:60,000</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Oct 2010</td>
<td>65,268</td>
<td>31,464</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Total (incl PR and Navajo)*</td>
<td></td>
<td>1,005,798</td>
<td>1,208,681</td>
<td>19</td>
<td>~1:58,000</td>
</tr>
</tbody>
</table>

* up to date to September 2011, courtesy of Anne Comeau and MA SCID Newborn Screening workgroup; Fred Lorey, Jennifer Puck, Mort Cowan in CA; Michelle Caggana in NY
Genotype distribution and treatment modalities of patients identified with SCID at birth

19 patients

17 SCID

- 5 IL2RG
- 3 ADA
- 2 RAG1, 1 RAG1/OS
- 2 IL7R
- 1 JAK3
- 1 SCID/MIA
- 2 undefined

2 other

BMT
Summary of treatment and outcome to date

19 patients identified:
  3 PEG-ADA/GT
  2 awaiting transplant

14 transplanted:
  follow-up 1 month-15 months
  13/14 alive, 1 died of VOD

  donors: 1 sib, 1 haplo/homozygous sib, 3 mother, 6 URD, 1 UCB

  conditioning: 2 none
                 2 ATG alone
                 9 myeloablation (bu/cy or bu/flu +/- ATG)

  graft failures: 3 (1 ATG, then bu/flu/ATG)
                 (1 Bu/flu/ATG TCD & T cell add-back, then DLI)
                 (1 rATG, then got boost)

personal communication SY Pai, M Cowan, TN Small, C Seroogy, J Routes, D Kohn, M Porteus
SCID Transplant Outcomes

- Mortality - 20-40%
- Inadequate antibody production - 30-60%
- Decline in T cell function?
- GvHD
- Autoimmunity
- Growth and development problems
- Cognitive problems
Gene Therapy for SCID
Gene Therapy for SCID: Rationale

• correct the disease at its roots by inserting one normal copy of the gene into the patient’s hematopoietic stem cells

• no risks of Graft-versus-Host Disease

• selective advantage expected for gene-corrected cells in T cell development

• no or little chemotherapy needed

• an alternative to MMRD-HCT for patients who lack HLA-identical donors

• stem cells readily available (patient’s own cells!)
Gene therapy for X-SCID: Experience in London and Paris

- 20 patients (10 at each site)
- 18 alive
- 17 showing persistent immune reconstitution as the result of GT

Insertional mutagenesis and clonal proliferation (5 out 20)
Retroviral insertion near oncogenes drove overexpression and leukemia

Out of 20 patients, 5 (4 in Paris, 1 in London) have developed T acute leukemia

<table>
<thead>
<tr>
<th>Insertion site</th>
<th>Clinical outcome</th>
</tr>
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<tbody>
<tr>
<td>P4 LMO2</td>
<td>1 patient died (P4)</td>
</tr>
<tr>
<td>P5 LMO2</td>
<td>4 patients treated successfully</td>
</tr>
<tr>
<td>P7 CCND2</td>
<td>now have normal T cell number and diversity</td>
</tr>
<tr>
<td>P10 LMO2, BMI1</td>
<td></td>
</tr>
<tr>
<td>P8 LMO2</td>
<td></td>
</tr>
</tbody>
</table>

Safer vector designed to reduce insertional mutagenesis

LTR-driven gammaretroviral vector: **MFG γC**

New gammaretroviral SIN vectors: **SRS11**

No gag, pol or env residues

Key modifications compared to Paris/London **MFG** vector:

- removal of viral LTRs to reduce transactivation of neighboring genes
- removal of all gammaretroviral coding regions
- cellular EF1α promoter to drive transgene expression
- modification of PRE (posttranslational regulatory element) to enhance expression
- other modifications to improve titer
Clonal dominance assay

Vector transduction → Primary recipients → 4 mos → analysis

Secondary recipients → 12 mos → analysis

PB and BM, deep sequencing

NO DONOR-DERIVED TUMORS (~100 MICE)

Insertion in Evi1
MFG: 8 out of 3621 insertions
SRS: 0 out of 2690 insertions

Chris Baum (Hannover MS)
Chad Harris (CHB)
Martijn Brugman (Hannover MS)
Gene transfer for SCID-X1 using a self-inactivating (SIN) gammaretroviral vector

A multi-institutional phase I/II trial evaluating the treatment of SCID-X1 patients with retrovirus-mediated gene transfer

Sites:
Great Ormond Street Hospital, UK
Hôpital Necker Enfants Malades, France
Children’s Hospital Boston, US
Cincinnati Children’s Hospital Medical Center, US
Mattel Children’s Hospital, Los Angeles, US
SCIDX1 gene transfer protocol

eligible if no sib donor, sick or no matched URD

SCF
IL3
TPO
Flt3L

autologous
BM harvest
CD34+ selection

3 rounds of transduction in retronectin coated bags

24h 24h 6h

infuse

d-4 d-2 d-1 d0

Upfront therapy
No conditioning
Observe for safety, reconstitution and clinical outcome
Patient P00001

4 mo old. Diagnosed at birth because of Family History
PMH: thrush, therapy-resistant oral ulcers

ALC: 2450 cells/μL
CD3: 5 cells/μL
CD19: 1866 cells/μL
CD16: 86 cells/μL
IL2RG: Y98C
No HLA-matched related or unrelated donor

Gene therapy at 5.5 months
Evolution of therapy-resistant oral ulcers

pre-GT

Neutrophilic infiltrate

day +50 post-GT

Mononuclear cell infiltrate
Infiltration of oral mucosa of GT00001 with T cells as a result of GT

CD3, original biopsy

CD3, day +50 biopsy

gene marking confirmed in peripheral T cells
full clinical resolution by day +70 after GT
Immune reconstitution after gene therapy for X-SCID in GT 00001
Normalization of PHA response after GT
Boston SCID-X1 00001

<table>
<thead>
<tr>
<th></th>
<th>Pre-GT</th>
<th>Post-GT day +90</th>
<th>Post-GT day +135</th>
</tr>
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<tbody>
<tr>
<td>PHA</td>
<td>206</td>
<td>8527</td>
<td>139,923</td>
</tr>
<tr>
<td>bkg</td>
<td>725</td>
<td>1056</td>
<td>775</td>
</tr>
<tr>
<td>SI</td>
<td>&lt;1</td>
<td>7.07</td>
<td>331</td>
</tr>
</tbody>
</table>
Normalization of $\gamma_c$ expression after GT
Boston SCID-X1 00001

day +171

CD4 T

CD8 T

CD56 NK

ND

GT 00001

$\gamma_c$
Conclusions (1)

• SCID represents a medical emergency. Early recognition and treatment are necessary to offer permanent cure to infants affected with this fatal group of disorders.

• A high index of suspicion must be used in infants with possible SCID. Simple laboratory tests (ALC, lymphocyte subsets) may disclose the diagnosis. Molecular analysis is important for genetic counseling and reproductive choices.

• Newborn screening for SCID is now possible and should result in significant improvement of outcome, allowing definitive treatment before severe infections develop. Variability in the frequency of the disease in different areas must be expected. Cost-benefit analysis remains to be done.
Conclusions (2)

• Hematopoietic cell transplantation is the mainstay of treatment for SCID. Advances in critical care and in development of novel drugs for prevention and treatment of infections, increasing availability of alternative donors, and high-resolution methods for HLA typing are key to promote better survival.

• There is a need to develop less toxic conditioning regimens and novel strategies to speed-up immune reconstitution.

• SCID has also offered proof of principle that gene therapy works, but carries significant risks. Development of safer vectors and novel approaches (true gene correction, safe harbors) may lead to improved outcome.
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Collaborators
Frederic Bushman, UPenn
Kenneth Cornetta, IU
Anne Comeau, MA DPH
Mission Statement

Founded in 1980, the Immune Deficiency Foundation (IDF) is the national patient organization in the United States dedicated to improving the diagnosis, treatment and quality of life of persons with primary immunodeficiency diseases through advocacy, education and research.

www.primaryimmune.org
US Immunodeficiency Network

The United States Immunodeficiency Network (USIDNET) was established in 2003 as a research consortium to advance scientific research in the field of primary immunodeficiency disorders.

The current focus of this initiative is on the Primary Immunodeficiency Disease Registry.
USIDNET Disease Registry

The objective of USIDNET is the collaboration of physicians from all over the United States to form a large Primary Immunodeficiency Diseases Registry which serves to:

- Improve understanding of the prevalence of these disorders and provide longitudinal patient data
- Evaluate measures of quality of life of patients
- Examine side effects of treatment protocols
- Determine the natural history of these disorders and establish genetic correlations
- Provide a centralized informational resource for clinical and laboratory research
USIDNET Disease Registry

Physicians with primary immunodeficient patients can contribute to this valuable tool by enrolling their patients in this national HIPAA compliant registry initiative. USIDNET will send you step-by-step instructions for you to review.

Steps include:

• Obtaining IRB approval
• Obtaining access to and training on the online registry
• Consenting your patients
• Enrolling your patients in the Registry
More information about the US Immunodeficiency Network and its programs can be obtained at

www.usidnet.org

Or by contacting the USIDNET Registry Manager at 866.939.7568

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