Hematopoietic Stem Cell Transplant for Primary Immune Deficiencies

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HSCT Can Also Cure Primary Immune Deficiencies

The Primary Immune Deficiencies involve defects of the blood cells that comprise the immune system (T cells, B cells, NK cells, granulocytes, macrophages), These blood cells are all made from HSC.

Therefore, providing normal HSC can cure the PIDs.

HSCT can also treat: Sickle Cell Disease, Thalassemia Metabolic (Gaucher disease, Lorenzo’s Oil disease), Fanconi’s anemia, Kostmann’s….
Hematopoietic Stem Cell Transplantation

Sources: bone marrow, peripheral blood stem cells (PBSC), umbilical cord blood, (hESC/iPSC?).

Donors:

Allogeneic – anyone but self or identical twin (syngeneic)
  HLA-matched sibling donor
  HLA-matched unrelated donor (adult BM, cord blood)
  Haplo-identical/-mismatched T cell-depleted parent

Autologous – self with gene addition or gene correction
  = Gene Therapy
HSCT has been successfully applied for the treatment of PID since 1968:
   --SCID, WAS, X-HIM, CGD, HLH, LAD, IPEX, etc.

In general, achieve high rates of immune recovery and good quality of life, with an HLA-matched sibling donor.

Can infuse HLA-matched sibling donor marrow, when available, without “conditioning” and restore T cell function in most SCID patients.
Bone Marrow Transplant - an example of Stem Cell Therapy

- Harvesting bone marrow from the Donor
- Processing Stem Cells
- Patient is “conditioned” with high dose chemotherapy
- Intensive medical support until stem cells grow
- Stem cells infused
Outcome after HSCT with Full, Partial or No Myeloablative Conditioning

Patient’s Bone Marrow HSC

Full Myeloablation

Donor or Auto/Gtx HSC

Donor Chimerism

Full

None

Minimal

Partial-Myeloablation

Mixed
## Effects of Pre-BMT Conditioning

<table>
<thead>
<tr>
<th>Conditioning</th>
<th>Myeloablative</th>
<th>Immune Ablative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body Irradiation</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>Busulfan/Melphalan</td>
<td>4+</td>
<td>No</td>
</tr>
<tr>
<td>Cyclophosphamide (cytoxan)</td>
<td>No (suppressive)</td>
<td>3-4+</td>
</tr>
<tr>
<td>Antibodies (Anti-thymocyte Globulin, mAB vs. CD3, CD52)</td>
<td>No.</td>
<td>2-4+</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>No</td>
<td>3-4+</td>
</tr>
<tr>
<td>Conditioning</td>
<td>“Full”</td>
<td>“Reduced”</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Total Body Irradiation</td>
<td>900-1,300 cGy</td>
<td>100-400 cGy</td>
</tr>
<tr>
<td>Busulfan</td>
<td>16 mg/kg</td>
<td>4-8 mg/kg</td>
</tr>
<tr>
<td>Melphalan</td>
<td>&gt;150 mg/kg</td>
<td>&lt;140 mg/kg</td>
</tr>
</tbody>
</table>
Average Gene Marking in Rhesus at Six Months After Non-Myeloablative Busulfan/Gene BMT

Selective Expansion of Specific Cells after HSCT without Marrow Conditioning

Patient’s Bone Marrow HSC (Mixed chimerism)

Blood Cells:
- Granulocytes
- Erythrocytes
- T Cells
- B Cells

No Selective Advantage

Blood Cells:
- Granulocytes
- Erythrocytes
- T Cells
- B Cells

SCID: T Cell Selective Advantage
Allogeneic HSCT

With the transplant of HSC from an allogeneic donor, donor T cells are also transplanted.

The donor T cells may:
  a. Cause graft versus host disease. 😞
  b. Eliminate residual leukemia (graft vs. leukemia). 😊
  c. Contribute to immune reconstitution. 😊
  d. Facilitate engraftment/eliminate endogenous HSC. 😊
Donor T Cells Can Eradicate Host Leukemia and HSC

Patient’s Bone Marrow HSC After Non-Ablative HSCT

Donor T Cells → Eradicate Host Leukemia and HSC

Granulocytes
Erythrocytes
T Cells
HSC
Graft Versus Host Disease (GVHD)

A major limitation to the use of HSCT to treat PID is the unwanted immune responses between a patient and the transplanted cells.

Patient can reject the transplanted HSC.

Donor T cells within the marrow can react against the recipient (GVHD).

Immune suppression is needed, but significant clinical complications may still occur.
Immune Responses in Clinical Transplantation

Major clinical approaches to prevent or control immune rejection and GVHD are:

1) **Matching of donor and recipient** as best as possible:

   - Identical twin
   - HLA-matched
   - ABO-matched
   - Minor antigen-matched
   - “cloned” (hESC/iPC)
Immune Responses in Clinical Transplantation

Major clinical approaches to prevent or control immune rejection and GVHD are:

2) Non-specific immune suppression:
   a) Corticosteroids
   b) Anti-metabolites: azathioprine, methotrexate, mycophenolate
   c) Calcineurin inhibitors (cyclosporin, tacrolimus)
   d) mTOR inhibitor (rapamycin=sirolimus)
   e) Antibodies:
      polyclonal – ATG
      monoclonal - anti CD3, CD2, CD25, CD52, CD20
   f) Cytokine blockade: anti-TNF: (Atanercept, Embrel)
   g) Co-stimulatory blockade: CTLA-4 Ig, anti-B7.1/B7.2
Graft Versus Host Disease (GVHD)

GVHD remains a major cause of morbidity and mortality after allogeneic HSCT, even with HLA-matched donor.
T Cell Depletion

When bone marrow is harvested from donors, it is mixed with blood from the donor, which adds T cells.

T cells are the donor cells that can cause graft versus host disease, especially if there are tissue-type (HLA) mismatches between the donor and recipient (e.g. parent).

Several methods have been developed that can significantly decrease the numbers of T cells that are in donor bone marrow and thereby decrease the risks for GVHD.

A drawbacks of T cell-depleted bone marrow is slower recovery of immunity (from square one).
T Cell Depletion

The first clinically acceptable method for T cell depleting human bone marrow performed E-rosetting of T cells with sheep red blood cells after removing cells clustered by soybean agglutinin (SBA-/E-).

This cell processing approach may have unexpected benefits, like retaining some cell types that facilitates engraftment and removing B cells that may carry EBV→PTLD. But, use of animal-derived reagent is not FDA-friendly.

Most centers use immuno-affinity with monoclonal antibody column to CD34+ cells to enrich for stem and progenitor cells. Effectively removes T cells, but may also eliminate other useful cell populations.
Use of Umbilical Cord Blood for Stem Cell Transplants

~50-100 ml of newborn’s blood in blood vessels of the umbilical cord and placenta.

Umbilical cord blood (UCB) has the same HSC as bone marrow and can be used for clinical stem cell transplants.

UCB normally wasted. Can be saved privately for a single child or family or can be saved in community banks to be publicly available.
Use of Umbilical Cord Blood for Stem Cell Transplants

**Advantages** of UCB vs. adult BM or PBSC:

- Easiest to obtain
- May be banked frozen and quickly available
- UCB storage can be private/directed or public.
- May use with less close match than adult sources, although GVHD and rejection are still possible, as in any allogeneic transplant
- Provides a good source of stem cells for infants and children needing transplant, especially when from sibling
Use of Umbilical Cord Blood for Stem Cell Transplants

**Disadvantages** of UCB vs. adult BM or PBSC:

- relatively low number of HSC
  - risks for engraftment failure (5-15%)
  - delayed recovery of blood cell production

- cannot use same donor if 2nd transplant needed

- naïve immune system (e.g. no antiviral T cells)
Choice of HSCT Donor

HSCT have the highest chances for success if the bone marrow comes from a closely matched donor, ideally a sibling (younger is better).

But, only ~15% of people have a sibling who is a full match.

BMT from unrelated donors (adults or cord blood) have higher risks for immune complications (GVHD and rejection) because they are less well-matched than matched siblings.
Allogeneic Transplants in Patients ≤20y, Registered with the CIBMTR, 1989-2006
- By Donor Type and Graft Source -
Inherited Immune Syndromes
Survival of Pediatric (Age < 18 Years) Marrow Recipients with All Preparative Regimens, by Disease – Unrelated Donors 1998–2006

- WAS (n = 48)
- SCID (n = 35)
To Condition or Not To Condition: That is the Question

HSCT products (BM, UCB, PBSC) are heterogeneous with long-lived stem cells at low frequencies and lineage-restricted progenitor cells of multiple stages and activity.

Immune reconstitution may result from combined effects of short-term lymphoid progenitor cells producing large numbers of T, B and NK cells early and rarer long-term stem cells serving as a sustained source of lymphocytes.

Without conditioning, may only get former effect. With conditioning, may get both effects and more complete immunity from multi-potent, long-term stem cells.
To Condition or Not To Condition: That is the Question

Should pre-transplant conditioning be given prior to HSCT for SCID?

**No:** May be highly toxic, acutely and long-term (G+D, CNS, dental...) May exacerbate pre-existing infections.

Survival is good without it, with sufficient long-term T cell function for good health in most.

**Yes:** Higher frequency of HSC engraftment with conditioning → greater probability of adequate B cell function, to D/C IVIg, make IgA.

Non-ablative HSCT leads to minimal engraftment of HSC, which is more likely to cause long-term deficit in B cells (antibody) and NK cells.

Which gives best outcome?

Survival, immune reconstitution, G+D...
To Condition or Not To Condition: That is the Question

Should pre-transplant conditioning be given prior to HSCT for SCID? If so, what combo? to whom?

“Full”: Busulfan/Cytoxan, Bu/flu +/- ATG, Campath, melphalan, treo
“Reduced”: Flu/ATG, “little bu”
“Serotherapy”: ATG, Campath
Nothing: Nothing

NK+ SCIDs, maternal engraftment of maternal T cells, Ommen’s?

What to do if radio-sensitive SCID (Artemis, DNA PK)? **NEONATES**

Based on HSC donor? Matched sib vs. MUD vs. Cord vs. haplo.

GVHD prophylaxis: what, to whom, how long…..
The central hypothesis of gene therapy using HSC is that by correcting the gene in a patient's own HSC, no immune reactions will occur after transplant and there will be better outcomes with fewer complications.

Normal gene
Map of Retrovirus and Retroviral Vector

A. Retrovirus

```
5' LTR  | gag | pol | env  | 3' LTR
       |     |     |      |
D      |     |     | A    |
```

B. Retroviral Vector

```
5' LTR  | Exogenous gene (s)  | 3' LTR
       |                    |
D      |                    |
E/P    |                    |
```

- Vector RNA
- Therapeutic Protein
Retroviral Transduction Leads To Stable Integration of the Transgene in the Target Cell Chromosomal DNA
Gene Therapy vs. BMT

Why might gene therapy be better than BMT?

1. Every patient has a perfectly matched donor - themself.
2. No immune mismatches $\Rightarrow$ no GVHD, rejection unlikely.
3. Less chemotherapy and immune suppression may be needed for gene therapy to be beneficial than is needed for successful BMT from another person.

So, some of the most severe side-effects from BMT may be absent or less with gene therapy.
Gene Therapy vs, BMT

Why might gene therapy be worse than BMT?

1. May not get the new gene into enough stem cells.
2. The new gene may not make enough hemoglobin to fix the red blood cells.
3. Handling the stem cells could hurt them so that they can’t continue to make new blood cells for decades.
4. The gene manipulation of the stem cells could make them grow too much, even cause leukemia (cancer of the blood stem cells).

So, some new problems and side-effects could occur.
Summary of ADA-Deficient SCID Patients
Retroviral Vectors, Myeloreductive Conditioning
– Milan/London/CHLA-NHGRI, NIH-UCLA

<table>
<thead>
<tr>
<th>Center</th>
<th># Pts</th>
<th>F/U (yrs)¹</th>
<th>Off Enzyme</th>
<th>Survival</th>
<th>DFS²</th>
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<tr>
<td>Milan</td>
<td>17</td>
<td>0.9 – 10.5</td>
<td>14/17</td>
<td>100%</td>
<td>82.4%</td>
</tr>
<tr>
<td>London</td>
<td>8</td>
<td>0.5 – 7.5</td>
<td>4/8</td>
<td>100%</td>
<td>50%</td>
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<tr>
<td>CHLA-NHGRI UCLA-NHGRI</td>
<td>6</td>
<td>2– 5</td>
<td>3/6</td>
<td>100%</td>
<td>50%</td>
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<tr>
<td></td>
<td>5</td>
<td>0.1-2</td>
<td>4/5</td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>36</td>
<td>0.1 – 8.0</td>
<td>25/36</td>
<td>100%</td>
<td>69.4%</td>
</tr>
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</table>

¹ As of April 2011
²DFS ≡ Alive without BMT or PEG-ADA re-start

Data: Courtesy HR Gaspar (London) and Alessandro Aiuti (Milan)
## Primary Immune Deficiencies
Target for Gene-Corrected/Engrafted HSC

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Lineages</th>
<th>Stable HSC</th>
<th>Conditioning Regimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>XSCID</td>
<td>T, B, NK</td>
<td>0.1-1.0%</td>
<td>none -</td>
</tr>
<tr>
<td>ADA SCID</td>
<td>T, B, NK</td>
<td>1-10%</td>
<td>Bu - ¼ str (4/kg)</td>
</tr>
<tr>
<td>WAS</td>
<td>T/B/Meg</td>
<td>10-20%</td>
<td>½ str busulfan (8/kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+/- fludarabine</td>
</tr>
<tr>
<td>CGD</td>
<td>granulocytes</td>
<td>~1-20%</td>
<td>½-3/4 str busulfan (8-12)</td>
</tr>
<tr>
<td>LAD</td>
<td>lymphocytes</td>
<td>10-20%</td>
<td>¼-1/2 str busulfan (4-8)</td>
</tr>
<tr>
<td>HLH</td>
<td>CTL</td>
<td>10-20%</td>
<td>¼-1/2 str busulfan (4-8)</td>
</tr>
<tr>
<td>XLA</td>
<td>pre-B/B cells</td>
<td>10-20%</td>
<td>½ str busulfan (8/kg)</td>
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</table>
Improved Vectors for Greater Safety

Major risk factor for insertional oncogenesis determined to be the strong enhancers of retroviral vectors that can \textit{trans}-activate adjacent cellular genes.

New retroviral and lentiviral vectors have been developed without the strong retroviral enhancers, instead using cellular gene promoters (\textit{EF1-\text{\textalpha}}, \textit{PGK}) that lack strong enhancer activity.

In pre-clinical models (in vitro and in vivo), these new vectors show >100-1,000-fold lower risks for trans-activation of adjacent genes.

Next generation of clinical trials are testing these vectors to assess efficacy and safety
1\textsuperscript{st} Generation Retroviral Vector

Cellular Gene → 5’ LTR → E/P → D → Exogenous gene (s) → Vector RNA → Therapeutic Protein → 3’ LTR → E/P → Cellular Gene

(+ ) Trans-activate

2\textsuperscript{nd} Generation Retroviral/Lentiviral Vector

Cellular Gene → 5’ LTR → Prom → Exogenous gene (s) → Vector RNA → Therapeutic Protein → 3’ LTR → Cellular Gene

Enhancer Deleted

(+ ) Trans-activate