SUCCESSFUL BONE MARROW TRANSPLANTATION FOR IPEX SYNDROME FOLLOWING REDUCED INTENSITY CONDITIONING.

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Running title: Reduced intensity conditioning in IPEX syndrome
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Abstract:

IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked disease) is a rare, fatal autoimmune disorder caused by mutations in the FOXP3 gene leading to disruption of signaling pathways involved in regulatory T lymphocyte function. Life-long multi-agent immunosuppression is necessary to control debilitating autoimmune manifestations such as colitis and food allergies. Allogeneic hematopoietic stem cell transplantation (HSCT) can restore T cell regulatory function but has been previously associated with poor outcome. We describe successful HSCT in 4 patients with IPEX syndrome using a novel reduced-intensity conditioning regimen that resulted in stable donor engraftment, reconstitution of FOXP3+ T regulatory CD4+ cells, and amelioration of gastrointestinal symptoms.

Key words: Immune dysregulation, IPEX syndrome, stem cell transplant and reduced intensity conditioning.
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Introduction:
The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is a life-threatening disorder associated with protracted diarrhea, severe food allergies, ichthyosiform dermatitis, endocrine insufficiency, and hemolytic anemia. Mutations of the FOXP3 gene result in loss of functional regulatory T cells and fatal autoimmune manifestations. Chronic immunosuppression is used to control symptoms. Allogeneic hematopoietic stem cell transplantation (HSCT) restores T regulatory function but has been associated with poor outcome. We describe successful HSCT in 4 boys with IPEX syndrome following reduced intensity conditioning consisting of campath-1H, fludarabine and melphalan.

Methods:
Patient details, pre-transplant immunosuppression, clinical indications for transplant, and FOXP3 mutation analysis are summarized in Table 1. Additional manifestations included elevated serum IgE levels, diabetes mellitus, hypothyroidism, hypoadrenalism and growth failure, necessitating hormone replacement. Before transplant, patient 3 had a Mycobacterium chelonae/abscessus induced thigh abscess resection and vancomycin resistant Enterococcus faecium infection. Patients 1, 2 and 3 had recurrent Clostridium difficile colitis.

Patients 2 and 3 were undergoing second transplants 6 and 33 months after rejecting previous grafts. Patient 2 previously received myeloablative conditioning with full dose busulfan, fludarabine, ATG and a 4/6 loci matched unrelated cord transplant. Patient 3
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received reduced intensity conditioning with 200 cGy TBI, fludarabine, and a cord transplant from a HLA matched sibling.

All recipients received campath-1H (48 mg if >10 kg; 33 mg if <10 kg - patient 2) (days -21 to –19), fludarabine (150 mg/m²) (day -8 to –4) and melphalan (day -3). Two patients received 140 mg/m² and two others received 70 mg/m² of melphalan (dose de-escalation strata were planned in this transplant protocol for non-malignant disorders as previously described) 7. All received bone marrow, three unrelated and one from the same matched sibling. The median total nucleated cell (TNC) dose was 9.3 x 10⁸ /kg (3.1 -12.6); CD34+ cell dose was 9.25 x 10⁶ / kg (5.04 - 34.7). GVHD prophylaxis consisted of cyclosporine or tacrolimus (levels maintained until day +100), methotrexate (10 mg/m² on day +1; 7.5 mg/m² on days +3 and +6), and methylprednisone (1 mg/kg/day) (day +7 to +28).

Supportive care included Pneumocystis prophylaxis, acyclovir if herpes virus positive, weekly monitoring for CMV reactivation, and antifungal prophylaxis until day +100.

Donor chimerism was determined by molecular analysis of blood or bone marrow. Immune reconstitution monitoring included lymphocyte subpopulations, serum immunoglobulin levels, and lymphocyte proliferation to phytohemagglutinin (PHA). For detection of FOXP3+ cells, whole blood was stained with CD4 and CD25 (Becton-Dickinson, San Jose, CA), permeabilized with FOXP3 kit reagents (eBioscience, San Diego, CA), and intracellularly stained with CD152 (Pharmingen, San Diego, CA) and FOXP3 (eBioscience) antibodies. Immunofluorescence positive cells were detected by a
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FacsCalibur Flow Cytometer. Proportion of FOXP3+ cells was determined using low side scatter gating for CD4+, CD25^{bright} and CD152+ cells.

Approval was obtained from the Institutional Review Boards of all participating centers listed in this study. Informed consent was provided according to the Declaration of Helsinki.

Results:

Campath-1H infusion was well tolerated and no toxicities were encountered during conditioning. Myeloid (ANC >0.5 x 10^9/L) and platelet (>50 x 10^9/L) engraftment occurred at a median of 12.5 (12-16) and 24.5 (13-26) days respectively (Table 2). Colitis and food allergies resolved post-HSCT. Endocrine dysfunction persisted, necessitating continued hormone replacement. Patient 3 developed hypotension episodes and malaise that corrected with mineralocorticoid therapy. Patient 2, a younger patient, had 50% decrease in insulin requirement to 0.4U/Kg/day (pre and post HSCT anti-GAD antibodies were 115.9 and 1.6U/ml respectively; normal 0-1.5U/ml). Transplant outcomes and immune reconstitution are summarized in Table 2. Immune reconstitution was robust after 6 months except when immunosuppression was continued for chronic GVHD. FOXP3+ cells were lowest in patient #3; his female sibling donor was not tested for FOXP3 mutation heterozygosity which may have resulted in lowered numbers of FOXP3+ cells. Campath-1H levels measured by ELISA (BioAnaLab, Cambridge, UK) in patient 3 were high (206-220ng/ml) until day +30, in contrast to other non-malignant disorder patients transplanted with a similar regimen (levels undetectable on day 0 -
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unpublished). This is likely due to fewer CD52+ cells in IPEX patients after pre-transplant immunosuppression. Campath-1H was undetectable on day +100.

Patient 2 developed acute respiratory distress following pulmonary hemorrhage of unclear etiology on day +7 but recovered completely after extra-corporeal membrane oxygenation for 72 hours. Infectious complications after HSCT included C. difficile colitis (n=3) 4-16 weeks post HSCT, Enterococcus faecalis (patient 2) between 8 and 12 weeks, Histoplasma capsulatum, Enterococcus fecium and Staphylococcus hominis infections (patient 3) five months following HSCT, and CMV reactivation (patient 1) at 6 months. No infections were encountered after 6 months post HSCT.

Patient 1 developed grade 2 acute GVHD of the gut 5 months after HSCT that resolved. At 9 months, he developed extensive chronic GVHD involving skin and joints, and is on therapy with tacrolimus and extracorporeal photopheresis with good response. No other recipient has developed GVHD to date. Patients 2 and 3 discontinued immunosuppression 10 and 5 months post HSCT. Patient 4 is on an immunosuppression taper.

Discussion:

IPEX is a lethal disorder of childhood due to mutations of FOXP3, loss of T regulatory and scurfin function, a FOXP3 gene product protein that is critical for control of T cell activation. Inactivity of scurfin or modification of target DNA-binding sites results in T cell proliferation, defective apoptosis, and autoimmunity in mice and humans. Without
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extensive immunosuppression the disease progresses to death in early childhood due to hemorrhage, sepsis, colitis, or diabetic complications.

HSCT in affected mice results in 20-50% survival even with mixed donor chimerism. Transplant experience in children with IPEX is limited. Of 4 previously reported sibling donor transplants, 3 died due to hemophagocytosis (1) and disease progression (2). The fourth survived a myeloablative transplant that resulted in mixed donor chimerism at 12 months (70% and 30% T and B cells respectively). Improvement in clinical symptoms paralleled donor engraftment and normalization of T cell function. Another report of myeloablative HSCT in 3 patients described 2 survivors with clinical improvement. There are no previous reports of successful reduced intensity HSCT in children with IPEX.

The reduced intensity conditioning regimen described here was well tolerated during administration, even after previous HSCT and myeloablative conditioning. It was successful in achieving donor cell engraftment with BM as the stem cell source in all patients after 2 previously rejected UCB grafts. In all recipients, colitis and allergies subsided post transplant. Transplant related complications were mainly early infections, and self-limiting, except for one patient with acute and subsequently chronic GVHD of the skin and joints. Infections likely receded following early immune reconstitution. Immune functions had significantly recovered after 9 months except in patient 1 (Table 2). FOXP3+ cells also recovered to normal numbers in patients 2 and 3 and were present in patient 4. Lower numbers in patient 1 were likely due to chronic GVHD therapy.
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Successful engraftment and amelioration of symptoms in all 4 patients suggests that HSCT should be considered early for all patients with IPEX syndrome. The potential advantages of early HSCT would be the avoidance of disease related organ toxicities, infection risks associated with chronic immunosuppression and the potential for preventing autoimmune endocrine organ destruction. This reduced intensity conditioning approach used campath-1H early pre-transplant to specifically immunoablate recipients and facilitate donor engraftment. It was well tolerated, supported stable engraftment and early immune reconstitution, and may reduce late toxicities associated with standard myeloablative conditioning regimen.

Bibliography:


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Table 1: Demographic details on HSCT recipients

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age (yrs)</th>
<th>Race</th>
<th>FOXP3 mutation analysis</th>
<th>Clinical indications for HSCT</th>
<th>Pre HSCT IS</th>
<th>Stem cell source (allele matched by HR typing)</th>
<th>Cell dose TNC ($10^6$/kg) / CD34 ($10^6$/kg)</th>
<th>Melphalan Dose ($m^2$)</th>
<th>Myeloid engraftment (days)</th>
<th>ANC &gt;0.5x10^9/L</th>
<th>Platelet engraftment (days)</th>
<th>ANC &gt;50x10^9/L</th>
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<tr>
<td>1</td>
<td>7</td>
<td>C</td>
<td>A&gt;G splice junction mutation in intron 9</td>
<td>Colitis Food allergies Eczema FTT AIHA RAD</td>
<td>Imuran CSA Prednisone</td>
<td>8/8 URD BM</td>
<td>12.6 / 34.7</td>
<td>70</td>
<td>12</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>AA</td>
<td>303_304 del TT</td>
<td>Colitis Food allergies Eczema FTT AIHA TPN</td>
<td>CSA Rituximab</td>
<td>7/8 A - antigen mismatched URD BM</td>
<td>6.0 / 5.8</td>
<td>140</td>
<td>16</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>C</td>
<td>1271 G&gt;A C 424 Y</td>
<td>Colitis Food allergies Eczema MGN</td>
<td>FK506 MMF Prednisone</td>
<td>8/8 MSD BM</td>
<td>3.1 / 5.04</td>
<td>70</td>
<td>13</td>
<td>26</td>
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</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>C</td>
<td>1226 A&gt;G C 409 G</td>
<td>Colitis TPN AIHA</td>
<td>FK506 Rituximab Prednisone Campath</td>
<td>8/8 URD BM</td>
<td>5.3 / 12.7</td>
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<td>12</td>
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Pt. – Patient
C – Caucasian
AA – African American
URD – Unrelated donor
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MSD – Matched Sibling Donor
TNC – Total Nucleated Cells
IS – immunosuppression
FTT – Failure to thrive
AIHA – Autoimmune hemolytic anemia
TPN – Total parental nutrition
RAD – Reactive airway disease
CSA – cyclosporine
MMF – mycophenolate mofetil
MGN – Membranous glomerulonephritis
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Table 2: Outcome and immune reconstitution following HSCT

<table>
<thead>
<tr>
<th>Patient</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
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<tbody>
<tr>
<td>Follow up (months)</td>
<td>25</td>
<td>19</td>
<td>11</td>
<td>6</td>
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<tr>
<td>% donor chimerism</td>
<td>100</td>
<td>100</td>
<td>89</td>
<td>84.6</td>
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<tr>
<td>GVHD (acute/chronic)</td>
<td>Grade 2 gut / extensive skin</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
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<tr>
<td>Immunosuppression</td>
<td>FK506 / Photopheresis</td>
<td>No</td>
<td>No</td>
<td>Tapering prophylaxis</td>
</tr>
<tr>
<td>Lansky score</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Absolute lymphocyte numbers/mm$^3$ CD4 / CD8 / B cells</td>
<td>446/1757/124 (1 year)</td>
<td>2005/792/1322 (1 year)</td>
<td>649/193/669 (9 months)</td>
<td>218/44/218 (6 months)</td>
</tr>
<tr>
<td>Lymphocyte proliferation to PHA (% normal control)</td>
<td>26.5 (1 year)</td>
<td>100.2 (1 year)</td>
<td>78.6 (9 months)</td>
<td>35 (6 months)</td>
</tr>
<tr>
<td>Immunoglobulin levels (mg/dl)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Ig G (N 608-1572)</td>
<td>681</td>
<td>502</td>
<td>769</td>
<td>1300</td>
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<td>Ig M (N 52-352)</td>
<td>149</td>
<td>42</td>
<td>48</td>
<td>79</td>
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<td>Ig A (N 45-312)</td>
<td>54</td>
<td>96</td>
<td>121</td>
<td>18.3</td>
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<tr>
<td>FOXP3+ cells (%) (N 76-91)</td>
<td>52</td>
<td>77</td>
<td>72</td>
<td>33</td>
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<tr>
<td>Absolute number (N 43-149 /mm$^3$)</td>
<td>29</td>
<td>99</td>
<td>17</td>
<td>30</td>
</tr>
</tbody>
</table>

N – normal range
Successful bone marrow transplantation for IPEX syndrome following reduced intensity conditioning

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