BMT for Cystinosis?

K30 Journal Club
Jan 26, 2009
Cystine

- Cystine is a dimeric amino acid formed by the oxidation of two cysteine residues
- Cystinosis is a lysosomal storage disorder
Cystinosis

- Autosomal recessive disorder
  - 1 in 100,000 live births
  - Ctns gene on 17p13 encodes a lysosomal membrane protein called cystinosin
  - Over 50 mutations have been described
  - Deletions loss of function --> severe disease
- Leads to complications of the kidneys, eyes, musculature, liver, spleen, pancreas, testes and GI tract
- Without intervention lifespan is 10 years
Biggest problem

- End-stage renal disease (ESRD)
- Cystine deposition in kidneys
  - Glomerular damage is evident at 2-5 years of age with ESRD at 9-10 years of age
  - Renal tubular Fanconi Syndrome
- There is a late onset form that presents with renal manifestations in adolescence
- Renal transplantation has helped to prolong lives
Other complications

- Became more apparent after renal transplantation allowed these patients to live longer
- Impaired growth, hypothyroidism
- Ophthalmologic: retinal, corneal,
  - Adult or “benign” cystinosis is of adult onset that presents with corneal and bone marrow disease
- Vacuolar myopathy---> muscle atrophy
- Pulmonary dysfunction
- Swallowing impairment
Medication?

- Cysteamine (Cystagon®)
  - Developed in 1970’s
  - Has pushed back the need for renal transplantation to 14-18 years old
  - FDA approved in 1994
- Free thiol traverses the lysosomal membrane to breakdown cystine to cysteine and cysteine-amine
  - Both can exit the cell without the cystinosin transporter
Cure?

- Can prevent all of the nonrenal manifestations
- Patients have been reported to live to 50 years old
- So what’s the problem?
- Now chronic disease
Cysteamine

- Needs to be taken every 6 hours to have its full benefits...not 4 times a day, every 6 hours
  - New formulation being tested for 12 hour dosing
- Tastes bad (sulfur)
  - 10-15% do not tolerate full dosing
- Still have renal Fanconi syndrome
  - Patients take oral ca, mg, phos which are multiple doses per day
  - If no donor for transplant, some still on dialysis
- Swallowing difficulties in older patients
- Cystinosis Research Foundation
More on Cystinosin

- Heterozygotes have half the normal cystine transporting capacity, but phenotypically normal
- Cellular cystine levels are near normal
Other Lysosomal Storage Disorders

- Treated by enzyme replacement, substrate depletion, HSCT
- Hurler’s, Krabbe, ALD have been treated successfully by HSCT
- Cystinosin is a transporter not an enzyme
  - Thus, proof-of-principle was needed that HSCT could work for this disease prior to in human testing
  - Mechanism is to be identified
Syres et al.

- Cherqui et al have developed a murine model
  - Ctns -/- mice
  - Cystinosin is truncated, mislocalized, and nonfunctional
  - Accumulate cystine in all organs tested
  - Ocular changes, muscular defects
- 4-8 week old mice transplanted.
  - Culled at 4 months
  - Some culled at 2 months for confocal tracking
- Engraftment 5-90% for mice transplanted with WT BMCs
  - 3-6% for transplanted by MSCs
- BMC transplanted with lethal irradiation had 93% survival
<table>
<thead>
<tr>
<th></th>
<th>Wild type control</th>
<th>Ctns-/- control</th>
<th>Ctns-/- treated w/WT BMC’s</th>
<th>Ctns-/- treated w/ Ctns-/-</th>
<th>Ctns-/- treated w/ Msc’s</th>
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<tbody>
<tr>
<td>Creatinine P&lt;0.5 */#</td>
<td>0.34</td>
<td>0.49*</td>
<td>0.35#</td>
<td>0.43*</td>
<td>0.38</td>
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<tr>
<td>Crt clearance</td>
<td>79.4 +/- 74.25</td>
<td>32.28 +/- 42.58</td>
<td>65.65 +/- 72.34</td>
<td>43.04 +/- 34.46</td>
<td>85.35+/- 64.60</td>
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Figures 1-4

- Slides using stains to locate transplanted GFP+ cells
Figure 1.
Kidney Sections

- TOP Ctns-/-
  Transplanted by -/-
  No GFP
- Mid:WT Tx by WT
  Few GFP
- Bottom
  Ctns -/- transplanted
  By WT
  Abundant GFP
Figure 2. Ctns-/- mice Tx w/ WT cells

- In proximal distal tubules
- some colocalized to tubular and glomerular basement membranes and with endothelial cells
Figure 3A-D

- Ophthalmologic complaints are significant in Cystinosis
- Less mice treated with WT BMC had orbital crystals
Figure 3  E-H

- Majority of GFP+ cells in brain were associated with blood vessels
Figure 4. Muscle and Spleen
<table>
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<th>Table 2</th>
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<tr>
<td><strong>2 months</strong></td>
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<td>Cystine content significantly less in:</td>
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<tr>
<td>WT BMC Tx Spleen, Liver</td>
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<tr>
<td>MSC Tx Hearts</td>
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<td><strong>4 months</strong> Compared to Ctns-/- Tx w/ Ctns-/-</td>
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<tr>
<td>WT BMC brain, eye, heart, kidney, liver, muscle, spleen</td>
</tr>
<tr>
<td>WT MSC brain, heart, kidney, Spleen</td>
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Table 3

- Ctns expression seen in all tissues evaluated s/p WT BMC transplant
  - At 2 and 4 mos
  - Not to control levels
- MSC transplanted with expression (except 2 mos in muscle), but very low
- Ctns-/- donors had none detected
Figure 6
Conclusion

- WT BMCs engrafted in major affected tissue
  - Brain, kidney, eye, hearts, muscle, liver, spleen
  - Reversed the disease (compared to Ctns-/- syngeneic transplant)
- MSCs did not integrate efficiently. Renal Cystine levels decreased at 2 mos, then increased thereafter
- Mechanism?
- Bystander, replacement?
Future Directions

- S. Cherqui also has a lentiviral vector
- Gene therapy could resolve this issue with acceptable toxicity
  - Less chemotherapy
  - Less neutropenia
  - Less immune compromise
  - No GvHD
- Many steps may need to be optimized
- Needs proof-of-principle that it has a can work
Future Directions (2)

- Prior to gene therapy
  - Trial for matched sibling gene therapy
  - Gahl paper shows mortality rate is 8%
  - Gahl, Schneider discussion
- Registry, survey
- Can they tolerate conditioning? Optimal conditioning?
  - Murine model
- How much correction needed?
  - Murine dilutional
Thank you
- Amount of cellular cystine does not correlate to problems
  - Lymph cell lines, fibroblasts with high levels do not have crystals
  - Liver and intestine of patients have crystals but less affected
- Nephropathy path unknown. Possibly caused by increased apoptosis
  - Lysosomes are involved with programmed cell death