Introduction to Cellular Therapy

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Goals

• Understand basics of the most common cellular therapy, bone marrow transplantation
• Become familiar with novel cellular therapies, both already in the clinic and still at the research bench
• Understand some basic technical aspects of cellular therapy
Bone marrow transplantation

- Currently most common cellular therapy treatment
- Bone marrow contains the “hematopoietic stem cell” responsible for blood production

**Locations of Somatic Stem Cells in the body**

- **Autologous**
  - For bone marrow recovery after high-dose chemo
  - Bone marrow may or may not be affected with disease (e.g. multiple myeloma, lymphoma)

- **Allogeneic**:  
  - To replace diseased with normal bone marrow from a donor
  - For graft versus tumor effect via lymphocytes

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Indications for Hematopoietic Stem Cell Transplants in the US, 2012

- Allogeneic (Total N=7,554)
- Autologous (Total N=11,145)

Number of Transplants

- Myeloma/PCD
- AML
- ALL
- CML
- NHL
- HD
- MDS/MPD
- CLL
- Aplastic Anemia
- Other Non-Malignant Dis
- Other Cancer
Annual Number of Transplant Recipients in the US by Transplant Type

* 2013 Data incomplete
Stem Cell Sources for Allogeneic Transplants by Year

- Bone Marrow (BM)
- Peripheral Blood (PB)
- Cord Blood (CB)

Transplants, %

<table>
<thead>
<tr>
<th>Year</th>
<th>Bone Marrow (BM)</th>
<th>Peripheral Blood (PB)</th>
<th>Cord Blood (CB)</th>
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<tbody>
<tr>
<td>2003-2007</td>
<td>25%</td>
<td>65%</td>
<td>10%</td>
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<tr>
<td>2008-2012</td>
<td>15%</td>
<td>75%</td>
<td>10%</td>
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CIBMTR
CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH
Peripheral blood versus bone marrow

- Faster neutrophil and platelet recovery
- Apheresis more practical
  - Operating room versus (G-CSF mobilization + apheresis)
  - No need for red cell reduction
- Caveat: Higher T-cell content results in higher rate of chronic GVHD in allogeneic transplant
Umbilical cord blood vs PB/BM

- HLA mismatch better tolerated (less GVHD)
- Readily available for use
- Usually CMV-negative
- Caveat: smaller hematopoietic stem cell numbers, prolonged engraftment time
PB hematopoietic stem cell “mobilization”
Apheresis principle

• Greek apairesos: “to take away by force”
Separation by density centrifugation

*Median measurements for separation by specific gravity
Target the cell of interest

Access

Return

Collect
Peripheral blood mononuclear cells via apheresis

Standard cell for BMT

Standard BMT immunotherapy

Emerging BMT immunotherapy

1-2% circulating cells: Dendreon “pulsed” dendritic cells
HSC: surface-marker CD34+

- At least $2 \times 10^6$ CD34+/kg required for long-term BM engraftment/blood production
T-lymphocytes: crucial for GVT

• “GVT” effect: prevent or treat disease relapse
• Caveat: also cause GVHD (graft versus host disease)
• Collected without need for mobilization: “Donor lymphocyte infusion”
• T-cell markers: CD3+, CD4+, CD8+
  – < 1 x 10^8 CD3+/kg allows GVT without excessive GVHD

Castor M, Front Pharmacol 2012
CD34+ selection

• To prevent GVHD
  – Haploidentical transplant (4/8 HLA match)
  – Later T-cell “donor lymphocyte infusion” as necessary for GVT effect
Transplant Recipients in the US, by Transplant and Donor Type

* 2013 Data incomplete
Survival after HLA Match Sibling Donor Transplants for AML, 2002-2012

- Early (n=7,607)
- Intermediate (n=2,124)
- Advanced (n=2,578)

p < 0.0001

Probability, %

Years

By Disease Status
Causes of Death after HLA Match Sibling Transplants done in 2011-2012

Gene therapy with autologous cells eliminates GVHD problem
**HSC gene therapy: option in congenital disease**

- **Patients treated**
  - **Congenital immunodeficiencies:**
    - X-linked SCID (severe combined immunodeficiency): 85% disease-free survival of 20 pts
    - Adenosine deaminase deficiency: 75% dz-free survival of 40 pts
    - Chronic granulomatous disease, Wiskott Aldrich syndrome
  - **Hemoglobinopathies:** β-thalassemia and sickle cell disease
- **No GVT effect needed:** replace enough “corrected” HSCs to provide adequate function
- **“Ex-vivo” gene therapy:** CD34+ cells undergo gene transfer using retro/lentiviral vectors to correct genetic deficiency

Chandrakasen S, Heme
Onc Clin North Am  2014
HSC gene therapy

- Transduced HSC injected back into autologous recipient

- Caveats
  - "Insertional mutagenesis" activation of cellular oncogenes leading to acute leukemia and MDS was observed in X-SCID, WAS, and CGD trials
  - "Gene silencing" by methylation of viral promoter

Gene Editing

• Way to create site-specific double stranded DNA breaks to remove diseased gene and replace with normal gene
Chimeric antigen receptors (CAR) T-cell therapy

• Unlike traditional TCR, Ag recognition is not HLA restricted
• Autologous T-cells collected by apheresis undergo gene transfer using retro/lentiviral vectors to express “CAR” against antigens overexpressed in tumors or tumor-specific antigens
• Unlike gene transfer into HSC, leukemic transformation has not been observed
CAR AND B-cell malignancies

• anti-CD19 CAR T-cells for B-cell malignancies:
  – 70% 2-year disease free survival in 30 ALL pts (Grupp, 2014)
  – Caveats
    • Requires lympho/myelo-depletion conditioning prior to infusion to increase CAR T-cell survival
    • “Cytokine release syndrome”: hypotension, fevers, fatigue, renal failure, and obtundation, severe in 30%
    • Depletion of normal B-cells: chronic IVIG therapy
    • Relapse with CD19- B-cells

Grupp S, NEJM 2013
Cell therapy processing

Cell phenotype/identity
Cell viability
Cell potency
Cell purity
Contamination free: bacteria, virus, fungi, endotoxin

somapps.med.upenn.edu/pbr/cvp/f/technology.html
Causes of Death after HLA Match Sibling Transplants done in 2011-2012

- Primary Disease: 48%
- Infection: 16%
- GVHD: 13%
- Organ Failure: 4%
- Second Malignancy: 18%
- Other: 1%
Anti-viral T-cells for transplant recipients

- Transplant recipients at risk for viral re-activation due to immunosuppression
  - EBV: post-transplant lymphoproliferative disease
  - CMV: pneumonia, hepatitis, retinitis, colitis
  - Adenovirus: pneumonia, hepatitis, colitis, haemorrhagic cystitis, keratoconjunctivitis
  - BK and JC polyomavirus: hemorrhagic cystitis, nephritis, multifocal leukoencephalopathy

- Expansion of donor (or recipient) CD4+ and CD8+ T-cells that are specific for these viruses

Eggermont, Trends Biotech 2014
Evolution of adoptive T-cell therapy

Moss and Rickinson, 2005
Peripheral blood mononuclear cells via apheresis

Standard cell for BMT

Standard BMT immunotherapy

Emerging BMT immunotherapy

1-2% circulating cells: Dendreon “pulsed” dendritic cells
NK lymphocytes: GVT without GVHD?

- 10-15% of circulating lymphocytes
- NK cell markers: CD56+CD3-
- Specific NK donor types mediate GVT possibly without increased GVHD in acute leukemia
  - Apheresis product depleted of CD3+ (T-cells) and CD19+ (B-cells)
  - Then cytokine expanded

Franco Locatelli et al. J Leukoc Biol 2013
Other current and future cell therapies

- **Mesenchymal cells (MSC)**
  - Umbilical cord or fat
- **Tumor-infiltrating lymphocytes**
  - Harvested tumor
- **Induced pluripotent stem cells (iPS)**
  - Skin cells or fibroblasts
- **Somatic cell nuclear transfer (SCNT)**
  - Fibroblast, female egg
Mesenchymal cells (MSC)

• “Mesenchyma”: the embryonic layer giving rise to bone marrow, but also bone/cartilage, muscle, fat
• Exist in all tissues: easily isolated from adipose tissue, umbilical cord
• Two types
  – Mature stromal cells: cells from tissue stroma that help to scaffold and sustain other cells
  – Multipotent stromal cells, “stem cells”: cells from bone marrow that can differentiate into a variety of mature cell types

Meragalli M et al
J Stem Cell Res Ther 2011
Uses of MSCs

- Non-immunogenic: do not need to be “matched”
- Over 300 current clinical trials
- Main functions
  - Tissue repair/injury healing via differentiation and production of trophic factors
  - Dampening immune response
  - Prochymal to treat GVHD and Crohn’s disease is FDA-approved

Xin Wei et al
Acta Pharmacol Sin 2013

- Myocardial infarction (22.9%)
- Diabetes (10.3%)
- Spinal cord injury (9.2%)
- Crohn’s disease (3.8%)
- Aplastic anemia (1.5%)
- Rheumatoid arthritis (1.1%)
- Brain injury (0.4%)
- Graft versus host disease (16.0%)
- Liver cirrhosis (10.3%)
- Osteoarthritis (8.0%)
- Multiple sclerosis (3.4%)
- Systemic lupus (1.1%)
- Parkinson’s disease (0.8%)
- Others (11.0%)
Tumor-infiltrating lymphocytes (TILs)

• Method of “adoptive cell transfer therapy” (ACT) involving infusion of autologous tumor-specific T cells (CD8+) harvested and expanded from resected metastatic tumor deposits
• Successful in melanoma treatment and being explored in other cancers also
  – Side effects include vitiligo, ureitis and retinitis

• Recipient lympho-depletion required:
  - Decrease competition from recipient lymphocytes for growth factors/ cytokines
  - Prevent rejection
Induced pluripotent stem cells (iPS)

- Can theoretically generate any cell type of the body
- No risk of rejection, as generated from autologous cells

- Teratoma formation
- “Reprogramming” introduces genetic modifications that could cause cancer

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Somatic cell nuclear transfer

- Like iPSC, can theoretically generate any cell type of the body
- Like iPSC, no risk of rejection; derived from autologous cells
- Reprogramming may be safer than iPSC

Teratoma formation
Thank you!

Questions?