Bone Marrow Transplantation for Epidermolysis Bullosa

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Stem Cells = Master Cells

- Divide many times.
- Do not get used up.
- Billions of older cells are shed every day and replaced.
Wide Range of Uses

- Arthritis
- Epidermolysis bullosa
- Burns
- Muscular dystrophy
- Kidney disease
- Stroke
- ALS
- Alzheimer’s
- Parkinson’s
- Traumatic spinal injury
- Macular degeneration
- Cardiovascular disease
- Diabetes
- Macular degeneration
Stem Cell Medicine

Genes

Cells

Tissues

Individuals

Society
Stem Cells: Self-renewing Parts

- Common myeloid progenitor
- Common lymphoid progenitor
- Thymus
- Natural killer (NK) cell
- Erythrocytes
- Platelets
- Basophils
- Eosinophils
- Neutrophils
- Monocytes
Bone Marrow Transplantation
Stem Cells Changing Their Plans

1. First stem cell therapy
   → Leukemia

2. First gene therapy
   → Lysosomal diseases

3. Using blood to regenerate skin
   → Epidermolysis bullosa
Medawar's Rules

• Start with a practical problem that can be solved.
• See inexplicable data as potential discovery in disguise.
• Draft and explanation and show the proof.
• Expand the theory.
• Make it clinically meaningful.
RDEB Skin

A Healthy skin

- Stratum corneum
- Stratum granulosum
- Stratum spinosum
- Stratum basale
- Basement membrane
- Dermis

B Skin in patient with recessive dystrophic epidermolysis bullosa

- Erosion of skin layers
- Blistering between the epidermis and dermis

Epidermal cell
- Hemidesmosome
- Lamina densa
- Anchoring plaque
- Anchoring fibrils (type VII collagen)

Damaged or absent anchoring fibrils

NEJM 2015
Cellular Therapy: Preclinical Model

Donor GFP → 8 million IV → Recipient EB

Epidermis

Type VII Collagen

Donor cell

Dermis

Blood 2009
HCT Increases Type VII Collagen

NEJM 2010
Anchoring Fibrils: 3 years after HCT

Electron Microscopy  Immuno-Gold
Oversight Committees

Colleen Delaney, MD
Gay Crooks, MD
John McGrath, MD
Alain Hovnanian, MD
Hiroshi Shimizu, MD/Katsuto Tamai, MD, PhD

Data Safety and Monitoring Board

External review of each patient’s eligibility

Web-based, password-protected transmission of patient information
Confidentiality agreement
Transplantation Regimens

**MAC (N=6)**
- BU BU BU BU BU CY CY CY CY
- HCT
- GvHD Prophylaxis: Cyclosporine + MMF

**MAC, MSC (N=7)**
- BU BU BU BU BU CY CY CY CY
- HCT + MSC
- GvHD Prophylaxis: Cyclosporine + MMF

**RIC, MSC (N=13)**
- Flu Flu Flu Flu Flu CY CY CY CY
- HCT + MSC
- GvHD Prophylaxis: Cyclosporine + MMF

**Details:**
- BU: Busulfan
- CY: Cyclophosphamide
- Flu: Fluorouracil
- TBI: Total Body Irradiation
Functional Test of Skin Fragility
Skin Fragility +1 Year after HCT

Blister Times - RII-1

- Father
- Mother
- Patient Day Pre-Tx
- Patient Day +31
- Patient Day +59
- Patient Day +186
- Patient Day +460

Time to Full Blister in Minutes
Increased Healing after HCT

“One of the major reasons we wanted to do transplant is because the wound on his knee has been open for the past three years. We knew this put him at increased risk of skin cancer and besides that it just HURT.”
Increased Healing after HCT

Parental statement:
“His skin is healing beautifully. He is standing, taking steps with support, and becoming active again.”

“All that purple on his knee...that is where there used to be no skin. He literally has 2 small wounds on his left leg. Each one the size of a dime. That is it.”
“Today he ate:

- nachos and cheese
- chips and salsa
- chocolate chip cookie
- oat milk
- Chex
- ice cream
- yogurt
- melon
- taco
- popcorn
Increased Healing after HCT

Parental statement:

- Chronic wounds
- All areas of body involved
- Healing time of large wounds 30 to 45 days

- All chronic wounds are healed
- Many areas never blistered again
- Healing time of large wounds 10 to 17 days
Increased Healing after HCT

Parental statement:

- Blistering 75% of body area
- Not able to stand up
- Hands gloved from day 1

- Blistering 30% of body area
- Walked unassisted at 11 months
- Half-finger gloves at night time
Increased Healing after HCT

Parental statement:

- Severe involvement of oral mucosa
- Weekly corneal abrasions
- Daily dressing changes (1.5-2 h)
- Weight gain stopped at 1 year
- Constant itch increased at nighttime, partially controlled only with medications

before transplant

after transplant

- Small blistering in upper palate
- No corneal abrasions
- Weekly dressing changes (20-30 min)
- Weight gain restarted at 9 months after transplant
- Itch disappeared 1 month after transplant
Grid for EB Therapy

1. Collateral damage
   → Physical and immune injury
2. Plasticity, harnessed
   → Reprogramming
3. Gene correction
   → Gene addition and gene editing
Grow Your Own Transplant: iPS cells

Transcription Factors → Weeks of factor induction → embryonic-like stem cells
Hatched: induced pluripotent stem (iPS) cells

Self renewal produces infinite supply of patient-matched cells

Diseased patient

Human Skin/Fat/Blood

Ectoderm: Skin, Brain, Nerve
Mesoderm: Blood, Heart, Kidney, Muscle, Bone
Endoderm: Lung, Stomach, Liver, Pancreas, Intestine
Blood from RDEB iPS Cells

Skin-Biopsy

Embryoid bodies

CFU-E

CFU-GM

Number of colonies per 100,000 cells

Patient 2
Patient 3
Bone Marrow
Therapeutic DNA Repair

- Nuclease-induced double-strand break
  - NHEJ
    - Deletions
      - Variable length indels
    - Insertions
  - HDR
    - Precise insertion or modification
    - Donor template
Site-specific Nucleases

TALEN

CRISPR/Cas9

Zinc Finger
Type VII Collagen Restored in Skin

Skin from human induced pluripotent stem cells

RDEB

RDEB Unmodified

RDEB TALEN Edited

1837 locus

Type VII collagen

Mol Ther 2013
The 3-body Problem of Regenerative Medicine
2020 Vision: Cells as Medications

- Decode determinants of cell fate
- Understand organogenesis
- C7 cells from induced pluripotent stem cells
- People with RDEB
- Combination therapy
Combination Therapy for RDEB

**Combination therapy**

- **Allogeneic cells**
- **Mosaic cells**
  - Autologous cells with natural reversion of COL7A1 mutation
- **Gene-corrected cells**
  - Autologous cells after gene editing or gene addition of COL7A1

**Cellular therapy**

- Skin
- Keratinocytes
- Fibroblasts
- Induced pluripotent stem cells (iPSCs)
- Bone marrow
- Cord blood
- Hematopoietic (stem) cells
- Mesenchymal (stem) cells

**Type VII collagen protein therapy**

**Local therapy**

- Intradermal injection (e.g., with microneedles)
- Skin grafts
- Healing effects on skin and mucosa (and potentially other organs)

**Systemic therapy**

- Intravenous injection
- Intraarterial injection

*CNEJM 2015*
Collaborators and friends

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