Andrew D. Leavitt, MD

BIOGRAPHY:

Andrew D. Leavitt, MD is a Professor with appointments in the Department of Laboratory Medicine and the Department of Medicine (Hematology). He received his MD from Harvard Medical School, and completed his internal medicine residency training at The University of Michigan. He then came to UCSF in 1988 to begin his Hematology Fellowship training and was appointed to the UCSF faculty in 1992. Dr. Leavitt is the Medical Director for the Adult Blood and Marrow Transplant (BMT) Laboratory, and Assistant Medical director for the UCSF Blood Bank. He is also Directs the Non-malignant Hematology section within the UCSF Department of Medicine, and is the Medical Director of the UCSF Adult Hemophilia Treatment Center, a federally funded center for patients with congenital bleeding disorders. He sees patients in the UCSF hematology clinic, and provides hematology consultation for patients in the hospital. Among his various teaching responsibilities, he is the director for the UCSF School of Medicine 2nd year Hematology course. His past research has focused on megakaryocyte development and platelet function, and his current laboratory research focuses on improving hematopoietic stem cell options for patients in need of a bone marrow transplant. He has served on the editorial boards for Blood, the leading professional journal in hematology, and the Journal of Clinical Investigation.
Stem Cells, and Blood Cells, and Blood Counts, Oh My!*
a walk down the hematologist’s yellow brick road

Andrew D. Leavitt, MD
UCSF Mini Medical School
June 4, 2013

*With apologies to L. Frank Baum and all the lions and tigers and bears…

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Our Road Over the next 90 minutes

- Stem cells – Definitions
Types: embryonic, adult, IPS

- Hematopoiesis is THE model for stem cell biology

- Hematopoietic development:
  Fetal & Adult
  Location, Potential, & Output

- The Bone Marrow:
  All about location: The Niche
  What it looks like

- Bone Marrow Transplant – Stem Cell Sources:
  Marrow, Peripheral Blood, Umbilical Cord Blood

- The Complete Blood Count (aka: CBC)

- Blood Donations & Transfusions

- What Makes a Stem Cell a Stem Cell?

- Conception in a Dish

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Embryonic stem cells & Early Development

Comes from undifferentiated cell from the inner cell mass of the early pre-implantation embryo (BLASTOCYST) that has the potential to become any cell type in the body.

Development Milestones and Terms

Days:
- 1-7: Zygote to blastocyst...
- 7-14: Uterine implantation
- 14: Three distinct layers begin to form
- 21-24: Beginning of future nervous system
- 21-3: Beginning of future face, neck, mouth, and nose
- 8: Beginning of organ formation
- ≥8: Called a fetus

Embryonic Development:

~1 day for a Zebrafish (3+ weeks for a human)

Keller et al. 2008

Starts at 64 cell stage for the Zebrafish
**Adult Stem Cell**

1. UNDIFFERENTIATED cell
2. Located within differentiated tissue
3. Can **renew itself** indefinitely AND,
4. With certain limitations, **give rise to all** the specialized cell types of the tissue from which it originated.

**BEST EXAMPLE:** Hematopoietic Stem Cell (HSC)

Tissues thought to contain adult stem cells: Skeletal muscle, Hair follicle, Heart muscle, Dental pulp, Colon, Liver, Prostate, Mammary gland, Fat, Pancreas...

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**Stem Cells & Potential...**

**(more definitions)**

- **Totipotent**: (whole) A cell that can give rise to all the cells of a new individual, including cells of the amniotic sac and the placenta.
  - Zygote (fertilized egg)
- **Pluripotent**: (more) A cell that can give rise to all types of adult tissue cells but not extraembryonic components of the trophoblast or the placenta.
  - Embryonic stem cell
  - Induced pluripotent stem (iPS) cell
- **Multipotent**: (many) A cell that can give rise to several types of mature cells within ONE mature organ, tissue or physiological system.
  - Adult stem cell (hematopoietic stem cell)

(HSCs are often incorrectly called pluripotent)

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**The Basic Developmental Paradigm:**

_A Unidirectional Path of More Options to Fewer_

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**Induced Pleuripotent Stem (iPS) Cell**

_A Paradigm Shift_
**Induced Pleuripotent Stem (iPS) Cell**

Some background biology #1

DNA \[\rightarrow\] RNA \[\rightarrow\] Protein

"Transcription Factor":
- binds to a specific DNA sequence
- regulates gene expression where it binds
- can regulate many (related) genes

**Induced Pleuripotent Stem (iPS) Cell**

Some background biology #2

- All cells of an organism have the same DNA.
- Cell-to-cell differences reflect different gene expression patterns among cells.
- Transcription factors regulate sets of genes.
- *Maybe... just maybe... one could find a set of transcription factors that 'defines' the stem cell state, & maybe that set of transcription factors expressed in a mature cell could re-establish the stem cell state.*

**Induced Pleuripotent Stem (iPS) Cell**

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazunori Takahashi* and Shinya Yamanaka**

*Department of Stem Cell Biology, National Institute of Medical Sciences, Yokohama, Japan
**Department of Stem Cell Biology, Kanagawa Institute of Medical Science, Tokyo, Japan

Cell 126:663-676, 2006

- Identified 24 candidate transcription factors
- Expressed them in various combinations in fibroblasts
- Identified a set of 4 factors that give a fibroblast many characteristics of an ES cell

**Induced Pleuripotent Stem (iPS) Cell**

"De-differentiation"

- 14 – Oct3/4
- 15 – Sox2
- 20 – KIF4
- 22 – c-Myc

No colonies
At day 10
So What?

- Basic Research
- Disease-specific research
- Possible therapies
**Better Living Through iPS cells...**

- iPSCs
- 6
- 7
- Compatible transplants
- Cells, e.g. skin

**Lets Shift Gears**

**World War II**

The ‘birth’ of hematopoietic stem cells & Bone marrow transplants

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**History of Hematopoietic Stem Cell Transplantation**

A report in 1950 that included classified studies performed in early 1940s by the atomic energy commission.

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**History of Hematopoietic Stem Cell Transplantation**

"The Effect of Splenic Protection of Bone Marrow Transplantation" by O. Jacobsen, B.D. E. Mann, and A. M. Spence, in *Archives of Surgery*. Chicago, Ill.
Bone Marrow Protects Against Radiation Injury

Evidence Favoring the Cellular Theory of BM Protection

The Radiation Sensitivity of Normal Mouse Bone Marrow Cells, Determined by Quantitative Marrow Transplantation into Irradiated Mice

A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells

Are the protective effects cellular or humoral?
One 'Source Cell' (aka, a Stem Cell) for All or Multiple Source Cells?

Therefore, A Hematopoietic Development Tree

The Changing Location of Hematopoiesis During Gestation & Post-partum

The Changing Location of Hematopoiesis in Utero
Hemogenic Endothelium

Lineage Tracing by Genetic Tagging in the AGM

Evolving Models

Zebrafish mouse

Zape & Zovein, 2011

The hematopoietic stem cell niche

(Evolve location, location, location. Hematopoietic at the right place)

Niches:
Endosteal, Perivascular

Interactions:
Matrix, Cells

Growth factors:
Soluble, Cell surface

Points:
Quiescent and dividing HSCs
Symmetric & Asymmetric divisions

Transcription factors & hematopoiesis

Circadian Rhythms and Hematopoietic Stem Cell Circulation

Yes, there is a nervous system of the bone marrow!
The Hematopoietic Stem Cell Circulation

- Circulate
- Retain
- Mobilize/Release

*NOTE: This just shows the endosteal niche for simplicity, but there is also a perivascular niche.

Wilson & Trumpp 2006

And now…the marrow & the cast of characters

- Bone Marrow Biopsy
- Bone Marrow Aspirate
- Peripheral Blood

Bone Marrow - How do we get?

- Biopsy
- Aspirate

Examples of bone marrow biopsies

- Normal
- Hypocellular
- Lymphoma
- Lymphoid nodule

Hoffbrand et al Essential Hematology 2001
The Hematopoietic Development Tree

<table>
<thead>
<tr>
<th>Stem Cells</th>
<th>Multipotent Progenitor Cells</th>
<th>Committed Precursor Cells</th>
<th>Mature Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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</tbody>
</table>

- Identity by functional assay
- Identity by routine marrow staining

Aspirate Gives the Best Cell Definition

Bone Marrow Aspirate - Myelopoiesis

- 1. (myelo?)blast
- 2. promyelocyte
- 3. myelocyte
- 4. metamyelocyte
- 5. Band
- 6. Mature Neutrophil (poly)

Chronic Leukemia

Acute Leukemia

The Hematopoietic Development Tree & Disease

<table>
<thead>
<tr>
<th>Normal</th>
<th></th>
<th>AnoLymphoch (AML)</th>
<th>WBC</th>
<th>RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem Cell</td>
<td>Multipotent Progenitor Cell</td>
<td>Committed Precursor Cell</td>
<td>Mature Cell</td>
<td></td>
</tr>
</tbody>
</table>

- eosinophil
- neutrophil
- monocyte
- basophil
- erythrocytes
- platelets
- T lymphocyte
- B lymphocyte

- Normal
- Chronic Leukemia
- Acute Leukemia
Hematopoietic Stem Cell Transplantation - The Start of Human Studies

- Mid 1950s: realized that BM transplant could be used to treat diseases of the marrow and not just to provide radiation protection
- Mid 1950s - 1960s: lots of failed attempts for BM transplant - interest drops
- 1968: First successful transplant to treat an inherited immunodeficiency using HLA matched sibling
- 1975: NEJM paper by Thomas et al reviewed the state of BMT, and helped define critical issues in the field, including HLA matching, infectious complications, GVHD etc. and showed clearly that one could use this therapy to cure people with leukemia.
- 1979: Better results if treated earlier, i.e., in first remission.

*E. Donnell Thomas awarded the Nobel Prize in 1990 for BMT

How Far We Have Come: Timeline for Blood and Marrow Transplantation

Diseases Commonly Treated with HSC Transplantation

<table>
<thead>
<tr>
<th>AUTOLOGOUS*</th>
<th>ALLOGENEIC**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cancers</strong></td>
<td><strong>Cancers</strong></td>
</tr>
<tr>
<td>Multiple Myeloma</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>Non-Hodgkin's lymphoma</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Myeloproliferative disorders</td>
</tr>
<tr>
<td>GvH</td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>Myelodysplastic syndromes</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td></td>
</tr>
<tr>
<td>Other Diseases</td>
<td><strong>Other Diseases</strong></td>
</tr>
<tr>
<td>Autoimmune disorders</td>
<td>Aplastic Anemia</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>Sickle cell anemia</td>
</tr>
<tr>
<td></td>
<td>Thalassemia major</td>
</tr>
<tr>
<td></td>
<td>Pancreatic endocrine hormone deficiencies</td>
</tr>
<tr>
<td></td>
<td>SCIDs</td>
</tr>
<tr>
<td></td>
<td>Idiopathic aplastic anemia</td>
</tr>
</tbody>
</table>

| *>30,000 annually worldwide; 2/3 for lymphoma and multiple myeloma |
| **>15,000 annually; >50% for acute leukemias |

HSC sources for clinical application - Bone Marrow

1. General anesthesia in operating room
2. 200-300 entries into posterior iliac crest yielding >1,000 ml
3. Overnight stay (or more)
4. Sore for a while
5. Net loss of a couple units of blood
6. Dose: ≥ 2 × 10^8 nucleated cells/Kg
HSC sources for clinical application: Peripheral Blood Stem Cells

1. Mobilization - G-CSF, CXCR-4 antagonist, Rx
2. Two ivs & a cell separator
3. 3-4 hours
4. Cytokine can give flu-like symptoms
5. Product is ~250-300 ml
6. “Dose”: 2-5x10^6 CD34+ cells/Kg

HSC sources for clinical application: Cord Blood

1. No pain
2. No cytokines
3. <100 ml
4. Dose: >2x10^7 nucleated cells
5. Permits greater immune (HLA) mismatch
6. A readily available unrelated source

Clinical limitations: Supply-Demand

• 80% of patients lack a related, HLA matched donor
• Data from NMDP suggests: 10-50% of patients needing a donor remain without one
• Highly pronounced in certain ethnic groups:
  African American (50%)
  Asian American (35%)
  Hispanic ancestry (35%)

So, we think….

Better in the bag than in the trash…

…and lets expand!!
Bone marrow is a very active place

~5,000,000 RBC's/μL
~5 L of blood (70 Kg person) = 5 x 10⁶ μL
So, ~ 25 x 10¹² RBC in body

If RBC life span = 120 days...
then we each make ~2 x 10¹¹ RBC's/day
Or
~8,000,000,000 RBC's/hour!
(Yes, 8 billion!)

That is a lot of cell divisions...

As if that is not impressive enough...

~8,000,000,000 RBC's/hour
RBC is ~ 8 microns in diameter
= ~64,000,000,000 microns
= ~40 miles if placed side-by-side

~25 x 10¹² RBC in body
RBC is ~ 8 microns in diameter
~2 x 10¹⁴ microns = ~124,000 miles!

But, we are visual animals:
124,274 miles means...

Earth's circumference = 24,901 miles
So, total body RBC side-by-side
CIRCLE THE EARTH 5 times

Red Blood Cell (RBC)

Delivers O₂ from your lungs to all your tissues
Takes CO₂ from your tissues to your lungs
Also - binds NO (a vasodilator)

Their size: ~8 μ [capillaries have ~3 μM diameter]
Their life-span: 120 days
Mature RBCs have no nucleus
Too few RBCs = anemia
What tells your body to make RBCs?

**EPO (erythropoietin):**
- Is not stored, but expressed in response to the kidney sensing oxygen in the blood
- Increases # of E-committed progenitors
- Increases GATA1 and FOG expression
- Enhances anti-apoptotic gene expression
- Increases transferrin receptor expression

**Hemoglobin**

Hemoglobin (Hgb) in the RBC carries O₂, which is poorly soluble in water, to tissues & CO₂ from tissues to lungs.

~640,000,000 Hemoglobin molecules/RBC

Heme: Iron in a Porphyrin ring

**Hemoglobin Switching**

ALWAYS: 2 alpha & 2 "beta-like" (3 "gamma" options)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Embryo</th>
<th>Fetus</th>
<th>Birth</th>
<th>6 mo.</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb</td>
<td>αβγε (75%)</td>
<td>αβεε (25%)</td>
<td>αβεε (96-98%)</td>
<td>αβγδ (2-3%)</td>
<td>αβεε (&lt; 1%)</td>
</tr>
</tbody>
</table>

Colors of a bruise are globin breakdown products

**Megakaryocytogenesis - making platelets**

SGF
- Epo, IL-6, LIF, IL-11, TPO

Proliferation
- Ento (Stage I)

Maturation
- (Stage II) & (Stage III)

**Colt (GPIb/IIIa)**
- GP IIb III, GP IIb, PTA, VWF, GPI

**EGF (GPIIb/IIIa)**
- GP IIb IIIa (P)
A quantitative perspective of blood

Normal Values for Peripheral Blood Counts (CBC)

- Red Blood Cells: $4.4 - 5.0 \times 10^9/\mu l$
- White Blood Cells: $3.4 - 10.0 \times 10^3/\mu l$
- Platelet Count: $150 - 400 \times 10^3/\mu l$

- >21 yrs old: Neutrophils: $1.8 - 6.8 \times 10^3/\mu l$
  Lymphocytes: $1.0 - 3.4 \times 10^3/\mu l$
  Monocytes: $0.2 - 0.4 \times 10^3/\mu l$
  Eosinophils: $0.0 - 0.4 \times 10^3/\mu l$
  Basophils: $0.0 - 0.1 \times 10^3/\mu l$

- 70 Kg adult has ~5L blood volume

- ~0.8 $\times 10^{10}$ RBCs/hr
- ~1 $\times 10^9$ Neuts/hr
- ~0.5-1 $\times 10^{10}$ platelets/hr
Marrow cells in the peripheral blood
CASE 4: A 42 year old man is brought to the ER by his wife because he is acting strange, has a fever. You get a CBC that shows Hgb 9.9, Platelet count 37K, and WBC 9.7.

Blood Donations: Infectious Disease Safety

A. Donor Intake: Plays a major role in maintaining a safe blood supply
B. Unit Testing: Tests performed on all units collected in the United States

1. VDRL (antibody) 1947
2. HbsAg (viral protein) 1971
3. HIV 1 (antibody) 1985
4. ALT* (enzyme) 1986
5. HbsAb (antibody) 1986
6. HTLV III (antibody) 1988
7. HCV (antibody) 1990
8. HIV 2 (antibody) 1992
9. HIV-1 p24 Ag* (antigen) 1996
10. NAT for HIV (DNA) 1999/2002
11. NAT for HCV (DNA) 1999/2002
12. NAT for West Nile (DNA) 2003
13. Bacteria (growth) 2003/4
14. Trypanosoma cruzi** (antibody) 2007

*blue = discontinued; **Chagas disease

Consent

Donor: Immigrated to US 17 yrs previously and is asymptomatic
Human Immunodeficiency Virus (HIV) and Hepatitis B (HBV) and Hepatitis C (HCV) Transfusion Transmission Risk

DISEASE TRANSMISSION: What is the risk?

<table>
<thead>
<tr>
<th>Disease</th>
<th>Units of Transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>1: 220,000</td>
</tr>
<tr>
<td>HCV</td>
<td>1: 1,800,000</td>
</tr>
<tr>
<td>HIV</td>
<td>1: 2,300,000</td>
</tr>
<tr>
<td>HTLV I(II)</td>
<td>1: 2,998,000</td>
</tr>
</tbody>
</table>

Bacteria*

- Sepsis: 1: 74,807
- Septic death: 1: 1,498,711

U.S. Blood Collections & Transfusions*

Collections:
- 10,877,000 allogeneic (WB/RBC) donors: 29% first time; 71% repeat donors
- 17,286,000 units WB/RBC collected
- 1,352,000 plateletpheresis collections

Transfusions:
- 15,014,000 whole blood/Red blood cell transfusions
- 2,021,000 platelet transfusions
- 23,668,000 total transfusions

*Estimates from the 2009 National Blood Collection & Utilization Survey, United States DHHS

PLEASE

Roll up your sleeve and become a blood donor if you can!
Thank you...Questions Please!