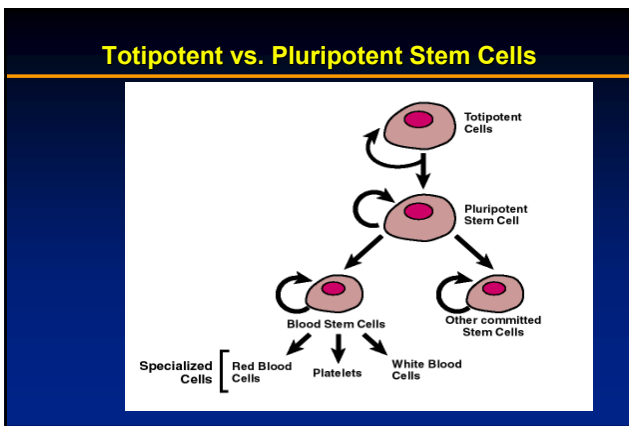


Hematopoietic Progenitor Cell Transplants
November 8th, 2008

Ronan Foley, Hamilton

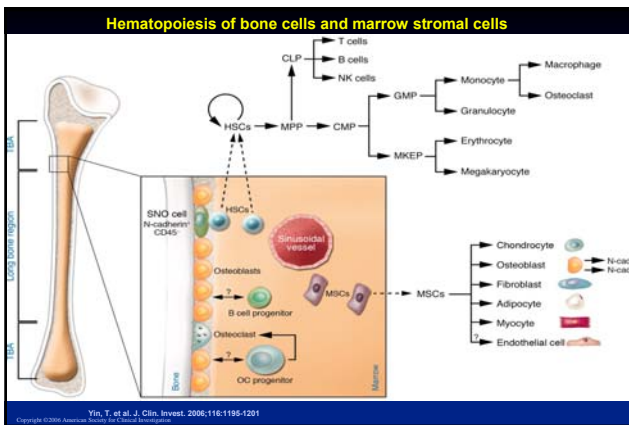
NIH Definitions

- Stem cells - cells that have the ability to divide for indefinite periods in culture and to give rise to specialized cells.
- Pluripotent - capable of giving rise to most tissues of an organism.
- Totipotent - having unlimited capability. Totipotent cells have the capacity to specialize into extraembryonic membranes and tissues, the embryo, and all postembryonic tissues and organs.



Aspirate

Core Biopsy



Schedules for Collection of Mobilized PBSC

Cyclo 2.5g/m²

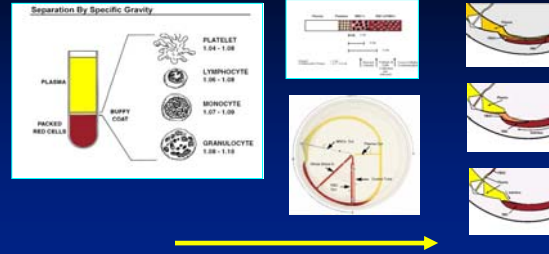
1. G-CSF (5-10ug/kg)
2. G-CSF +SCF (5-10 ug/kg 20ug/kg)
3. G-CSF (5-10ug/kg)
4. G-CSF (16ug/kg)
5. G-CSF +SCF (5-10 ug/kg 20ug/kg)

N.B.:
 1. Filgrastim 300ug, 480ug vials dose rounded off
 2. large volume apheresis 25L

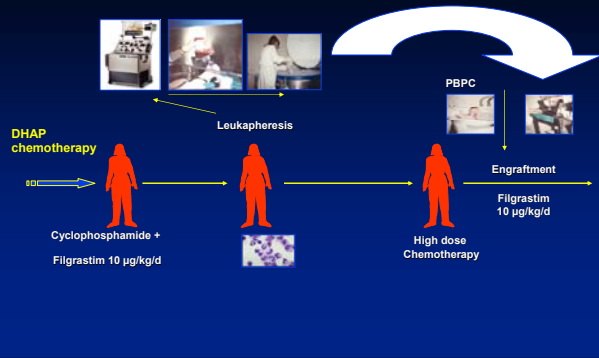
Advantages of PBSC Support Compared to BMT

- Outpatient procedures
- No OR
- Platelets engraft faster
- Decreased cost of treatment
- Decreased chance of tumor contamination in peripheral blood
- Faster recovery of WBC
- Reduced infection, antibiotic use and hospitalization

Cell Separation



Autologous Transplant Synopsis



Indications for Autologous Transplant

- Relapsed Aggressive histology NHL
- Relapsed Hodgkin's Disease
- Myeloma
- **Experimental**
- Low grade lymphoma with marrow purging
- Aggressive (high-grade) lymphoma

Steps to PSCT

1. Insertion of pheresis catheter
2. Mobilization of stem cells
3. Stem Cell Harvest
4. Transplant chemo "High dose chemo"
5. Re-infusion of stem cells
6. G-CSF and recovery

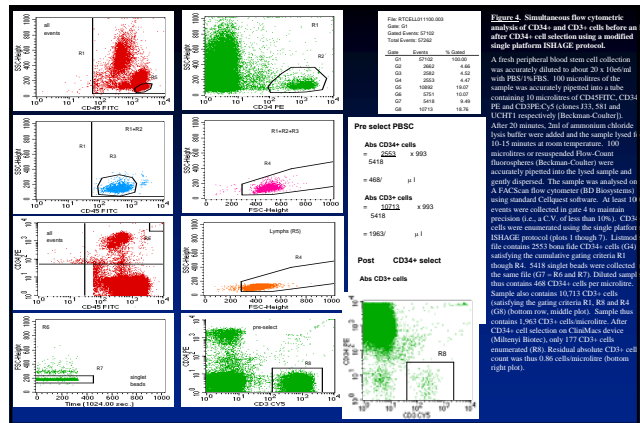
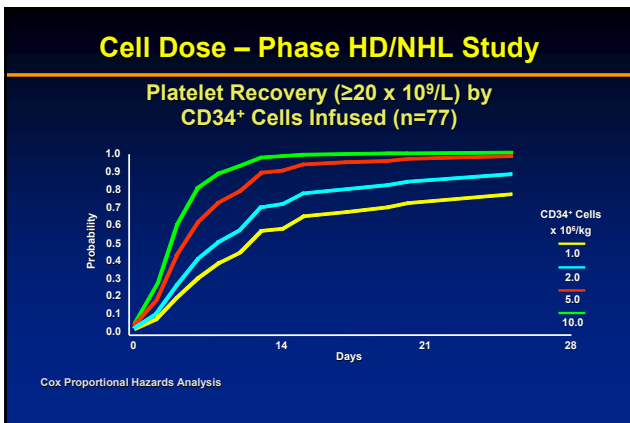
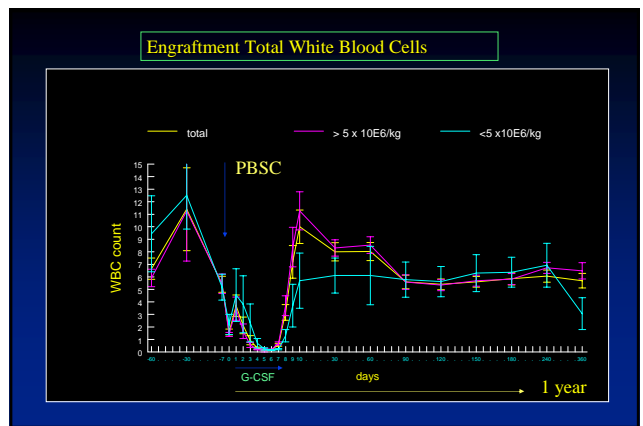
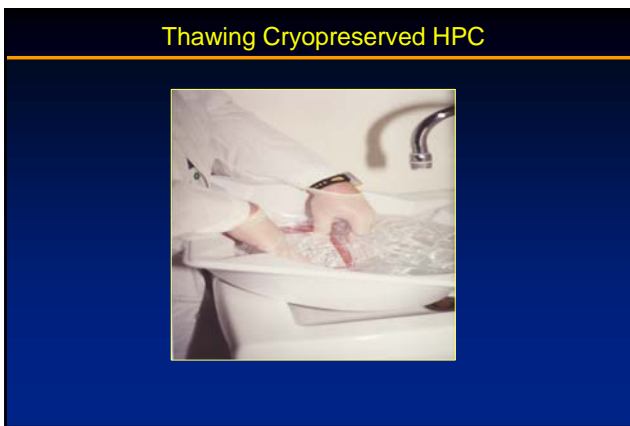
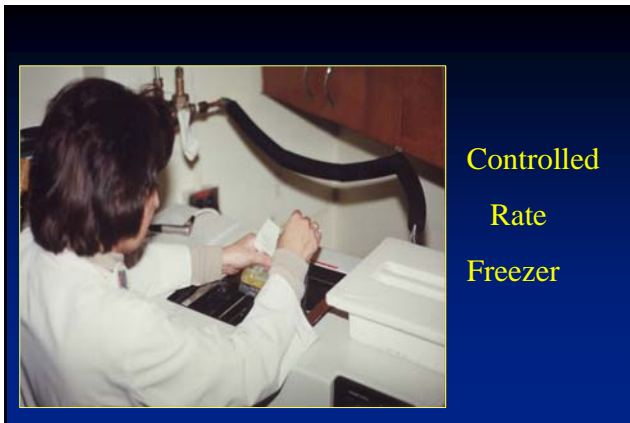


FIGURE 2. Simultaneous flow cytometric analysis of CD34 and CD33 cells before and after CD34+ cell selection using a modified single platform SNA-MAC protocol. A fresh peripheral blood stem cell collection was accurately diluted to about 20 x 10⁶/ml with PBS/PAGE. 100 microliters of the sample was accurately pipetted into a tube containing 10 microliters of CD34-FITC, CD33-FITC and CD33PE-Cy5 (clone-F5, S8 and UCHL1) respectively (Beckman-Coulter). After 20 minutes, 2ml of ammonium chloride lysis buffer were added and the sample lysed 4-10-15 minutes at room temperature. 100 microliters or resuspended Flow-Count fluorophores (Beckman-Coulter) were accurately pipetted into the lysed sample and gently dispersed. The sample was analyzed on a FACScan flow cytometer (BD Biosciences) using standard Cellquest software. At least 10 events were collected in gate 4 to minimize precision (i.e., CV of less than 10%). CD34 cells were enumerated using the single platform SNA-MAC protocol (gate 4 through 7). Lymphoid file contains 2553 bona fide CD34+ cells (G4) including the cumulative gating events R1 through R4. 5418 single beads were collected the same file (G7 - R6 and R7). Diluted sample thus contains 465 CD34+ cells per microliters. Sample also contains 10713 CD33+ cells (enumerated in R5). Residual absolute CD33+ cell count was thus 0.86 cells/microliters (bottom right plot).



Number of CD34 cells infused after high-dose chemotherapy

Minimum: $2 \times 10^6/\text{kg}$ body weight

Optimal: $5 \times 10^6/\text{kg}$ body weight

“Poor Mobilizers”

- bone marrow harvesting
- remobilization with same or more intensive regimen
- dose escalation of available cytokines
- combination of cytokines

Influence of Patient Characteristics on PBSC Mobilization

Variable	Average	No.	CD34 cell/kg* Median	P†
Mobilization G-CSF		119	1.52	.0001
Chemotherapy + CSF		124	3.93	
No. of chemotherapy cycles				
<6	181		2.42	.0227
>6	62		1.98	
Diagnosis				
Breast Cancer	87		2.61	.0211
Non-Hodgkin's Lymphoma	77		2.44	.0758
Other	79		1.71	
Marrow disease				
Yes	58		1.71	.0338
No	185		2.77	
Prior radiation				
Yes	86		1.79	.0455
No	157		2.77	
Disease status				
Remission	65		2.15	NS
Relapse	178		2.41	

Abbreviation NS: Not significant

*Total number of CD34+ cells collected per kilogram divided by number of collection days

†By regression analysis $Y1 = B_0 + B_1X_1 + B_2X_2 + E$

Bensinger et al. Journal of Clinical Oncology Vol 13, No. 10 (October) 1995; pp 2547-2555

Previous Therapy Scoring System

Toxicity factor

0	→	Prednisone, Dexamethasone
1	→	Vincristine, Vinblastine, Bleomycin, IFN α
2	→	Cyclophosphamide, Anthracyclines, Cisplatin, Etoposide
3	→	Chlorambucil, Procarbazine
4	→	Melphalan, Carmustine, Mechlorethamine, Lomustine

TF x N (# courses) = < 60 or > 60

P = 0.02

Any exposure to TF 4 drugs

P = 0.038 ***

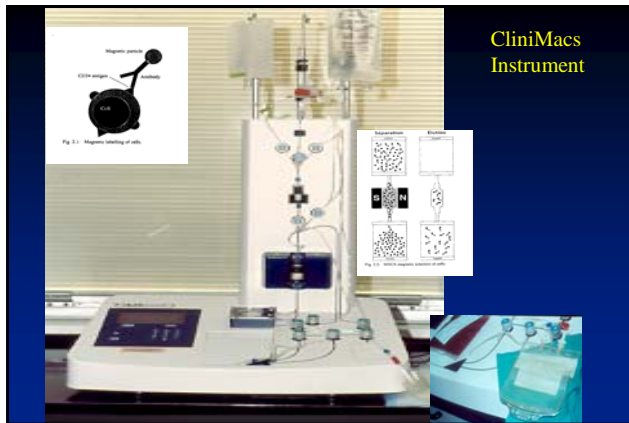
Drake et al, *BJH* 1997

Stem Cell Factor

Description and Biology

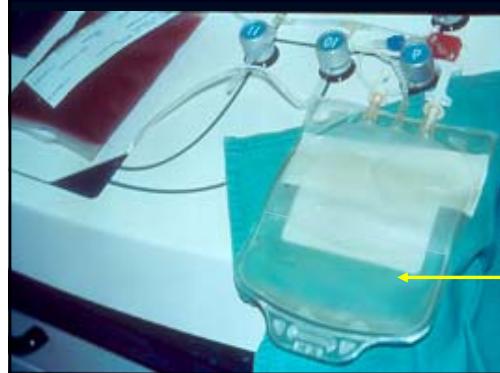
- Glycoprotein growth factor produced in bone marrow stromal cells
- Also called *c-kit* ligand or steel factor
- Found in soluble and membrane-bound forms
- Acts on primitive and mature blood cell progenitors
- SCF receptors widely distributed in normal tissues and some tumor cells

Galli SJ, et al. *Adv Immunol.* 1994;55:1-96.

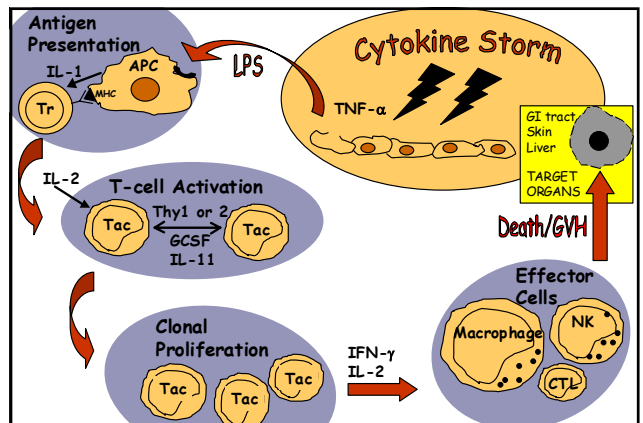
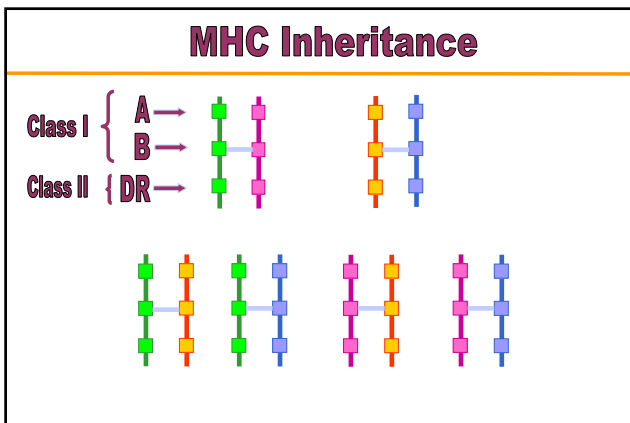
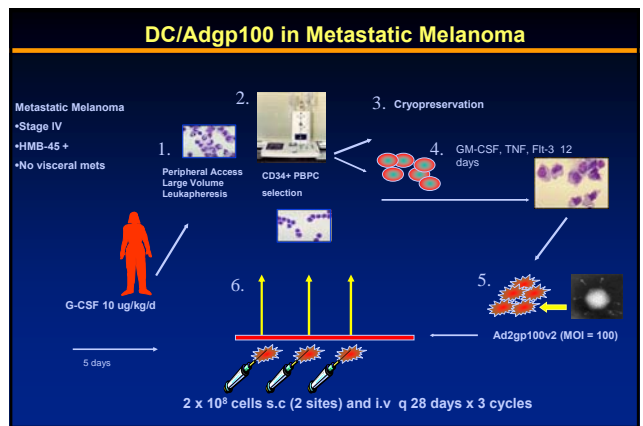
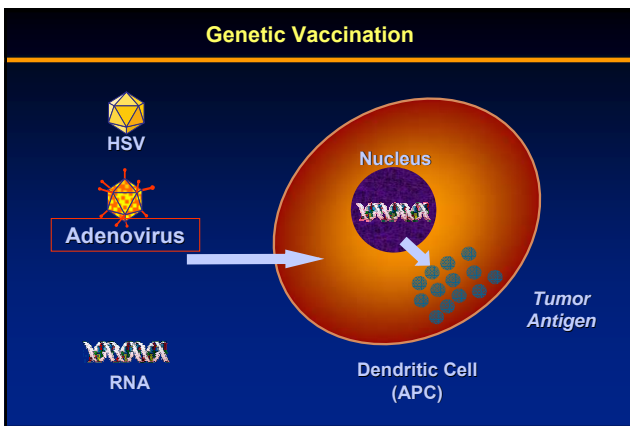
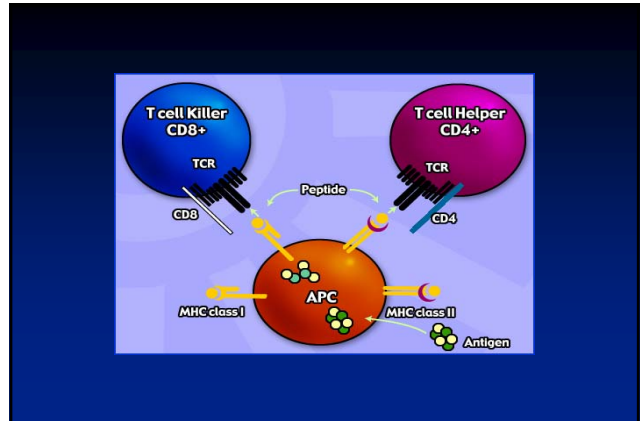
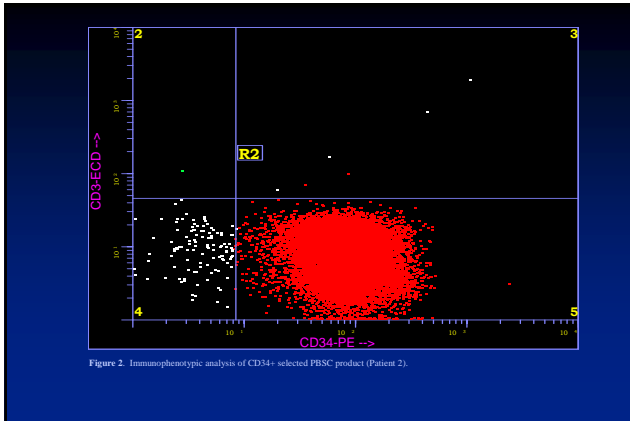


CliniMacs Instrument

CD34 Positive Concentrate



50ml CD34+ Concentrate



Examples of Successful Immunotherapy cGVHD



Graft vs. Leukemia

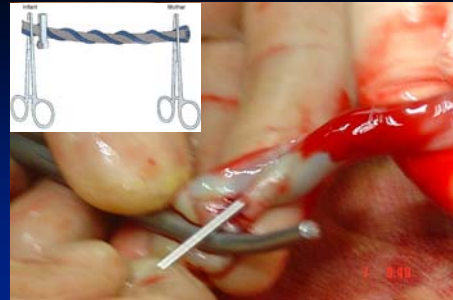
- Immune system very important to prevent growth of cancers
- Major part of the efficacy of allogeneic transplantation is the donor immune system killing any residual cancer cells
- GvHD is a marker of GvL
- Little bit of GvHD is a good thing

Umbilical Cord Blood

- High concentration of blood stem/progenitor cells
- Immunologic immaturity of lymphocytes
- Risk-free collection
- Banked cord blood units – 'off-the-shelf' substitute for blood or marrow units for transplantation



Cord blood - transplantation



Cord blood - transplantation

- Approches to increasing stem cell number
 - ex vivo expansion
 - combination of several cord units
- Approches to reducing GVHD
 - depletion of host-reactive t-cells from graft
 - treatment of graft with irradiated recipient cells and anti-B7:CD28 antibody

Characteristics of Stem Cells

Properties

CD34++
Thy-1+

SSC-low
c-kit+
HLADR-
CD45RA-
CD38-
lin-
CD13-
CD33-

in vitro Assays *in vivo* Assays

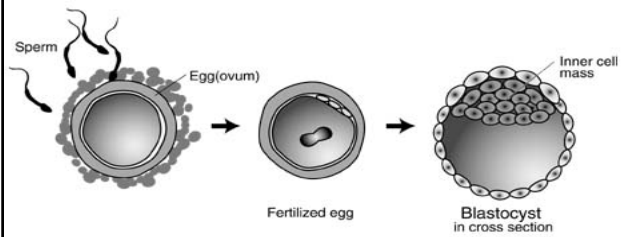
LTC-IC
CAFC
CFU-Blast

Rh-123low
FSC-low

Tx sheep *in utero*

SCID mice
SCID-Hu
Primates
Human BMT

What are Stem Cells?



Potential therapeutic uses of embryonic stem cells

- Traumatic injuries
 - Spinal cord and head trauma
 - Bone fractures
 - Osteoarthritis
 - Skin (burns, ulcers, lacerations)
- Degenerative, ischemic and autoimmune diseases
 - Parkinson's disease
 - Myocardial infarction
 - Diabetes mellitus
 - Multiple sclerosis
 - Muscular dystrophy

Risks and potential pitfalls of embryonic stem cell therapeutics

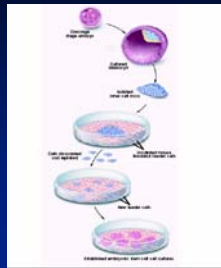
- Reliability of differentiation uncertain
- Transplantation and engraftment
 - ? Requirements for tissue typing
 - ? Uncontrolled growth
 - ? Stability and regulation of therapeutic functions

Ethical controversies in stem cell research

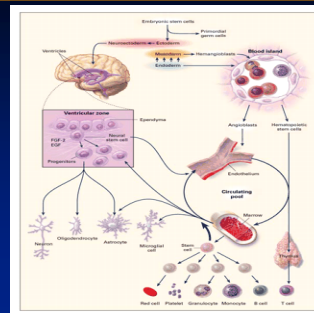
- Sources of embryos
 - Extra embryos from clinical *in vitro* fertilization
 - Intentional fertilization for research
 - Cloning
- Is an oligocellular embryo in a 'test tube' actually a human being?
 - Genetic instructions and protoplasmic substrates
 - Hurdles to generation of whole organism
 - Implantation
 - Organogenesis
 - High risk of spontaneous abortion

Derivation of embryonic stem cell lines

- Isolation of cells
 - Mechanical and enzymatic disruption of zygote
 - Excision of inner cell mass
- Culture on feeder cell layer in dish
- Differentiate into specialized cells
 - Growth factors
 - Gene transfection
 - Transplantation



Post-embryonic stem cells



- Bone marrow
- Blood
- Muscle
- Brain
- Fat
- Liver
- Pancreas
- Other

What about Adult Stem Cells?

- Some Stem cells live in the body of humans.
- Most of these cells are called Multipotent (most adult stem cells). They can be induced to become fewer tissues than Pluripotent Stem cells that are derived from Embryos.
- New research is showing that we can induce adult stem cells to become more pluripotent.

Potential Benefits

- Organ shortage (long term)
- Tissues for Treatments (shorter term)
 - Valves, Skin, Nerves for Spinal Cord Injuries
 - Diabetes (Type 1)

