Notch-mediated Expansion of Human Cord Blood Progenitor Cells Capable of Rapid Myeloid Reconstitution

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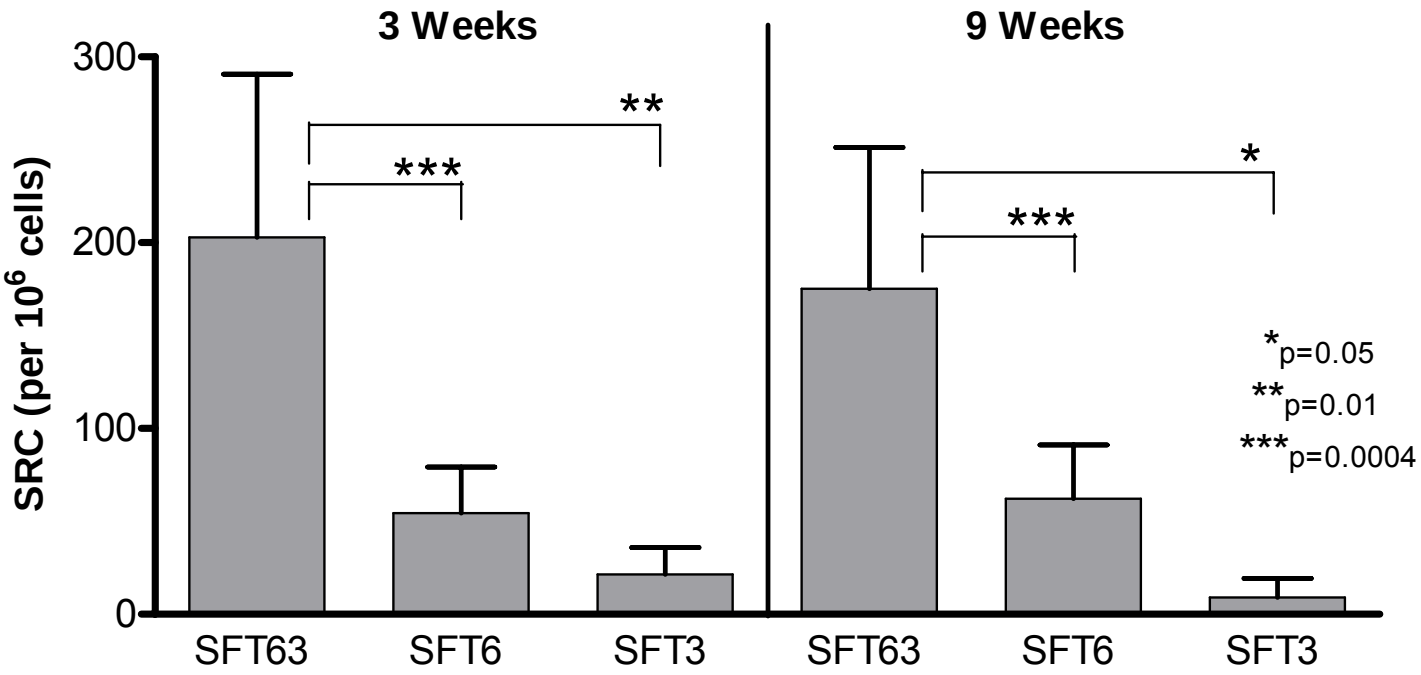
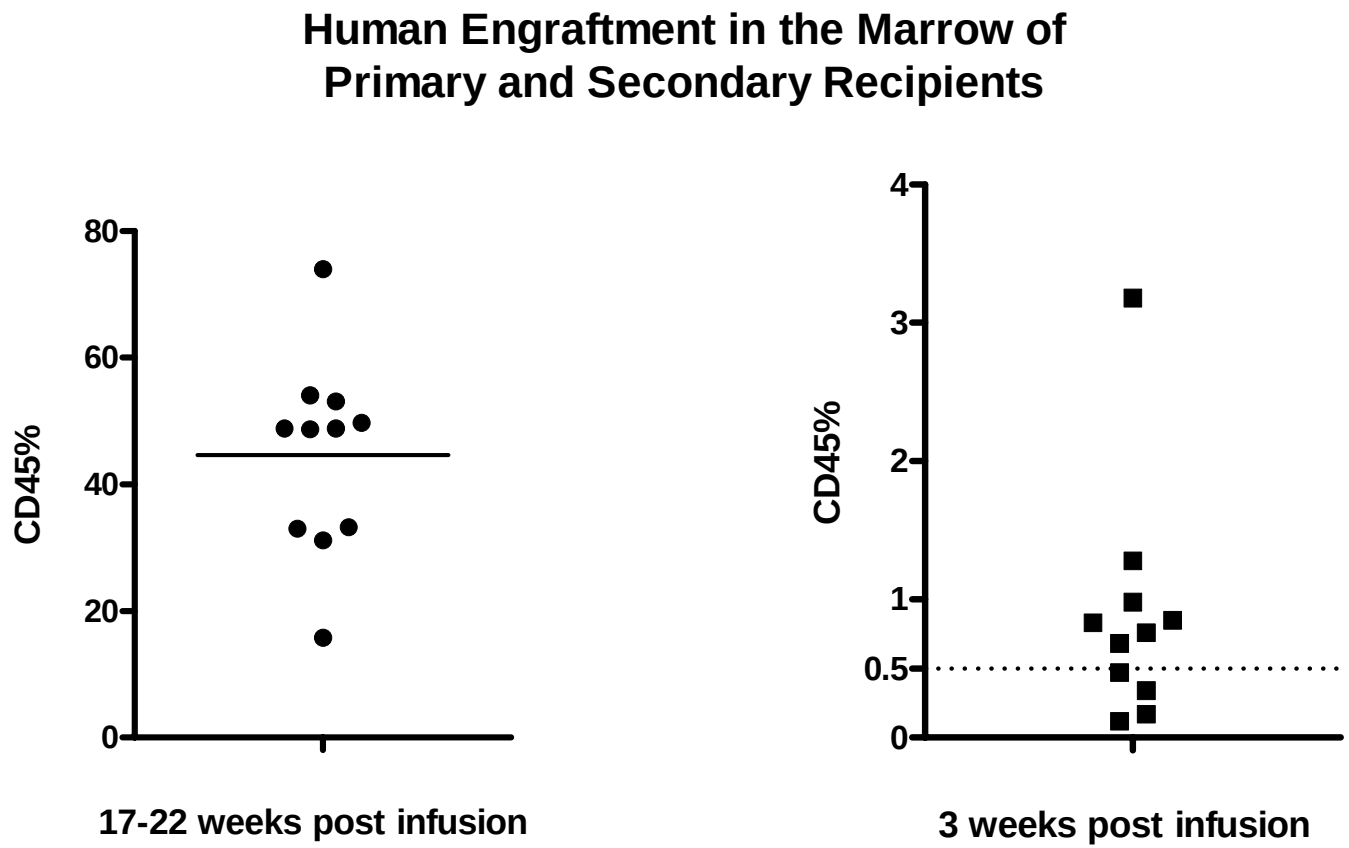
Supplementary Figure 1



5GF cytokine combination results in increased frequency of SRC. CD34+ cord blood progenito
were cultured for 17 days in varying cytokine combinations and limiting dilution transplants perform
described previously with 5GF versus 4GF to determine SRC frequency (SRC frequency per 10^6 st
cells). *SFT63 is reference group for p values.

(SFT=SCF, Flt3L, TPO; **SFT6**=SFT + IL6; **SFT3**= SFT + IL-3; **SFT63 (5GF)**= SCF, Flt3L, TPO, IL-6

Supplementary Figure 2; Delaney et al



Long-term repopulating cells are maintained after Notch-mediated ex vivo expansion of cord blood CD34$^+$ progenitor cells. CD34$^+$ cells were cultured for 16 days as described and infused into sublethally irradiated NOD/SCID IL-2Rγ^{null} mice. (a) Overall human engraftment in primary recipients at the time of sacrifice (17 – 22 weeks) and collection of whole marrow for secondary transplantation. Bar at the mean. (b) Overall human engraftment in the marrow of secondary NOD/SCID IL-2Rγ^{null} recipients at 3 weeks post infusion of primary recipient marrow. Mice were considered engrafted if they had ≥0.5% human CD45+ cells.

Supplemental Table 1: Demograpahics, HLA and Infused Cell Doses

	Ex vivo Expansion (N=10)	Conventional Double (N=20)
Male/Female	5/5	9/11
Median Age in years (range)	27.5 (3 to 43)	21.5 (10 to 42)
Median Weight in kg (range)	61.5 (16 to 79)	69.5 (32 to 110)
Disease		
AML	8	9
ALL (Ph^+)	1 (1)	7 (4)
Bi-phenotypic	1	0
CML	0	3
MDS	0	1
Disease Status		
CR1 (morphologic)	5	7
≥ CR2 (morphologic)	5	9
Chloroma	3	0
MRD (flow)	1	7
HLA Matching (HLA-A, B and DRB1)		
Unmanipulated CBU: patient		
4/6	7	26
5/6	3	11
6/6	0	3
Expanded CBU: patient		
4/6	6	N/A
5/6	4	N/A
Infused Cell Doses		
Non-manipulated units		
Total TNC/kg $x10^7$; avg (range)	3.3 (1.5 to 6.3)	4.3 (2.2 to 11.2)
Total CD34/kg $x10^5$; avg (range)	2.4 (0.6 to 5.4)	3.1 (1.3 to 5.4)
Ex vivo Expanded Unit		-
TNC/kg $x10^7$ per unit; avg (range)	4.6 (0.6 to 9.1)	N/A
CD34/kg $x10^5$ per unit; avg (range)	60.3 (9.2 to 133)	N/A

Supplementary Table 2: Expanded Unit Characteristics

Patient #	Pre-Freeze		Post-selection	Recovery	Starting Purity	Post-expansion		
	TNC ($x 10^9$)	CD34 ($x 10^6$)	Abs # CD34 ($x 10^6$)	CD34 (%)	CD34 (%)	Viability	Abs # CD34 ($x 10^6$)	Fold Increase
1	1.5	12.5	5.5	44	61	70	1440	261
2	1.3	7.9	1.6	20	45	86	154	96
3	1.3	7.2	1.5	21	83	77	60	41
4	1.3	3.4	1.5	44	37	73	113	74
5	1.2	3.6	1.3	36	54	66	111	87
6	2.4	8.2	2.6	32	42	87	977	382
7	1.2	3.6	2.0	56	46	73	145	72
8	1.9	n/a	4.1	n/a	60	76	229	57
9	1.7	5.8	1.7	29	41	81	810	471
10	3.6	28.0	5.4	20	54	76	537	99
Mean	1.6	7.6	2.7	36	52	76	457	164
Sem	0.14	1.2	0.52	4.5	4.3	2.1	149	48

*n/a = not available

Supplementary Table 3: Expanded Unit Selected Immunophenotyping

	Percent (range)	Cells/kg (range)
CD34	14.5 (6.2 - 26)	6.1×10^6 (0.9 -13.6)
CD7	8.1 (5.9 – 12)	3.9×10^6 (0.3 - 9.1)
CD14	11.3 (1.8 – 23)	5.6×10^6 (0.1 - 14.6)
CD15	20.5 (6 – 36)	9.0×10^6 (1.1 - 23)
$CD34^+/56^+$	2.9 (1.4 – 5.8)	1.7×10^6 (0.08 – 5.3)
$CD3^-/CD16^+/56^+$	5.4 (2.2 – 13.6)	2.7×10^6 (0.1 – 12.4)
CD20	0.1 (0 – 0.2)	3.6×10^4 (0 – 14)
CD3	0.2 (0 – 0.6)	4.8×10^4 (0 – 13)
$CD4^{lo}/CD3^{neg}/CD8^{neg}$	40.6 (16 – 67)	1.7×10^7 (0.2 – 6.1)
CD8	0.1 (0 – 0.5)	3.4×10^4 (0 – 17)

SUPPLEMENTARY METHODS (Delaney, et al)

cGMP purification of stable Delta1$^{ext\text{-}IgG}$

Generation of Cell Bank. A candidate cell line was quarantined within the cGMP production facility. Following screening for specific isotype and freedom from microbial contamination cells were weaned for growth with HyQ SFM4MAb-Utility media (Hyclone) supplemented with 2 mM L-glutamine (JRH Biosciences) and 1% irradiated fetal bovine serum (Hyclone). Both a master working cell bank and working cell bank were generated, tested, and qualified. Cell banks were then stored in controlled and isolated liquid nitrogen freezers reserved exclusively for qualified clinical production cultures.

Cell Culture Process. To initiate a production run, vials from the working cell bank were thawed then placed into culture in an 125 ml spinner flask containing 50 ml complete media (HyQ SFM4MAb-Utility media, Hyclone) supplemented with 2 mM L-glutamine (JRH Biosciences) and 1% irradiated fetal bovine serum (Hyclone). The culture was grownat 37oC with a 5% CO2 modified atmosphereand expanded into increasingly larger spinner flask cultures as required to provide a minimal initial seed of 30 liters at 3.5 x 10^5cells/ml to initiate production within a 110 liter stainless steel bioreactor (Applikon). Bioreactor control set points were adjusted to 37o C, pH 7.0, dissolved O2 at 10%, and impeller agitation at 60 RPM. When sufficient cell density was reached the bioreactor culture was expanded to 85 liters. At the end of the bioreactor growth cycle, as indicated by a decline in cell viability approaching 20%, the run was terminated and bulk crude product (conditioned medium) harvested. At harvest quality control testing was performed for mycoplasma, potential viral contaminants by electron microscopy, and sterility (bioburden).

Purification. Cell removal and clarification steps were conducted by harvesting the conditioned medium from the Applikon bioreactor and processing through bulk filtration (Cuno Maximizer filters, 90M05 Cartridge and 90ZA BioCap). Clarified harvest was then stored frozen until further processing. Samples of clarified harvest were tested for product concentration (ELISA) and endotoxin (Charles River Endosafe KTA LAL).

The clarified, conditioned media was then thawed, pooled, adjusted (pH and conductivity to match Protein A chromatography equilibration buffer; 50 mM Sodium Phosphate, 220 mM Sodium Chloride, pH 7.2), andloaded onto an equilibrated Protein A affinity column (Pharmacia Index 100, 10 cm diameter column, packed to a bed height of 6 cm with rProtein A Sepharose Fast Flow, Amersham Biosciences). Post high salt elution (10mM Tris, 4M MgCl2, pH 6.7) the eluate was adjusted to pH 3.8+/-0.05, held for 30 minutes for potential viral contaminant inactivation, and then adjusted to pH 7.0+/-0.2.

An Index 100 column (Pharmacia) packed with G25 Sephadex (GE Healthcare) equilibrated with 10 mM Tris, 5% glycerol, pH 7.5 was then used for an additional purification step and buffer exchange. This was followed by filtration for potential viral contaminants through a Viresolve NFR size exclusion filter (Millipore). Tangental flow filtration was performed to exchange product into final buffer formulation (10 mM Tris, 4 M Mg Cl2, pH 6.7) and a target concentration of 0.35 mg/ml. Product was 0.2 um filtered and aseptically filled into single dose glass vials then stored at -20o C until use. Quality control testing of bulk purified and final vialed product included assays for purity, identity, functionality, sterility, and endotoxin.