Parallela SIdEM-GITMO

Strategie citometriche per la valutazione dell'efficacia della criopreservazione

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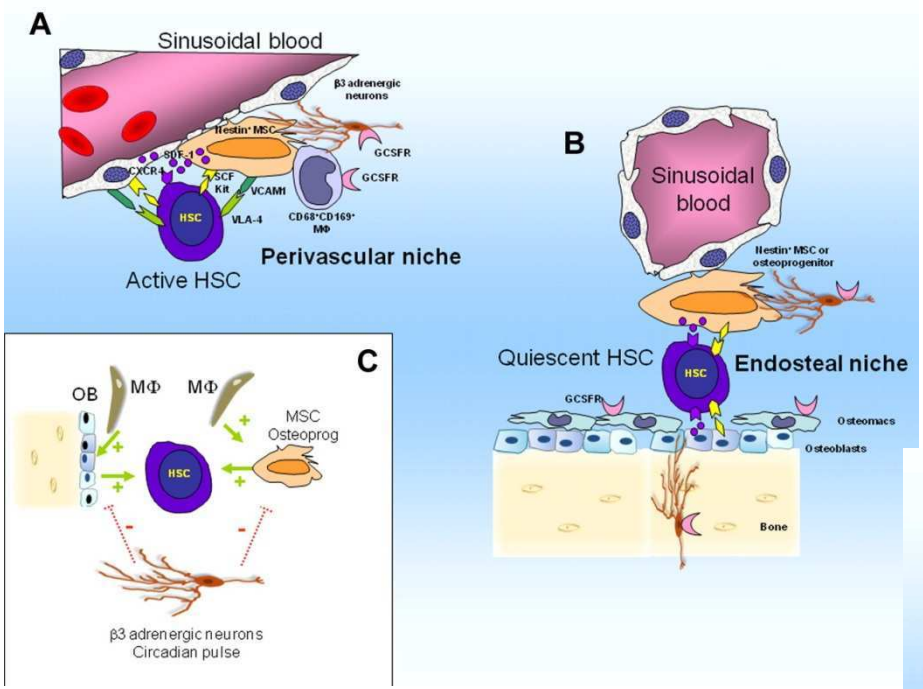
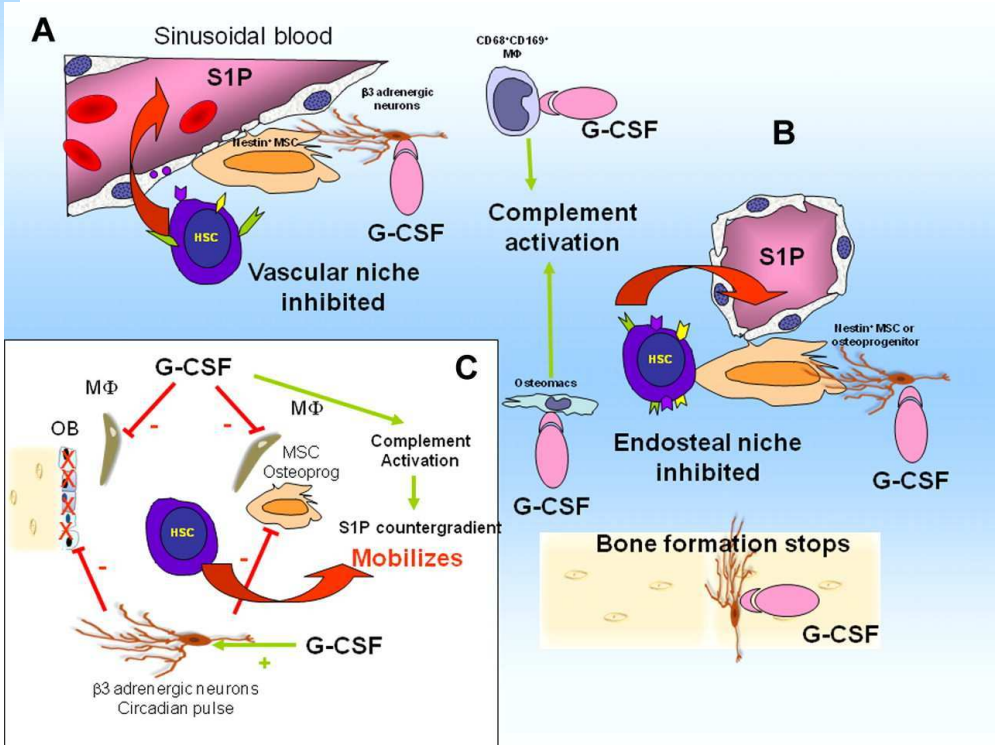
20 Ottobre 2012

HSC niche regulation in steady state



G-CSF deregulates niches and causes HSC mobilization



THE JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY
blood

LB To et al. Blood 2011;118:4530-4540

Engraftment kinetics as a function of CD34 content of PBSC

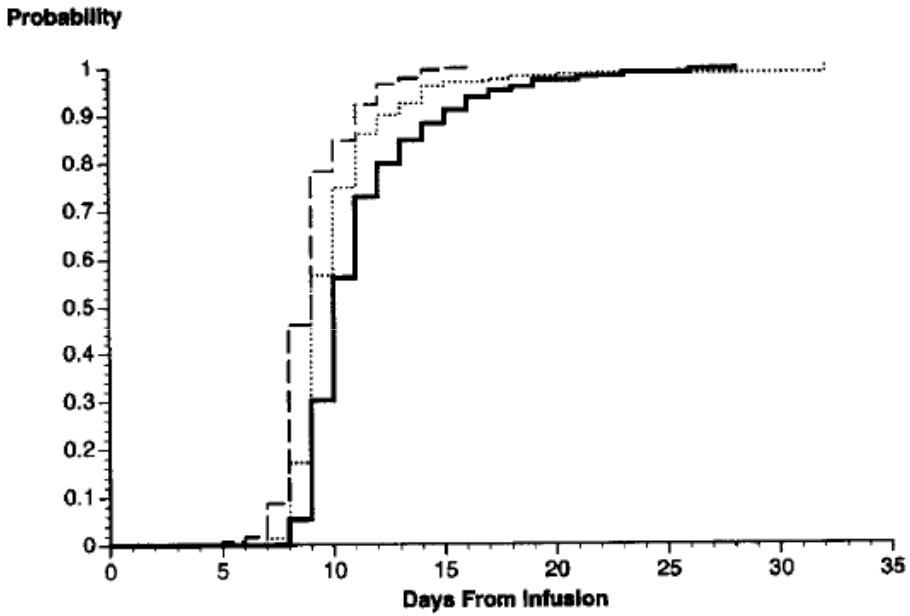


Fig 2. The Kaplan-Meier probability of achieving ≥0.5 × 10^9 neutrophils/L for <5.0 × 10^6 (—), >5.0 to 10 × 10^6 (· · ·), and >10 × 10^6 (---) CD34⁺ cells/kg (P = .0001).

CH Weaver Blood 1995;86: 3961-3969

Characteristics	
Patients	692
Age	46 (15-67)
Gender (M/F)	100/592
CD34*10^6/kg	
>5*10^6/kg	150
<5*10^6/kg>10	196
>10*10^6/kg	346

Cox regression analysis

Take	**p**
CD34 dose	0.001
G-CSF post-transplant	0.001
CTCb	0.006

Categorical variable analysis

Take	**p**
CD34 doses	0.0001
>5*10^6/kg	
<5*10^6/kg to <10*10^6/kg	
>10*10^6/kg	

Number of viable CD34+ 7AAD- cells infused predicts engraftment

Patients	36
Age	41 (16-68)
Product	
CD34/kg harvested	3.6 (3.6-182)
CD34/kg post thaw	2.0 (0.7-110)
MNC viability harvested	99%(99-100)
MNC viability post-thaw	76%(46-91)
Take	**day**
ANC>0.5x10^9/l	13 (9-22)
PLT>20x10^9/l	14 (9-42)
Antibiotics	8 (2-19)
Hospitalization	15 (5-44)

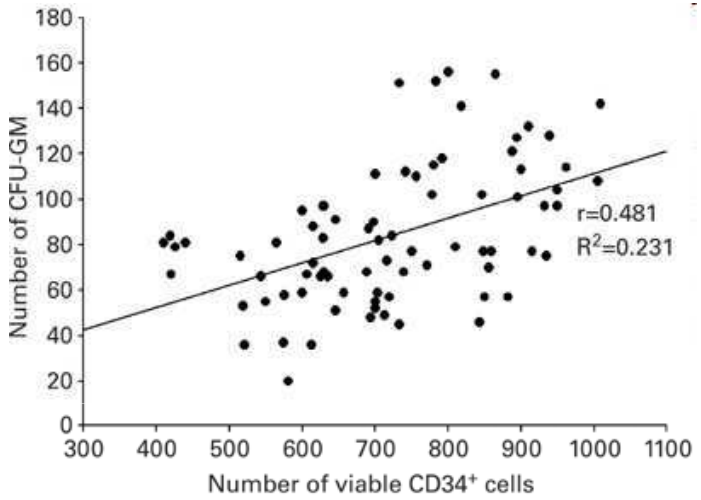
% of patients and viable CD34+ cells/kg at the time of engraftment

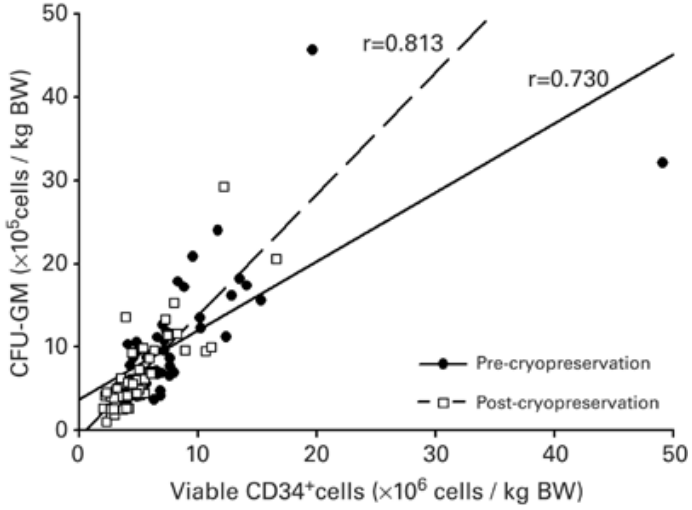
ANC take	**day >13**	**<13**	**p**
CD34/kg>2*10^6	57%	33%	ns
CD34/kg>5*10^6	29%	0%	0.03

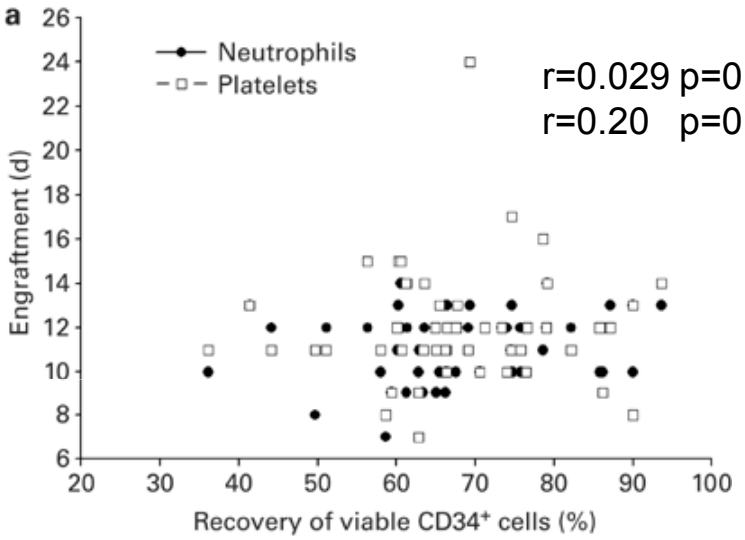
PLT take	**day >14**	**<14**	**p**
CD34/kg>2*10^6	68%	14%	0.002
CD34/kg>5*10^6	27%	0%	0.06

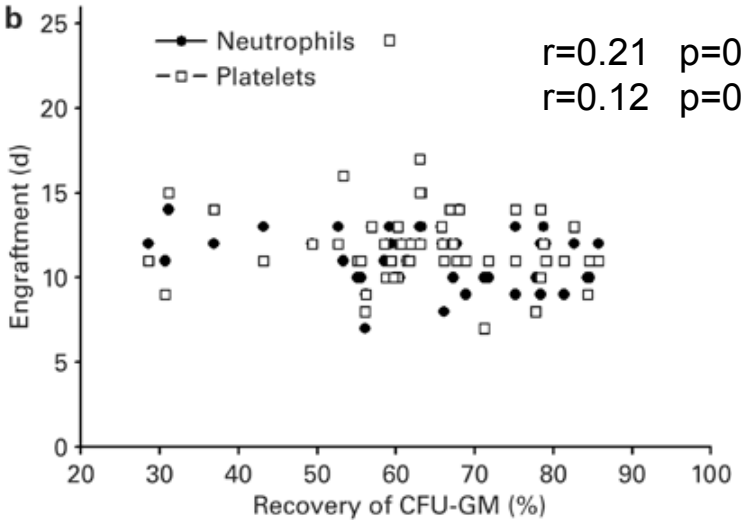
DS Allan BMT 2002;29:967-972

Association of post-thaw viable CD34+ cells and CFU-GM with time to hematopoietic engraftment





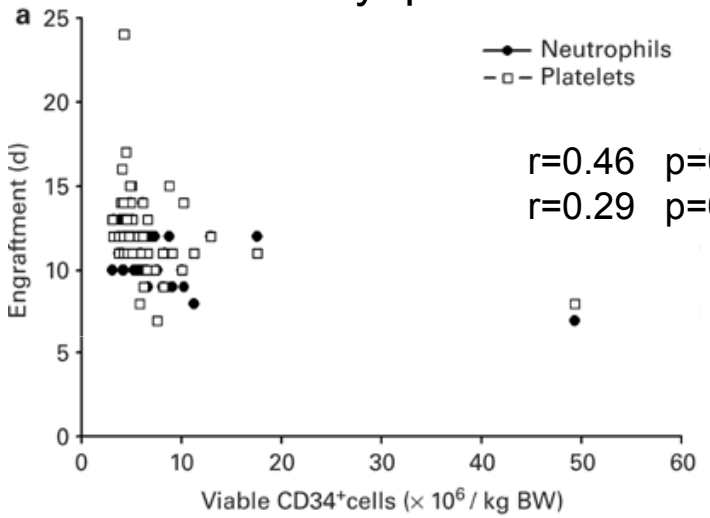


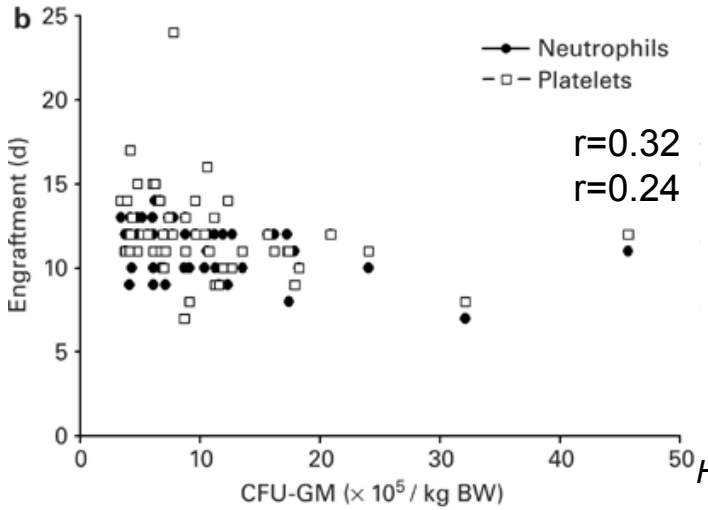


H Yang BMT 2005;35:881-887

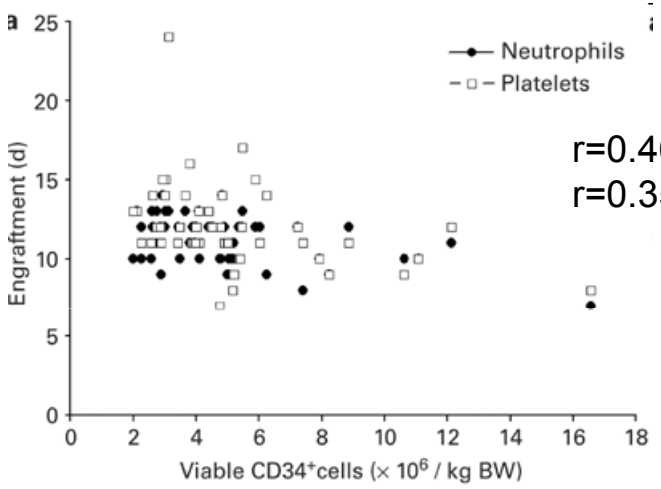
Association of post-thaw viable CD34+ cells and CFU-GM with time to hematopoietic engraftment

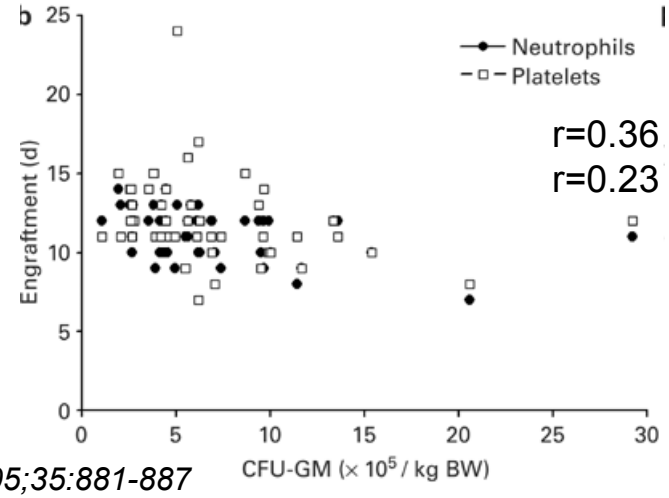
Pre-cryopreservation





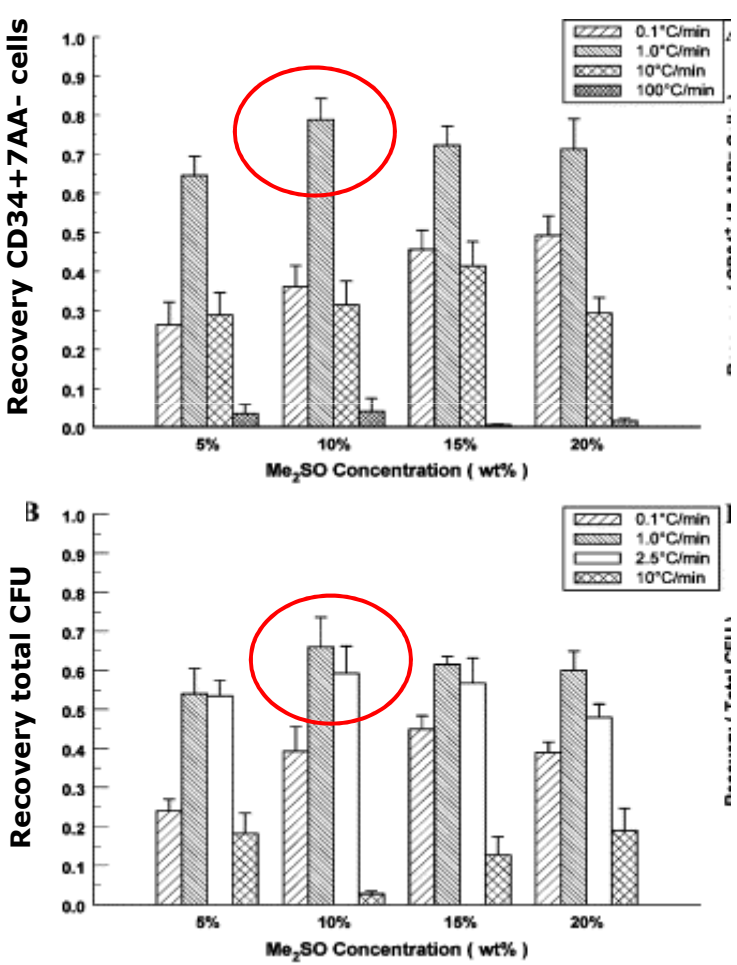
Post-cryopreservation

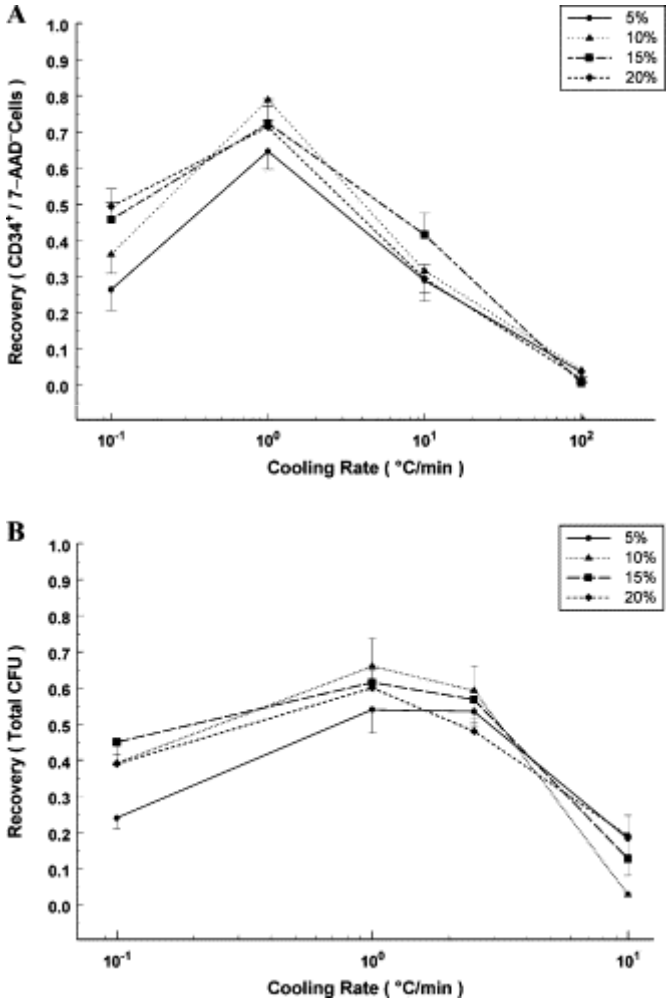




H Yang BMT 2005;35:881-887

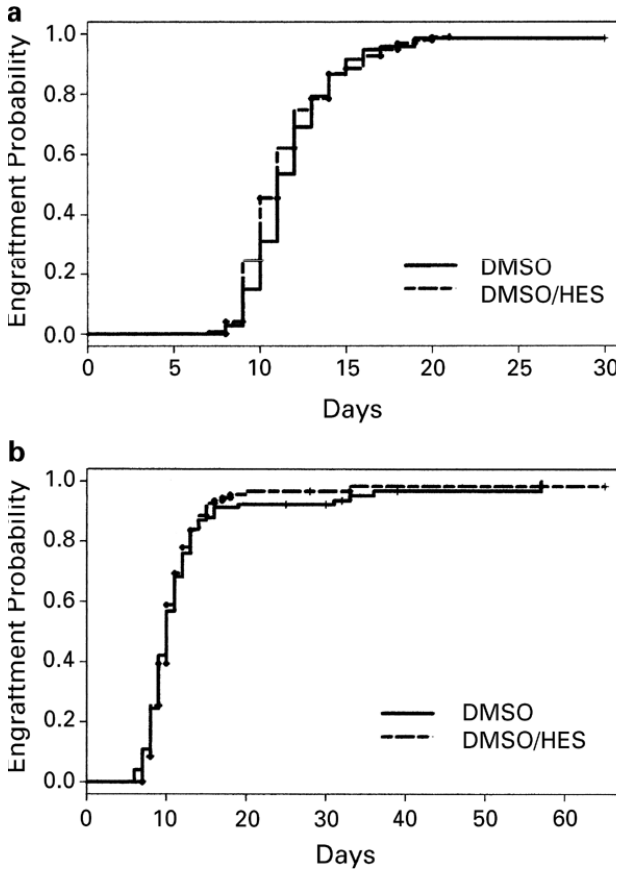
Effect of DMSO doses and cooling rate on cryopreservation of hematopoietic stem cell





JA Charles Cryobiology 2003;46:61-87

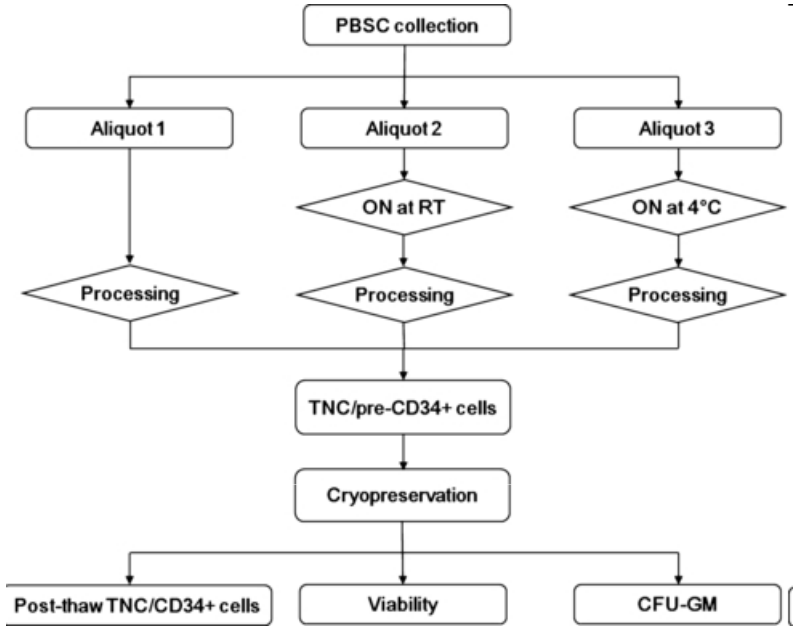
Comparaison cryopreservation using DMSO vs DMSO hydrossyethylstarch

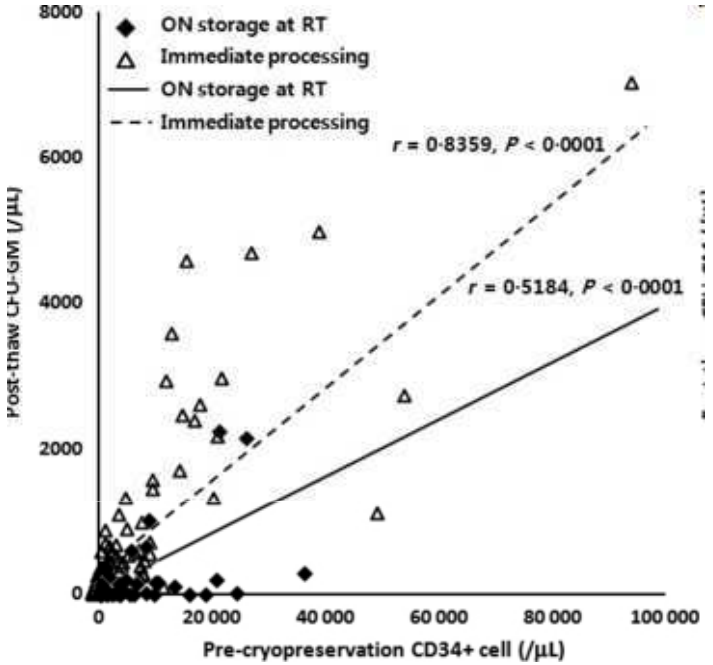


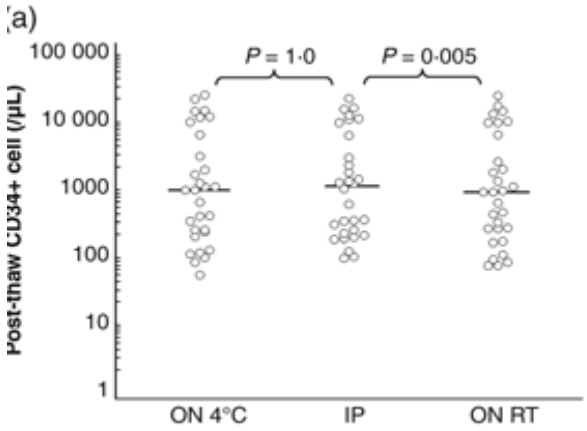
Patients 294	5% DMSO/HES 146	10% DMSO 148	p
CD34+ infused	6.5 (1.9–55.6)	6.4 (3.6–82.0)	0.91
TNC infused	7.3 (1.1–76.2)	7.6 (0.9–59.8)	0.69
WBC>1.0x10^9	10	11	0.04
ANC>500x10^9	11	11	0.05
PLT>20x10^9	10	10	0.99
Hospital dischar	16	17	0.54
Ant stoppage	13	14	0.04
RBC (Units)	4 (0-20)	4 (0-60)	0.98
PLT (Units)	14(0-276)	14(0-202)	0.57
TRM	8	7	0.98

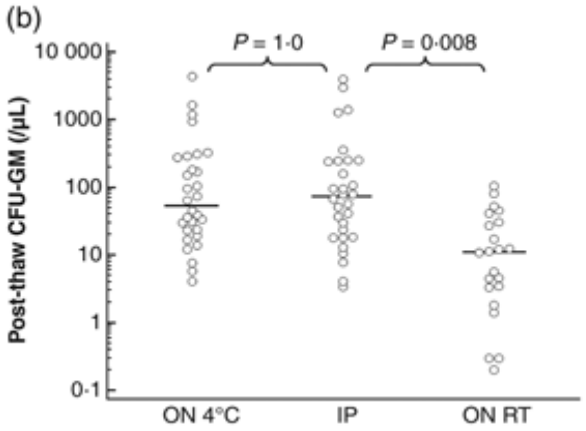
SD Rowley BMT 2003;3:1043-1053

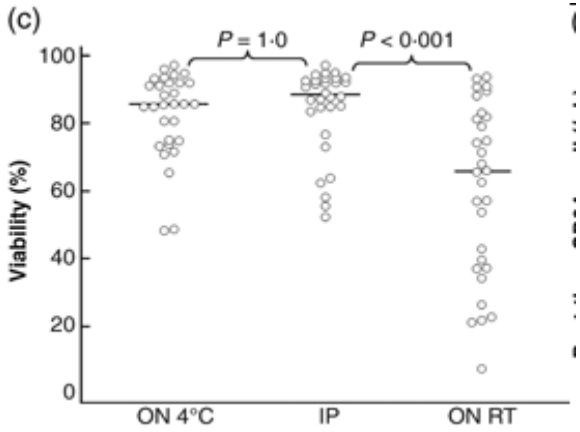
Evaluation of overnight storage conditions for autologous peripheral blood stem cell products: comparison of three different conditions





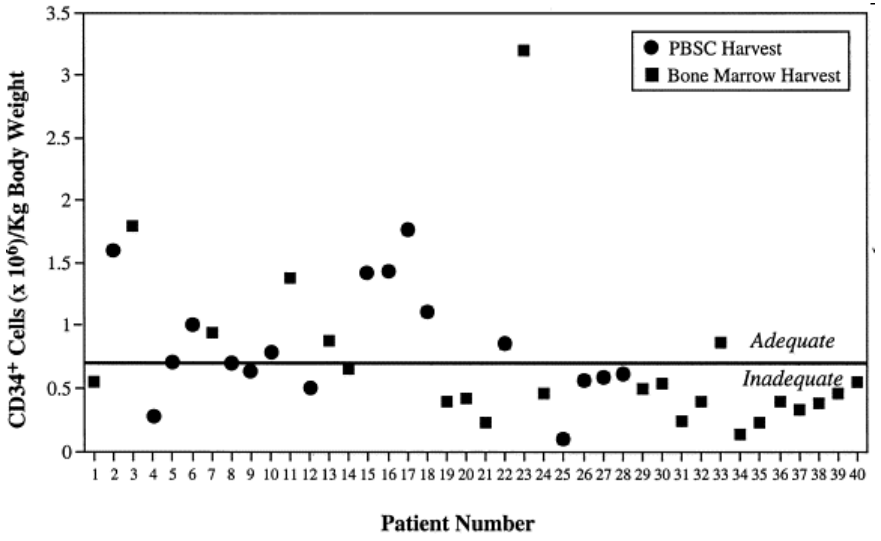


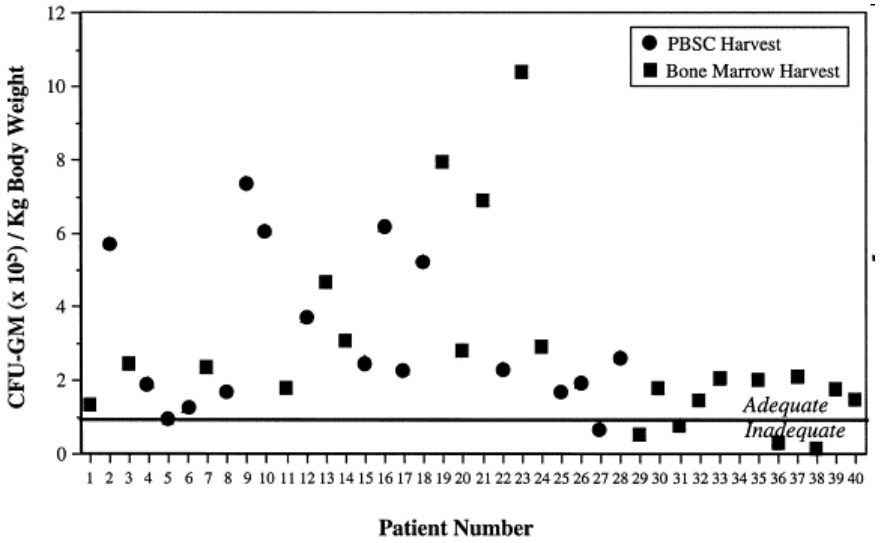


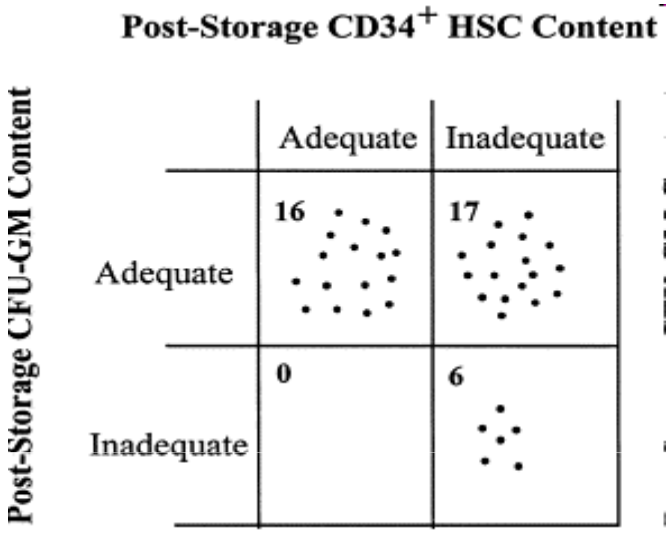


JY Seo Vox Sanguinis 2012;103(2):1-6

Cryopreserved HSC evaluation of extended (5-14 years) cryostorage





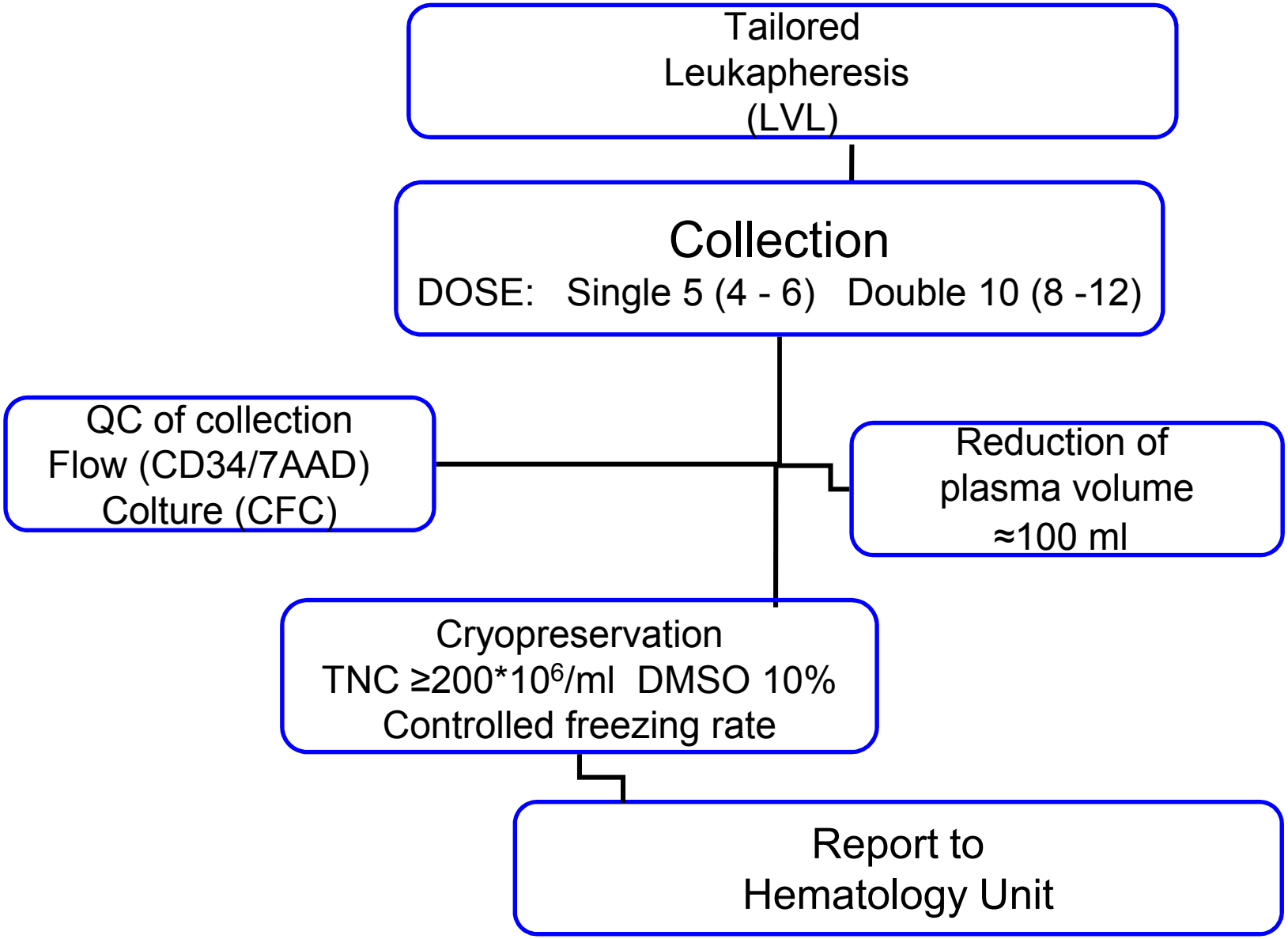


	BM	PB
Samples	23	17
Adequate	6	16
Inadequate	17	7

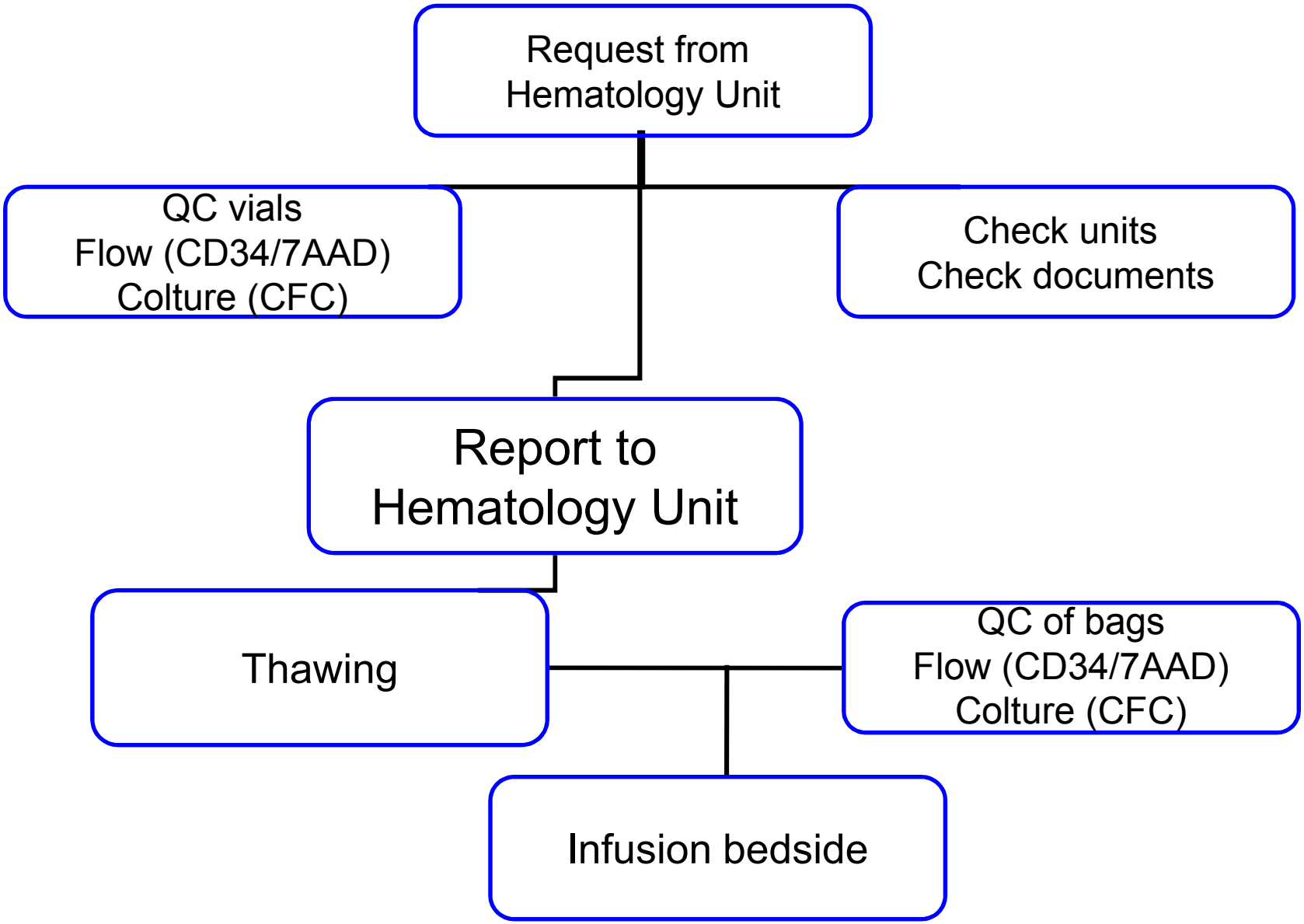
Adequate CD34 $\geq 0.7*10^6$/kg
CFU-GM $\geq 1*10^5$/kg

EE Spurr Cryobiology 2002;44:210-217

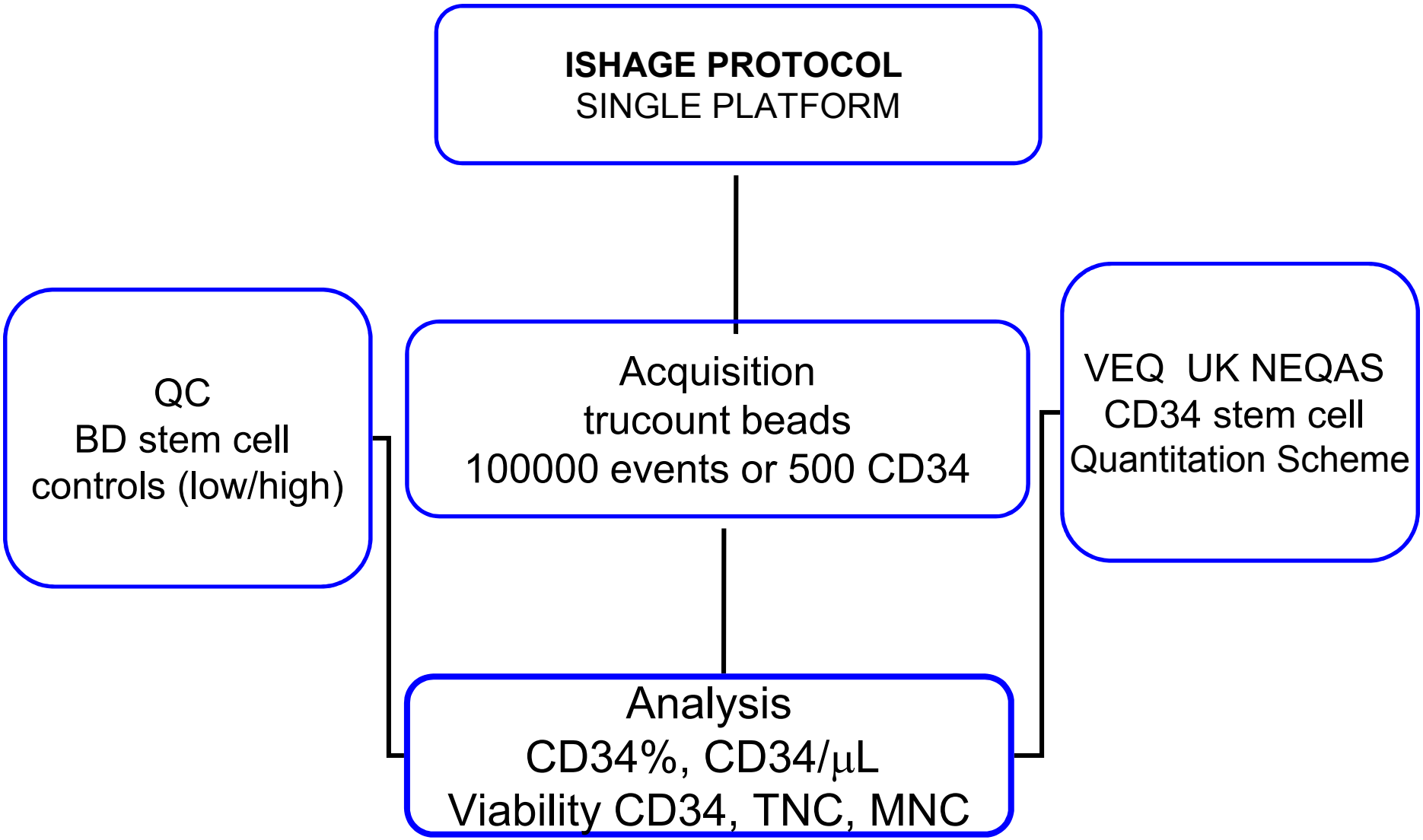
FLOW CHART COLLECTION



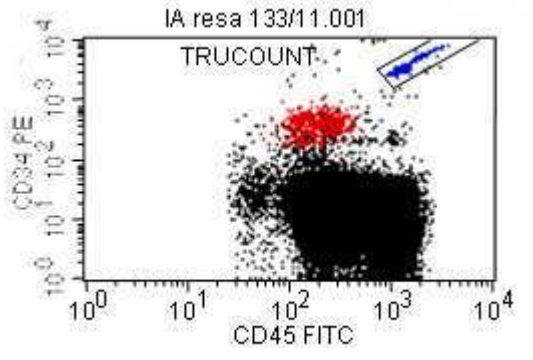
FLOW CHART TRANSPLANTATION

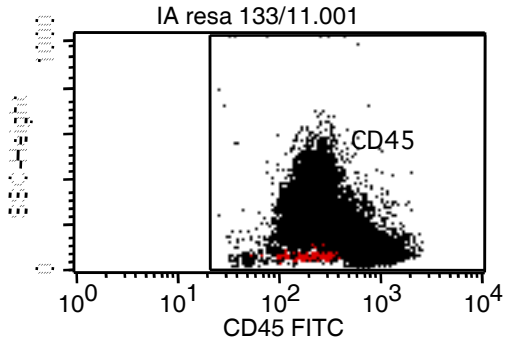


FLOW CHART FLOW CYTOMETRY

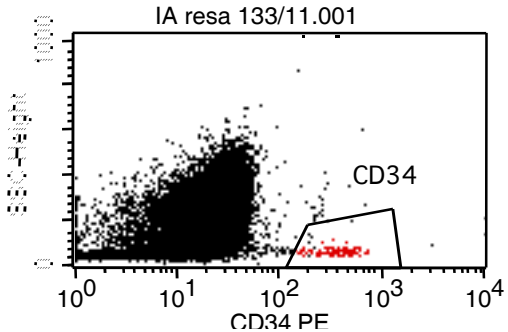


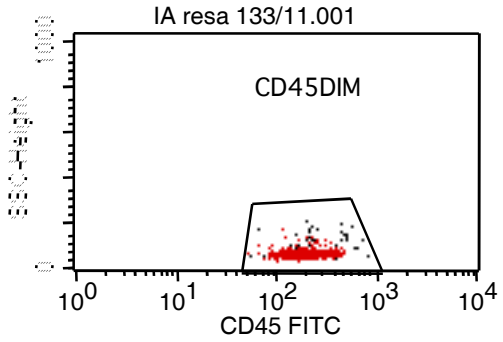
ISHAGE Protocol: Are we doing it correctly?

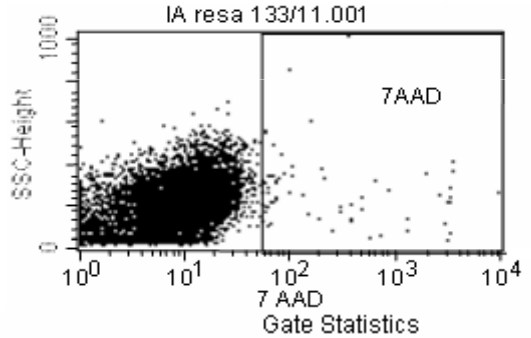




PMN	66.5%
MNC	33.5%
MNC viability	99.6%
CD34/µL	1121385
CD34/kg	$4.2*10^6$



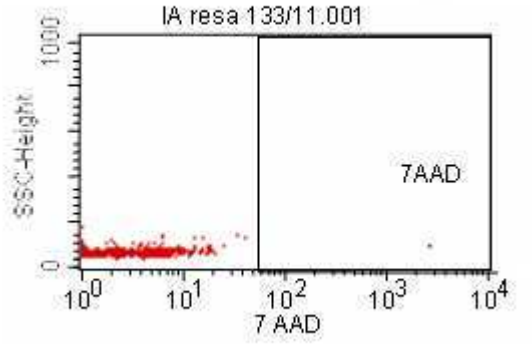


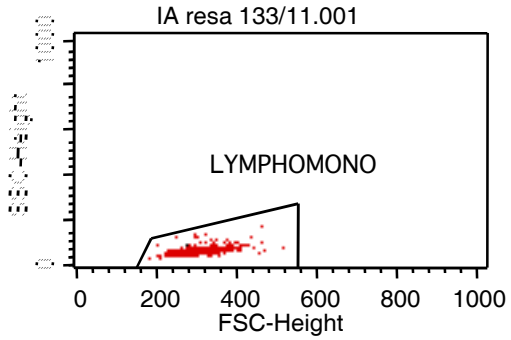


Gate Statistics

File: IA resa 133/11.001 Log Data Units: Linear Values
Gate: Total Events: 100000

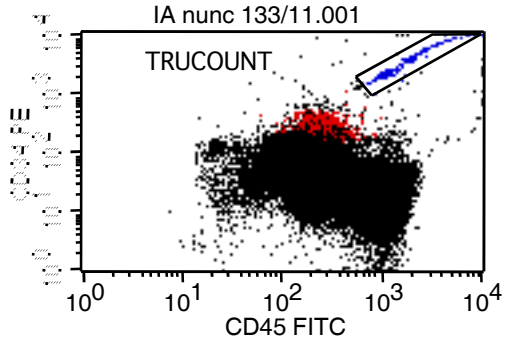
Gate	Events	% Gated	% Total
ALIVE STEM CELLS	697	99.86	0.70
TOTAL STEM CELLS	698	100.00	0.70
DEAD STEM CELLS	1	0.14	0.00
WBC	93840	93.84	93.84
ALIVE WBC	93261	93.26	93.26
BEADS	5994	5.99	5.99

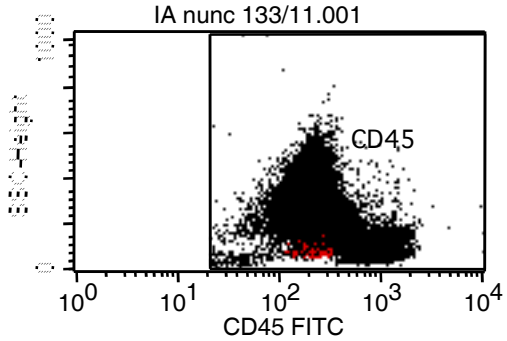




A Whitby Cytometry 2012;82B:9-17

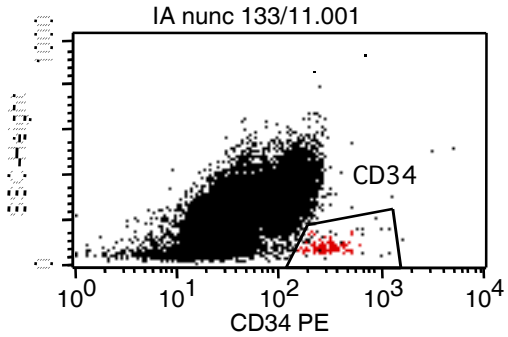
QC of cryopreserved hematopoietic progenitors cells

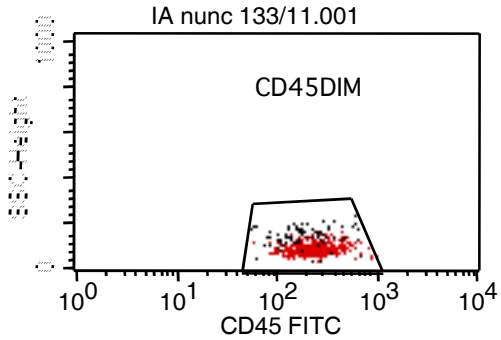


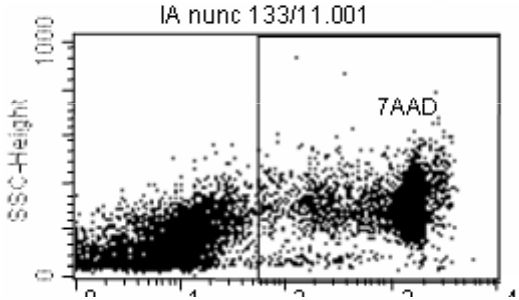


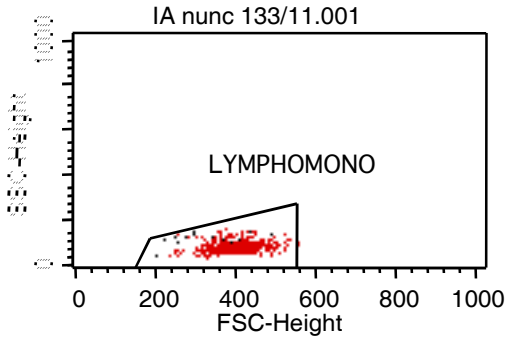
QC ½ week before release

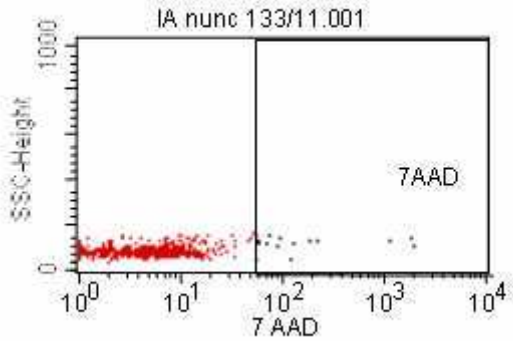
PMN	66.1%
MNC	33.9%
PMN viability	24.1%
MNC viability	96.2%
CD34 rec	78%
CFU-GM rec	75%











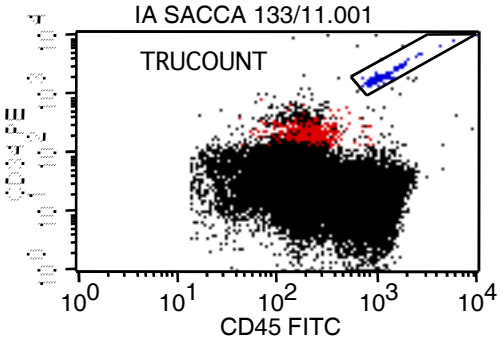
Gate Statistics

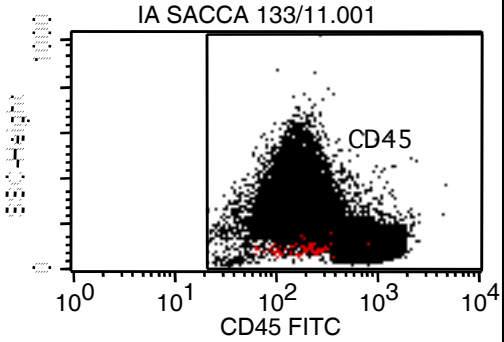
File: IA nunc 133/11.001 Log Data Units: Linear Values
Gate: Total Events: 100000

Gate	Events	% Gated	% Total
ALIVE STEM CELLS	610	97.44	0.61
TOTAL STEM CELLS	626	100.00	0.63
DEAD STEM CELLS	16	2.56	0.02
WBC	96767	96.77	96.77
ALIVE WBC	47798	47.80	47.80
BEADS	3106	3.11	3.11

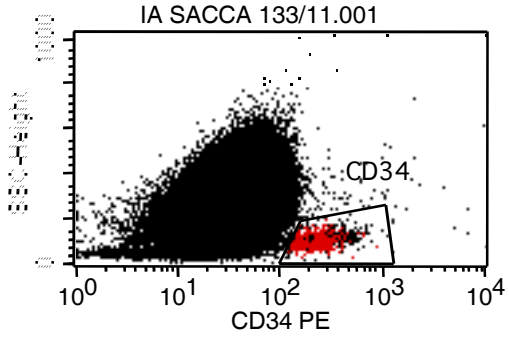
P Accorsi Vox Sanguinis 2011;101:255-75

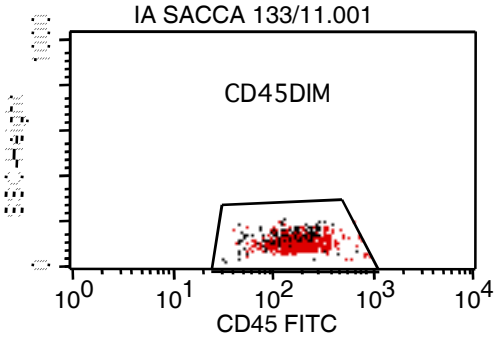
QC of thawed hematopoietic progenitors cells

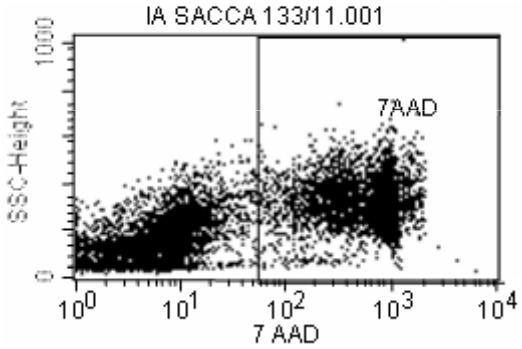


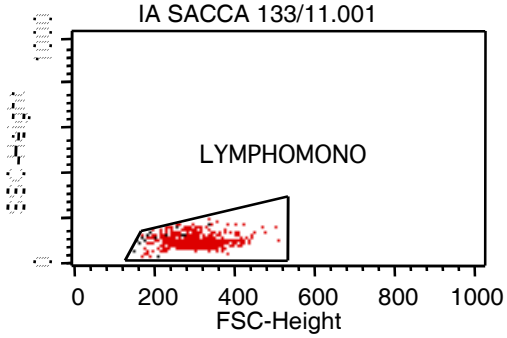


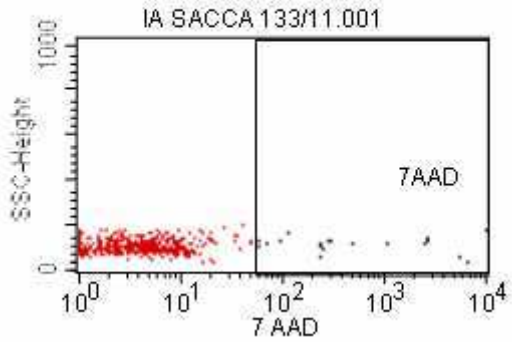
Collection	dec 2011
Thaw	oct 2012
MNC recovery	96%
CD34 rec	77%
CFU-GM rec	72%
CD34/kg BW	$5.87*10^6$











Gate Statistics

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Gate: TOTAL STEM CELLS Total Events: 100000

Gate	Events	% Gated	% Total
ALIVE STEM CELLS	525	96.33	0.53
TOTAL STEM CELLS	545	100.00	0.55
DEAD STEM CELLS	20	3.67	0.02
WBC	96627	96.63	96.63
ALIVE WBC	48235	48.23	48.23
BEADS	3239	3.24	3.24

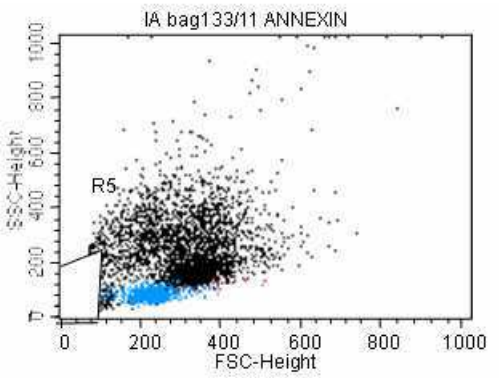
Freezing/Thawing Damages

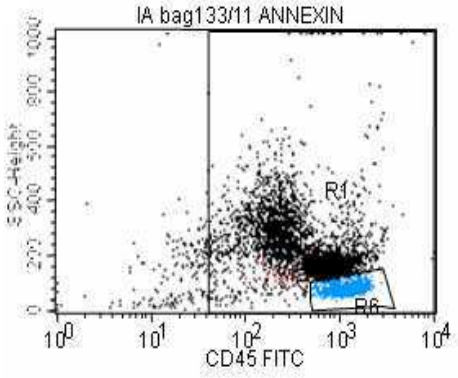
- Ice Formation
- Rise in solute concentration
- Changes in solution tonicity
- Toxic damage of DMSO

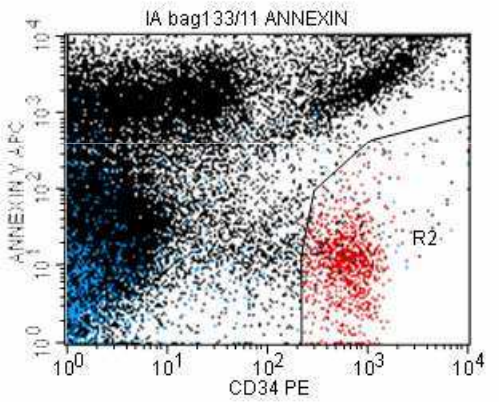
- Concentration of all solutes and biomolecules that aggregate
- 'Salting out' of protein molecules
- Marked changes in solution pH
- Disruption of sulfur bonds
- Concentration of potentially toxic impurity above a toxic threshold

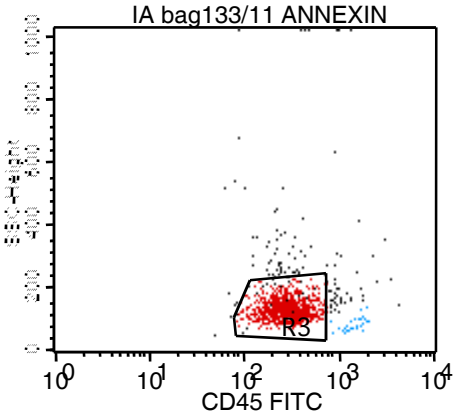
SM Watt SM, E Austin, S Armitage in *Cryopreservation and Freeze-Drying Protocols 2007*

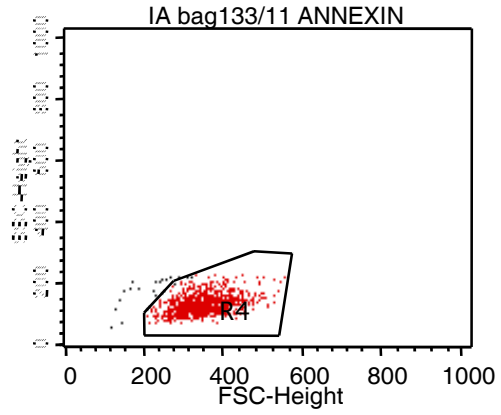
Flow cytometry assesment of apoptotic CD34+ by annexin V

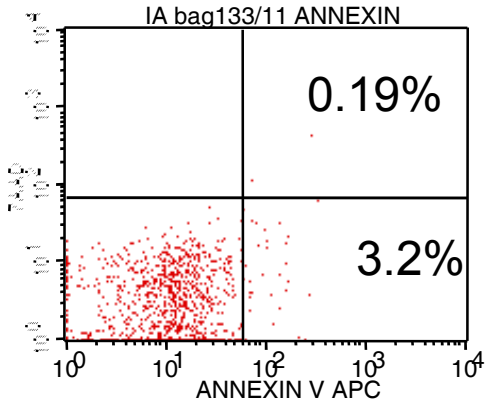










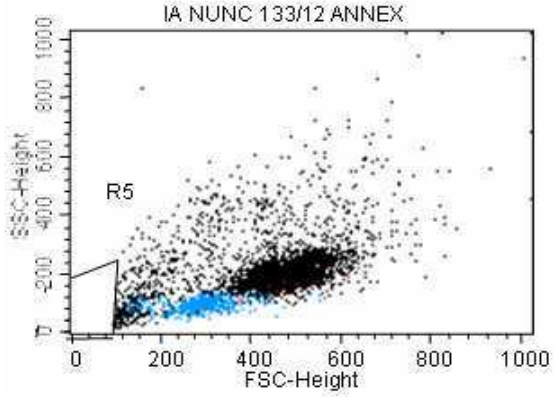


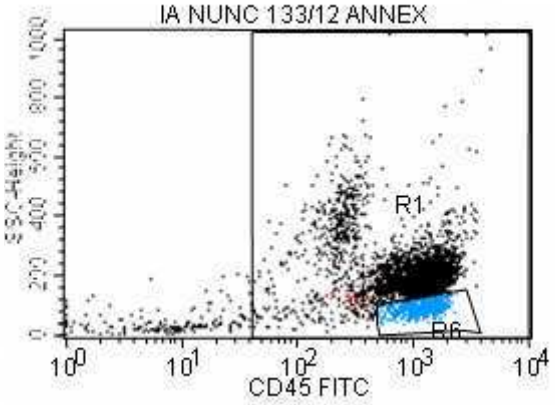
Induction of apoptosis in response to in vivo irradiation

	CD34+38- Annex V+	CD34+38+ Annex V+
Non irradiated BM	6.9±1.8	7.6±4.2
Irradiated BM	33.3±9.7	17.9±6.0
Fold increase in apoptosis	**4.8**	**2.3**

RC Duggleby Transfusion 2011;101:255-75

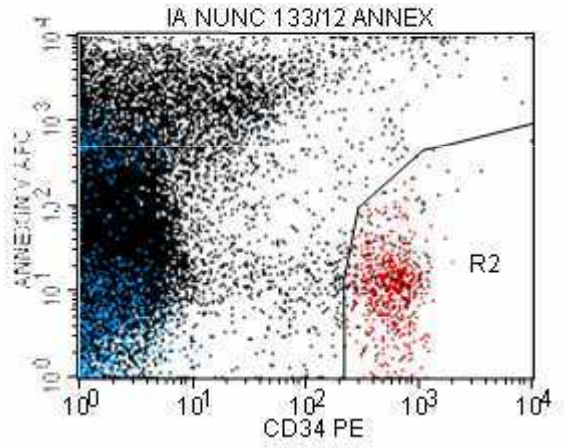
Annexin V evaluations in cryopreserved samples

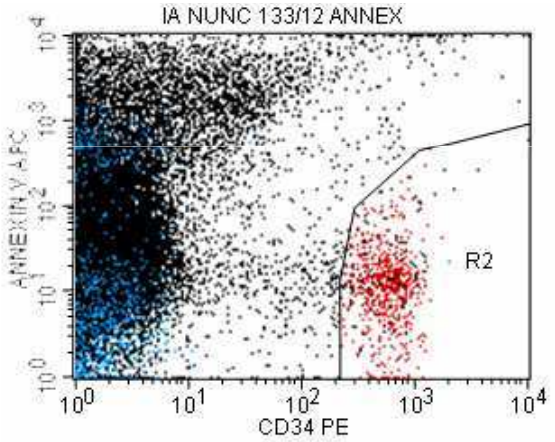




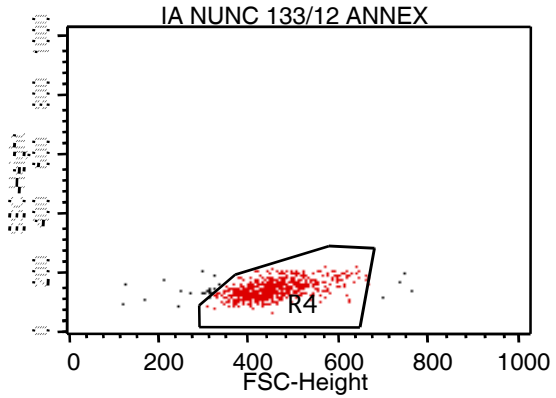
PANELS

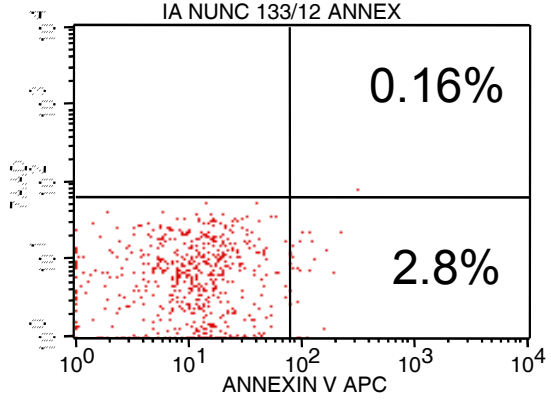
Annexin VFITC
CD34PE
7AAD
CD45APC





CD45FITC
CD34PE
7AAD
Annexin V APC





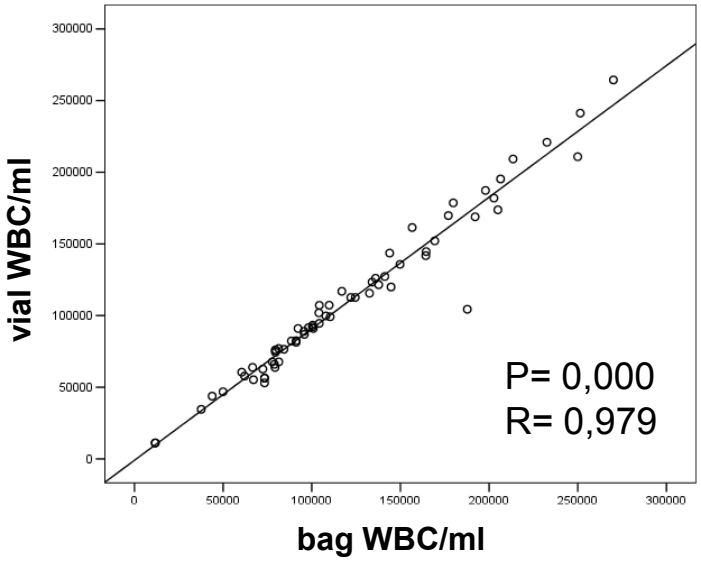
RESULTS

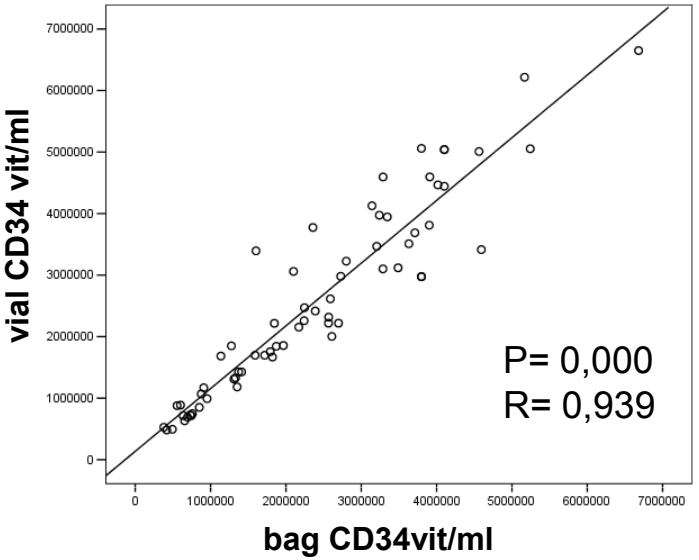
BAG	7.7 (1.9-16.3%)
NUNC	4.5 (2.5-15%)

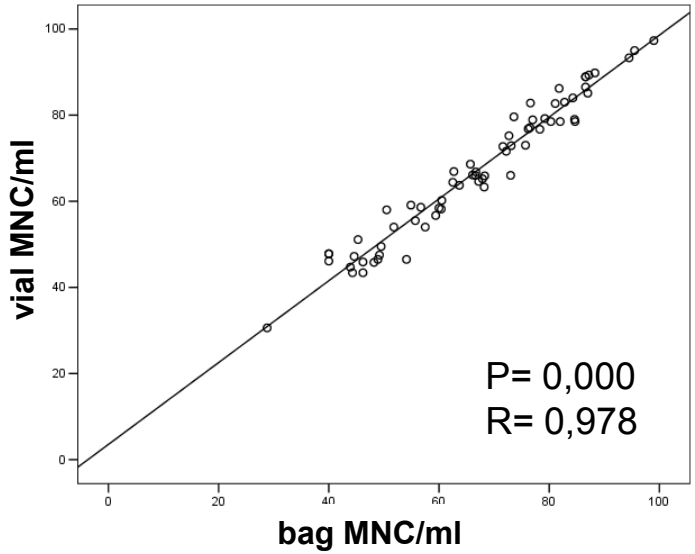
PESCARA year 2012

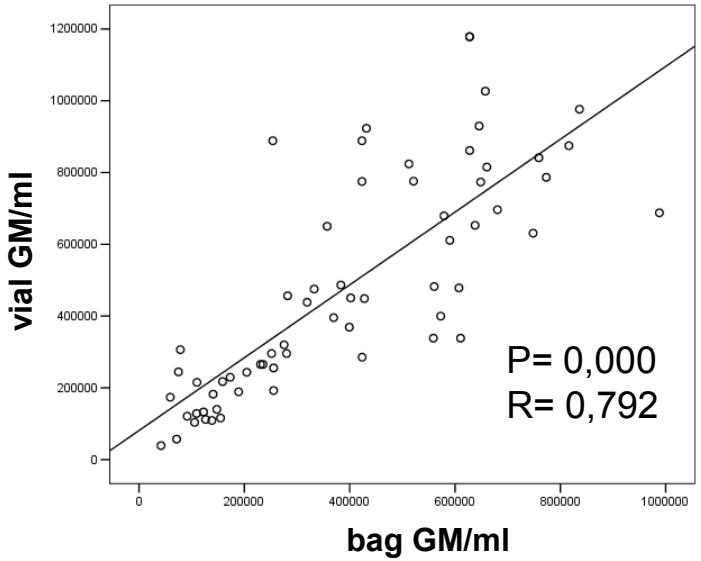
Patients	52
Age	57(19-69)
Gender(M/F)	25/27
NHL/MM	13/39
Trasplant	34 single 18 double
N° of apheresis	72 (1-3)
TNC viability	66% (11-92.2%)
MNC viability	85.8% (56.7-98%)
CD34 viability	96.3% (56.7-98%)
CD34/10*^{6}kg frozen	5.6 (3.2-19.1)
CD34/10*^{6}kg infused	4.5 (0.7-11.1)
CFU-GM 10*$^{4/}$kg	102 (51-805)
Take PMN	9 (7-25)
Take PLT	12 (7-21)

Correlation of QCs post cryopreservation

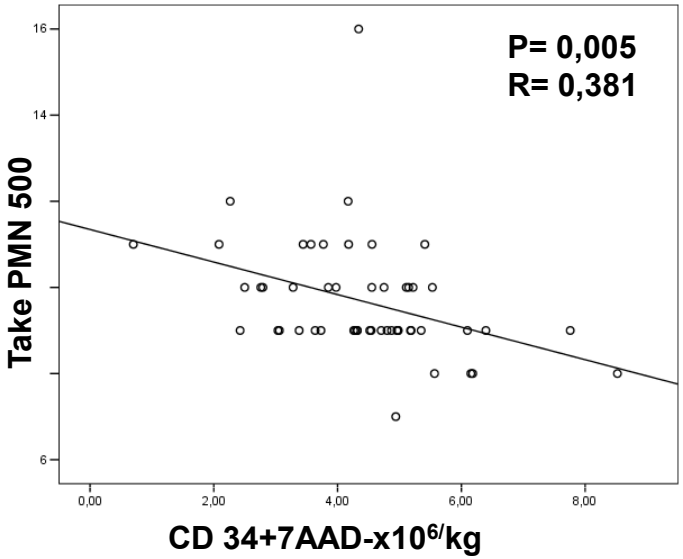


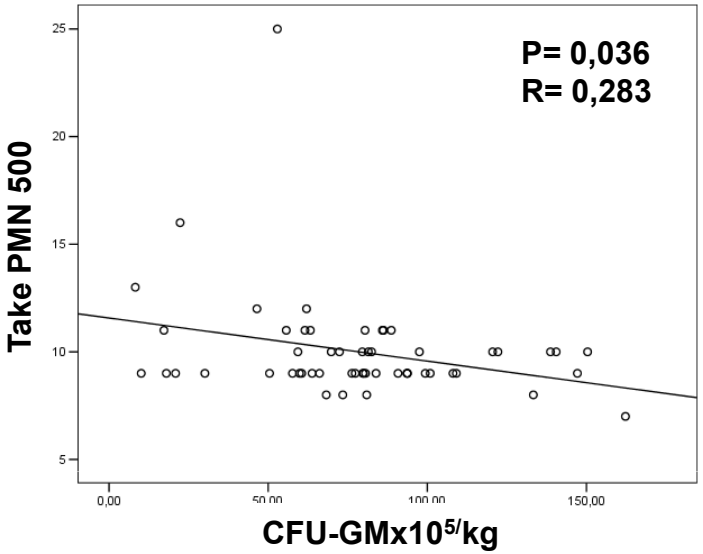


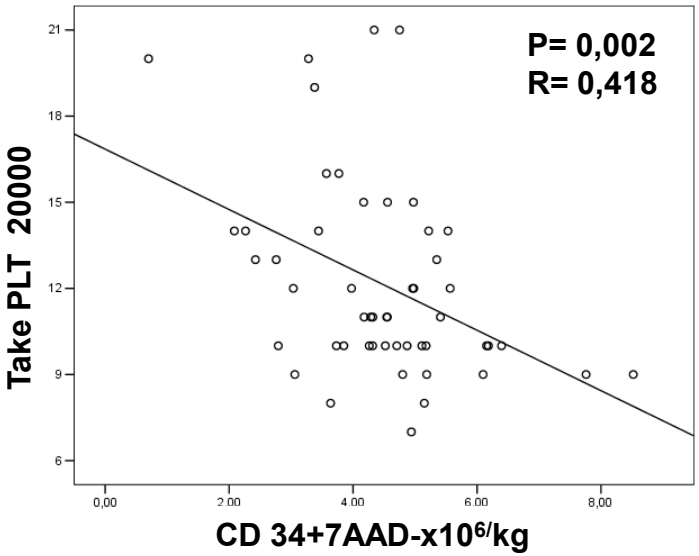


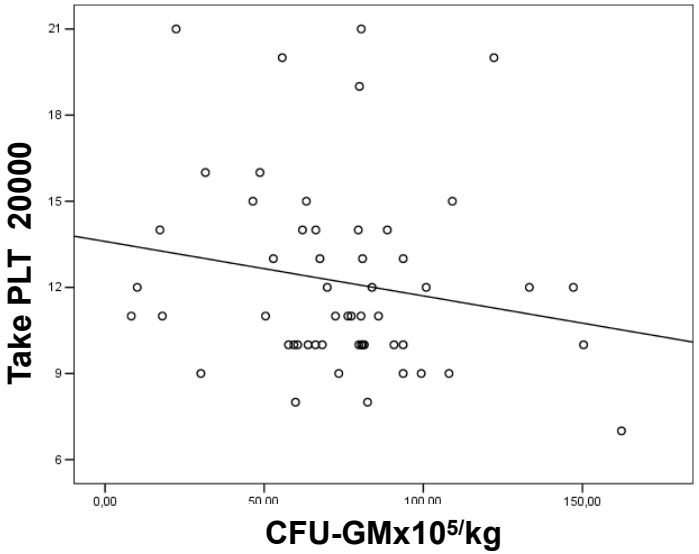


Correlation of viable CD34 and CFU-GM and PMN and PLT engraftment

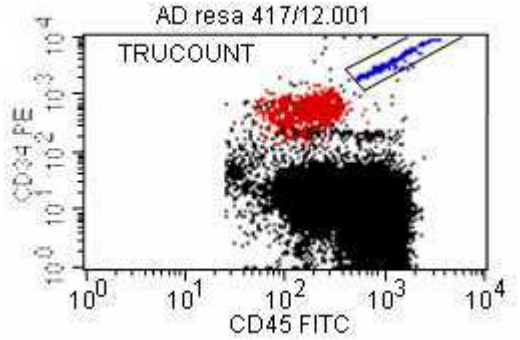


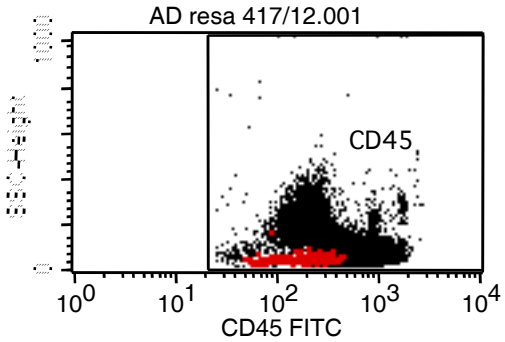


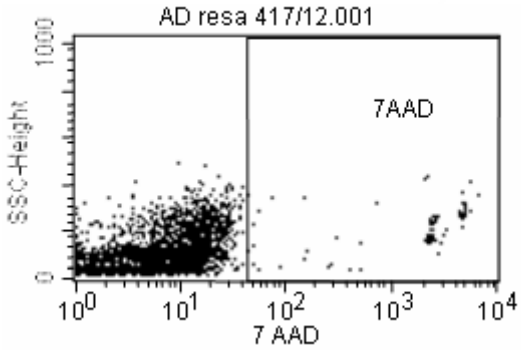


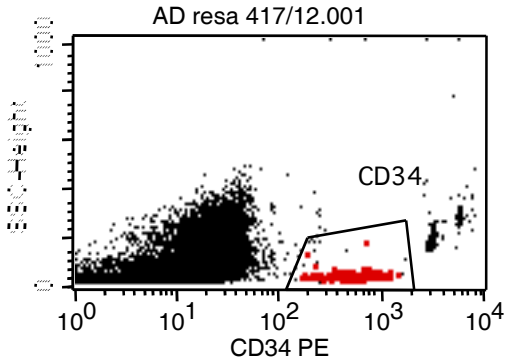


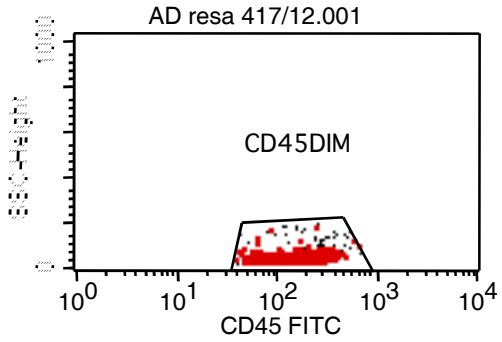
HPC-A 80417-NHL G-CSF + CHT
aa 56 weight 91kg







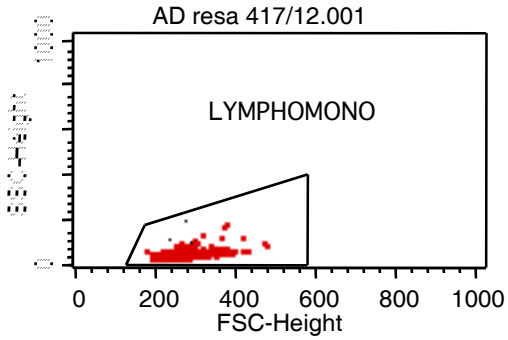


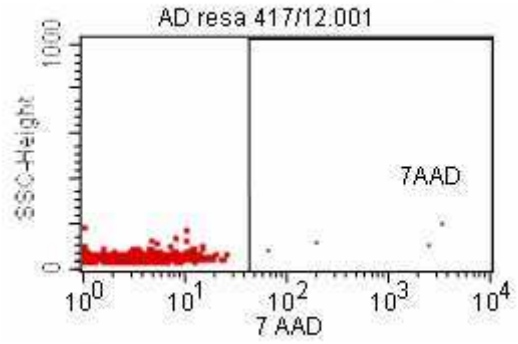


Gate Statistics

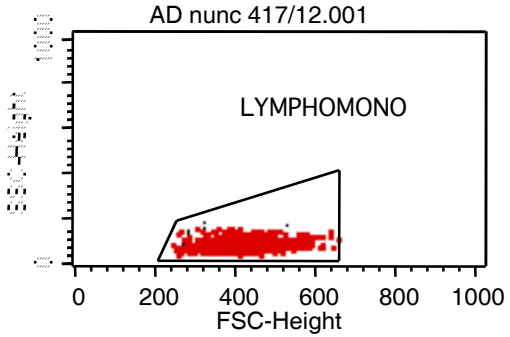
File: AD resa 417/12.001 Log Data Units: Linear Values
Gate Total Events: 100000

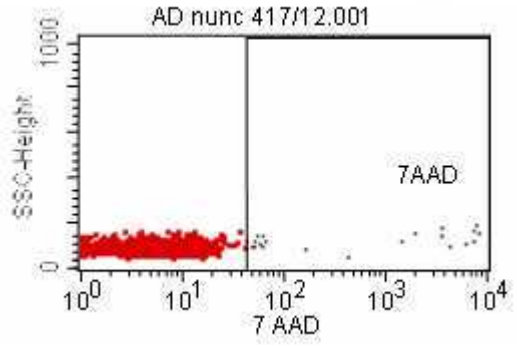
Gate	Events	% Gated	% Total
ALIVE STEM CELLS	1523	99.74	1.52
TOTAL STEM CELLS	1527	100.00	1.53
DEAD STEM CELLS	4	0.26	0.00
WBC	96421	96.42	96.42
ALIVE WBC	94215	94.22	94.22
BEADS	3198	3.20	3.20

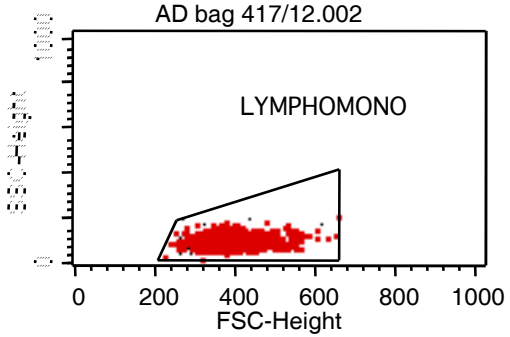


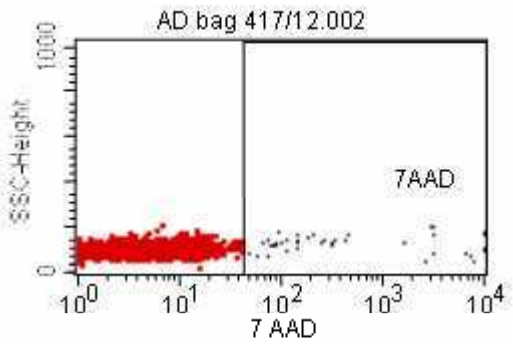


PMN	17.6%
MNC	82.4%
CD34	1399730/µL
CD34	$4.5*10^6$/kg









Gate Statistics

File: AD nunc 417/12.001 Log Data Units: Linear Values
Gate: Total Events: 100000

Gate	Events	% Gated	% Total
ALIVE STEM CELLS	1494	98.48	1.49
TOTAL STEM CELLS	1517	100.00	1.52
DEAD STEM CELLS	23	1.52	0.02
DEAD STEM CELLS	23	0.02	0.02
WBC	95614	95.61	95.61
ALIVE WBC	78572	78.57	78.57
BEADS	3976	3.98	3.98

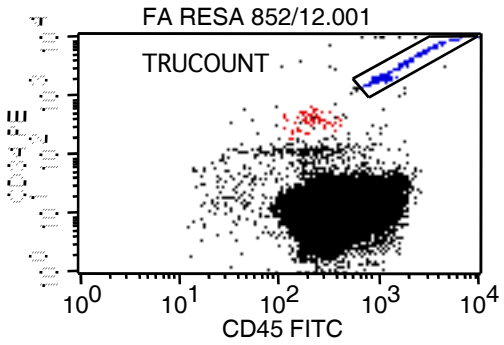
Gate Statistics

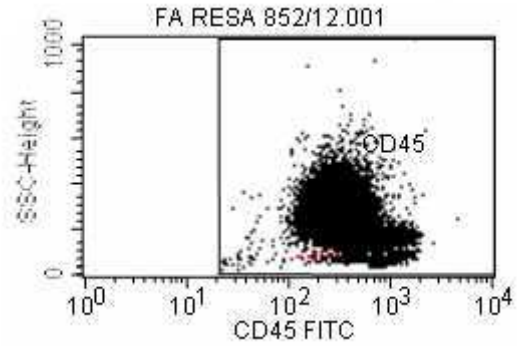
File: AD bag 417/12.002 Log Data Units: Linear Values
Gate Total Events: 100000

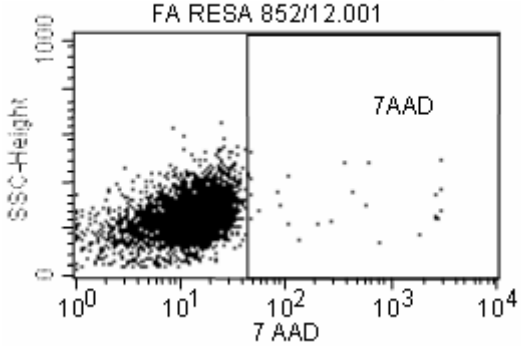
Gate	Events	% Gated	% Total
ALIVE STEM CELLS	1351	97.05	1.35
TOTAL STEM CELLS	1392	100.00	1.39
DEAD STEM CELLS	41	2.95	0.04
WBC	95697	95.70	95.70
ALIVE WBC	77045	77.05	77.05
BEADS	3359	3.36	3.36

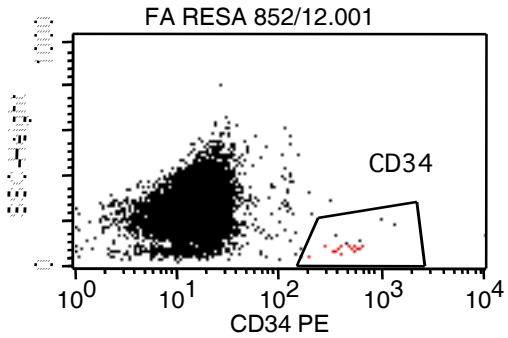
Thaw	Jul 2012
PMN	16%
PMN viability	22%
MNC	84%
MNC viability	95.5%
CD34	$4.3*10^6$/kg
CD34 recovery	95%
CFU-GM recovery	99%

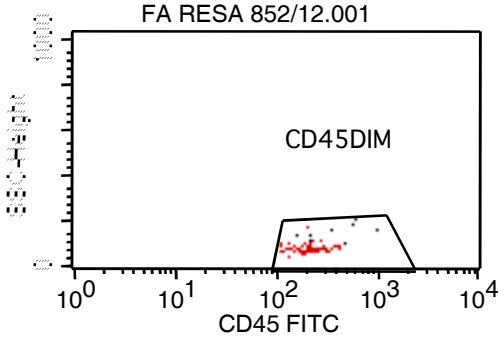
HPC-A MM G-CSF + plerixafor
aa 54 weight 50kg





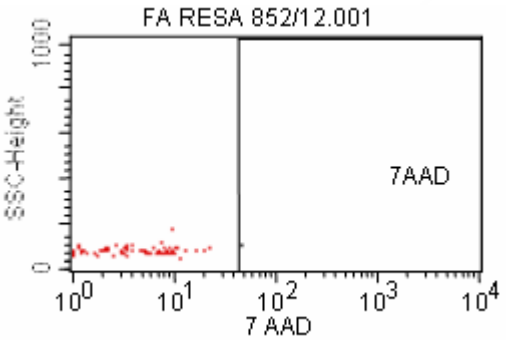


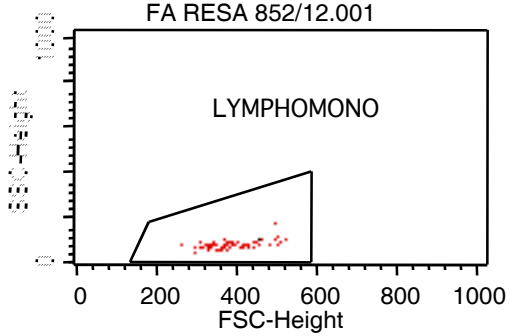




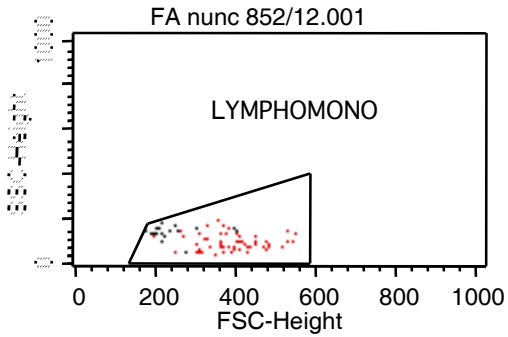
File: FA RESA 852/12.001 Log Data Units: Linear Values
Gate: Total Events: 100000

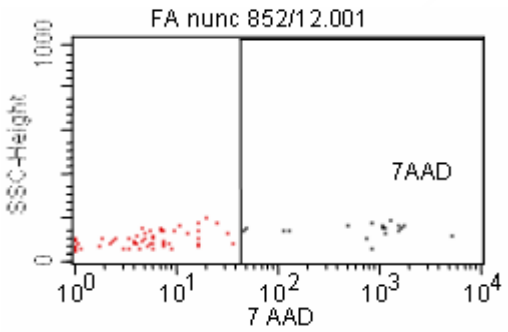
Gate	Events	% Gated	% Total
ALIVE STEM CELLS	69	98.57	0.07
TOTAL STEM CELLS	70	100.00	0.07
DEAD STEM CELLS	1	1.43	0.00
WBC	95847	95.85	95.85
ALIVE WBC	95208	95.21	95.21
BEADS	4132	4.13	4.13





Bag 852/12
CD34/kg $1.9*10^6$
Bag 857/12
CD34/kg $1.1*10^6$
PMN 87.2%
MNC 12.8%

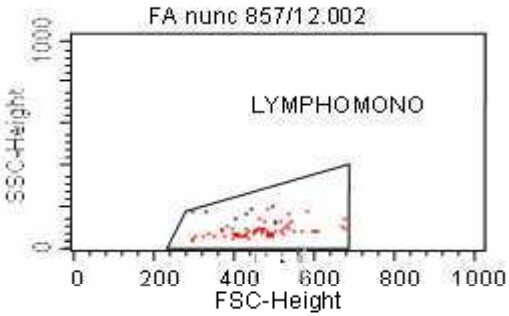


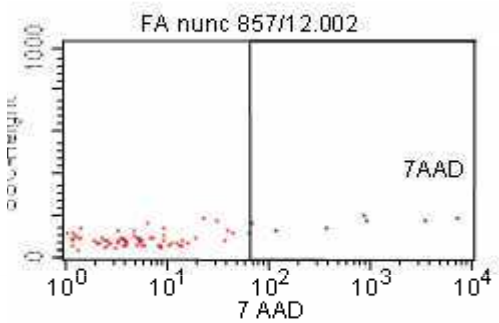


Gate Statistics

File: FA nunc 852/12.001 Log Data Units: Linear Values
Gate: TOTAL STEM CELLS Total Events: 100000

Gate	Events	% Gated	% Total
ALIVE STEM CELLS	58	76.32	0.06
TOTAL STEM CELLS	76	100.00	0.08
DEAD STEM CELLS	18	23.68	0.02
WBC	96625	96.62	96.62
ALIVE WBC	11149	11.15	11.15
BEADS	3328	3.33	3.33





Gate Statistics

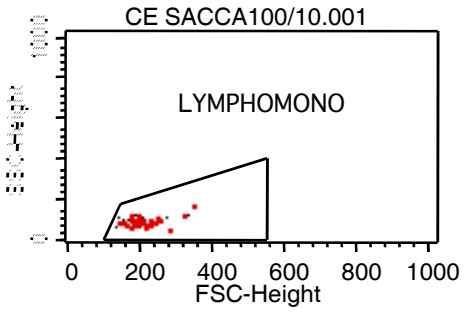
File: FA nunc 857/12.002 Log Data Units: Linear Values
Gate: TOTAL STEM CELLS Total Events: 100000

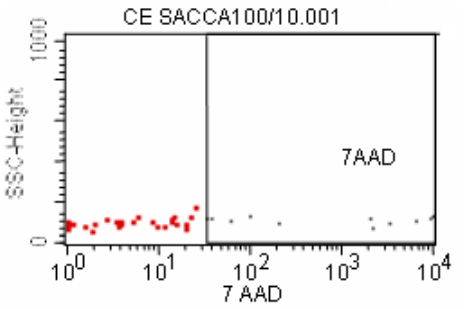
Gate	Events	% Gated	% Total
ALIVE STEM CELLS	72	91.14	0.07
TOTAL STEM CELLS	79	100.00	0.08
DEAD STEM CELLS	7	8.86	0.01
WBC	97064	97.06	97.06
ALIVE WBC	28660	28.66	28.66

nunc 852/12	**Sett 2012**
PMN	88.7%
PMN viability	4.2%
MNC	11.3%
MNC viability	60%
CD34 rec/ml	60%
CFU-GM rec/plate	65%

nunc 852/12	**Sett 2012**
PMN	88.7%
PMN viability	22.3%
MNC	12.1%
MNC viability	77.6%
CD34 rec/ml	68%
CFU-GM rec/plate	84%

Delay at processing sample





Gate Statistics

File: CE SACCA100/10.001 Log Data Units: Linear Values

Gate: TOTAL STEM CELLS Total Events: 100000

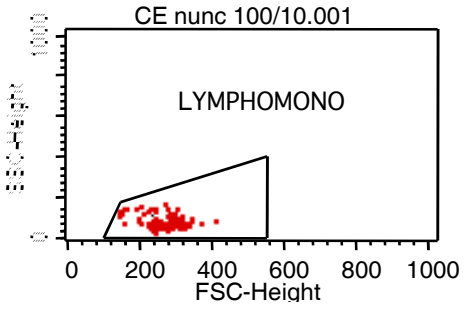
Gate	Events	% Gated	% Total
ALIVE STEM CELLS	30	73.17	0.03
TOTAL STEM CELLS	41	100.00	0.04
DEAD STEM CELLS	11	26.83	0.01
WBC	97741	97.74	97.74
ALIVE WBC	31897	31.90	31.90

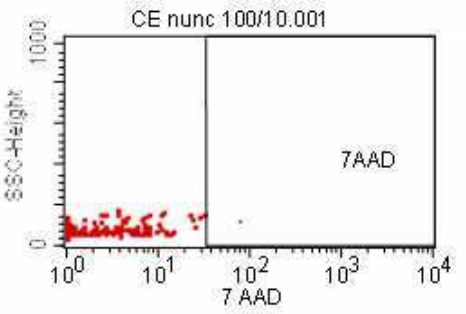
PMN 77.0%

PMN viability 27.8%

MNC 23.0%

MNC viability 49.2%





Gate Statistics

File: CE nunc 100/10.001 Log Data Units: Linear Values

Gate: TOTAL STEM CELLS Total Events: 100000

Gate	Events	% Gated	% Total
ALIVE STEM CELLS	158	99.37	0.16
TOTAL STEM CELLS	159	100.00	0.16
DEAD STEM CELLS	1	0.63	0.00
WBC	97991	97.99	97.99
ALIVE WBC	34772	34.77	34.77

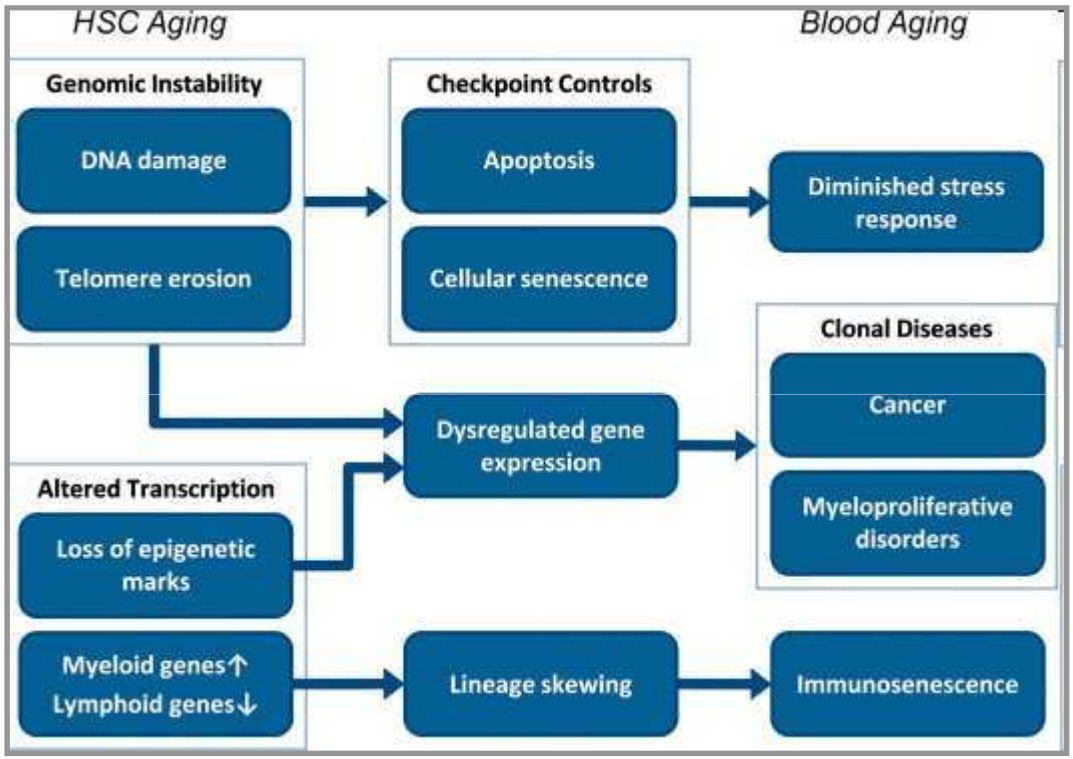
PMN 84.3%

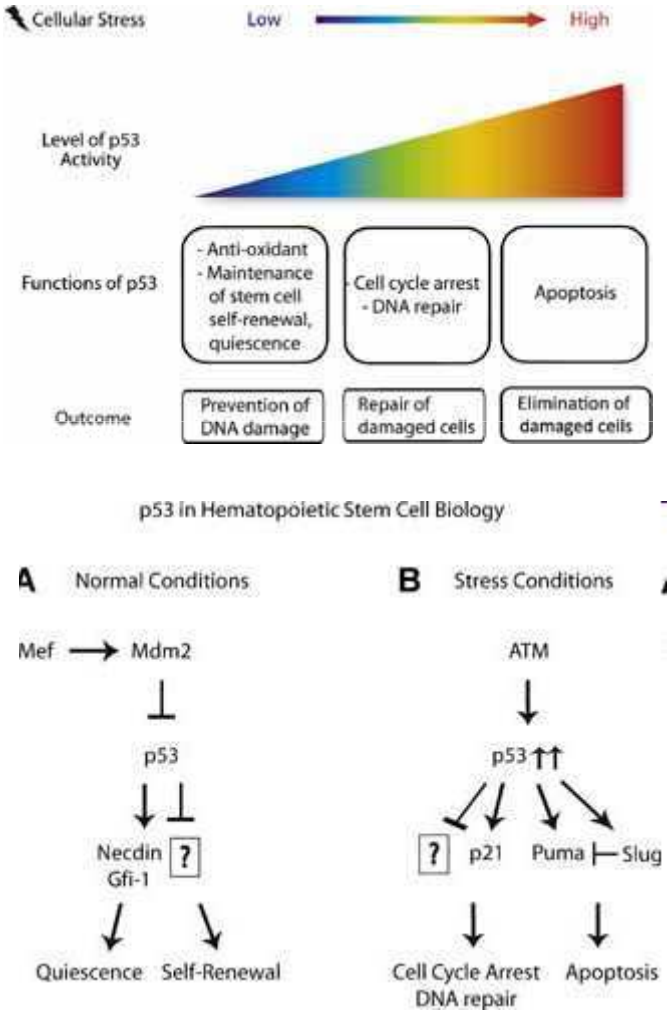
PMN viability 26.1%

MNC 15.7%

MNC viability 82.6%

CLONAL SUCCESSION MODEL



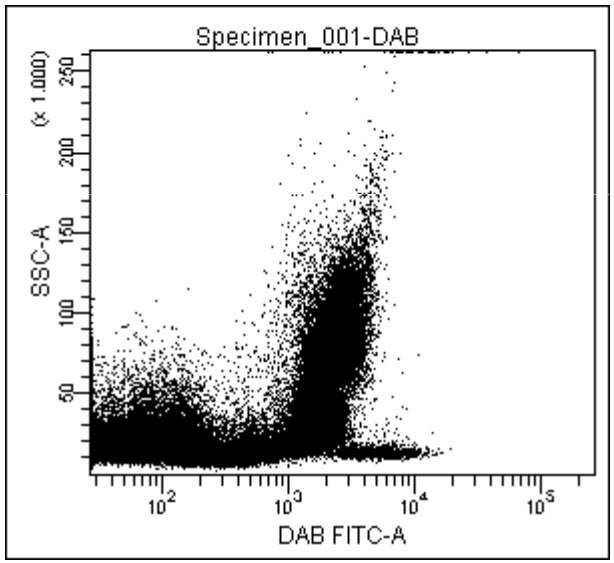


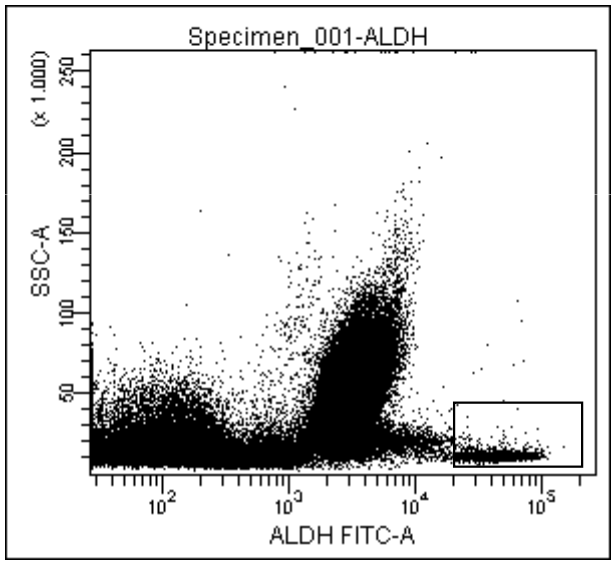
Lin-Sca+CD150+CD48-

- **90% in G0**
- **6% in G1/S G2/M**

LA Warren, DJ Rossi Mechan Ageing and Dev, 2009; 130:46-53

ALDEHYEDE DEHYDROGENASE ACTIVITIES





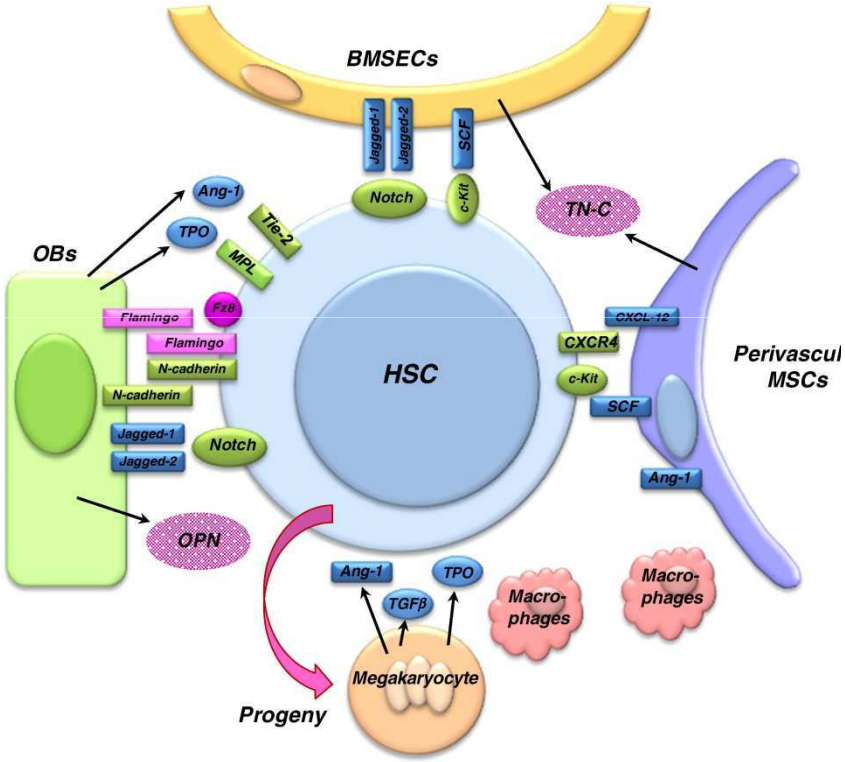
The ALDH enzyme is expressed at high level in primitive HSC

negative control

ALDH^{brigh} positive cells

DA Hess Hematologica 2007; 92:1165-1172

Hematopoietic stem cell in its place



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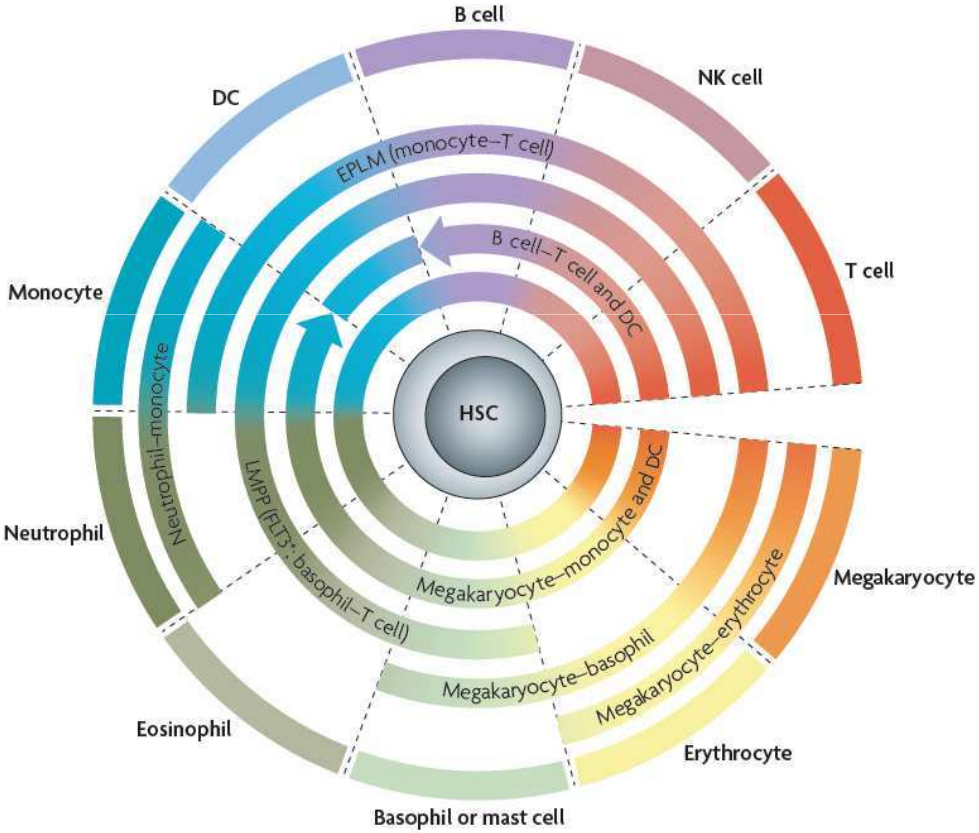
Parwise relationship models of hematopoiesis



Nature Rewiev Immunol, 2009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