Mobilization of Normal and Leukemic Stem Cells

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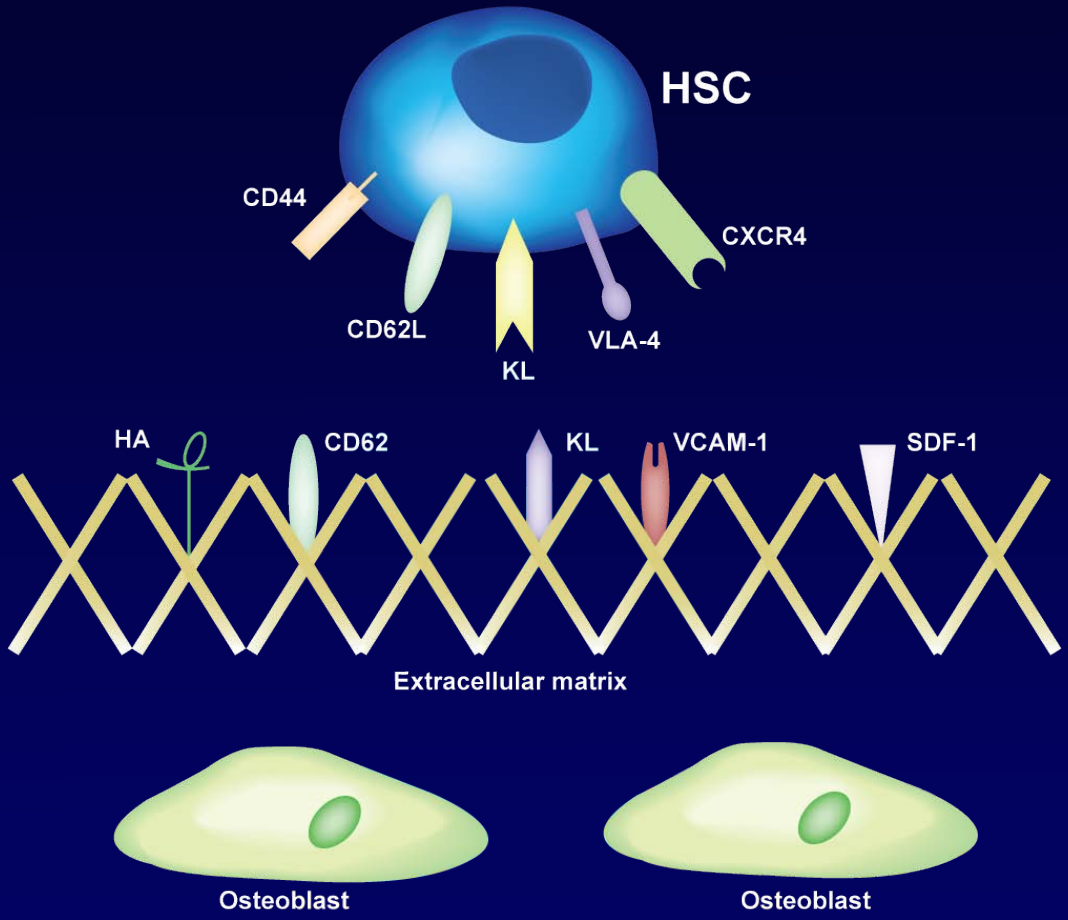
Normal Bone Marrow Microenvironment

- Composed of diverse population of stromal cells, osteoblasts, and osteoclasts[1]
- Extracellular matrix rich in fibronectin, collagens, and proteoglycans[1]
- To enter circulation, stem cells must migrate through vascular barriers[1]
- Adhesion molecules (eg, SDF-1 and VCAM-1) tether stem cells to the bone marrow[1]

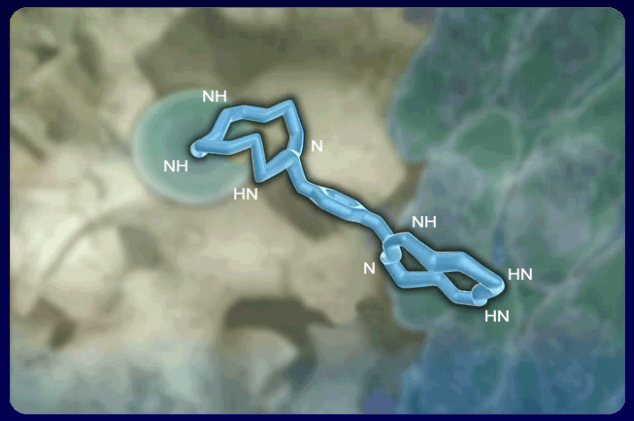


CXCR4, chemokine receptor 4; HA, hyaluronic acid; HSC, hematopoietic stem cell; KL, kit ligand; SDF-1, stromal cell-derived factor-1; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

1. Pusic I, et al. *Curr Pharm* Des. 2008;14:1950-1961. 2. Cashen AF, et al. *Future Oncol.* 2007;3:19-27.

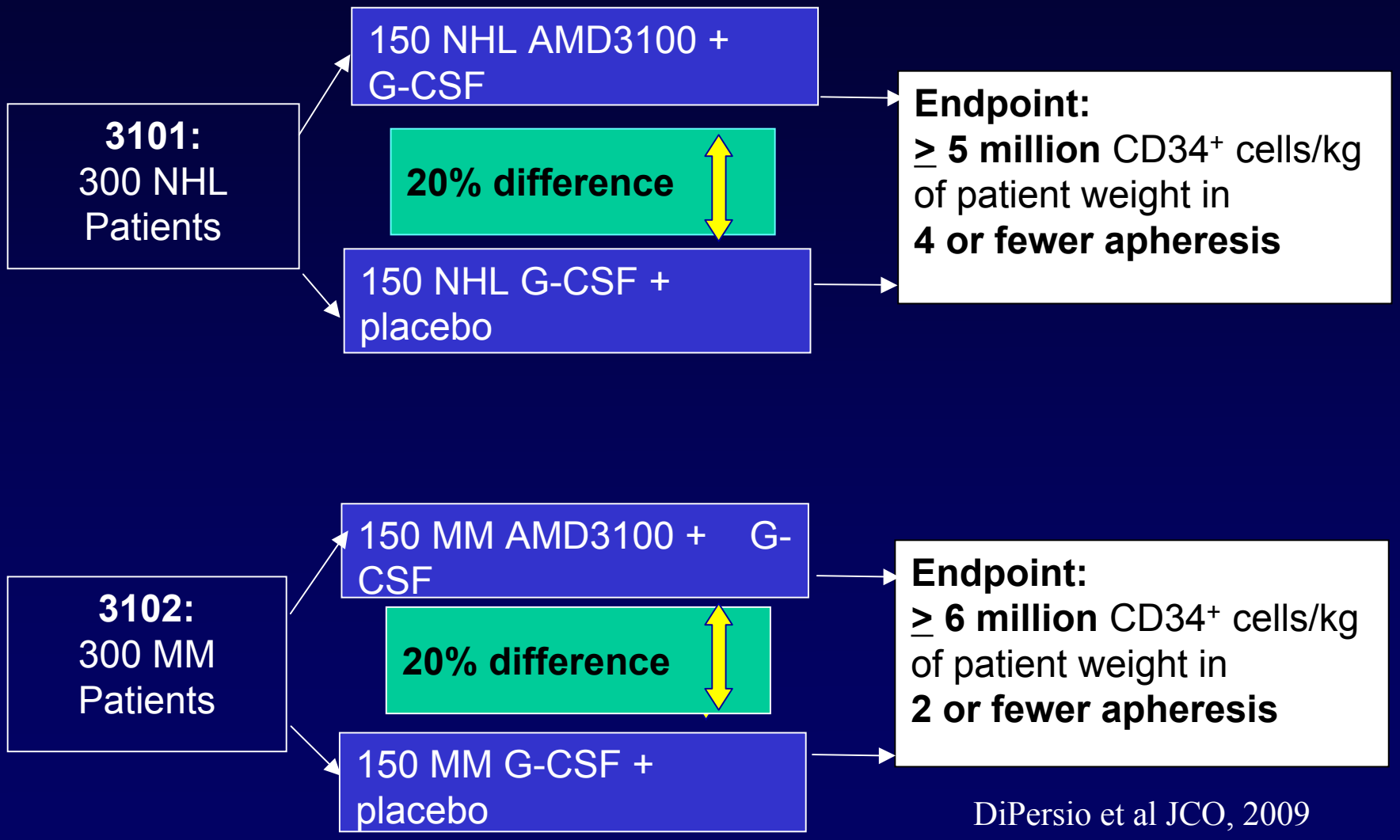
AMD3100 (plerixafor)

- A bicyclam molecule
- Reversibly binds to CXCR4 receptor and blocks SDF-1 interaction
- Very water soluble
- Highly charged
- Low molecular weight (MW = 502)
- Rapidly increases mobilization of CD34+ hematopoietic stem cells



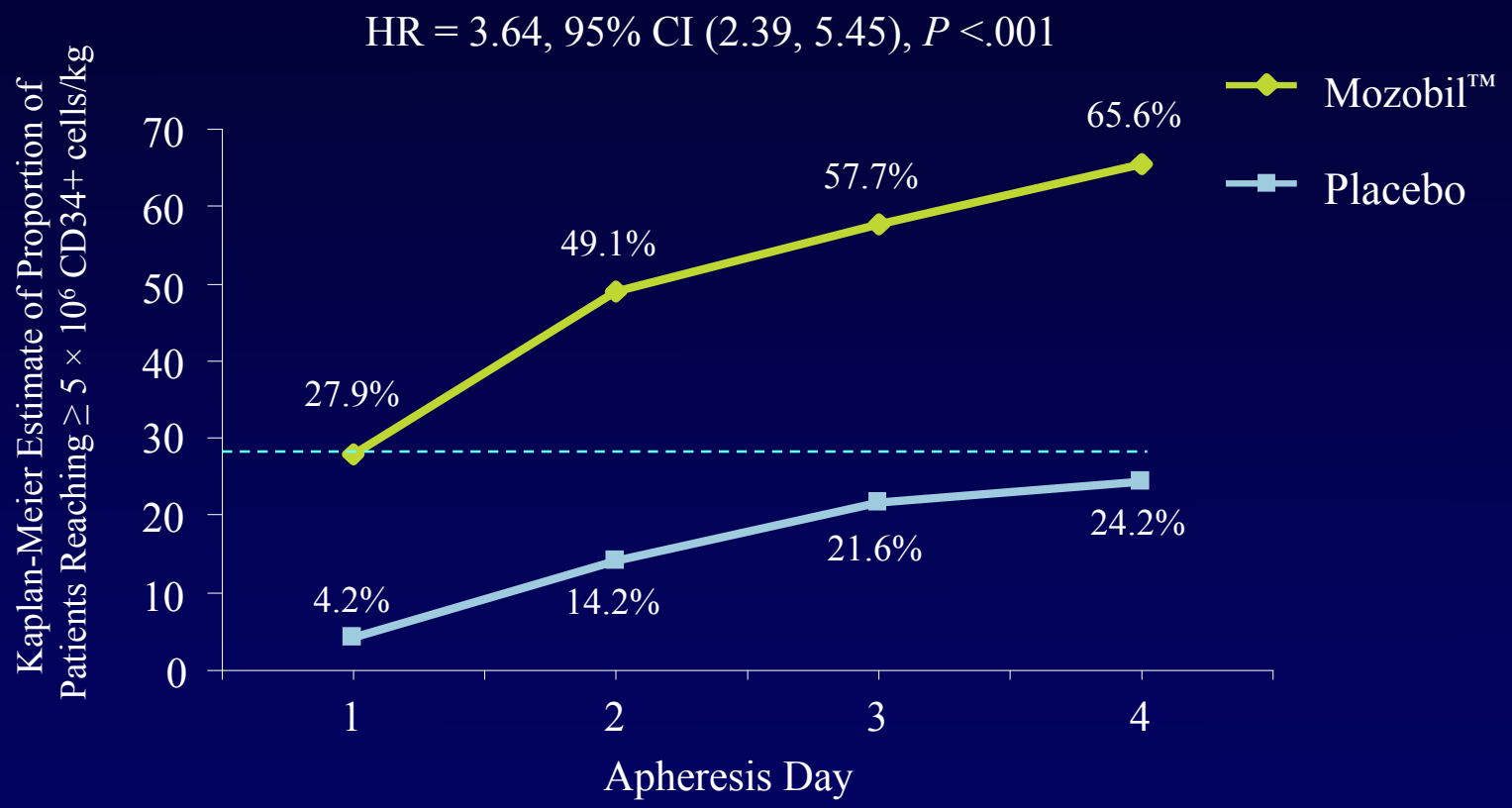
1. Pusic I, et al. *Curr Pharm Des*. 2008;14:1950-1961. 2. De Clercq E. *Nat Rev Drug Discov*. 2003;2:581-7.

Phase III trials in the US, Canada and Germany*



DiPersio et al JCO, 2009
DiPersio er al Blood, 2009

NHL Patients (%)[a] Achieving ≥ 5 × 10^6 CD34+ Cells/kg by Apheresis Day – ITT Population



CI, confidence interval; HR, hematologic response; ITT, intention-to-treat; NHL, non-Hodgkin's lymphoma.

[a] Percentage of patients achieving collection goal expressed as Kaplan-Meier estimate.

DiPersio JF, et al. *JCO, 2009*

Secondary Efficacy: Patients Achieving ≥ 2 million CD34+ cells/kg in 4 Days of Apheresis

Intent-to-Treat Population

Outcome	Plerixafor (n=150)	Placebo (n=148)	Estimate of Treatment Effect [95% CI]	p-value*
Success	130 (86.7%)	70 (47.3%)	39.4%[A] [29.7%, 49.1%]	<0.0001

[A] Treatment effect estimated using difference in chance of success

* p-value of difference in proportions using Pearson's Chi-Squared test

DiPersio et al JCO, 2009

What about AMD3100 to mobilize allogeneic PBSC? Questions:

- *How will AMD3100 affect homing properties of CD34+ cells?*
  - Will engraftment be impaired?
  - What is the half-life of AMD3100 on CXCR4?
  - Will there be downregulation of CXCR4 and dysfunctional homing?
  - How will other cells expressing CXCR4 be affected?
    - T-cells (GvHD, immune reconstitution)
    - Dendritic cells

Murine Competetive Repopulation Studies:

G-CSF Mobilized PBSC

G-CSF treated:
B6 H-2^{b}, Ly5.1
250 PB KLS cells
(Kit^{+}, Lin^{-}, Sca^{+})

BM

BM Donor:
B6 Ly5.1/5.2
1x10^{6} unmanipulated cells

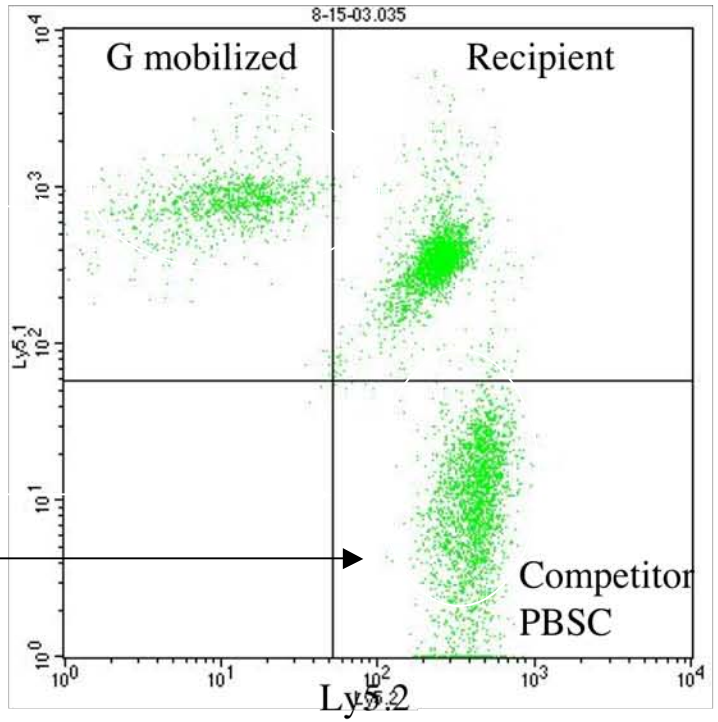
Competitor PBSC

1) G treated
2) AMD treated
3) G+AMD treated
B6 H-2^{b}, Ly5.1
250 KLS cells(Kit^{+}, Lin^{-}, Sca^{+})

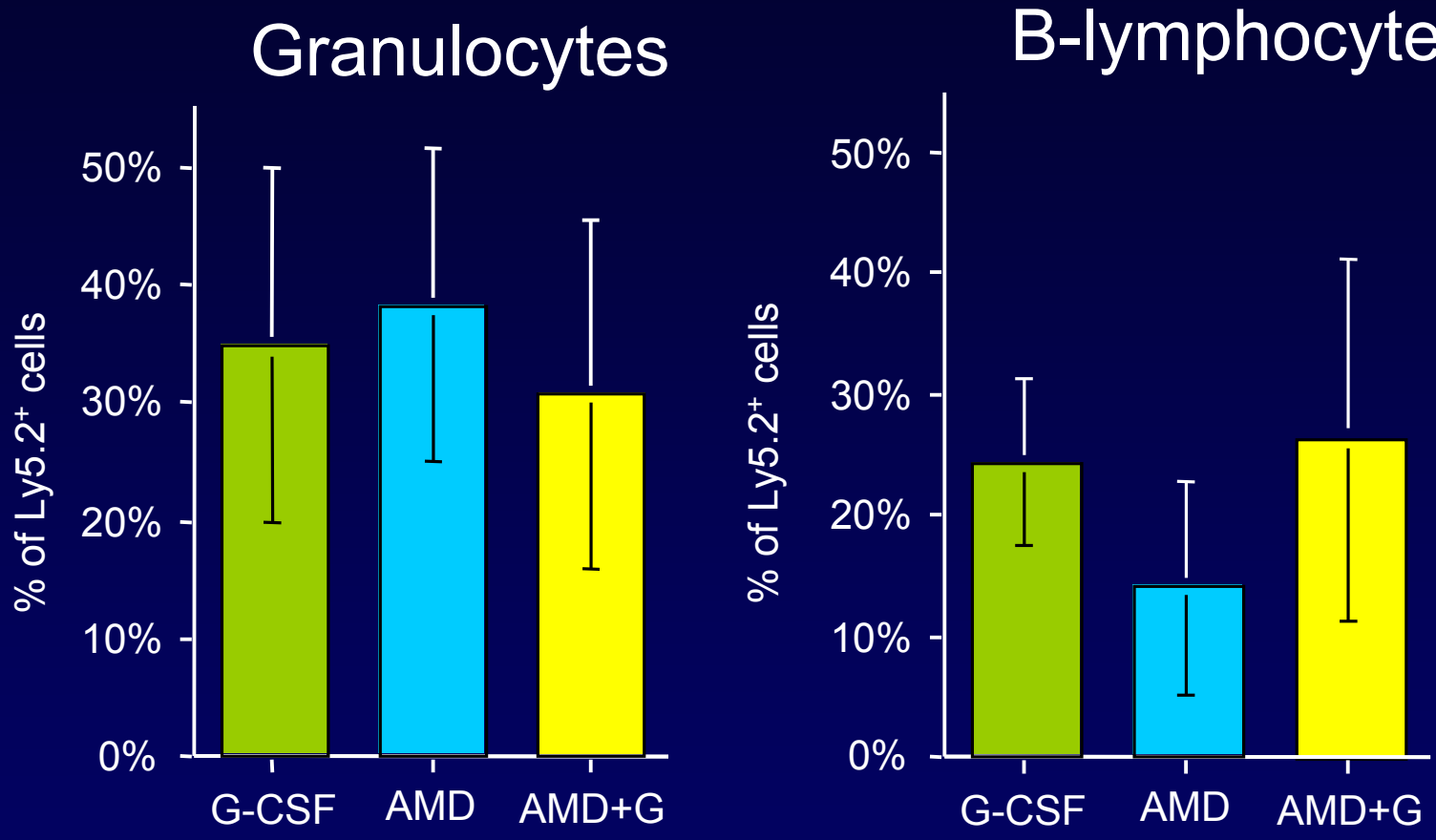
900 cGy

Recipient:
B6 H-2^{d}, Ly5.1/5.2

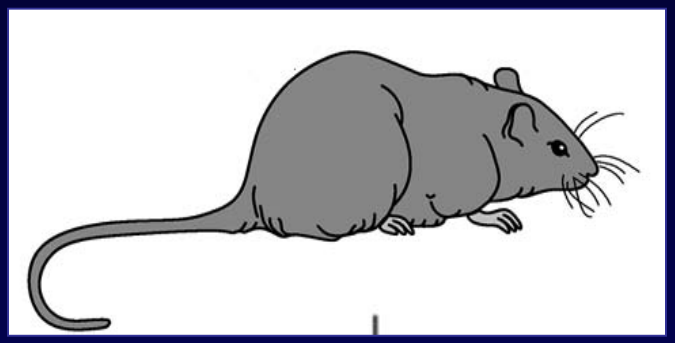
AMD3100



Lineage Specific Chimerism after Competitive Repopulation

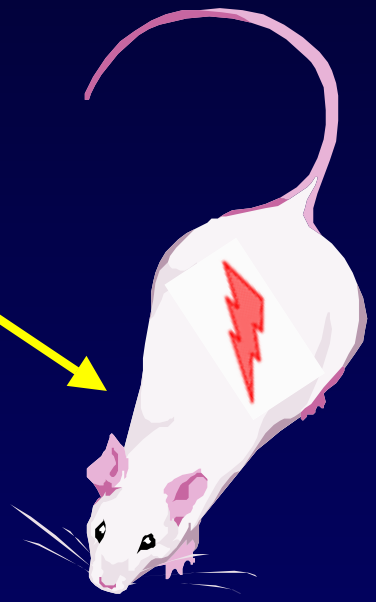


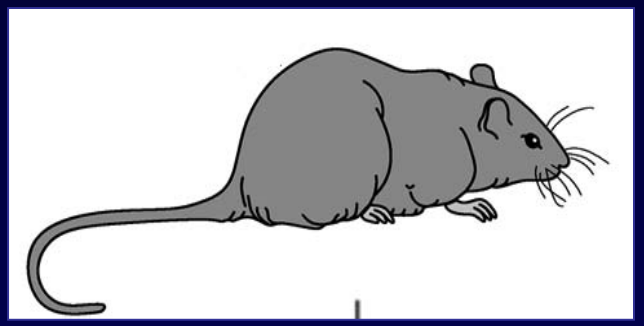
G-CSF, AMD3100, and G-CSF plus AMD3100 on Engraftment and GVHD



T Cell Donor: B6 H-2b
Splenic T cells (Ly5.1)

1. Naïve untreated
2. G-CSF (10ug x 4 days)
3. AMD 3100
4. G+AMD 3100





Bone Marrow Donor: B6 H-2b
4 x 10^6 TCD BM (Ly5.2)

Recipient BALB H-2d; Ly5.2

Model A (severe GVHD): 900 cGy irradiated recipients and 2 x 10^6 allogeneic T cells

Model B (moderate GVHD): 900 cGy irradiated recipients and 5 x 10^5 allogeneic T cells

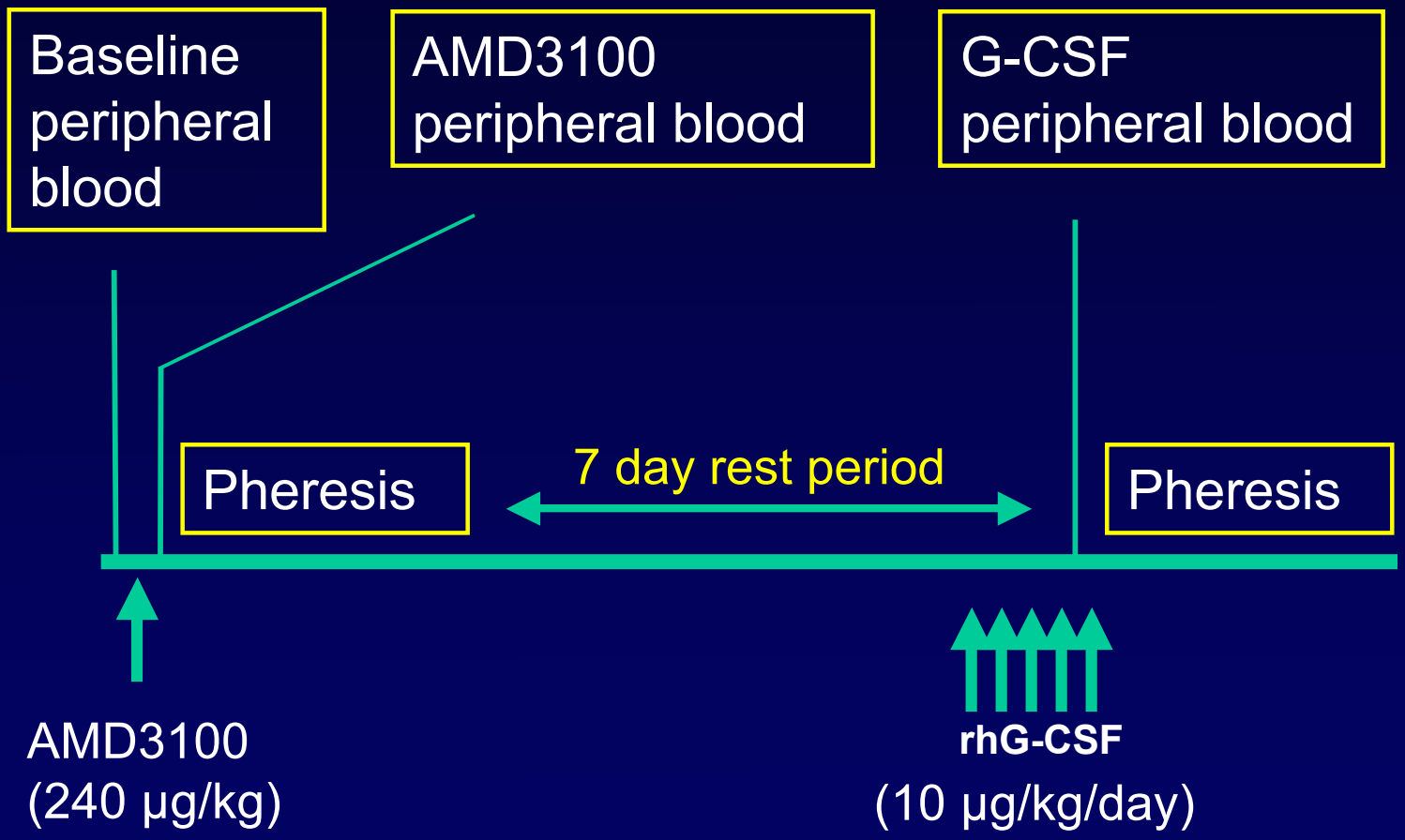
Model C (graft failure): 500 cGy irradiated recipients and 2 x 10^6 allogeneic T cells

Conclusions

1. ***Stem cells mobilized with G vs. G+A vs. A have identical early repopulation capacity***

2. ***T cells mobilized with G vs. G+A vs. A have identical GvHD and engraftment potential***

Design: AMD3100 Allogeneic Stem Cell Mobilization Study



Devine SM, et al. *Blood.* 2008;112:990-998.

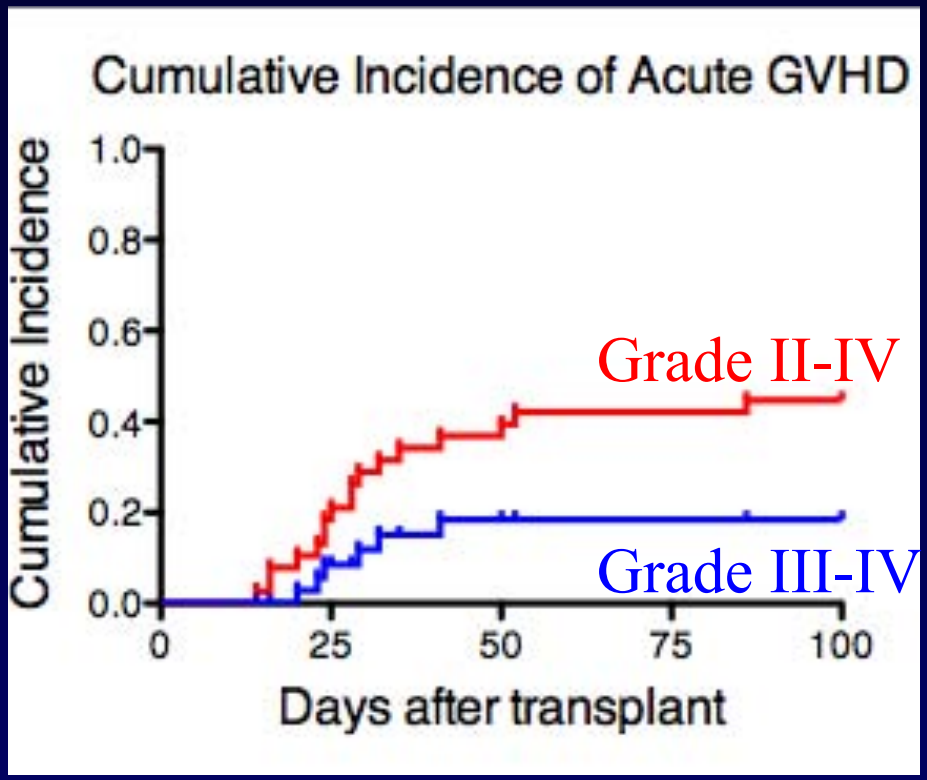
Allograft Composition (n = 22)

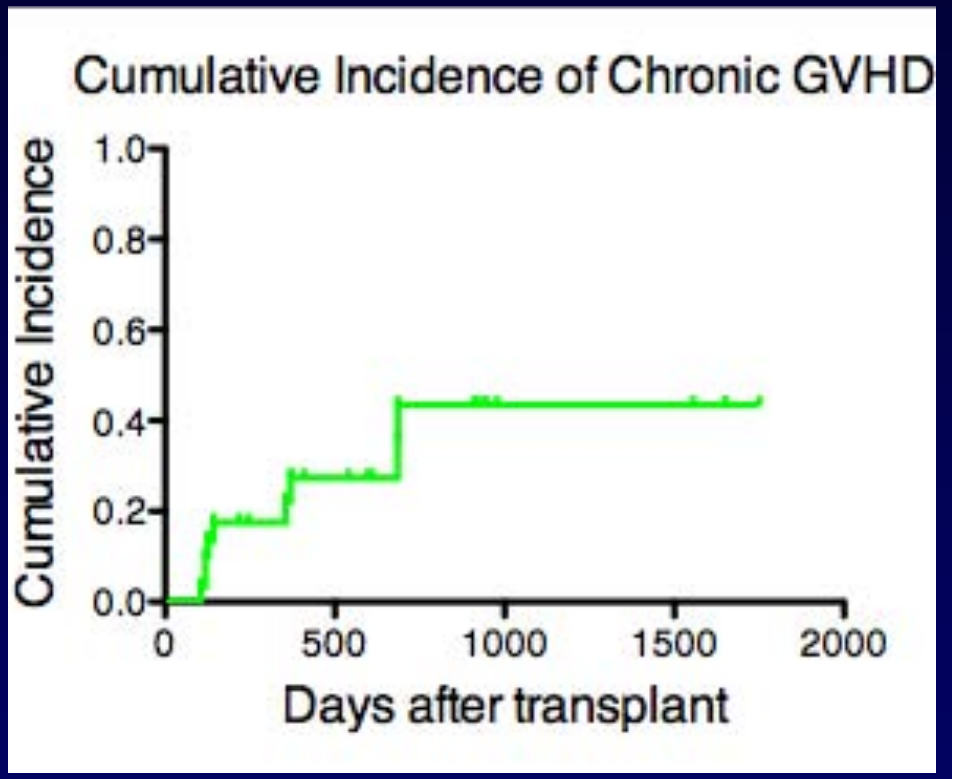
	AMD3100	G-CSF*	*P* Value
CD34 ($x10^6$/kg)	3.1 (1.2-6.3)	4.2 (2.5-18.7)	0.006
CD3 ($x10^8$/kg)	4.7 (1.5-7.8)	1.5 (1.2-6.8)	0.006
CD4 ($x10^8$/kg)	3.1 (1.0-5.7)	1.1 (0.7-3.2)	0.002
CD8 ($x10^8$/kg)	1.3 (0.4-3.4)	0.4 (0.3-3.4)	0.08
CD56 ($x10^7$/kg)	2.8 (0.2-4.6)	0.2 (0.1-0.5)	0.2

***includes 8 donors mobilized by both AMD3100 and G-CSF**

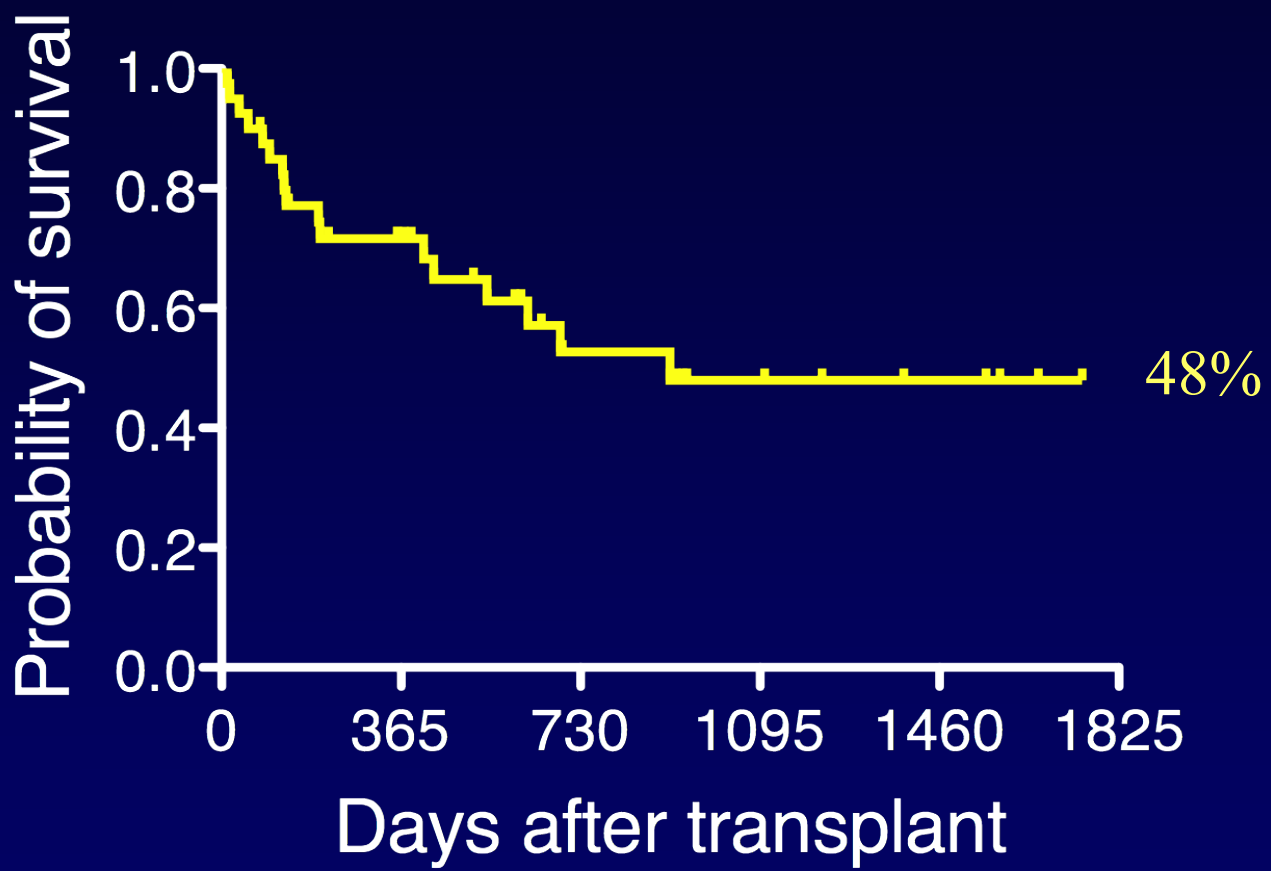
Devine et al Blood, 2008

AMD-ALLO: GVHD (n = 38)

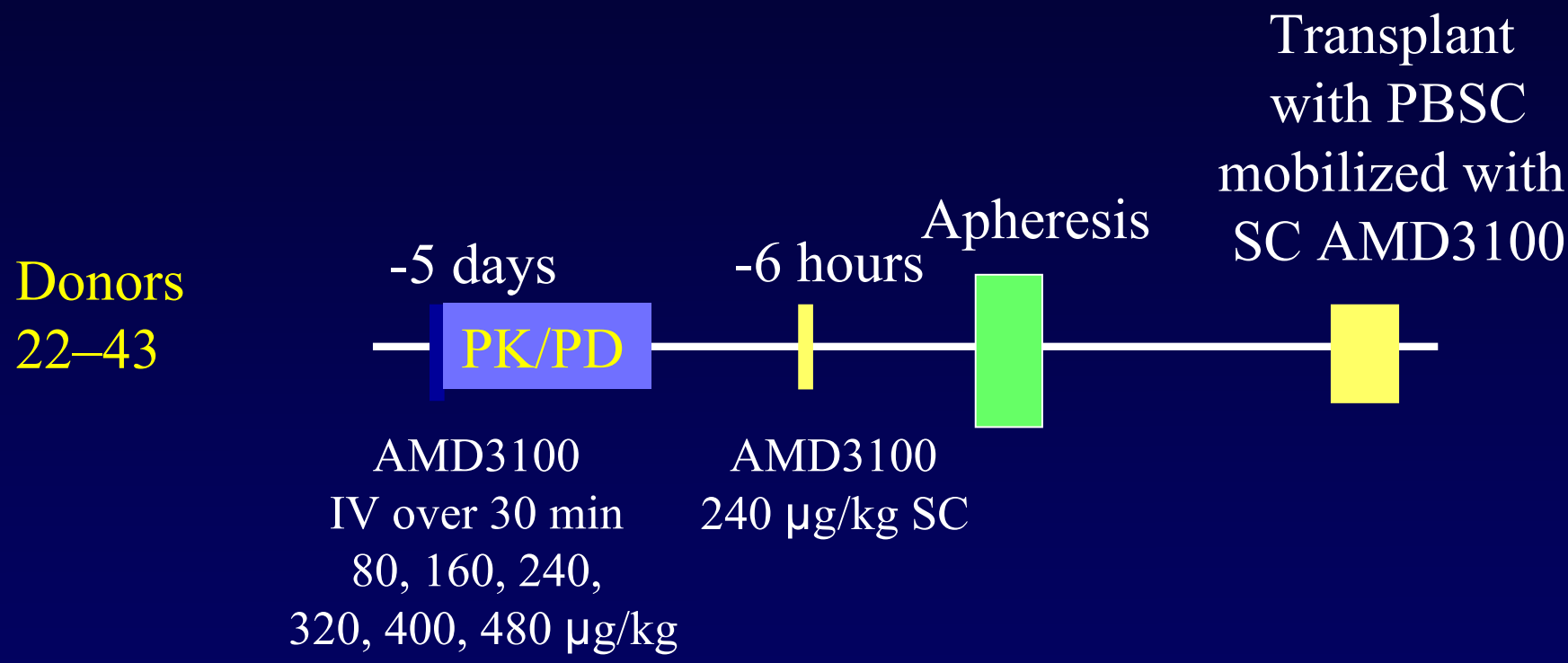




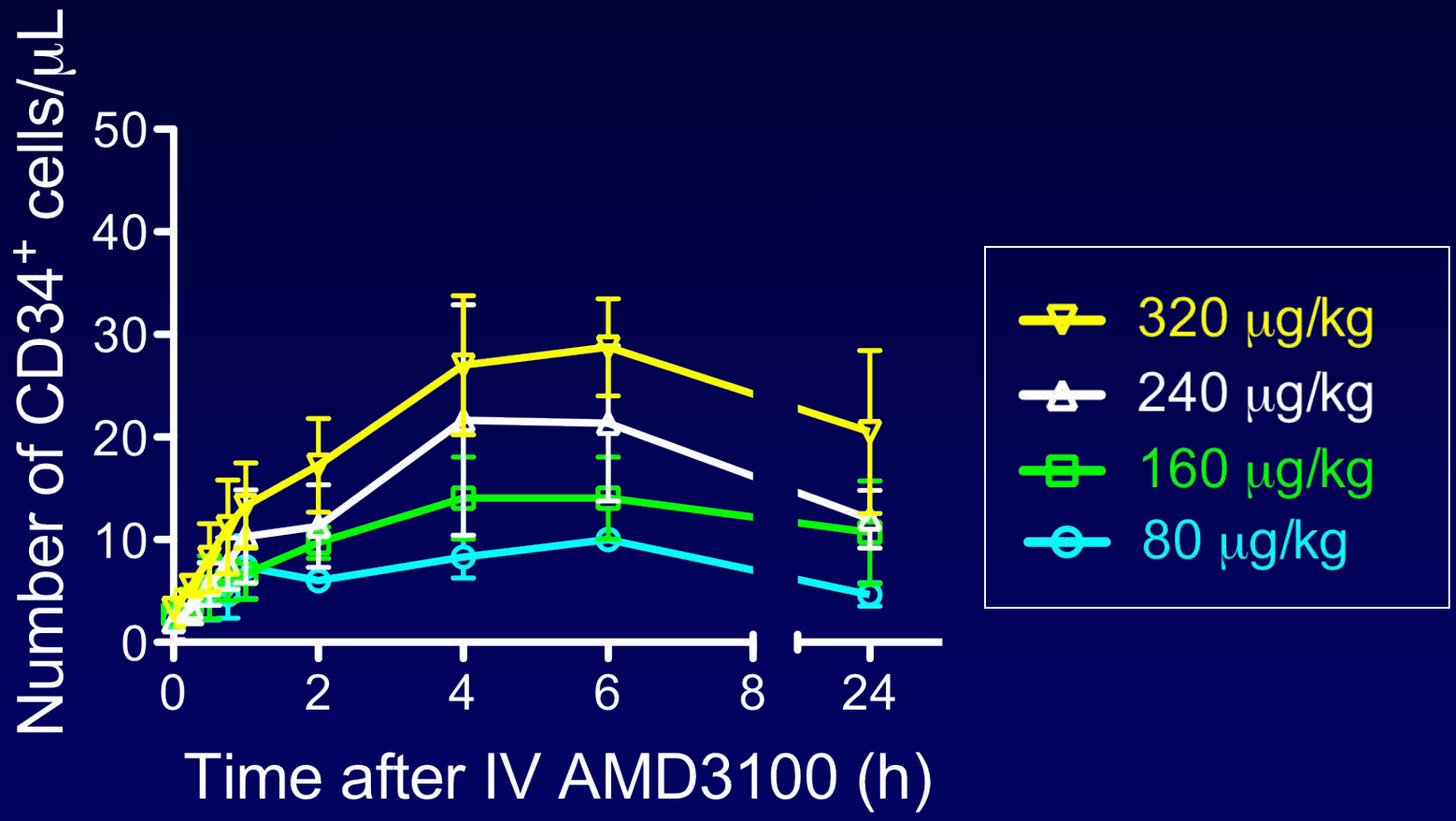
AMD-ALLO: Overall Survival (n = 38)



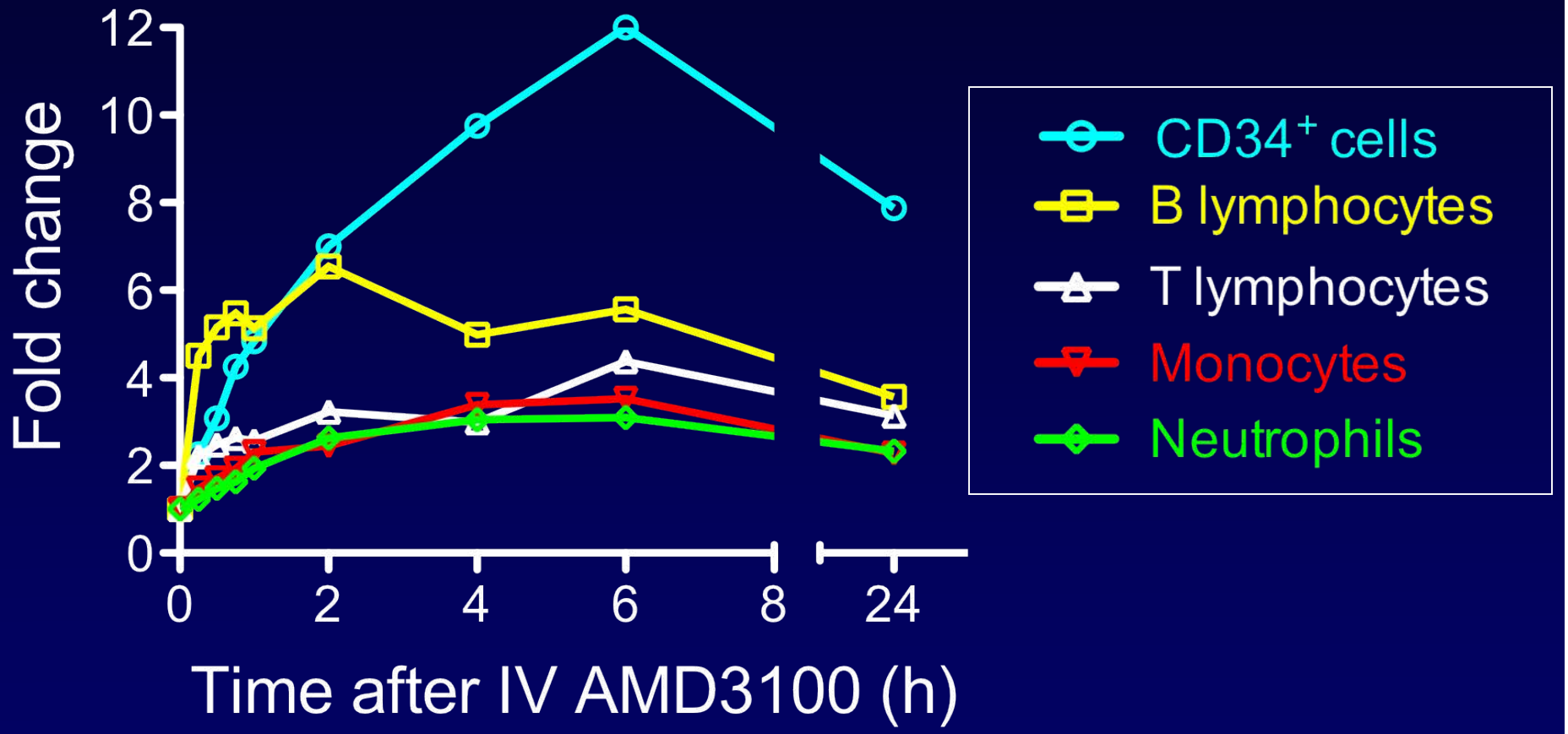
Optimal Effect of AMD3100: Cmax vs AUC



Dose Response of CD34^{+} Cell Mobilization by IV AMD3100



320 µg/kg IV AMD3100: Subset Analysis

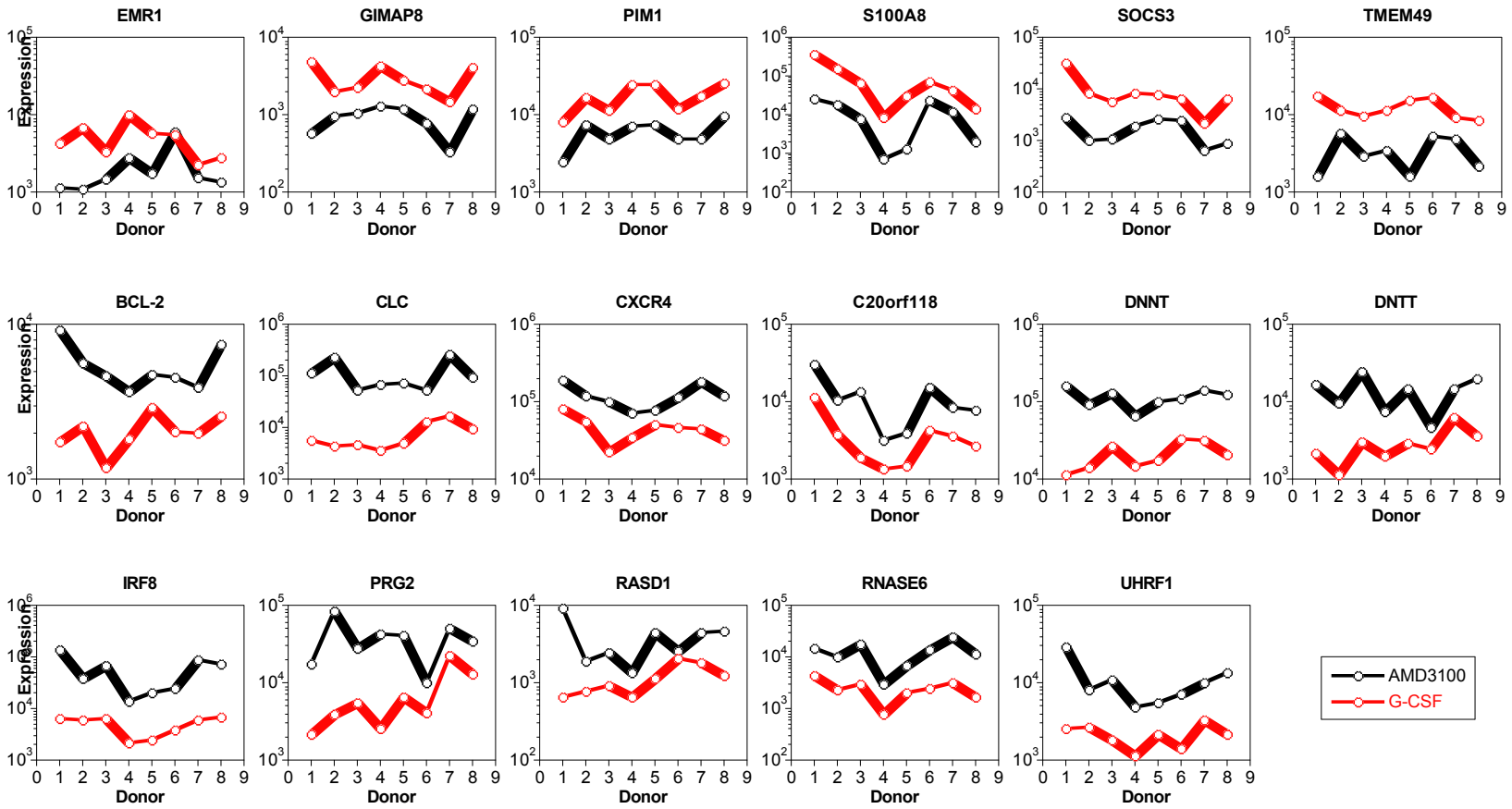


Blood cell counts, total CD34 cells, and SRC frequencies after administration of AMD3100 (240μg/kg) or G-CSF (10 μg/kg/day).

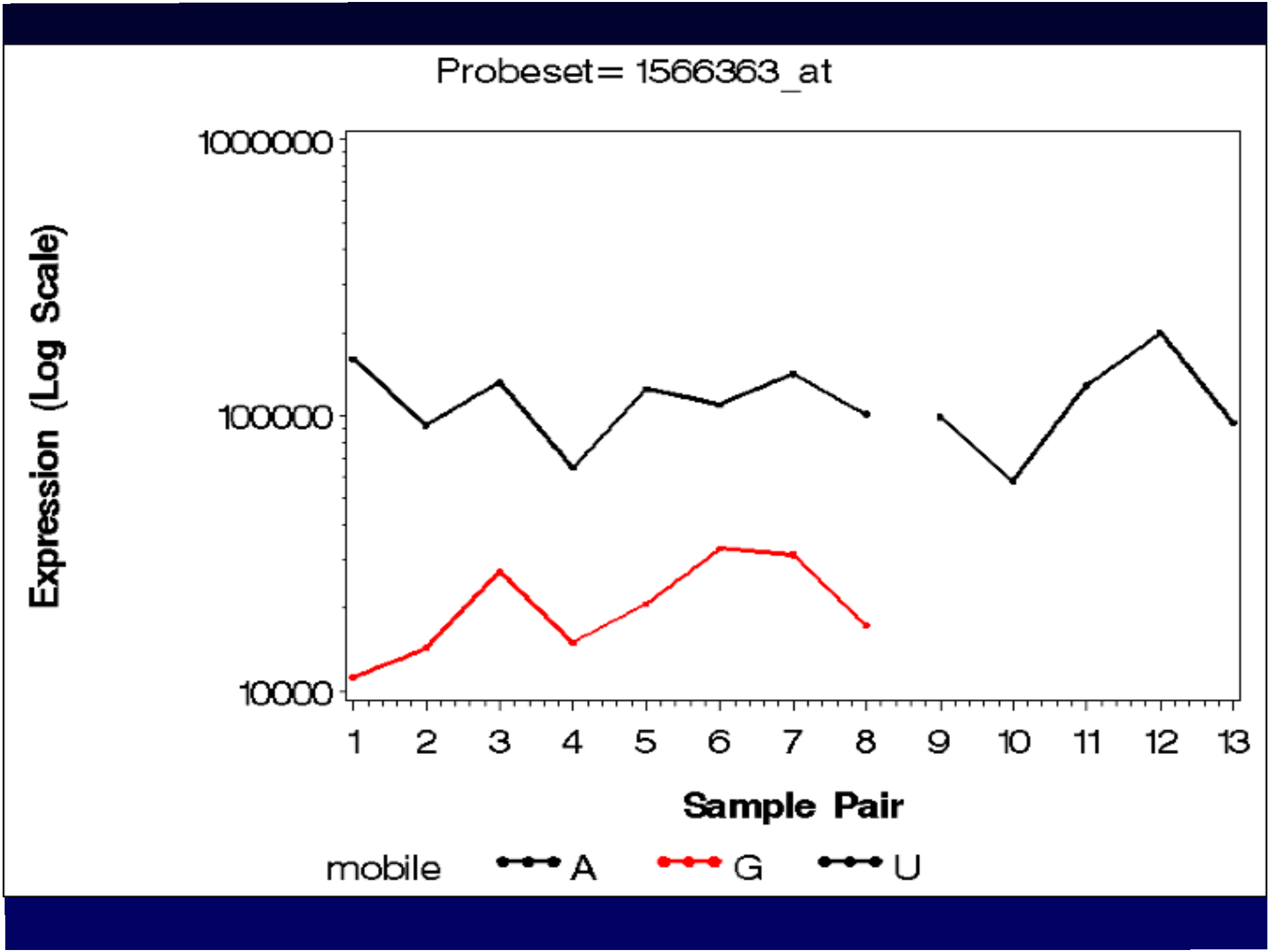
Mobilizing agent	WBC, $x10^3$/mL		Total $CD34^+$, Cells/μL SRC Frequency, $x10^6$ MNC		SRC Frequency $x10^3$ $CD34^+$
	0 hours	4 hours			
AMD3100	**6.5±0.3**	**19.1±0.6**	**2.9±1.0**	**1 in 8.7**	**1 in 117**
G-CSF	7.0±0.5	39.0±3.0	6.2±3.2	1 in 29	1 in 177

ND indicates not determined; Eng., engrafted; SRC, NOD/SCID repopulating cells

Genes Differentially Expressed in Human Peripheral Blood CD34+ Cells Following Mobilization with AMD3100 or G-CSF



GTPase, IMAP family member 8; PIM1 = Pim-1 oncogene; S100A8 = S100 calcium binding protein A8; SOCS3 = Suppressor of cytokine signaling 3; TMEM49 = Transmembrane protein 49; BCL-2 = B-cell CLL/lymphoma 2; CLC = Charcot-Leyden crystal protein; CXCR4 = Chemokine (C-X-C motif) receptor 4; C200rf118 = Chromosome 20 open reading frame 118; DNTT = Deoxynucleotidyltransferase, terminal; IRF8 = Interferon regulatory factor 8; PRG2 = Proteoglycan 2; RASD1 = RAS, dexamethasone-induced 1; RNASE6 = Ribonuclease, RNase A family, k6; UHRF1 = Ubiquitin-like, containing PHD and RING finger domains, 1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Expression (Log Scale)
1000000
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Sample Pair
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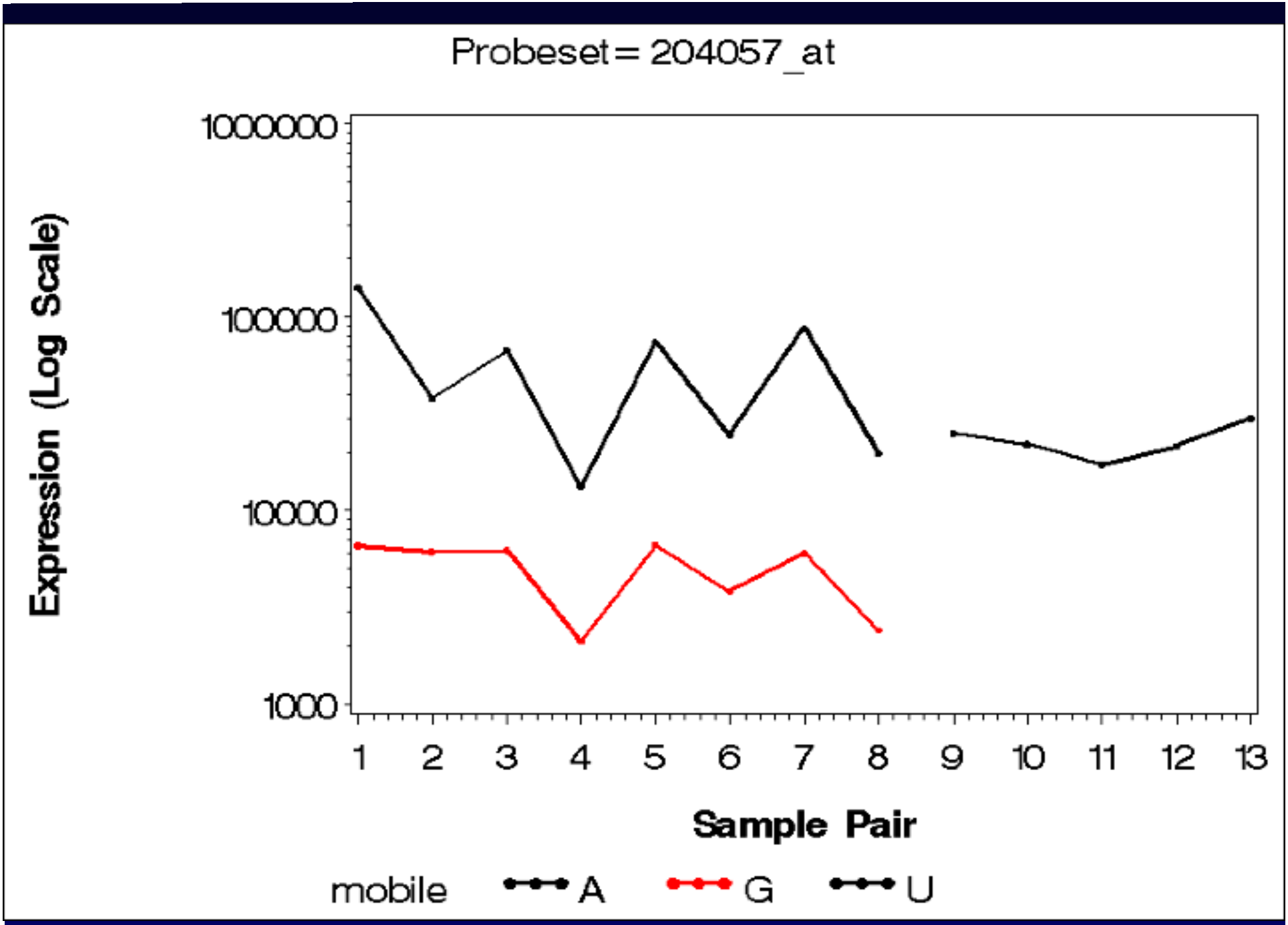
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Sample Pair
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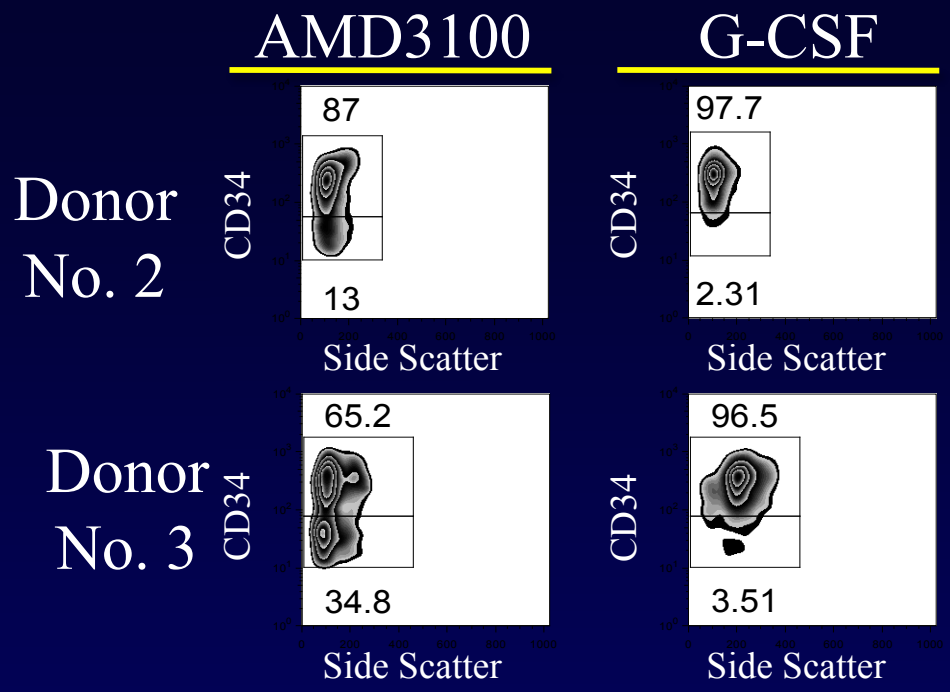
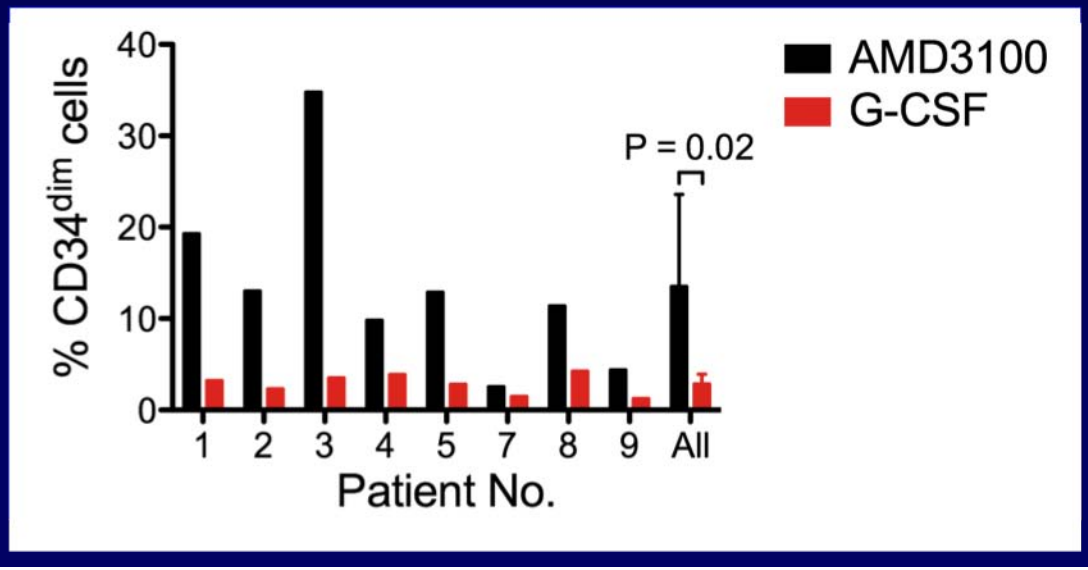
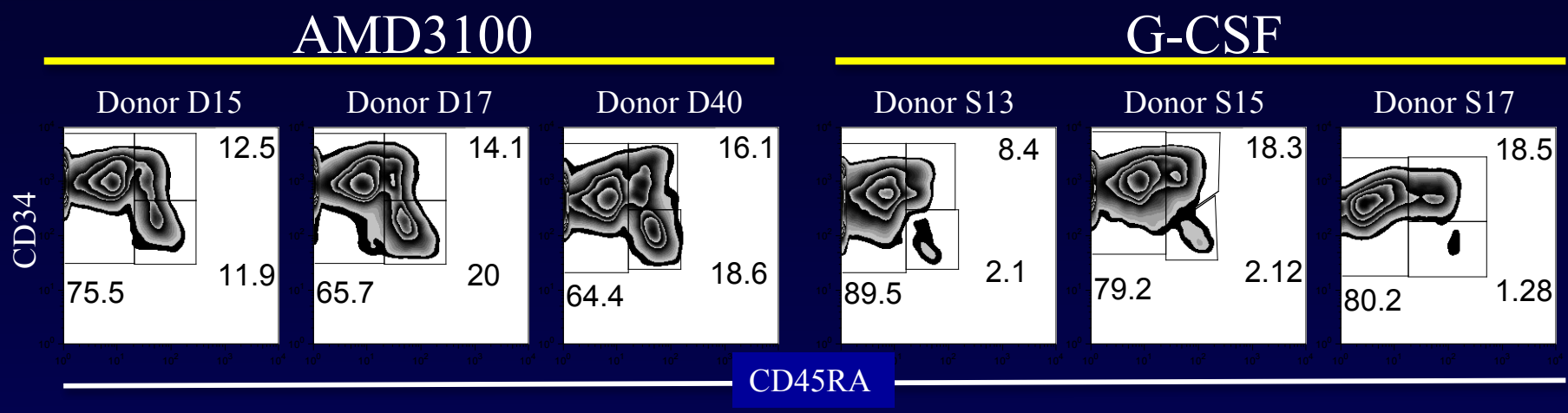
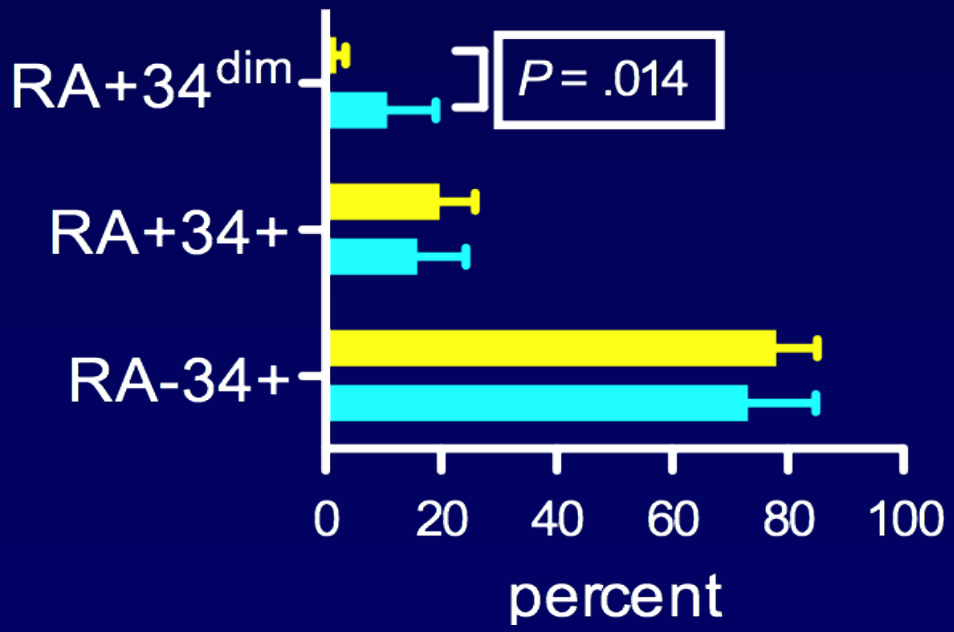
AMD3100 Preferentially Mobilizes a CD34^{dim} Subset



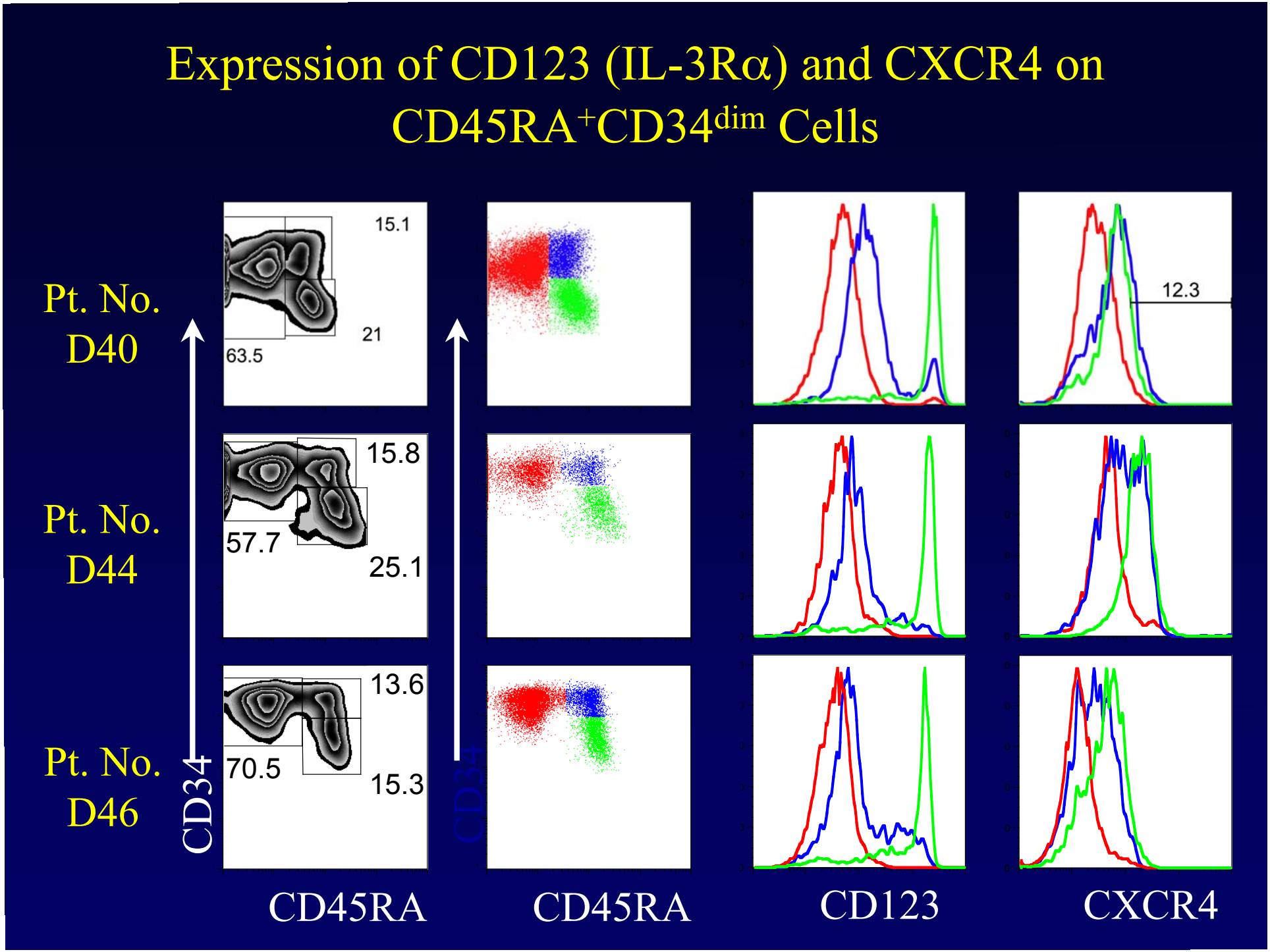


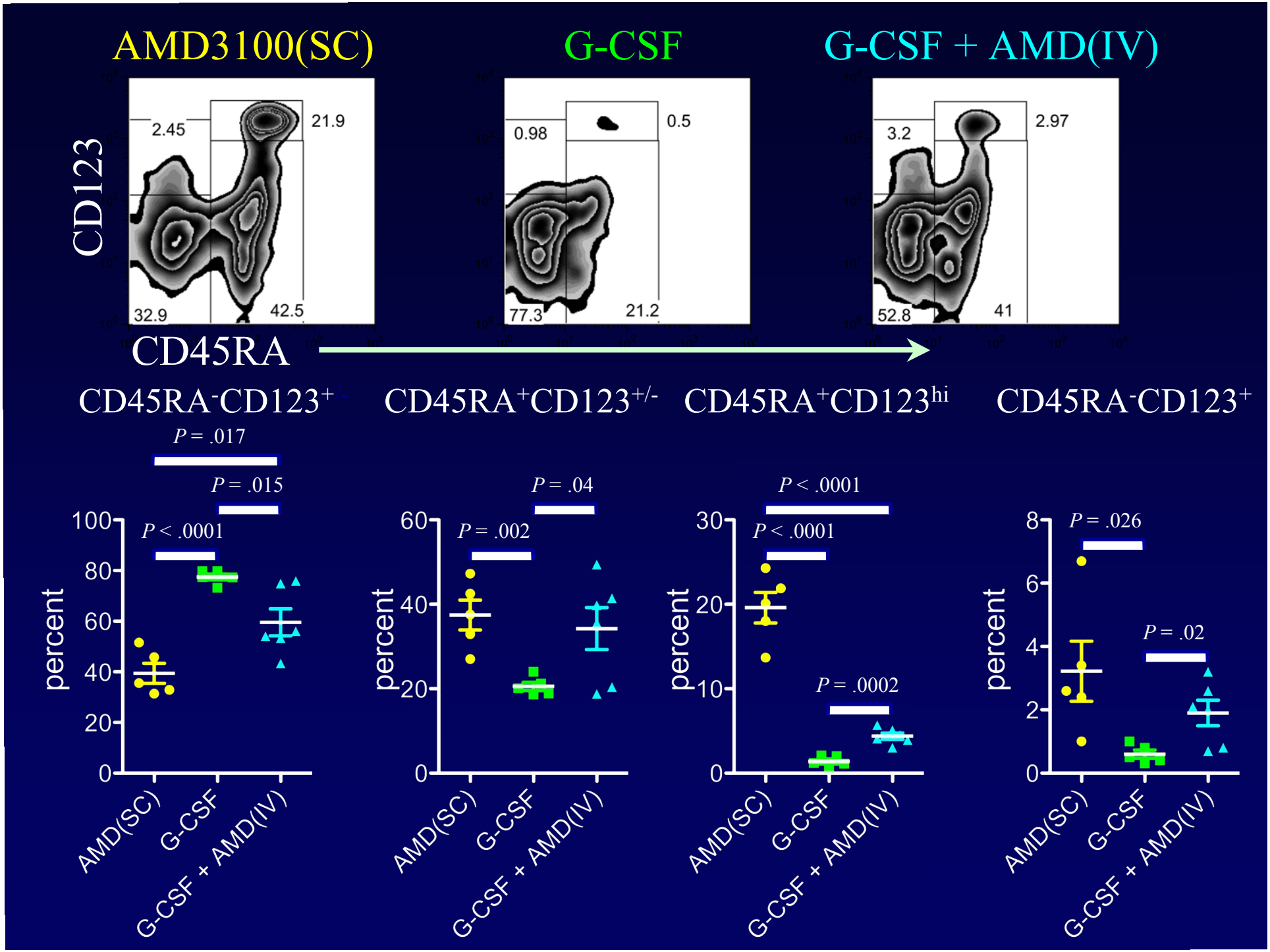
Co-expression of CD45RA on CD34^{+} cells identifies the CD34^{dim} subset





Expression of CD123 (IL-3Rα) and CXCR4 on CD45RA^{+}CD34^{dim} Cells




AMD3100(SC)
G-CSF
G-CSF + AMD(IV)
CD123
CD45RA
2.45
21.9
32.9
42.5
0.98
0.5
77.3
21.2
3.2
2.97
52.8
41
CD45RA-CD123+/-
CD45RA+CD123+/-
CD45RA+CD123hi
CD45RA-CD123+
percent
P = .017
P = .015
P < .0001
P = .04
P = .002
P < .0001
P < .0001
P = .0002
P = .026
P = .02
AMD(SC)
G-CSF
G-CSF + AMD(IV)

AMD3100 vs. G-CSF RNA Profiling

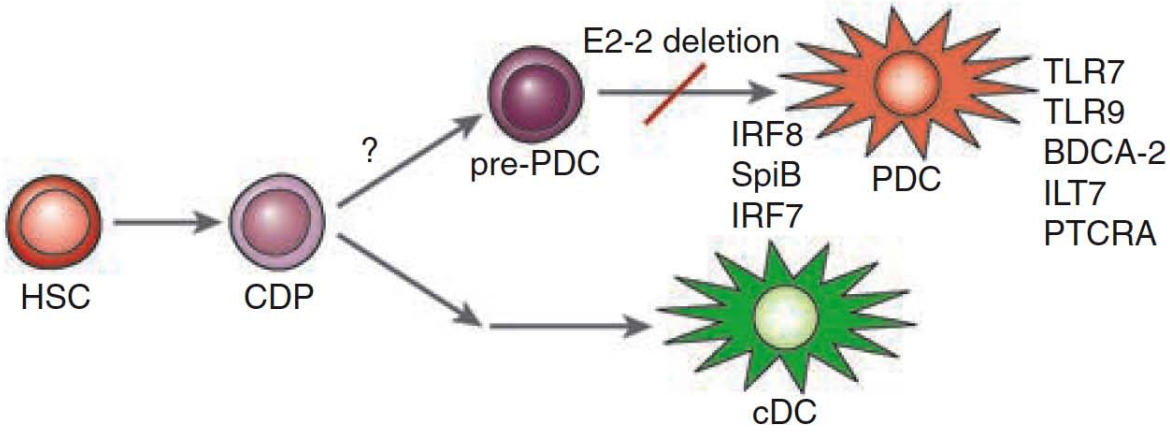
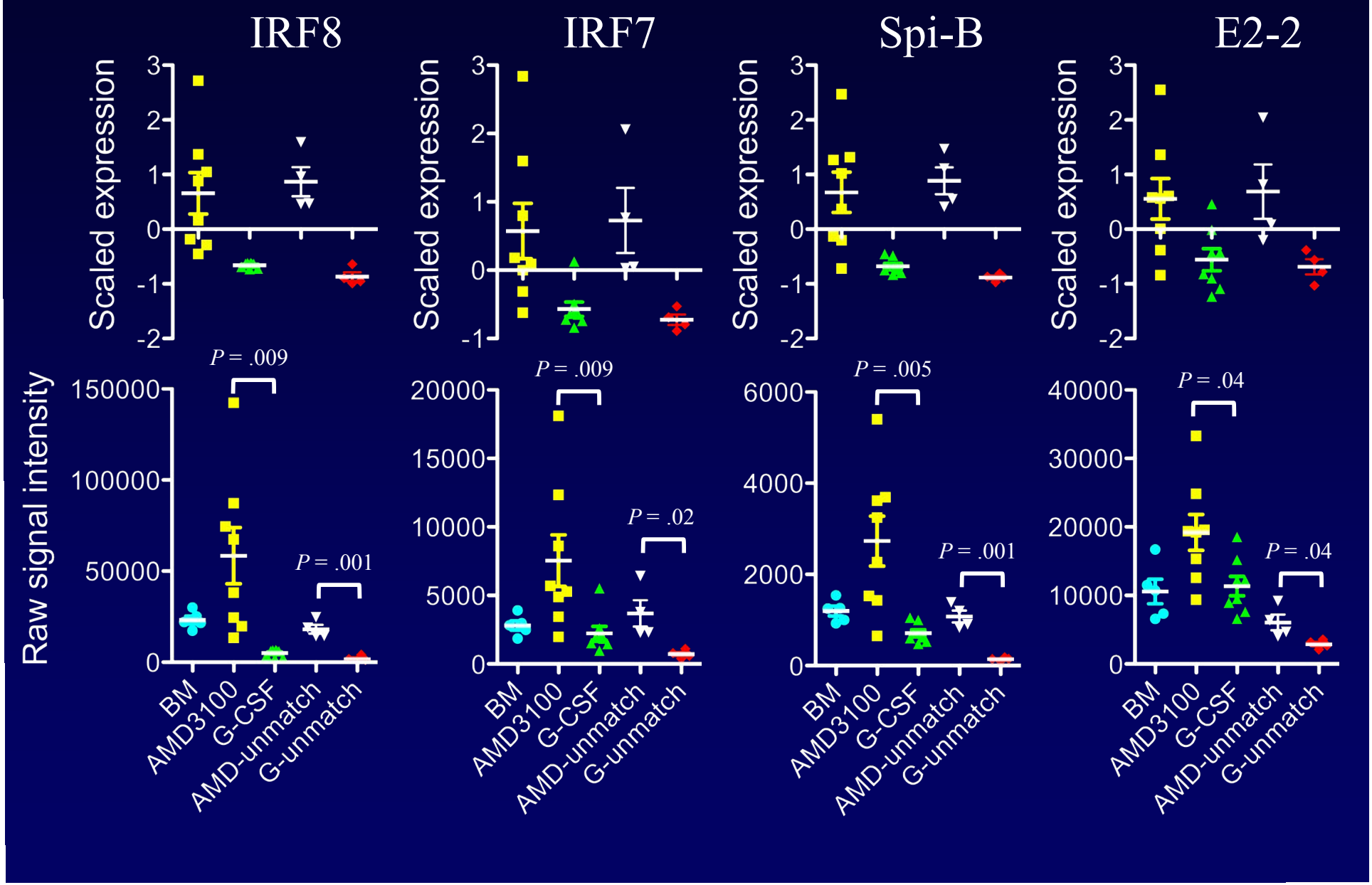
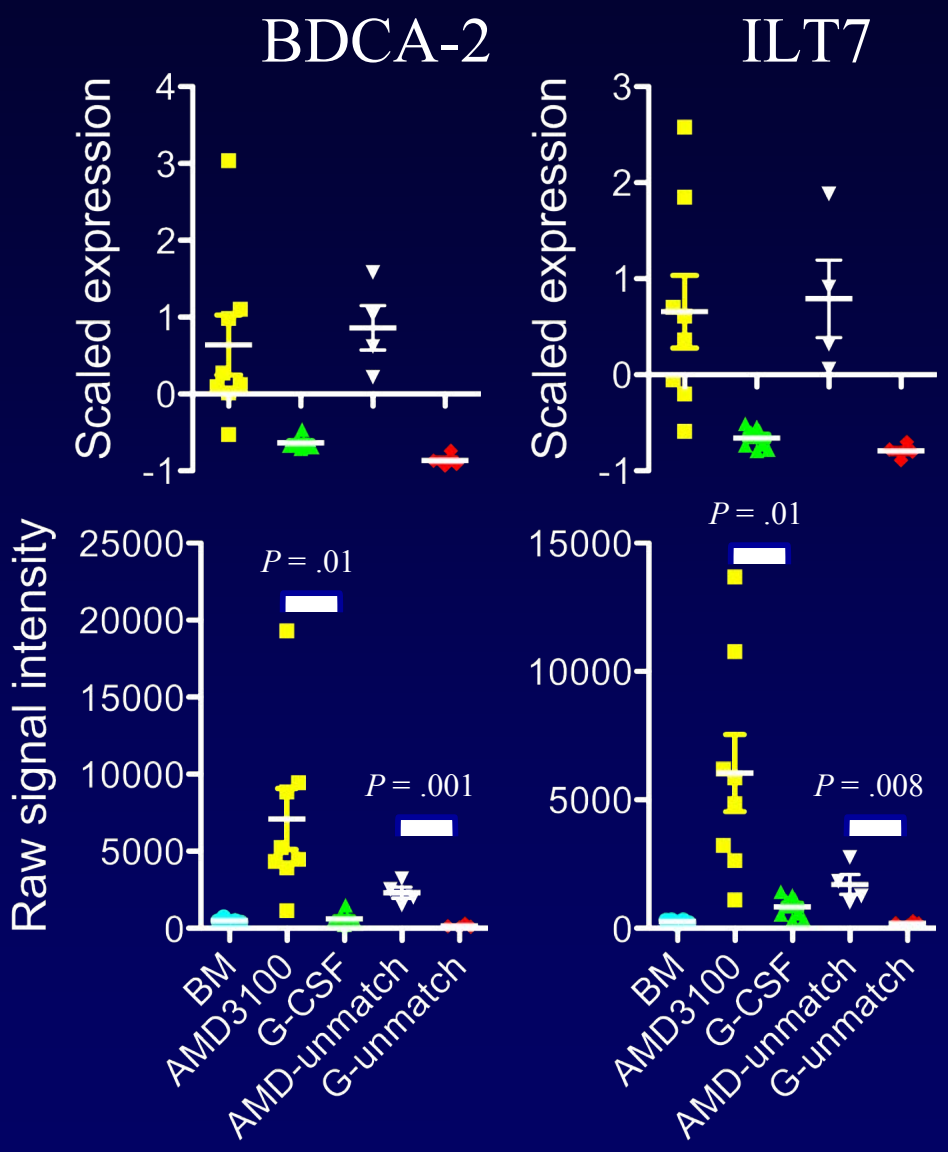


Figure 1 E2-2 function in the plasmacytoid dendritic cell (PDC) developmental pathway. DC development proceeds from hematopoietic stem cells (HSCs) through a common DC progenitor (CDP). Hypothetical precursors for PDCs (pre-PDC) may be specified by the differential expression or activation of transcription factors within CDPs. In the PDC lineage, E2-2 regulates terminal development and interferon production by controlling PDC-related genes.

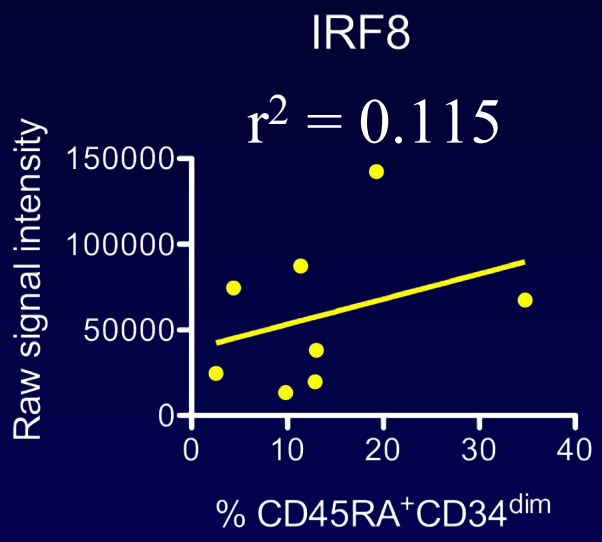
AMD3100 vs. G-CSF RNA Profiling

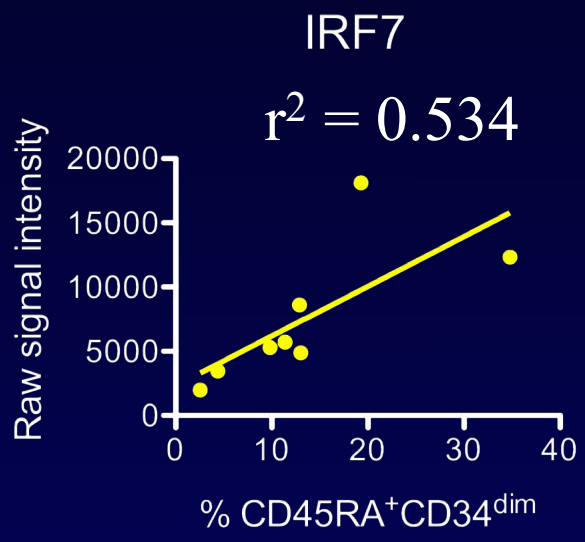


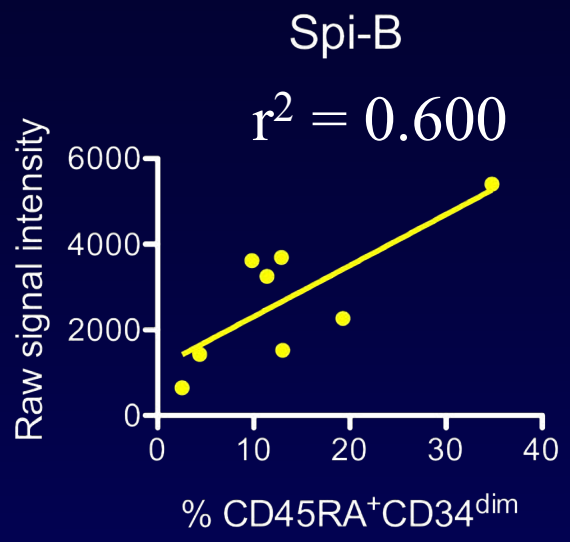
AMD3100 vs. G-CSF RNA Profiling

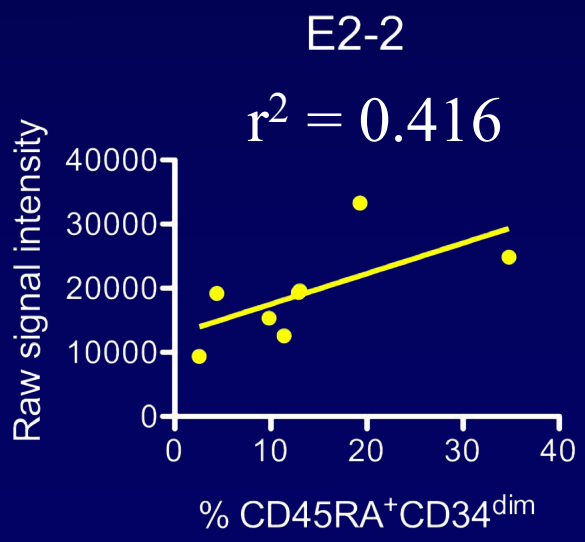


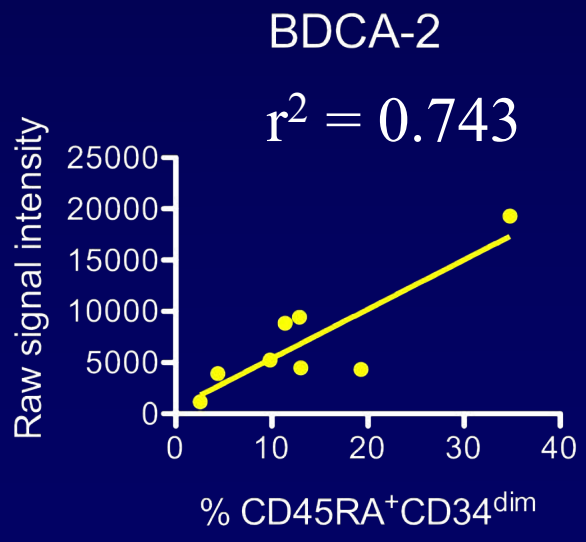
AMD3100 vs. G-CSF RNA Profiling

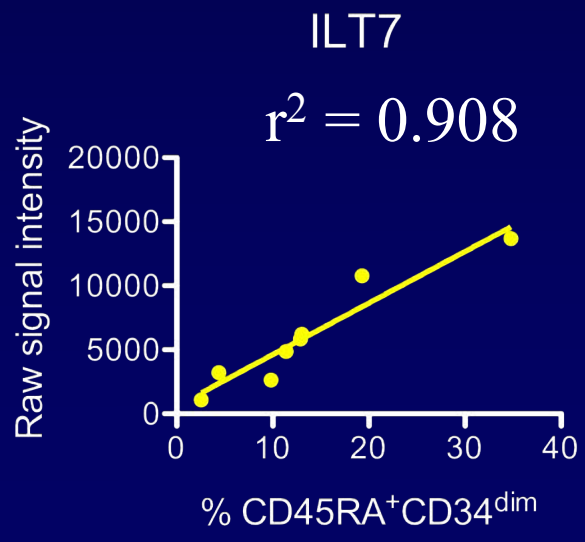






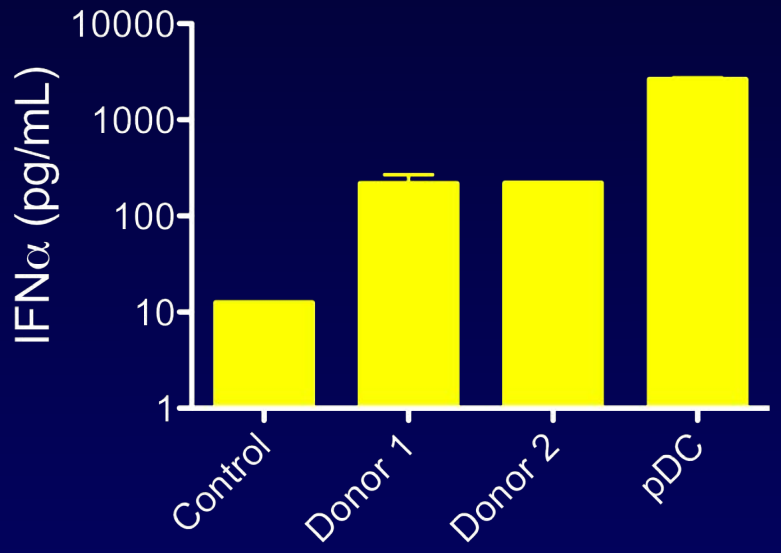






In vitro IFNα assay

Control = GpC
TLR9 = CpG



N=46 allografts
N=23 (50%) of patients at risk for CMV
N=5 (21%) developed viremia
N=0 (0%) developed CMV disease

CD62L expression on T lymphocytes

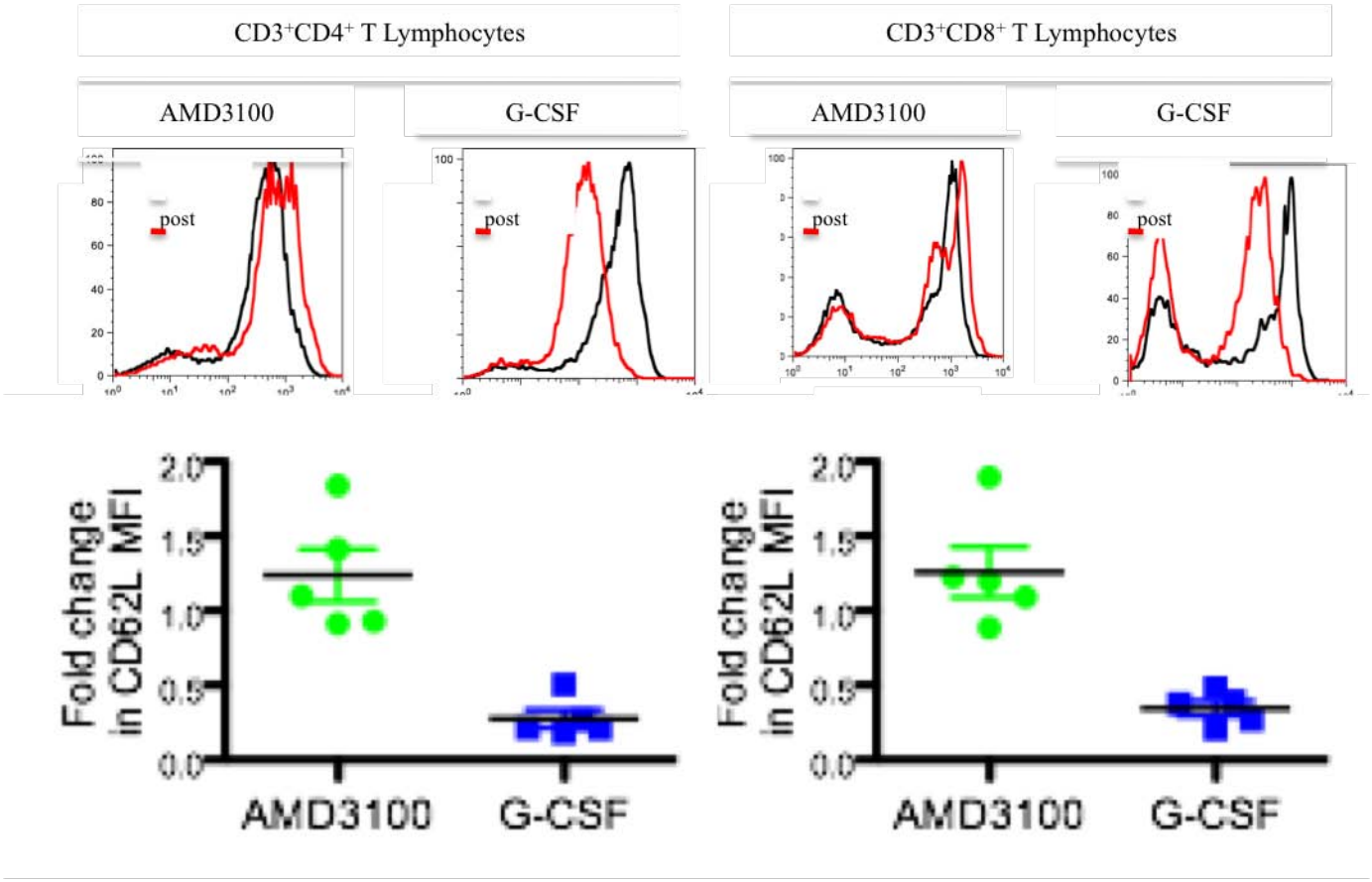
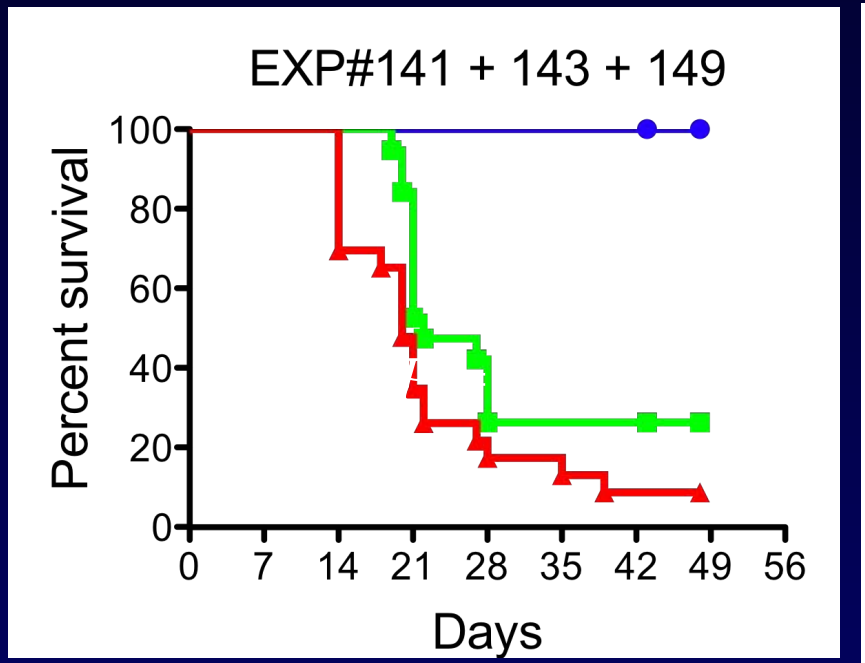
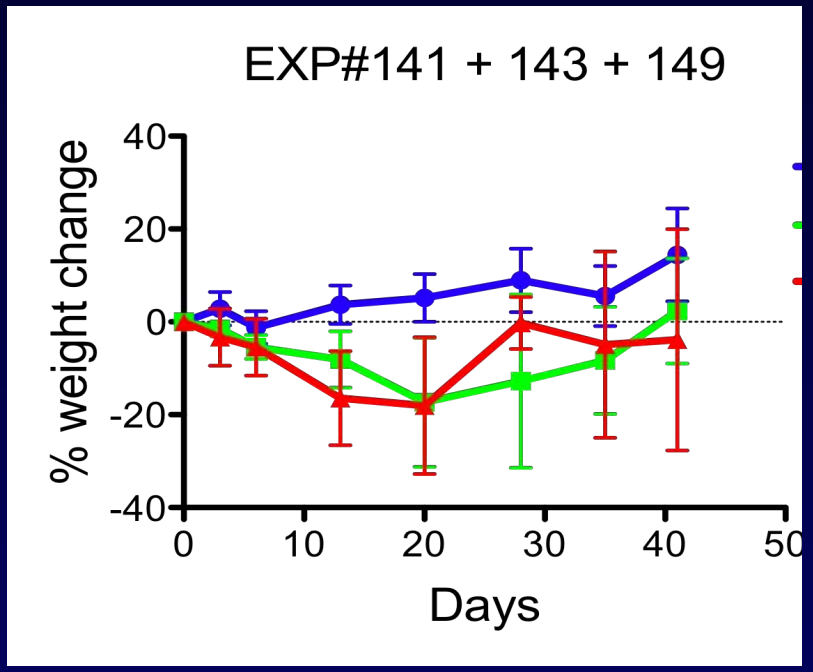


Figure 12. CD62L expression on T lymphocytes. In 5 donors, CD62L expression was assessed on donor T cells before and after treatment with AMD3100 (6 h after 240 µg/kg) or G-CSF (250 µg/kg/d x 5d). Flow cytometric analysis was performed using monoclonal antibodies to CD3, CD4, CD8, and CD62L. The fold change in CD62L mean fluorescence intensity (MFI) was calculated relative to pre-treatment values.

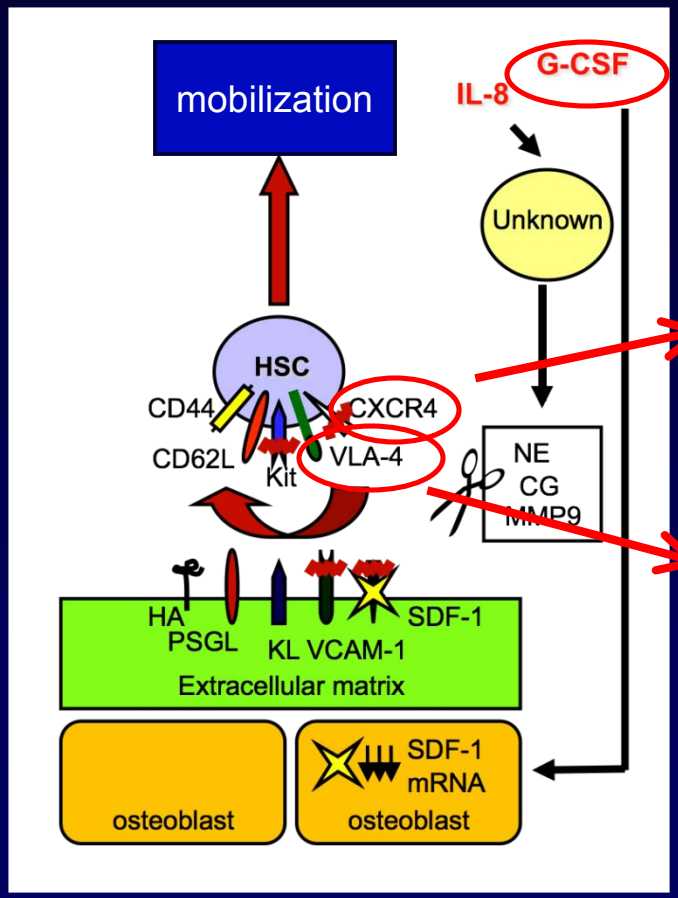
AMD3100 Allogeneic Stem Cell Transplant Trial: Overall Survival





XRT only (n=6)
pre-AMD3100 (n=19)
post-AMD3100 (n=23)

Adhesion Molecules and HSC Mobilization

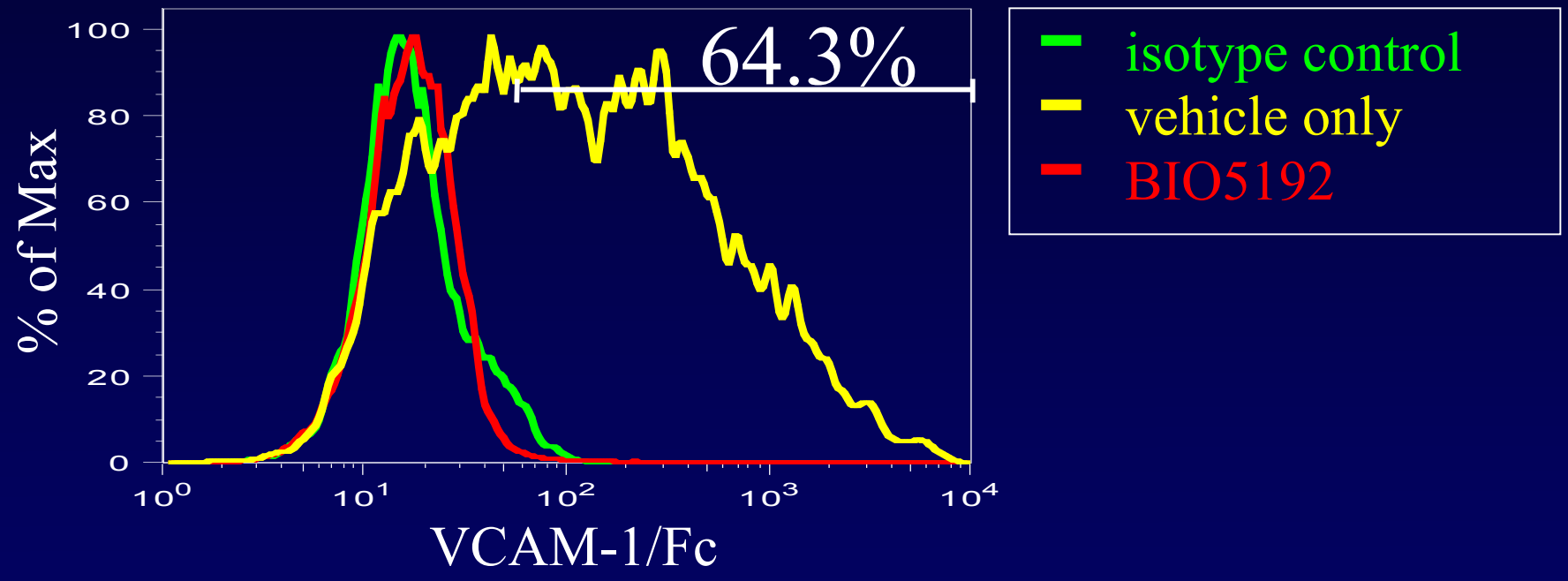


Plerixaflor

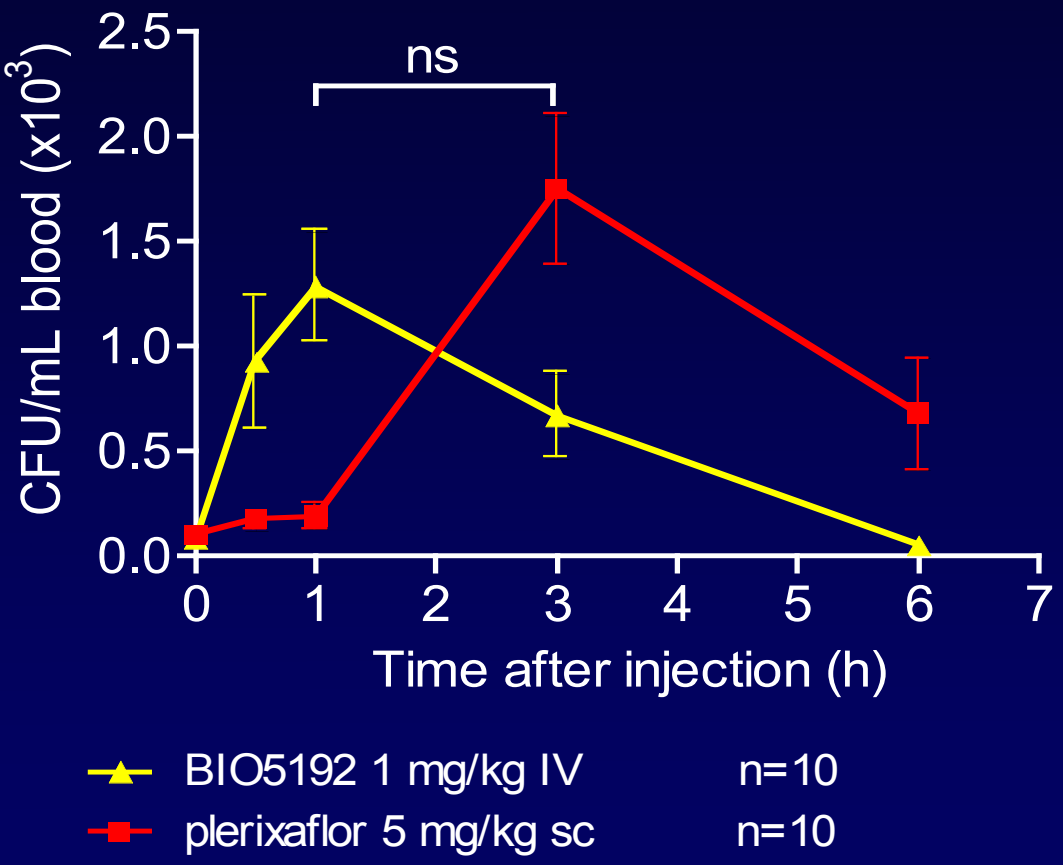
Natalizumab
BIO5192

Nervi B, Link DC, DiPersio JF. *J Cell Biochem*. 2006;99:690-705.

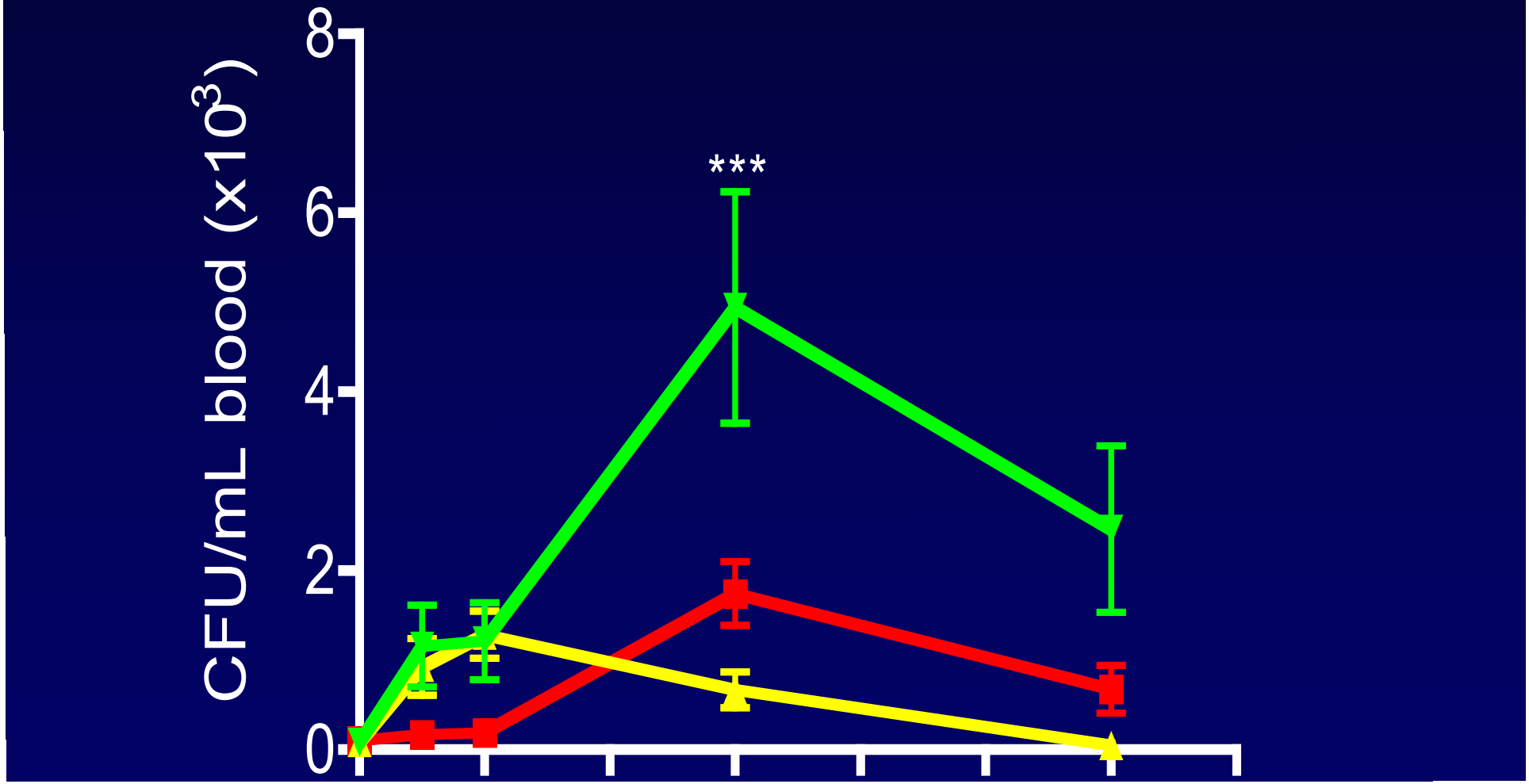
BIO5192 Blocks Binding of Jurkat Cells to Soluble VCAM-1



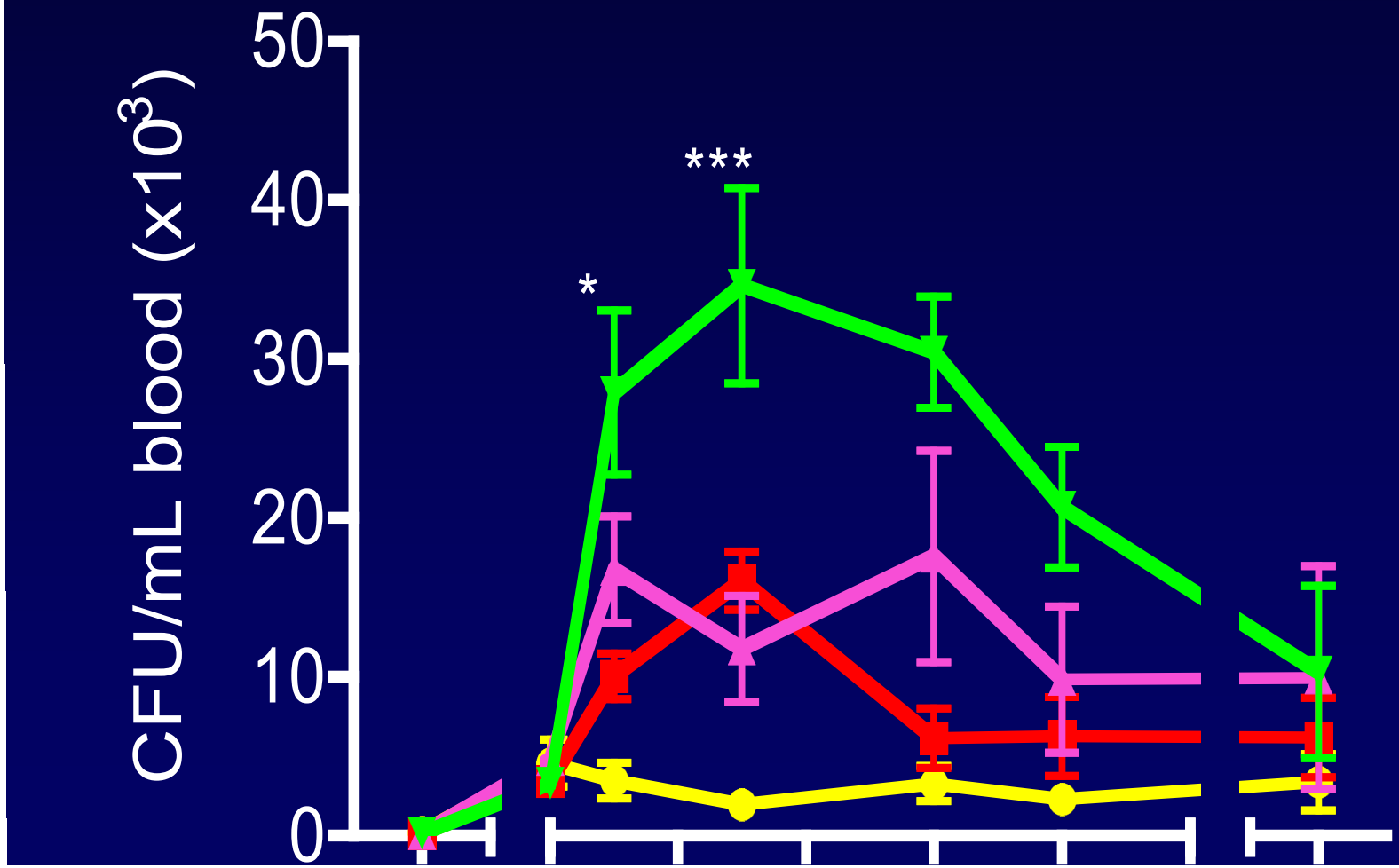
Kinetics of Murine Progenitors Mobilization in Response to BIO5192 and Plerixafor



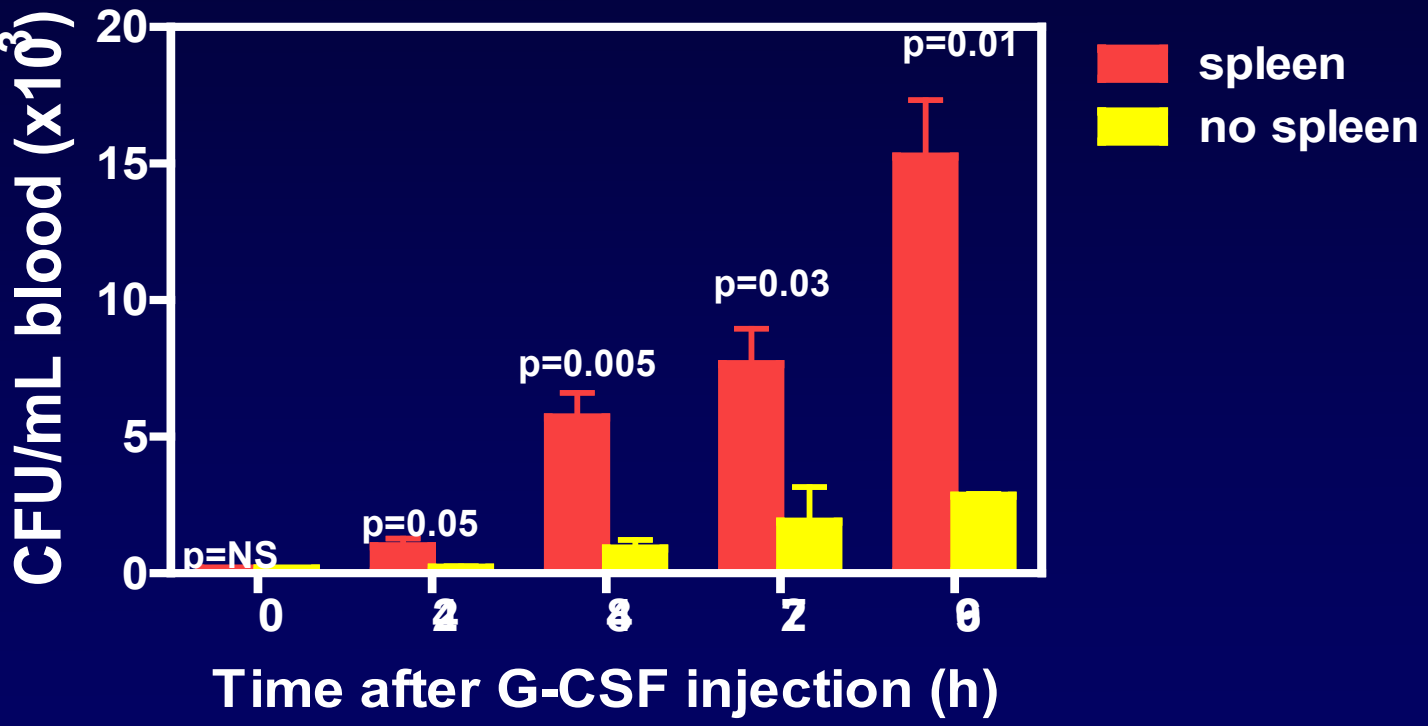
Additive Mobilization of Murine Progenitors After Combination of Plerixaflor SC and BIO5192 IV



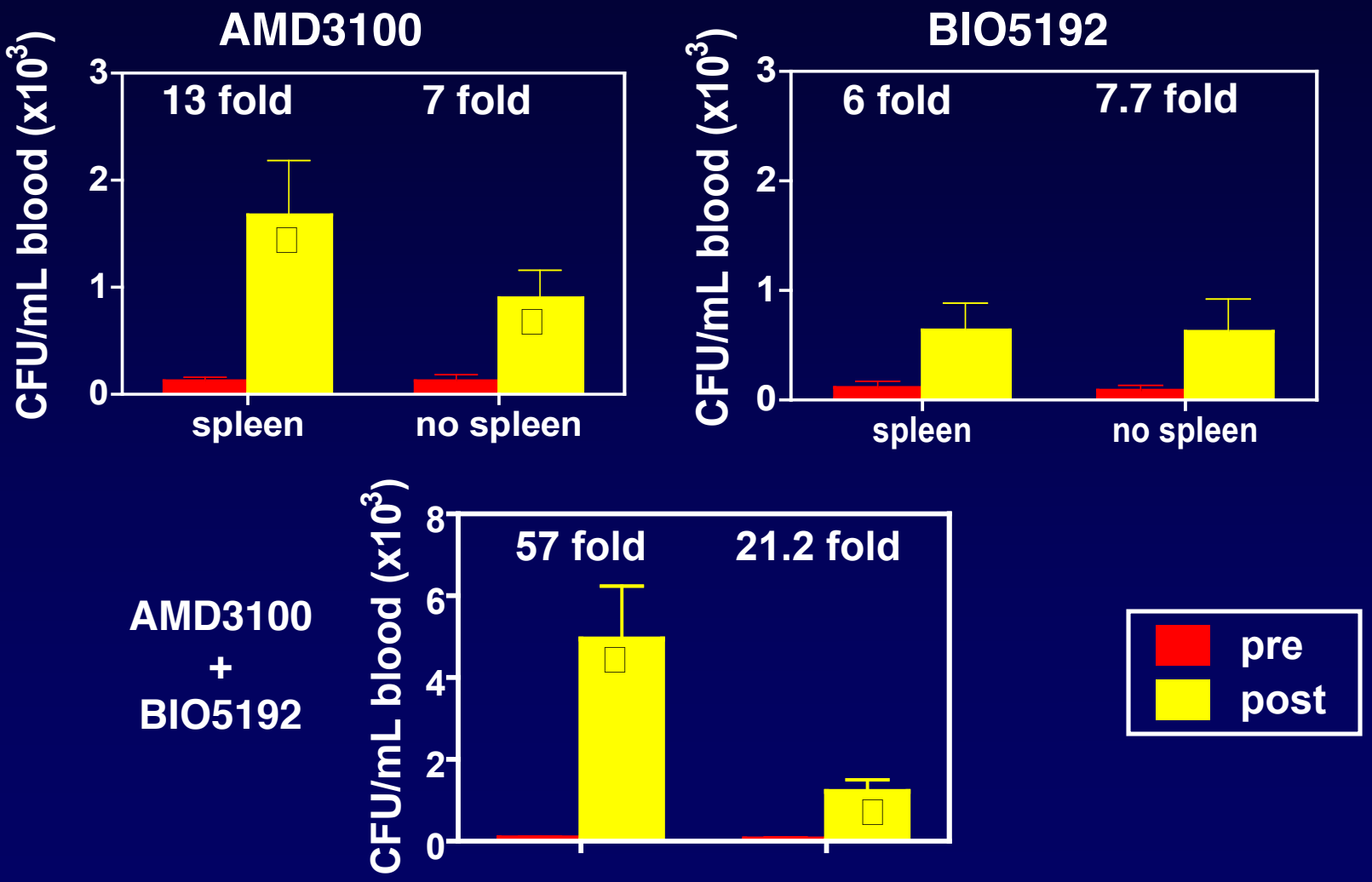
Additive Effect on Murine Progenitor



Increased mobilization of progenitors by G-CSF in splenectomized mice



Effect of Splenectomy on AMD3100 and BIO5192 Induced HSC mobilization



Long term repopulating assay

Peripheral blood mobilized HSC competitors

B6 5.2
Mobilization

BM competitor

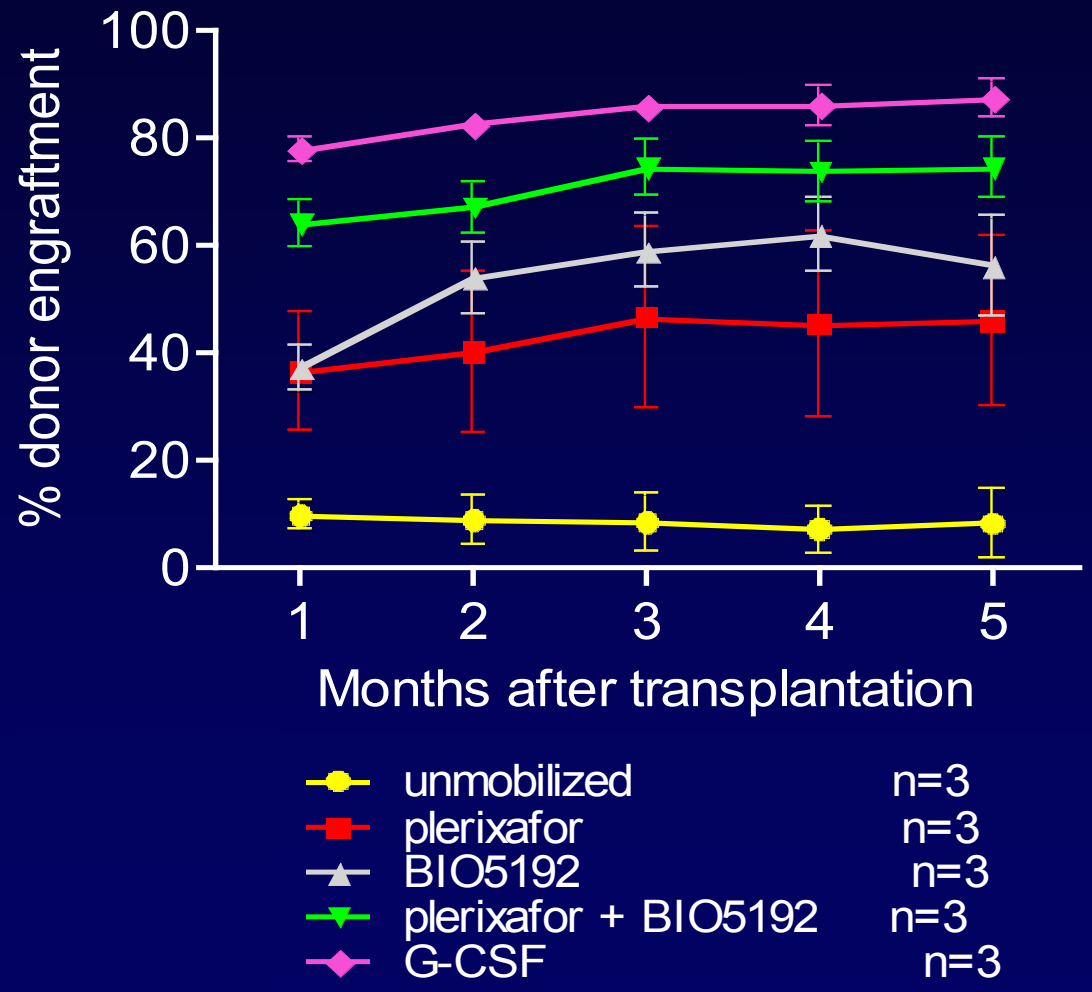
B6 5.1
Bone marrow mononuclear cells

Collection of PBMCs

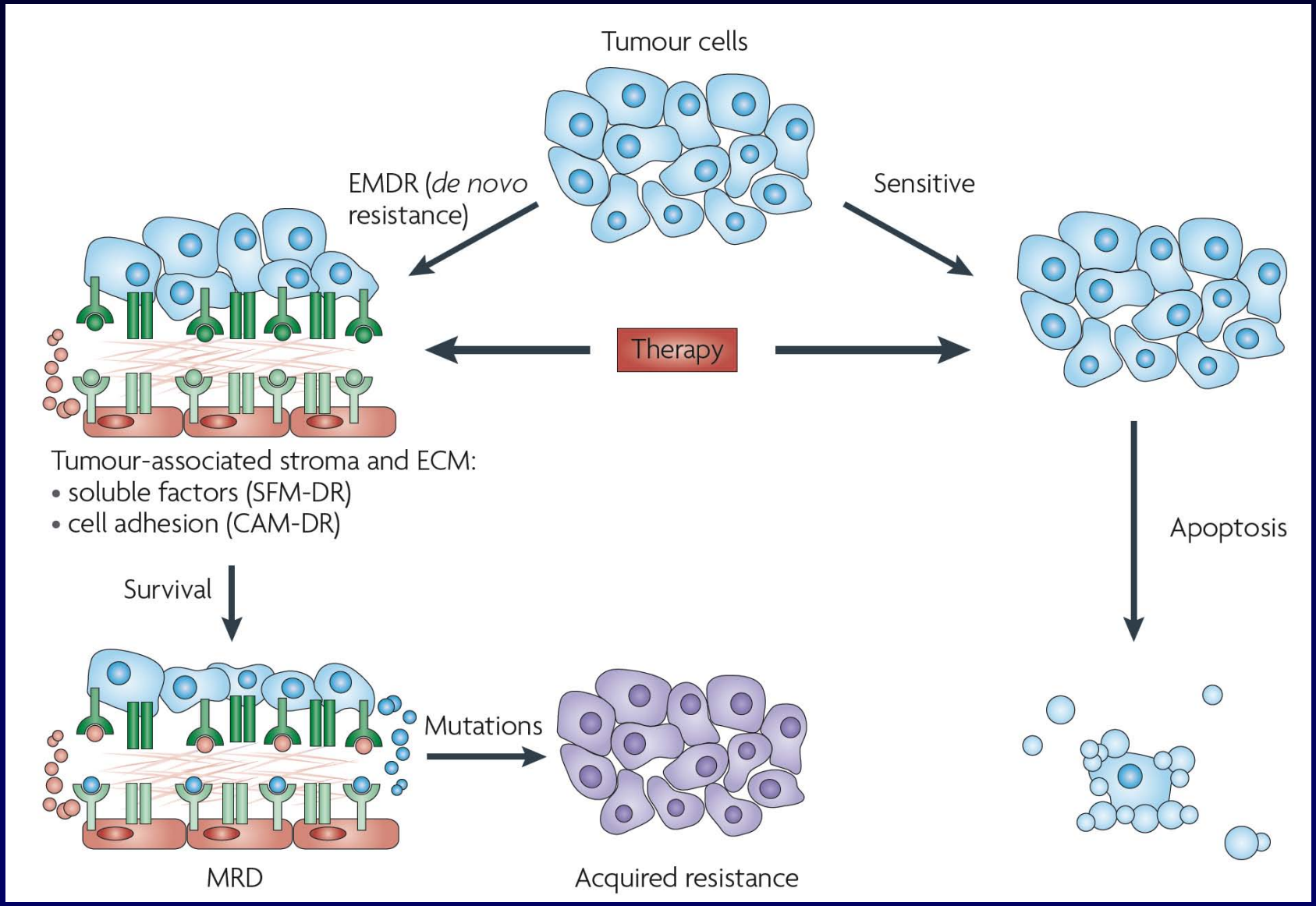
Recipient
B6 5.1/5.2

950 cGy

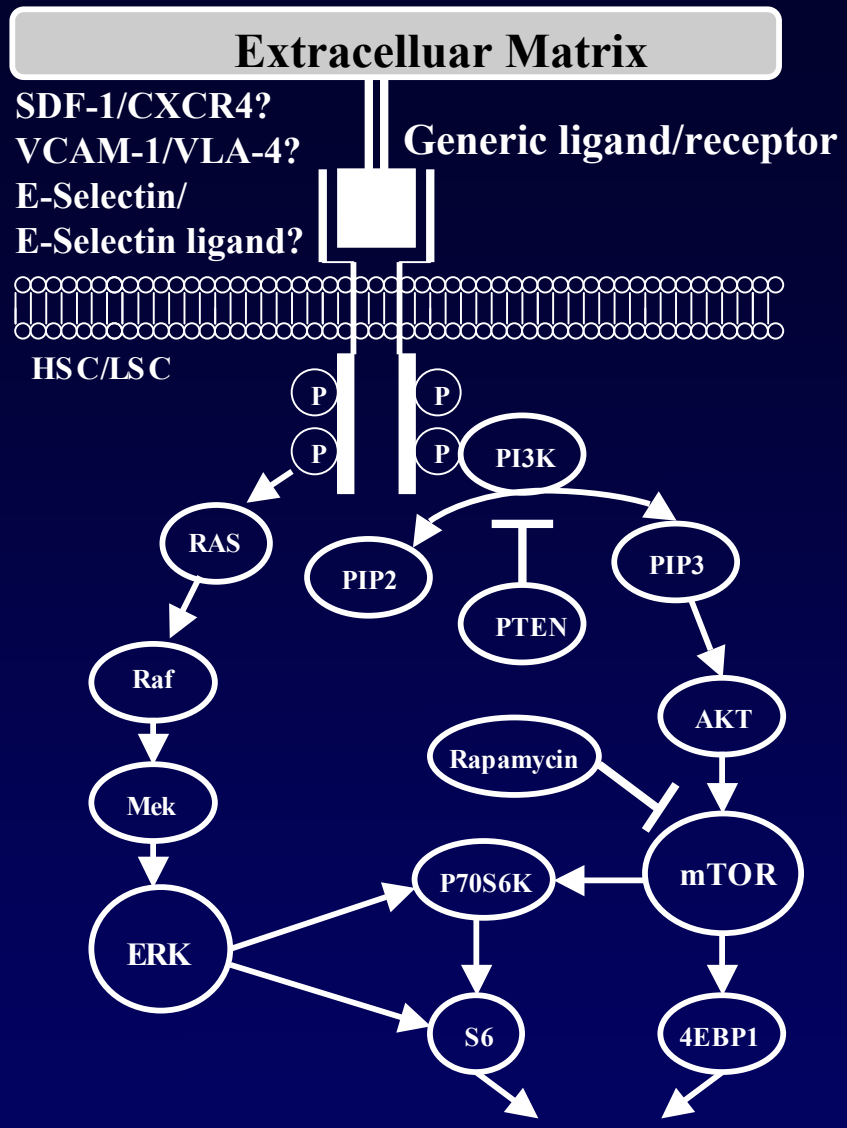
Engraftment of Mobilized HSC Primary Recipients



Environment-Mediated Drug Resistance



Meads M, et al. *Nature Reviews Cancer* 2009;9:665-674.



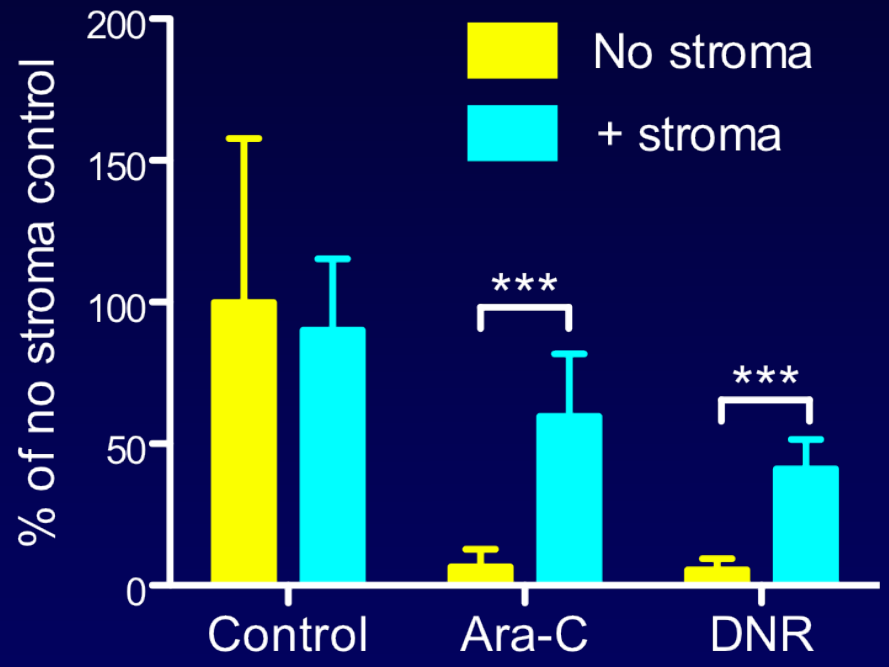
Stroma-leukemia contact

Hypothesis: Interruption of Stroma-leukemia cell contact and/or inhibition of stroma-induced signaling in leukemia cells will result in proliferation apoptosis, differentiation and *sensitization to genotoxic stresses such as chemotherapy*

Stroma-leukemia signaling

Anti-apoptosis
Anti-proliferation
Anti-differentiation

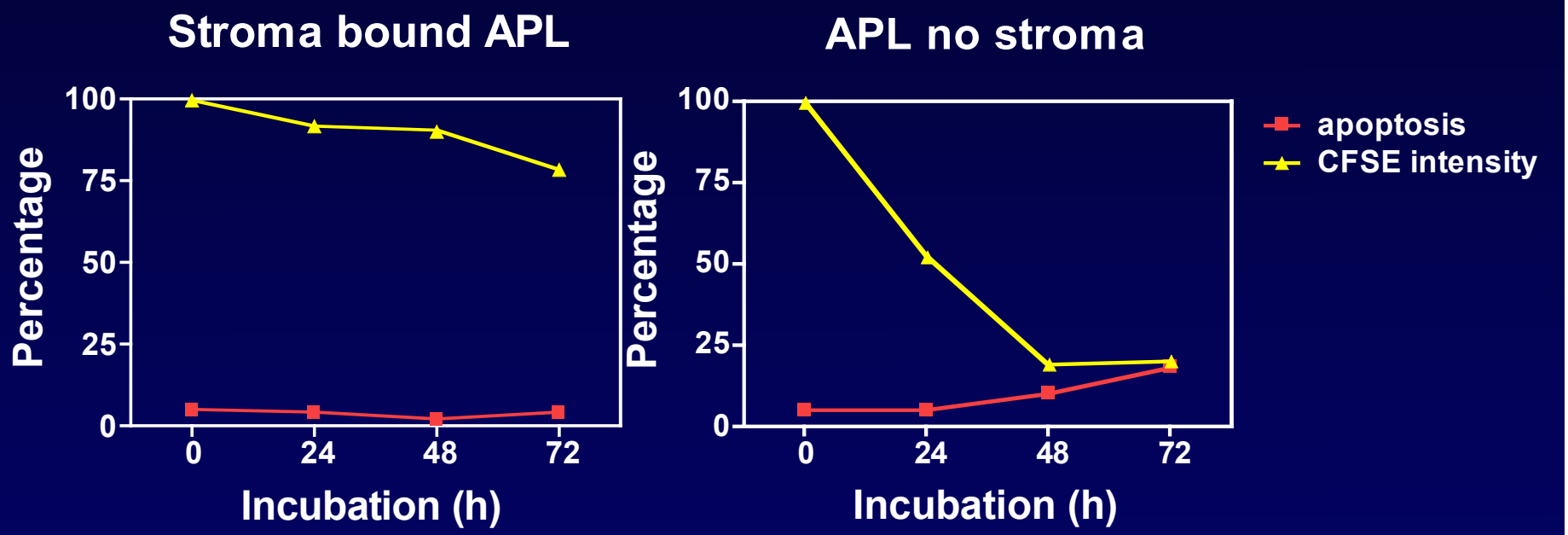
M2-10B4 Stromal Cell Line Provides In Vitro Chemoprotective to APL Cells



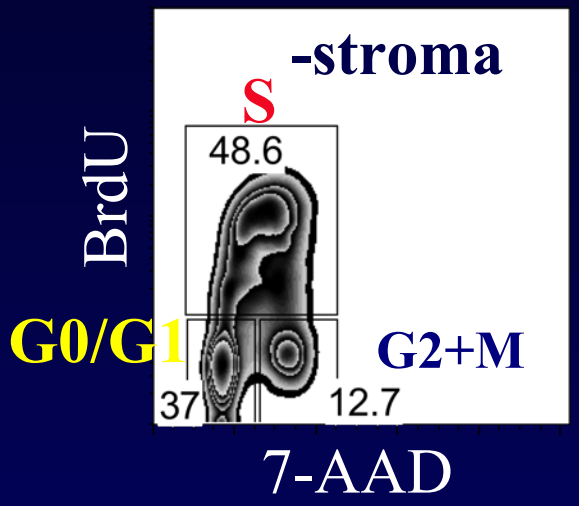
*** P < 0.0001

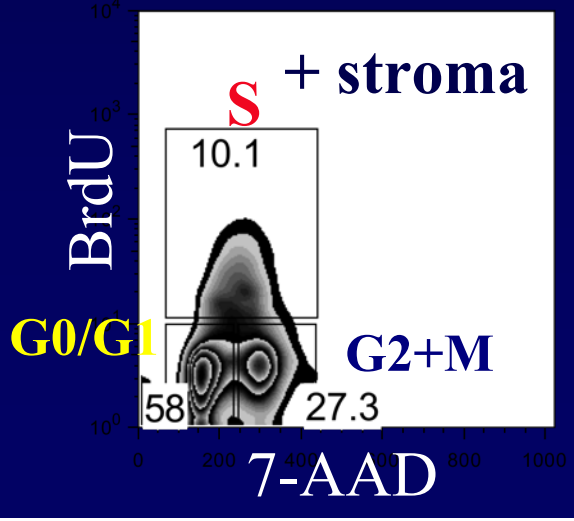
Nervi B, et al. *Blood* 2009;113:6206-6214.

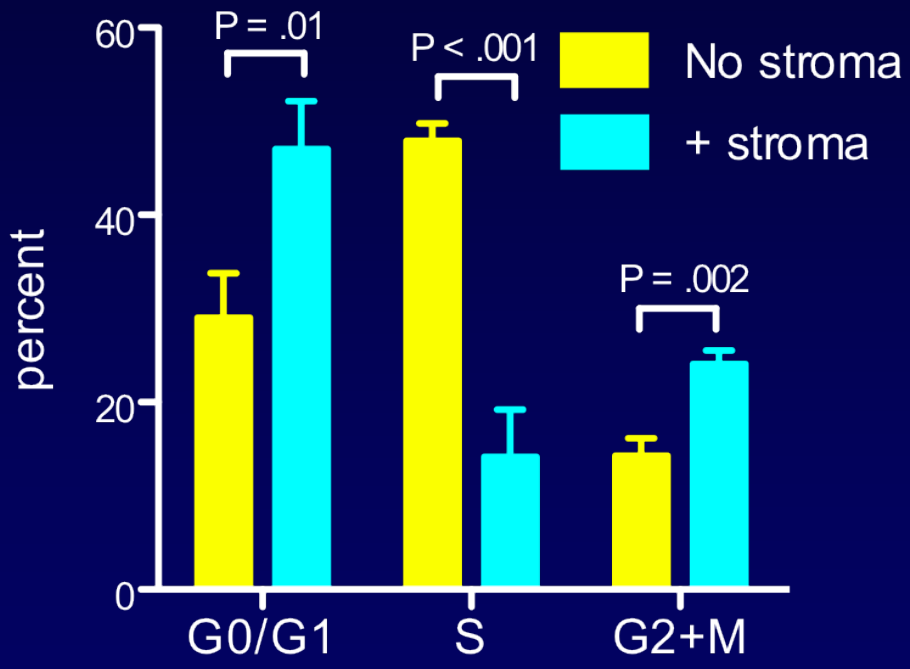
Effect of stroma on APL proliferation and spontaneous apoptosis



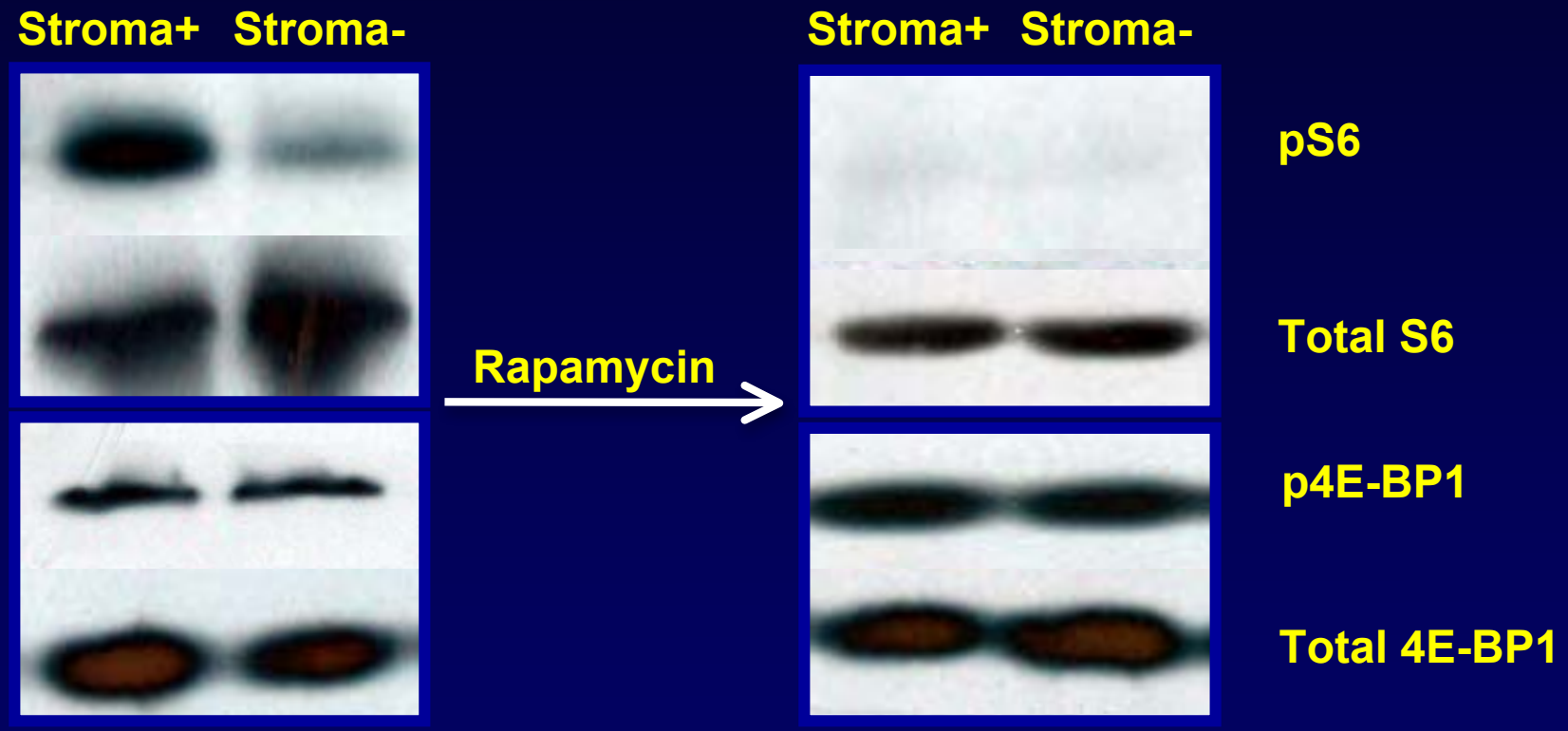
Reduced Proliferation of APL Cells in the Presence of M2-10B4 Stromal Cells



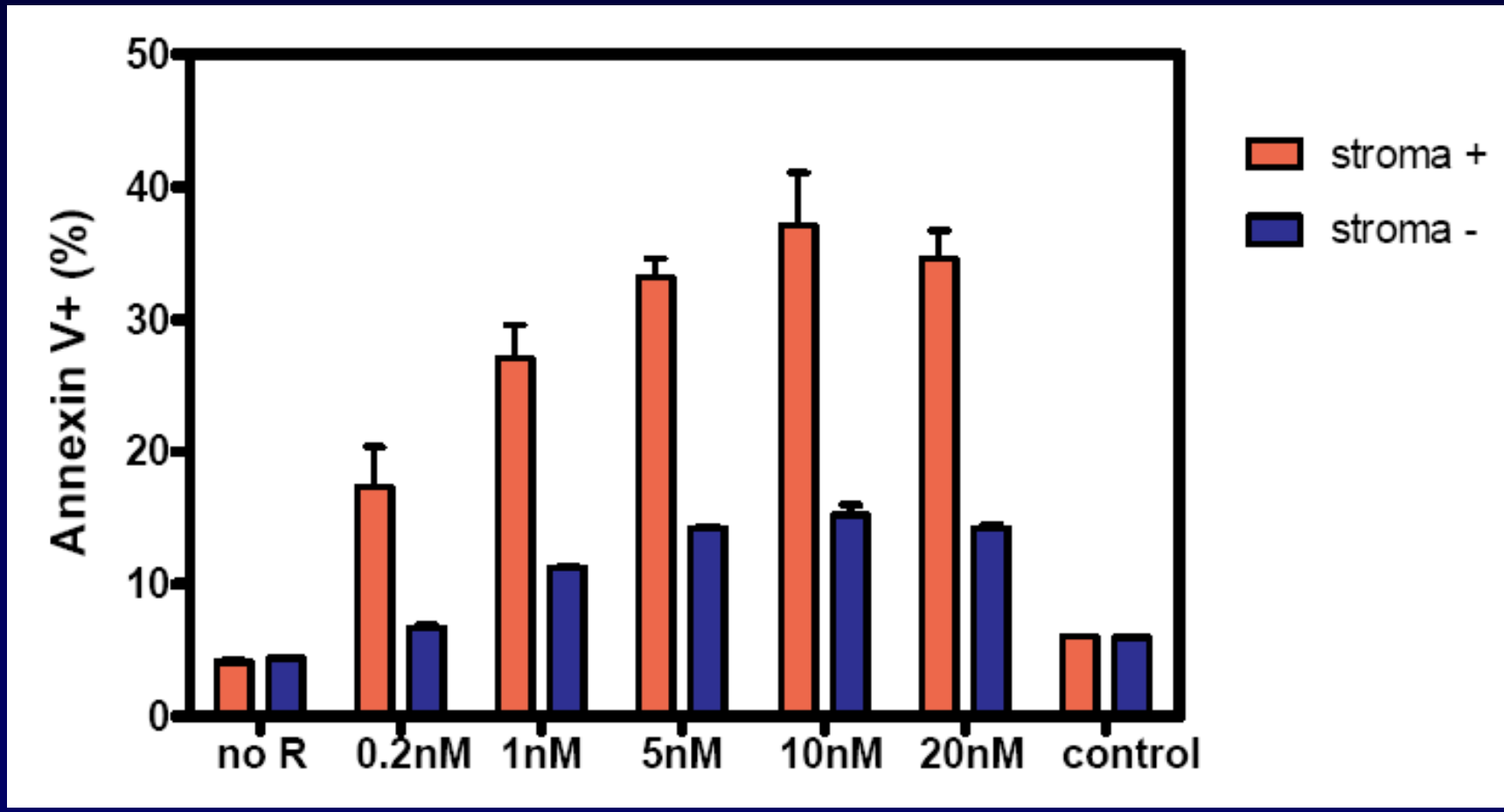




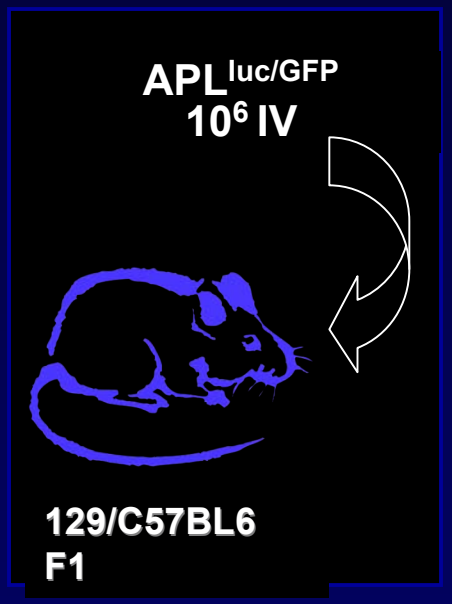
Effect of stroma on mTOR pathway phosphorylation



Increased sirolimus-induced APL apoptosis in presence of stromal cells

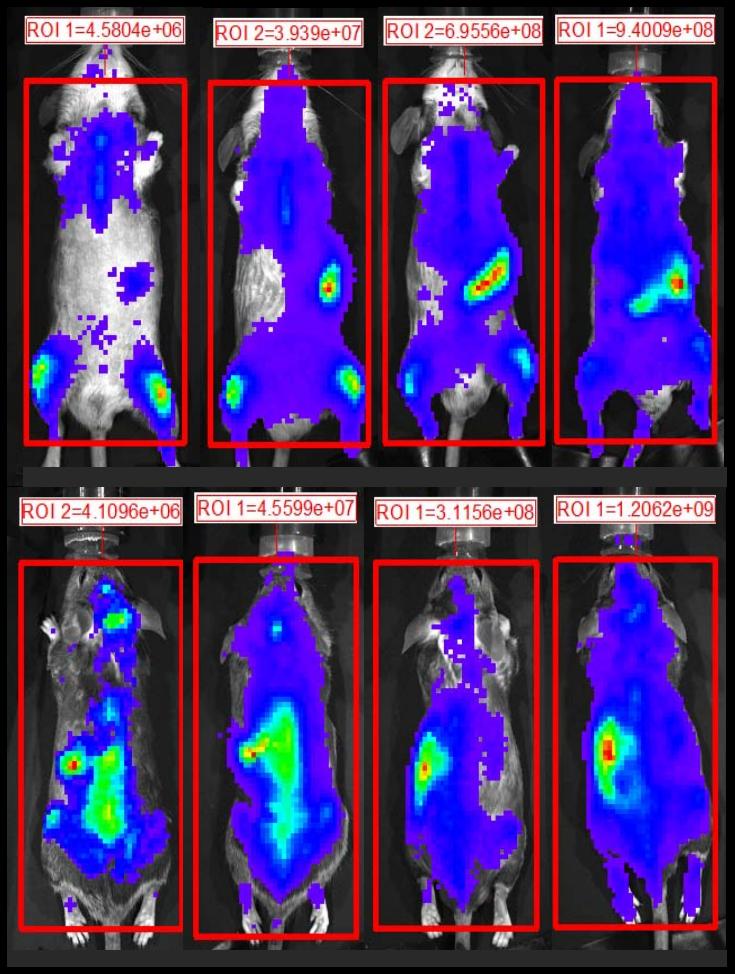


APL ENGRAFTMENT



Ventral view

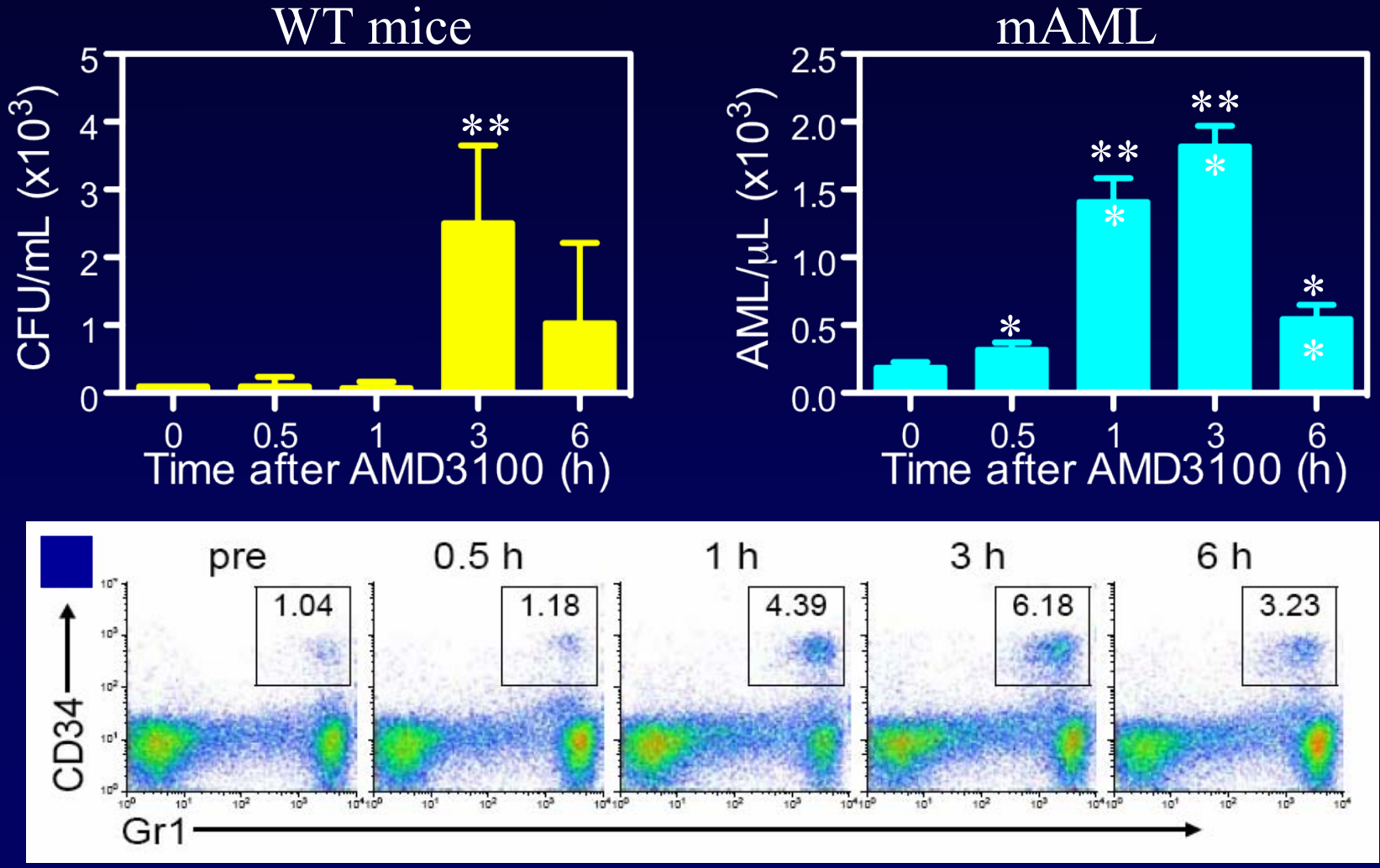
Dorsal view



day 4 7 11 14

	%blasts	%blasts	%blasts	%blasts
Blood	0%	2%	10%	40-50%
Spleen	1-2%	10%	40%	80-90%
BM	1-2%	20%	40%	90%
WBC/ul	5,000	8,000	15,000	75,000
Spleen	50 mg	150 mg	750 mg	1000 mg

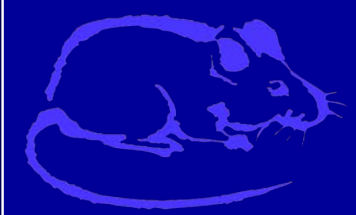
AMD3100 Mobilization of mAPL



Nervi B, et al. *Blood* 2009;113:6206-6214.

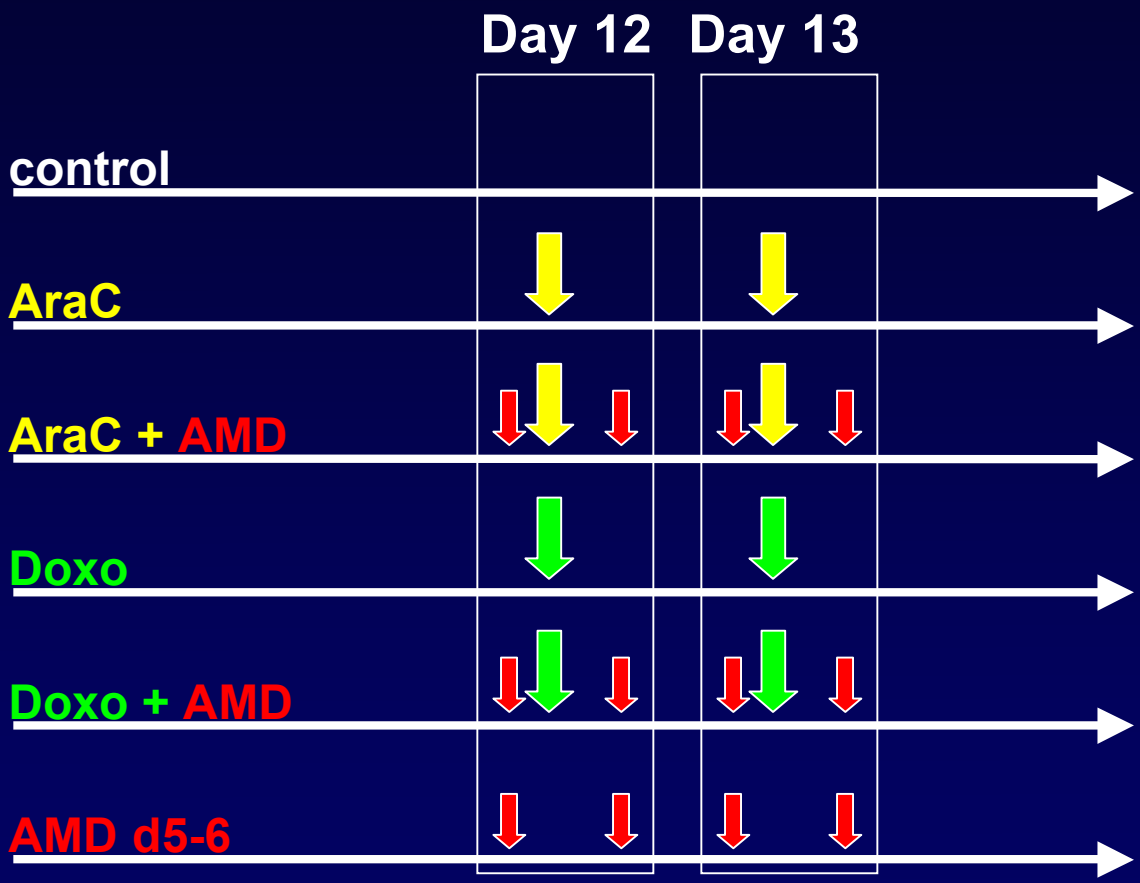
Mobilization of leukemia cells increases the efficacy of anti-leukemic chemotherapy

10^6 APL IV



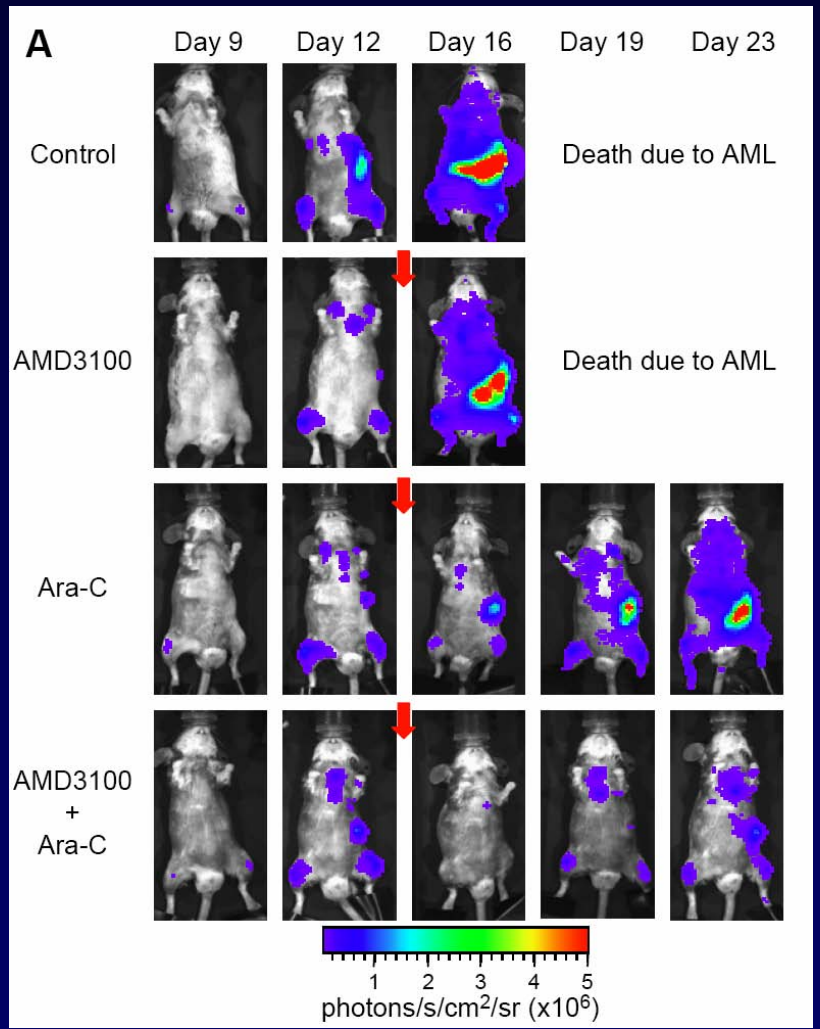
129/C57BL6 F1

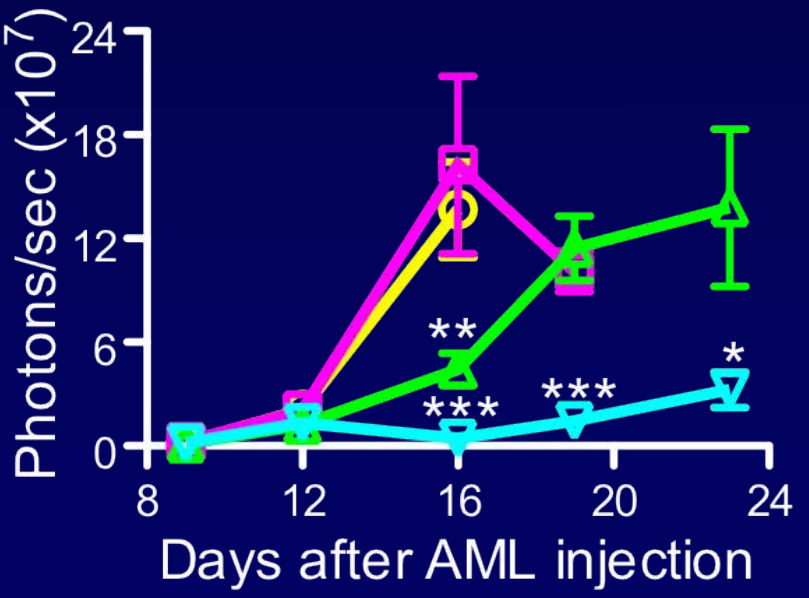
n=6 mice/group



AraC (500mg/kg/sc); Doxo (10mg/kg/sc); AMD (5mg/kg/sc)

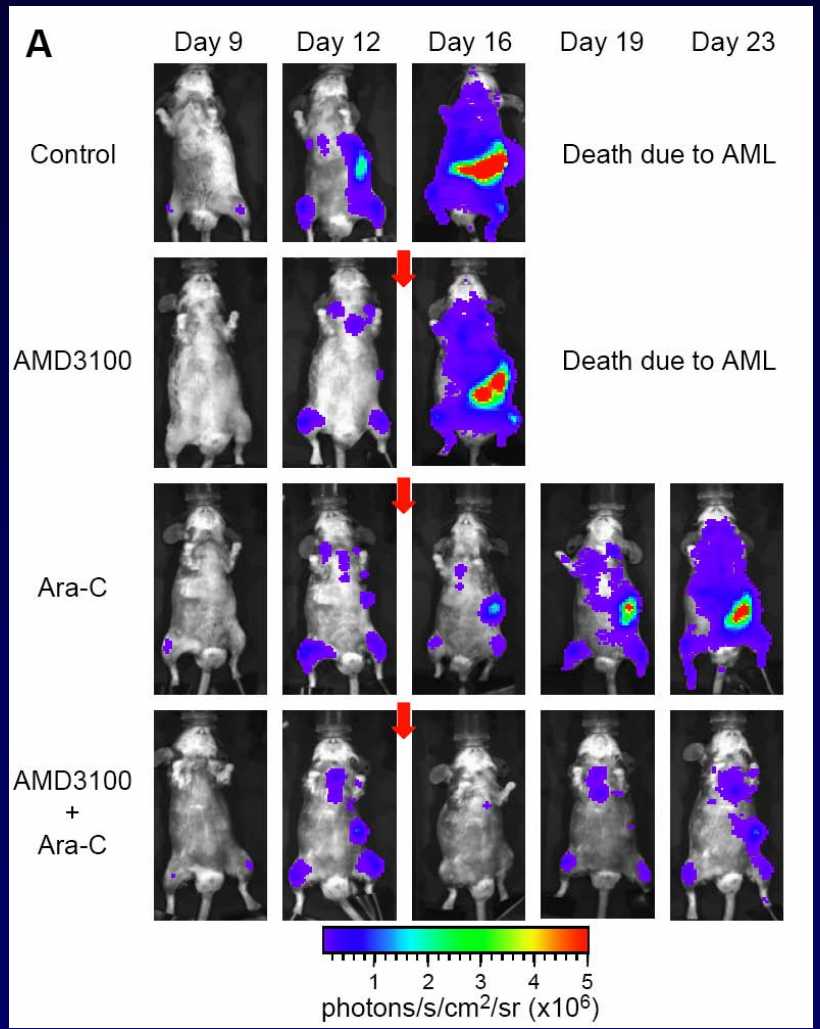
AMD3100 enhances effect of chemotherapy

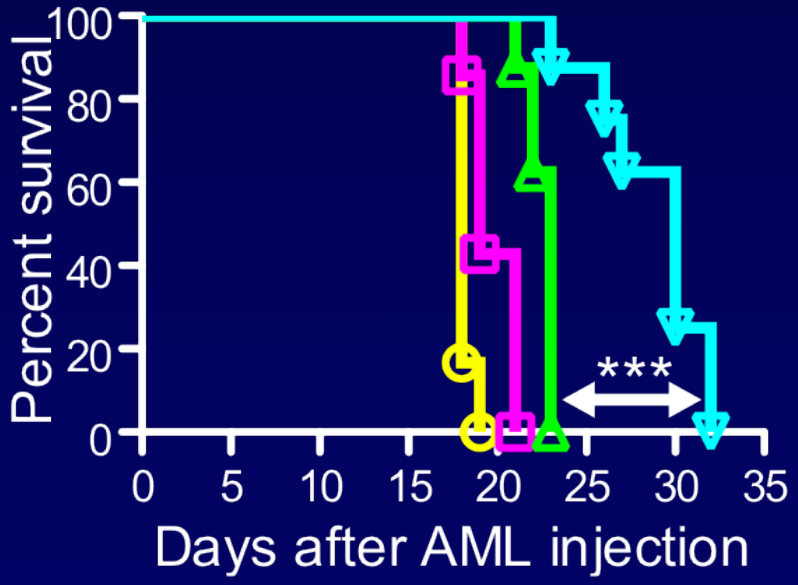




Nervi B, et al. *Blood* 2009;113:6206-6214.

AMD3100 enhances effect of chemotherapy





Nervi B, et al. *Blood* 2009;113:6206-6214.

Phase I/II Study of AMD3100 + MEC in Relapsed or Refractory AML

Eligibility Criteria

1. Dx of AML and either 1° refractory or relapsed disease (For pts in 1st relapse, must have CR1 duration < 12 mo)
2. Age 18-65, ECOG PS ≤ 2
3. Blast ct < 30,000/mm^3
4. No previous MEC salvage

AMD3100

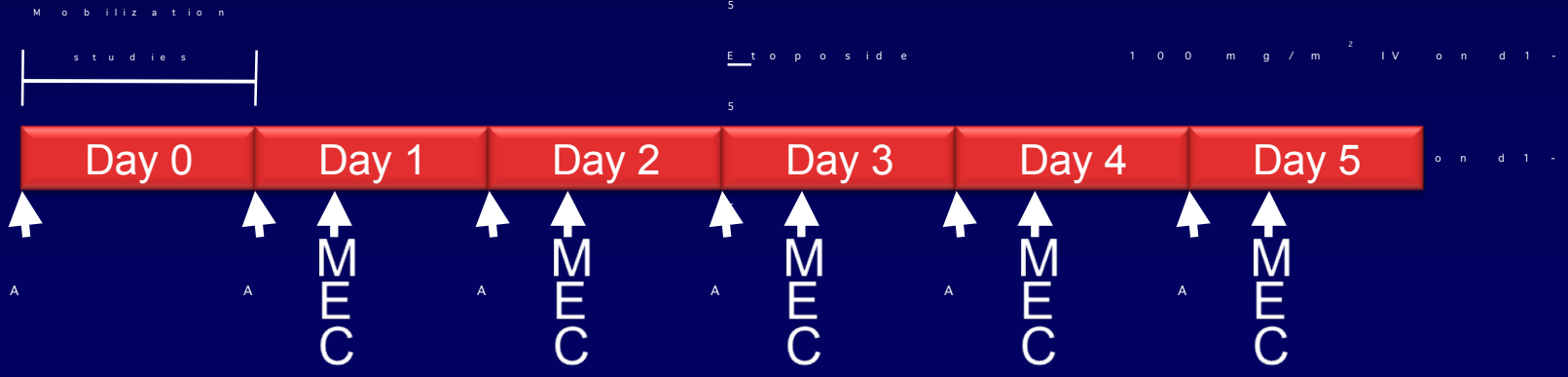
Dose level 1 80 mcg/kg SQ on d0-5

Dose level 2 160 mcg/kg SQ on d0-5

Dose level 3 240 mcg/kg SQ on d0-5

Mitoxantrone 8 mg/m^2 IV on d1-5

Etoposide 100 mg/m^2 IV on d1-5



Patient Demographics (n=40)

Age, median yrs (range)	49 (19-71)
Gender	
Male	19
Female	21
Cytogenetics	
Core Binding Factor	6
MLL translocation	4
Normal	24
Complex (>3 abn)	6
Treatment Indication	
1st relapse, 1st salvage	30 (median CR1 224 days)
1° refractory	8
1st relapse, 2nd salvage	2

Toxicities from Phase I

- No dose limiting toxicities have been observed, no hyperleukocytosis
- Neutrophil recovery at median 29.5 days (range 19-37)
- Platelet recovery at median of 26 days (range 21-40)
- Nonhematologic toxicities
  - 12/12 Febrile neutropenia
  - 1 pt with Grade 4 opportunistic infection (Aspergillus sinusitis) requiring debridement
  - 1 pt Grade 2 bradycardia
  - Grade 3 hypotension, diarrhea, colitis, confusion, back pain observed in 1 each and thought to be unrelated to administration of AMD3100
  - Grade 3 hypokalemia (<3) observed in 2 patients

Response Evaluation

	All doses (n = 43)	240 mcg/kg (n = 37)
CR	17	15
CRi	3	3
Total Responses	**20**	**18**
Persistent Disease	21	17
Early Death	2	2
Total Treatment Failures	**23**	**19**
Overall Response Rate	**46%**	**49%**

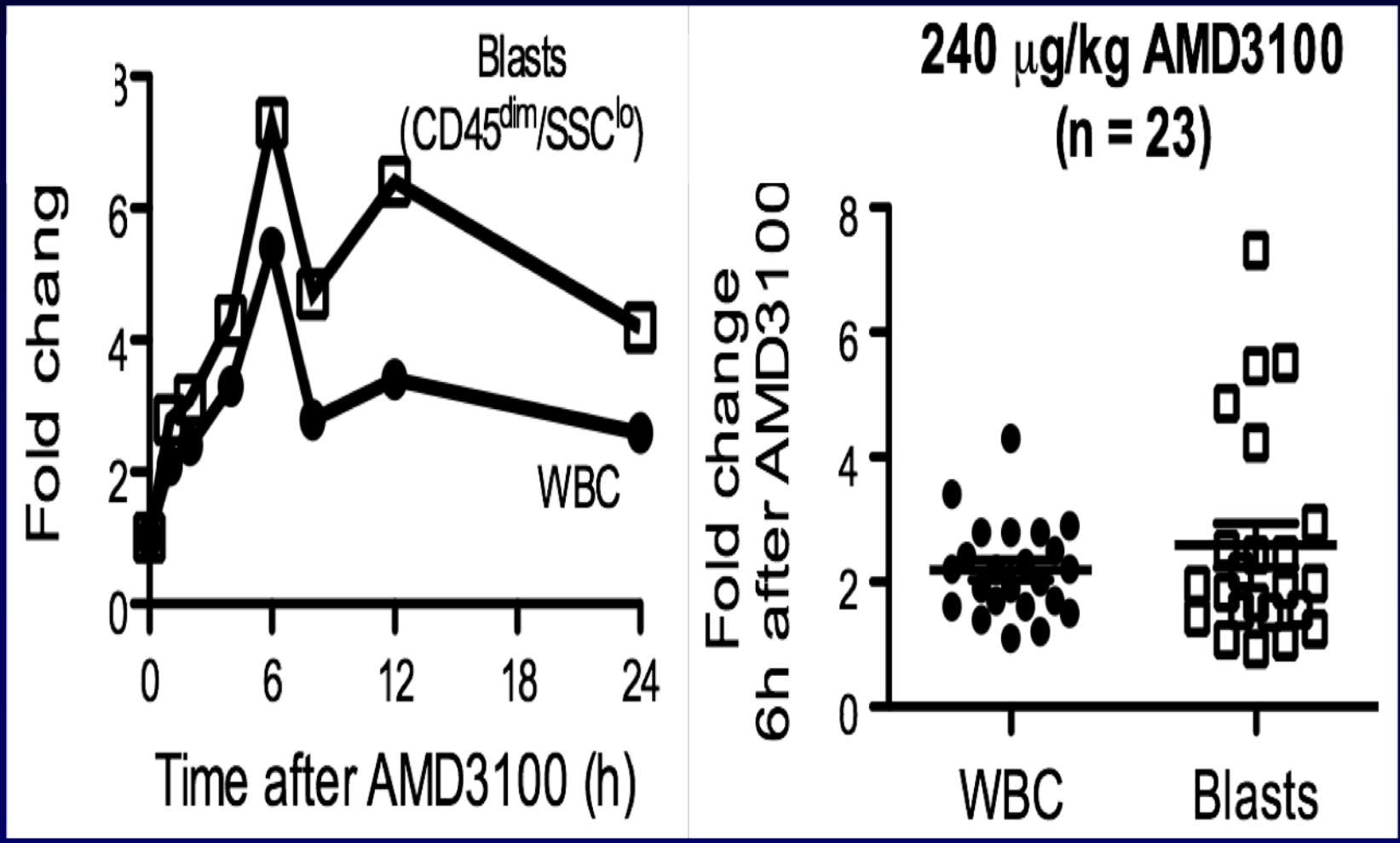
*Response Evaluation per IWG Criteria for AML, Cheson et al., JCO 2003

Historical RR with MEC

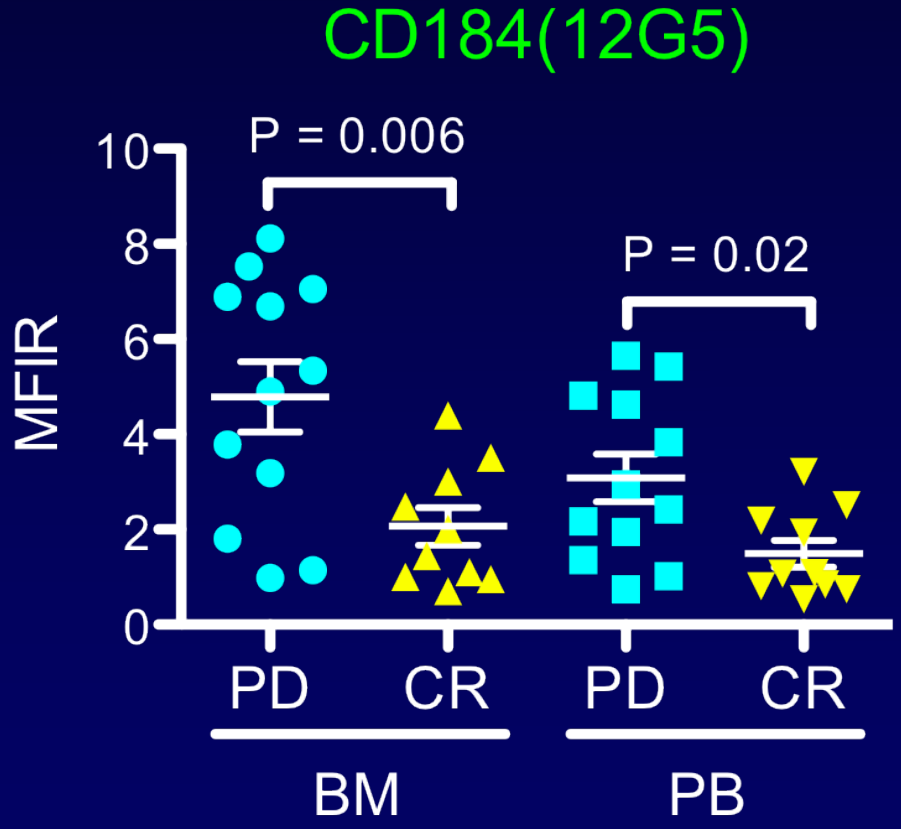
	Patients	CR Rate	OS	MEC Regimen
Tallman MEC +/- CSP Cancer 1999	38 elig/49 Rel/Ref ~47% Refractory	24%	2.5-3.5 mos	10 mg/m2 x5 or 6 100 mg/m2 x5 or 80
Greenberg MEC +/- Valspodar 2004	63 vs 66 pts 51% Early Rel/Ref	17 vs 25%	4.6 vs 5.4 mos	8 mg/m2 x5 100 mg/m2 x5 1 g/m2 x5
Feldman Lintuzumab +/- MEC JCO 2005	191 Refractory or < 1 yr CR duration	28% vs 36% CR + CRP	156d = 5.5 mos	8 mg/m2 x6 100 mg/m2 x6 1 g/m2 x6
Giles Laromustine + HDAC Vs. HDAC	177 vs. 86 pts First relapse ~50:50 < 60; > 60	35% vs 19% CR + CRP	128 vs 176 days	Laromustine 600 mg/M2 on day 2 HDAC 1.5 gm/M2 days 1-3 CVI
Uy/DiPersio MEC + AMD3100	First Ref/Rel N-47	49%	NR	8 mg/m2 x5 100 mg/m2 x5

1 g/m2 x5

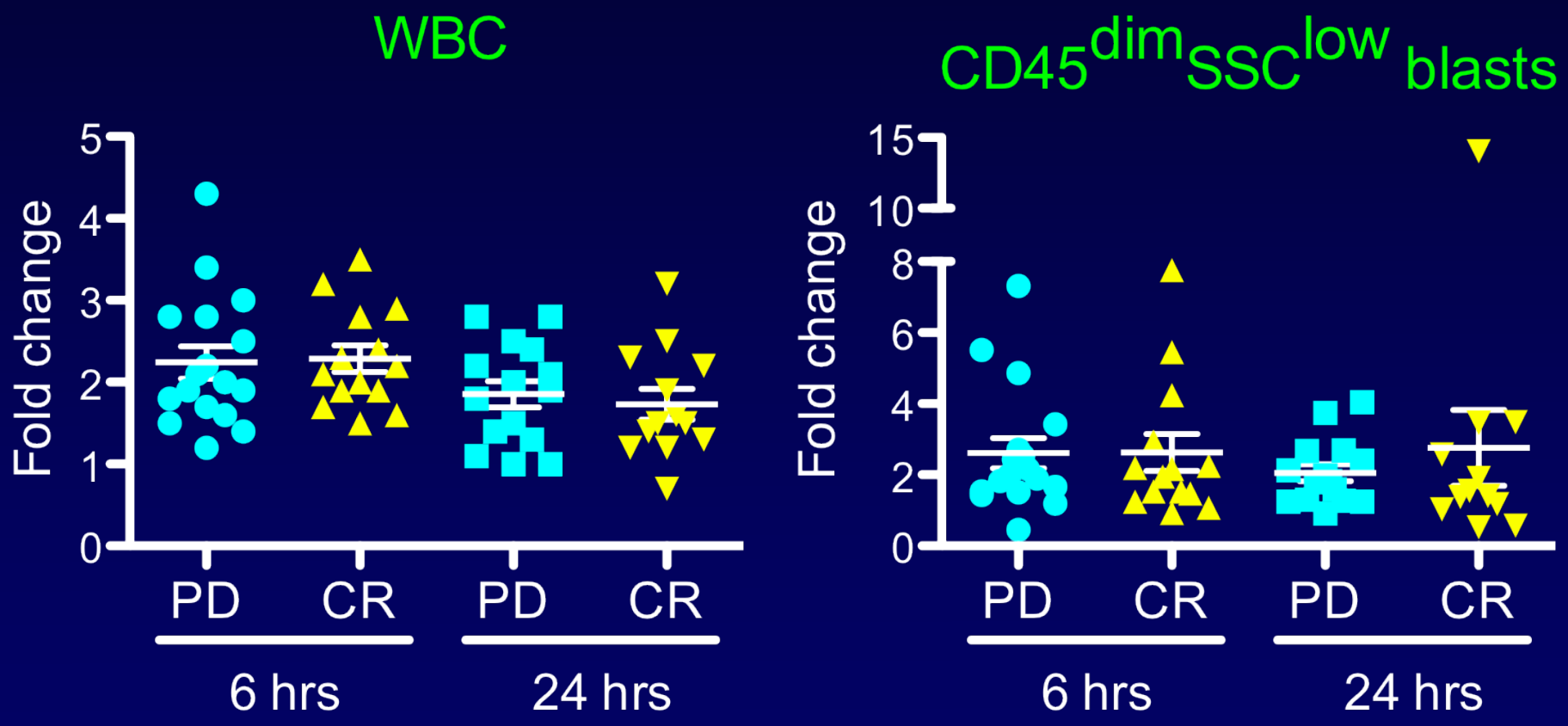
Effect of SC AMD3100 (240 mcg/kg) on AML Blast Mobilization



Surface CXCR4 Expression & Response



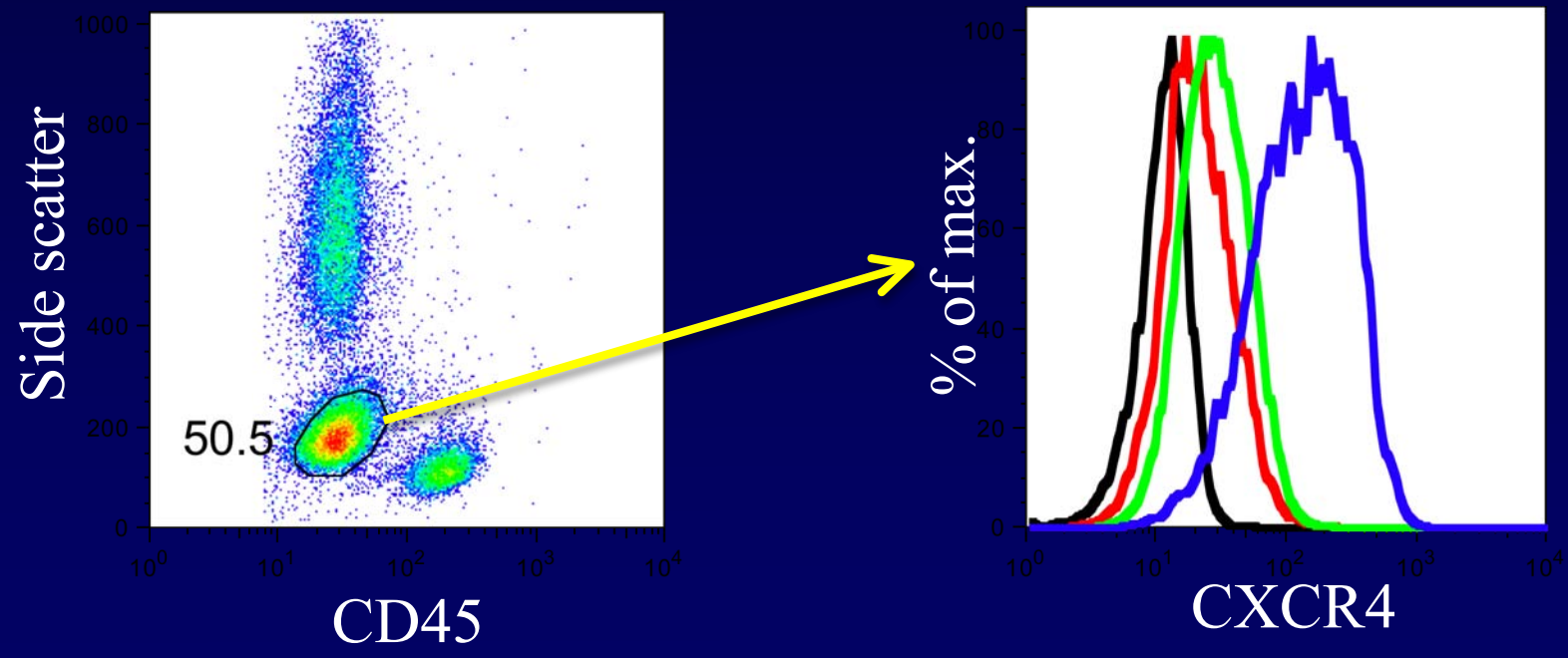
Mobilization of AML Blasts & Response



Peripheral Blood FISH post AMD3100 Mobilization

Patient #	Probe	Time Post AMD3100 (# FISH+ cells/ total)		
		0 HRS	6 HRS	24 HRS
#1	MLL	142/200	130/200	120/200
#2	MLL	128/200	106/200	136/200
#3	CBFB	12/200	15/200	11/200
#4	AML-ETO	151/200	150/200	162/200
#5	AML-ETO	47/200	33/200	41/200

Increased Surface CXCR4 Expression after AMD3100



Phase I/II Study of G-CSF + AMD3100 + MEC in Relapsed or Refractory AML

Eligibility Criteria

1. Dx of AML and either
   1° refractory or relapsed disease
2. Age 18-65, ECOG PS ≤ 2
3. Blast ct < 30,000/mm^3
4. No previous MEC salvage

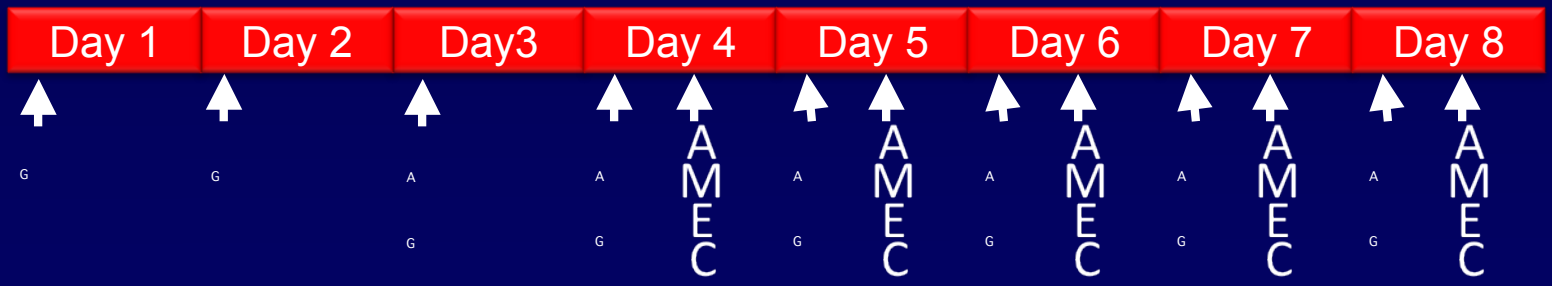
AMD3100: 240-480 mcg/kg IV qd/bid on d3-8

G-CSF 10 mcg/kg SQ on d1-8

Mitoxantrone 8 mg/m^2 IV on d4-8

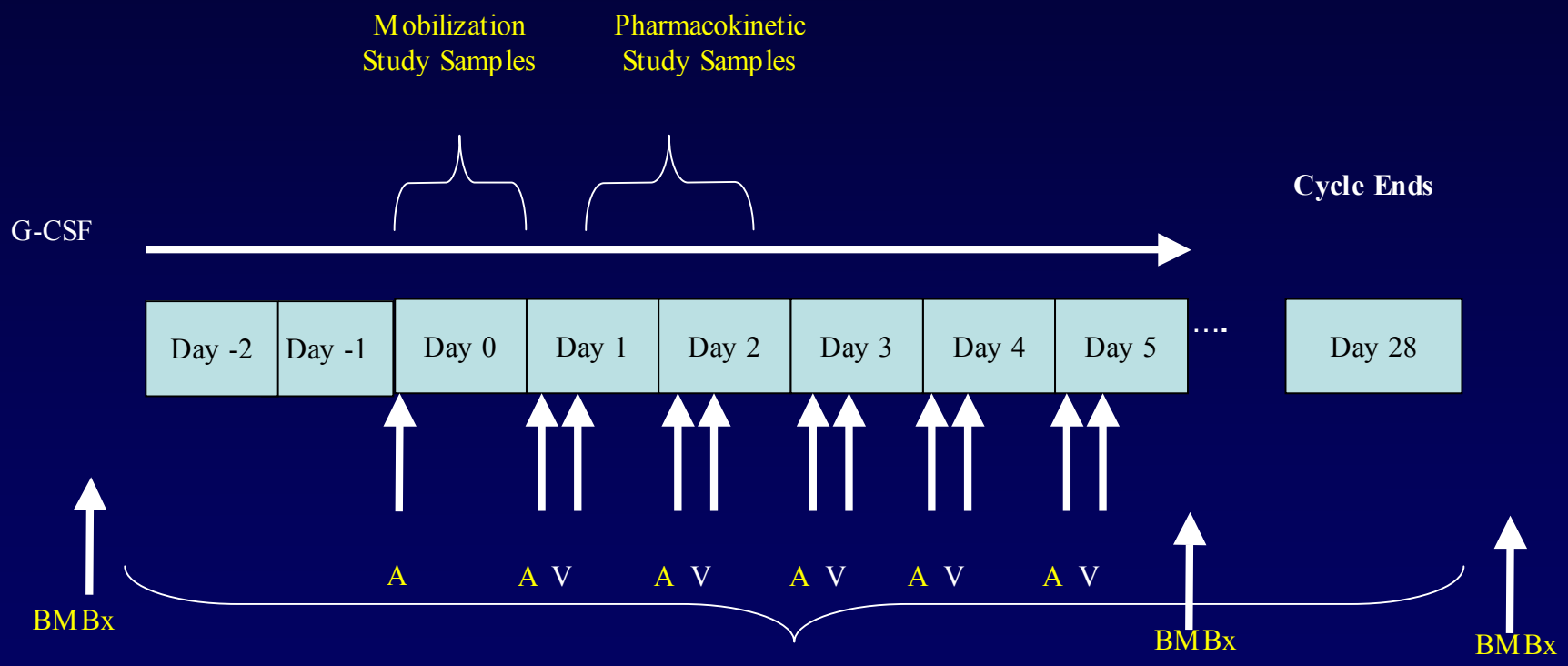
Etoposide 100 mg/m^2 IV on d4-8

Cytarabine 1000 mg/m^2 IV on d4-8



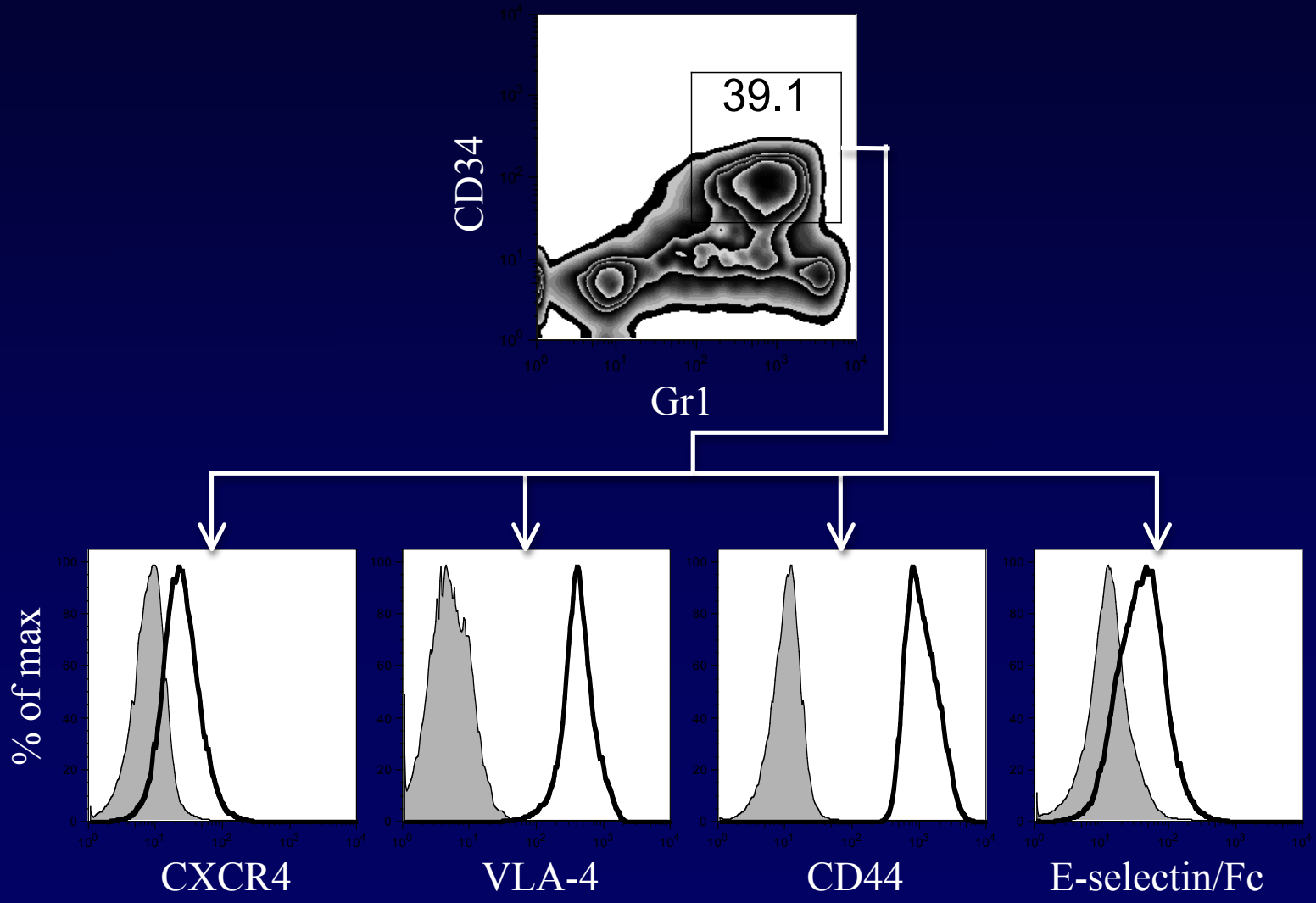
MDS Protocol Design

- Phase I dose-escalating trial
  - AMD3100 (**A**) + G-CSF (10mcg/kg) + azacitidine (**V**) (75mg/m^2)



Repeat for minimum 2 cycles

Expression of CXCR4, VLA-4, CD44, and E-Selectin Ligand on APL 18-4c clone



Mobilization of mouse APL in vivo by the VLA-4 inhibitor, BIO5192

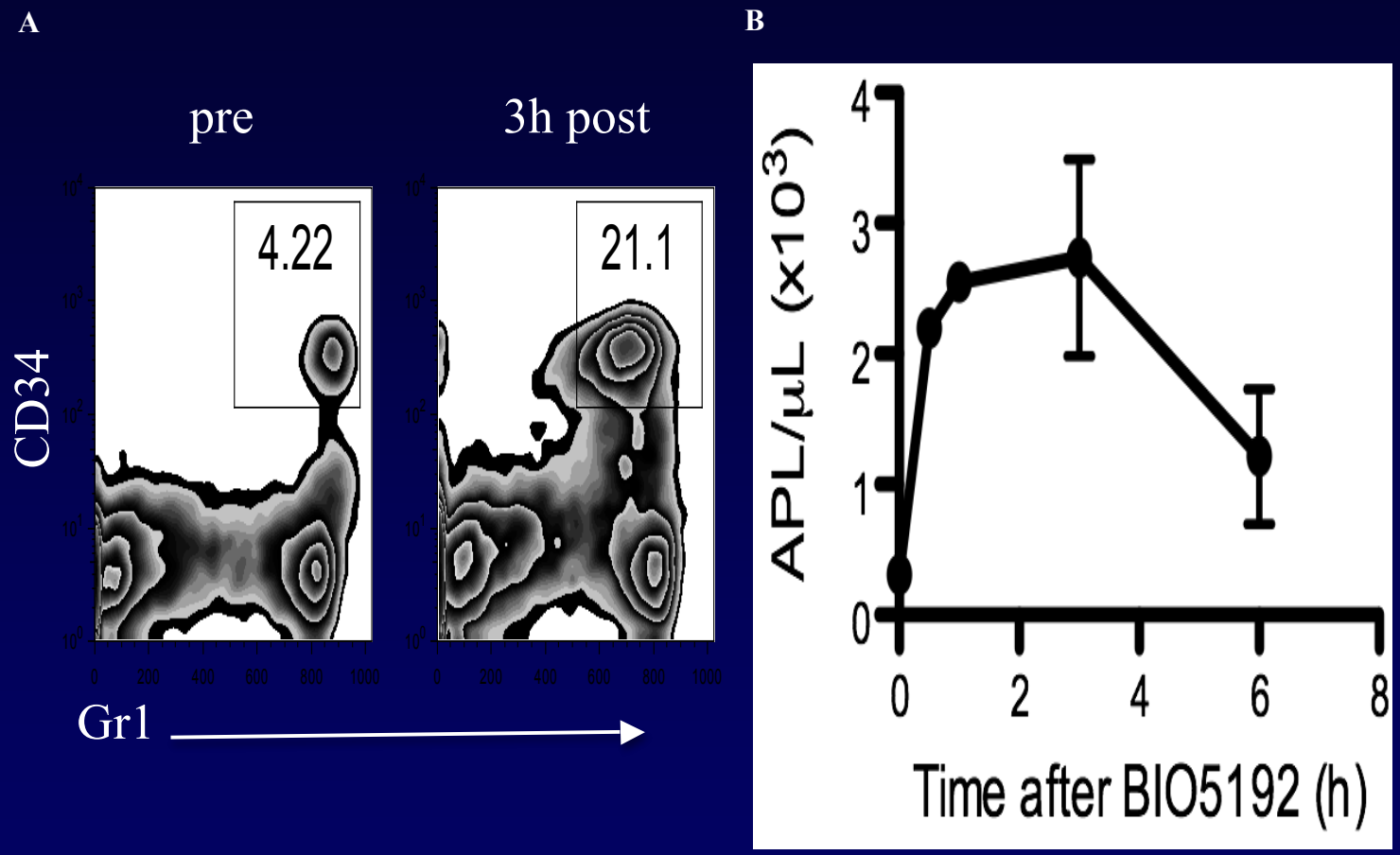
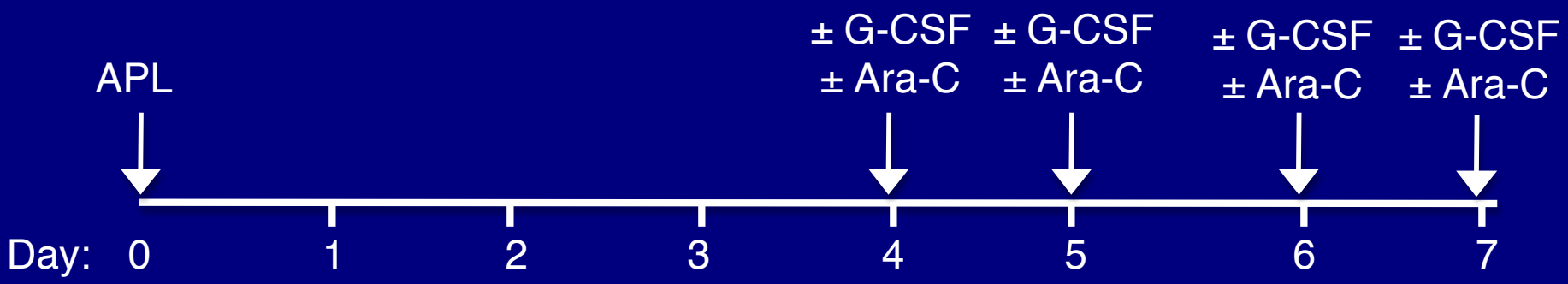
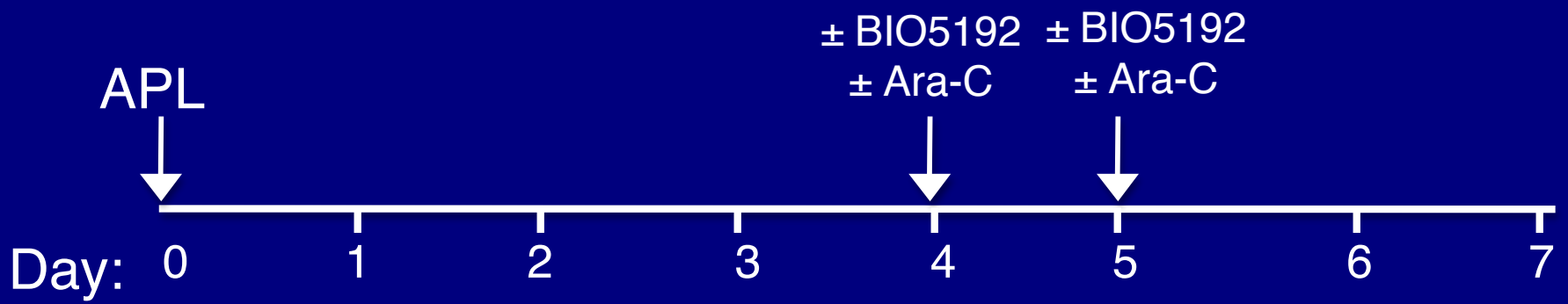


Figure 21. BIO5192 induces a rapid and transient mobilization of APL blasts into the peripheral blood. Syngeneic B6129F1 recipient mice were injected with 10^6 APL cells. Twelve days after APL injection, mice were treated with a single IV dose of 1 mg/kg BIO5192. Peripheral blood samples were collected immediately before and 0.5, 1, 3 and 6 hours after BIO5192 administration. (A) Representative FACS profiles showing mobilization of $Gr1^+CD34^+$ APL blast cells. (B) APL blast cell counts.

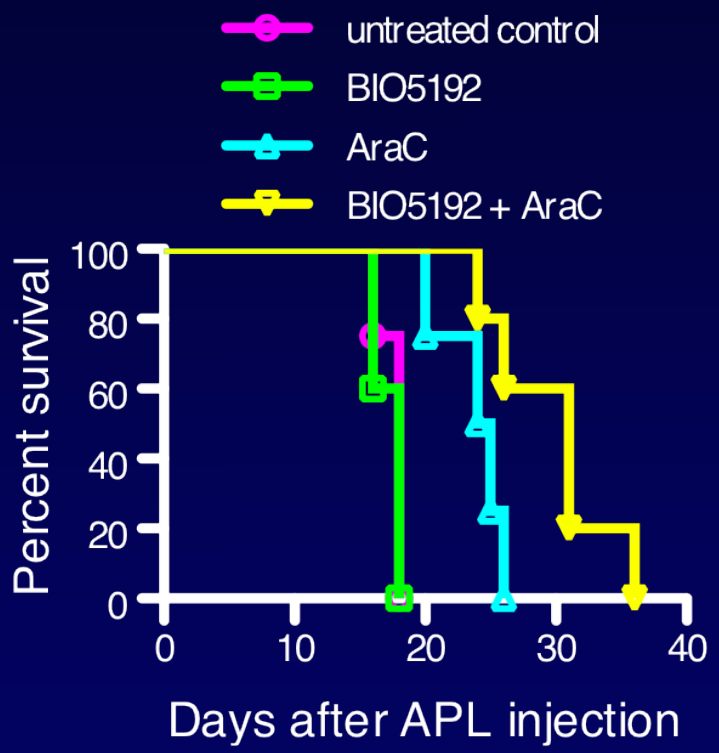
APL Chemosensitization by G-CSF & BIO5192

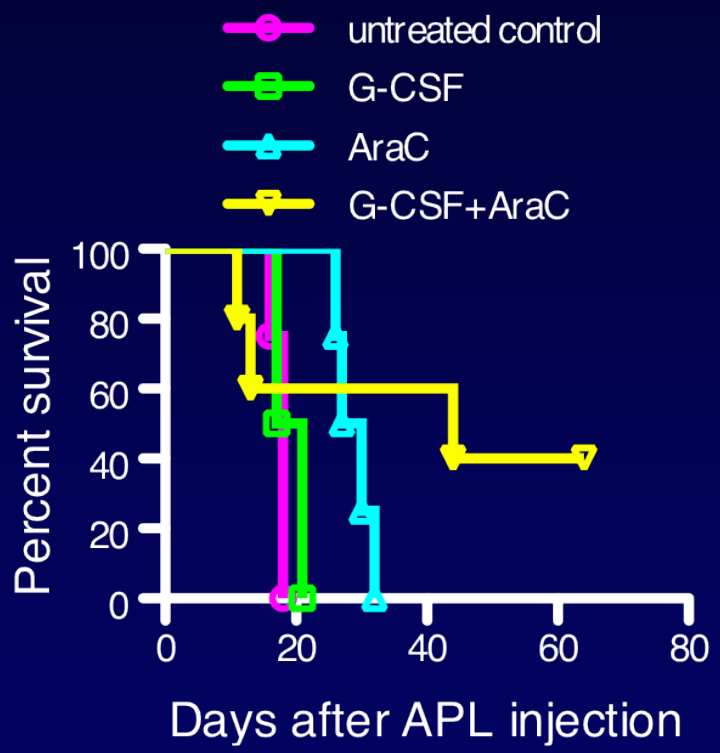




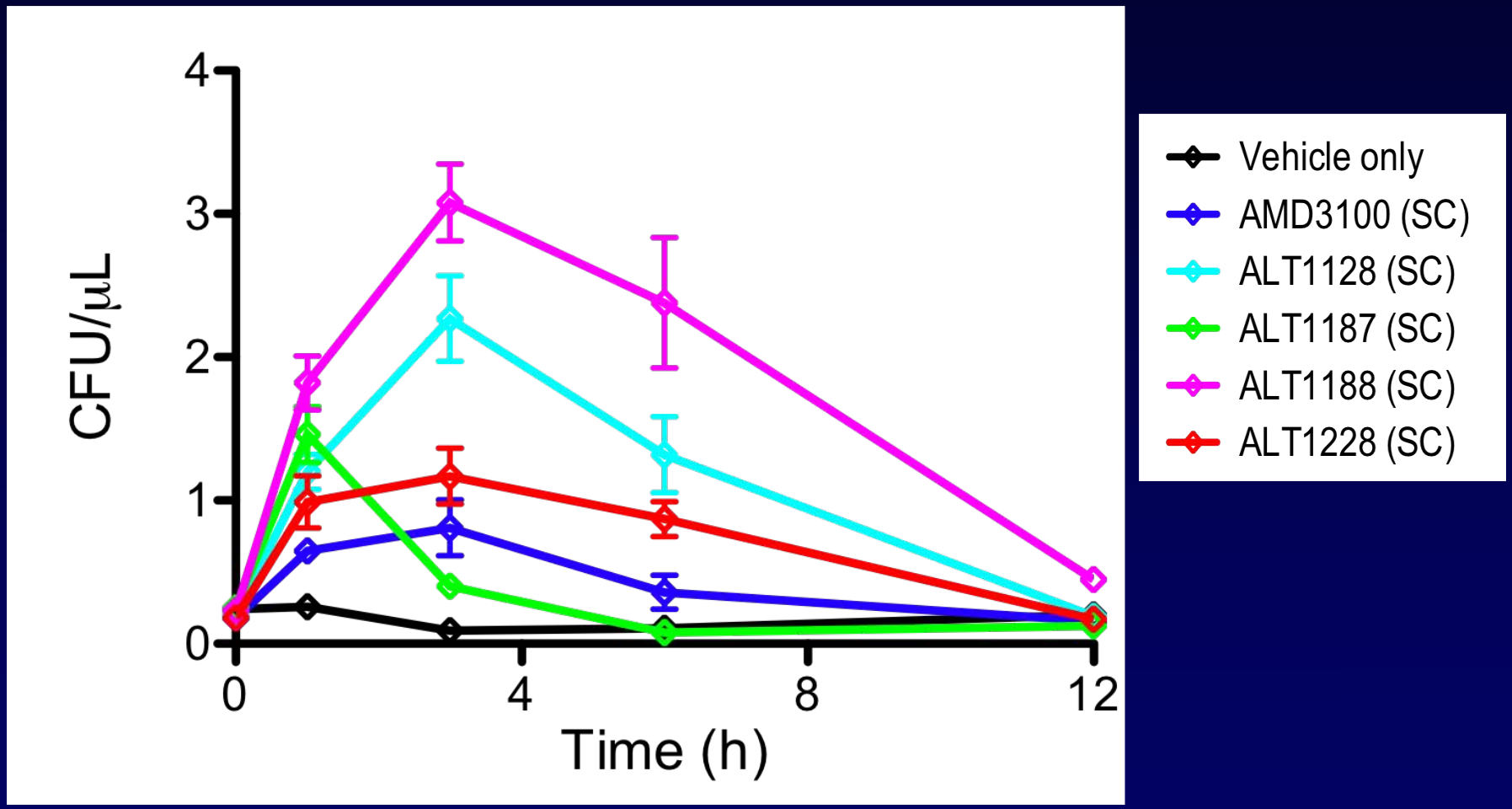
1. APL^{luc} = 10^6 cells/mouse IV
2. G-CSF = 250 μg/kg/day SC
3. BIO5192 = 1 mg/kg IV
4. Ara-C = 500 mg/kg SC at 4h after G-CSF/AMD injection

BIO5192 and G-CSF Enhance the Effect of Chemotherapy





Effect of 3rd Generation CXCR4 Inhibitors



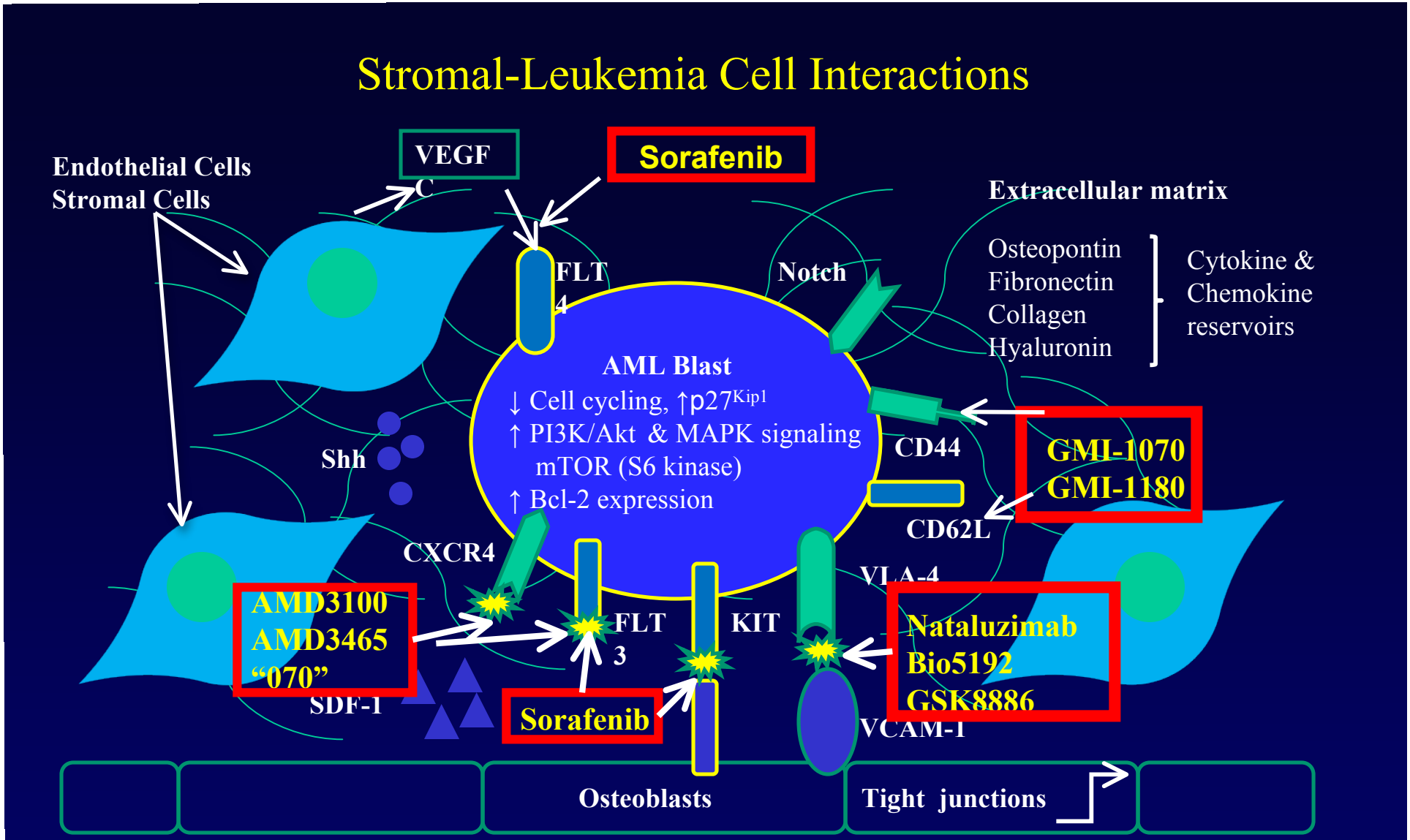


Figure 1. Protective effect of marrow microenvironment. The Hematopoietic Inductive Microenvironment (HIM) niches include osteoblasts, stromal / mesenchymal cells, endothelial cells and extracellular matrix components. AML blast quiescence , proliferation and apoptosis are influenced by receptor kinases, adhesive receptors and signaling via matrix mediated / bound chemokines and cytokines. AMD3100 and AMD3465 CXCR4 antagonist s +/- sorafenib or inhibitors of VLA-4/VCAM-1 interactions chemosensitize AML blasts within the HIM niches. Shh: sonic hedgehog , Notch, vascular endothelial factor and other adhesion receptors signals promote leukemic stem cell survival and expansion and can be targeted also to overcome AML chemoresistance.

CONCLUSIONS

1. AMD3100 is a safe and effective and rapidly mobilizes stem cells from mouse and man.

2. AMD3100 mobilized stem cells and T cells function normally in mouse competitive repopulation and GvHD models.

3. Infusion of AMD3100 mobilized human stem cell products into HLA matched sibling recipients can be performed in the majority of recipients after a single collection and is associated with outcomes, kinetics of engraftment and GvHD that are not dissimilar to those seen with G-CSF mobilized PBSC products.

4. APL cells were mobilized from the BM into PB after plerixafor administration.

5. Blockade of CXCR4 (via AMD3100 or G-CSF) and VLA-4 in in vivo sensitizes APL cells to chemotherapy.

6. We hypothesize that we can overcome resistance to chemotherapeutic agents by mobilizing AML from the BM into the PB. Mouse models and human clinical trials will test this hypothesis.

7. Interruption of the VLA-4/VCAM axis synergizes with CXCR4 blockade resulting in enhanced HSC and leukemic cell mobilization.

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Rettig Lab

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