STEM CELL CLUB

October 18, 2001 12:00 Noon

Y6.228 Conference Room

STEM CELLS, NK CELLS, and FRIEND VIRUS LEUKEMIA

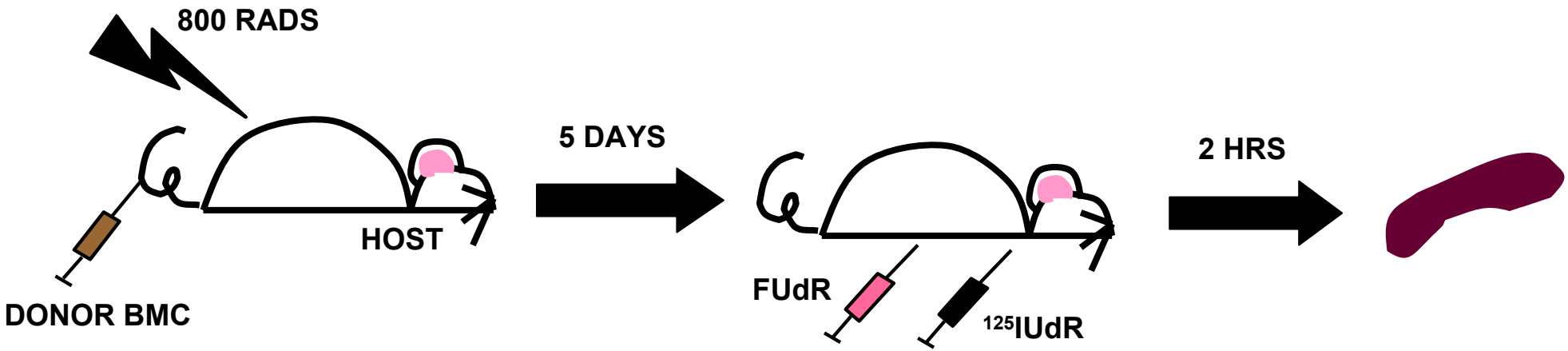
Michael Bennett, Department of Pathology

Stem Cells: Gustavo Cudkowicz, Don Metcalf, Roger Foster, and Vinay Kumar

Friend Virus: Gustavo Cudkowicz, Richard Steeves, Bob Eckner, Ed Mirand, and Vinay Kumar

NK Cells: Vinay Kumar, Dorothy Yuan, Wayne Lai, PA Mathew, and John Schatzle, (faculty) Differentiation: Edwardo Luevano, John Hackett, Michelle Tutt, Tom Moore, Igor Puzanov, PV Sivakumar, Noelle Williams, Susan Stepp, and Jennifer Klem (fellows/students) Bone marrow transplantation: Jean Eastcott, Pam Rodday, Pat Fitzgerald, John Lust, Sami Affifi, Anwar Mikhael, Rick Rembecki, Bill Murphy, Charles Sentman, Colleen Davenport, Lawrence Yu, Earl Stoneman, Jingxuan Liu, Tad George, Mesha Taylor, Maggie Morris, Gene Devora, Namita Ghandi, Crystal Koh, and Jill Rooney (fellows/students)

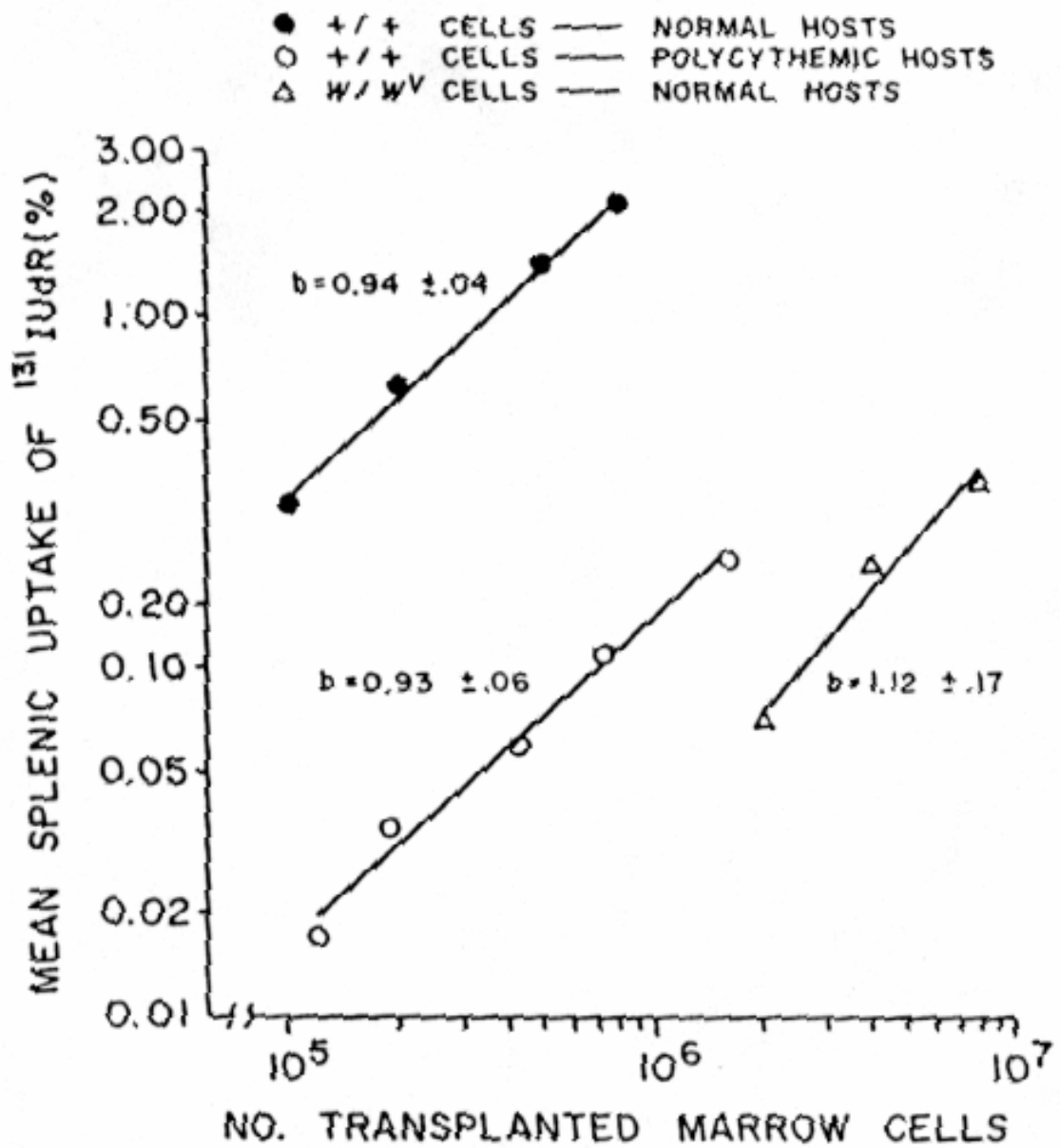
Bone marrow cell transplant assay

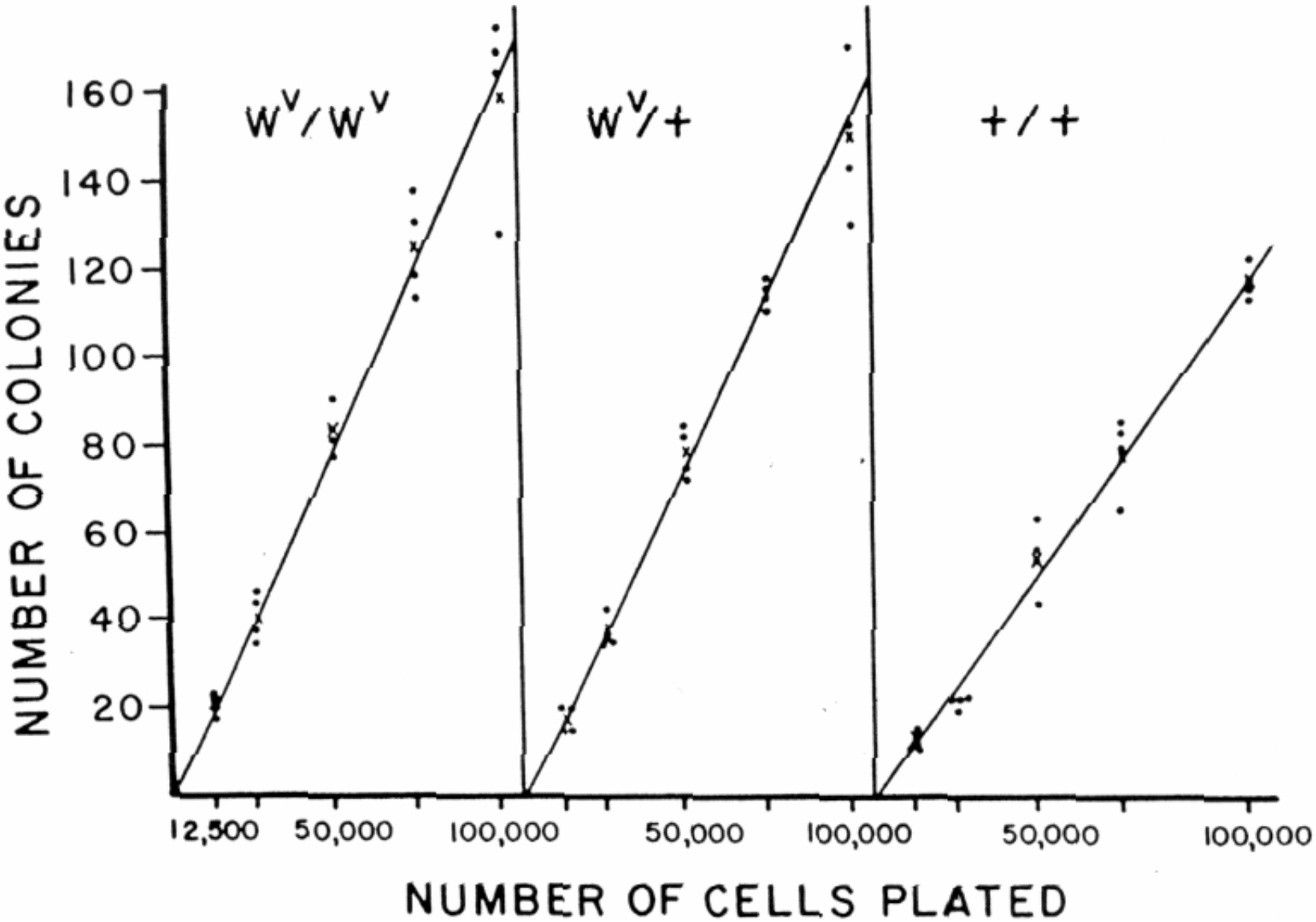


The effect of glass wool filtration on erythropoietic and proliferative competence of marrow and on frequency of various cell types

Method of assay	Proliferative Competence		
	U[1]	F[1]	F:U
Uptake of ^{131}IUdR	0.69±0.05	1.17±0.13	1.69
Uptake of ^{59}Fe	4.22±0.41	8.29±1.43	1.96
Nodular colonies	4.68±0.23	7.79±0.51	1.67
			1.77±0.09

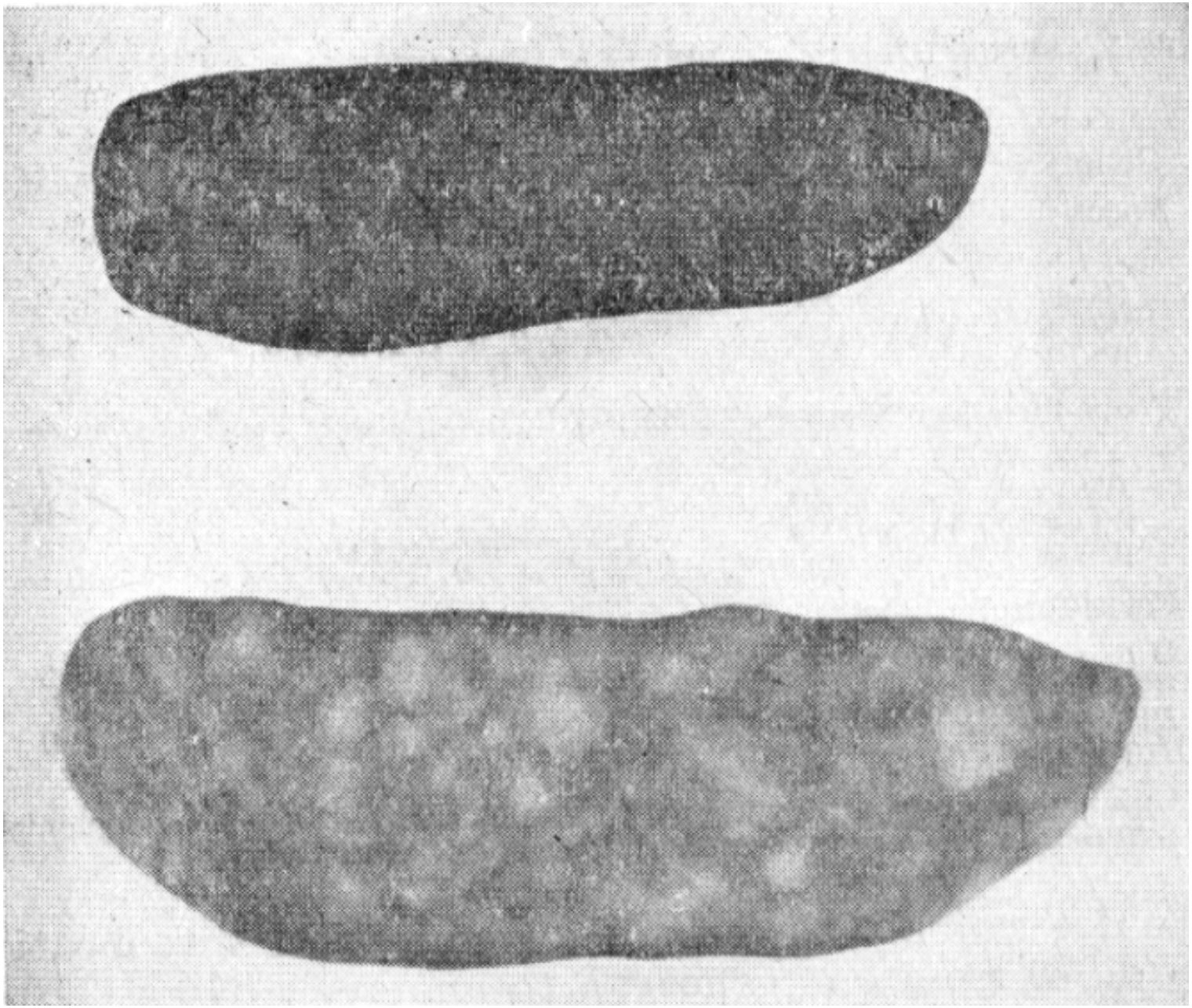
	Frequency of cells (%)(n)		
Cell type	U	F	F:U
SML	23.2	41.4	1.78
B	0.30	0.10	0.33
RE	1.32	0.59	0.45
	(2357)	(2028)	

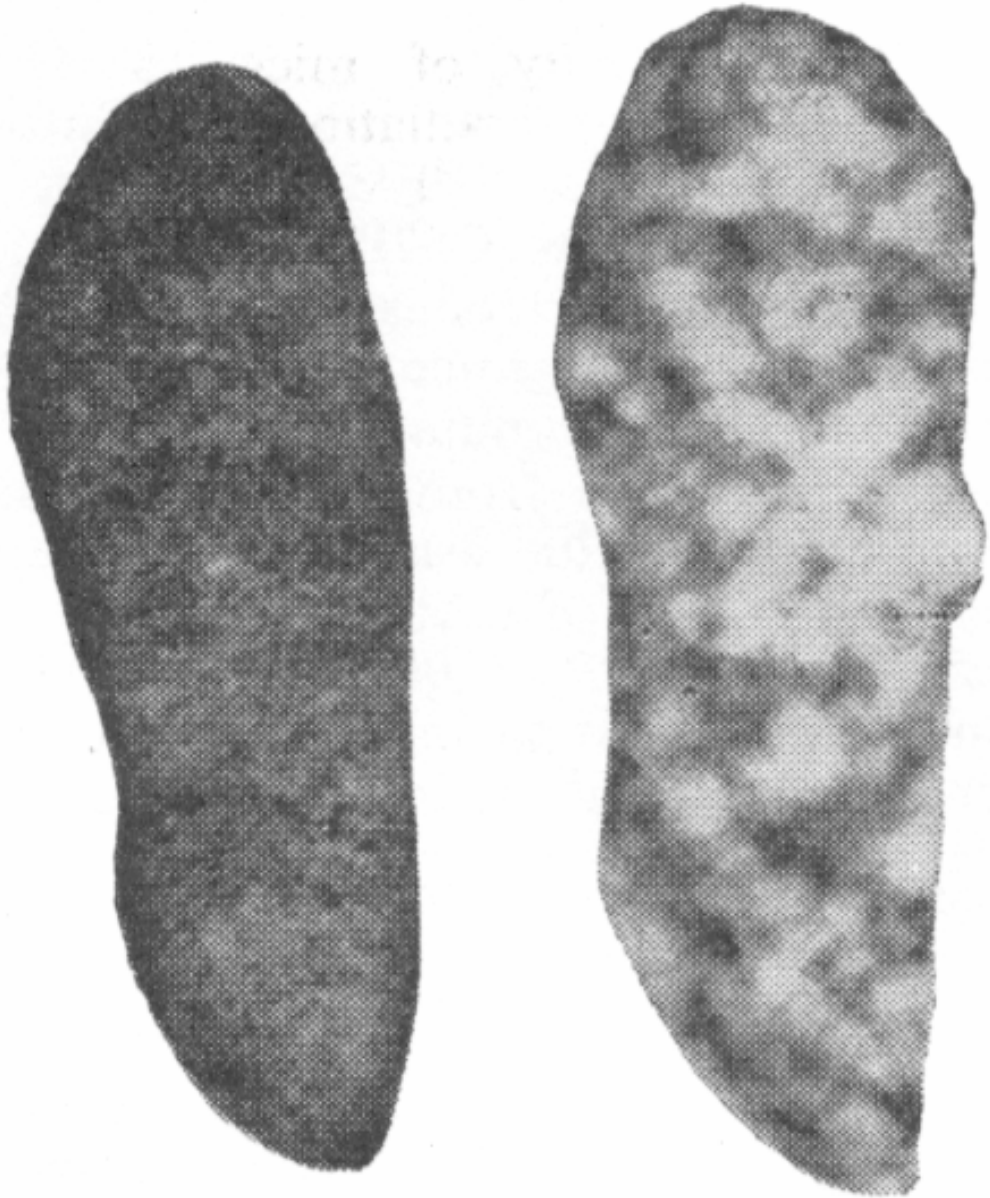

● +/+ CELLS —— NORMAL HOSTS
○ +/+ CELLS —— POLYCYTHEMIC HOSTS
△ W/W^V CELLS —— NORMAL HOSTS
MEAN SPLENIC UPTAKE OF 131IUdR(%)
3.00
2.00
1.00
0.50
0.20
0.10
0.05
0.02
0.01
b=0.94 ±.04
b=0.93 ±.06
b=1.12 ±.17
10^5
10^6
10^7
NO. TRANSPLANTED MARROW CELLS

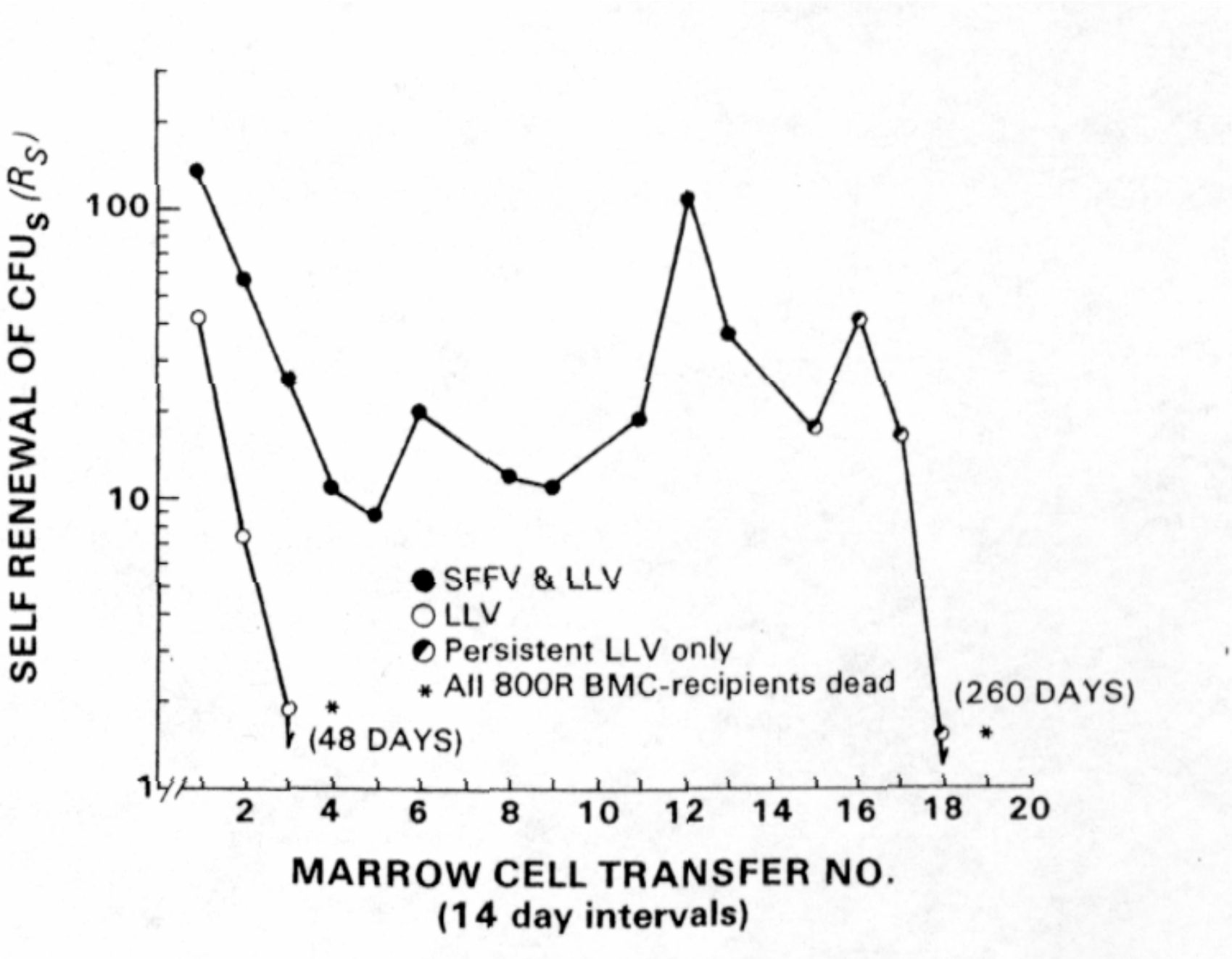

NUMBER OF COLONIES
160
140
120
100
80
60
40
20
W^v/W^v
W^v/+
+/+
12,500
50,000
100,000
50,000
100,000
50,000
100,000
NUMBER OF CELLS PLATED

Friend Virus Induced Erythroleukemia

1. Helper virus, F-MuLV, is responsible for growth of the virus complex.
2. F-MuLV can only produce lymphomas if injected into neonatal mice.
3. SFFV is defective/interfering virus that expresses gp55 env protein that binds to EpoR to produce erythroblastosis; has no oncogene; late events: p53□ PU.1□
4. *Fv2* resistance gene (StkR). *Fv2*^{s}-truncated, *Fv2*^{r} not so, StkR responsible for proliferation of erythroblasts in response to gp55
5. Do NK cells affect early stem/progenitor cells to inhibit leukemia?



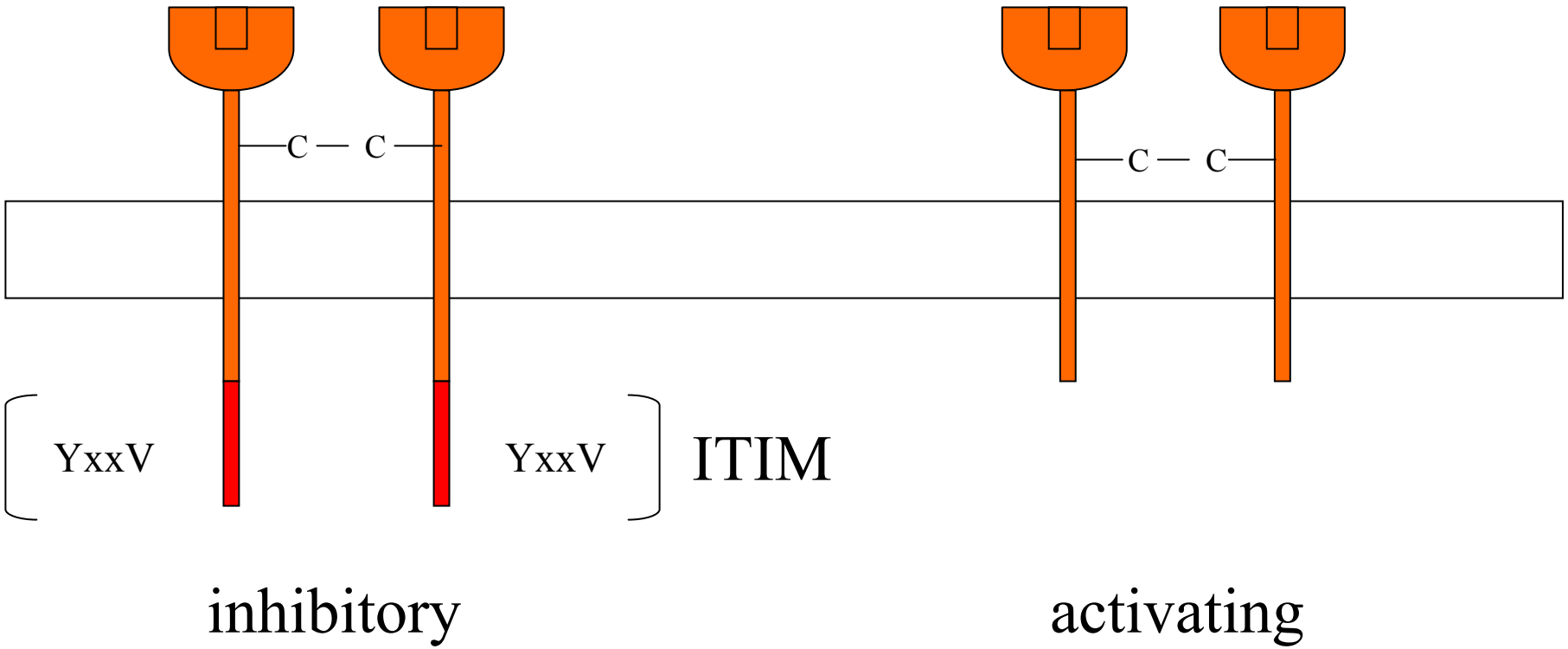



SELF RENEWAL OF CFU$_S$ (R_S)
100
10
1
SFFV & LLV
LLV
Persistent LLV only
All 800R BMC-recipients dead
(48 DAYS)
(260 DAYS)
2
4
6
8
10
12
14
16
18
20
MARROW CELL TRANSFER NO.
(14 day intervals)

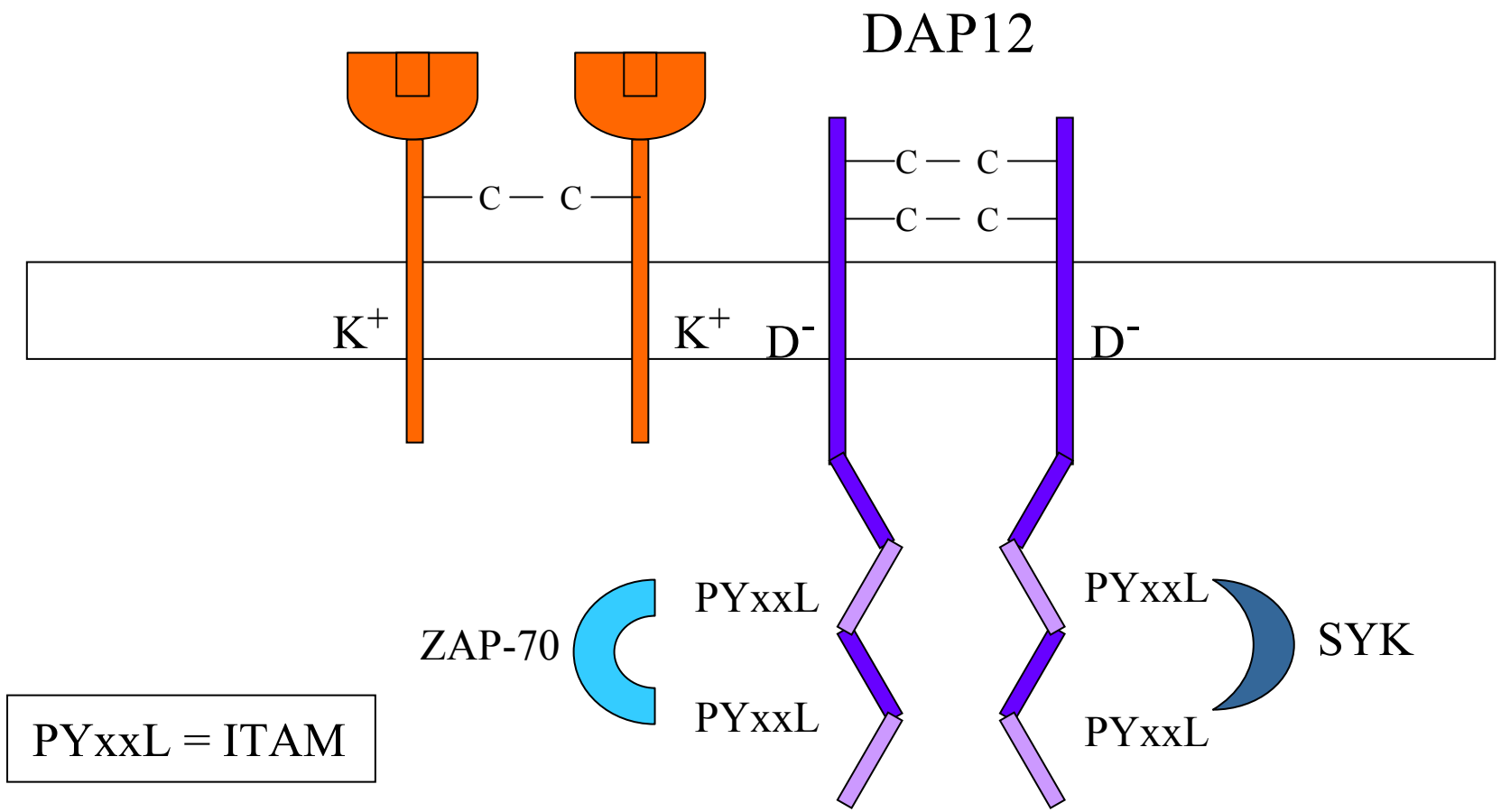
NK Cells

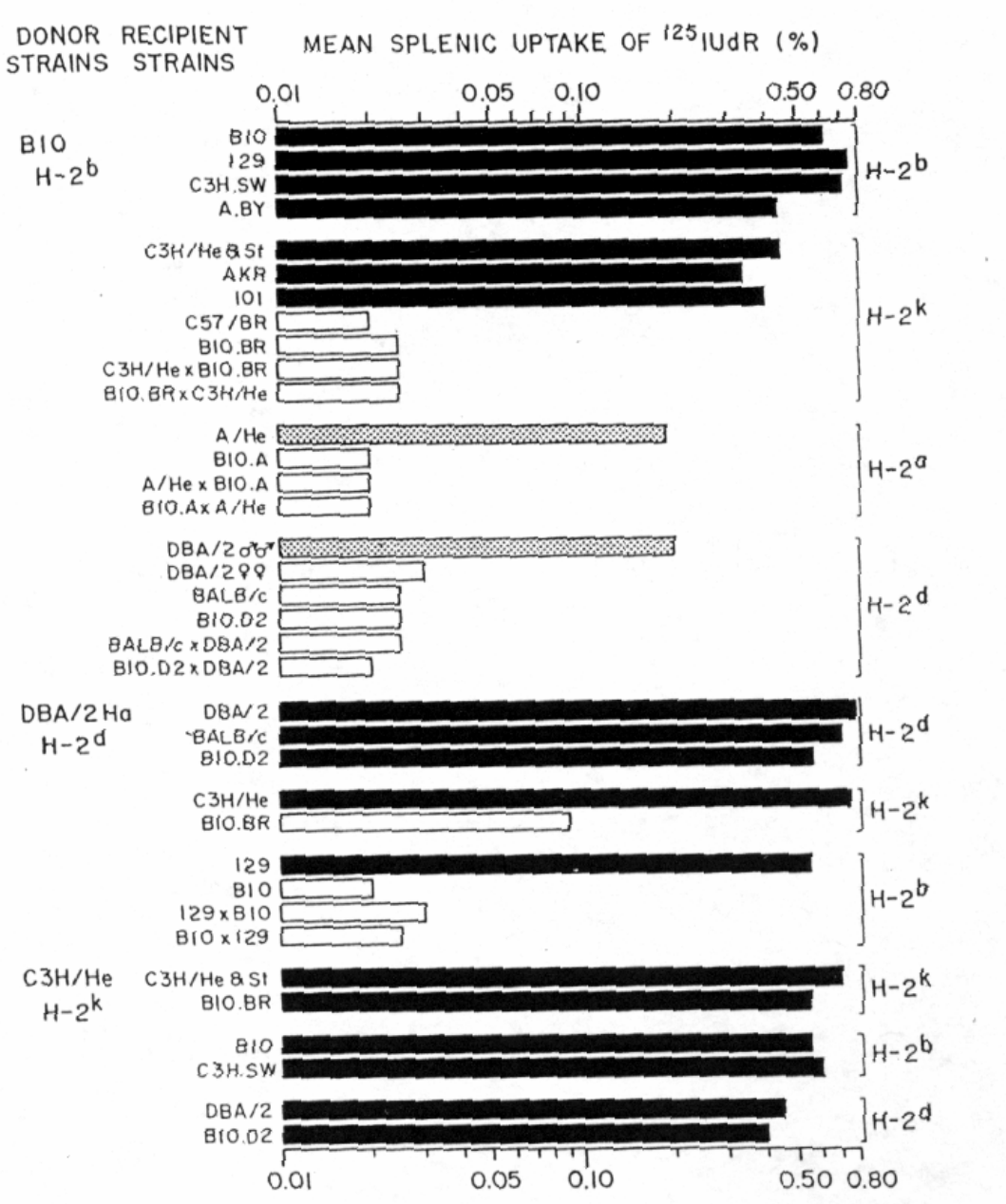
- Large granular lymphocytes
- Secrete cytokines that modulate immune responses
- Capable of lysing tumor cells, virally infected cells that are deficient in host (self) MHC class I
- Antigen receptor genes are in germline configurations
- Lack T cell markers (CD3, CD4, CD5)
- Function through the expression of surface receptors that either stimulate or inhibit NK cell activity

Activating Ly49 Receptors Lack an ITIM (Immunoregulatory Tyrosine-Based Inhibitory Motif)



Activating LY49 Receptors Associate with ITAM Containing DAP12 Adaptor Molecules

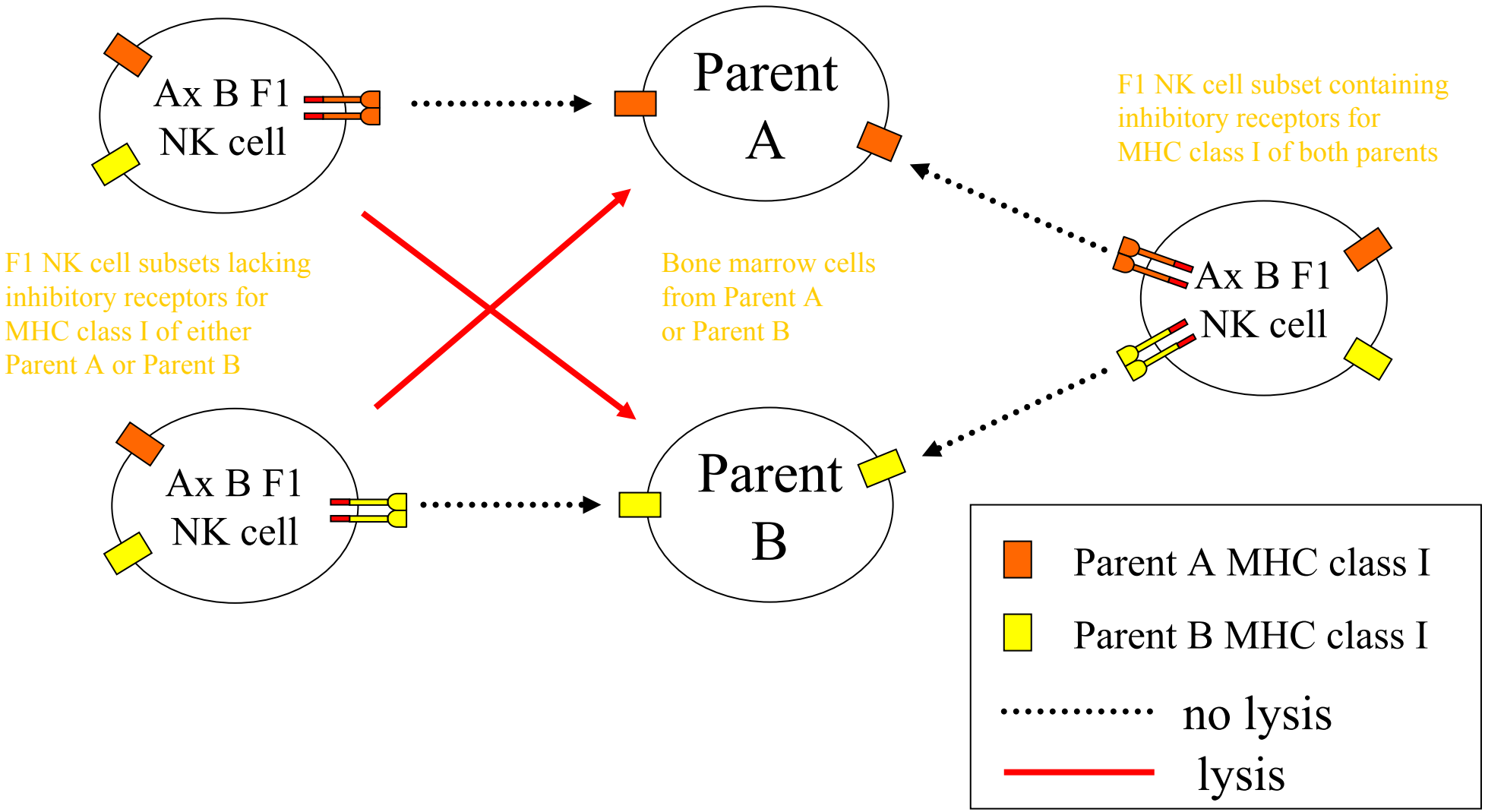


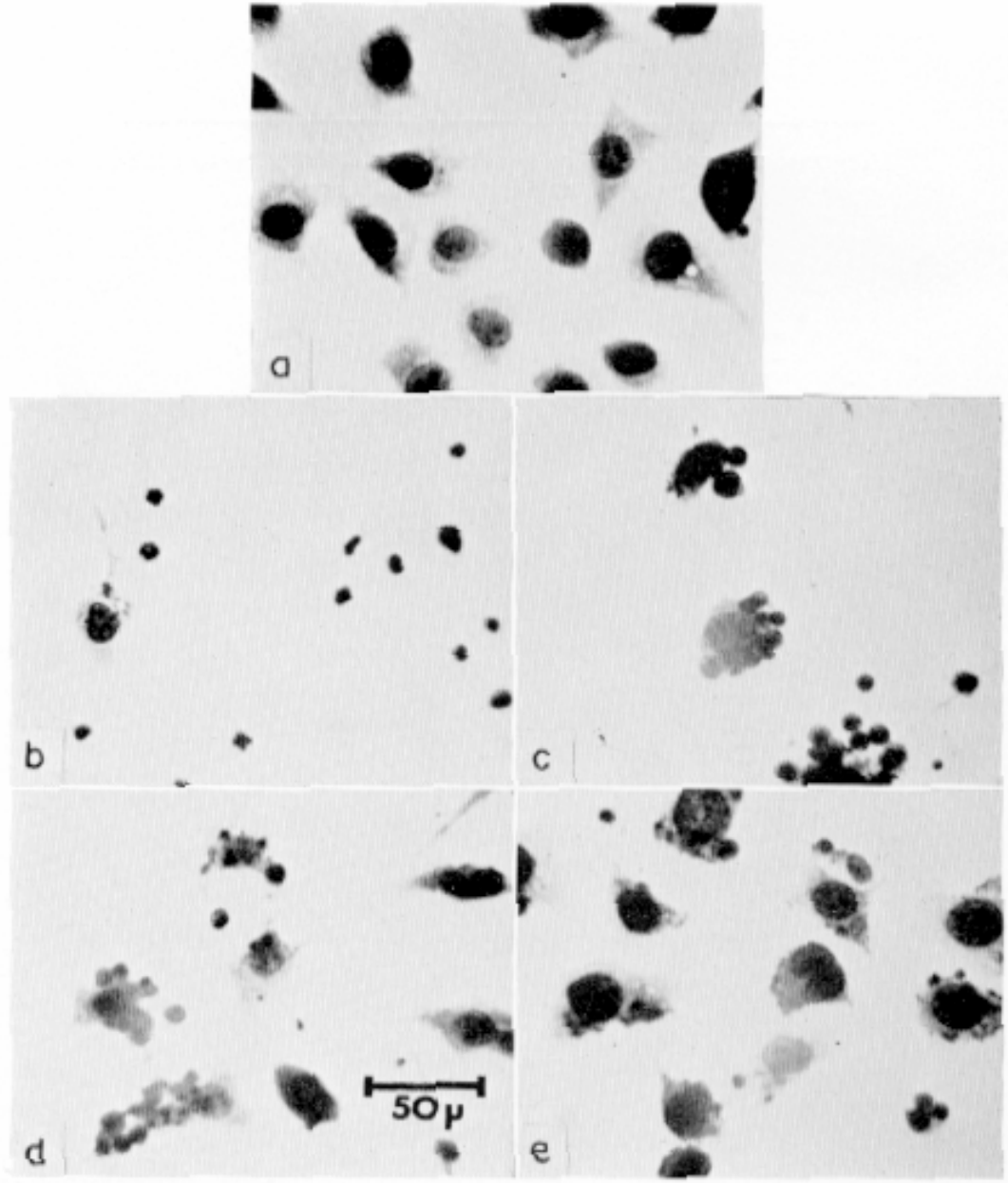

DONOR STRAINS
RECIPIENT STRAINS
MEAN SPLENIC UPTAKE OF ^{125}IUdR (%)
0.01
0.05
0.10
0.50
0.80
B10 H-2b
B10
129
C3H.SW
A.BY
H-2b
C3H/He&St
AKR
101
C57/BR
B10.BR
C3H/He x B10.BR
B10.BR x C3H/He
H-2k
A/He
B10.A
A/He x B10.A
B10.A x A/He
H-2a
DBA/2 ♂♂
DBA/2 ♀♀
BALB/c
B10.D2
BALB/c x DBA/2
B10.D2 x DBA/2
H-2d
DBA/2Ha H-2d
DBA/2
BALB/c
B10.D2
H-2d
C3H/He
B10.BR
H-2k
129
B10
129 x B10
B10 x 129
H-2b
C3H/He H-2k
C3H/He&St
B10.BR
H-2k
B10
C3H.SW
H-2b
DBA/2
B10.D2
H-2d
0.01
0.05
0.10
0.50
0.80

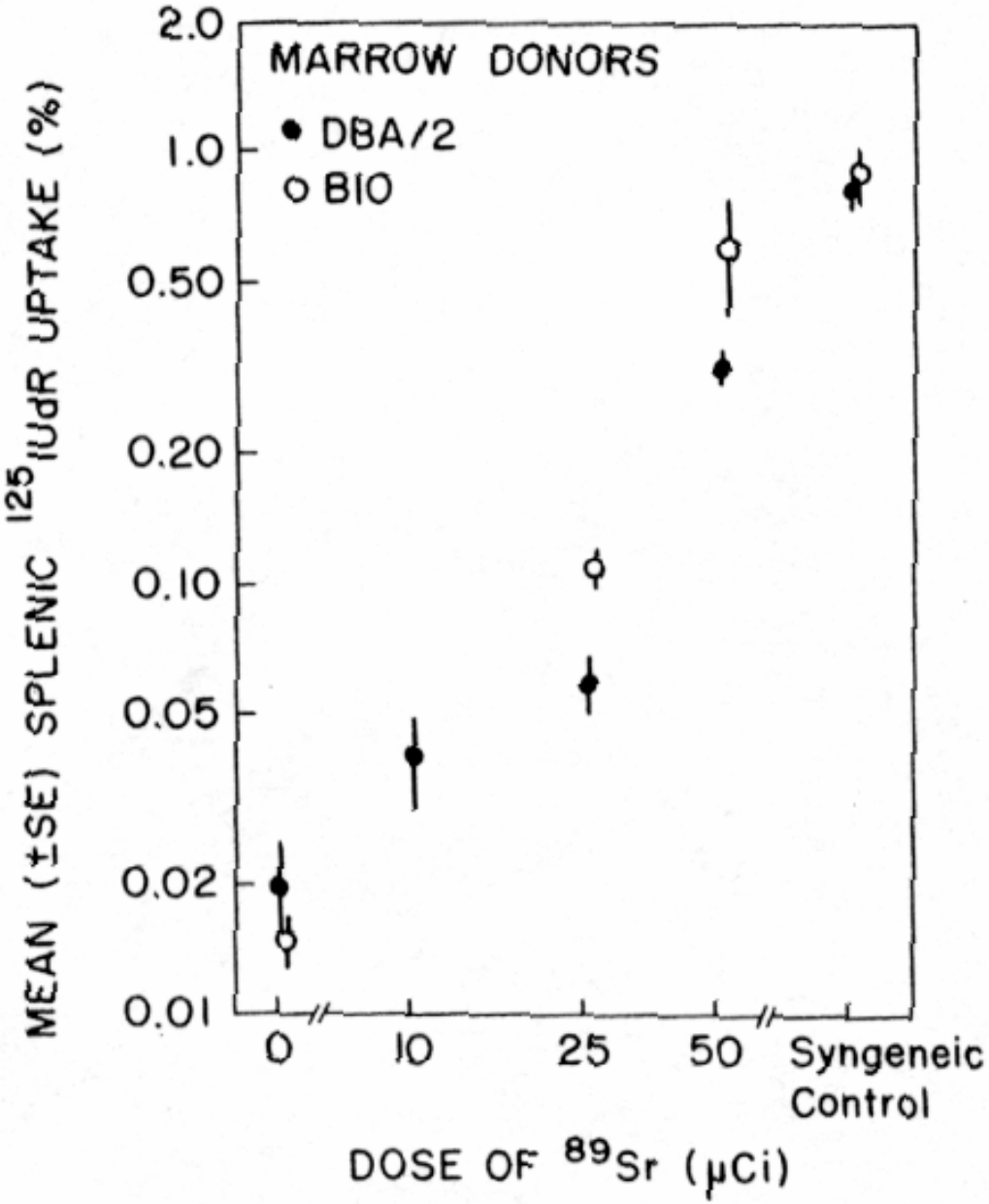
Donor Strain	Host Strain	Solid Tissue Graft	Bone Marrow Graft
A	A	Accept	Accept
B	B	Accept	Accept
A	B	Reject	Reject
B	A	Reject	Reject
A or B	(A X B)F1	Accept	Reject (hybrid resistance)
(A X B)F1	A or B	Reject	Accept

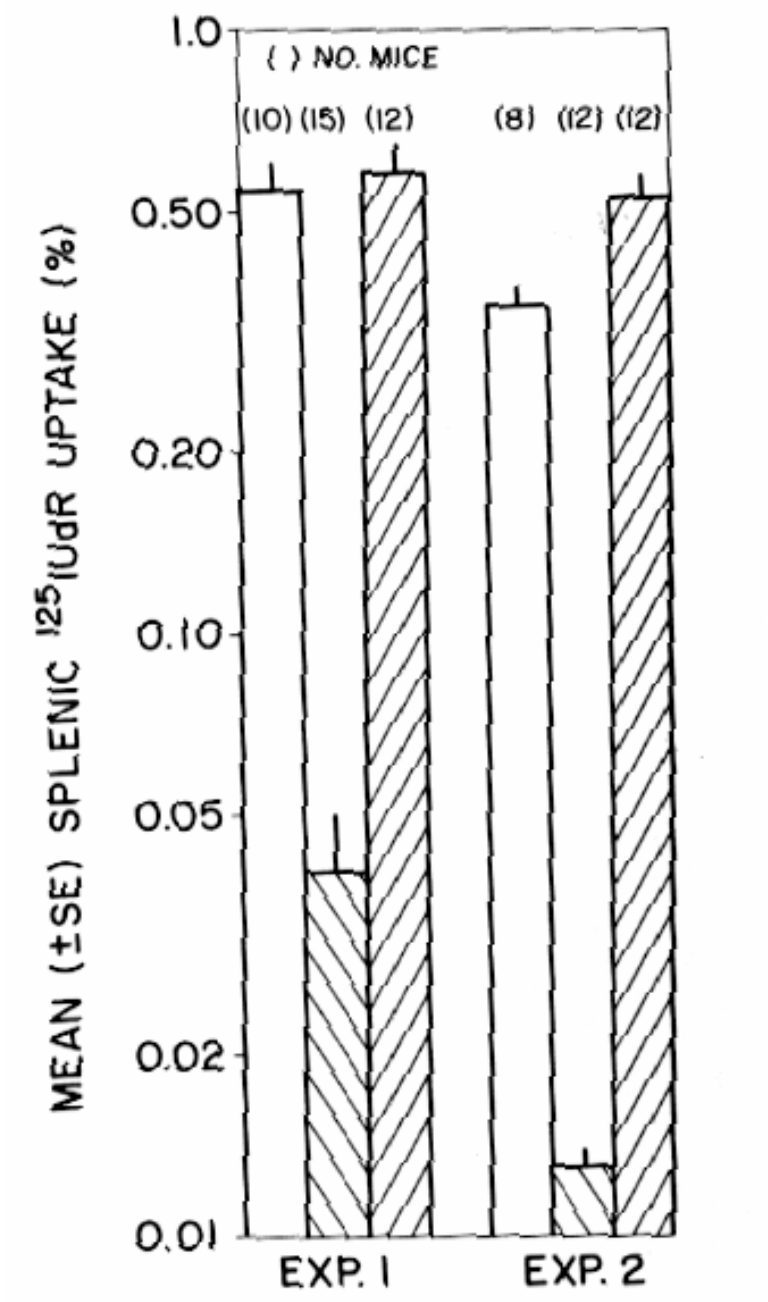
Antigens recognized (or not)	MHC I, II	MHC I
Antigen specific effector cells	CD8, CD4 T cells	NK cells
Activating receptors used	TCR	Ly49 ITIM^{neg}
Inhibiting receptors used	CTLA4	Ly49 ITIM^{pos}

Hybrid Resistance Explained by Missing Self




a
b
c
d
50μ
e


MARROW DONORS
DBA/2
B10
MEAN (±SE) SPLENIC ^{125}IUdR UPTAKE (%)
2.0
1.0
0.50
0.20
0.10
0.05
0.02
0.01
0
10
25
50
Syngeneic Control
DOSE OF ^{89}Sr (μCi)

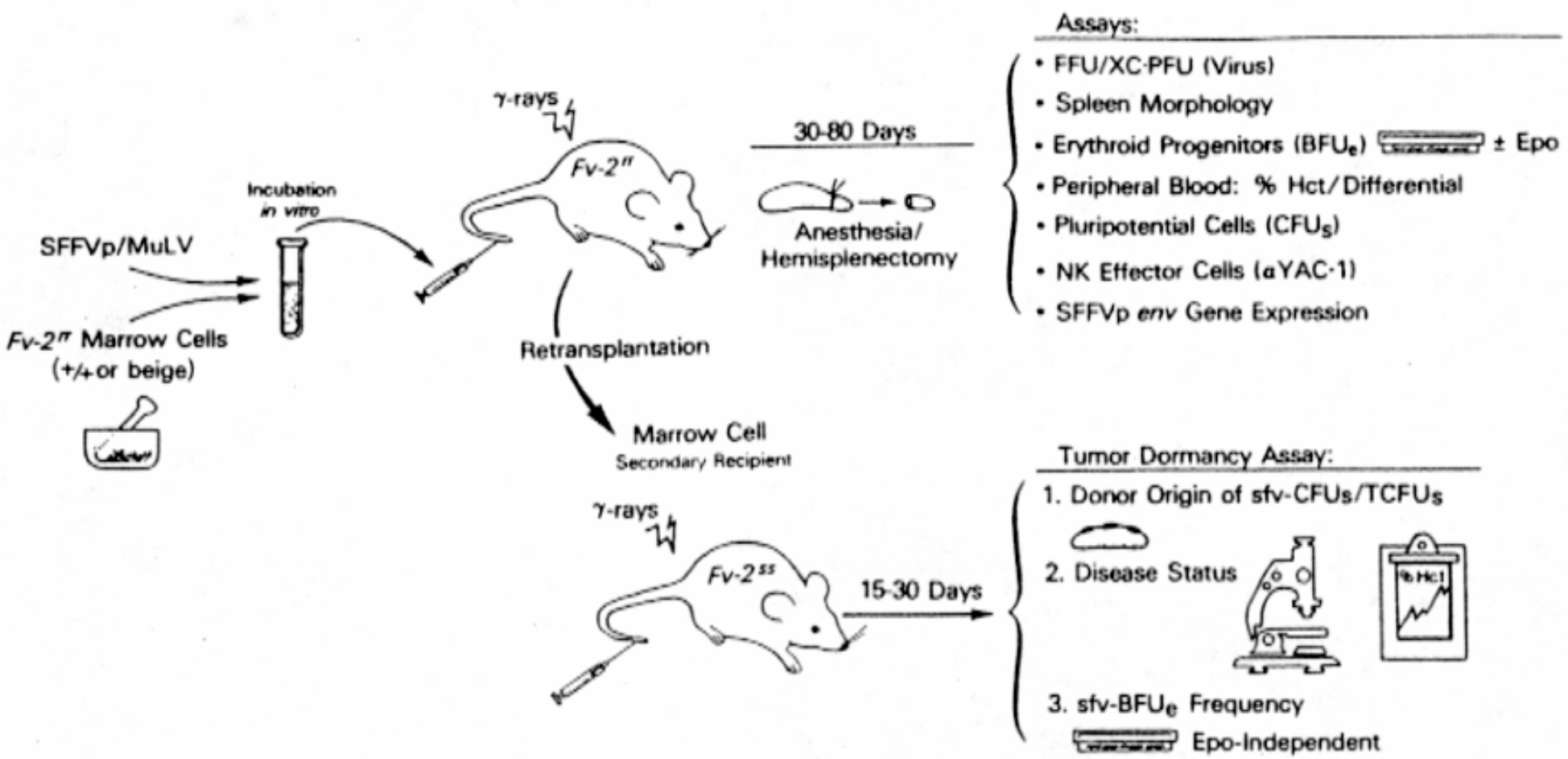

1.0
0.50
0.20
0.10
0.05
0.02
0.01
( ) NO. MICE
(10) (15) (12) (8) (12) (12)
MEAN (±SE) SPLENIC ^{125}IUdR UPTAKE (%)
EXP. 1
EXP. 2

Effect of ^{89}Sr on Genetic Resistance of C57BL/6 Mice to FV-Induced Malignant Erythropoiesis

Treatment	Infection	Erythroid cells in spleen				Virus recovery			
		Polycythemic		Not Polycythemic		Plasma		Spleen	
89Sr	FV	%	(n)	%	(n)	FFU/ml	(n)	FFU/spleen	(n)
yes	yes	95	(3)	91	(4)	$>10^5$	(7)	$>10^5$	(7)
yes	no	3	(2)	NT‖		0	(4)	NT	
no	yes	1.5	(3)	12	(4)	0	(7)	0	(3)
no	no	0.3	(2)	13.2	(4)	NT		NT	

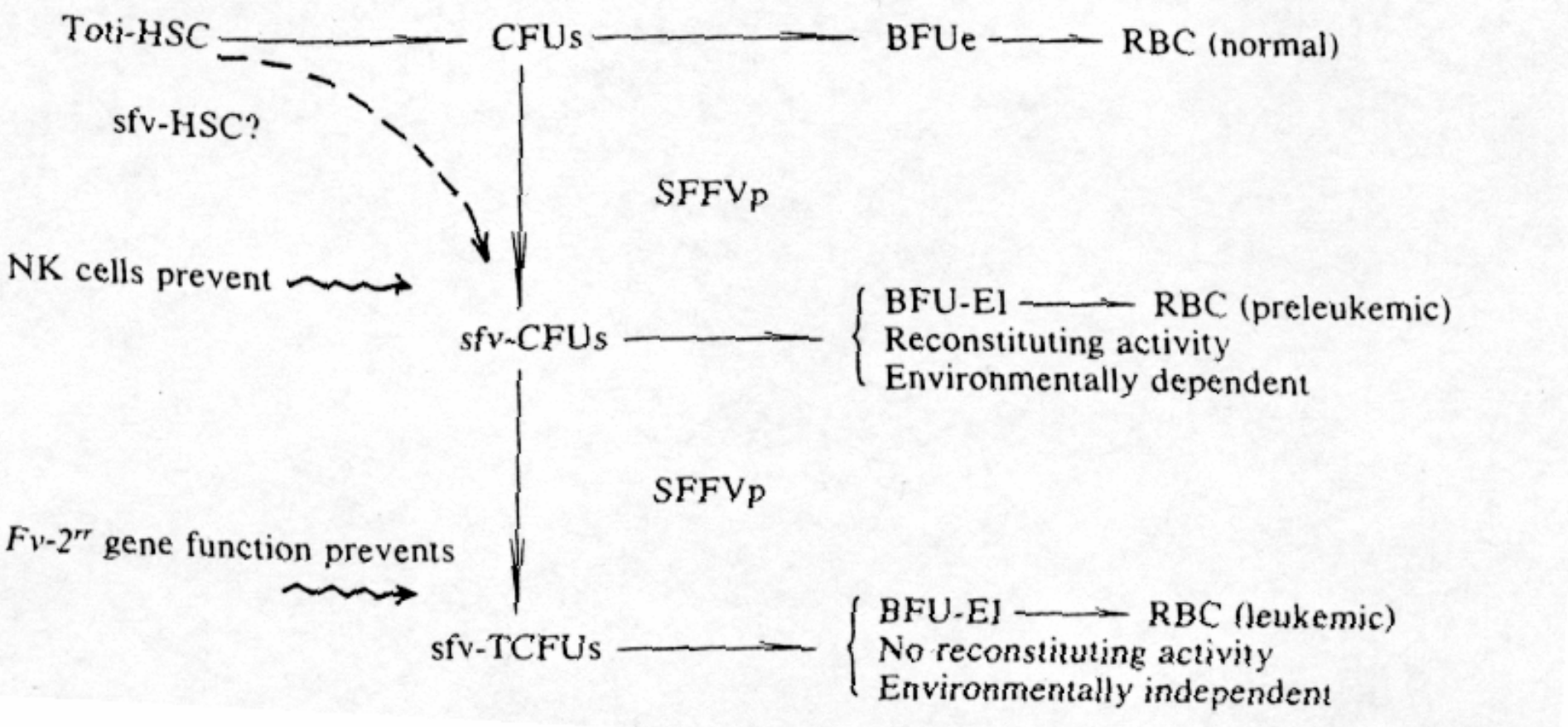
Effect of Varying Doses of ^{89}Sr on Genetic Resistance of C57BL/6 Mice to FV

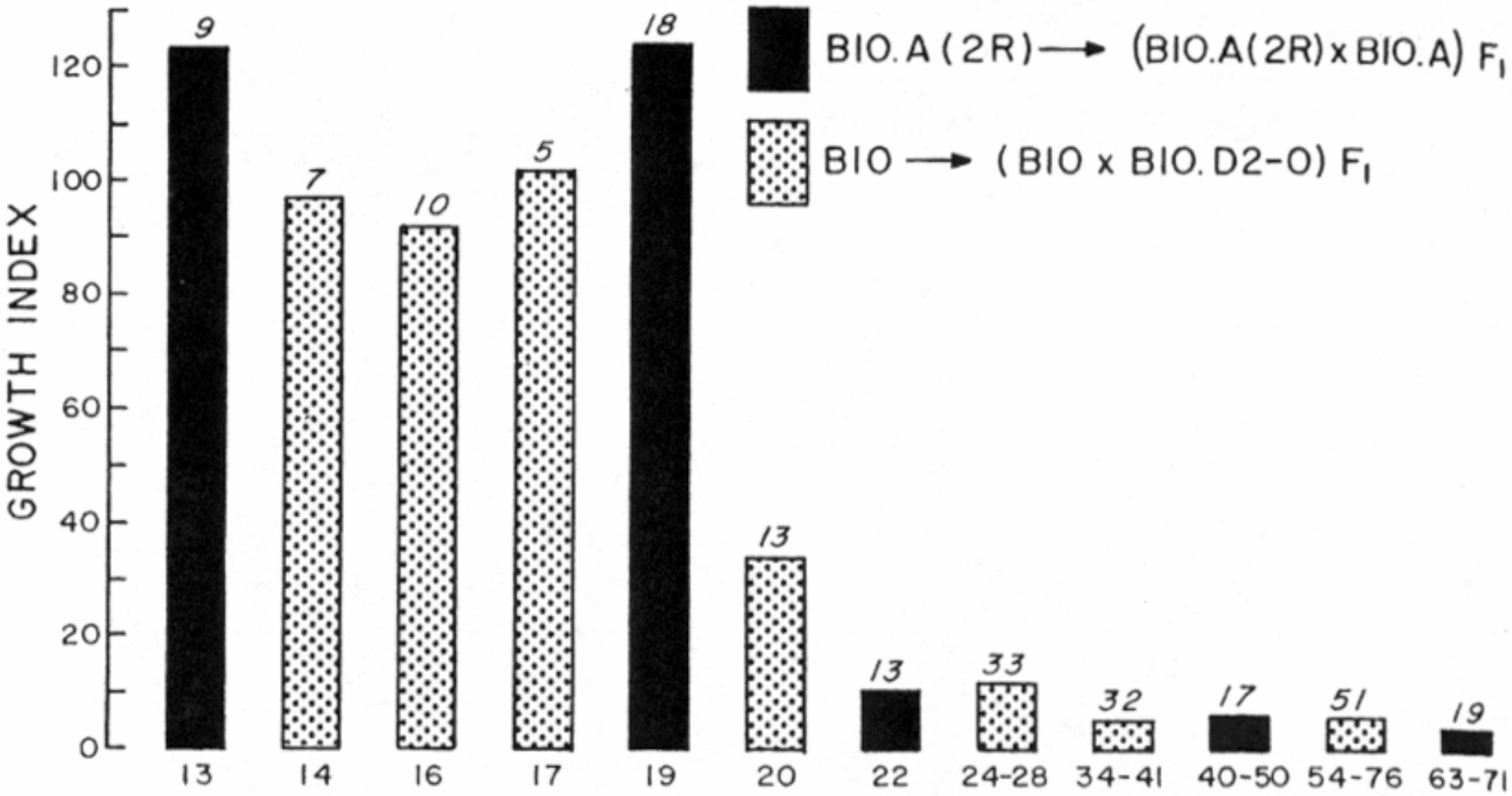
Dose of ^{89}Sr	Erythroid cells in spleen	SFFV spleen (X 10^5)
μCi	%	Mean ± SE
0	13.5	0.6 ± 0.39
25	32	15.2 ± 2.43
50	90	69.8 ± 5.64
100	93.5	76.4 ± 4.15

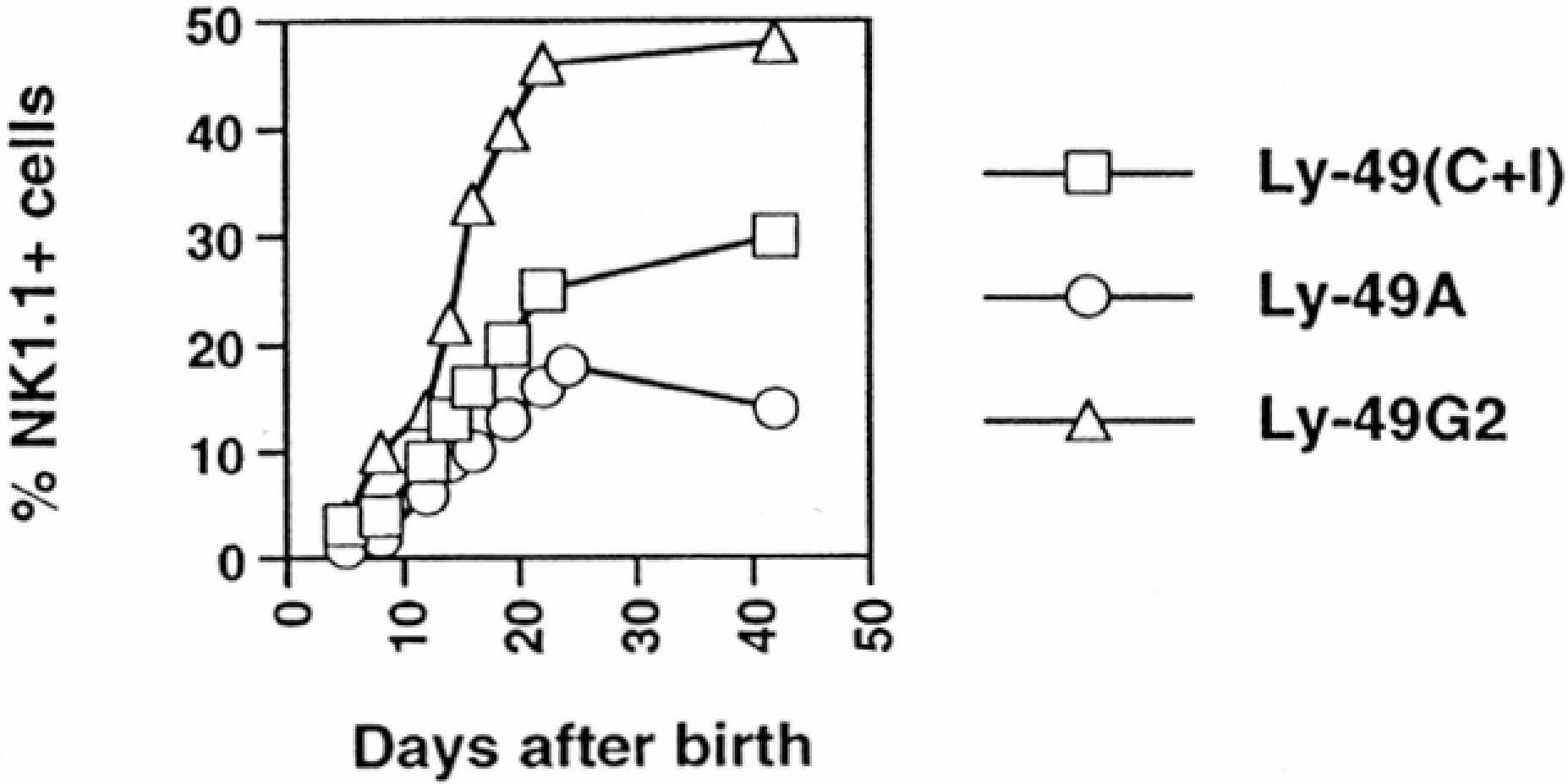

γ-rays
Fv-2ʳʳ
Incubation
in vitro
SFFVp/MuLV
Fv-2ʳʳ Marrow Cells
(+/+ or beige)
30-80 Days
Anesthesia/
Hemisplenectomy
Assays:
• FFU/XC-PFU (Virus)
• Spleen Morphology
• Erythroid Progenitors (BFU$_e$) ± Epo
• Peripheral Blood: % Hct/Differential
• Pluripotential Cells (CFU$_S$)
• NK Effector Cells (αYAC-1)
• SFFVp *env* Gene Expression
Retransplantation
Marrow Cell
Secondary Recipient
γ-rays
Fv-2ˢˢ
15-30 Days
Tumor Dormancy Assay:
1. Donor Origin of sfv-CFUs/TCFU$_S$
2. Disease Status
% Hct
3. sfv-BFU$_e$ Frequency
Epo-Independent

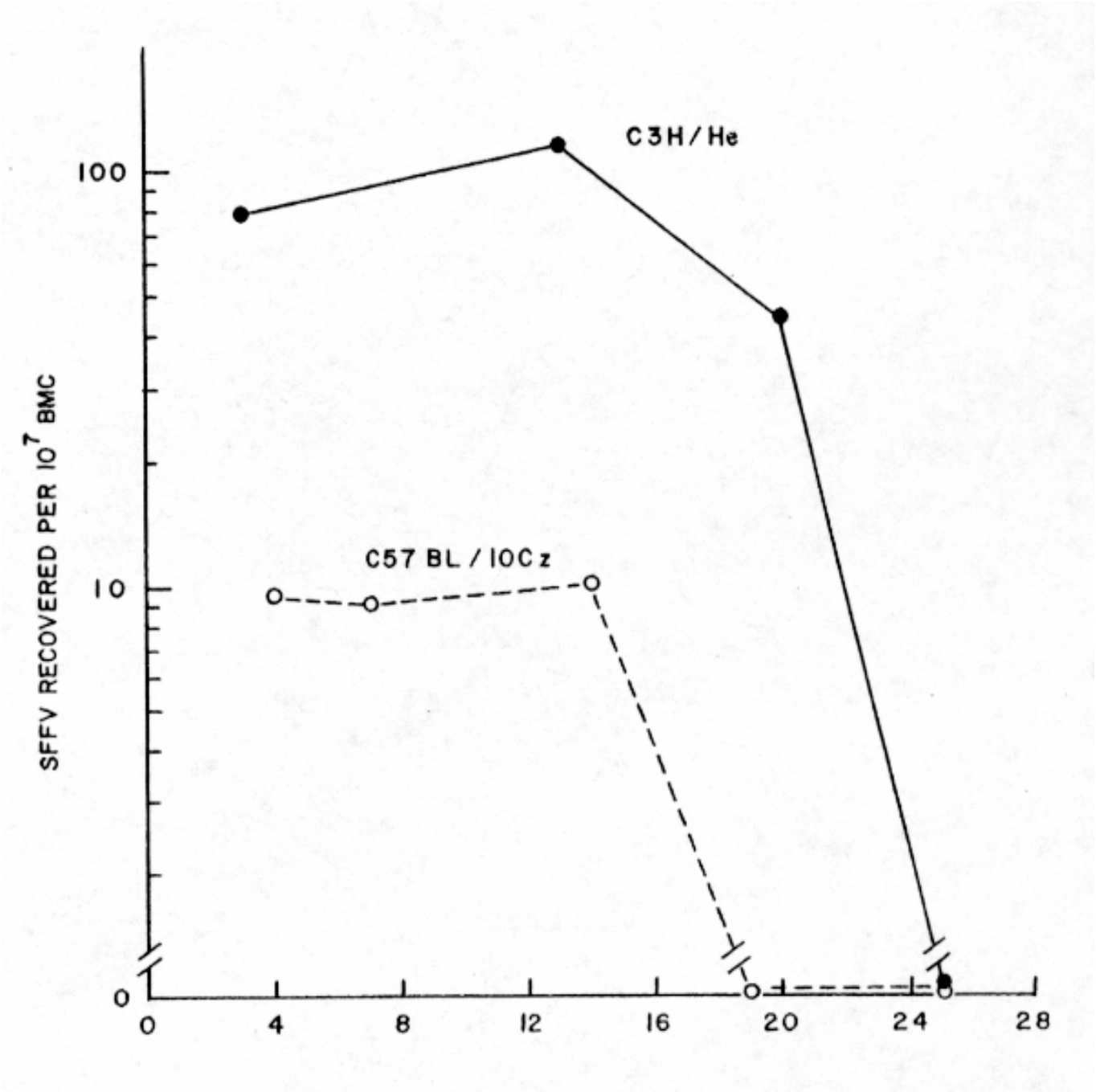
NK Cell Depletion results in more erythroleukemia and dormant tumors in B6 mice

Type and Number of 1° chimeras	Leukemias in 1° Hosts	2° Host Strain	Normal (N) or Malignant (M) Erythropoiesis	Number of donors tumor	% dormant
1. Myk ⇒ +/+ (38)	6/48	B6.C	N or ME	8	(20%)
2. *bg*/*bg* ⇒ +/+ (47)	5/56	B6.C	N or ME	33	(70%)
		B6+/+	N (*beige* PMN)	0	(0%)
3. *bg*/*bg* ⇒ *bg*/*bg* (24)	6/30	B6.C	N or rapid M	17	(63%)
4. My/K ⇒ +/+ (18) + anti-asialo GM1 serum	NT	B6.C	N or very rapid M	14	(78%)

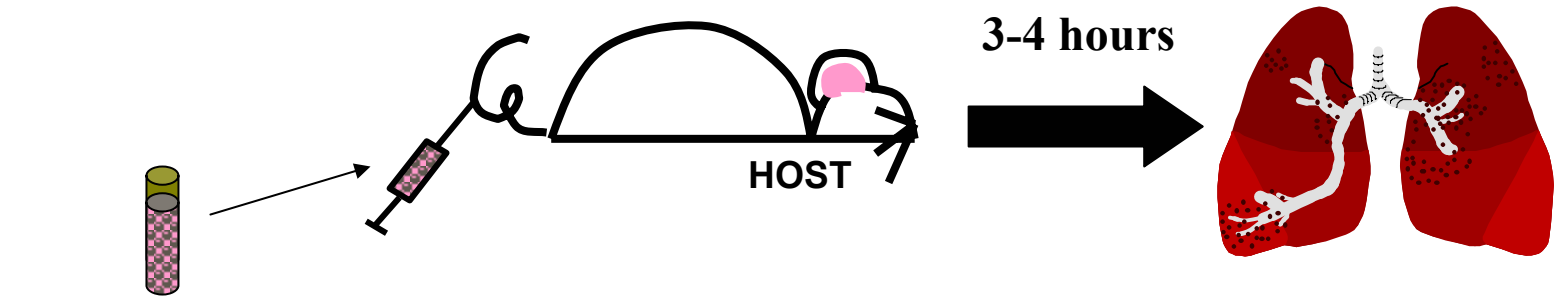

Toti-HSC
CFUs
BFUe
RBC (normal)
sfv-HSC?
SFFVp
NK cells prevent
sfv-CFUs
BFU-El
RBC (preleukemic)
Reconstituting activity
Environmentally dependent
SFFVp
Fv-2r gene function prevents
sfv-TCFUs
BFU-El
RBC (leukemic)
No reconstituting activity
Environmentally independent


GROWTH INDEX
0
20
40
60
80
100
120
9
7
10
5
18
13
13
33
32
17
51
19
13
14
16
17
19
20
22
24-28
34-41
40-50
54-76
63-71
BIO. A (2R) → (BIO.A(2R) x BIO.A) F1
BIO → (BIO x BIO. D2-0) F1


% NK1.1+ cells
50
40
30
20
10
0
0
10
20
30
40
50
Days after birth
Ly-49(C+I)
Ly-49A
Ly-49G2


C3H/He
C57 BL/10Cz
SFFV RECOVERED PER 10^7 BMC
100
10
0
0
4
8
12
16
20
24
28

Lung Clearance Assay



^{125}IUdR-labeled tumor cells

Transplantable Pluripotent Stem Cells (PPSC)

Lin^{neg} Ly6/Sca1^{pos}, ckit^{low}, CD43^{high}, Fall3^{high}, AA4.1^{low}, Rh123^{low}, Flt3^{neg}, Sca2/TSA-1^{neg}

⇓

Transplantable NK Progenitor Cells

Lin^{neg} Ly6/Sca1^{pos}, ckit^{low}, CD43^{high}, Fall3^{high}, AA4.1^{low}, Rh123^{high}, Flt3^{pos}, Sca2/TSA-1^{pos}

⇓

IL-2/15 Unresponsive NK Precursor Cells (in vitro)

Thy-1$^{pos/neg}$, Asialo GM1^{neg}, IL-2/15R^{neg}, CD8^{neg},

⇓ + IL-1

IL-2/15 Responsive NK Precursor Cells (in vitro)

Thy-1^{pos}, Asialo GM1^{neg}, IL-2/15R^{pos}, CD8^{neg}, Mac-1^{neg}, 2B4^{pos}

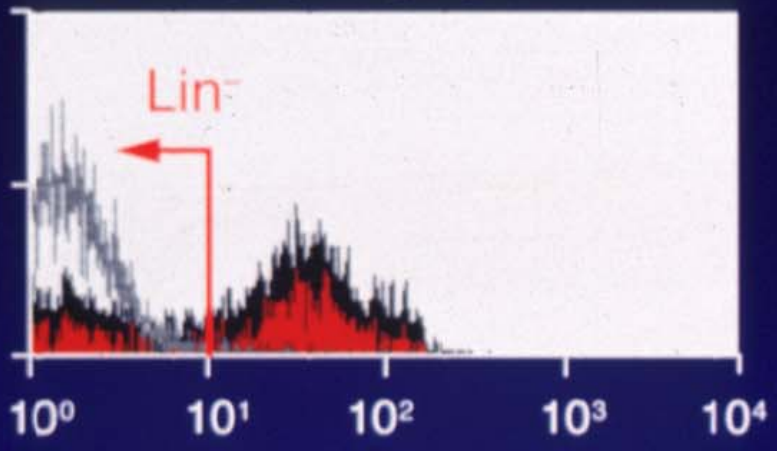
⇓

Regeneration of NK activity from C.B-17 scid bmc: Analysis by the lung clearance assay

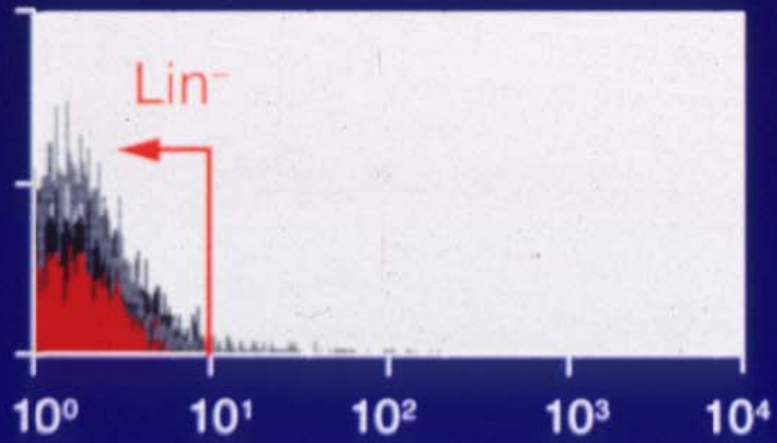
Bone marrow cells transferred (x 10^{6})	Geometric mean (95% CL) % ^{125}IUdR retained in lungs*	
None	21.8	(17.5-27.1)
1 C.B-17 scid	10.0	(6.5-15.5)
1 C.B-17 +/+	8.3	(5.8-12.0)
5 C.B-17 scid	1.9	(1.0-3.4)
5 C.B-17 +/+	1.4	(0.8-2.4)

Multipotent Progenitors: Lin⁻, c-kit⁺, Sca-2⁺

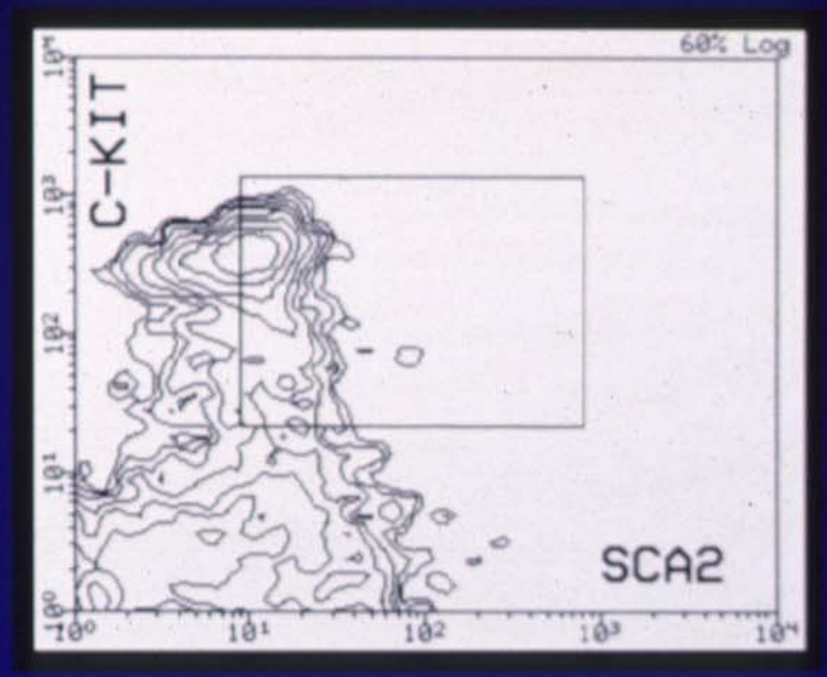
Bone Marrow



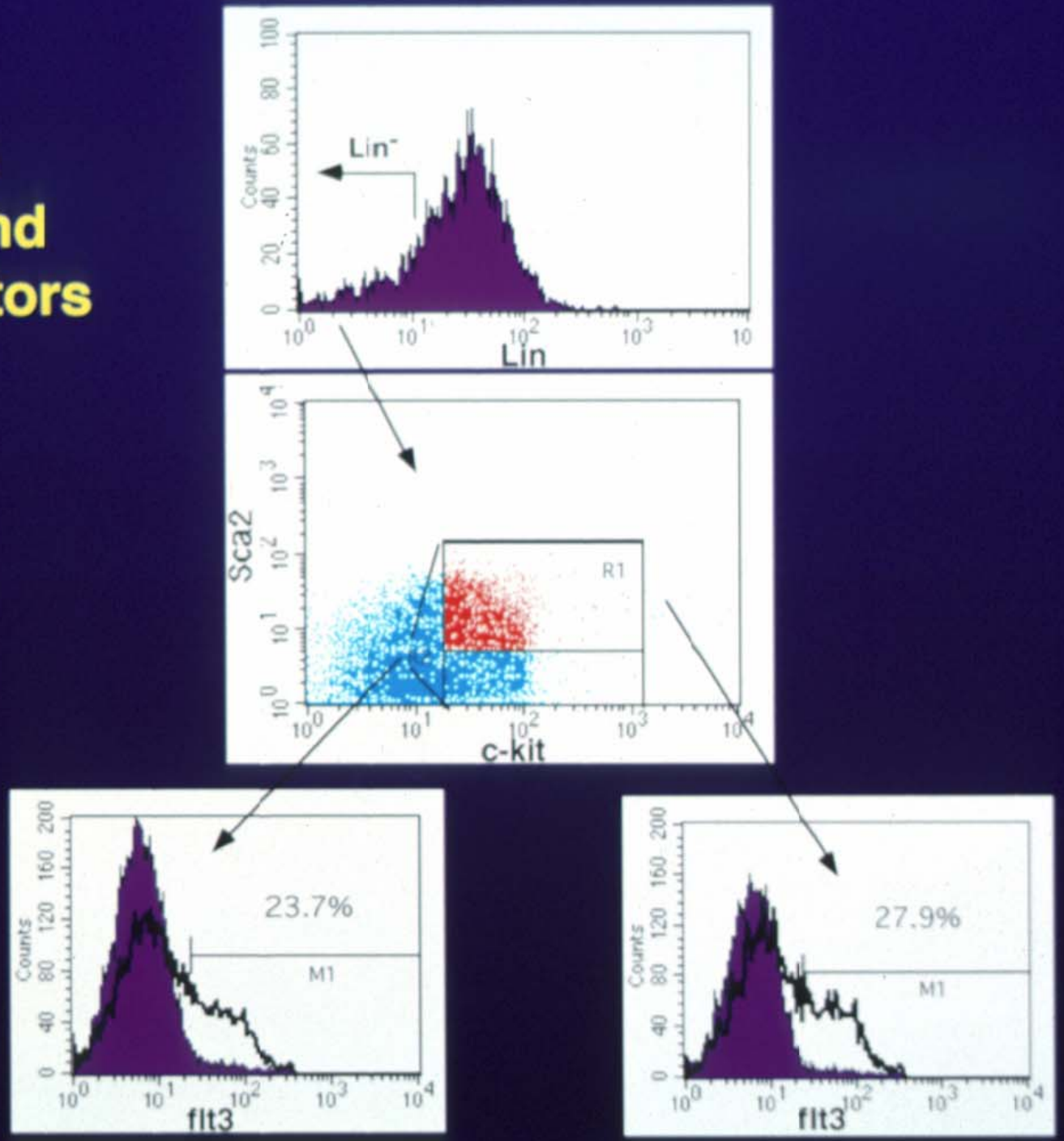
Lin depleted Bone Marrow



Lin⁻



Flt3 expression on Lin$^-$,c-kit$^+$, Sca2$^+$ and Lin$^-$, c-kit$^+$ progenitors



Rh123 retention by NK progenitors

Group		% IUdR Retention geometric mean (95% confidence limits)	
No bmc		18.1	(12.1-27.3)
10^6 unsorted bmc	1.4	(0.4-4.9)	
840 Ly6+ Lin- Rh123 ^{low} sorted bmc		13.2	(8.8-19.8)
980 Ly6+ Lin- Rh123^{high} sorted bmc		4.9	(3.2-7.5)
Normal controls		0.8	(0.7-1.0)

Lin- ckit+ Sca2+ Marrow Progenitor cells

Primary culture (1˚)			Secondary culture (2˚)				
Cytokines		Yield*	Cytokines		Yield*	1˚x 2˚	%NK1.1+
IL-15		0.3	IL-15		1.0	0.3	NT
6/7/SCF/15	9.4		6/7-SCF	8.7	81.8	2	
6/7/SCF/15	9.4		6/7-SCF/15	3.8	35.7	85	

*fold expansion

Immature NK Cells (in vitro or in vivo)

Ly49^{neg}, NK1.1^{pos}, Asialo GM1^{pos}, CD94/NKG2^{pos}, IFNα/β Unresponsive

Can lyse Class I^{neg} but not Class I^{pos} Target Cells

⇓

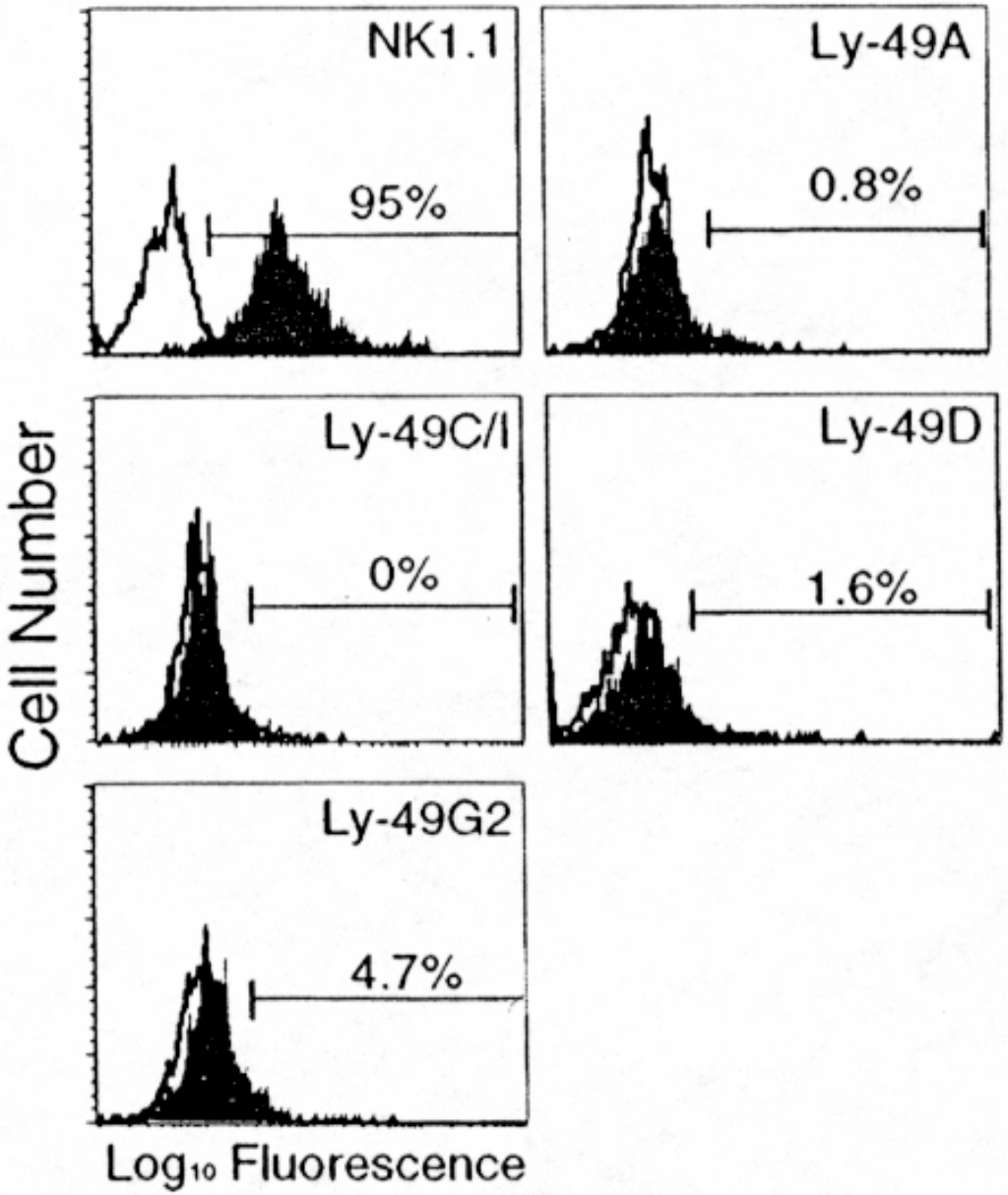
^{89}Sr —| Marrow microenvironment |— Estradiol

⇓

Mature NK Cells (in vitro or in vivo)

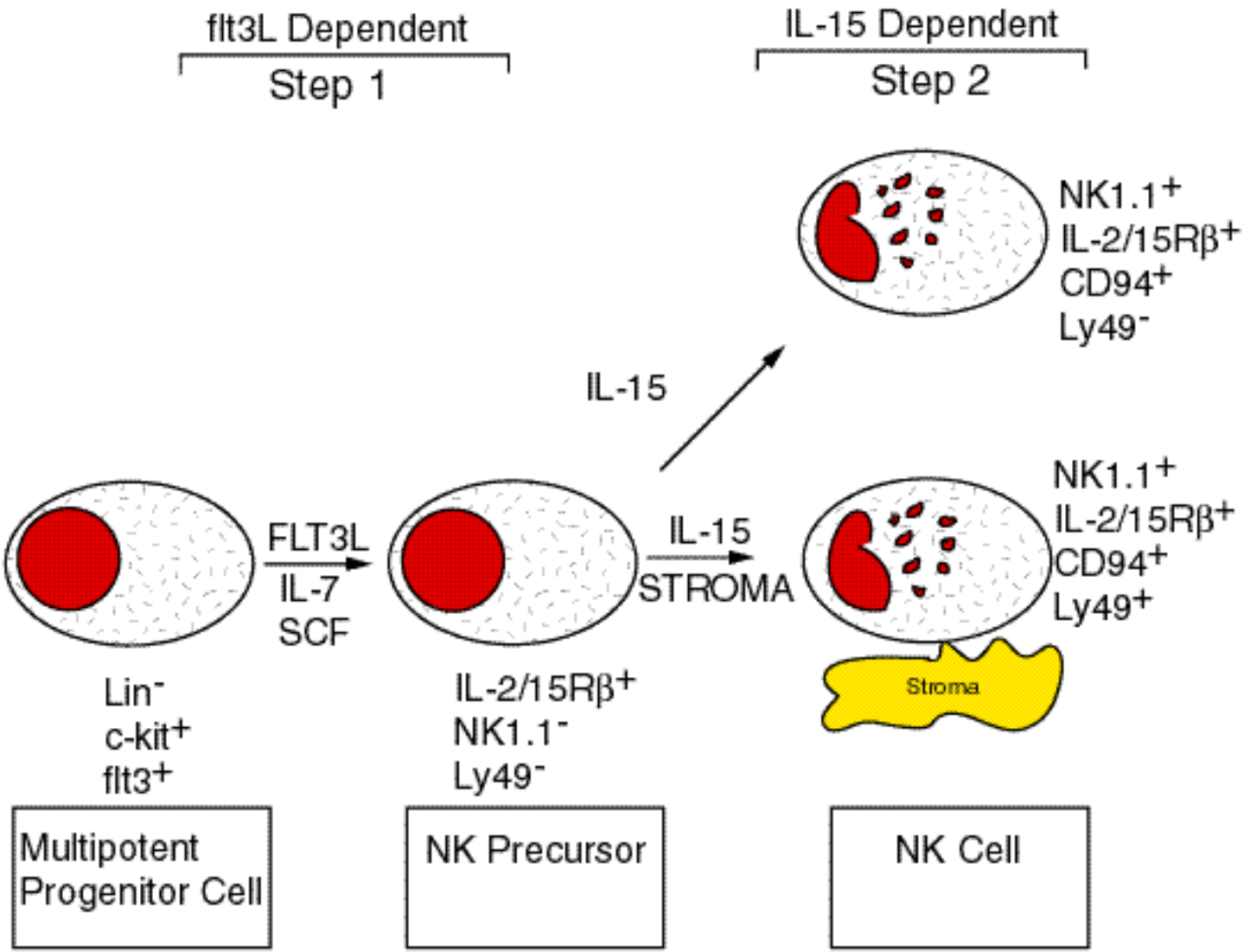
Ly49^{pos}NK1.1^{pos}, Asialo GM1^{pos}, CD94/NKG2^{pos}, IFNα/β Responsive

Can lyse Class I^{neg} and Class I^{pos} Target Cells


NK1.1
95%
Ly-49A
0.8%
Ly-49C/I
0%
Ly-49D
1.6%
Ly-49G2
4.7%
Cell Number
Log10 Fluorescence

Lin- ckit+ progenitors:
Effect of OP9 stromal cells in 2° culture

1° culture	2° culture	(IL2) Stroma	% NK1.1	Ly49G2	Ly49/IC	Ly49D	Ly49A
IL7/SCF/ftl3L (5 days)	6	+	47.4	18.5	10.9	0.4	5.6
	10	+	95.7	35.3	23.6	0.7	6.2
	6	-	72.4	0.3	4.4	0	0.3
	10	-	96.9	0.1	9.9	0	0
Marrow	NK1.1 + CD3 NK cells		100	45.3	62.8	36.1	18.2


flt3L Dependent
Step 1
IL-15 Dependent
Step 2
NK1.1+
IL-2/15Rβ+
CD94+
Ly49-
IL-15
NK1.1+
IL-2/15Rβ+
CD94+
Ly49+
FLT3L
IL-7
SCF
IL-15
STROMA
Stroma
Lin-
c-kit+
flt3+
IL-2/15Rβ+
NK1.1-
Ly49-
Multipotent Progenitor Cell
NK Precursor
NK Cell