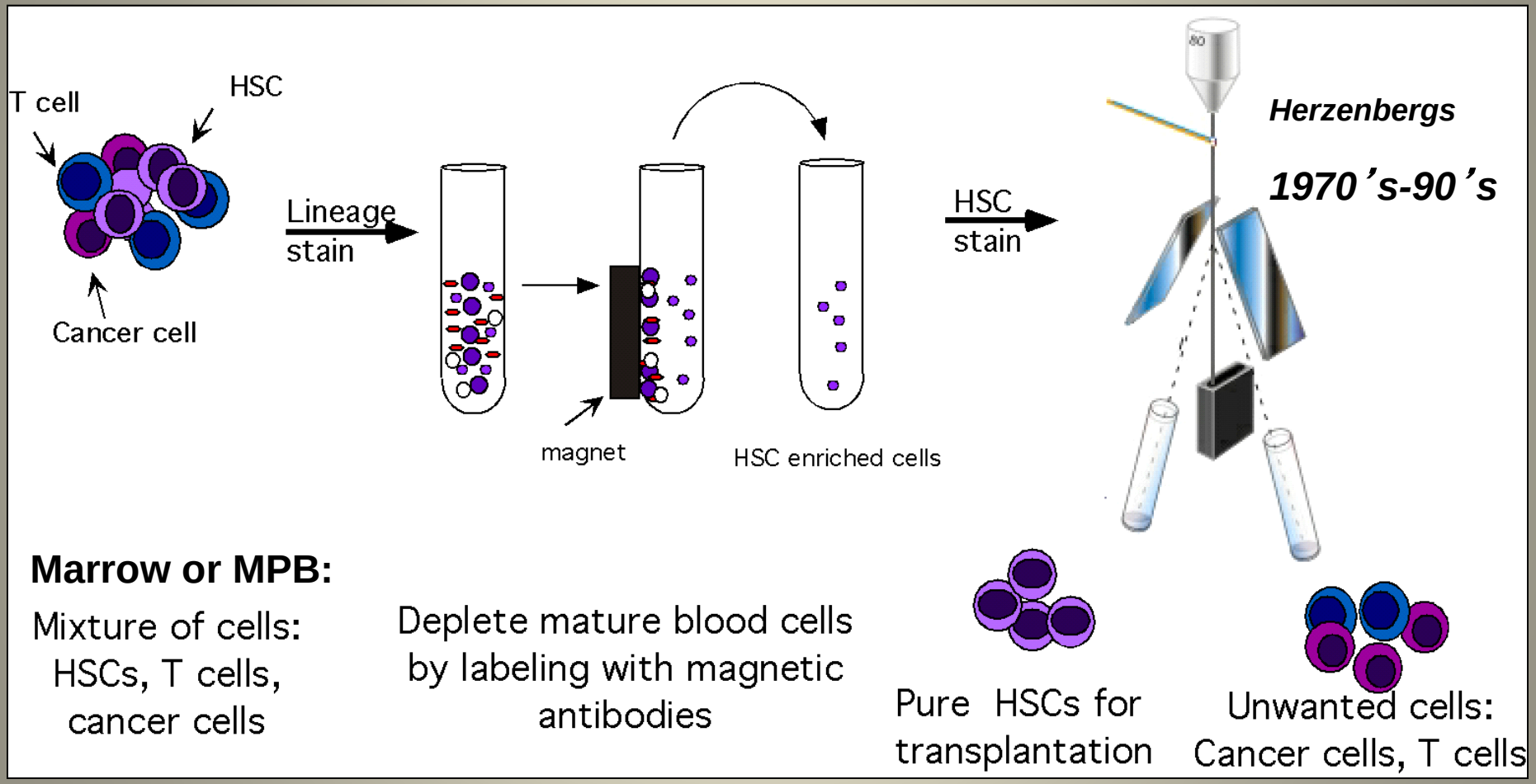
Removal of Contaminating Cancer Cells and T cells from Stem Cell Grafts

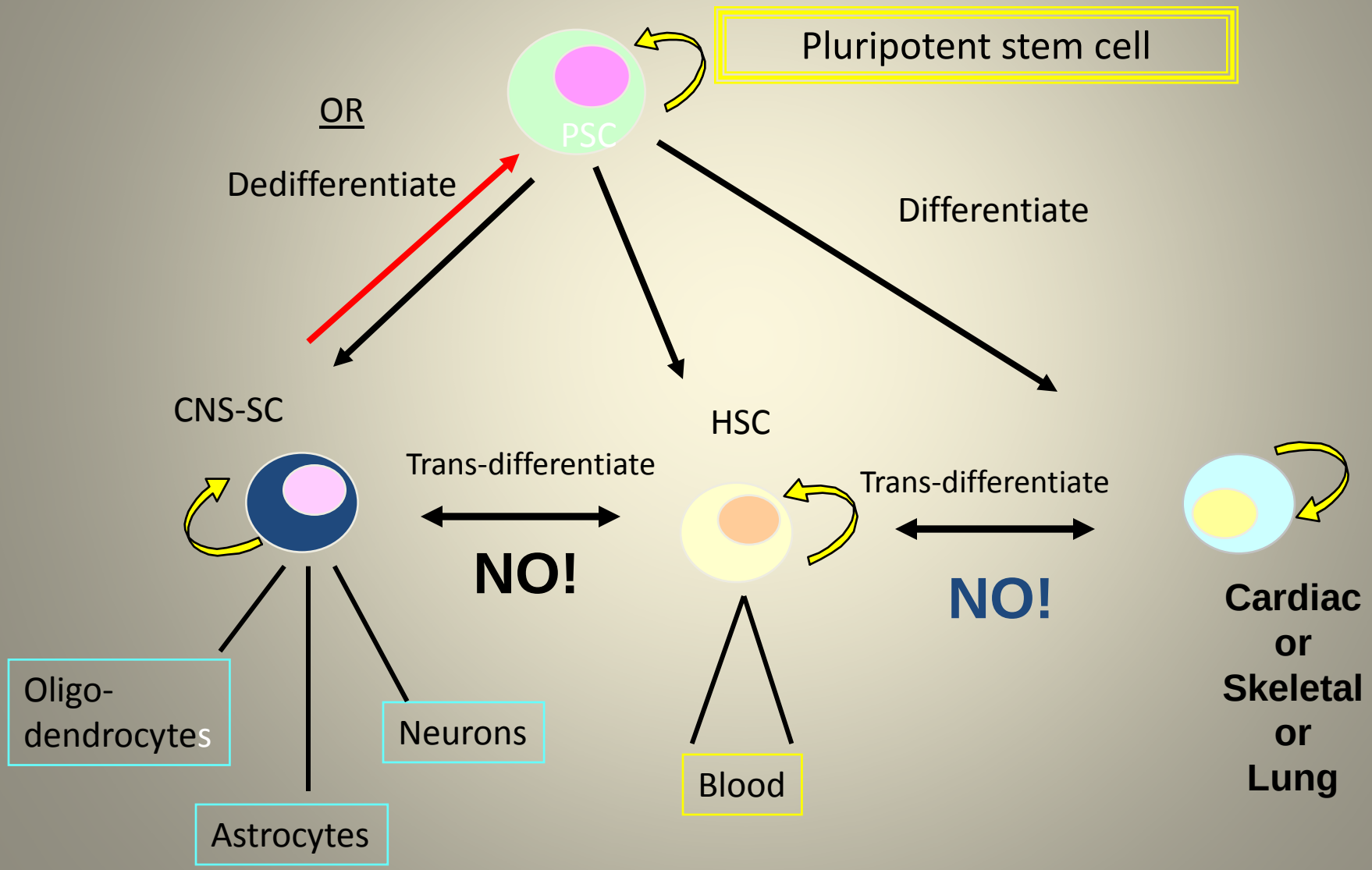


The semantics of hematopoietic stem cells

- Before 1988, they were called bone marrow transplants, later mobilized peripheral blood transplants
- By 2000 all are called hematopoietic stem cell transplants; but they are bone marrow and mobilized peripheral blood transplants

- Pure or enriched HSC transplants lack other cells, including cancer contaminants for autologous transplants, and T cells that cause GvH.
- The fault lies with us; BMT units, journal editors, and reviewers: no reputable journal should publish transplants that are not accurately named.

Can Tissue Stem Cells Transdifferentiate?



Print 02/16/99

Generally accepted requirements to establish that a discovery is a scientifically acceptable fact

- **The findings are published in a peer-reviewed journal wherein the peers are experts in the field…but that is not enough**
- **The findings are replicated in several independent laboratories..but that is not enough**
- **The principles that derive from the findings lead to new kinds of experiments and new approaches, both of which independently validate the original findings and the conclusions from the findings…but that is not enough**
- **To use the findings in human clinical medicine, the transplantation must be robustly regenerative.**

CLAIM: Umbilical cord blood cells are pluripotent

- Transplantation of UCB cells cures not only blood diseases, but a number of non-hematopoietic diseases such as cerebral palsy, heart septal defects, pulmonary fibrosis, etc.
- Our results: UCB and UCB HSC make blood and only blood

Reconstitution of sublethally irradiated NSG pups transplanted with human CB HSC

16 weeks post-transplant	Number of CB samples	Median Cells Injected	Number of Mice Injected	% Mice with Human Engraftment	Average % Human Chimerism	Average % Myeloid Cells	Average % Lymphoid Cells
PB	10	1000	20	100%	6.1%	7.4%	69.2%
BM	10	1000	20	100%	29.5%	26.7%	68.2%

Tissue	# sections examined	~# cells examined	# CD45-human cells
Brain	20	4000000	0
Liver	20	500000	0
Kidney	10	400000	0
Gut	20	200000	0
Skeletal muscle	10	1000	0
Cardiac muscle	10	2500	0
Lung	10	20000	0

Cultured bone marrow stromal cells are often called mesenchymal stem cells with *no evidence of the presence or frequency of self-renewing cells* that include in their clonal progeny mesenchymal progeny such as adipocytes, osteocytes, and chondrocytes; or bone marrow niche cells. Their use in cell transplants are a form of cell therapy that *does not include regeneration as an out*come, but are believed to ameliorate inflammatory processes.

CLAIM: VSEL CELLS ARE SMALL CELLS IN BLOOD AND BONE MARROW AND RESTORE HEMATOPOIESIS AND ARE PLURIPOTENT: RATAJSZACK ET AL

Background of VSELs

Masanori Miyanishi and Yasuo Mori and IW

VSEL don’t exist in mice as published

Accepted steps to clinical therapies

Discovery that is independently replicated

Preclinical and published proof of principle

Application to national or other body for successive trials to demonstrate safety and efficacy, e.g., FDA in US

Approval from that body that the trial was safe and efficacious

Toronto

ISSCR Response



Patient Handbook, 2008

— 7 languages

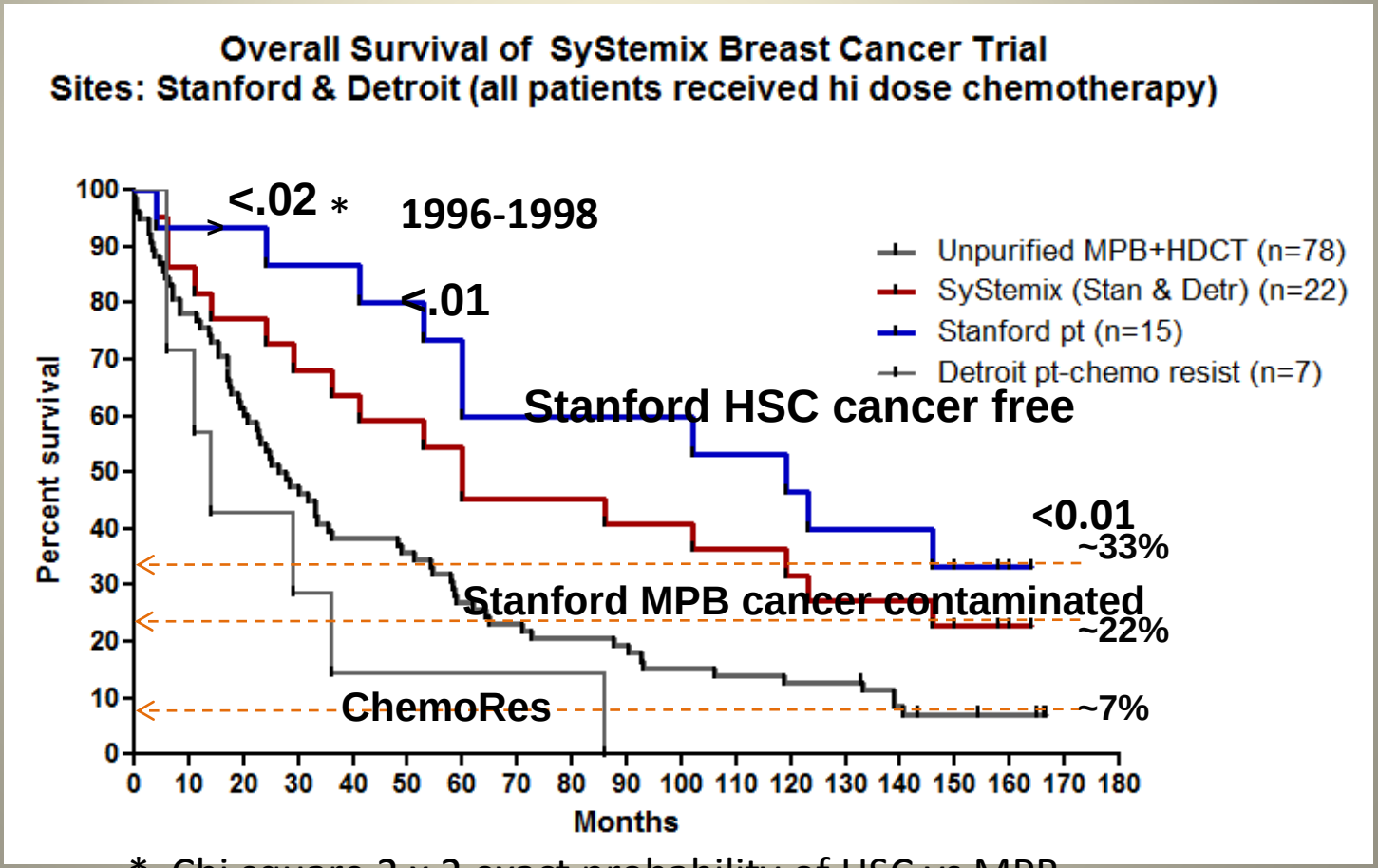


A Closer Look at Stem Cell Treatments, 2010

— English and Spanish

Cancer Free Stem Cell Grafts Improves Survival

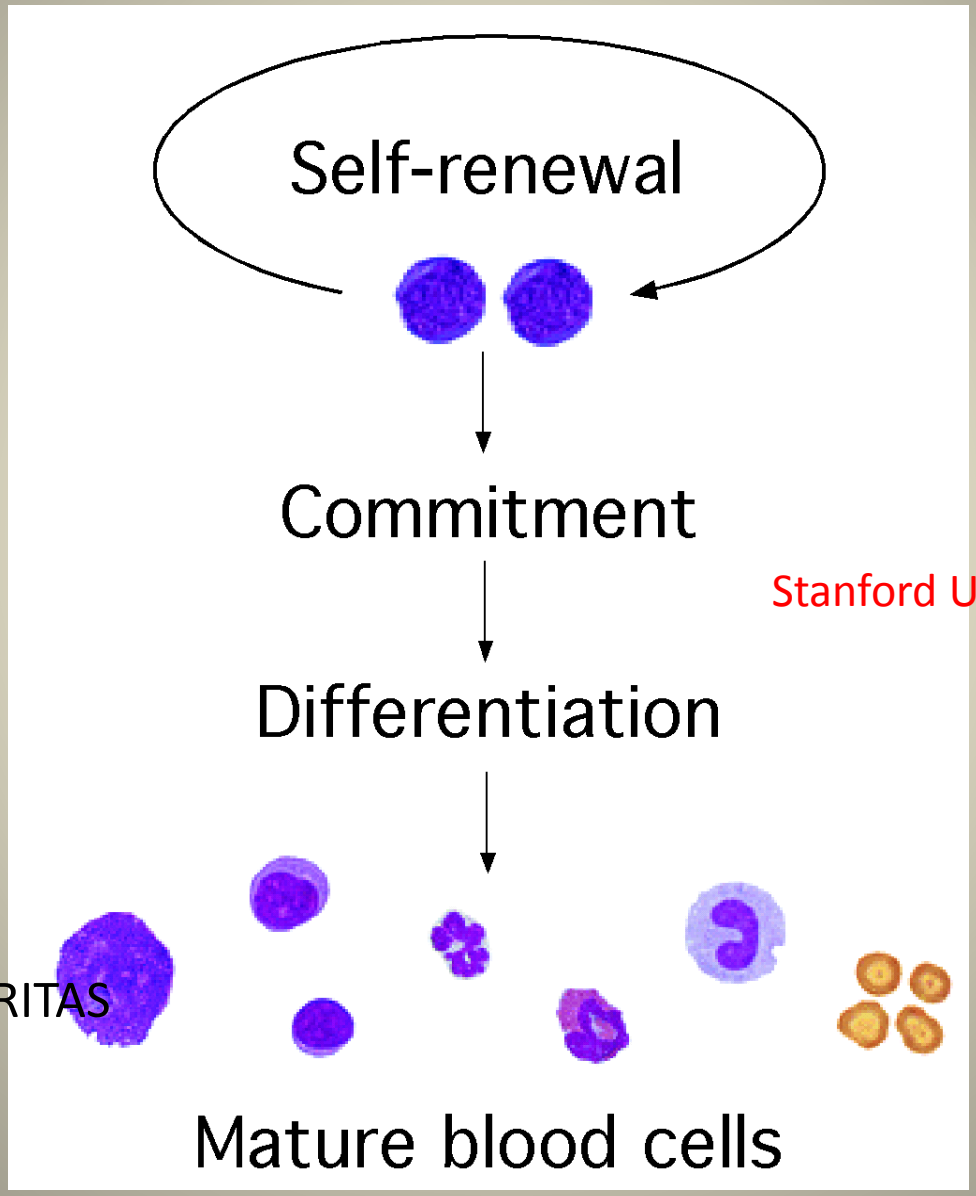
- Purified HSC show 3-fold higher survival vs non-purified MPB
- Stage 4 metastatic patients – failed all other therapies



* Chi square 2 x 2 exact probability of HSC vs MPB

A. Mueller, I.Weissman, R. Negrin, and J. Shizuru, 2011

Blood-Forming Stem Cells



1 Development

2 Maintenance

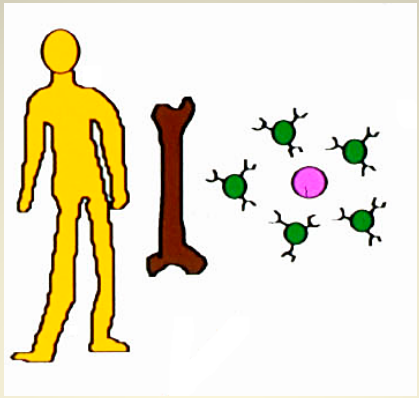
3 Malignant transformation

Ludwig Center at Stanford University

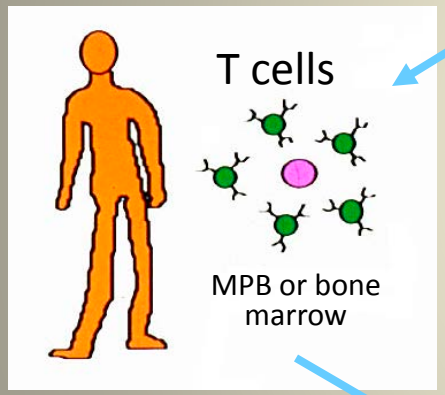
IN VIVO VERITAS

Healthy Donor Transplant

Healthy Donor

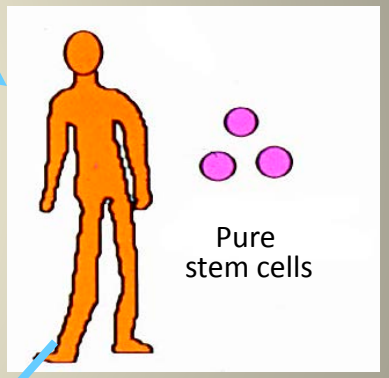


Recipient



RADIATION DAMAGE OR THERAPY

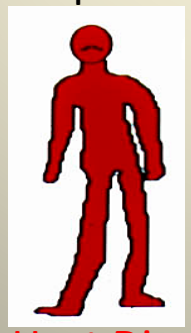
Recipient



GvH

- Dependent on Immunosuppressants
- Risk of Infections (fungus, bacteria)

Recipient



Graft vs Host Disease

Recipient

No GvH

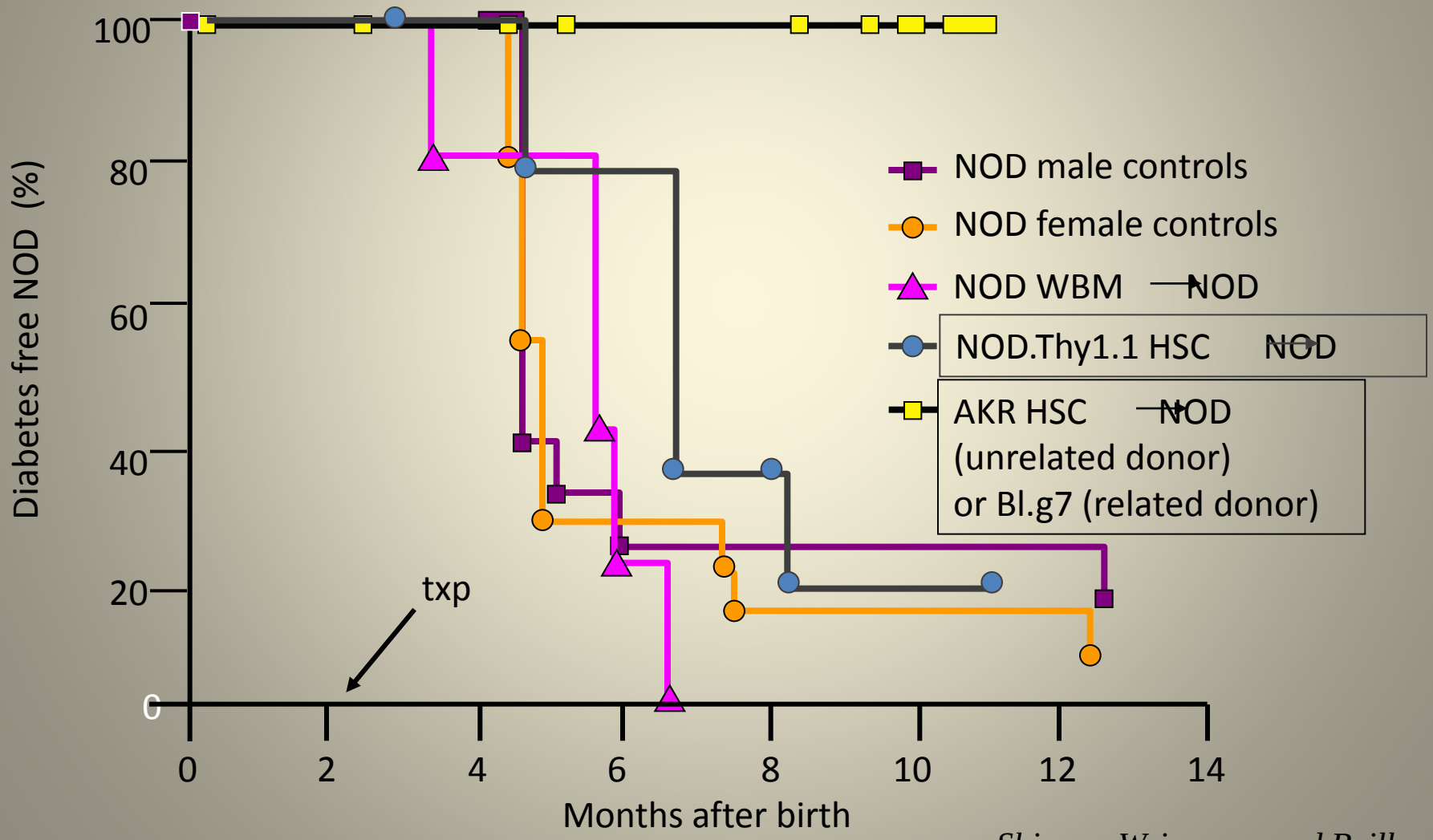


Healthy

- Donor blood-forming and immune system
- Induce permanent transplant tolerance
- Reverse genetic autoimmune disease

Shizuru, Beilhack, Weissman

Rescue of Diabetic Mice with HSCs

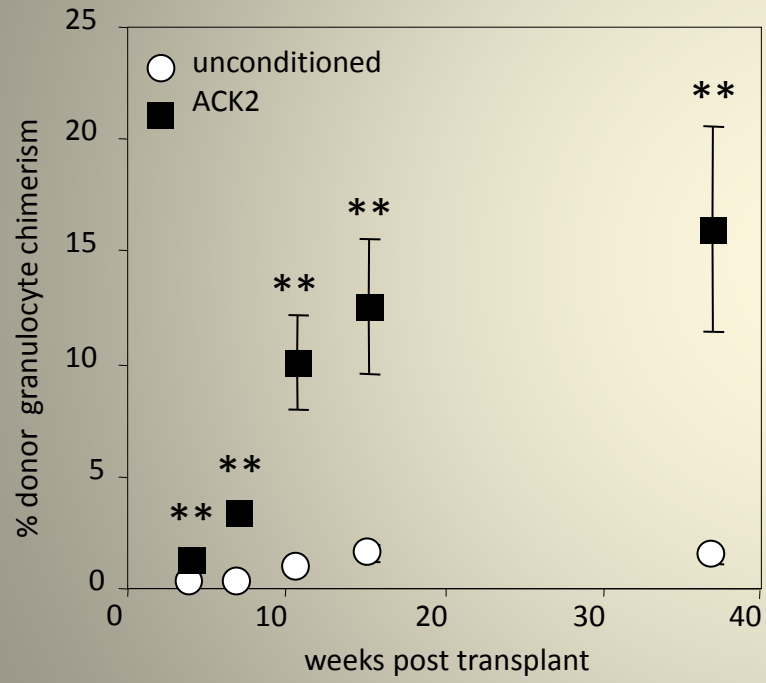


Shizuru, Weissman, and Beilhack

Anti-ckit Mab that deplete HSC condition the host for congenic HSC engraftment

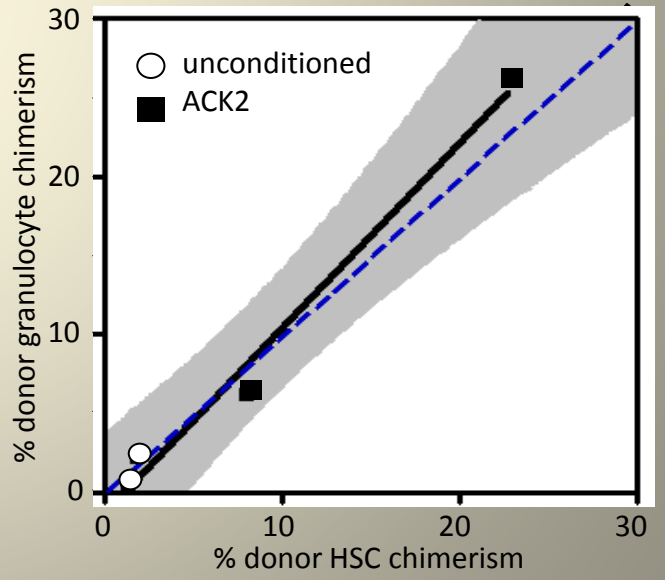
-Conditioned Rag2GCDKO mice with 500µg ACK2 IV

-Day 9 transplanted with 5000 LT HSCs (KLS $CD34^{-}$ $CD150^{+}$)



ACK2: anti-ckit that blocks SLF signal
Shin-Ichi Nishikawa

Donor granulocyte chimerism accurately measures donor BM HSC chimerism

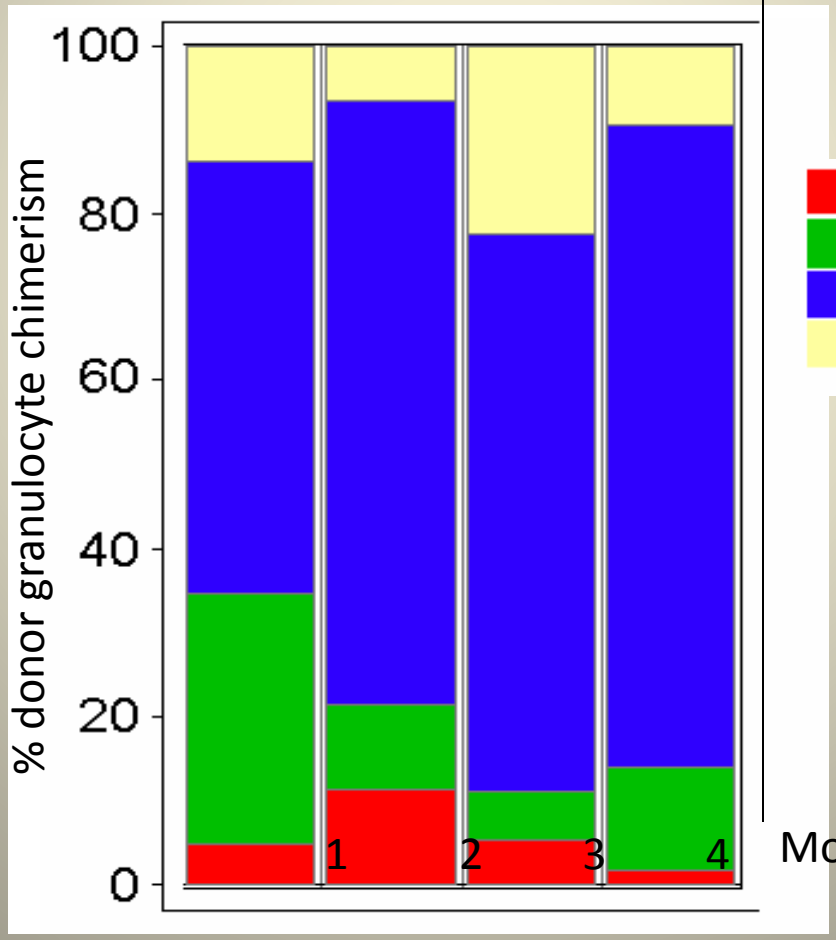


Czechowicz, Bhattacharya et al

Antibody clearance of host HSC to enable cure of SCID mice with healthy HSC: no radiation

D0 ACK2 | D7 1st | D14 ACK2 | D21 2nd | D28 ACK2 | D35 3rd

3x ~5000 LT HSCs

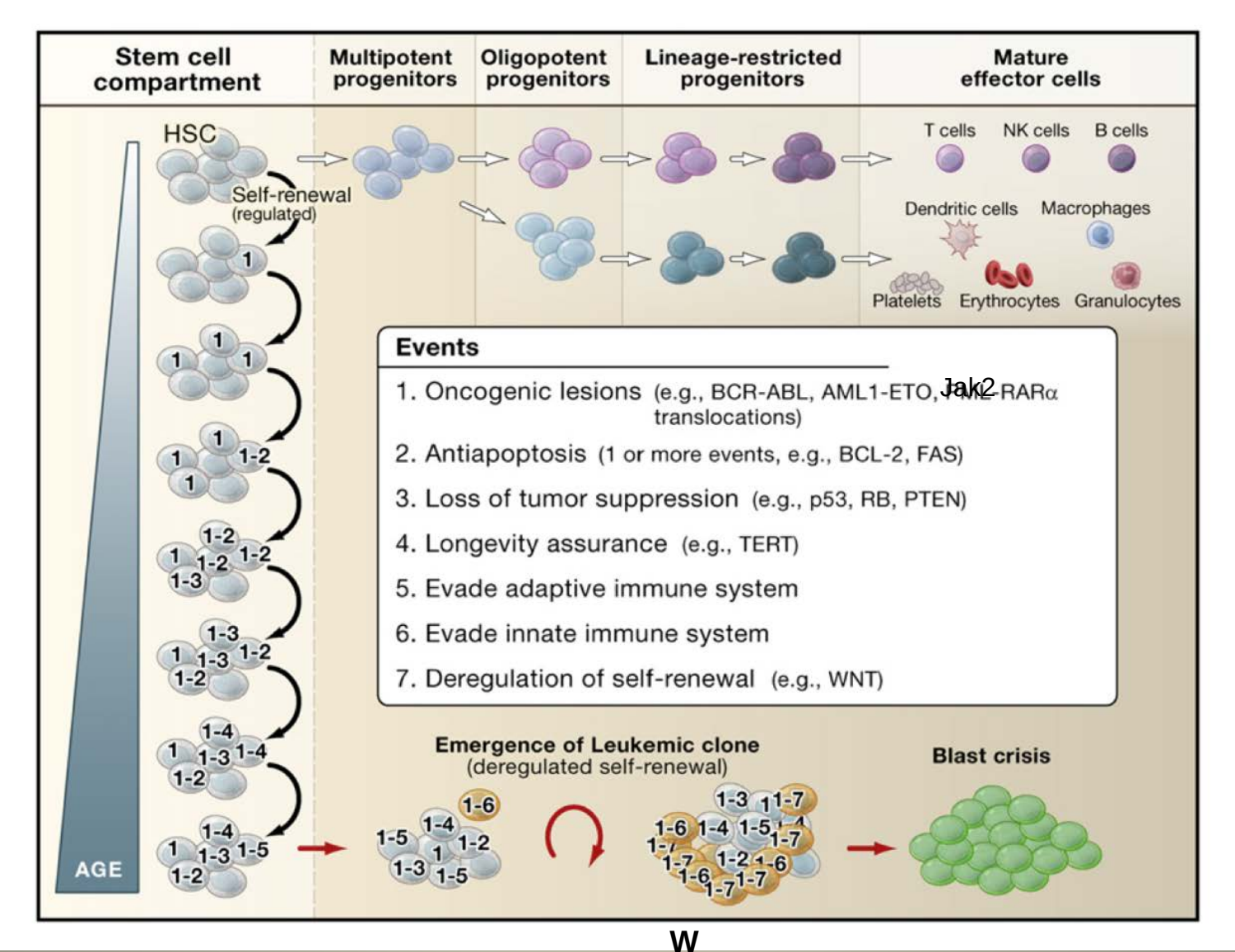


Czechowicz, Bhattacharya et a

HSC Transplants In The Future

- Limited or no use of radiation or cytotoxic drugs
- Targeted removal of host HSC, T cells, and NK cells with Mabs
- In the far future [>5-10 years], ES or iPS derived HSC will be co-transplanted with other tissue stem cells from the same donor line to create tolerance, stop auto-immune destruction and repair the lesions and/or replace lost cells

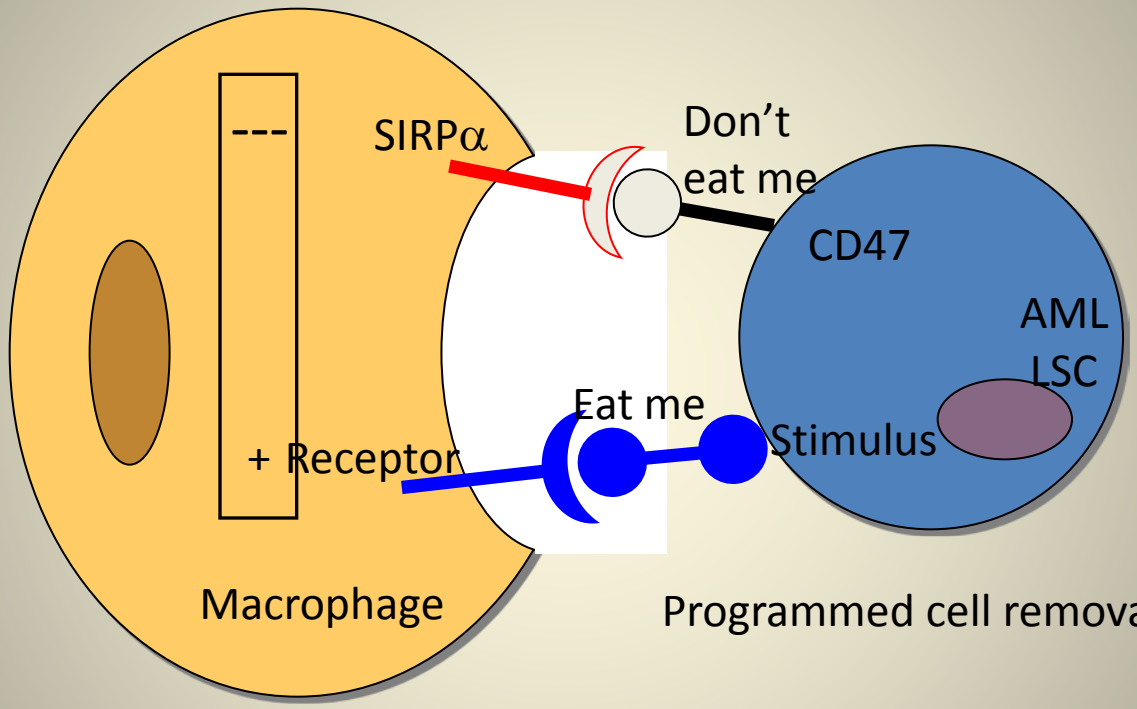
Cell of origin – progression to leukemia



We

CD47 was discovered as a marker of aging RBC by Oldenborg. We found it on m/h AML LSC

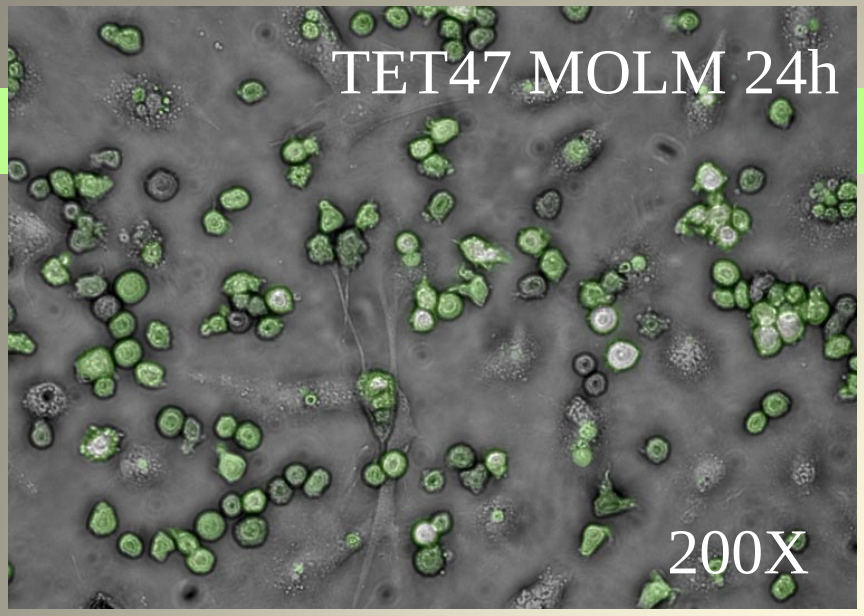
Hypothesis: Increased expression of CD47 on myeloid leukemia cells contributes to pathogenesis by facilitating evasion of phagocytosis



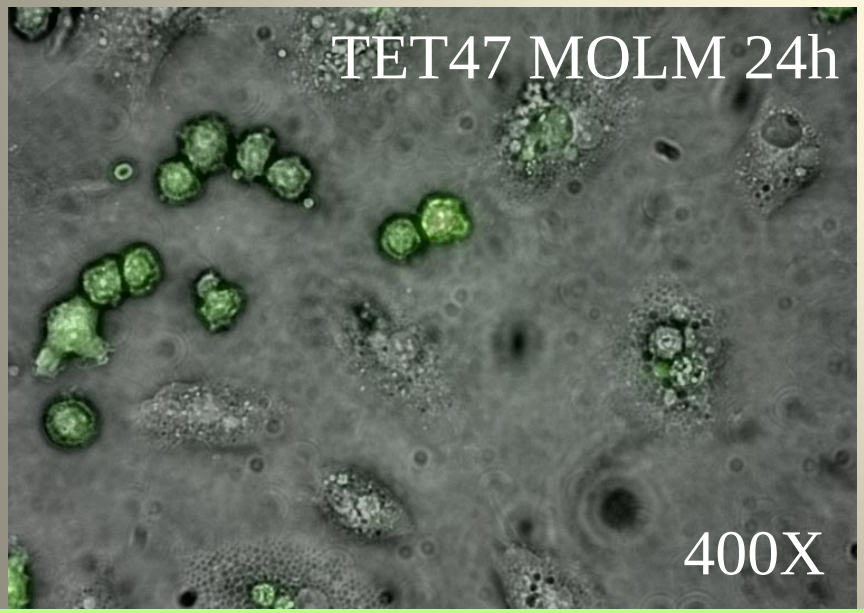
Net Result: No Phagocytosis

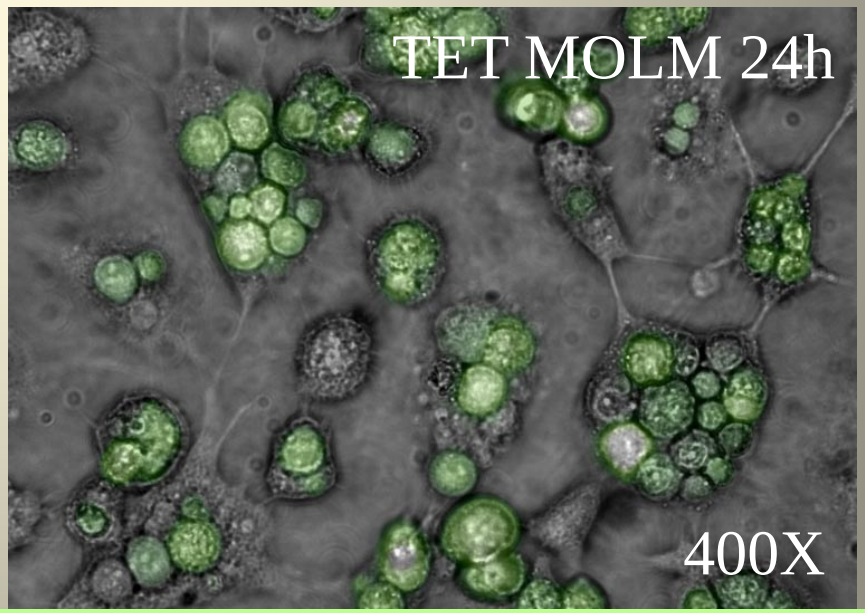
Prediction: Increased expression of CD47 on human AML is associated with a worse clinical outcome

Traver and IW 1998; Jaiswal, Majeti, Chao,and IW 2008.

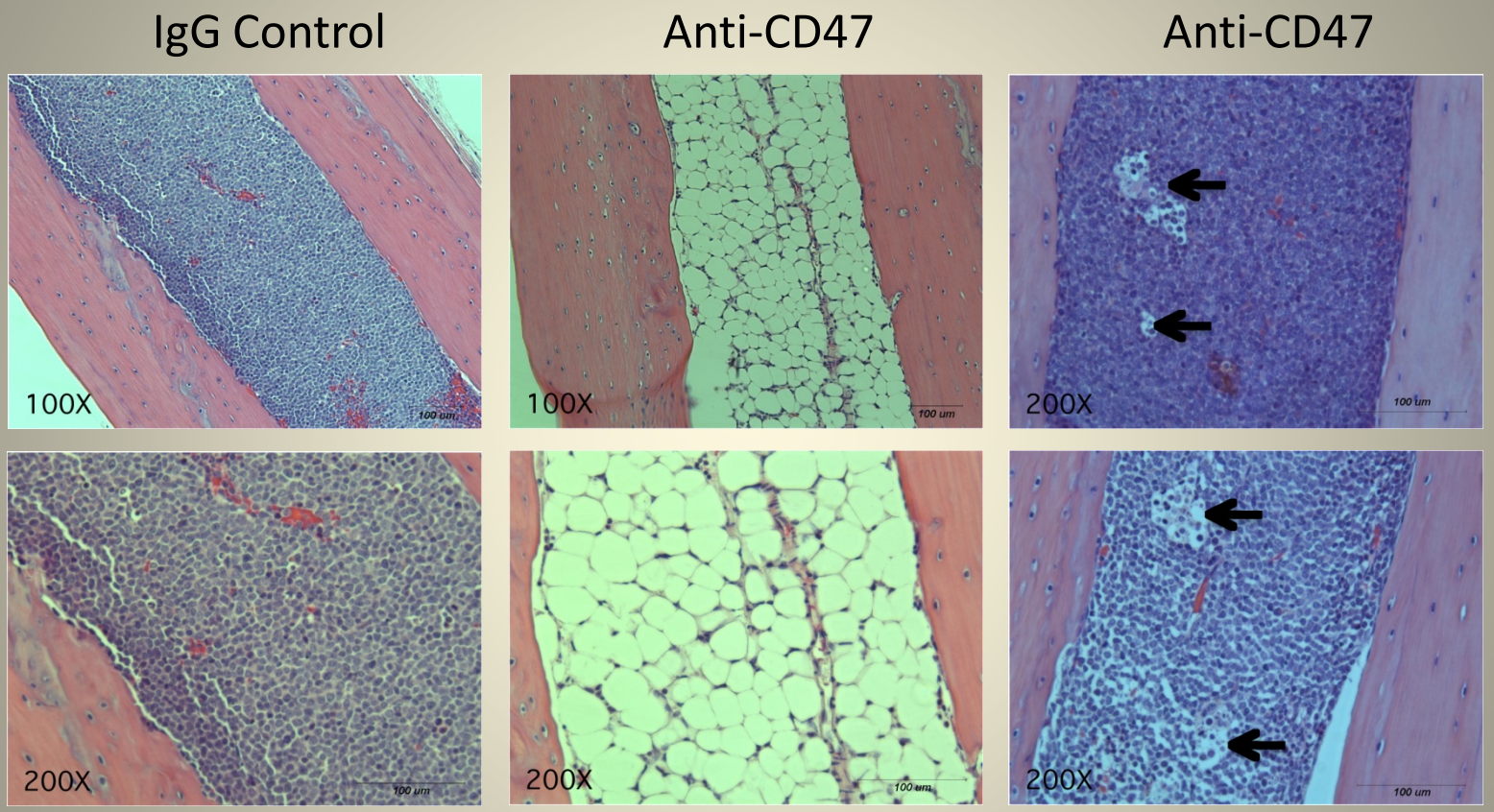

TET47 MOLM 24h
200X


TET MOLM 24h
200X


TET47 MOLM 24h
400X


TET MOLM 24h
400X

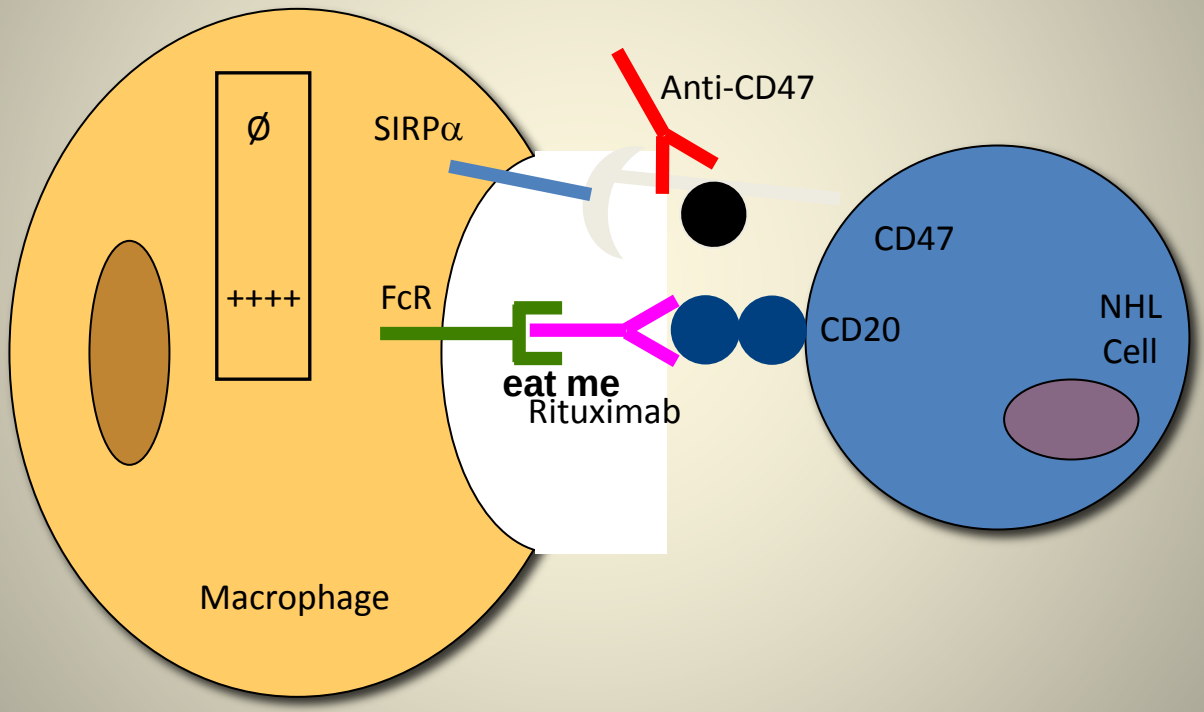
Anti-CD47 Antibody Depletes AML in the Bone Marrow



Mark Chao, Majeti et a

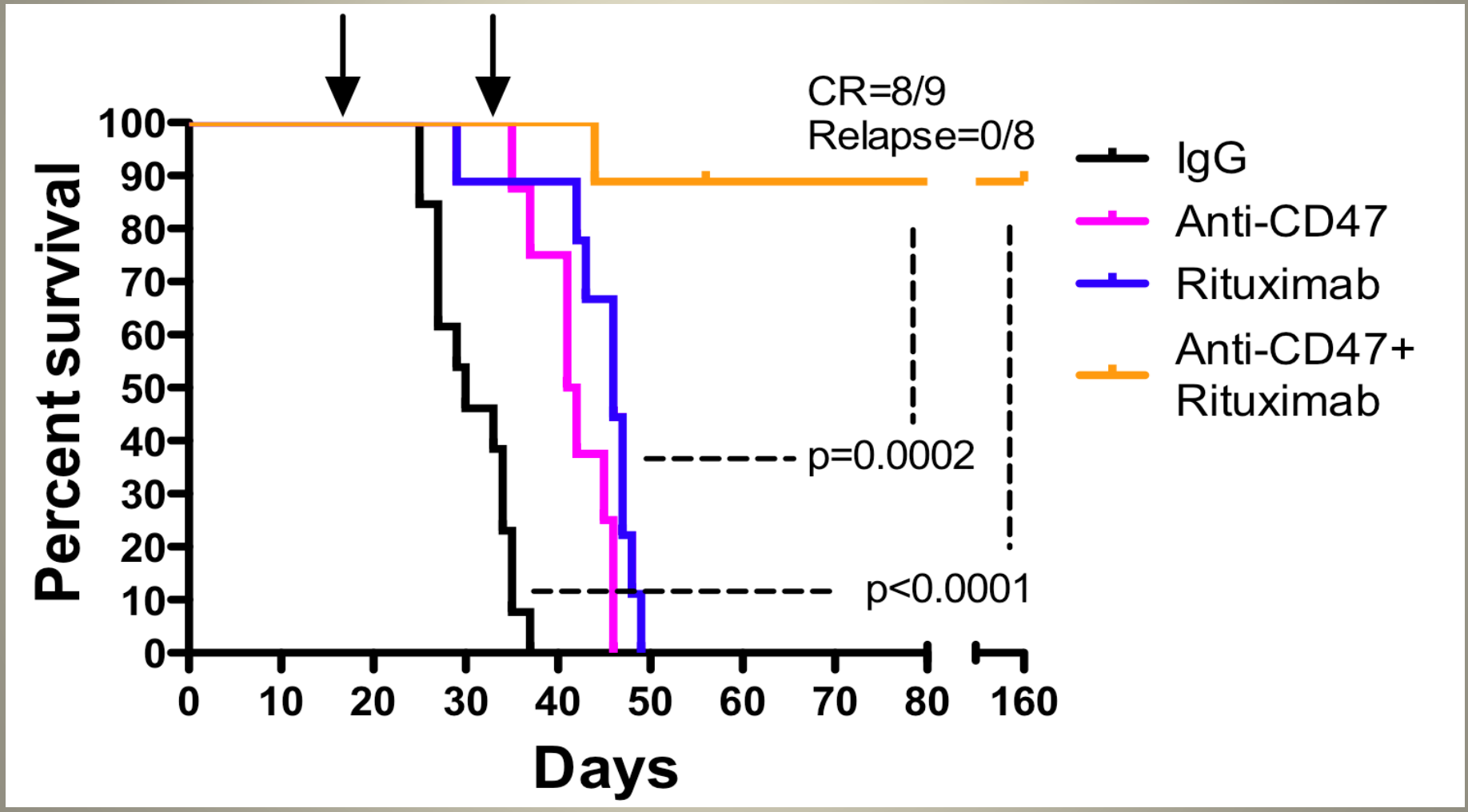
Model for Synergy of Anti-CD47 with Rituximab

Investigate the combination of anti-CD47 antibody with rituximab for synergy in eradicating NHL.



Net Result: Potent Phagocytosis

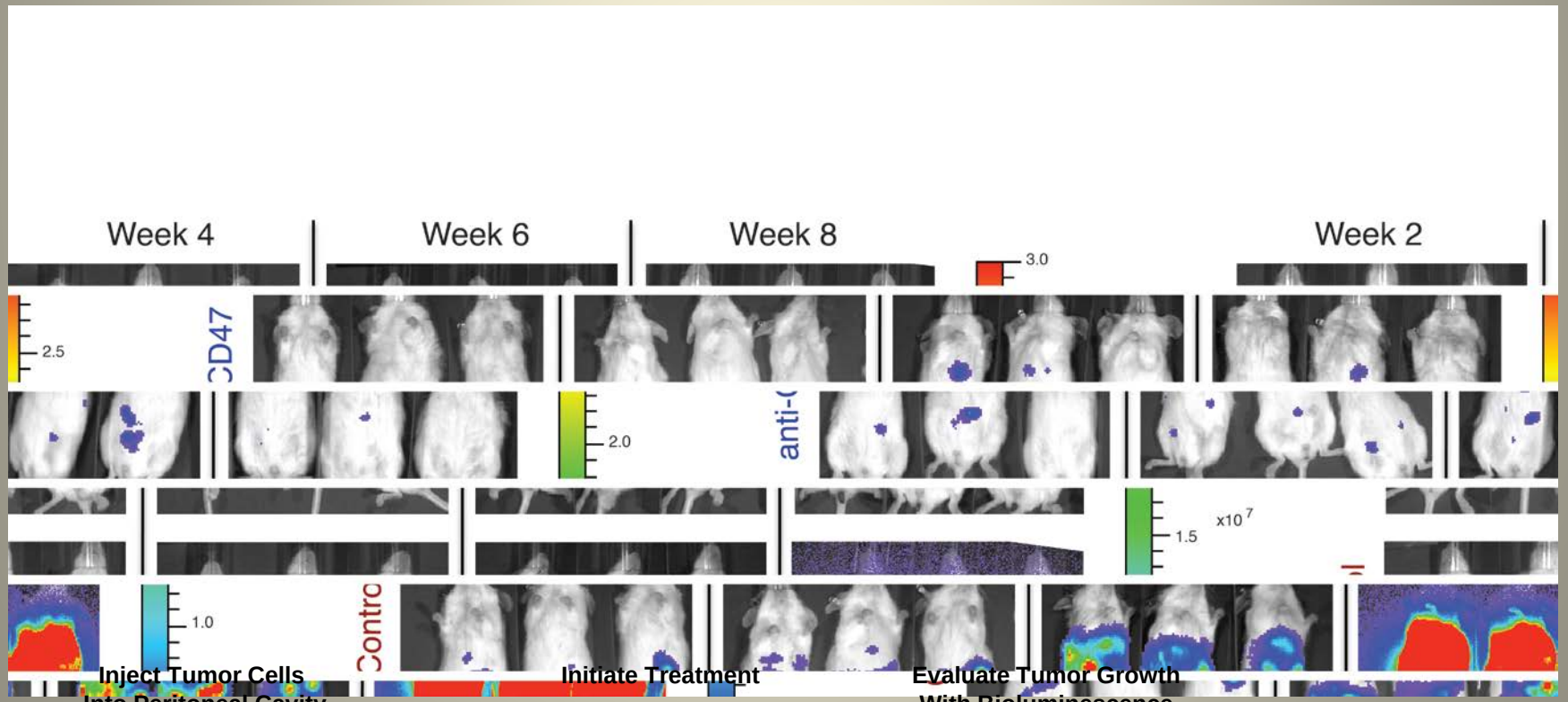
Combination antibodies eliminate a primary human lymphoma from immune deficient mice



Chao, Majeti, Alizadeh, and Weissman, 20

Anti-CD47 Antibodies Inhibit Growth Of Xenotransplanted Patient Tumors

Ovarian Cancer



Human primary solid

tumor xenografts

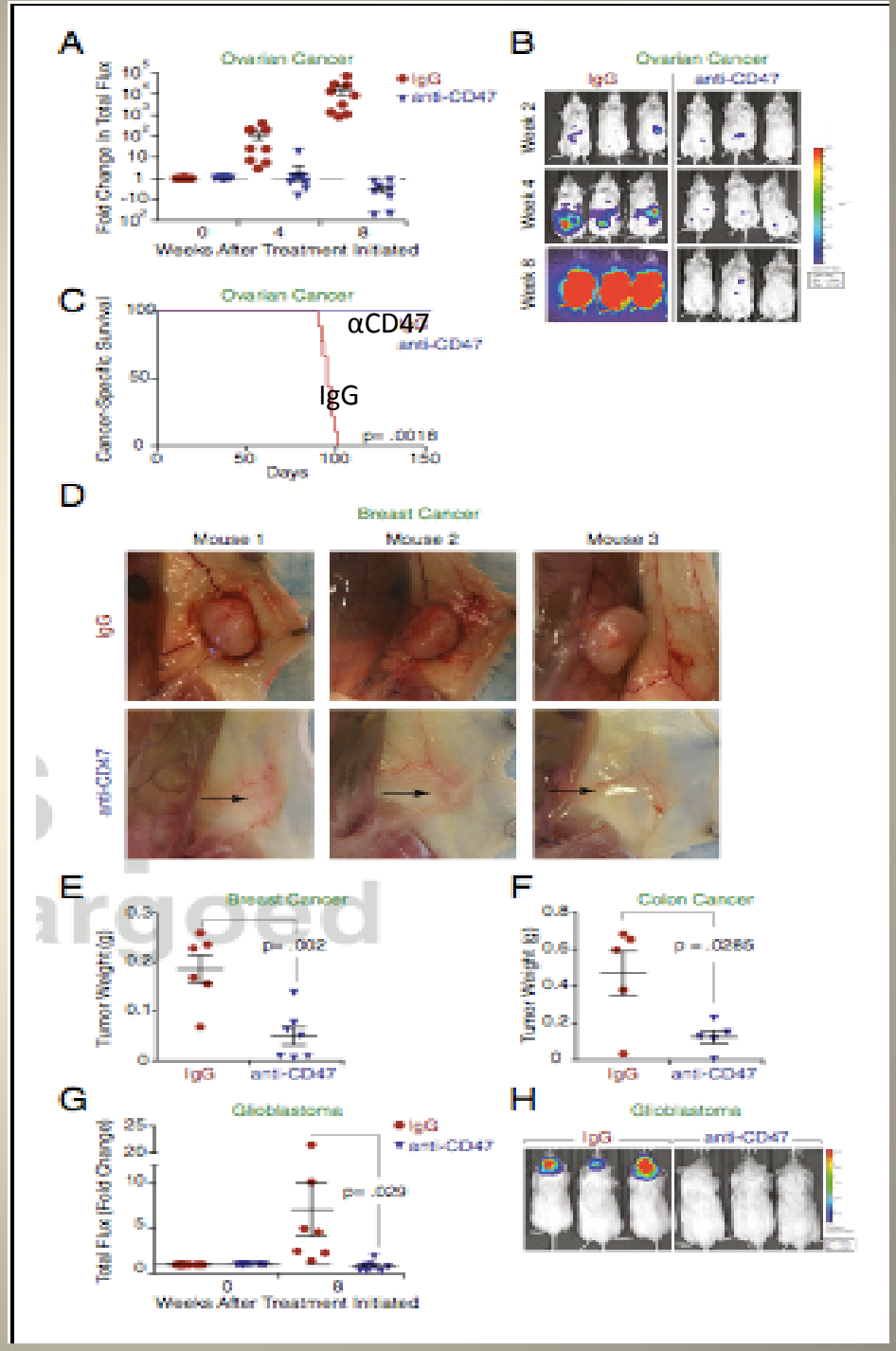
treated with anti-CD47

mabs beginning 2-4

weeks after

transplantation.

Jens Volkmer, Stephen Willingham, Sidd Mitra, Matt van de Rijn, Sam Chehshier Robert Chin, Ferenc Scheeren, Mike Clarke and IW



Investigation and Targeting of CD47 in Human Cancers

Breast	Glioblastoma
Ovarian	Medulloblastoma
Bladder	Oligodendroglioma
Pancreatic	Hepatocellular Carcinoma
Colon	Gastric Cancer
Prostate	Multiple Myeloma
Lung	Chronic Myeloid Leukemia
Kidney	Acute Myeloid Leukemia
Leiomyosarcoma	Non-Hodgkin's Lymphoma
Head & Neck	T-Acute Lymphoblastic Leukemia
Melanoma	B-Acute Lymphoblastic Leukemia

What stage of stem cell vs progenitors carry cancer stem cells ?

Patient Tumor Xenotransplantation Model
Treatment Data Available

The 5F9g4 antibody is humanized and headed to an AML trial and an all comers solid tumors in California and the UK.

- Paresh Vyas and Alan Burnett have an MRC grant to do part of the trial: single payer.

- IW and Ravi Majeti have a California Institute of Regenerative Medicine grant of $20 M to take the antibody through phase 1/2 trials. Disease Team of Maureen Howard, Susan Prohaska, Jens Volkmer, Jie Liu, students, MD/PhD trainees to do it.

Lokey Stem Cell Institute at Stanford



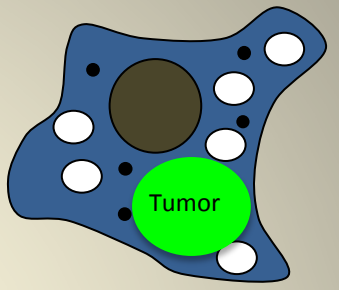
Institute for Stem Cell Biology and Regenerative Medicine

Ludwig Center at Stanford

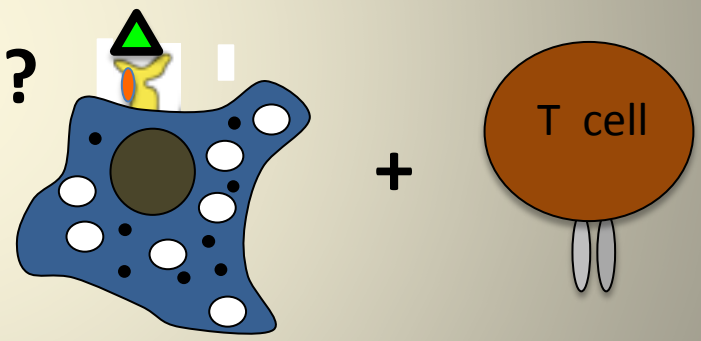
Supported grants from NCI, NHLBI, Calif Inst Reg Medicine, Lacob Foundation, Siebel SCI

Antigen Presentation Assay

Day 0:
10K RFP macrophages +
10K DLD1-cOVA-GFP cells

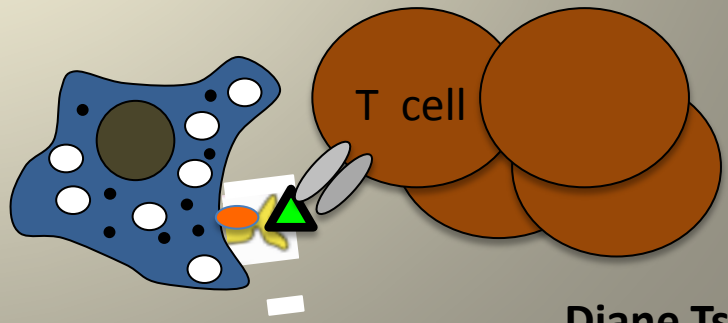


Add Ab:
-anti-CD47 (B6H12)
-anti-CD47 (2D3)
-mouse IgG1



Day 1:
Add 50K CFSE-labeled CD4+ magnetic bead-enriched OT 2 T cells to cultures

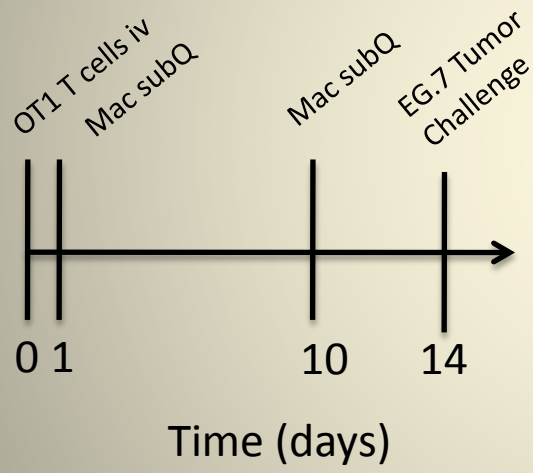
Day 5:
Analysis of CFSE dilution in OT2 T cells

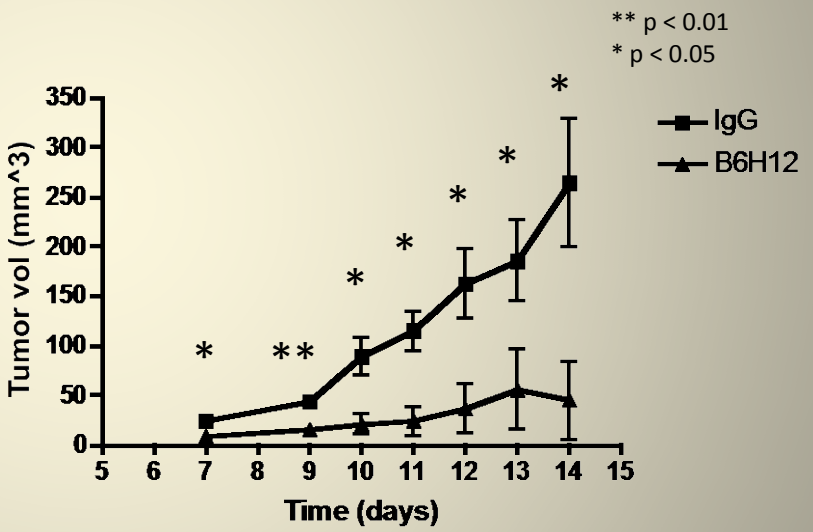


Diane Tseng

Following anti-CD47-mediated phagocytosis of cancer, macrophages prime an effective anti-tumor CD8 T cell response *in vivo*

1) CD4 T cells
2) CD8 T cells
3) Tumor control

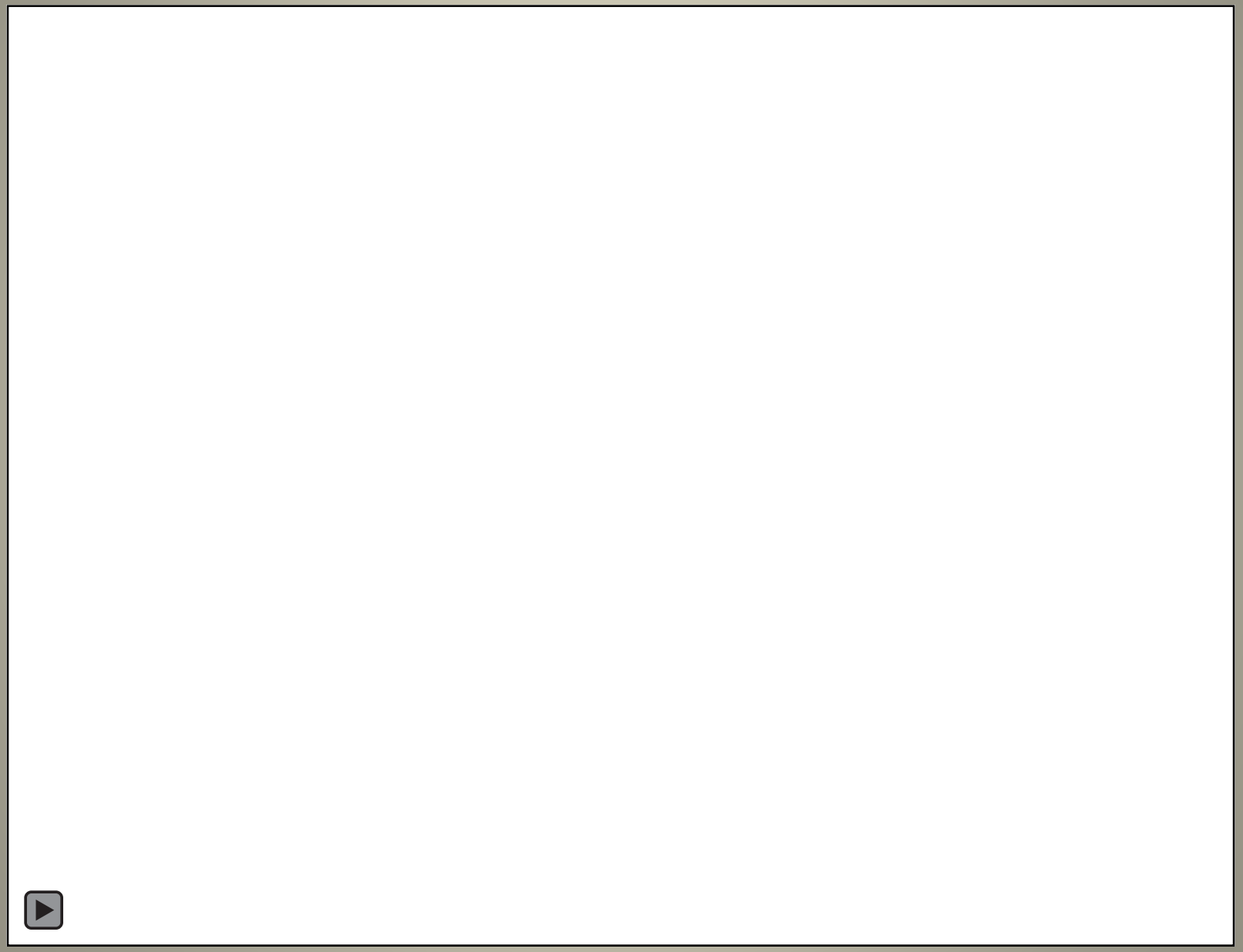




Conclusion: Following anti-CD47-mediated phagocytosis, macrophages prime an effective T cell response that protects against tumor challenge

Diane Tseng

Maximal phagocytosis is observed with FD6-hIgG4

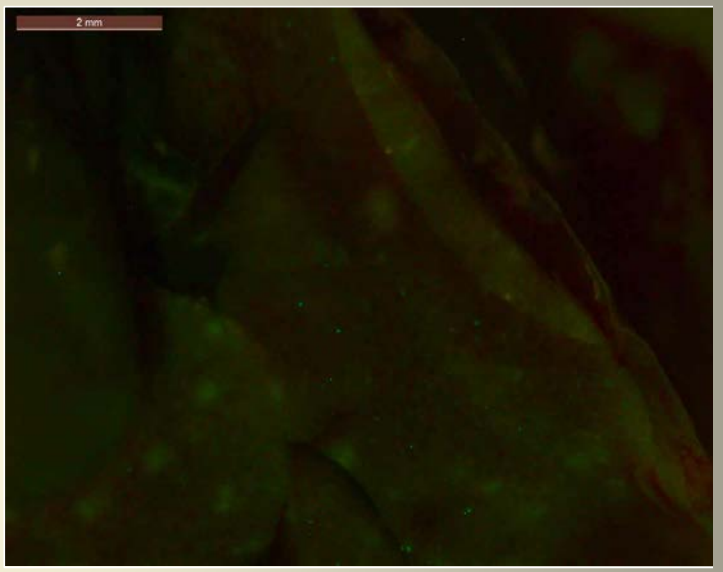


Hu5F9-G4 inhibits Tumor Growth & eliminates Metastases

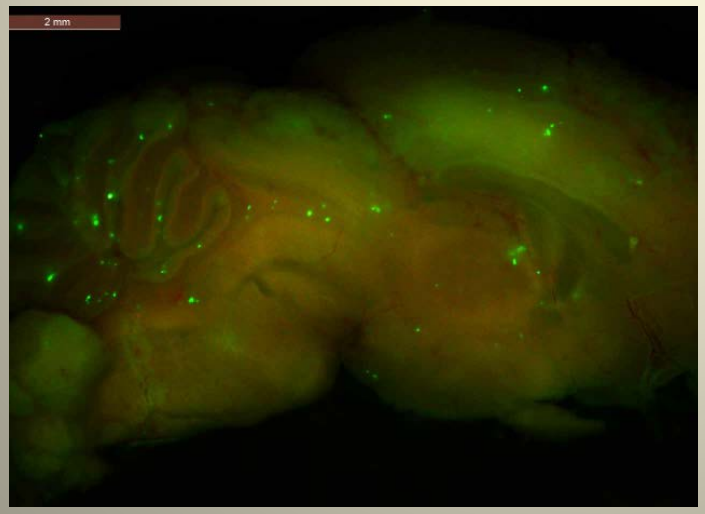
PBS

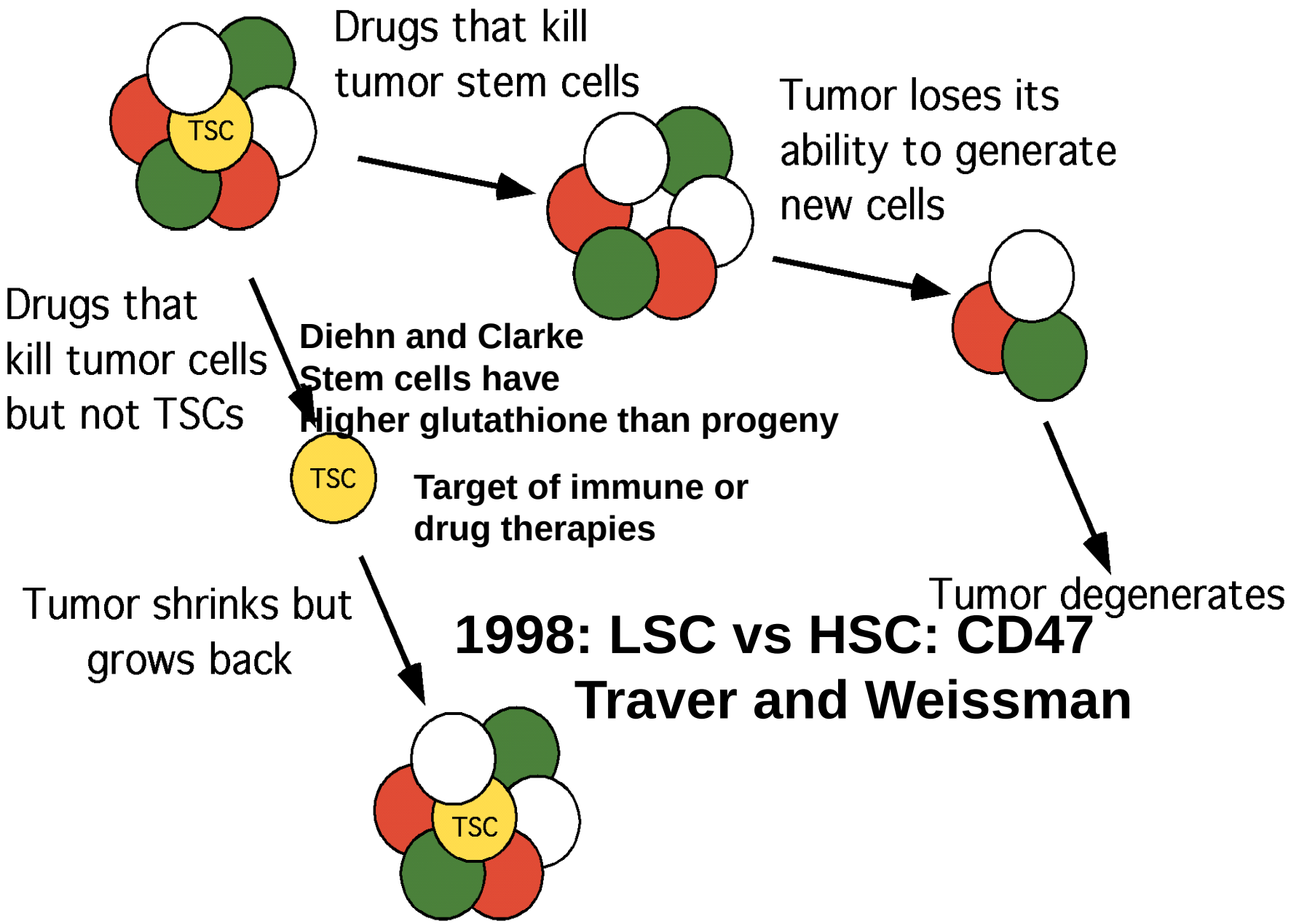
hu5F9-G4

Lung



Brain



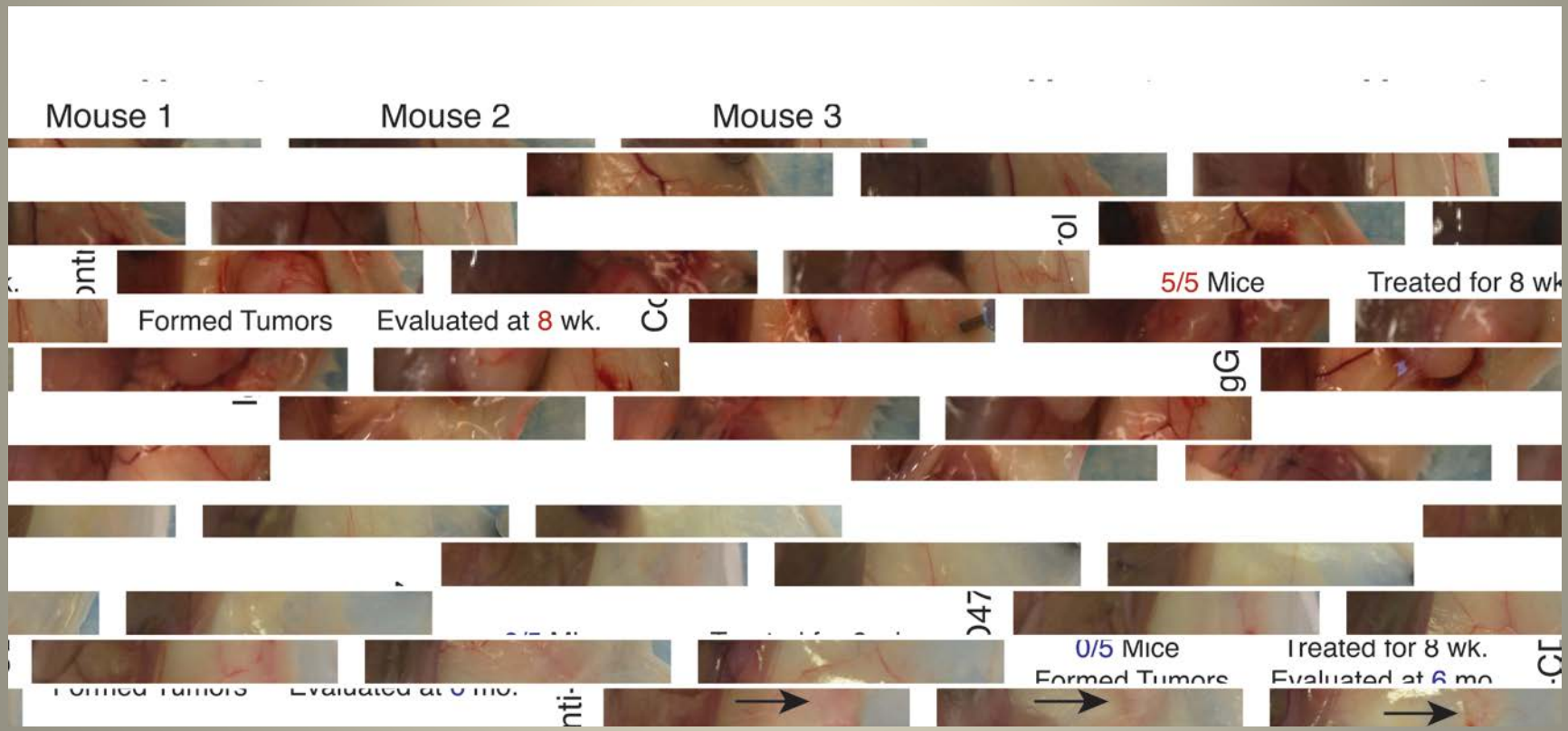


Reya, Morrison, Clarke, and Weissman, Natu

Scheeren, Clarke, Volkmer, Willingham, and IW

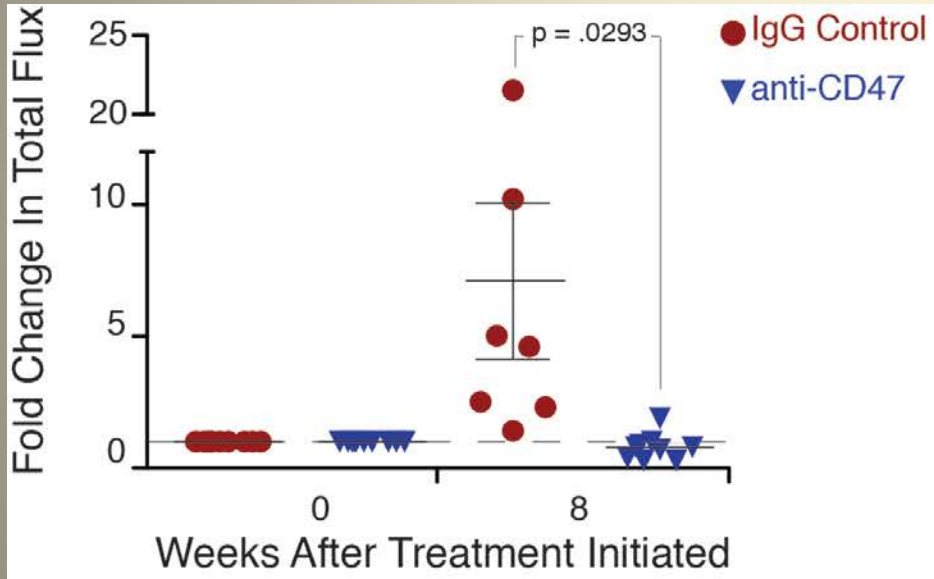
Anti-CD47 Antibodies Inhibit Growth Of Xenotransplanted Patient Tumors

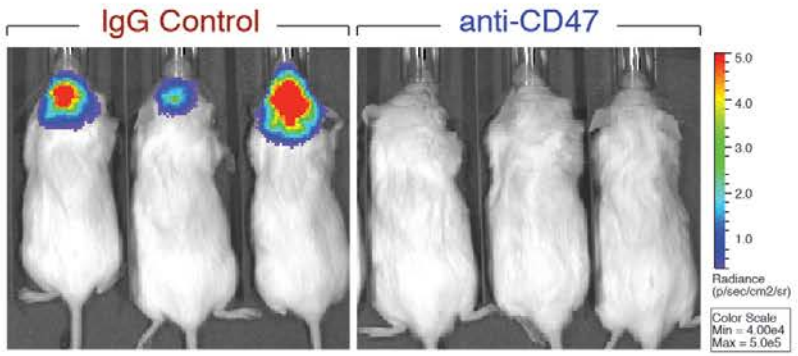
Breast Cancer



Anti-CD47 Antibodies Inhibit Growth Of Xenotransplanted Patient Tumors

Glioblastoma





MITRA, JIANG, CHESHIER, RAVEH, IW

Inject Tumor Cells Into Left Hemisphere — **Initiate Treatment** — **Monitor Tumor Growth With Bioluminescence imaging** — **Evaluate Tumor Formation**

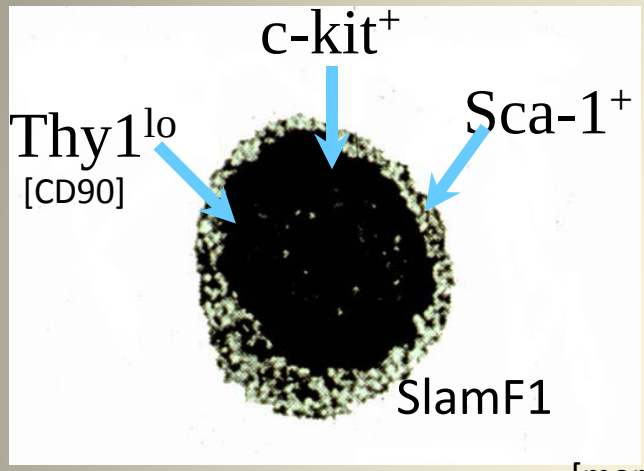
2 Weeks | **8 Weeks**

Blood-Forming Stem Cells

STANFORD

SYSTEMIX/NOVARTIS/CELLERANT

MOUSE



[morrison]

Stanford

Negative for:

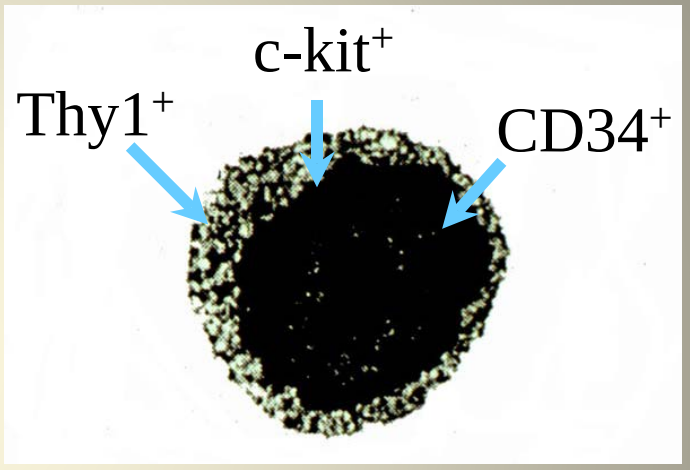
B220
Mac-1
Gr-1
CD3, 4, 8
Ter119
Flk2
CD34 [nakauchi]

1988

Spangrude, Muller, Heimfeld,IW

[CD90]

HUMAN



Novartis

Negative for:

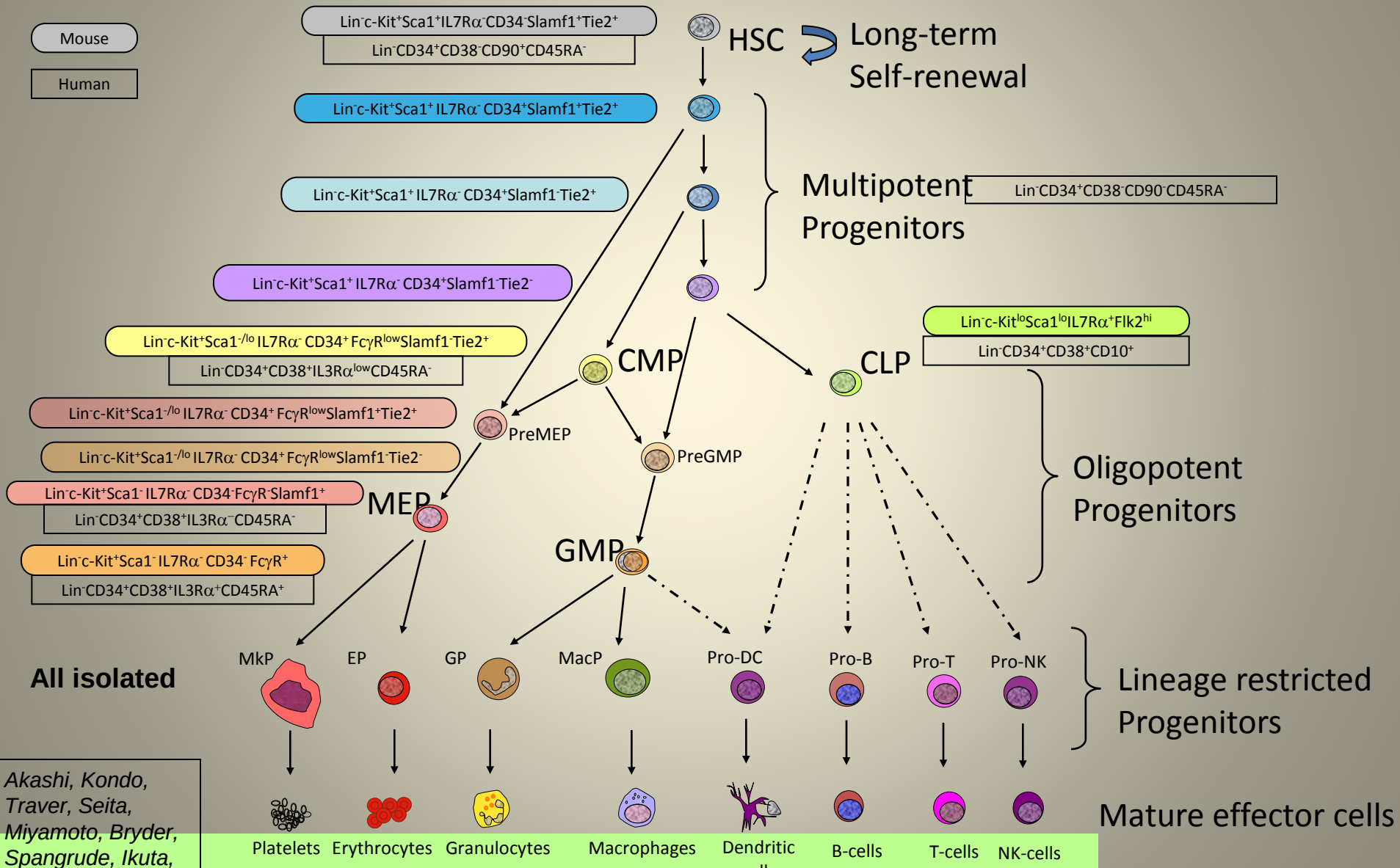
CD10
CD14
CD15
CD16
CD19
CD20
CD 38

CD 3,4,8
Glycophorin A

1992

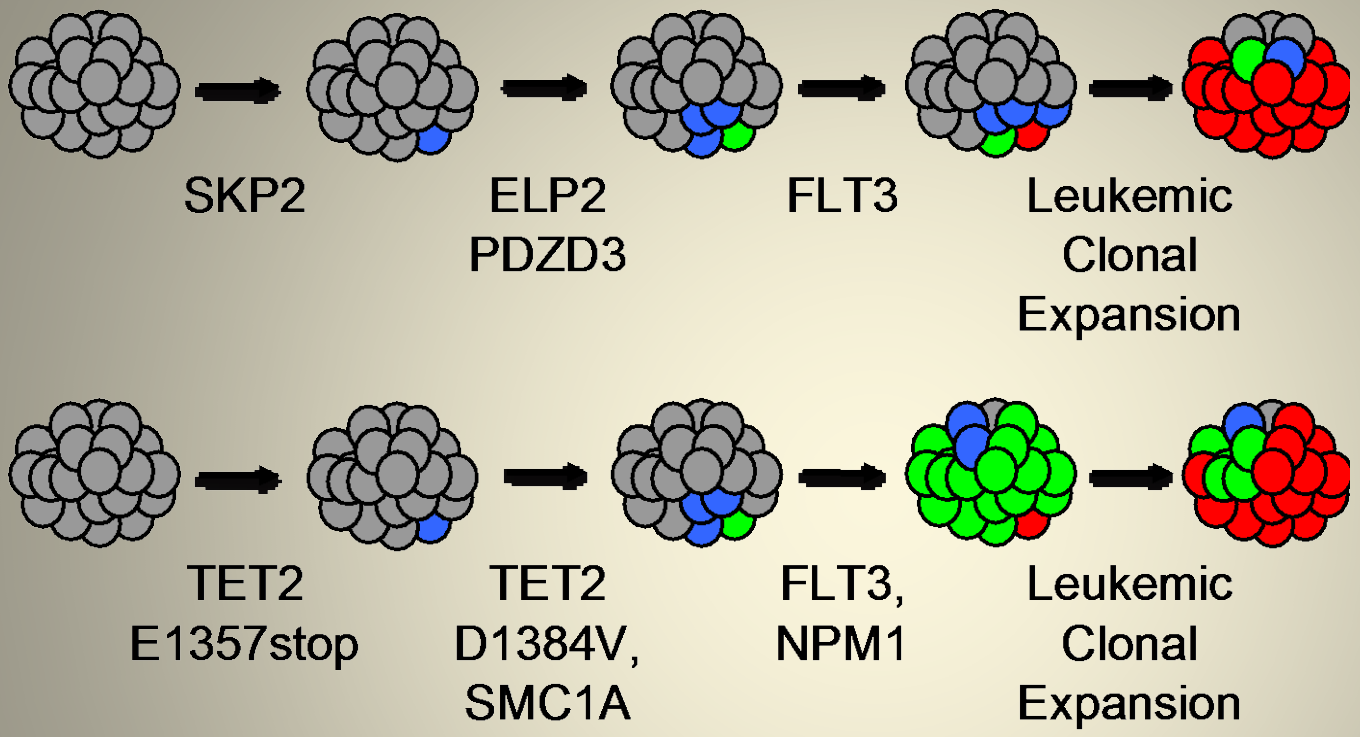
Baum, Buckle, Peault, Tsukamoto,IW

Hematopoietic Hierarchy



Akashi, Kondo, Traver, Seita, Miyamoto, Bryder, Spangrude, Ikuta, Morrison, et al

Analysis of Single HSC to Identify Pre-Leukemic Clones



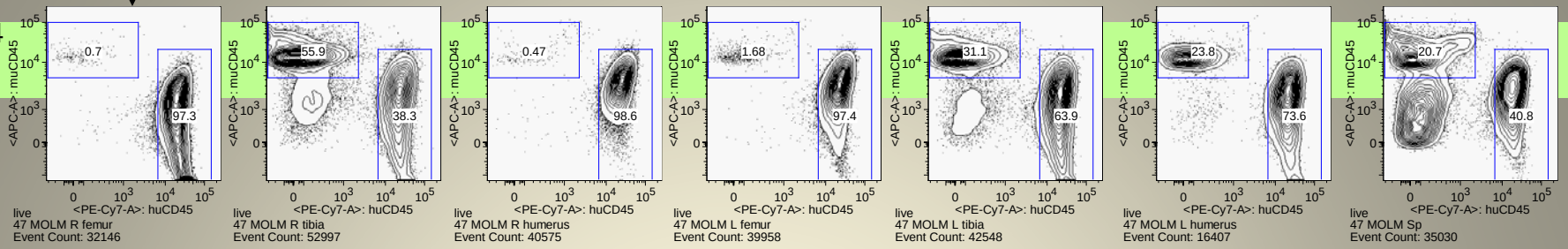
3 Mutations
5 Mutations
10 Mutations
Control Samples
Leukemia Cells
T Cells
No Template

Max Jan
Thomas Snyder
Ryan Corces-Zimmerman
Steve Quake
Ravi Majeti

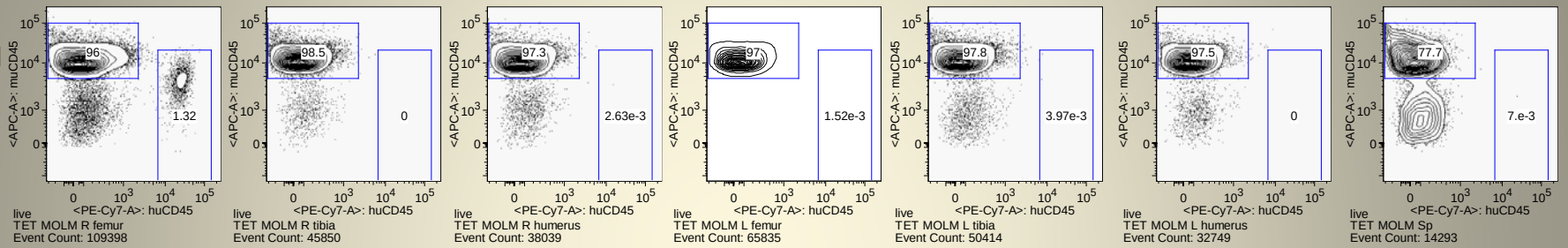
R-femur R-tibia R-humerus L-femur L-tibia

Injection site

TetCD47 MOLM

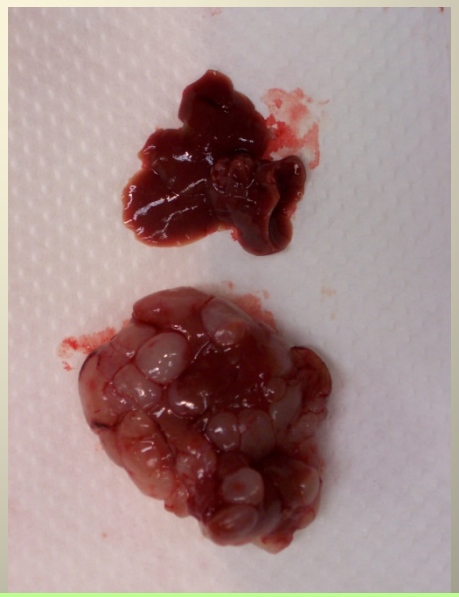


Tet MOLM



muCD45





Jaiswal et al

Leukemic cells in AML patients

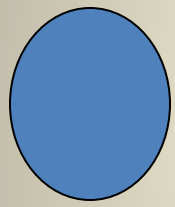
LT STEM CELLS

$CD34^+CD38^-Thy^+Lin^-$

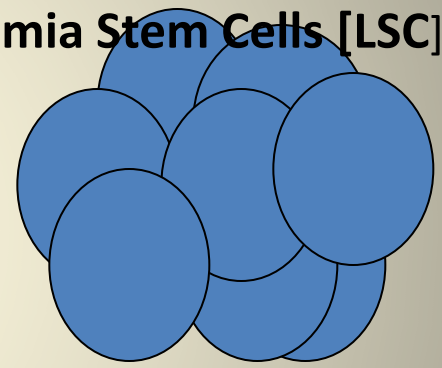
MPP

$CD34^+CD38^-Thy^-Lin^-$

Leukemia Stem Cells [LSC]



5-40%
AML-1/ETO$^+$

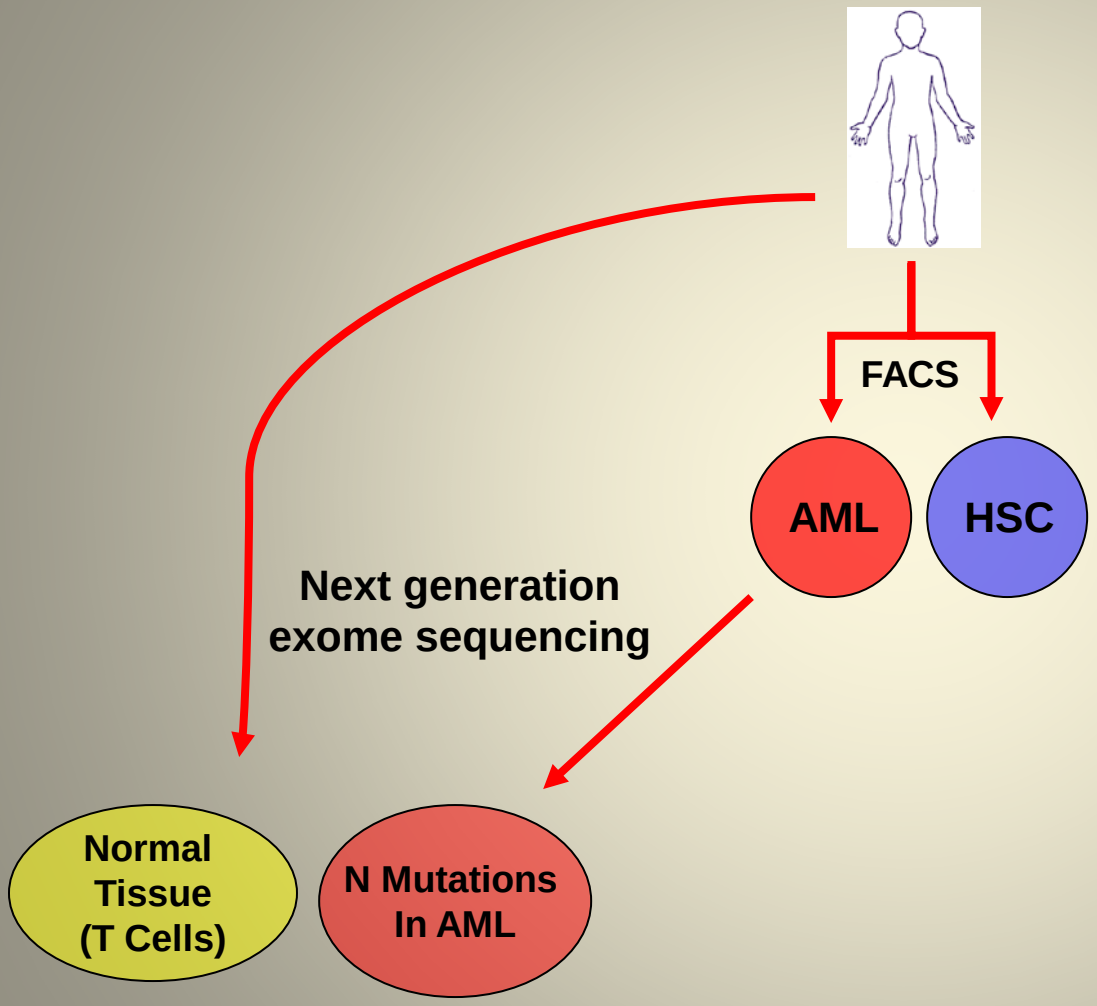


Normal colonies

Leukemic blast colonies

Miyamoto, Akashi, Weissman
PNAS 2000: 97: 6924

Identification of Somatic Mutations by Exome Sequencing

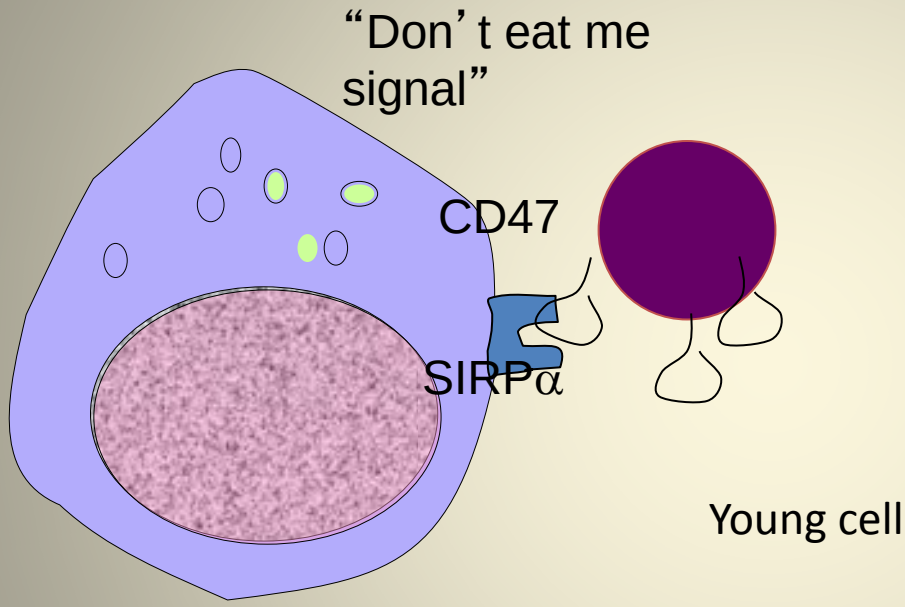


AML	Gene	Annotation
SU070	PXDN	V616I
	KALRN	S44P
	TET2	Y1649stop
	TET2	T1884A
	TMEM8B	nt G471A
	NCRNA00200	nt G354A
	TMEM20	A143T
	ZRANB1	nt G4659A
	SCN4B	H227N
	GABARAPL1	nt C1583T
	DOCK9	A1475V
	PLAG2G4D	P246A
	CACNA1H	R1069stop
	CTCF	R339Q
	GZF1	nt G3835C
	PRPF6	R527H
	CXorf36	I225L
	CXorf66	G321S
	FLT3	599-610 ITD

Max Jan
Thomas Snyder
Ryan Corces-Zimmerman

Steve Quake
Ravi Majeti
Irv Weissman

Young cells overexpress CD47 to evade phagocytosis



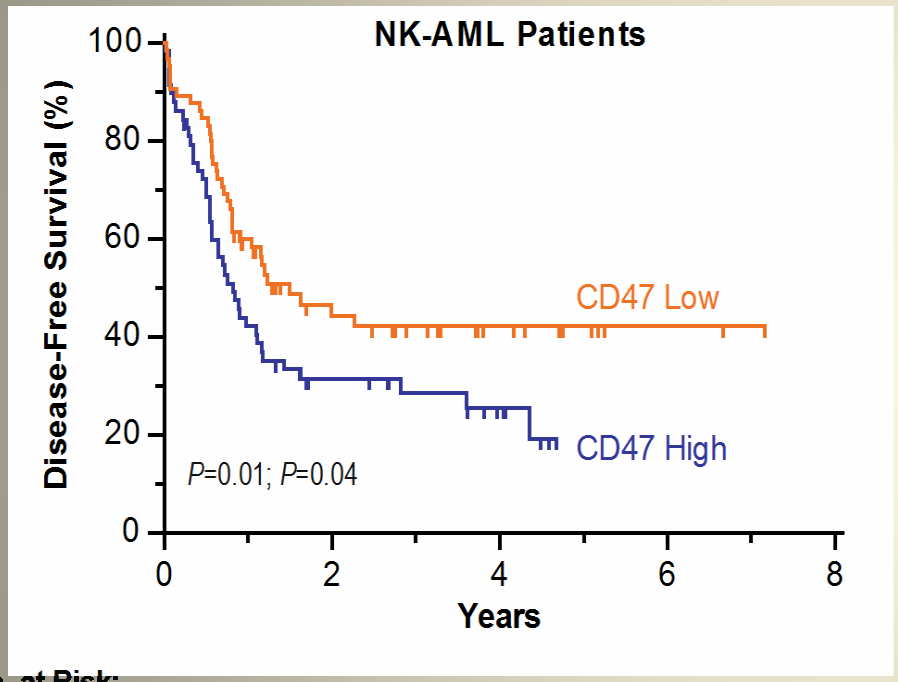
Macrophage

Oldenberg et al, 2000-2006

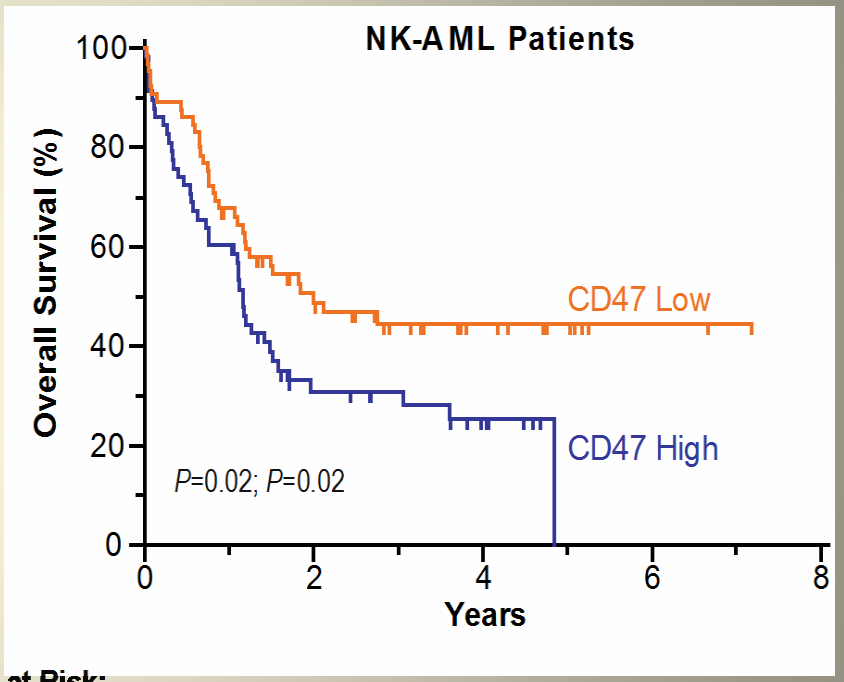
..and AML stem cells also overexpress CD47 [1998 mice;2009 human]

Jaiswal, Jamieson, Traver, and IW

Increased CD47 Expression is Associated with Worse Survival in Normal Karyotype (NK) AML Patients



No. at Risk:					
CD47 Low:	65	20	9	2	1
CD47 High:	58	14	6	0	0



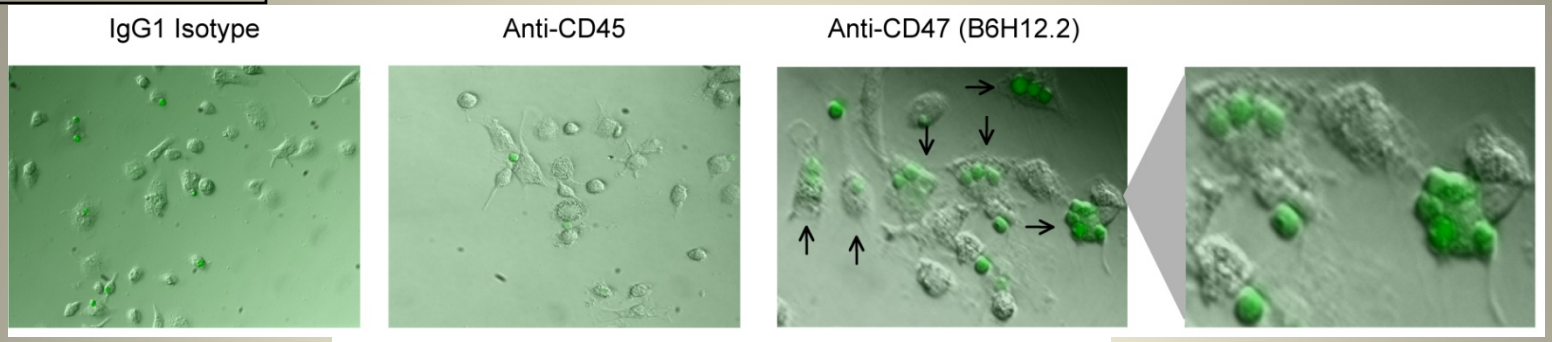
No. at Risk:					
CD47 Low:	65	26	10	2	1
CD47 High:	58	14	6	0	0

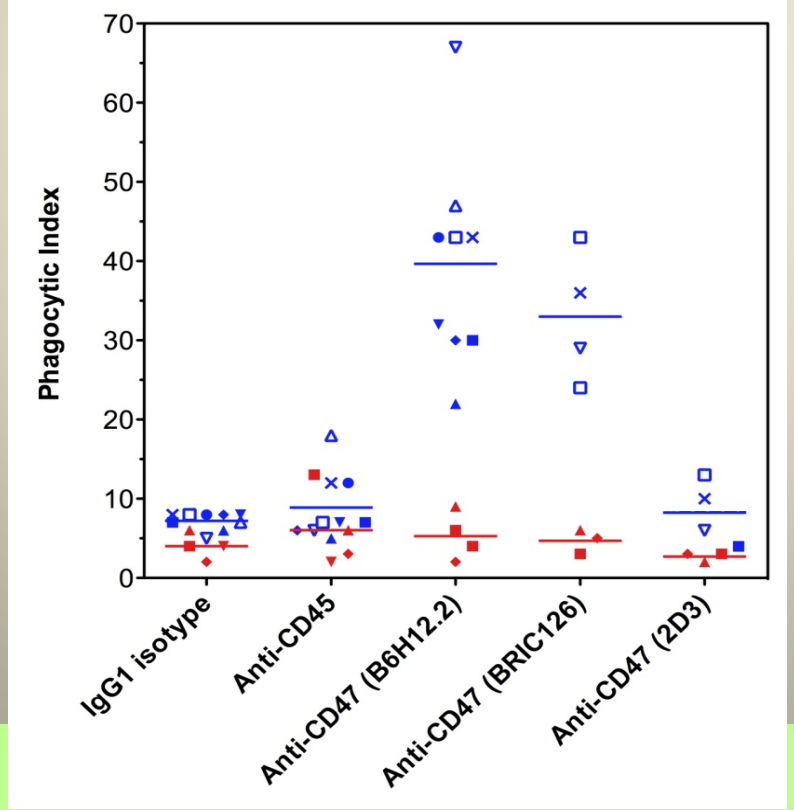
ASH ALIZADEH, RAVI MAJETI

Data analyzed from Bullinger L, et al. Blo

Anti-CD47 Antibodies Enable Phagocytosis of AML LSC

Human Macrophages

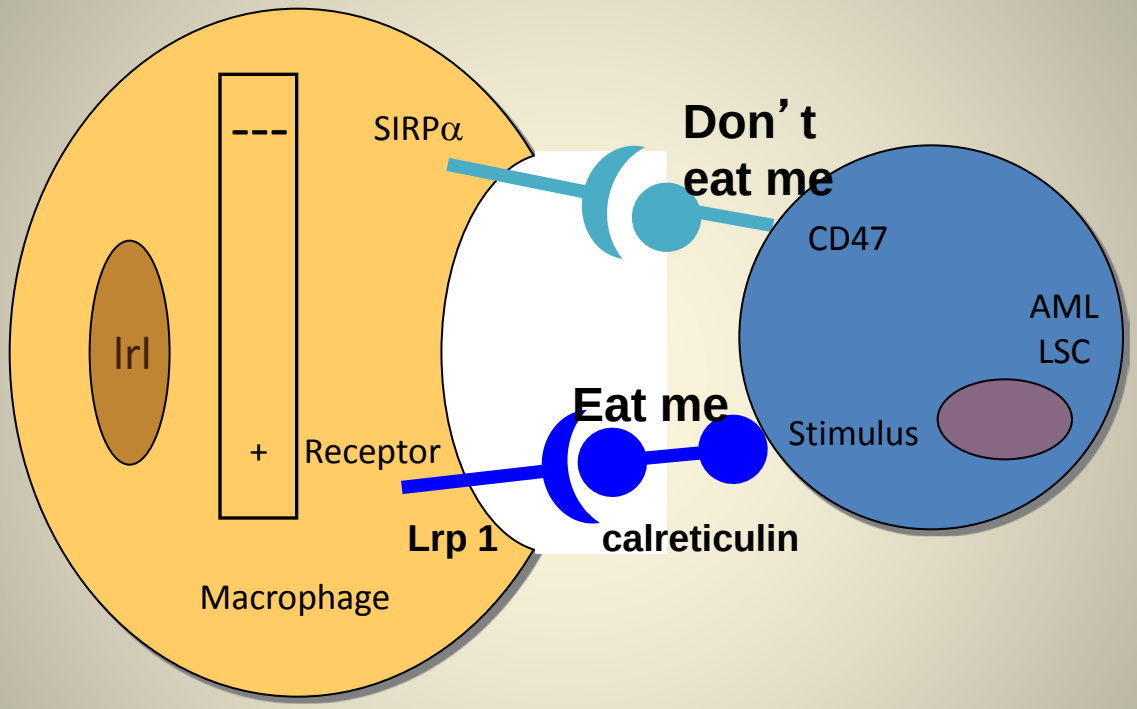




Mark Chao, Majeti et al

Precancer cells express calreticulin, and emergent cancer clones overcome this with CD47

Increased expression of CD47 on myeloid leukemia cells contributes to pathogenesis by facilitating evasion of phagocytosis



Net Result: No Phagocytosis

Chao, Jaiswal, Weissman-Tsukamoto, Majeti, and IW

Anti-CD47 Antibodies Can Eliminate Established Metastasized Tumors

Inject LMS Subcutaneous

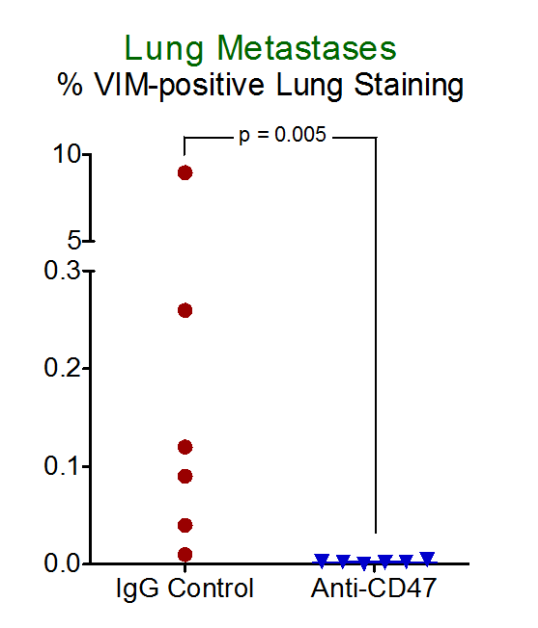
Resect Primary Tumor

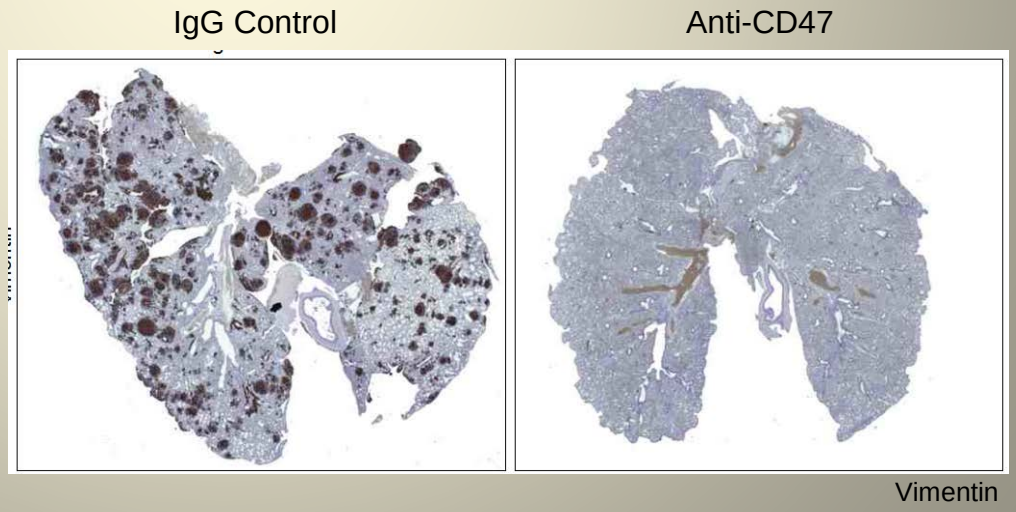
Anti-CD47 Therapy

Evaluate growth of metastases

Week 7

Week 11



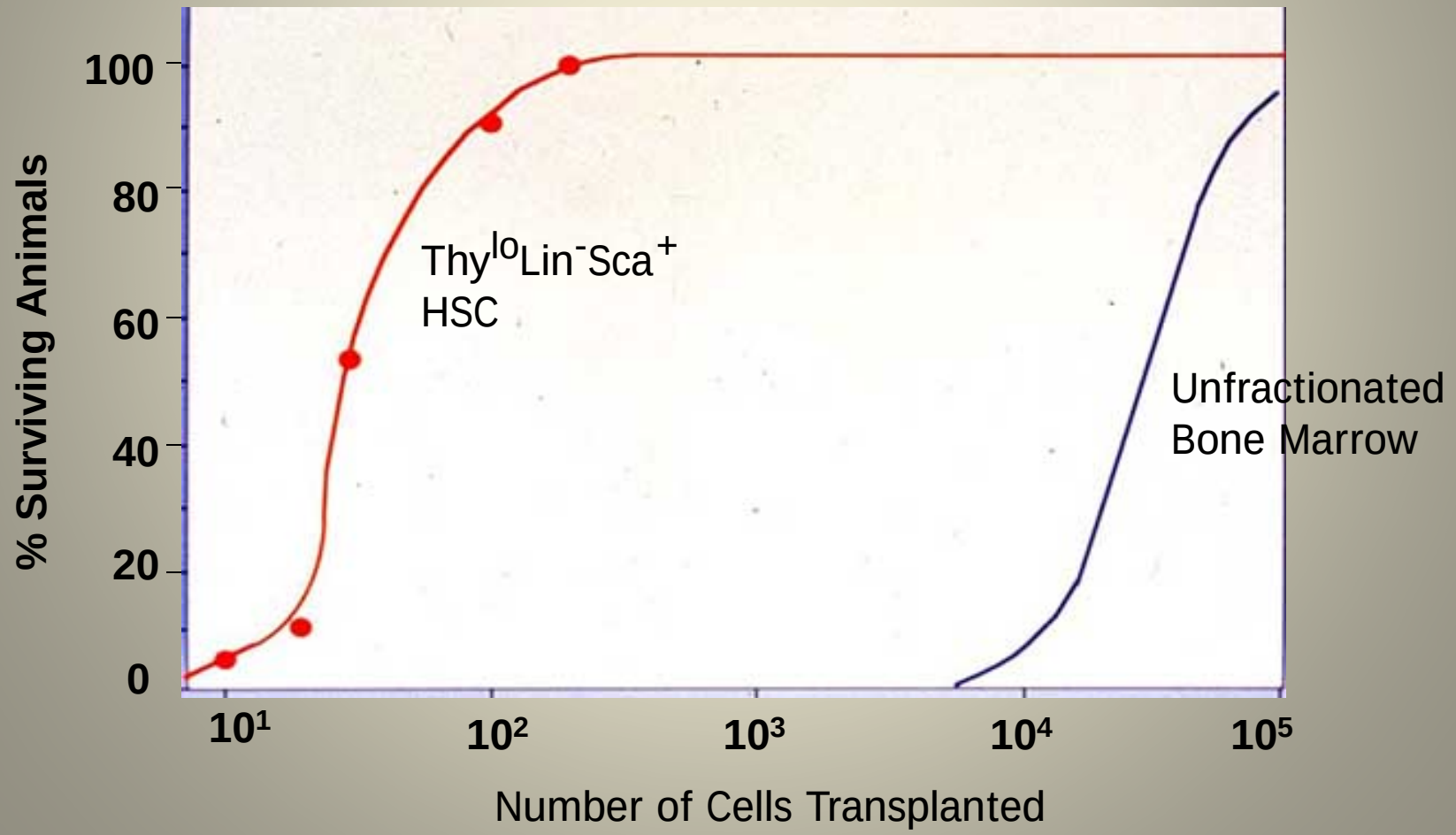


B Edris, M Rijn, J Volkmer, and IW

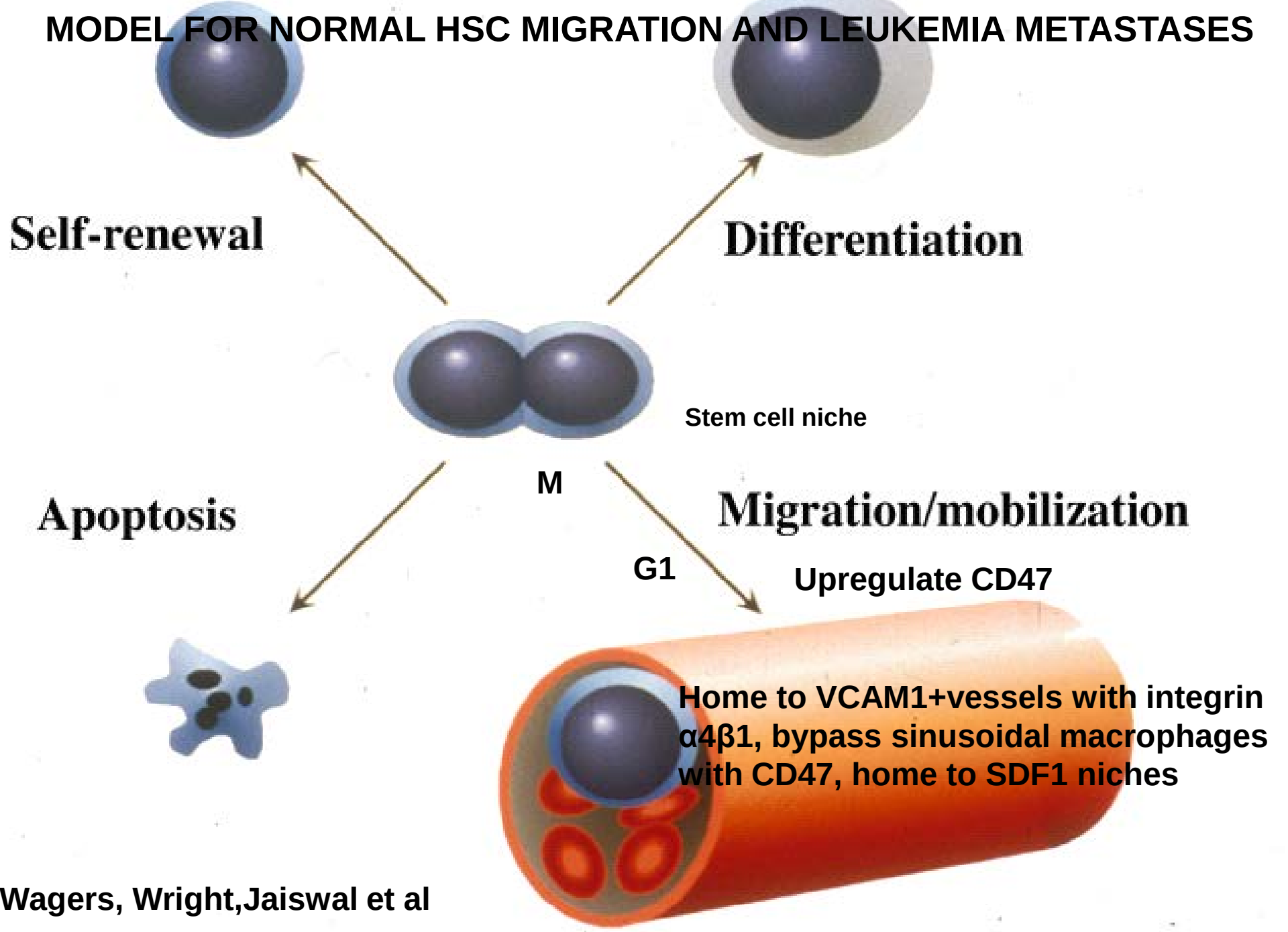
Hematopoietic Stem Cells (1988)

Spangrude, Heimfeld, and Weissman

- Represent 1 in 2000 bone marrow cells
- 2000 times more effective in radioprotection and restoring blood formation

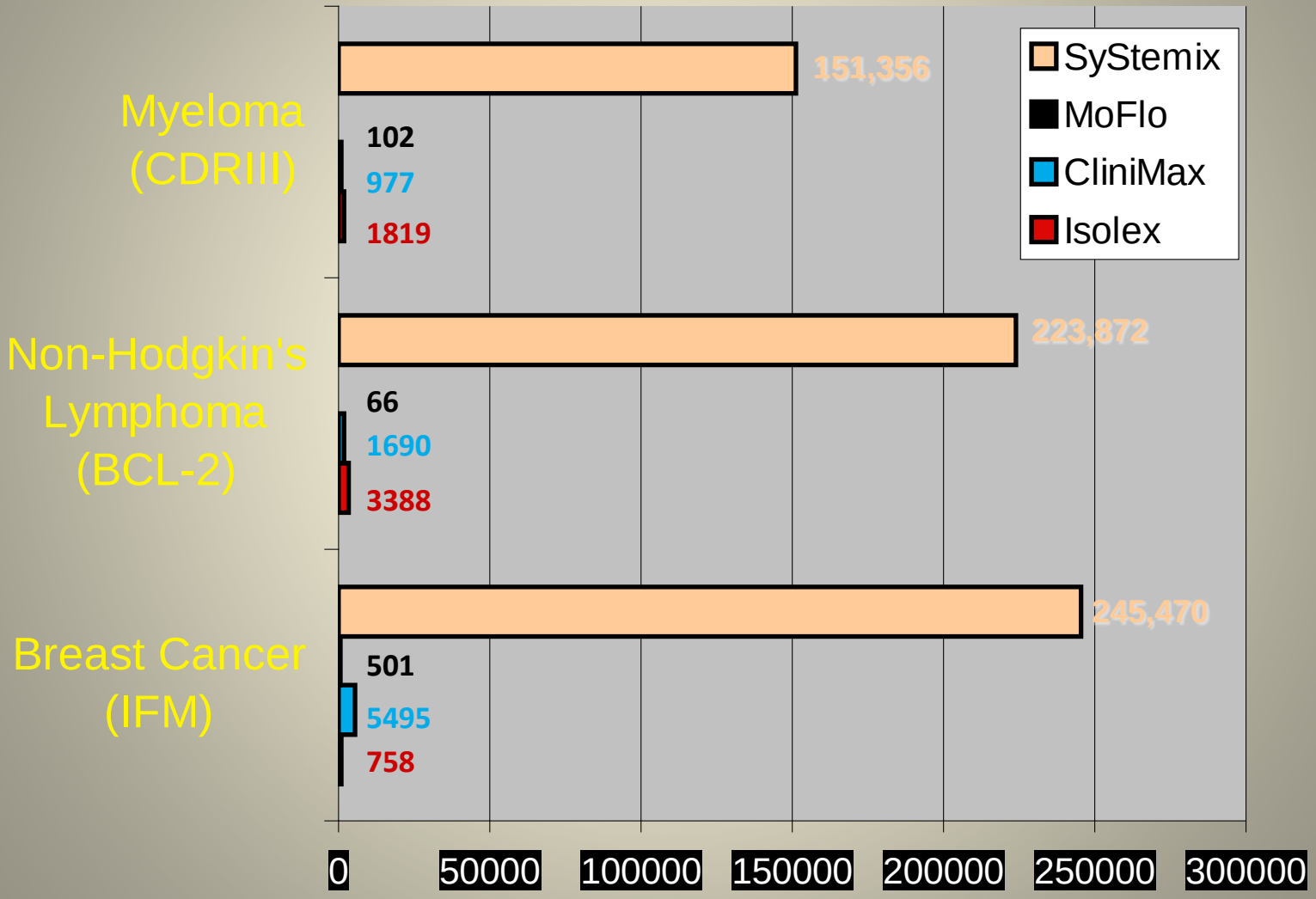


MODEL FOR NORMAL HSC MIGRATION AND LEUKEMIA METASTASES



Wagers, Wright,Jaiswal et al

Comparison of Fold Depletion of Cancer Cells from Mobilized Peripheral Blood (MPB)



Hanania

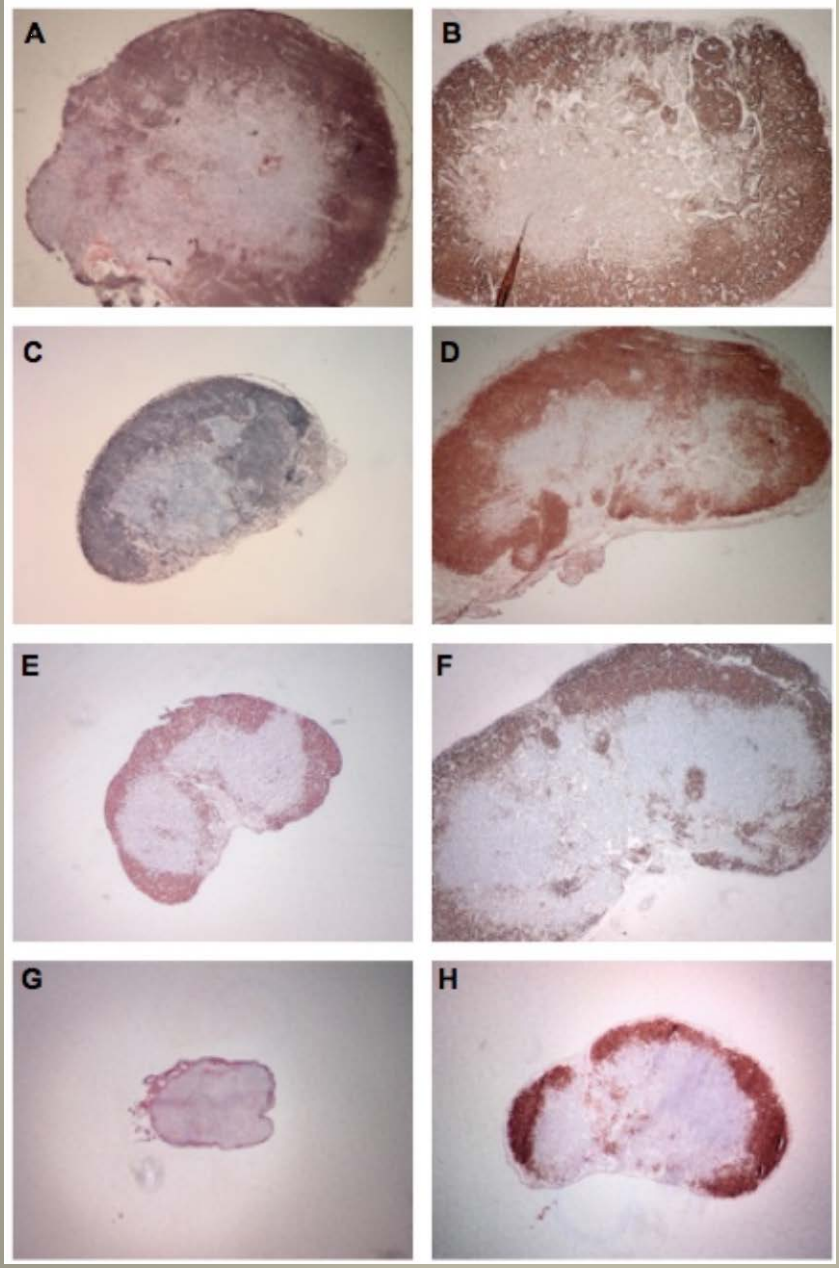
WBM HSC

Congenic

MHC-matched

Haplo

MHC-mismatched



Why transplant pure HSC?

A. Muller and J. Shizuru

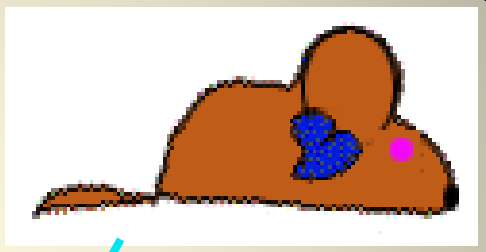
Do HSC chimeric mice demonstrate donor specific tolerance?

Neonatal Heart Donors

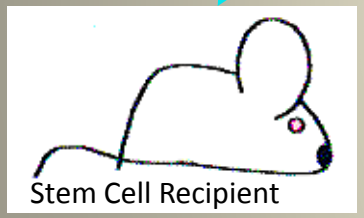
Stem Cell Donor



Third Party (G3H)

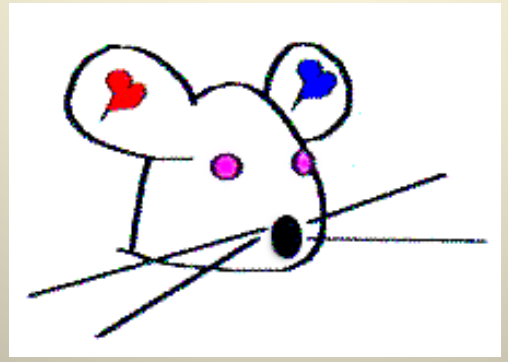


Irradiation or TLI/ATG: Strober



White mouse (Balb) with C57BL
Mouse blood and immune system

Stem cell donor heart is accepted



Stem Cell Recipient

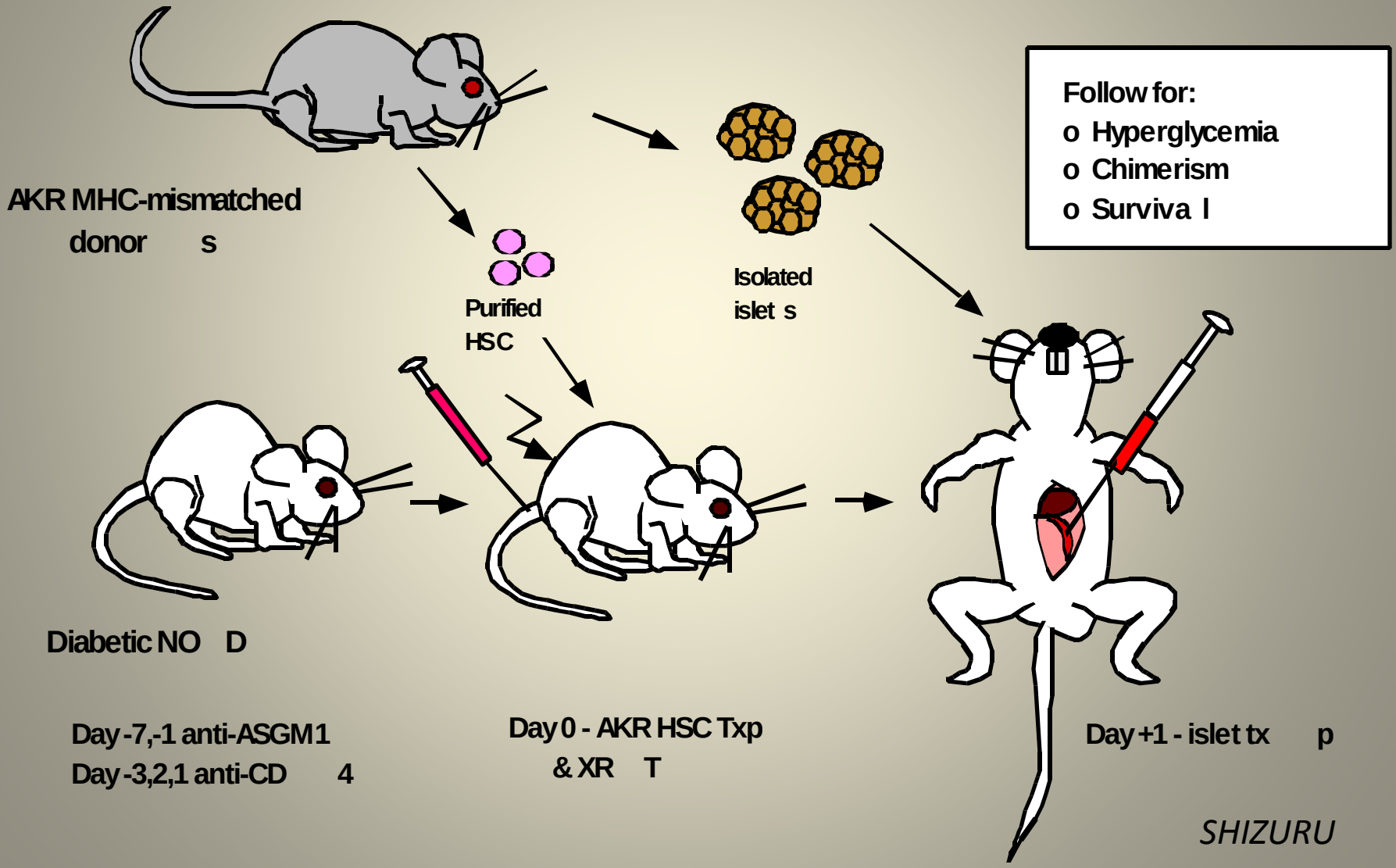
Third Party heart is rejected

Donor stem cells allow heart, kidney, pancreas,etc to be transplanted with no ongoing immune suppressingdrugs

Shizuru, Gandy

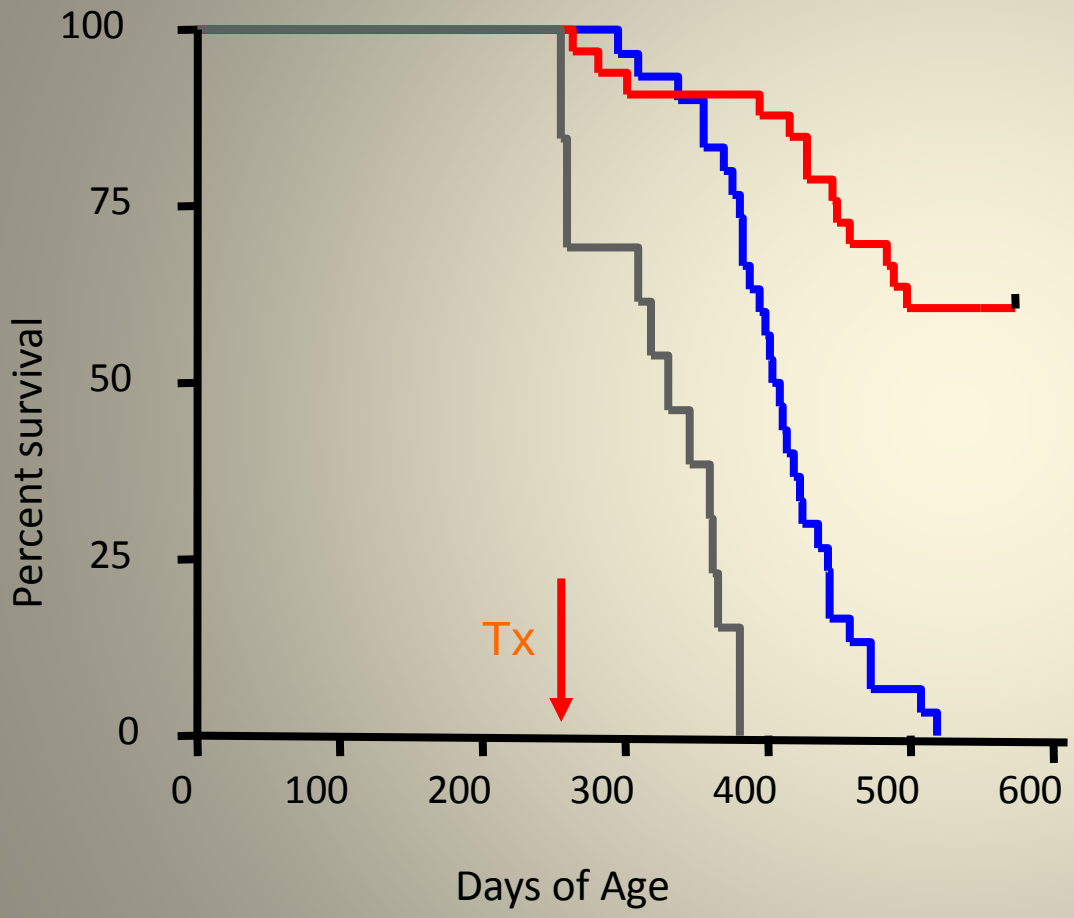
Weissman

Combined HSC & islet transplantation



Survival of NZBW [SLE] mice transplanted at 8.5 months with non- myeloablative conditioning

Mice conditioned with sublethal radiation +anti-CD4, anti-ASGM1[NK]



Age Matched Controls n = 13

Conditioned Only n = 30

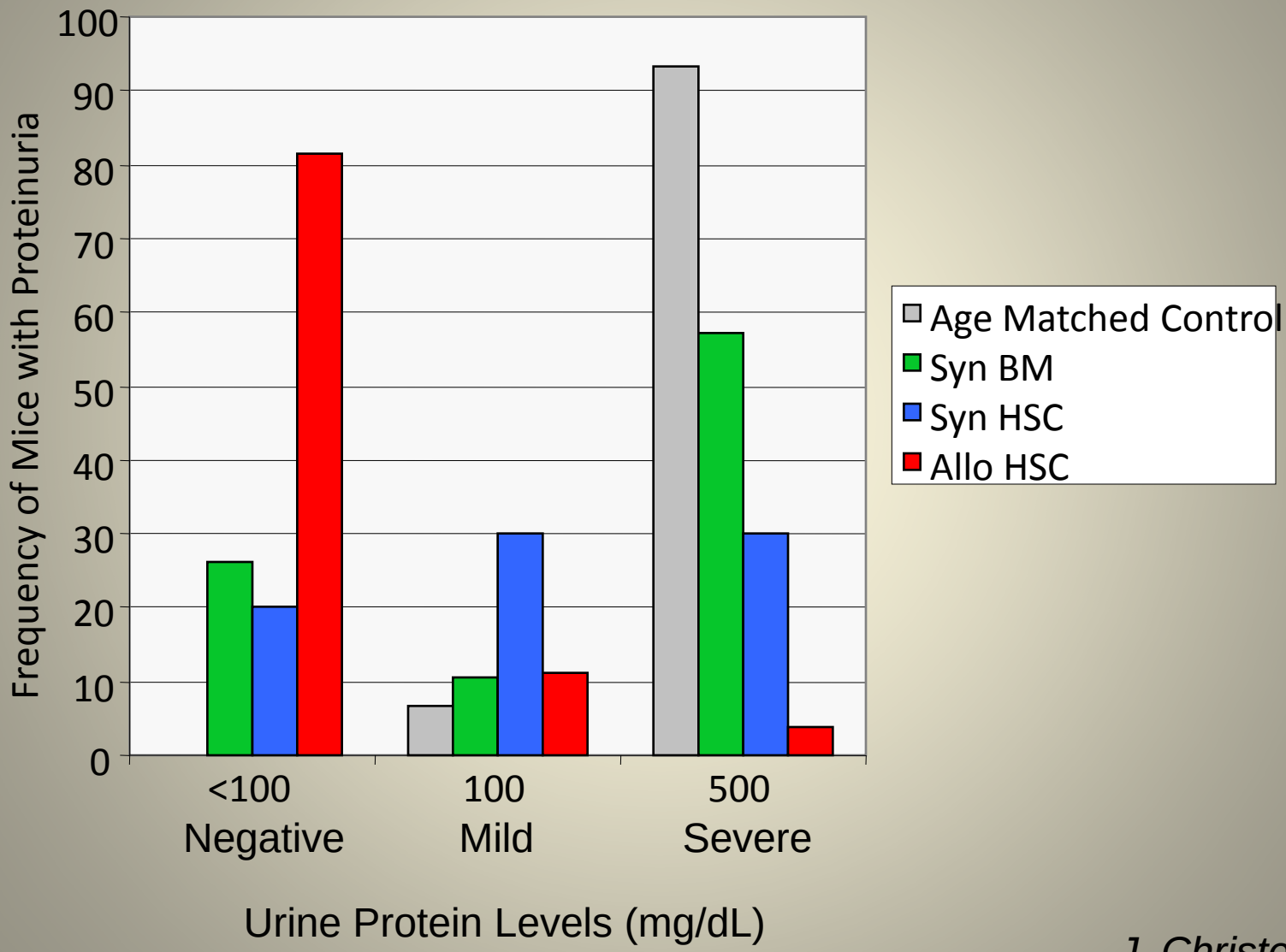
Allo HSC n = 33

donors =d/b [DBAxC57],

hosts = d/z [NZBxNZW]

Julie Christensen, IW et
Cellerant, Inc

Analysis of Proteinuria in Transplanted NZBW



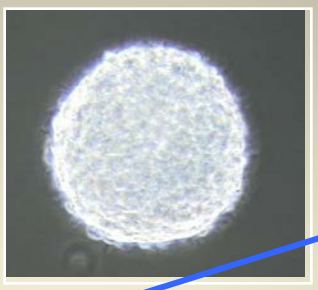
J. Christensen et al: Cellerant, Inc

Human CNS-SC Neurosphere Cells Engrafted, Migrated & Differentiated Into Neurons, Astrocytes and Oligodendrocytes

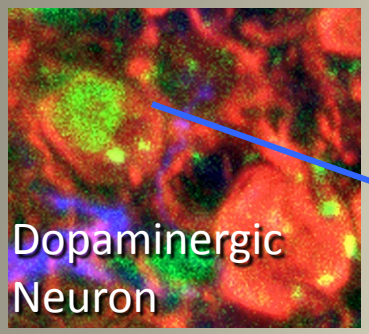
Uchida,Tsukamoto, Gage, Weissman

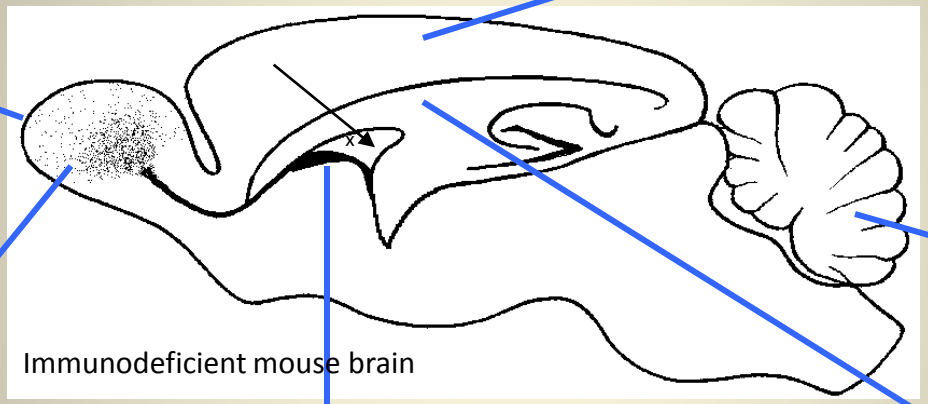
CD34-45-133+24-

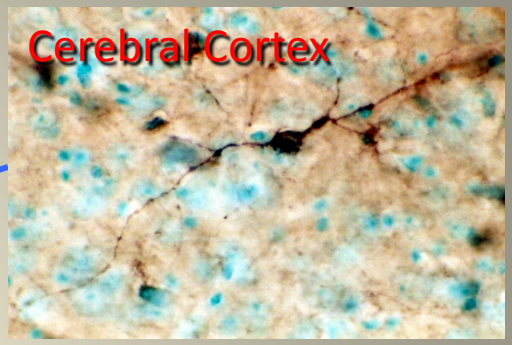
In Vitro Expansion

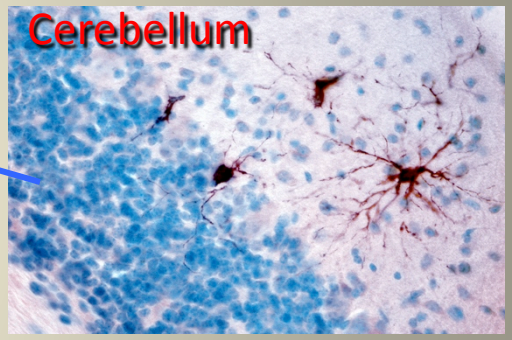


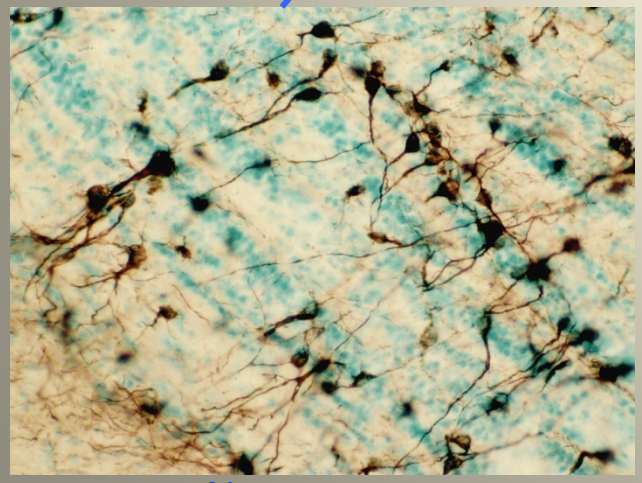
Transplantation



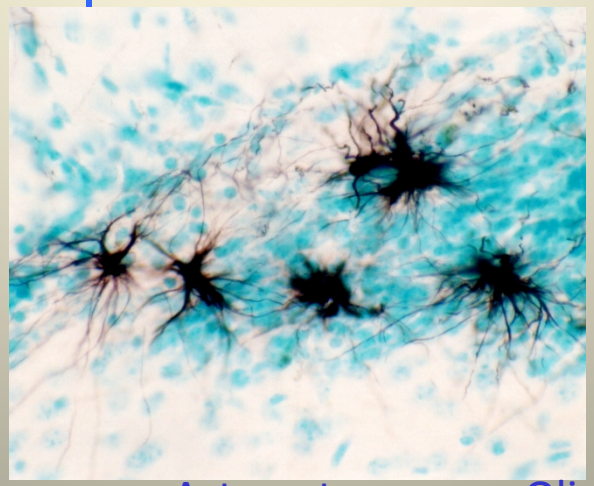




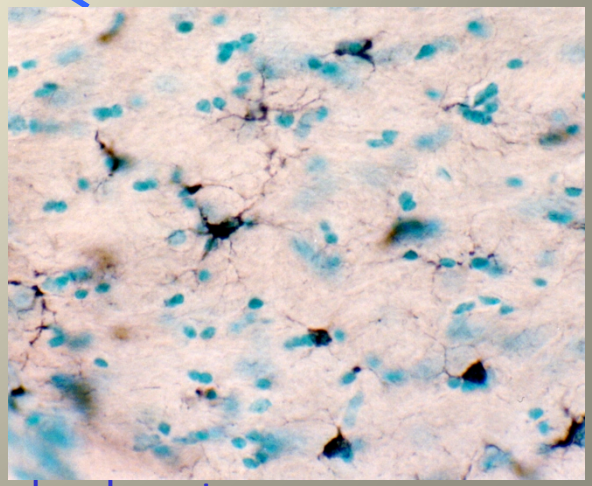




Neurons



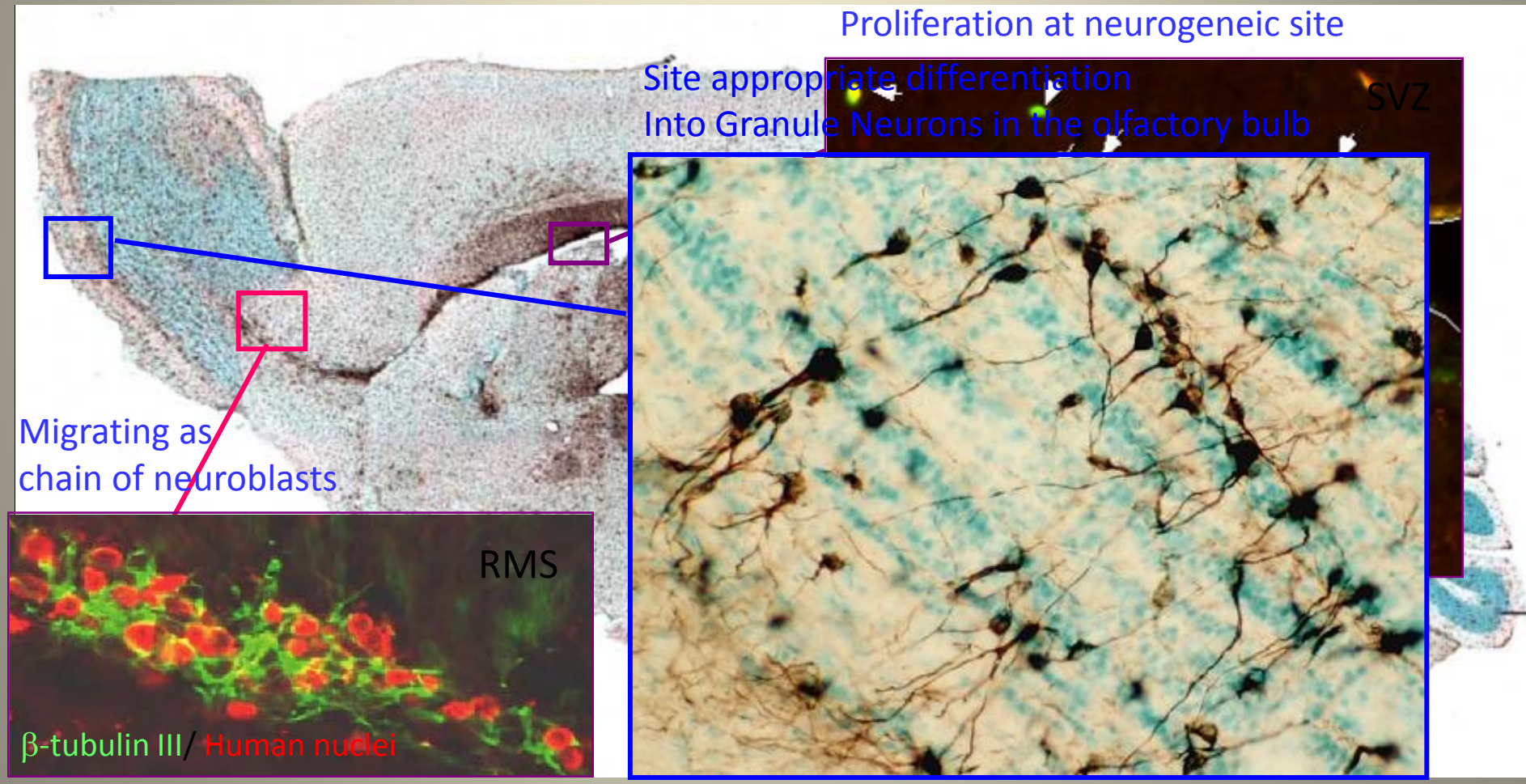
Astrocytes



Oligodendrocytes

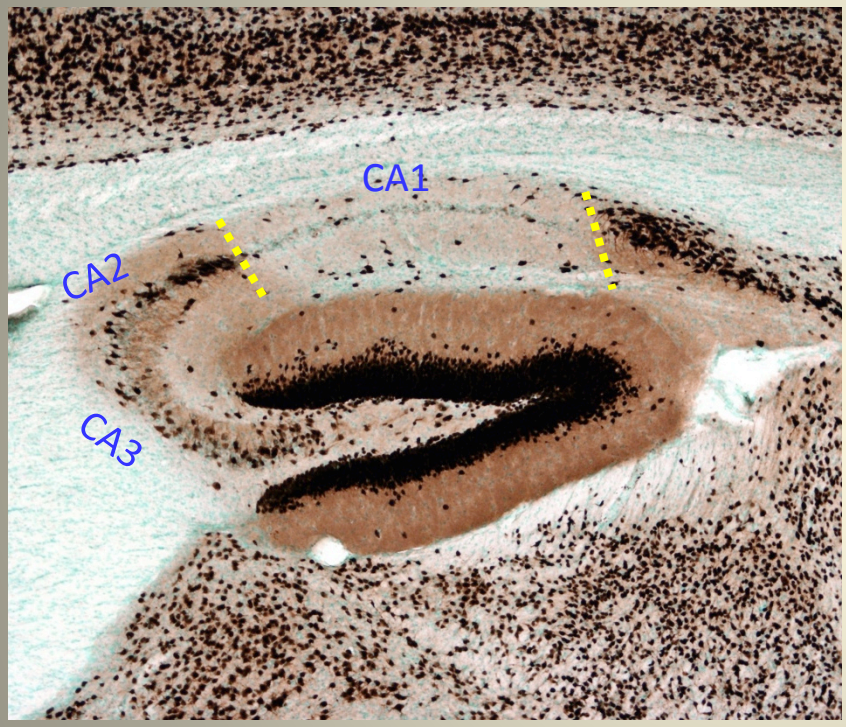
HuCNS-SC Engraft, Migrate & Mature

Human cells derived from HuCNS-SC transplant (brown)

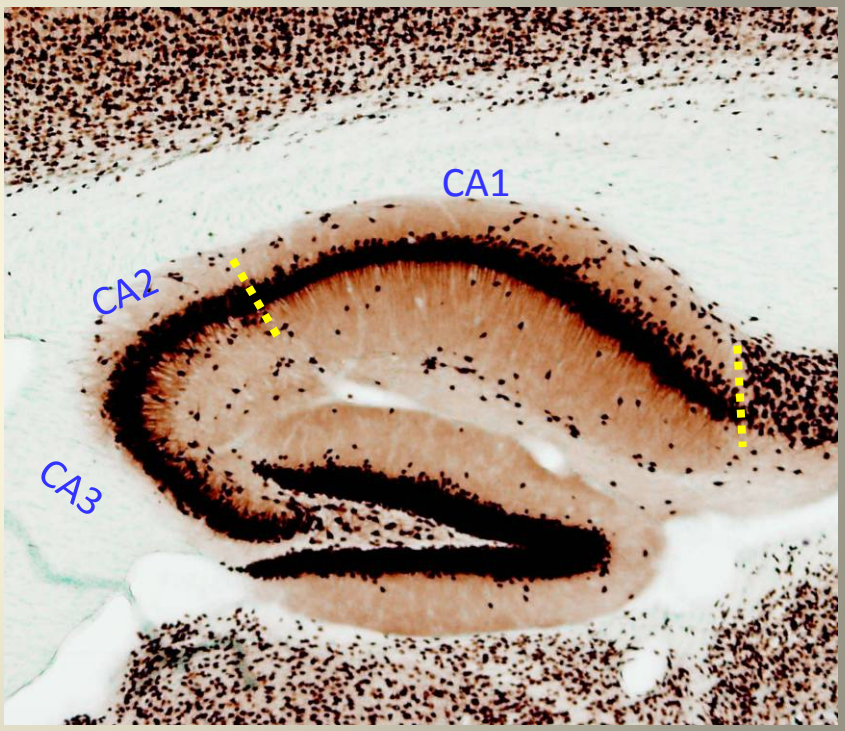


Mouse Model that Mimics Human NCL

PPT 1-/-



Normal (NOD-scid)

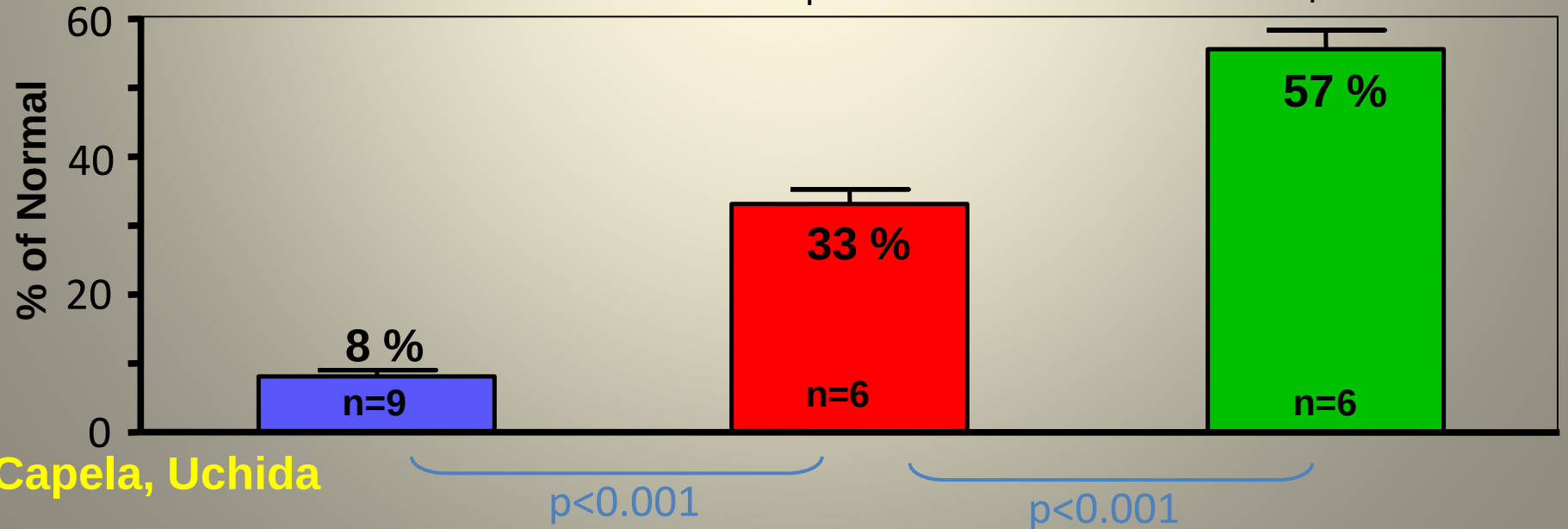


NCL mouse brain: CA1 region only 8% of neurons were detected compare to normal: S.Hoffman UTSW Dallas

HuCNS-SC Protects Neurons in Batten Mouse Hippocampus

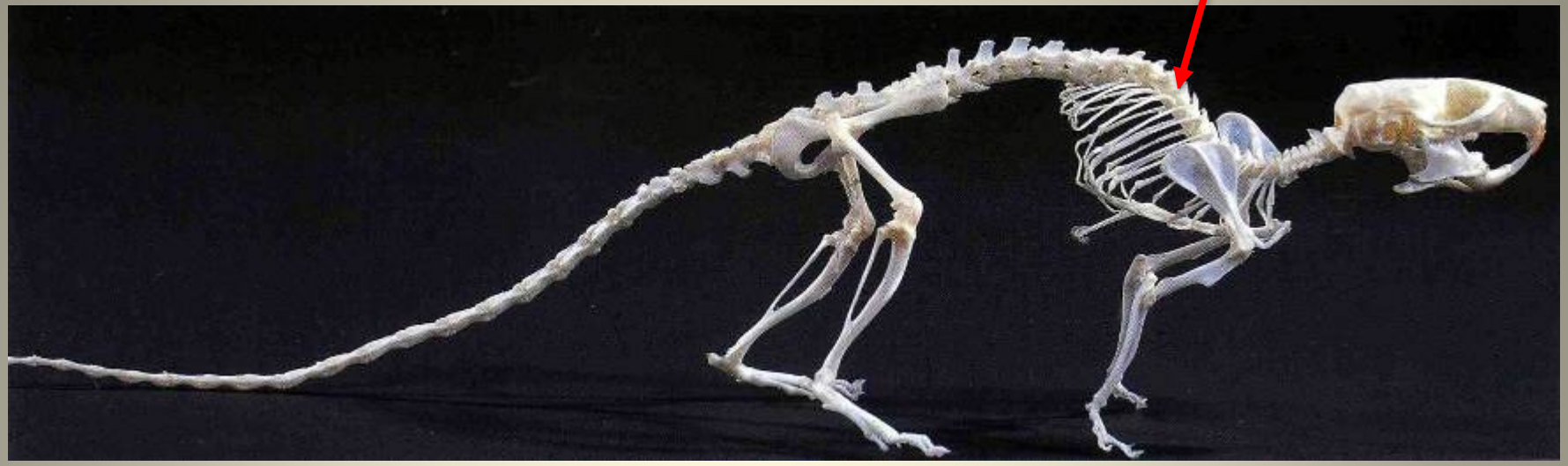


Not transplanted | Low Dose Transplanted | High Dose Transplanted

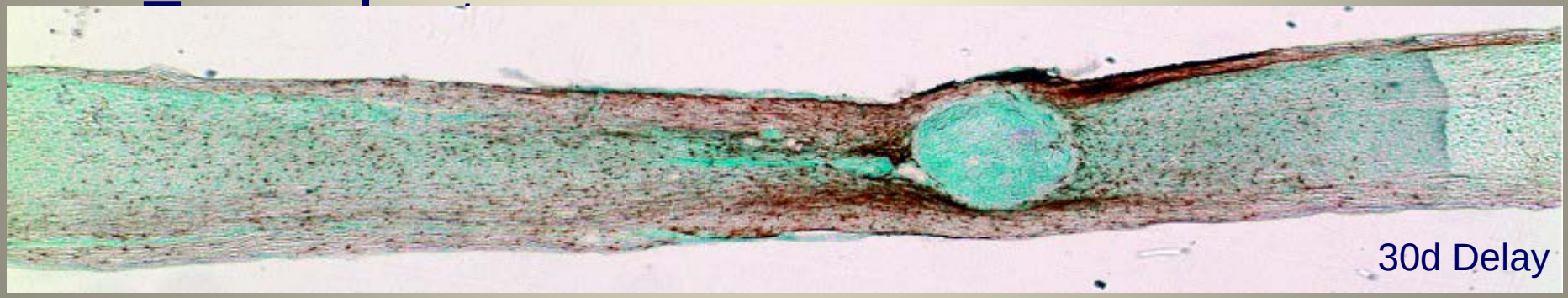


Capela, Uchida

HuCNS-SC: Thoracic Cord Contusion

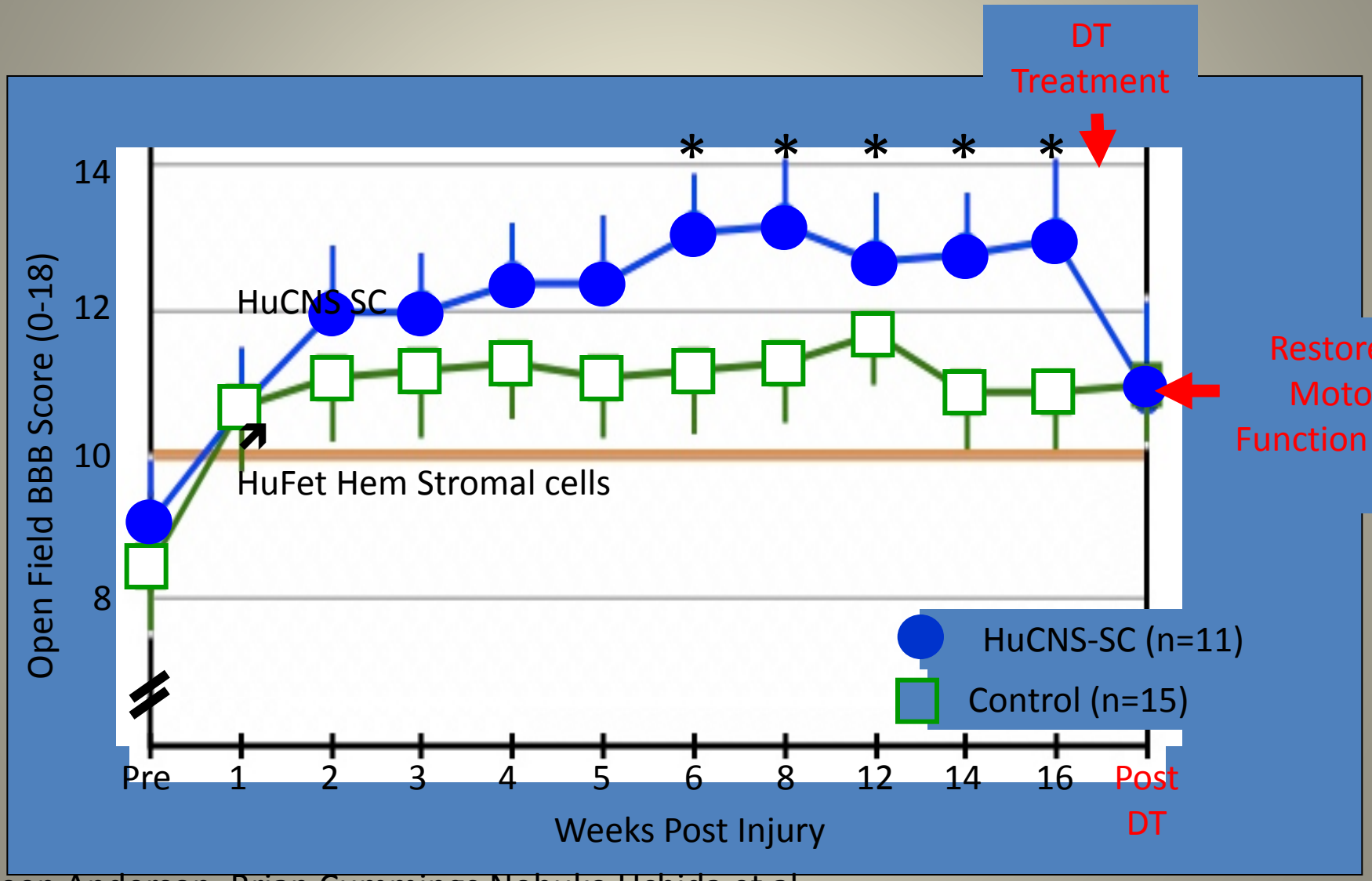


HuCNS-SC



Astrocytic markers (GFAP and SC123): 2.9%
Neuronal markers (Beta-tubulin III, GAD-67, NeuN): 26.4%
Oligodendrocyte markers (anti-NG2, CC-1/APC): 64.1%

HuCNS-SC Restores Motor Function in Spinal Cord Injured Mice

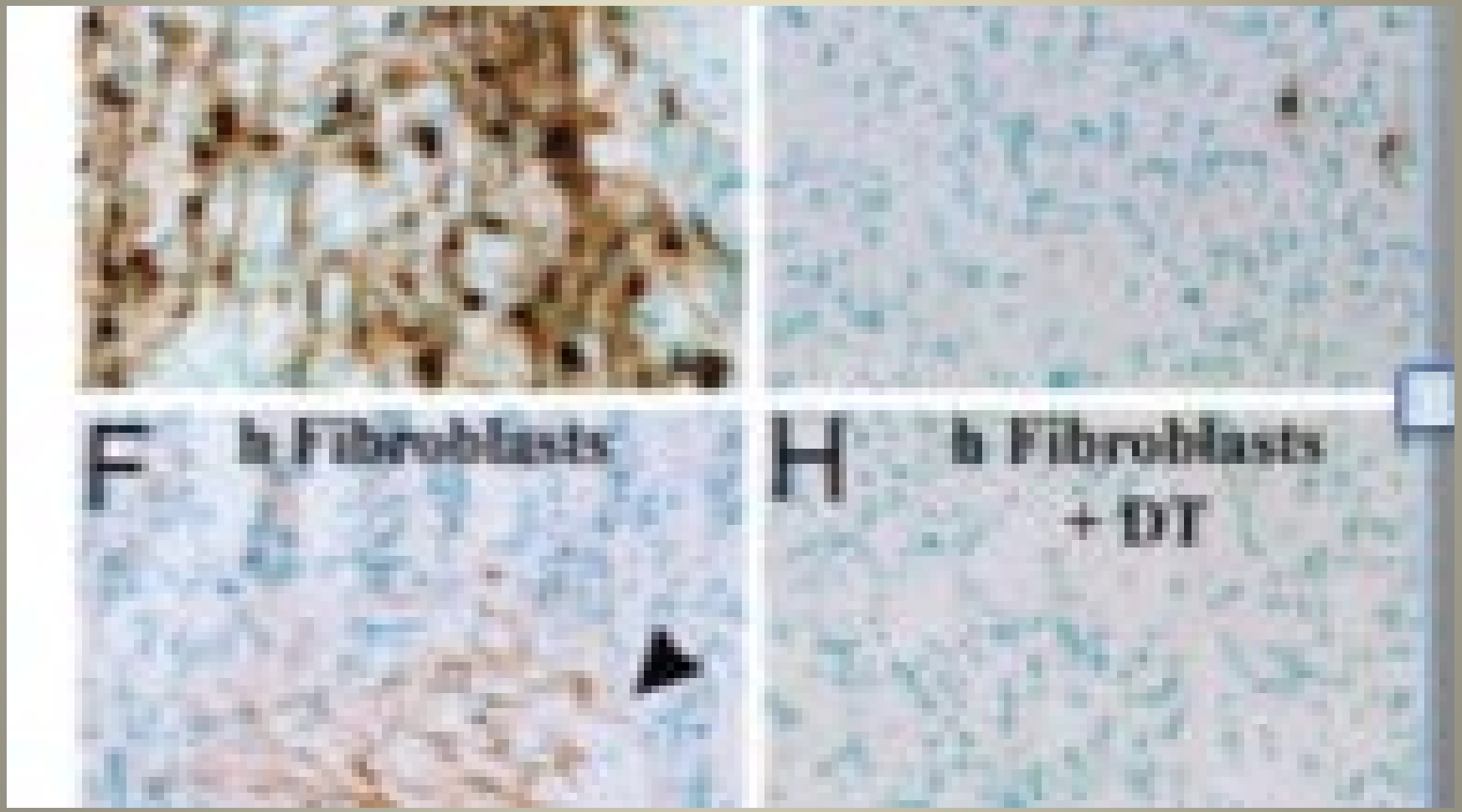


Aileen Anderson, Brian Cummings,Nobuko Uchida et al

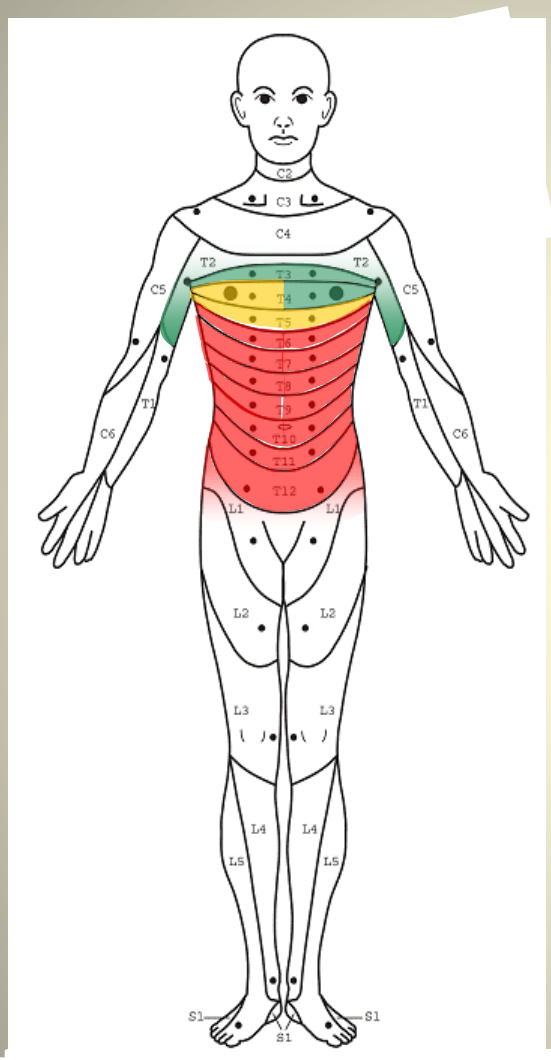
Repeated Measures ANOVA $p<0.01$

* individual time points $P<0.05$

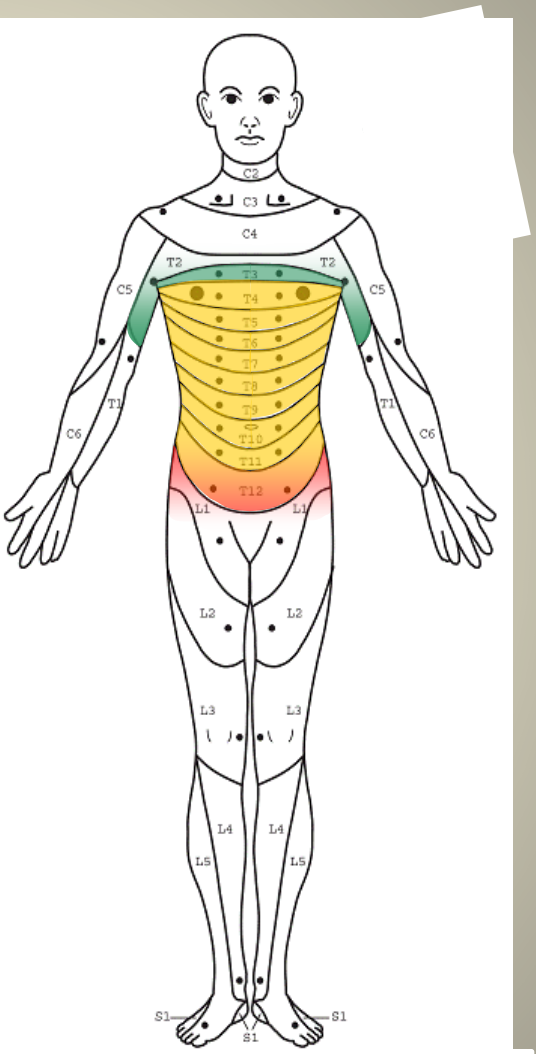
Extensive engraftment with CNS SC, local deposit with MSC; both DT sens



Subject 3:
Light touch examination



Dec 12 11 (Pre-TX)



Jun 28 12 (6m Post-TX)

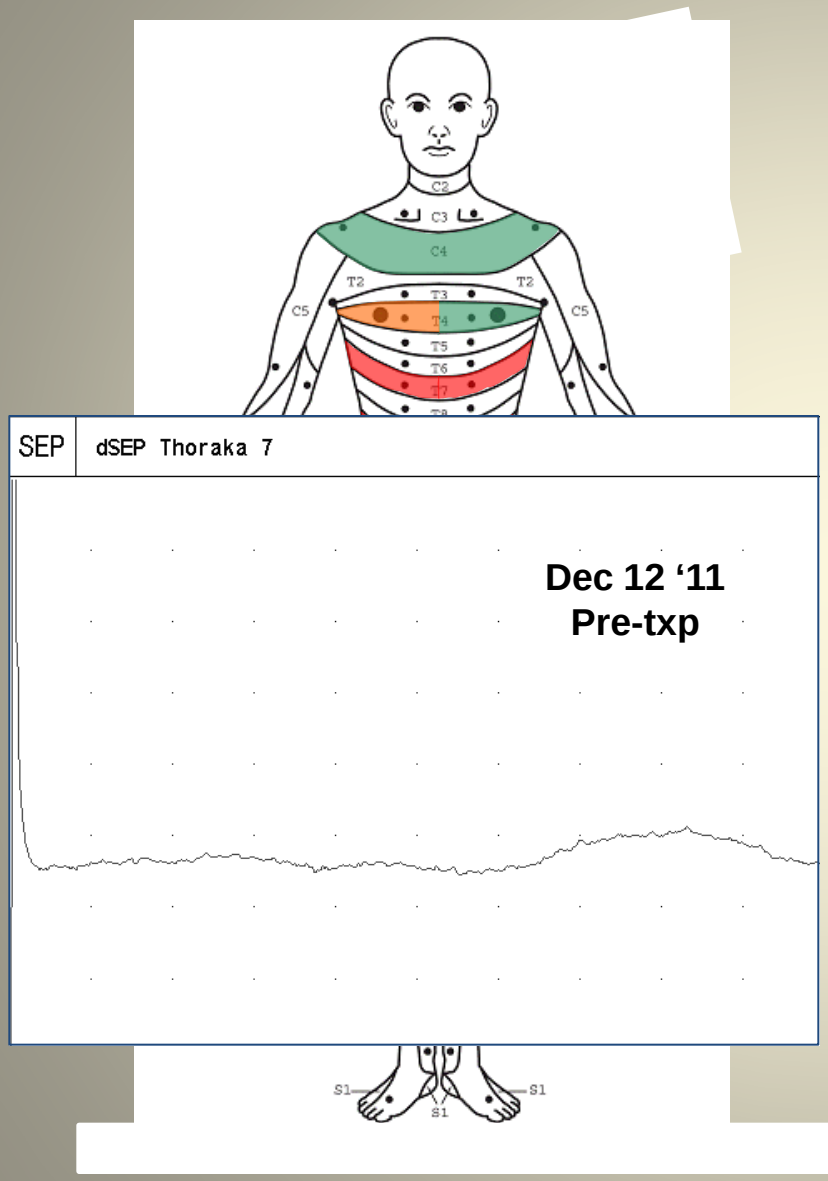
normal

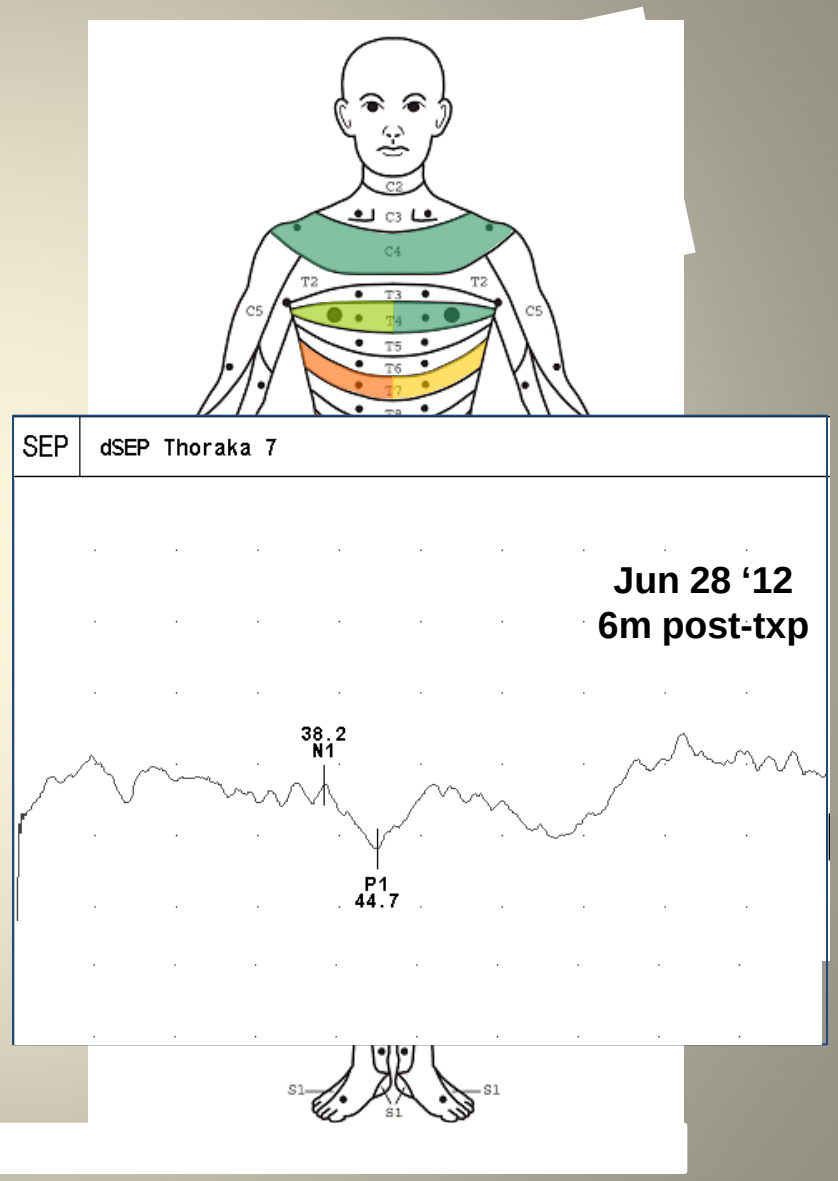
impaired

absent

© StemCells, Inc. 2012

Subject 3: EPT and dSSEP (T7 Left)





© StemCells, Inc. 2012

Clinical Trials with CNS SC derived human neurospheres

- Batten Disease, a lysosomal storage disease with fatal neurodegeneration OHSU
- Pelizeus Mehrbacher, a fatal dysmyelinating disease due to PLP mutation UCSF
- Thoracic spinal cord contusion injury Zurich
- Stem Cells Inc [IW a Director and consultant]

Reconstitution of sublethally irradiated NSG pups transplanted with human CB mononuclear cells

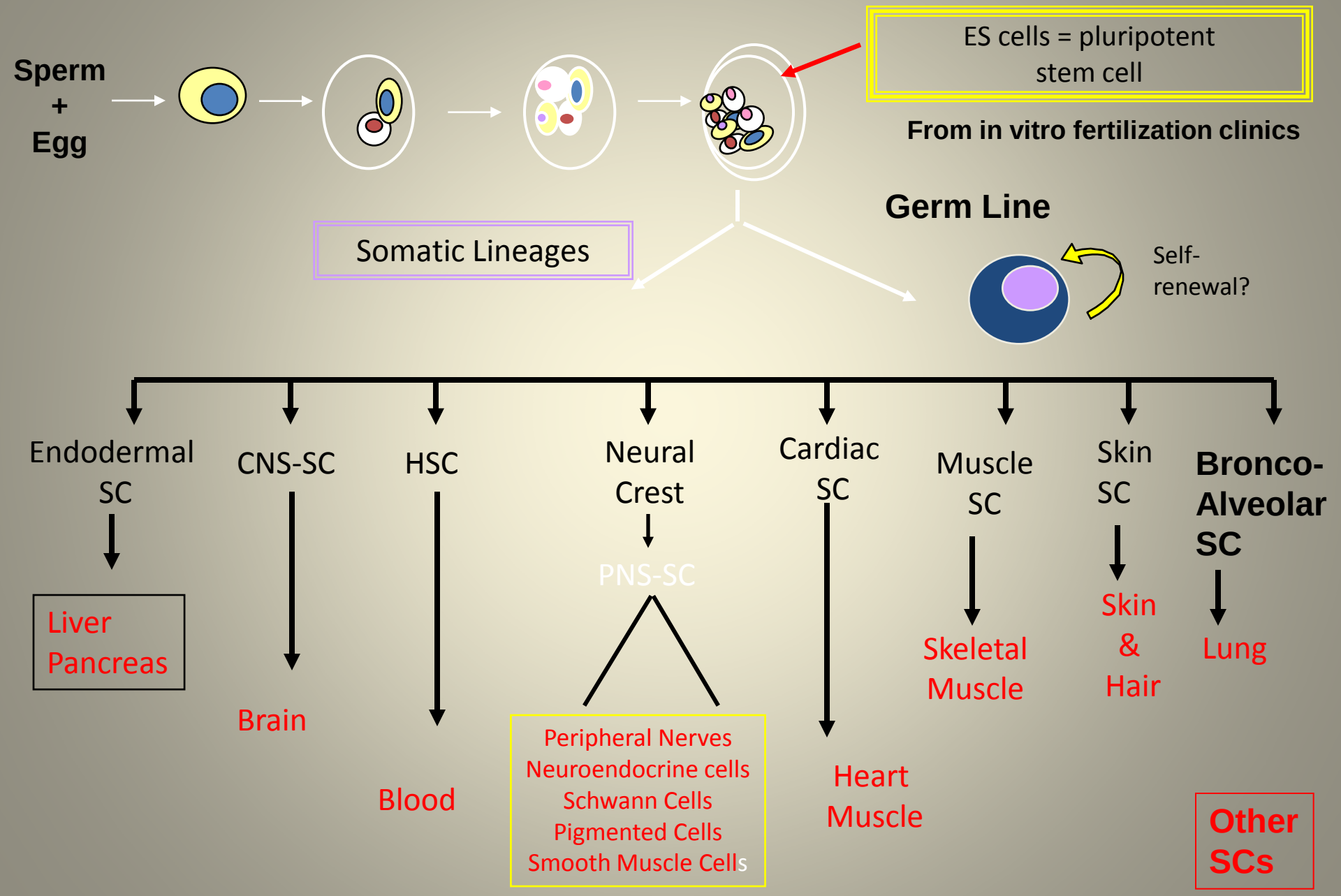
16 weeks post-transplant	Number of CB samples	Median Cells Injected	Number of Mice Injected	% Mice with Human Engraftment	Average % Human Chimerism	Average % Myeloid Cells	Average % Lymphoid Cells
PB	10	10000000	10	100%	34.6%	3.4%	89.2%
BM	10	10000000	10	100%	39.8%	10.2%	88.3%

Unproven stem cell treatments

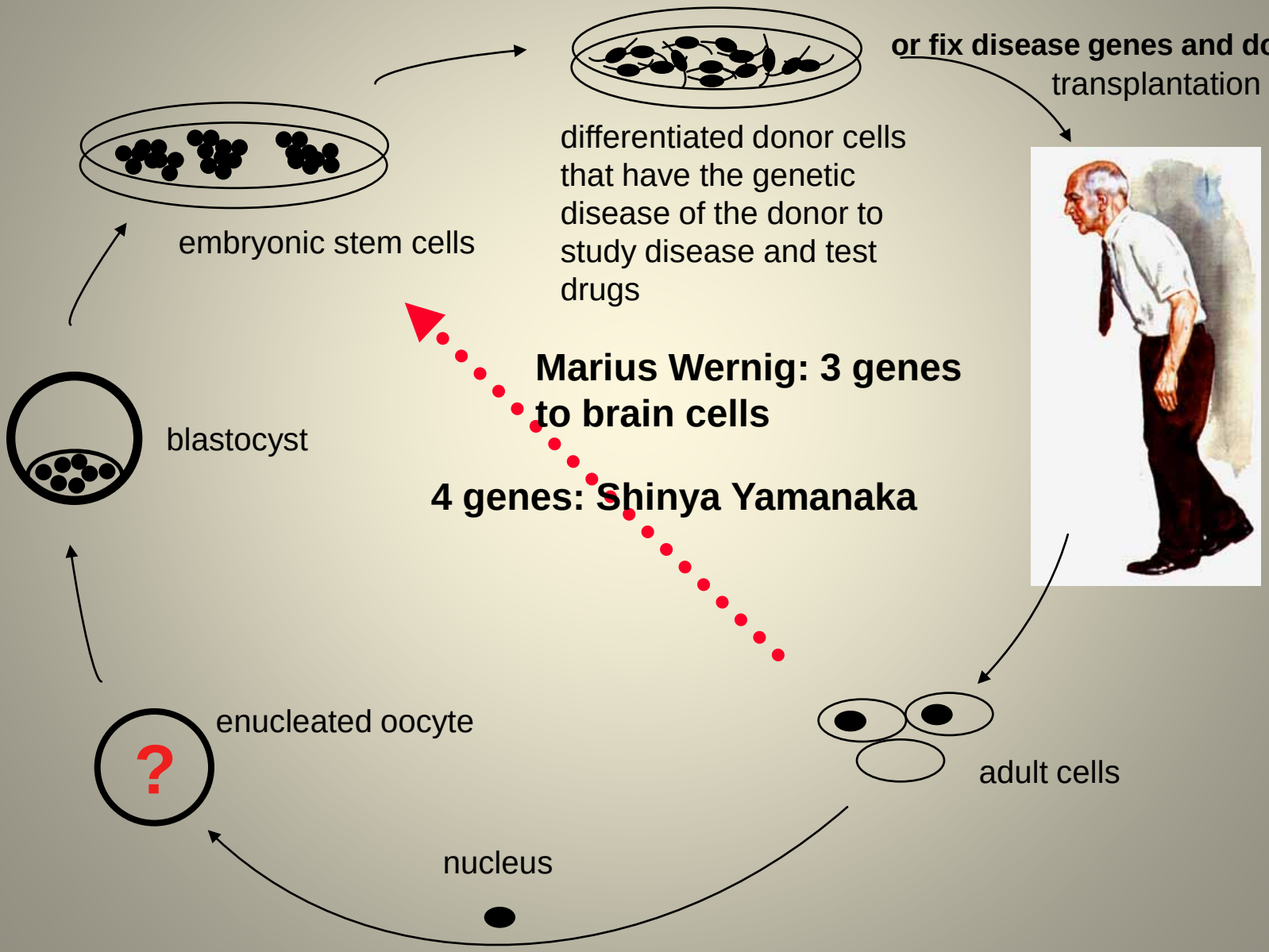
- **Beware of advertised treatments where one kind of stem cell is used to regenerate blood, brain, heart, lung and other damaged tissues; none can.**
- **There are plenty of these fraudulent treatments advertised to save lives.**
- **They mainly deplete patients with hopeless diseases of their money without any tissue regeneration. They do so while they risk your lives, rob you of the time better spent with family and friends, and rob your children of any assets you wished to pass on.**
- **Go to the ISSCR website:http://www.closerlookatstemcells.org//AM**

Given in a lecture to 180 farmers, ranchers, business people in Great Falls Montana-Two volunteered after the lecture that they had spent $50-90,000 to treat CNS diseases with stem cell treatments

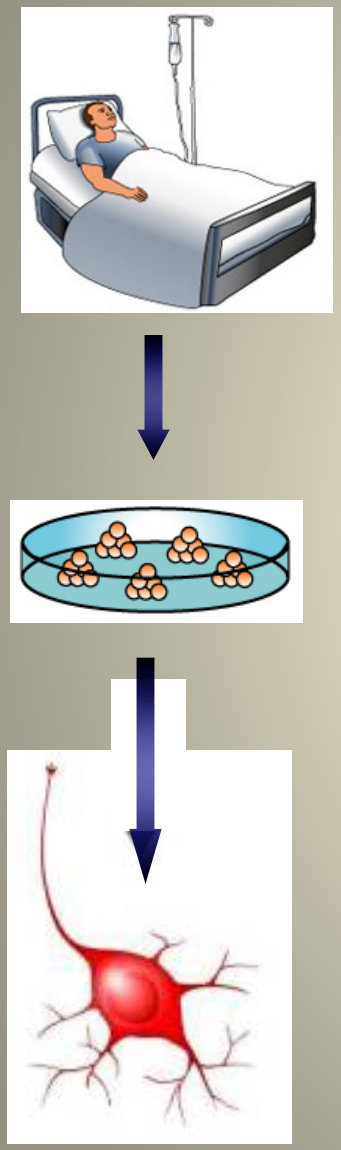
Pluripotent Embryonic Stem Cells



Genetic reprogramming



Patient-specific stem cells



- Human disease model in the dish
- Test disease model in SCID mouse by transplantation of each affected tissue's stem cells into the tissue of origin in the mouse.
- Epidermolysis Bullosum Team: Tony Oro, Marius Wernig, Al Lane
- Parkinson's Disease: Renee Reijo Pera
- Heart stem cells from ES cells: Weissman/Ardehali, Wu, and Robbins

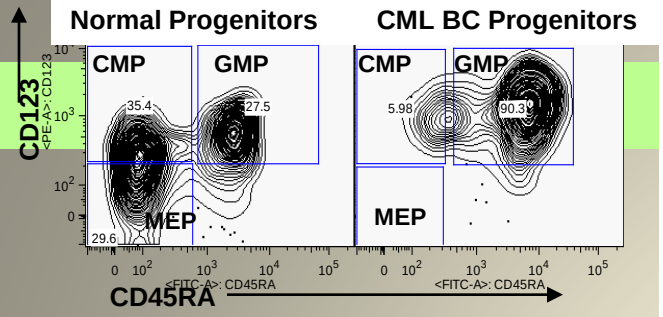
CML

- Fialkow: clonal disorder in G,M.E,B cells; Rowley/Nowell bcr-abl translocation; fusion protein in chronic, myeloproliferative phase; LSC proposed to be HSC or MPP; Jamieson and Weissman HSC.
- Myeloid blast crisis is at the stage of GMP, and overexpress activated β-catenin;axin inhibits them.
- 4/7 pts overexpress β-catenin by mis-splicing GSK3 the other inhibitor of β-catenin

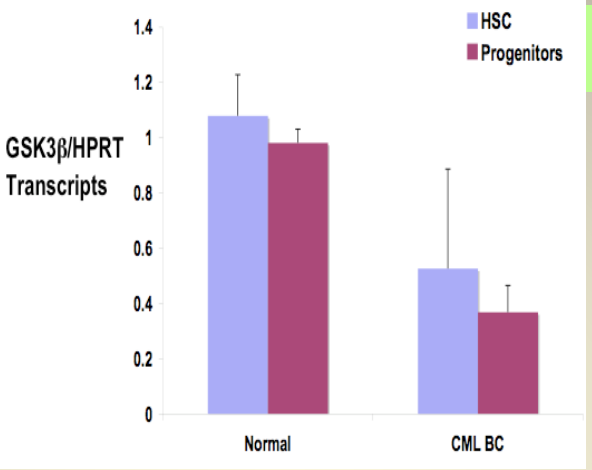
Jamieson and Weissman, 2004, 2009

Figure 3. Aberrant GSK3β Expression by Blast Crisis CML Progenitors

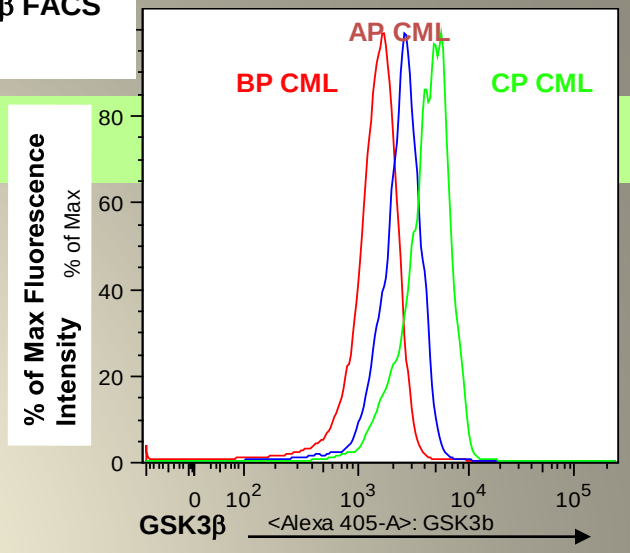
A. Progenitor FACS Analysis



B. Q-PCR of GSK3β Expression

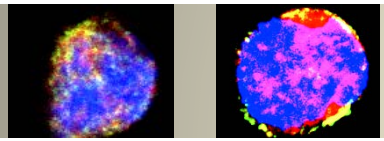


C. GSK3β FACS

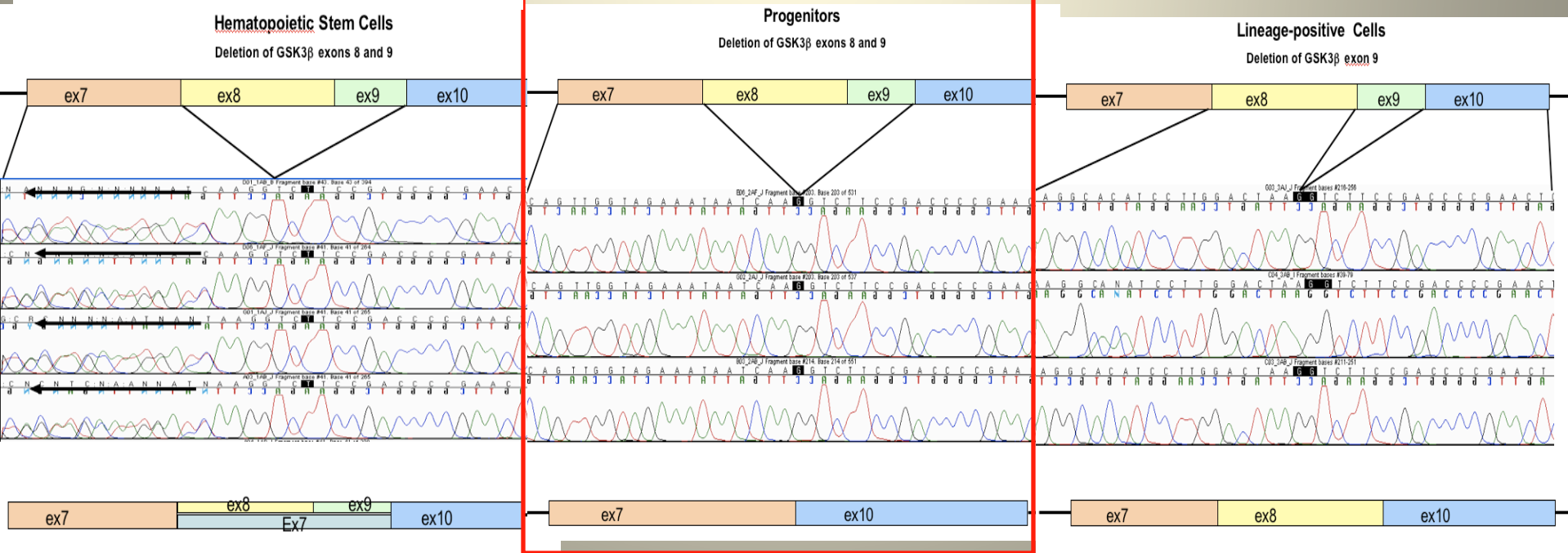


D. Confocal Analysis of β-catenin Expression

Normal GMP CML GMP



E. GSK3β cDNA Sequencing Analysis



Cell surface markers on LSC but not HSC

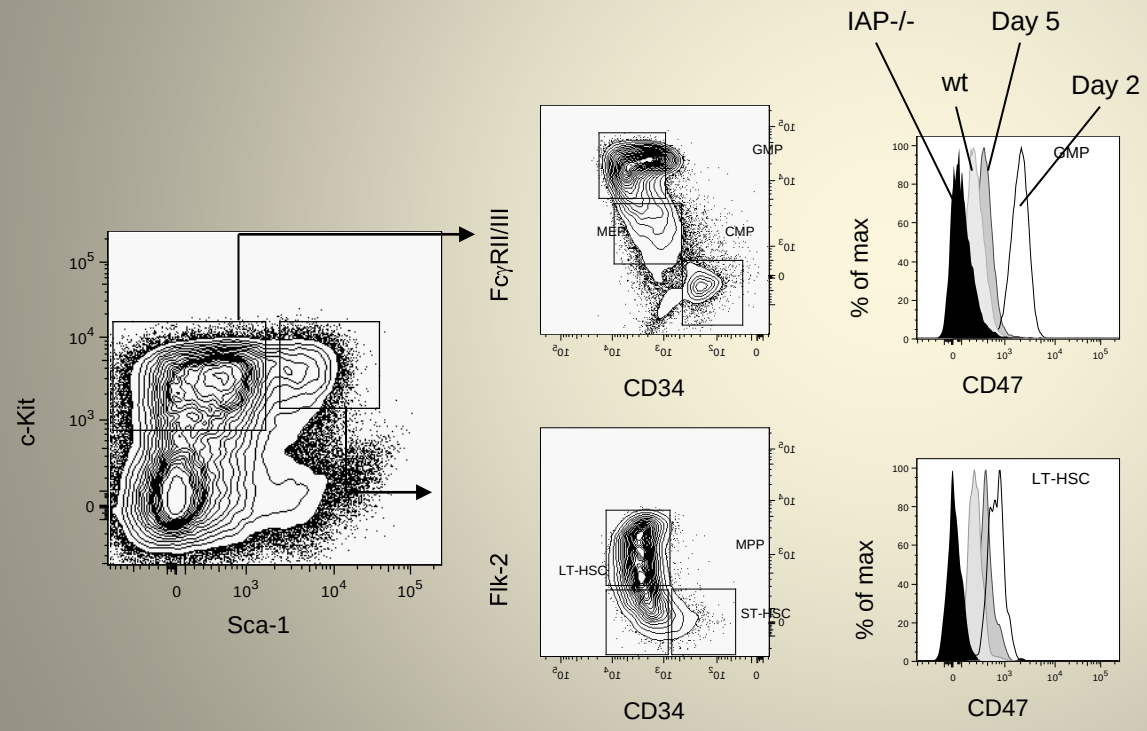
CD 96 > 40 fold in ~ 50% of AML samples

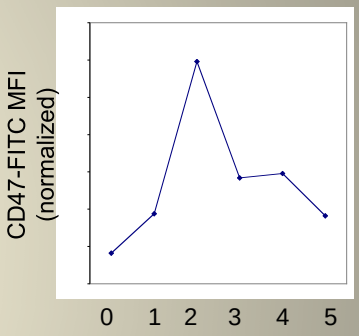
CD 44 ~ 3 fold in all tested AML

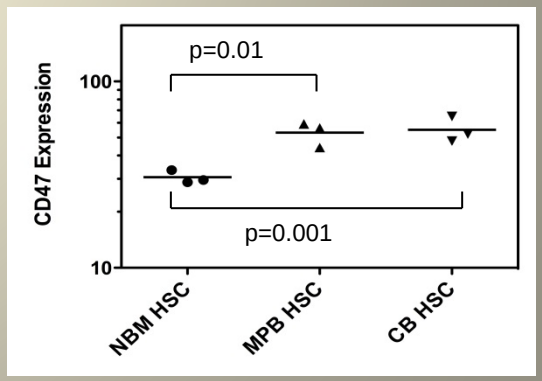
CD47 > 5 fold in all mouse AML LSC [1998]
and all tested human AML LSC[2008]

Traver and Weissman; Hosen, Majeti, Alizadeh and Weissma

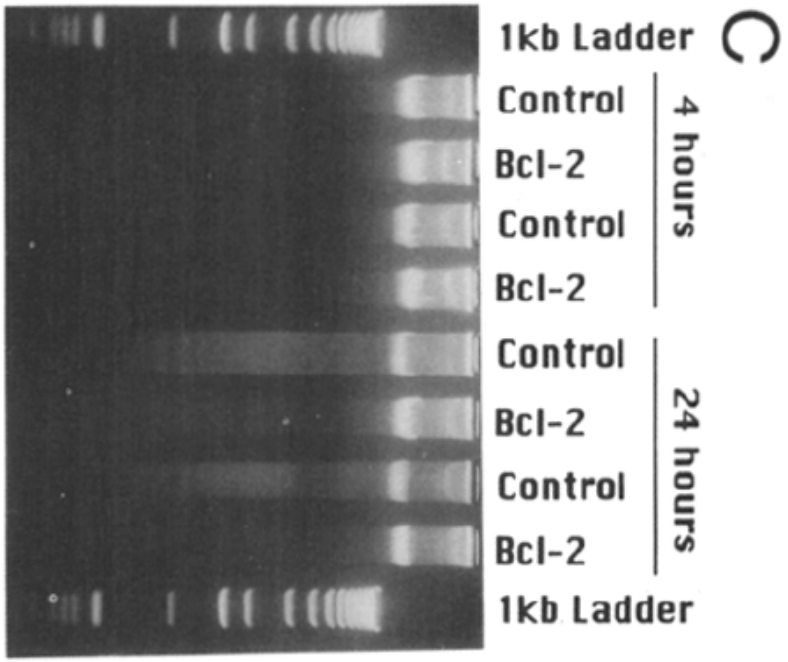
CD47 is up-regulated in HSC during mobilization and inflammation







BCL2 blocks apoptosis, but not programmed cell removal of neutrophiles: Lagasse and Weissman JEM 1994



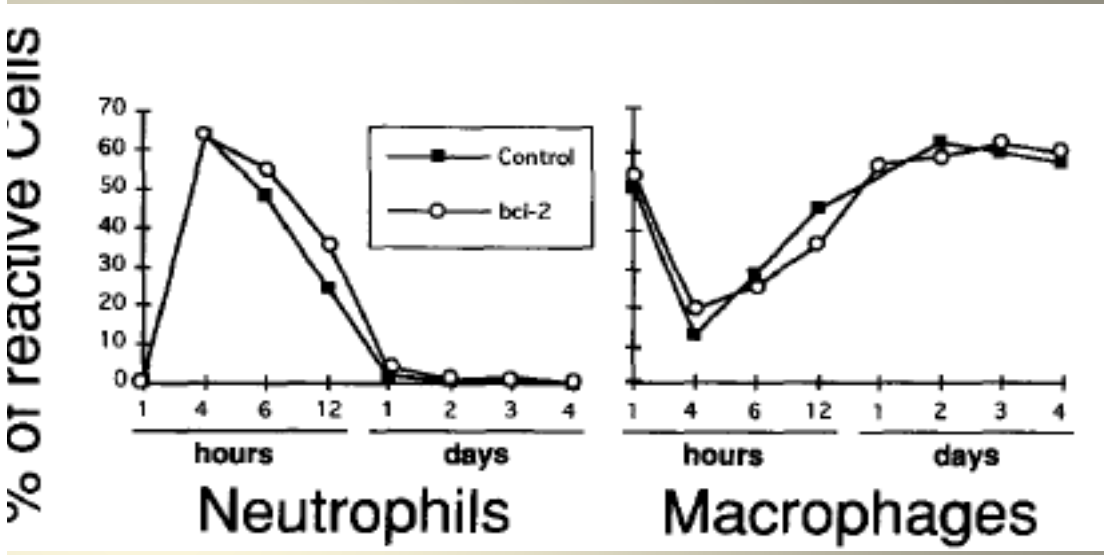
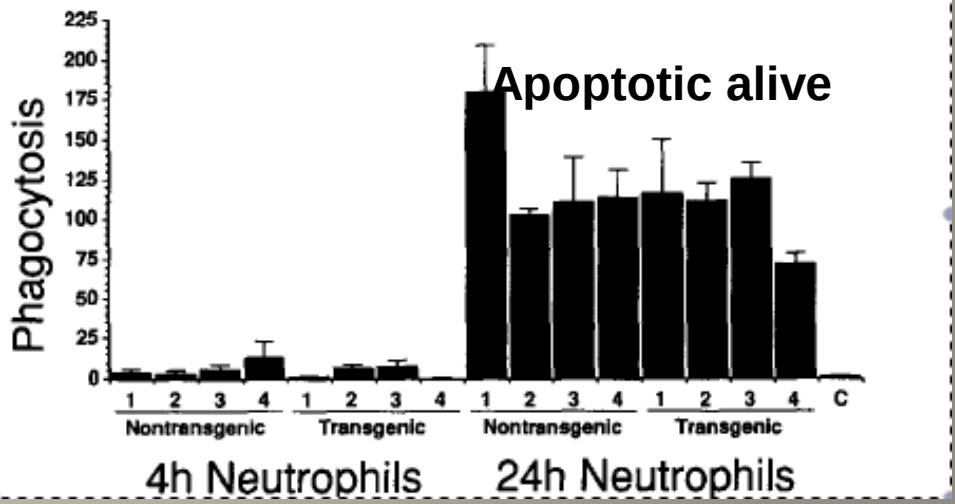


Table 1. *Neutrophil Content in the Bone Marrow, Blood and Spleen of Control and Transgenic Mice*

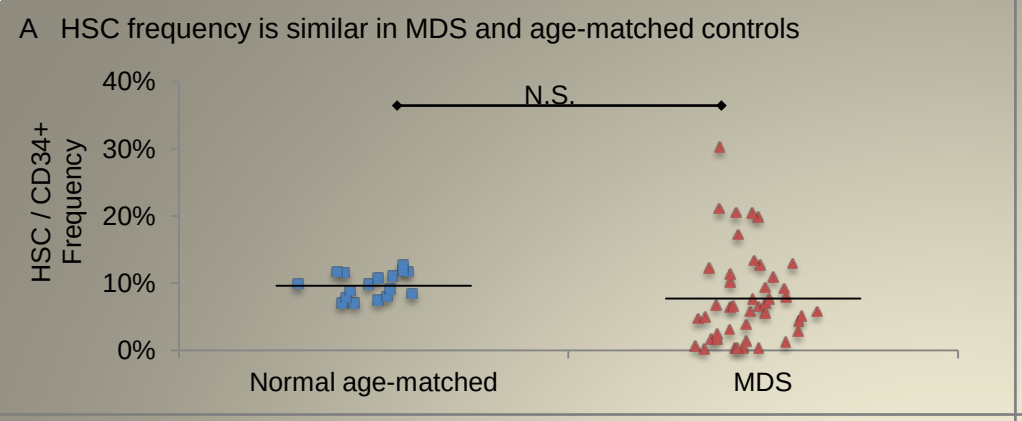
Mice	Bone marrow	Blood	Spleen
Nontransgenic	30.4 ± 4.0	5.8 ± 2.8	1.8 ± 0.4
Transgenic	30.3 ± 8.3	12.6 ± 3.8	2.2 ± 0.4

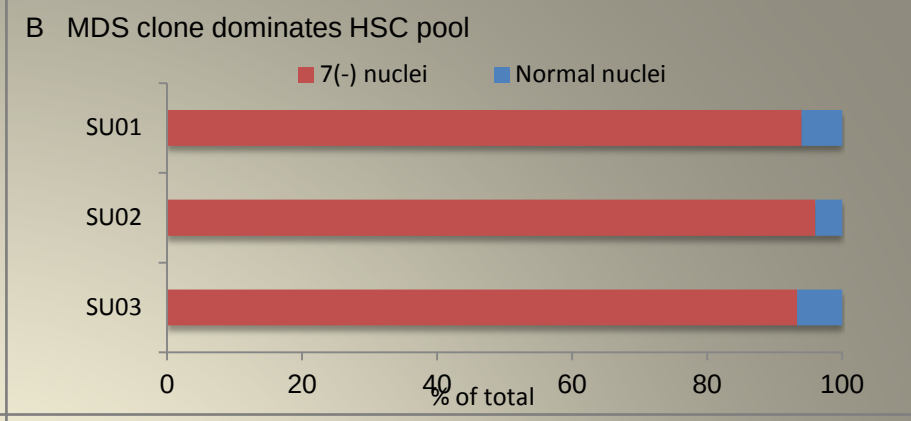
Neutrophils were counted by flow cytometric analysis of cells bearing Mac-1 and Gr-1 using two-color immunofluorescence. The results are expressed as arithmetic means (three mice) ± SD.

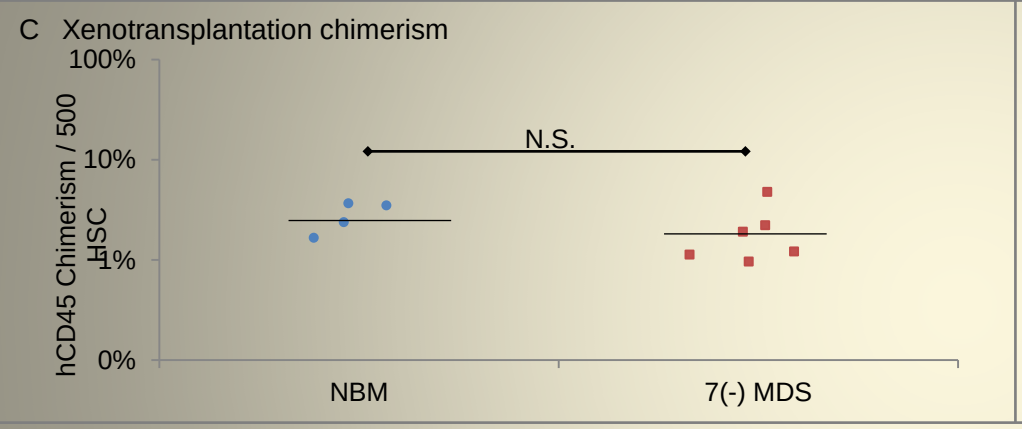


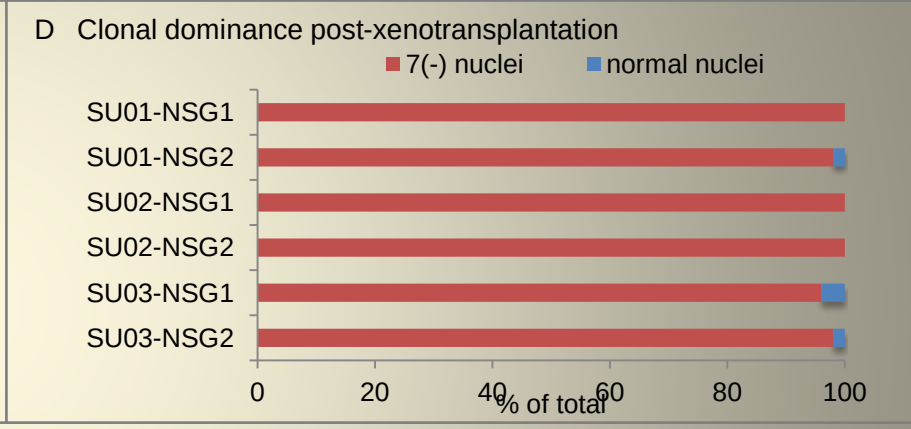
PCDeath and PCRemoval

- PCD is accompanied by PCR; blocking PCD with bcl2 does NOT block PCR [Lagasse and Weissman 1994, JEM]. PCR prevents inflammation.
- All cancers defeat PCD: p53, bcl2, bax, etc
- All cancers defeat PCR: calreticulin, Ph-serine, asialoglycoprotein ‘eat me’ and CD47 ‘don’t eat me’
- Stimuli that induce PCD and/or PCR develop cell competition/selection in pre-cancer lineages that can result in cancer clones







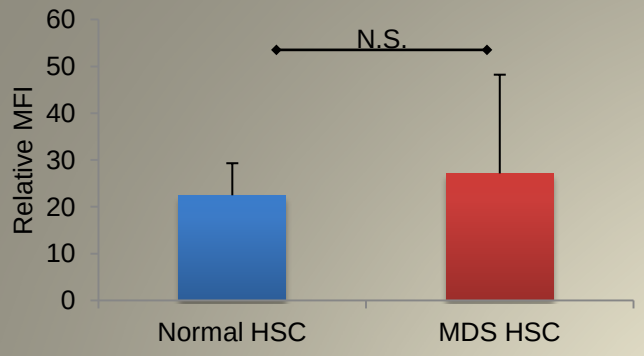


MDS is a pre-AML disease of older patients in which a cytopenia Precedes leukemia.

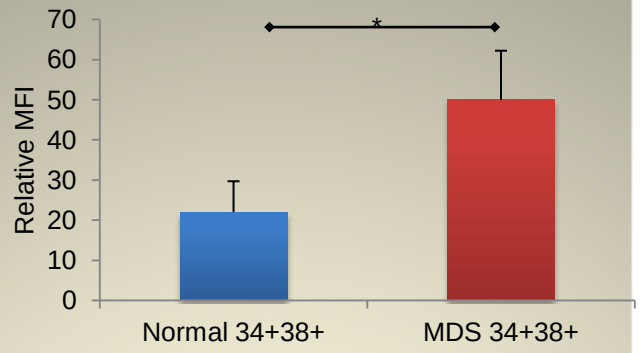
MDS HSC outcompete normal HSC in MDS patients and in transplanted NSG mice

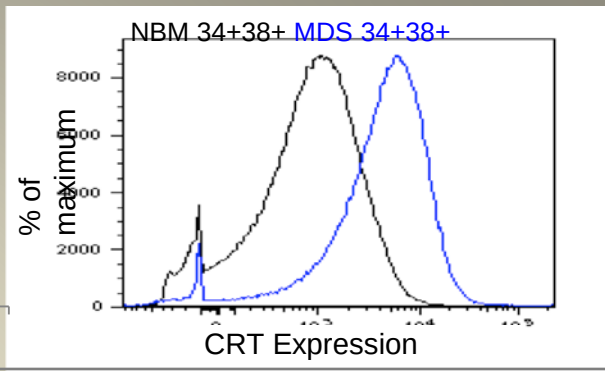
Wendy Pang, John Pluvinage, Chris Park, IW, et al

A CRT not increased on MDS HSC

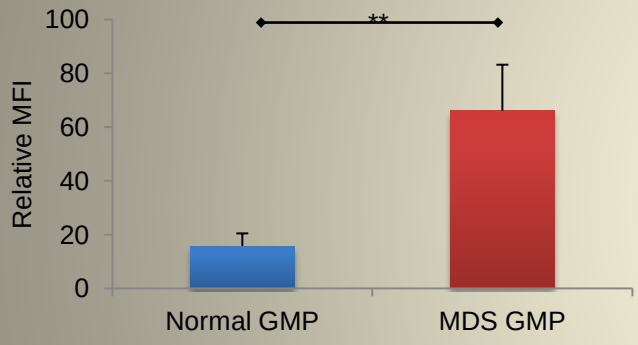


B Increased cell-surface CRT on MDS CD34+CD38+ cells

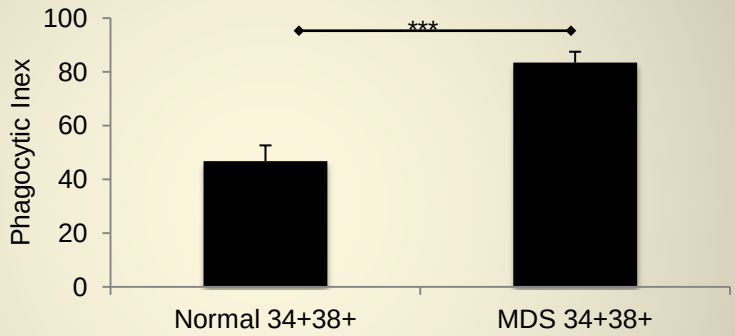


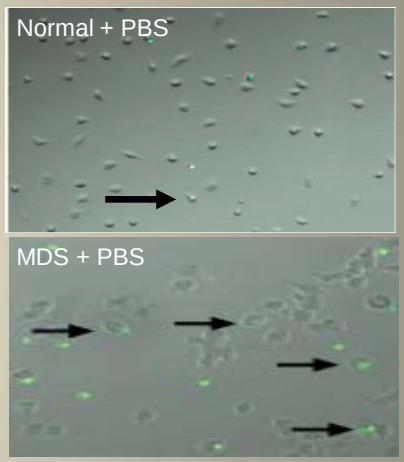


C Increased CRT on MDS GMP



D Increased phagocytosis of MDS CD34+CD38+ cells

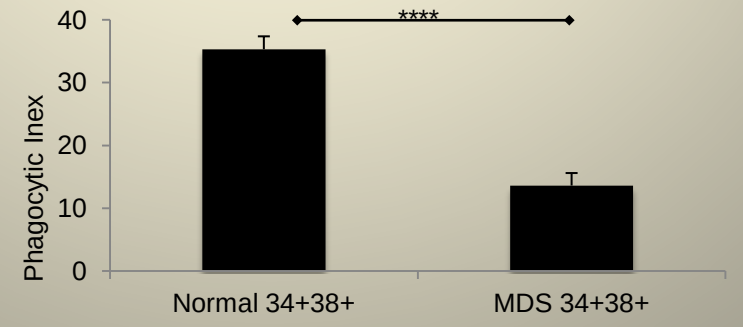


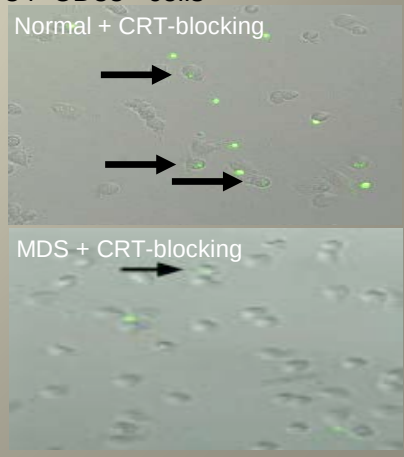


Calreticulin expressed on MDS progenitors, but not HSC, causes programmed cell removal

Wendy Pang, John Pluvinage, Chris Park, IW, et al

E CRT-blocking peptide abrogates phagocytosis of MDS CD34+CD38+ cells





Conclusions

MDS HSC outcompete normal HSC in patient and xenotransplant

The MDS-initiating cell resides in HSC compartment

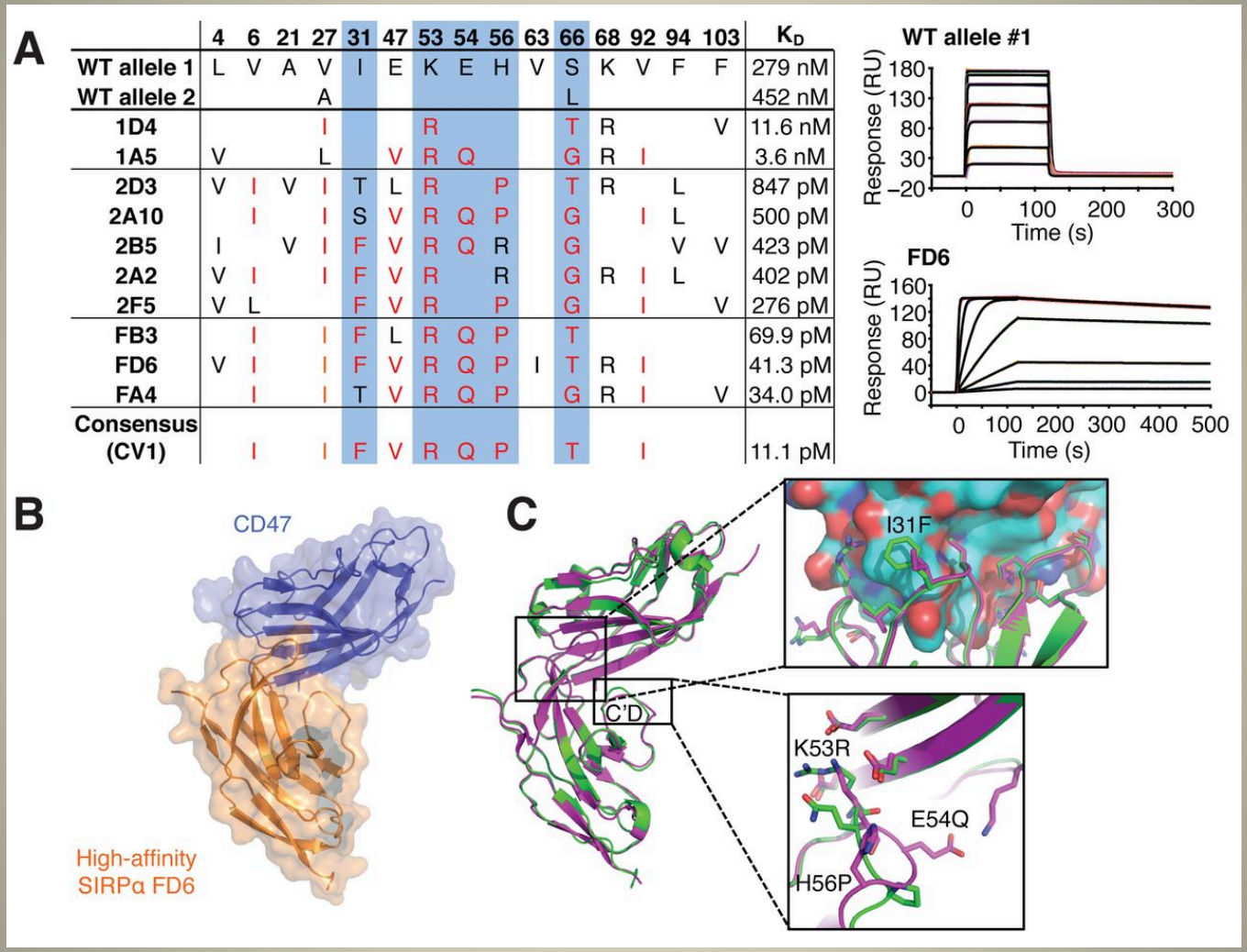
High CRT predisposes MDS myeloid progenitors for programmed cell removal

Increased CD47 expression is a crucial step in the progression from MDS to AML

- Calreticulin is a potential therapeutic target

Wendy Pang, John Pluvinage, Chris Park, IW, et al

Fig. 1 Directed evolution of high-affinity SIRPα variants.(A) Summary of sequences and SPR affinity measurements of engineered SIRPα variants.



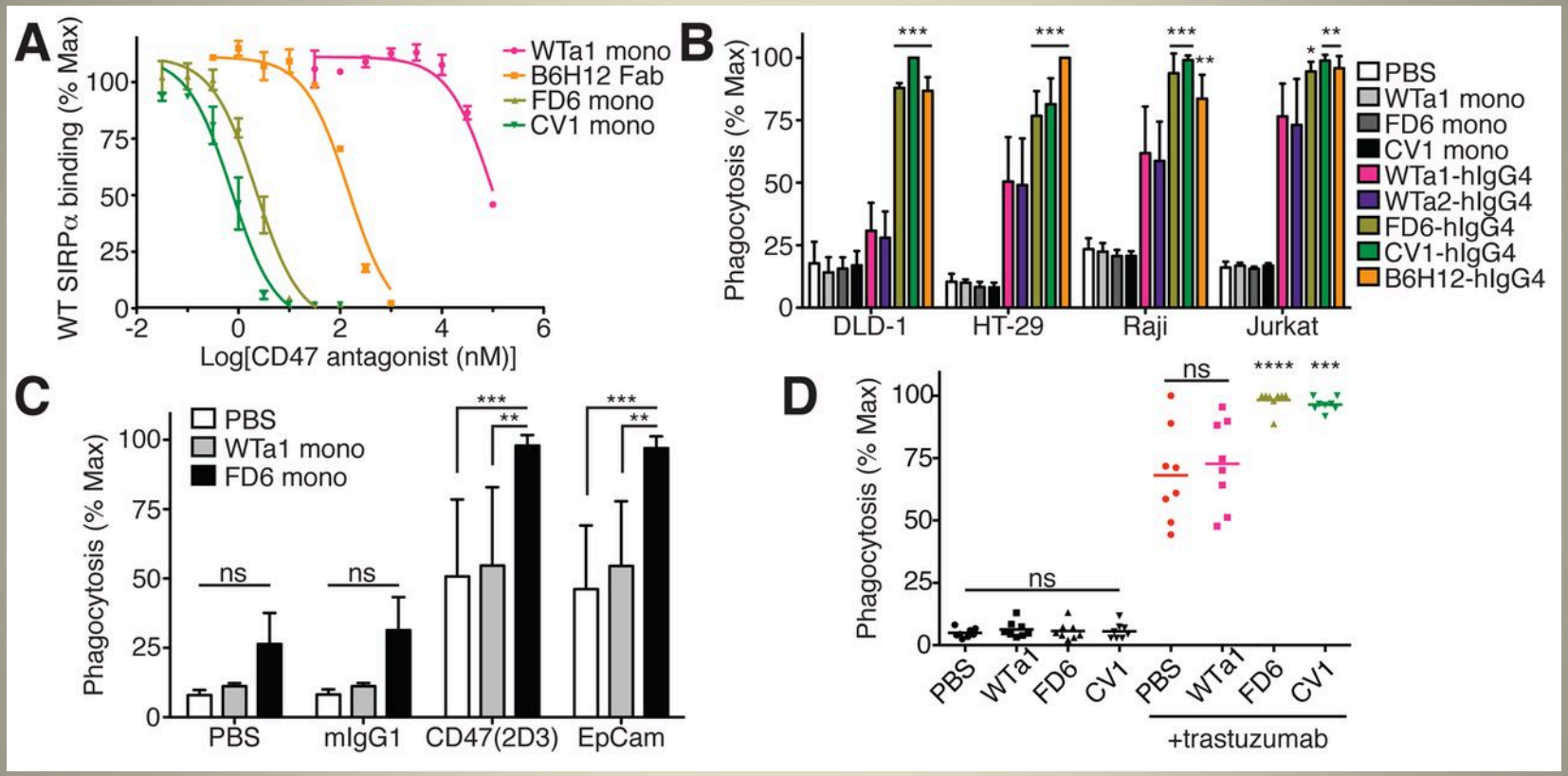
	4	6	21	27	31	47	53	54	56	63	66	68	92	94	103	K_D
WT allele 1	L	V	A	V	I	E	K	E	H	V	S	K	V	F	F	279 nM
WT allele 2				A							L					452 nM
1D4				I			R				T	R			V	11.6 nM
1A5	V			L		V	R	Q			G	R	I			3.6 nM
2D3	V	I	V	I	T	L	R		P		T	R		L		847 pM
2A10		I		I	S	V	R	Q	P		G		I	L		500 pM
2B5	I		V	I	F	V	R	Q	R		G			V	V	423 pM
2A2	V	I		I	F	V	R		R		G	R	I	L		402 pM
2F5	V	L			F	V	R		P		G		I		V	276 pM
FB3		I		I	F	L	R	Q	P		T					69.9 pM
FD6	V	I		I	F	V	R	Q	P	I	T	R	I			41.3 pM
FA4		I		I	T	V	R	Q	P		G	R	I		V	34.0 pM
Consensus (CV1)		I		I	F	V	R	Q	P		T		I			11.1 pM

K Weiskopf et al. Science 2013;science.1238856

Weiskopf, Ring, Volkmer, IW and Garcia

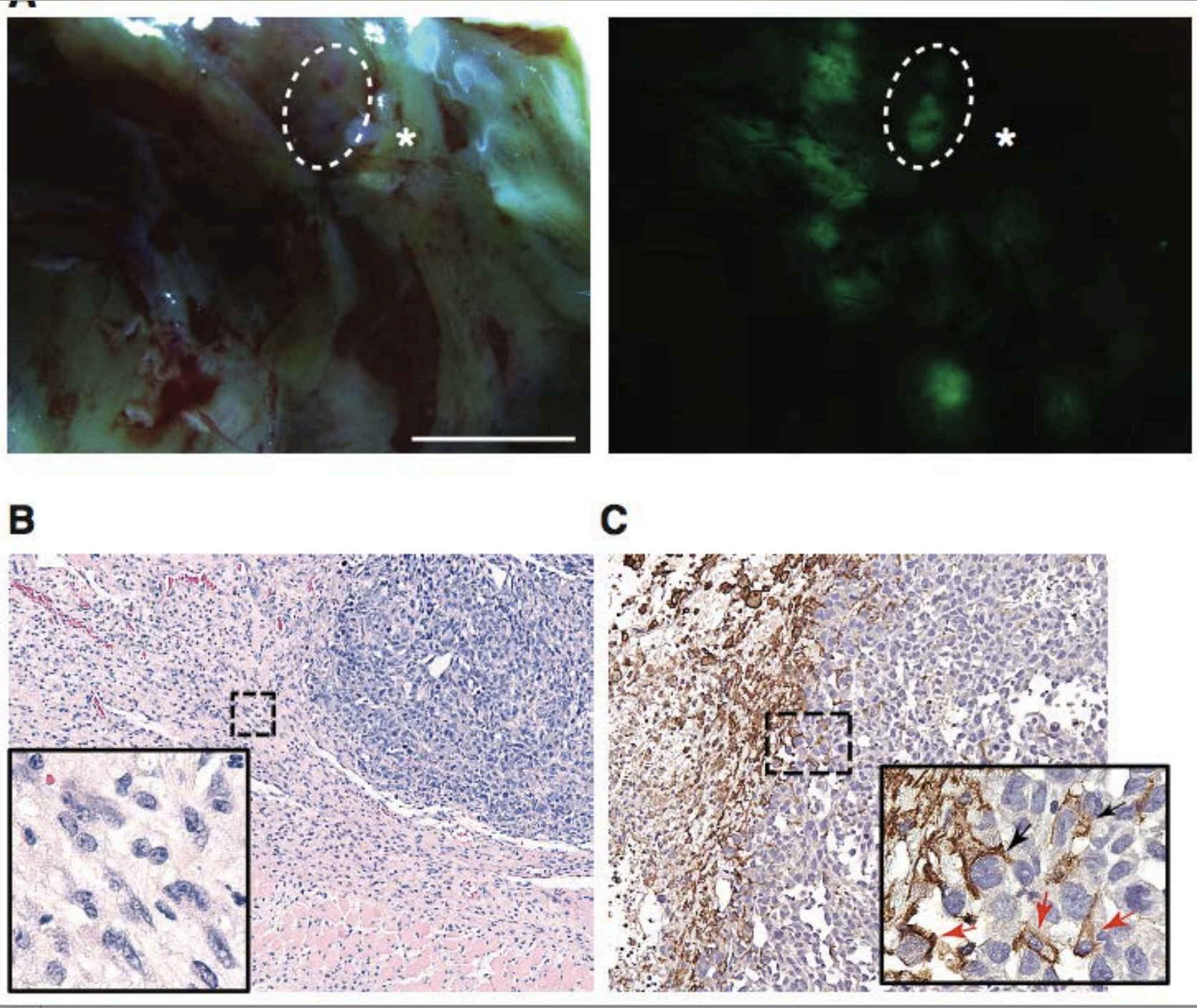
Published by AAAS

Fig. 2 High-affinity SIRPα variants lower the threshold for macrophage phagocytosis.(A) Dose-response curves of CD47 antagonism on Raji lymphoma cells with wild-type SIRPα allele 1 (WTa1), Fab fragments produced from the blocking anti-CD47 antibody clone B6H12, or two high-affinity SIRPα variants as monomers (FD6, CV1).



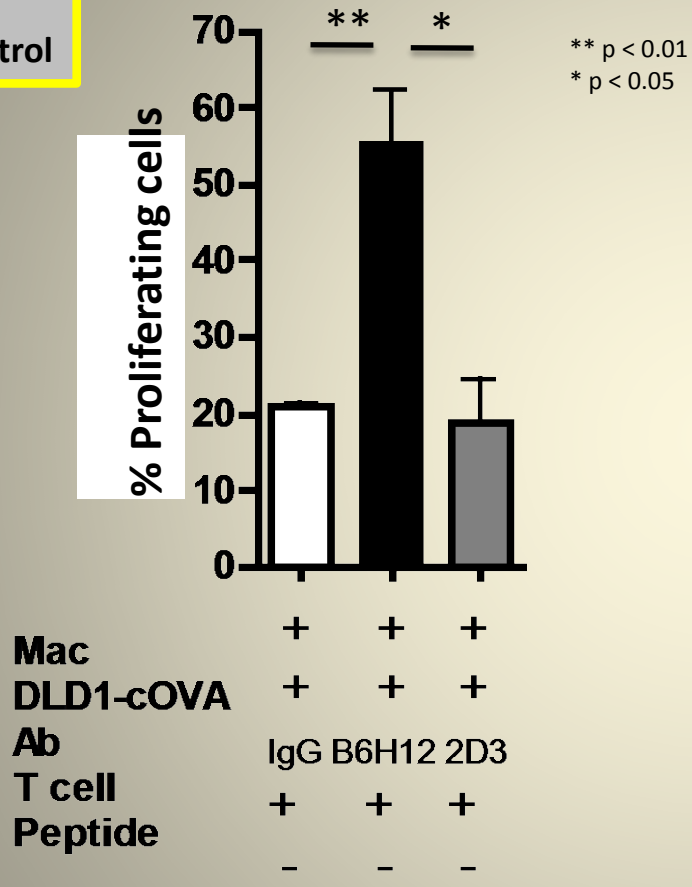
K Weiskopf et al. Science 2013;science.1238856

Published by AAAS

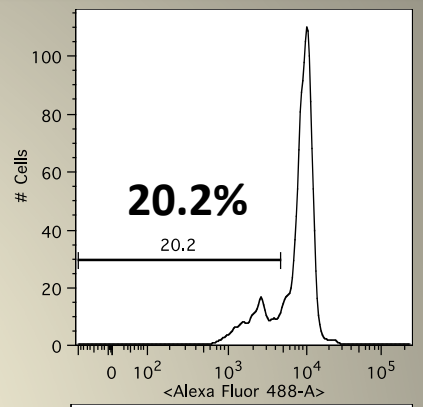

A
B
C

Anti-CD47-mediated phagocytosis of tumors by macrophages leads to increased MHC I antigen presentation

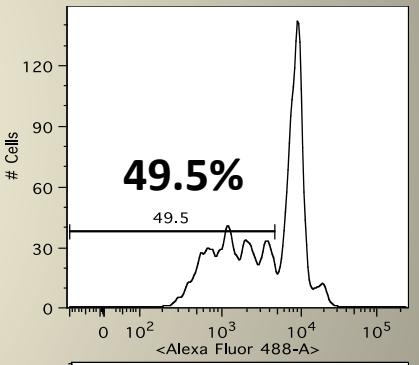
1) CD4 T cells
2) CD8 T cells
3) Tumor control



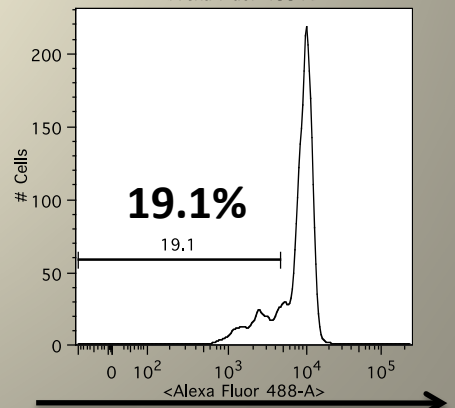
IgG



Anti-CD47
B6H12



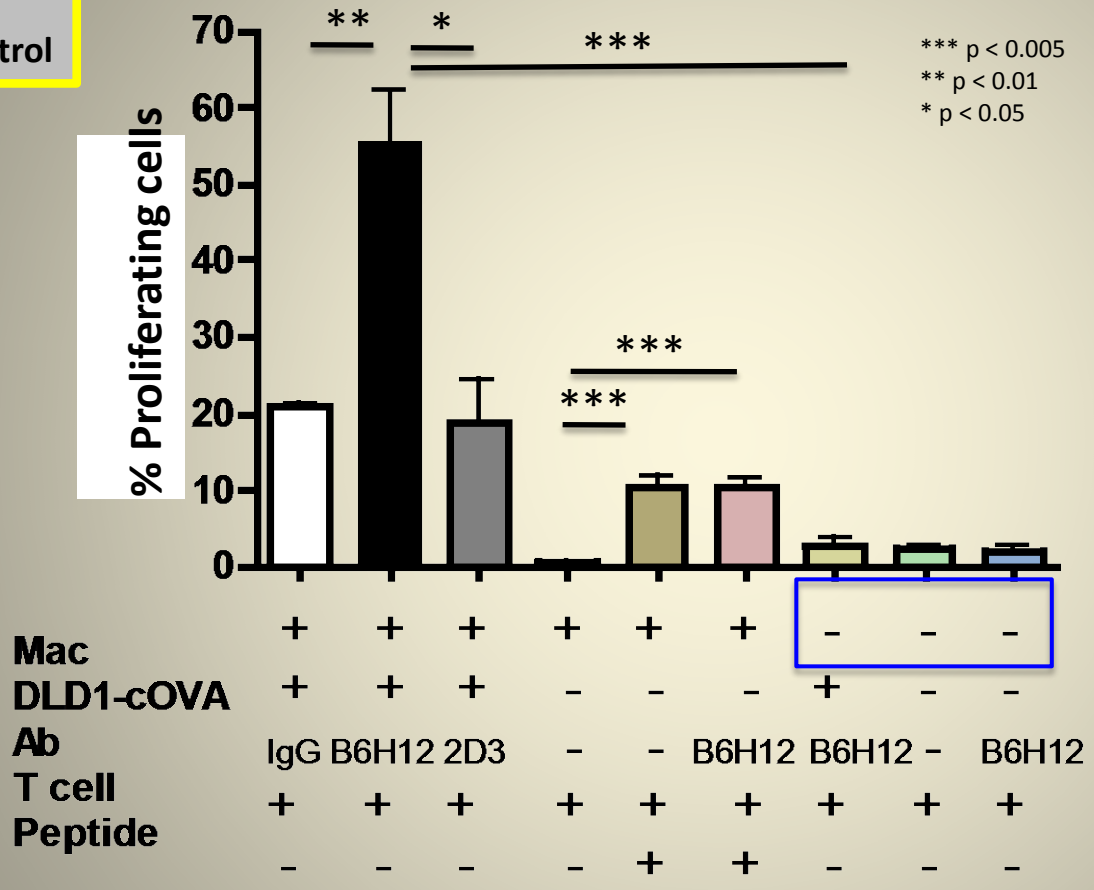
Anti-CD47
2D3



Diane Tseng

Anti-CD47-mediated phagocytosis of tumors by macrophages leads to increased antigen presentation of CD8+ T cells

1) CD4 T cells
2) CD8 T cells
3) Tumor control

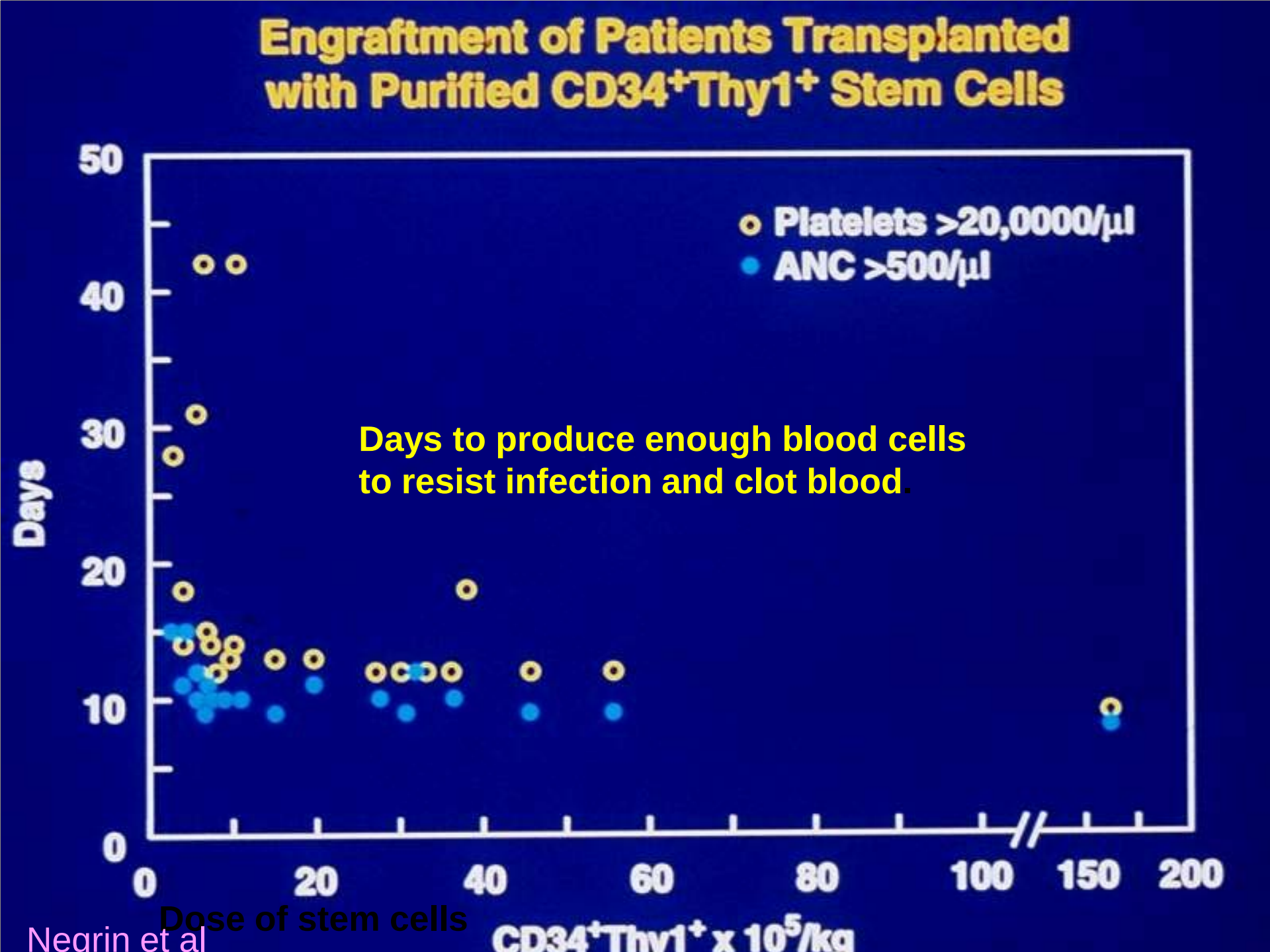


Conclusion: Following anti-CD47-mediated phagocytosis, macrophages activate the CD8+ T cell response

Diane Tseng

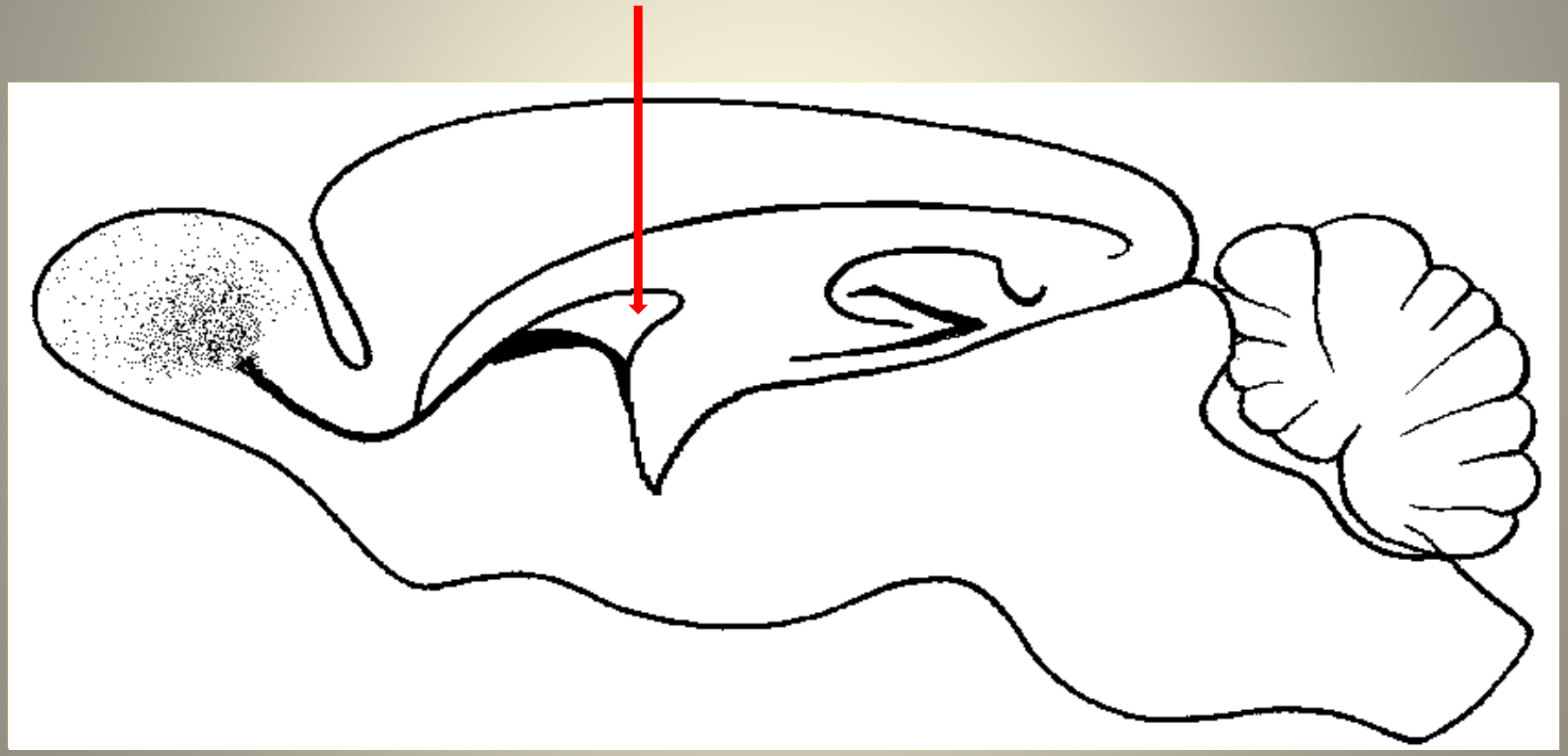
Adult (tissue) stem cells are useful in tissue and organ regeneration:Examples

- Blood-forming stem cells to regenerate blood and immune systems.
- Brain-forming stem cells to regenerate neuroprotective potential in brain.
- Skin-forming stem cells/burns.
- Limbal cells: cornea
- Skeletal muscle stem cells [not myoblasts] for muscular dystrophies in mice.
- Lung?, liver?, kidney? Heart? Etc.

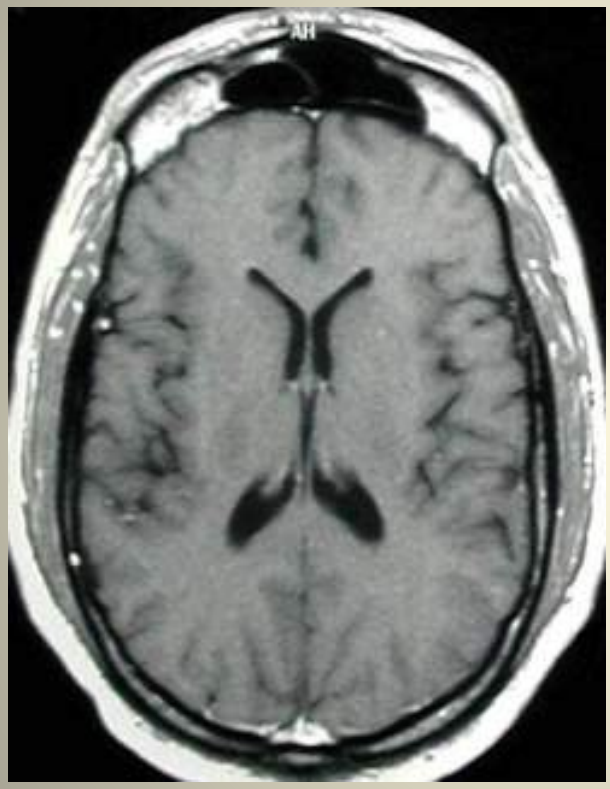

Engraftment of Patients Transplanted with Purified CD34+Thy1+ Stem Cells
Platelets >20,0000/µl
ANC >500/µl
Days to produce enough blood cells to resist infection and clot blood.
Days
50
40
30
20
10
0
0
20
40
60
80
100
150
200
Dose of stem cells
CD34+Thy1+ x 10^5/kg
Negrin et al

Testing Biological Activity of Human Cells in Animal Models: Xenogenic Transplant

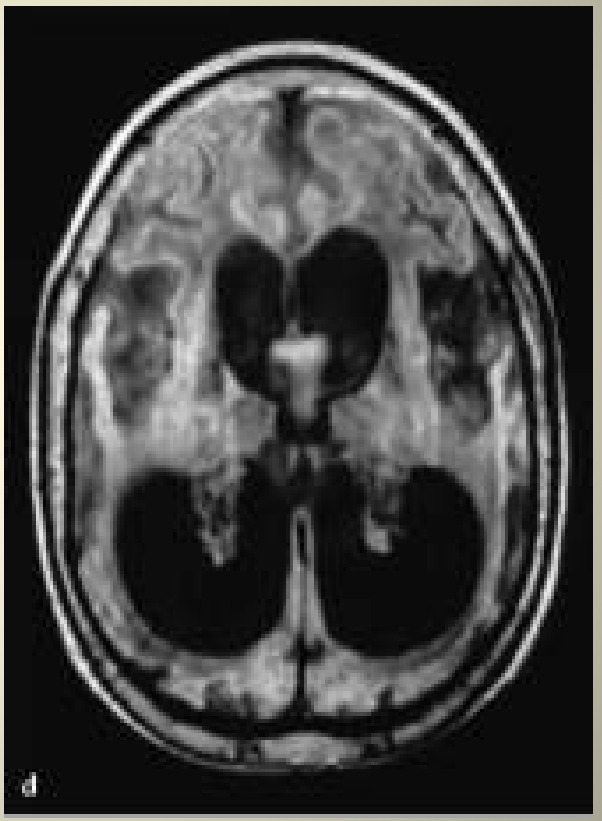
- **Immunodeficient mice: NOD-*scid***



MRI of End Stage Batten Brain



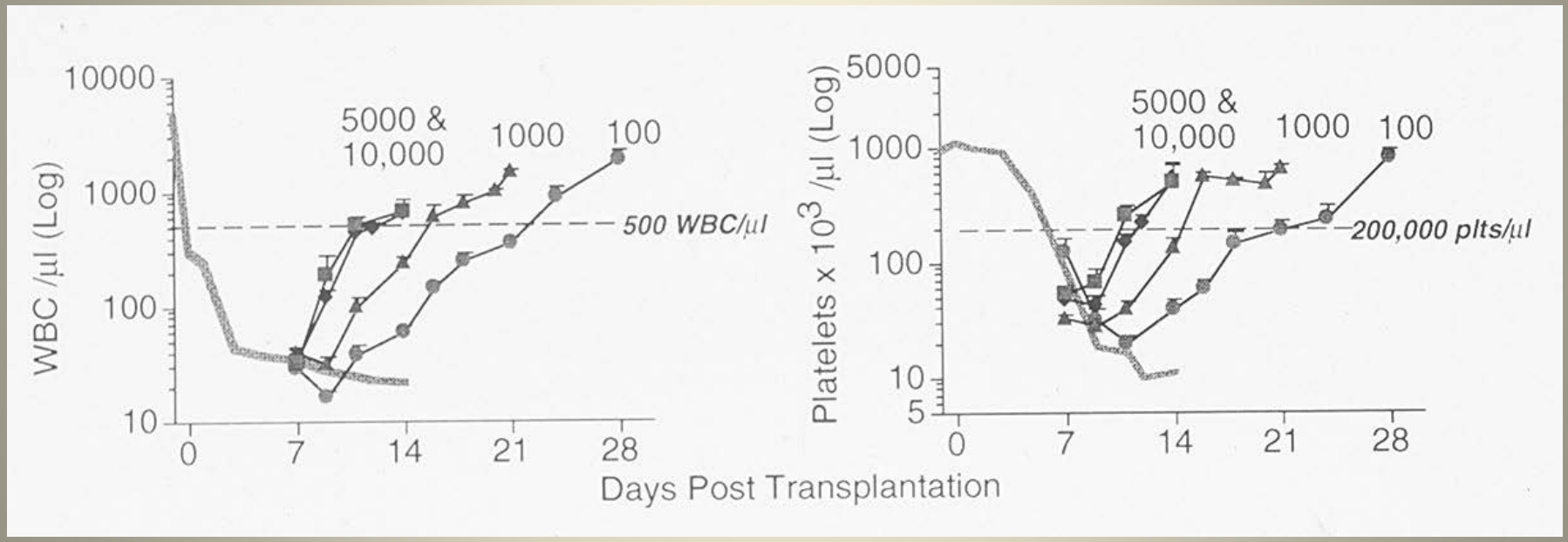
Normal Human Brain



7 yr old Batten child

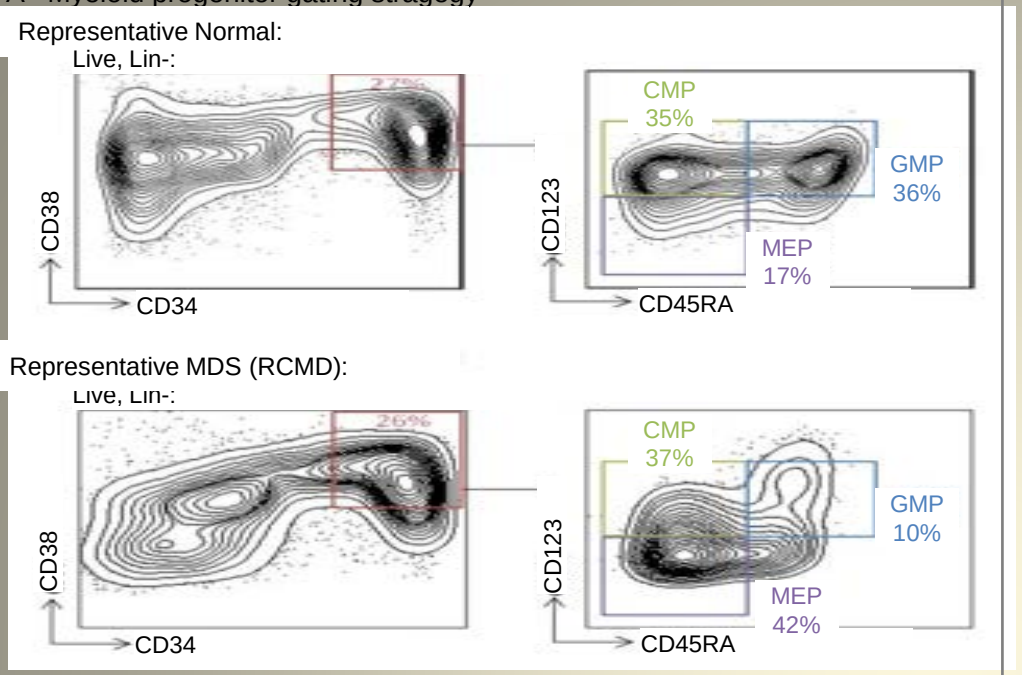
Vanhanen, S. et al, Neuropediatrics 35:27-35, 2004

Purified HSC transplantation can result in rapid engraftment

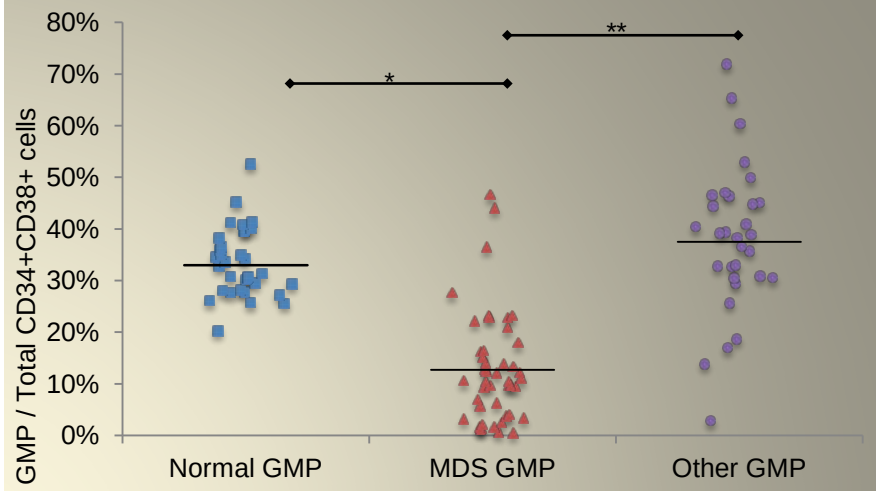


10,000 HSC = $4x10^5$/kg

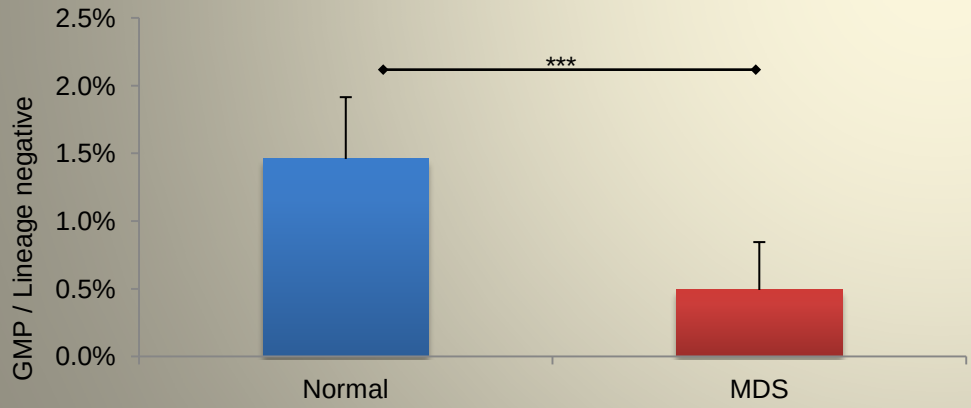
A Myeloid progenitor gating stragegy



B Decreased frequency of GMP in low-risk MDS



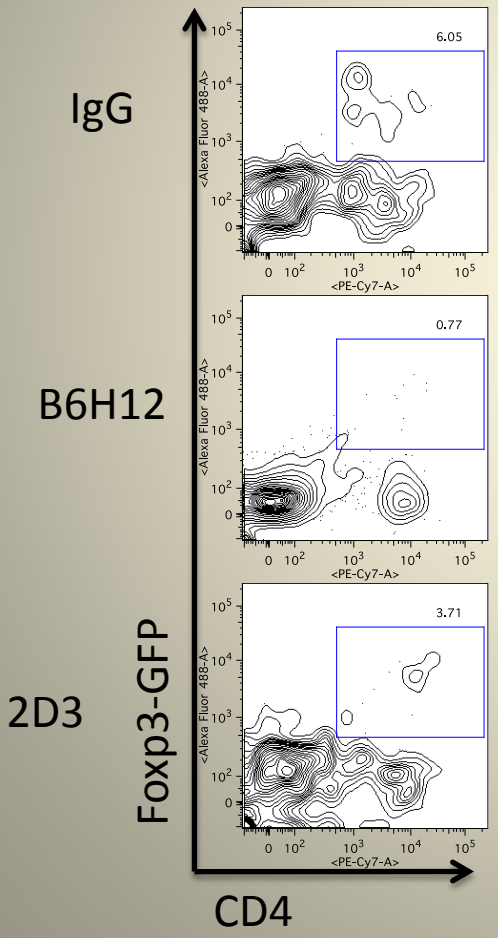
C Decreased frequency of GMP in low-risk MDS

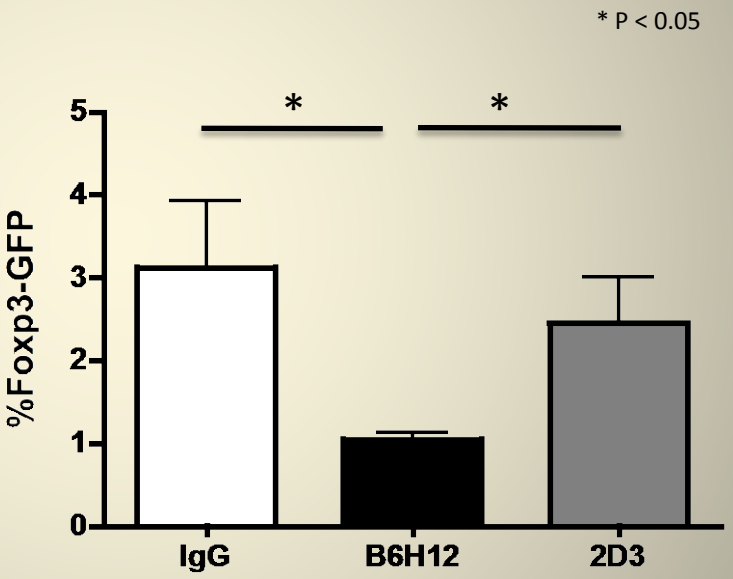


Decreased frequency of GMP is diagnostic for MDS

Wendy Pang, John Pluvinage, Chris Park, IW, et al

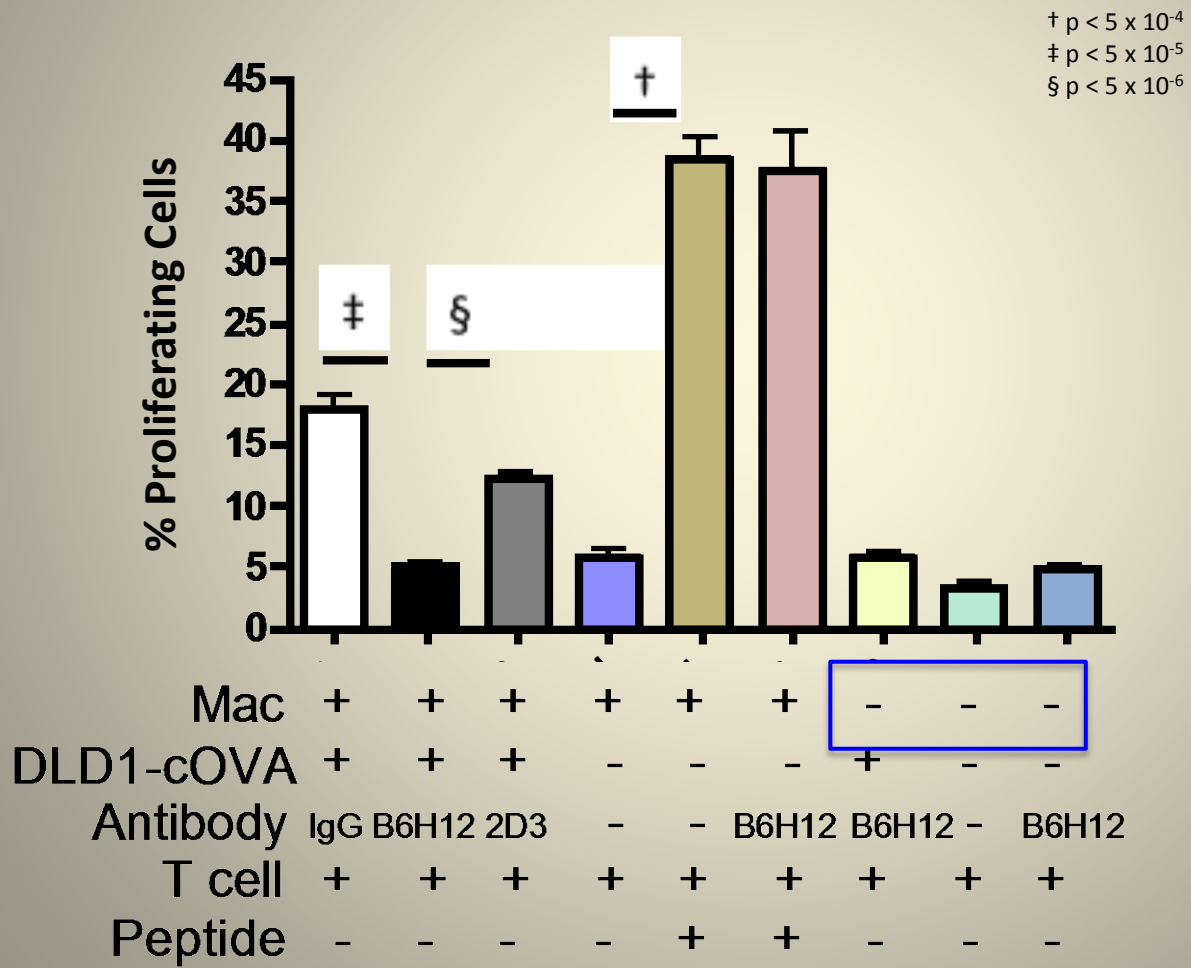
Anti-CD47 antibody leads to less efficient regulatory CD4+ T cell generation





Diane Tseng

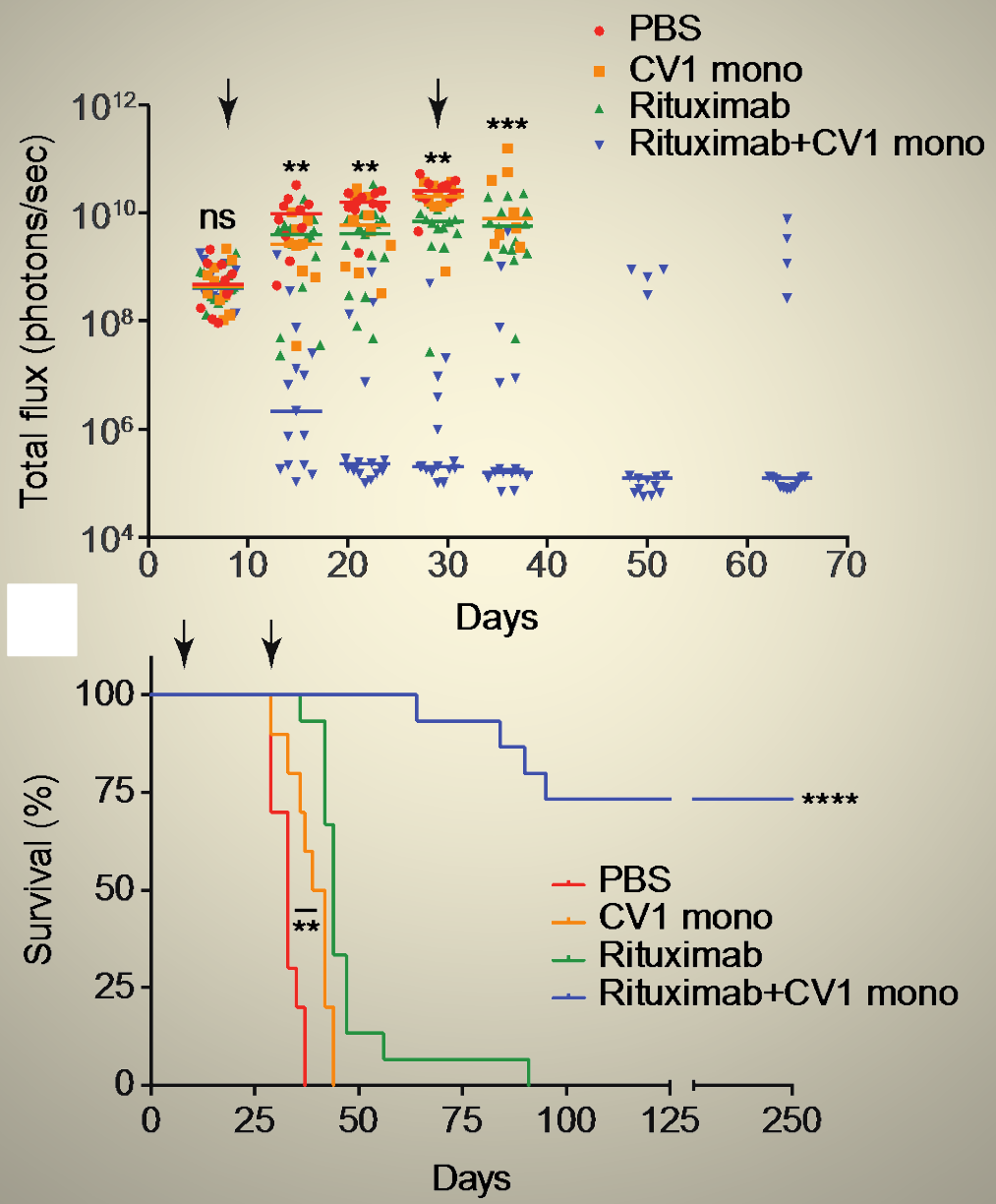
Anti-CD47-mediated phagocytosis of cancer by macrophages do not present antigen to OTII (CD4) T cells



Diane Tseng and
Jens Volkmer

Conclusion: Following anti-CD47 mediated phagocytosis of cancer, antigen presentation to CD4+ T cells is reduced below baseline levels

High-affinity SIRPα monomers enhance the efficacy of rituximab *in vivo*



Engraftment of human fetal blood-forming tissues in the SCID mouse

Ethics and politics in 1986-88





Bruno Peault, **Mike McCune, Reiko Namikawa, Ann Tsukamoto**
Irv Weissman

The changes we have seen are ones that are known
To be genetic: many more, such as tert, CD47, perhaps
Bcl2 up and Fas down, etc, are epigenetic, and will require epigenetic analyses for chromatin, CpG methyl. Dominant splice isoforms, etc.

Percentage of donor derived cells in human fetal bone segments injected with 10^4 donor cells

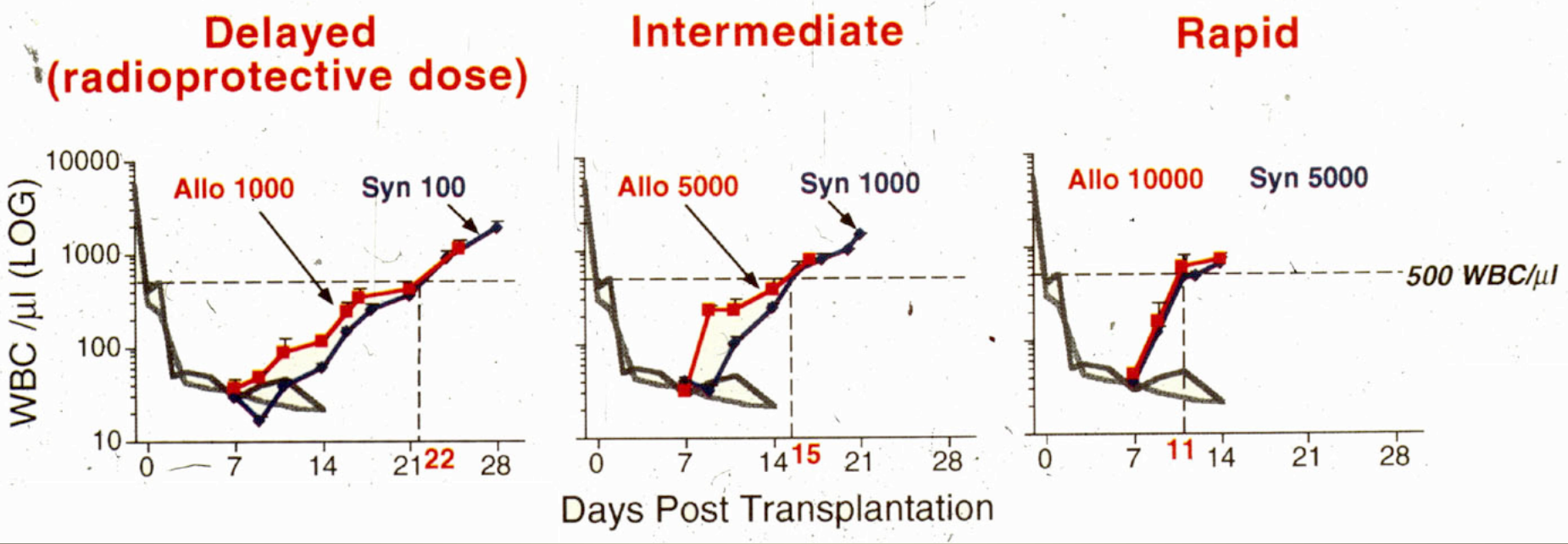
Population	2 months		3 months	
	% myeloid	% B	% myeloid	% B
Thy-1$^+$CD34$^+$	36	47	45	86
	13	20	6	1
	19	25	0	0
	ND	6	21	29
Thy-1$^-$CD34$^+$	0	0	0	0
	0	0	1	0
	0	0	0	0

Percentages of myeloid and B cells were determined 2 and 3 months after engraftment. Values represent percentage of donor B or myeloid cells from individual bone fragments from one experiment.

HSC Cell Dose Effects in "Autologous" vs. Allogeneic Transplants in Mouse

Allogeneic barrier=H-2 differences at both major and minor loci

WBC engraftment kinetics



NO GVHD OR HOST ANTI DONOR IMMUNE REACTIONS

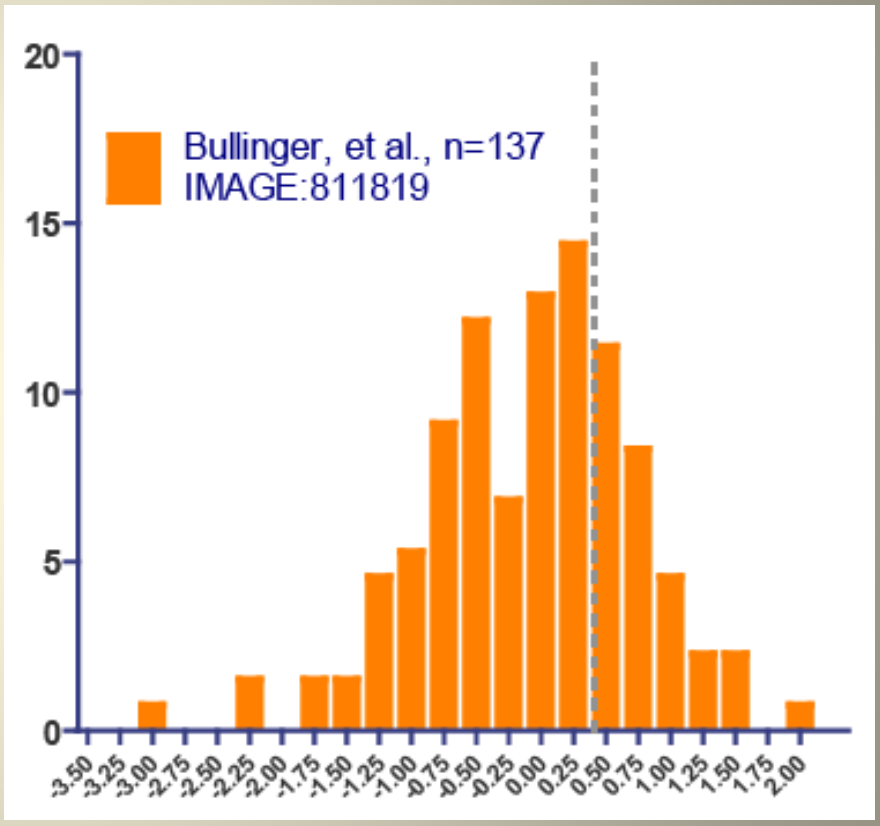
Mesenchymal Cells

- Cultured bone marrow stromal cells: what fraction are stem cells [self-renewing single cells that give rise to clonal progeny of a number of mature cell types]?
- Prospectively isolated mesenchymal stem cells should self-renew, and one type, that gives rise to bone, cartilage, fat, and hematopoietic stroma, are very rare.
- Claims for progeny of other cell types, including pluripotency exist but replication difficult.
- Accurate language with names based on evidence would help: most are mesenchymal stromal cells, not mesenchymal stem cells. Other functions in transplantation may be valuable when determined and reported.

- VSEL don’t exist as described

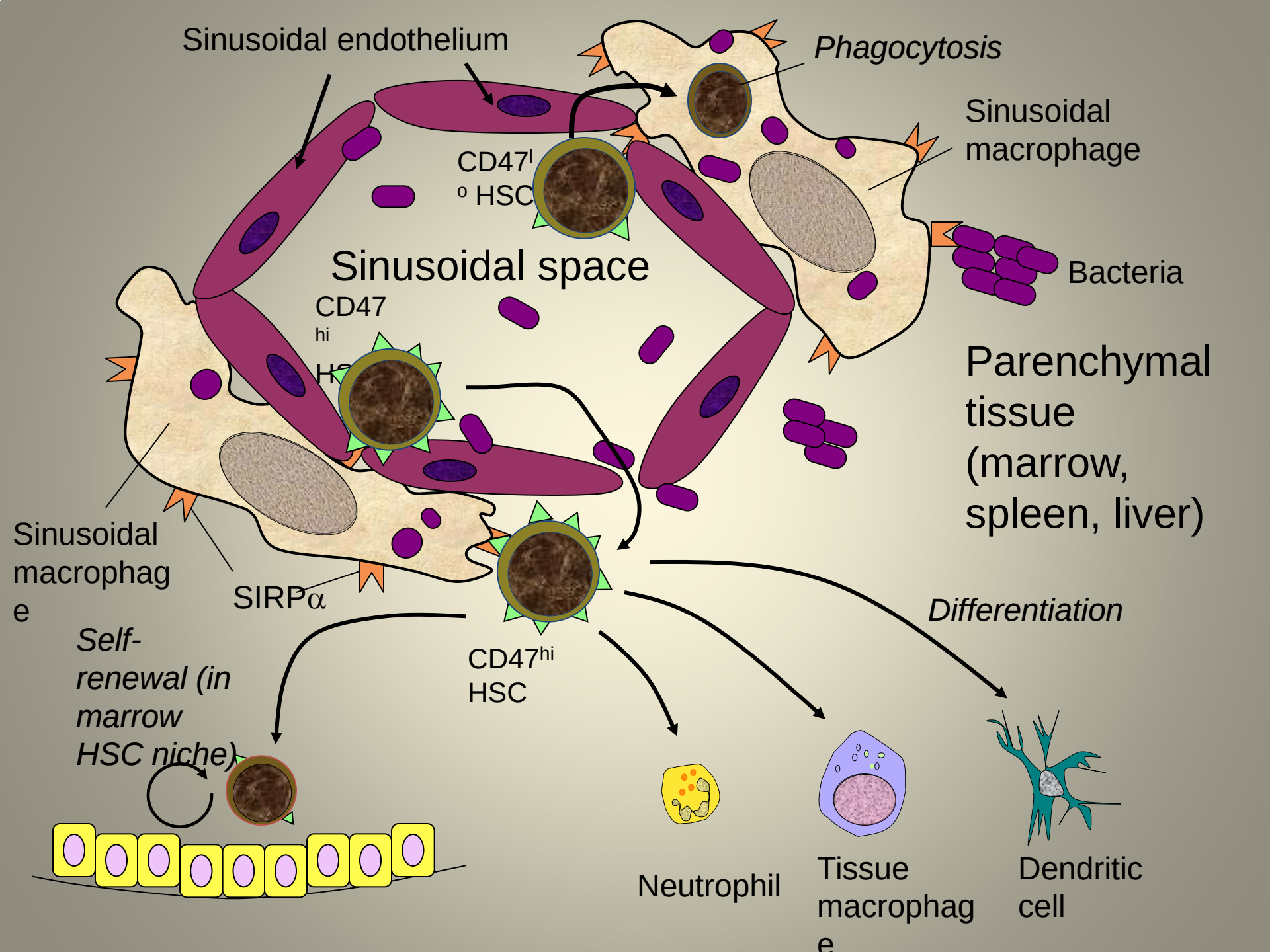
A Validation Cohort of 137 AML Patients with Normal Karyotypes

Set	Validation Set
Reference	Bullinger, L, et al. (2008) *Blood* 111:4490 PubMed ID 18309032
Cohort	Adult NK-AML (n=137)
Median age – yrs, (range)	46 (16-60)
Cooperative Group	German-Austrian AML Study Group (AMLSG)
Treatment Protocol(s)	AML HD98A (n=137)
Microarray Platform(s)	Stanford cDNA
NCBI GEO Accession	GSE8043

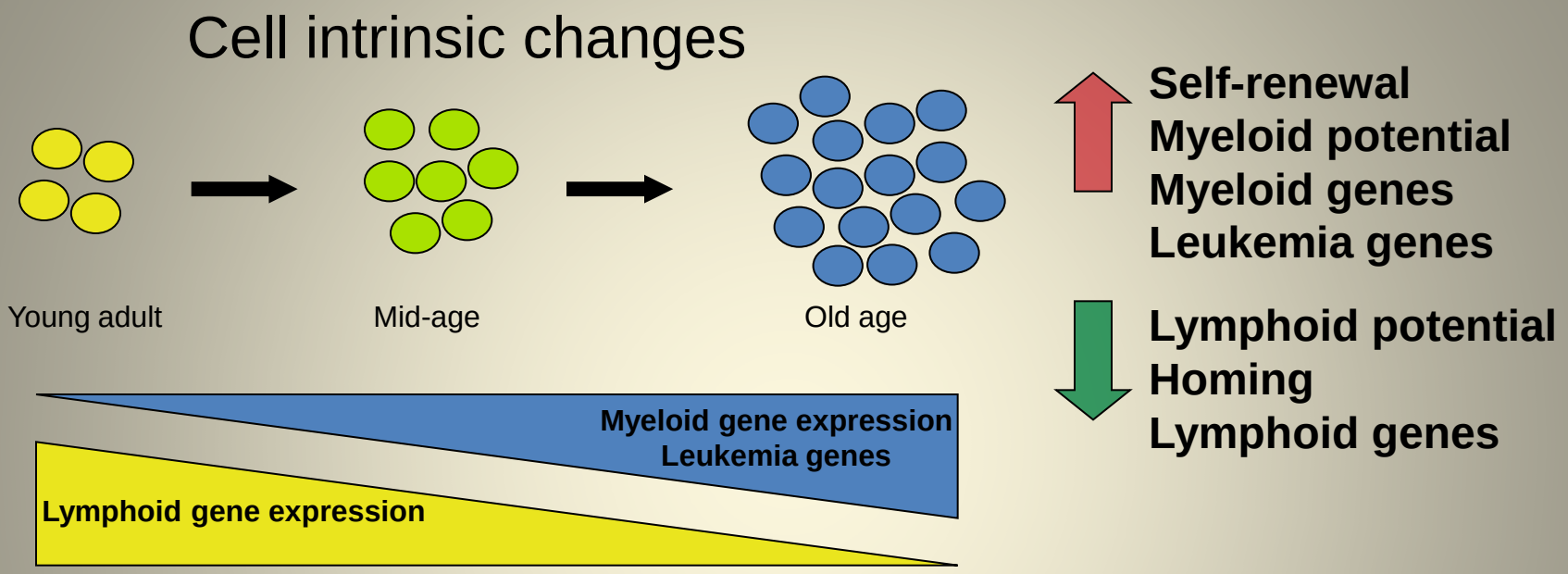


Relative Expression ($\log_2$)

Alizadeh, Majeti, and W


Sinusoidal endothelium
Phagocytosis
Sinusoidal macrophage
CD47lo HSC
Sinusoidal space
Bacteria
CD47hi HSC
Parenchymal tissue (marrow, spleen, liver)
Sinusoidal macrophage
SIRPα
Differentiation
Self-renewal (in marrow HSC niche)
CD47hi HSC
Neutrophil
Tissue macrophage
Dendritic cell

A Model of HSC Ontogeny



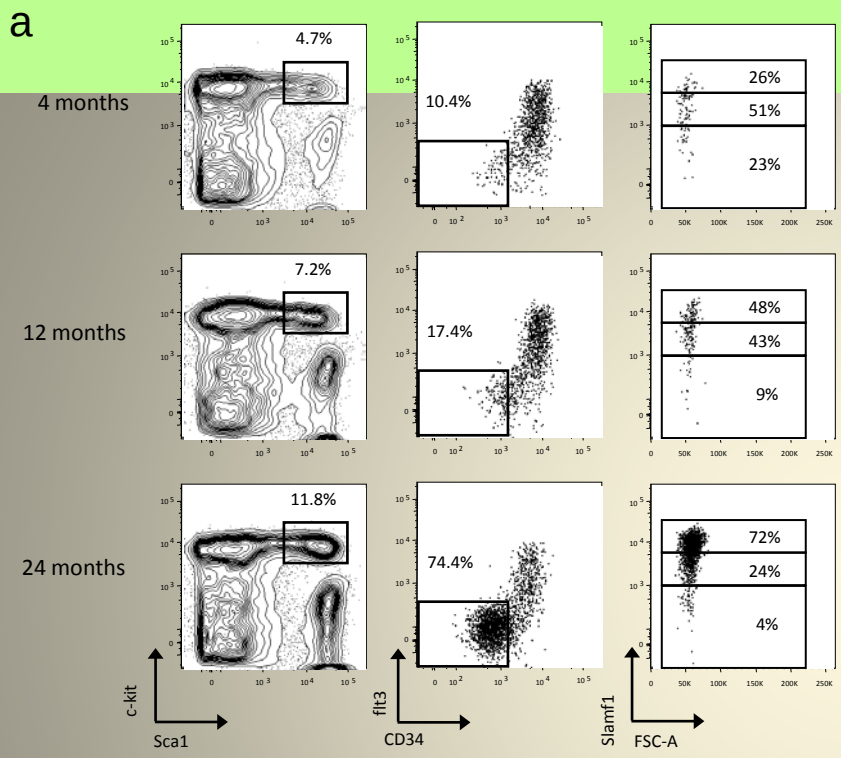
1) The cell-intrinsic functional and molecular properties of aging LT-HSC places the cellular and molecular rationale for the decreased immuno-competence that accompanies aging at the level of the LT-HSC.

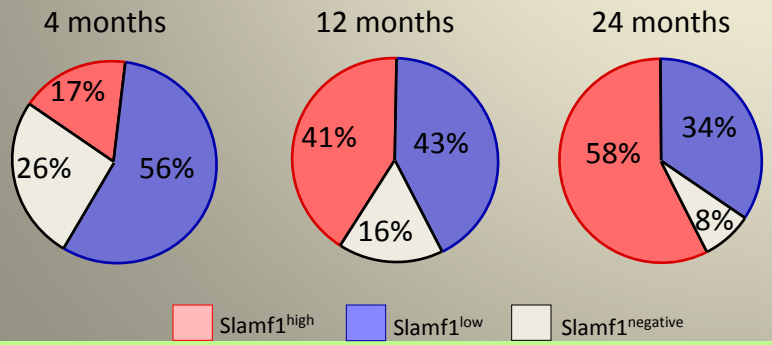
2) Age-dependent upregulation of genes frequently activated as proto-oncogenes in leukemogenesis (17/32 highest.)

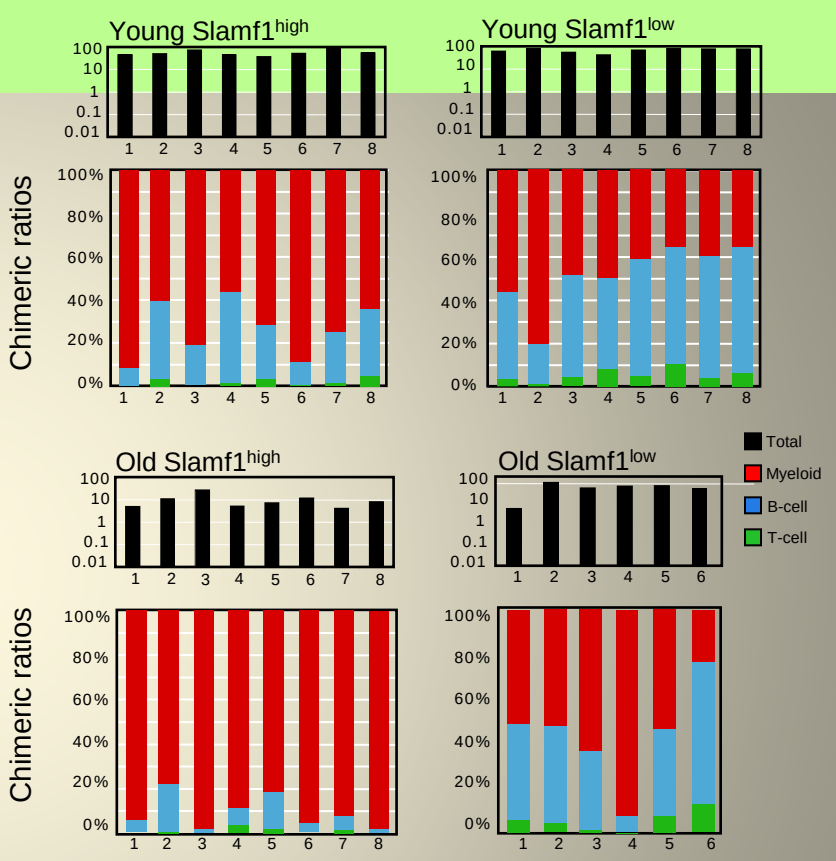
3) Is this maturation of all HSC, or clonal selection of some HSC?

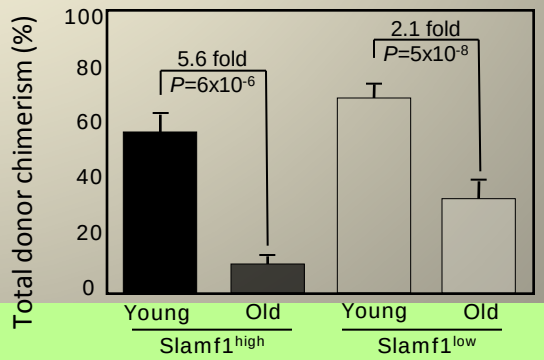
Rossi, Bryder, Weissm

Bryder, Beerman, Weissman, and Rossi

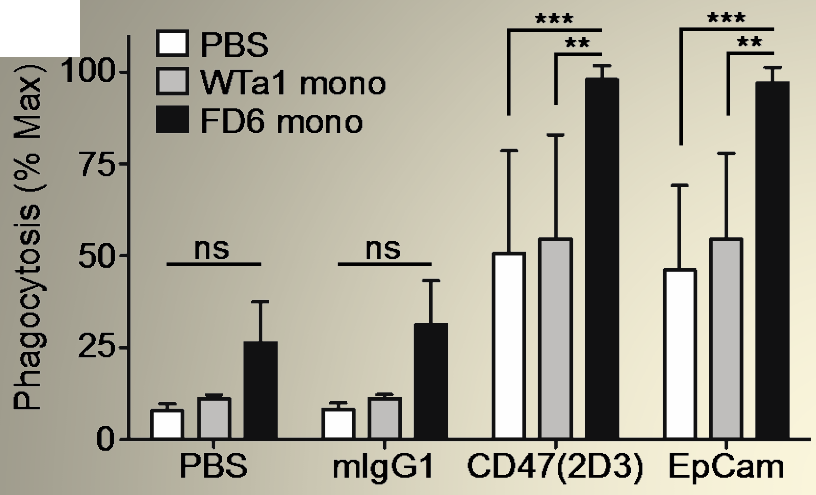


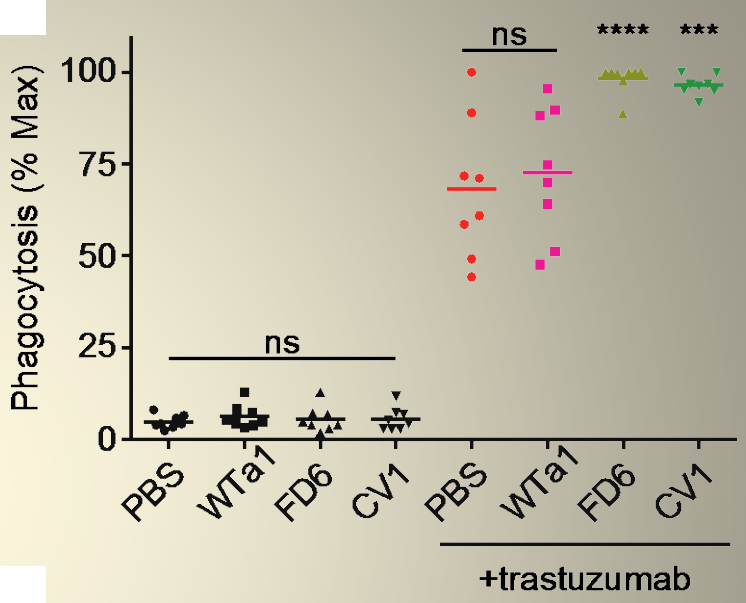


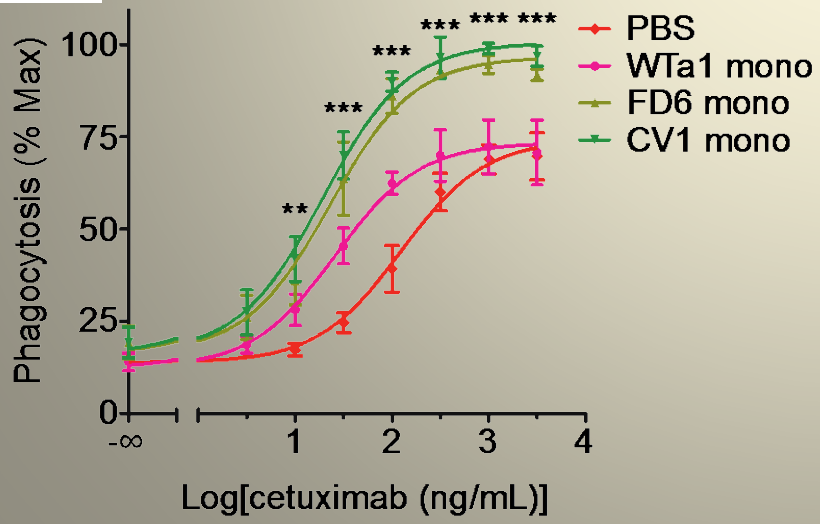


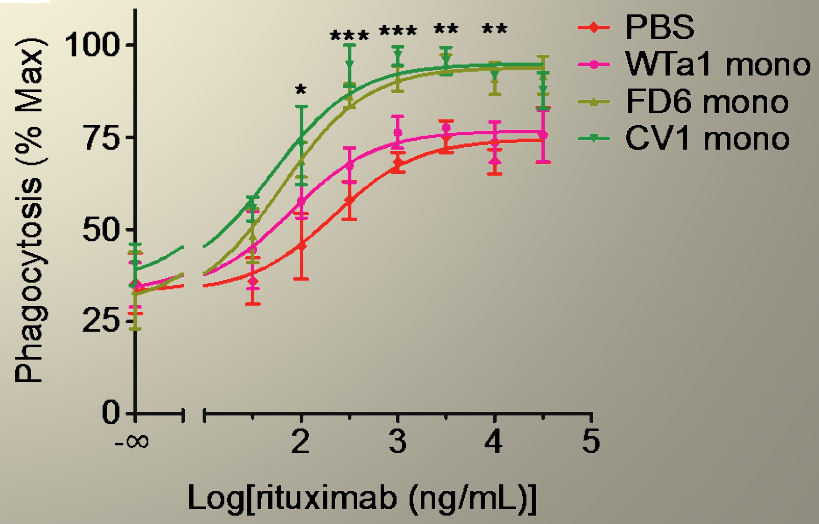


High-affinity SIRPα monomers enhance the efficacy of therapeutic monoclonal antibodies

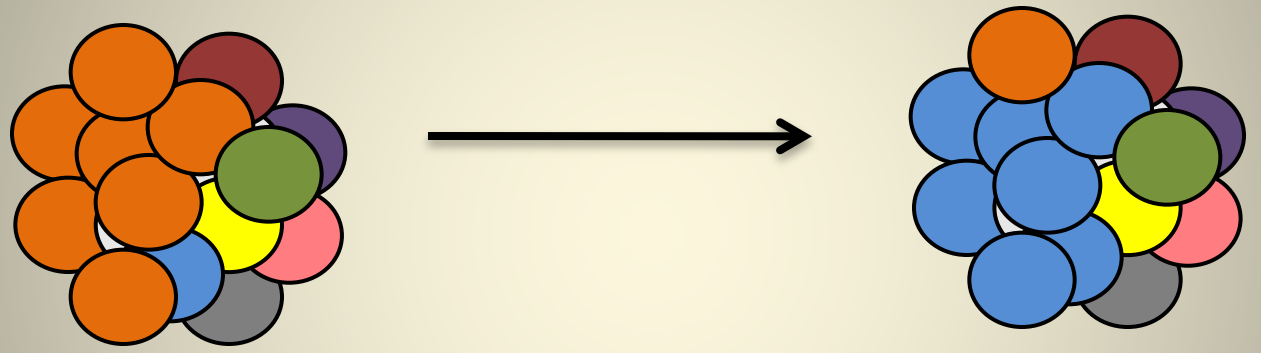






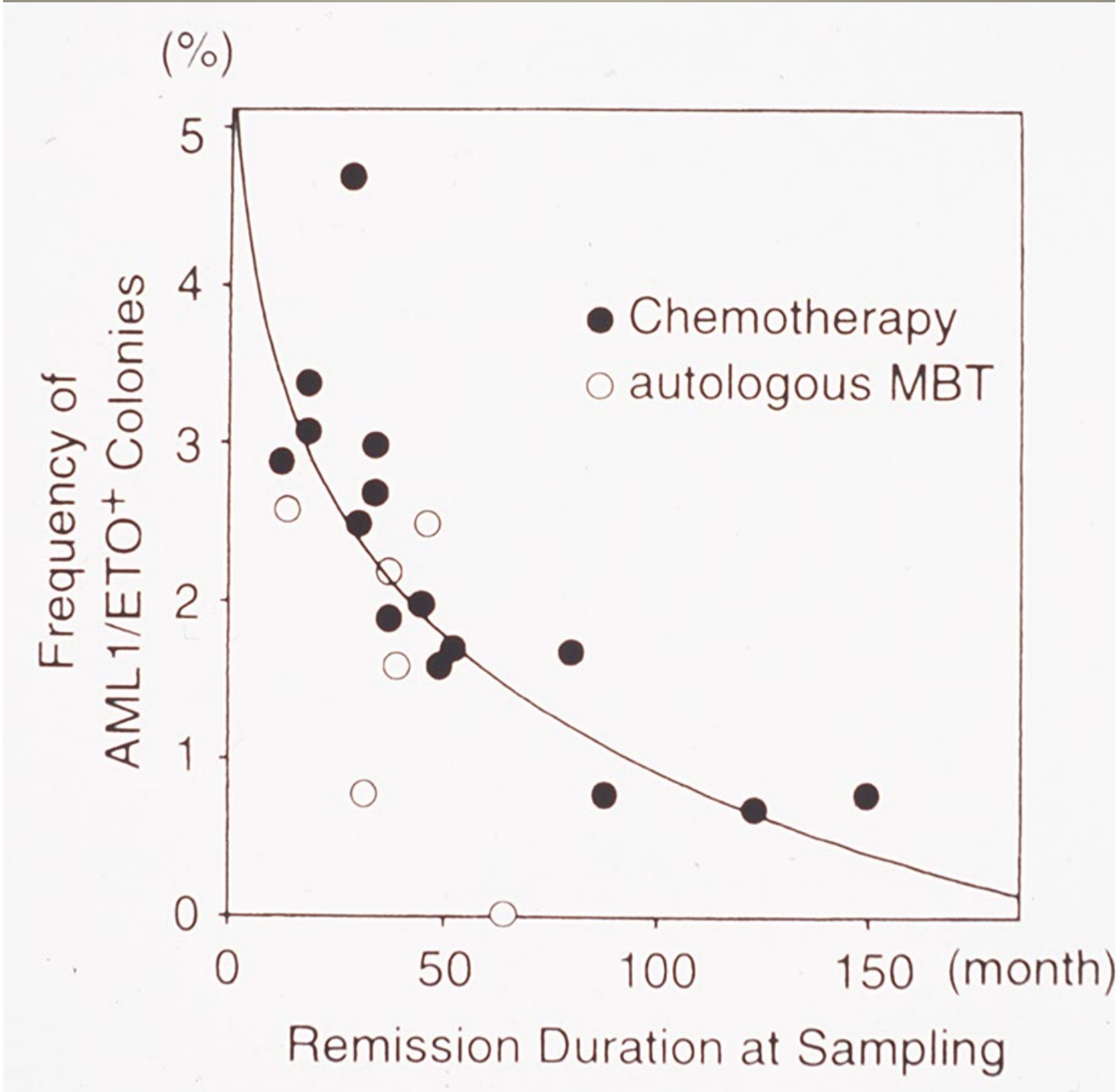


Clonal Selection or epigenetic programming of HSC with Aging

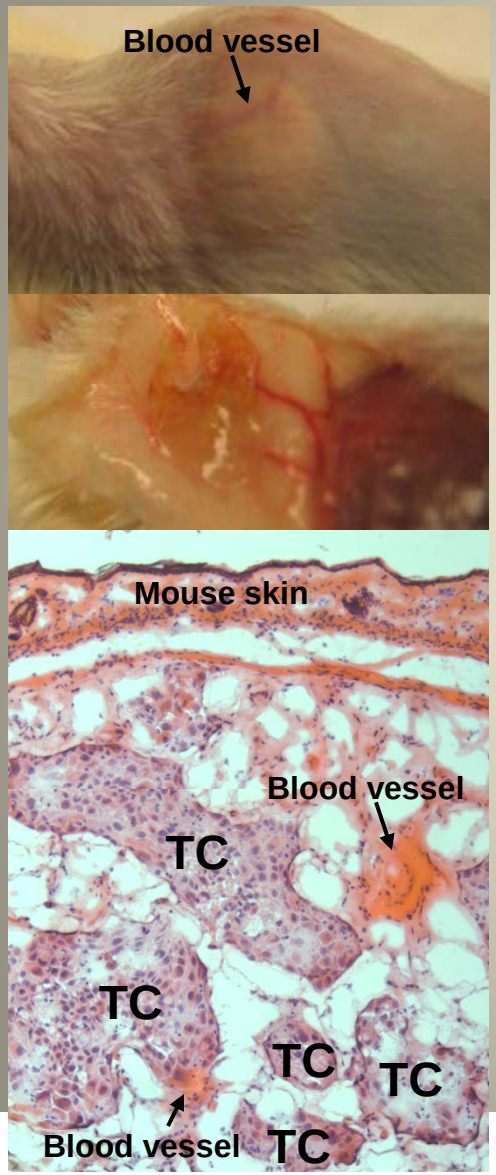


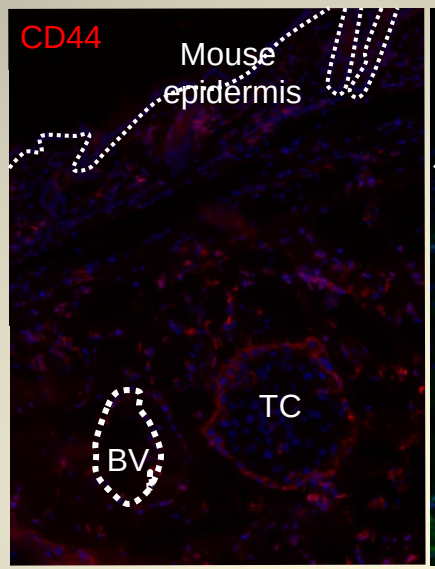
Normal HSC 2 mo **Normal HSC 2 years**

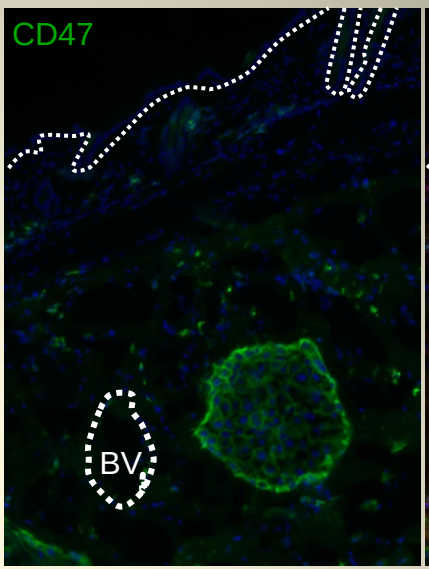
Hypothesis and data: Rossi, Eaves, Muller-Sieburg, Nakauchi, Bryder, Beerman, and Weissman

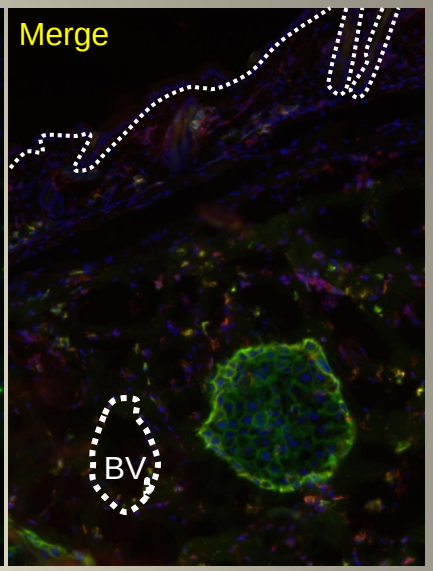

(%)
5
4
3
2
1
0
Frequency of
AML1/ETO+ Colonies
Chemotherapy
autologous MBT
0
50
100
150
(month)
Remission Duration at Sampling

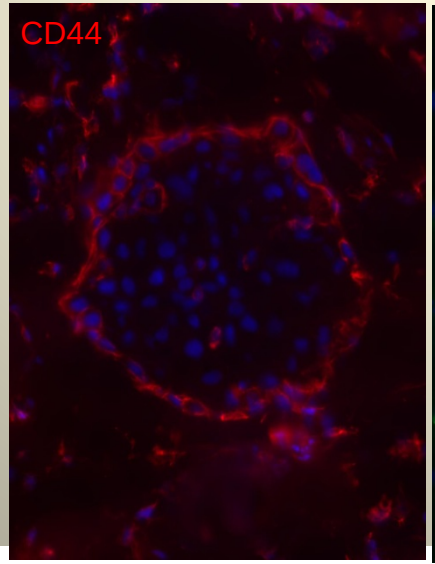
Localization of CD44 & CD47 in early stage bladder cancer xenograft

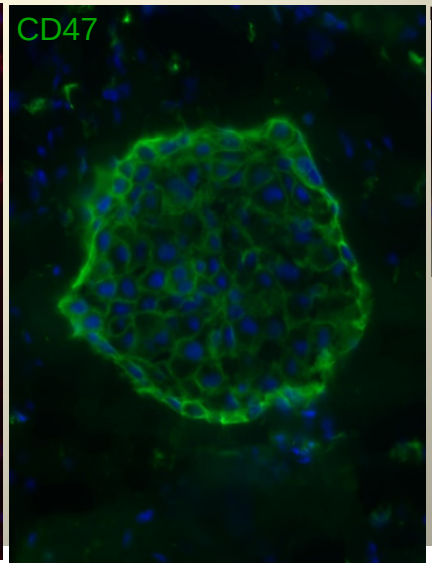


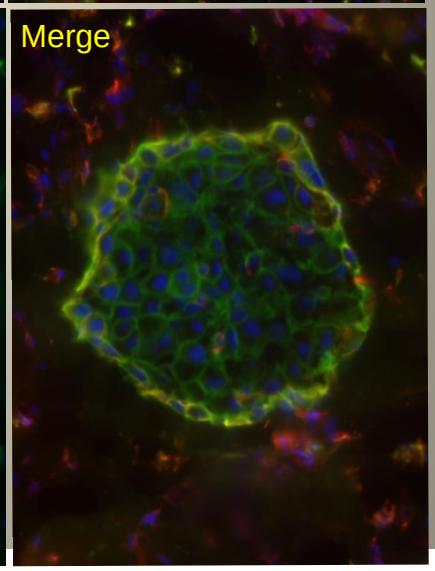










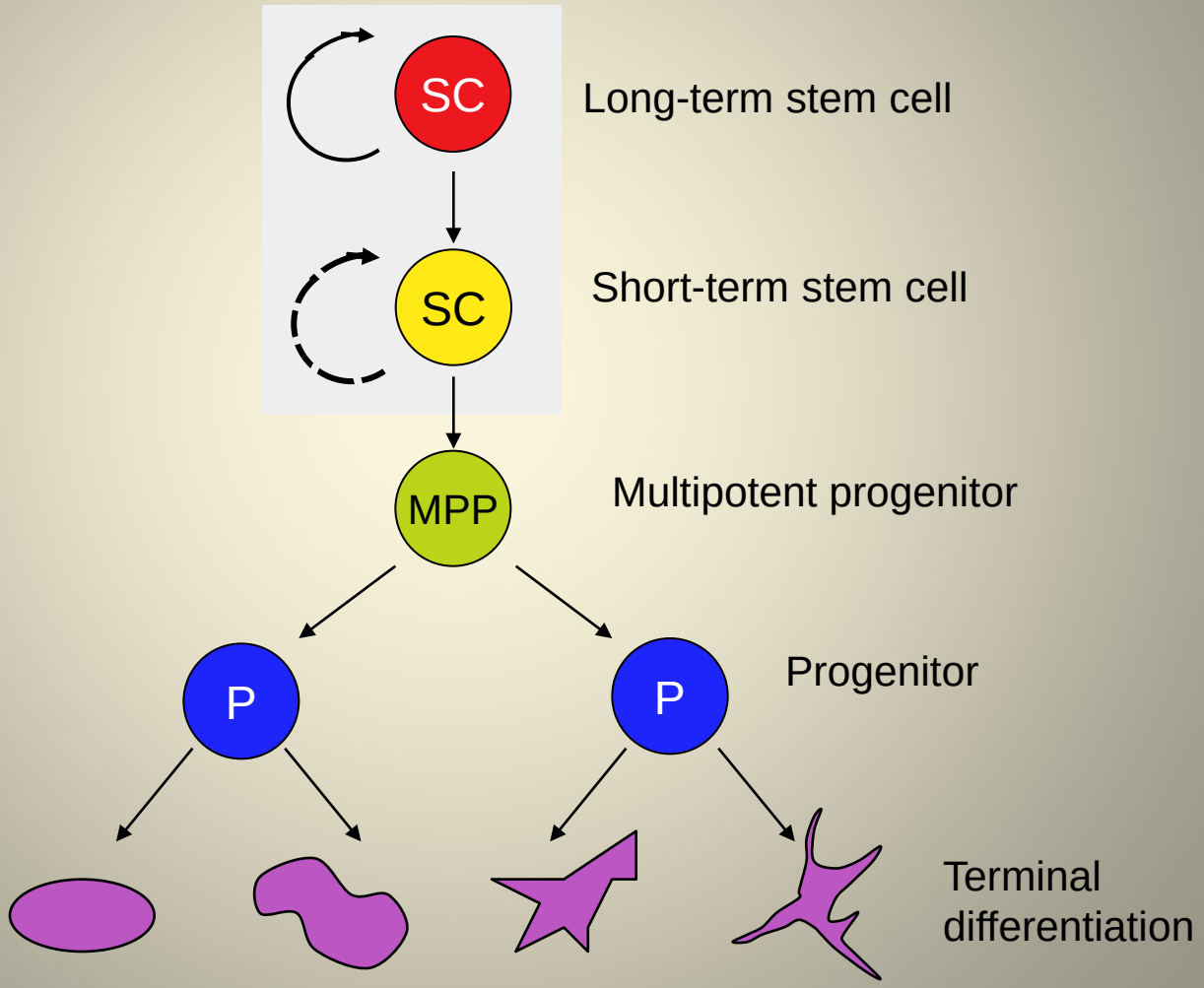


Keith Chan

Clinical Trials with CNS SC derived human neurospheres

- Batten Disease, a lysosomal storage disease with fatal neurodegeneration OHSU
- Pelizeus Mehrbacher, a fatal dysmyelinating disease due to PLP mutation UCSF
- Thoracic spinal cord contusion injury Zurich
- Dry AMD
- Stem Cells Inc [IW a Director and consultant]

The stem cell paradigm



Single –cell embryo

~ Day 3-4

~ Day 6



Embryonic Stem Cell
Totipotent

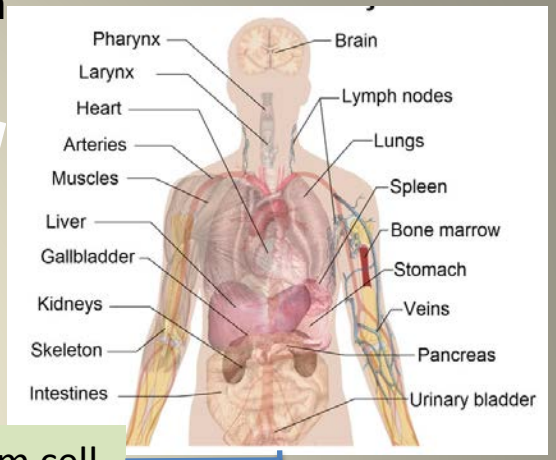
Epiblast Stem Cell
Pluripotent

Embryo – Fetal Transition

First tissue stem cells







Somatic / adult stem cell
Multipotent

Cord blood & placenta

Hematopoietic (HSC)
&
Mesenchymal (MSC)

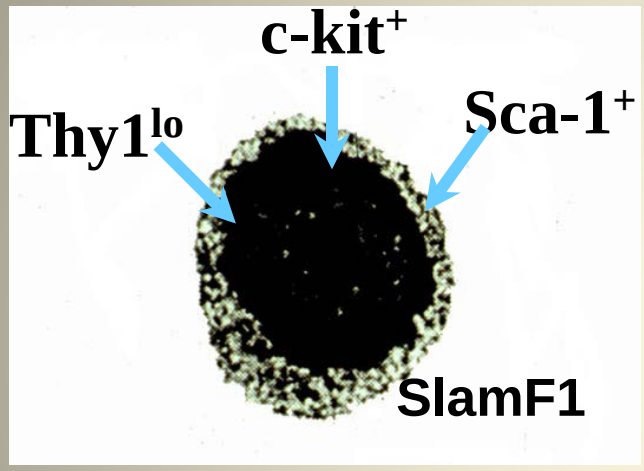
Other tissue stem cells

Reprogrammed iPS
Pluripotent

Blood-Forming Stem Cells

STANFORD

MOUSE



Stanford

Negative for:

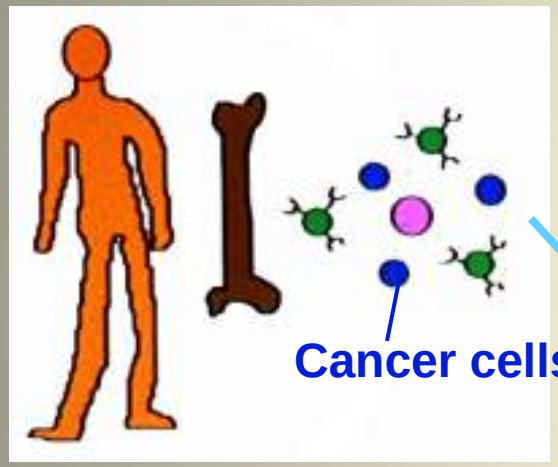
B220
Mac-1
Gr-1
CD3, 4, 8
Ter119
Flk2
CD34

Spangrude, Muller, Heimfeld

Self-Donor Transplant

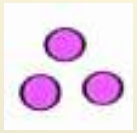
RADIATION EXPOSURE OR CANCER THERAPY

Donor / Recipient

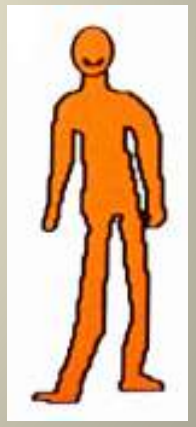


TREAT PATIENT TO KILL CANCER CELLS AND THIS KILLS BLOOD-FORMING CELLS

Donor / Recipient



Purified Stem Cells



Classified by FDA as unmanipulated grafts

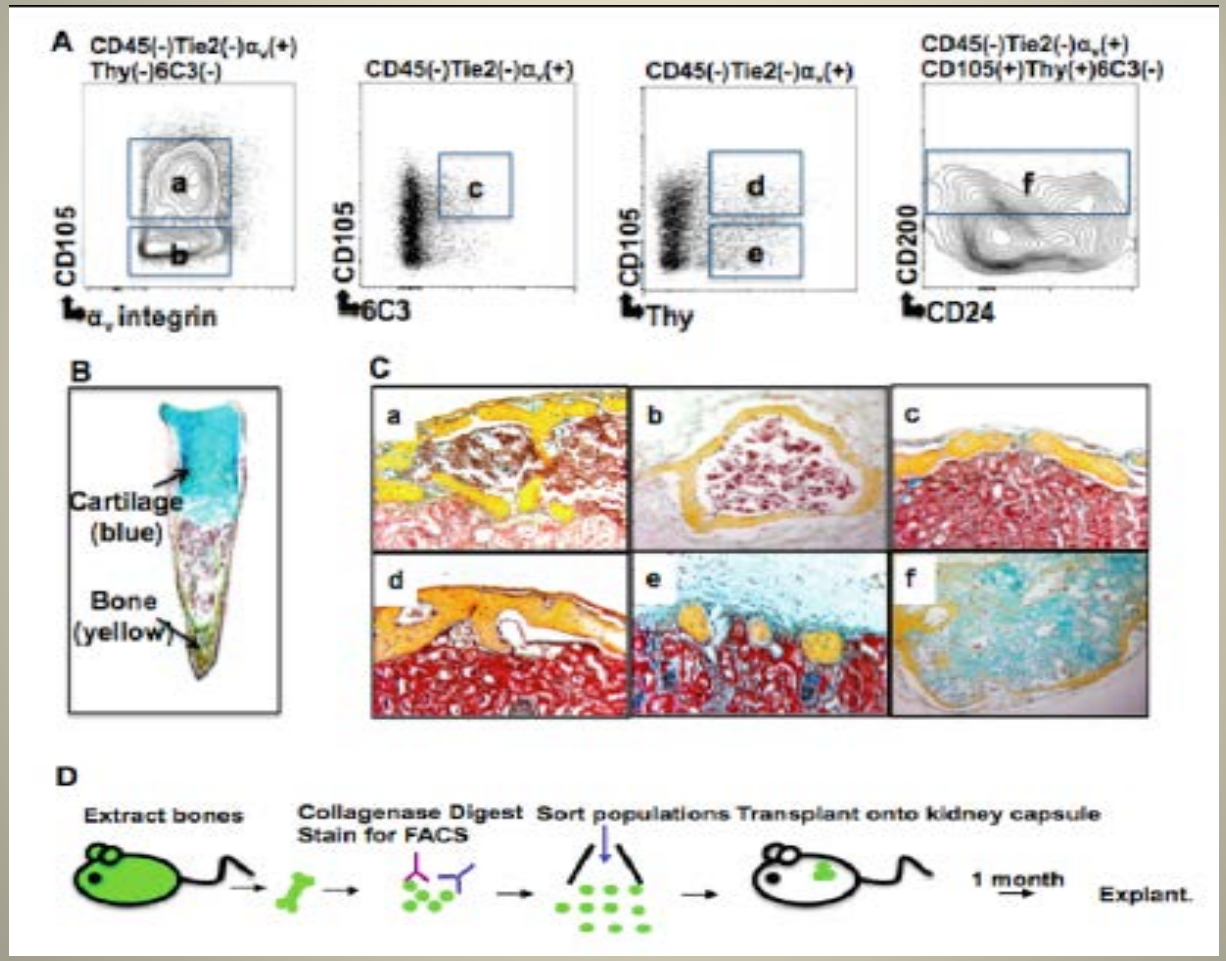
Cancer free blood regeneration

SyStemix/Novartis/Celler
IW a Director and consul

Should you save umbilical cord blood for the child?

- Umbilical cord blood contains blood forming stem cells and some mesenchymal stem cells
- Blood forming stem cells only make blood;mesenchymal stem cells only make bone, fat, cartilage, and scar tissue
- Umbilical cord blood has the same genetic disease potential that the child has; only very rarely has a person needed their own cord blood
- Public cord blood banks are a blessing for others who don’t have a ‘match’

Mouse bone, cartilage and bone marrow stromal stem cell



The BCS-SC is a clonal progenitor

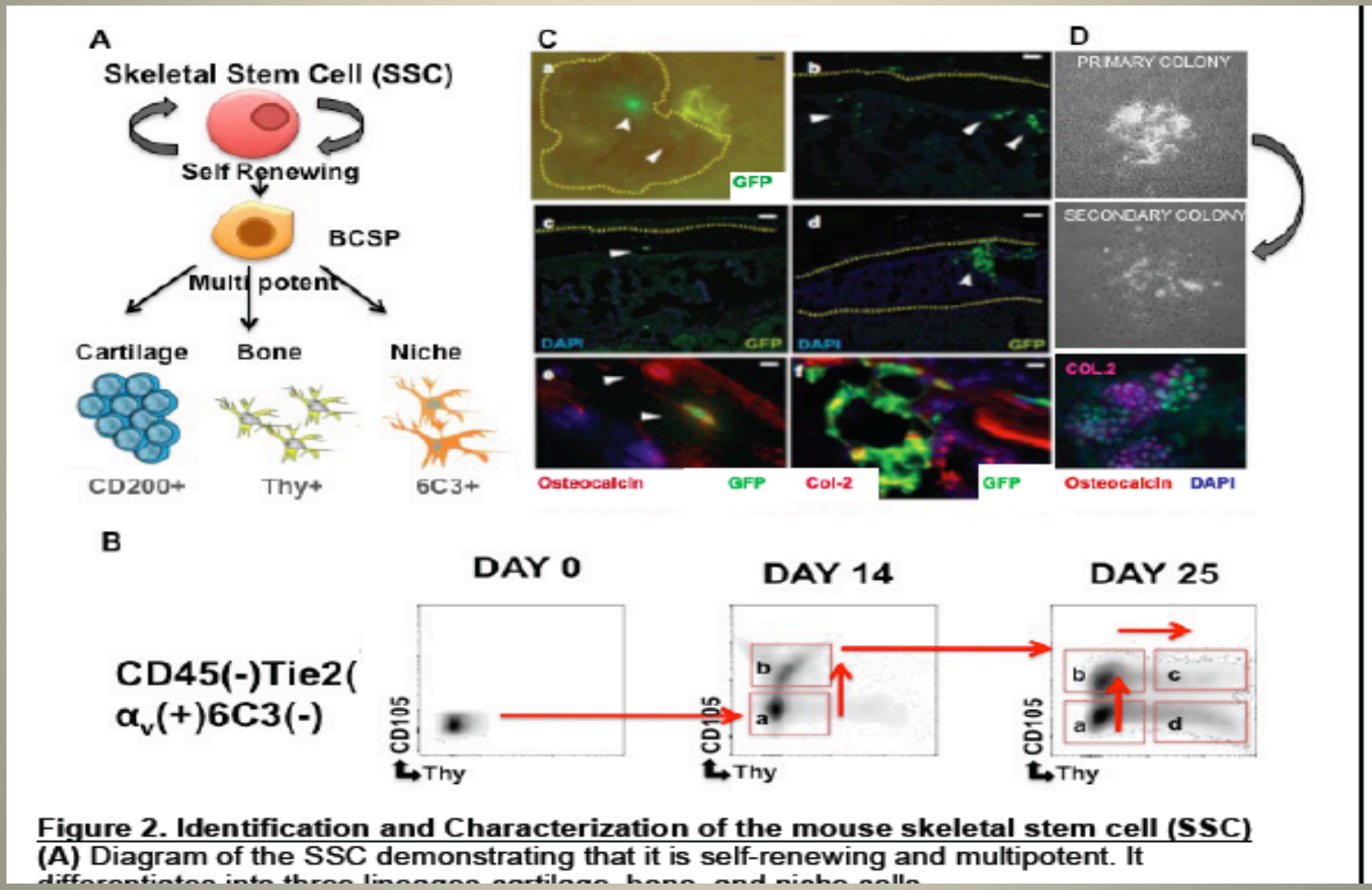
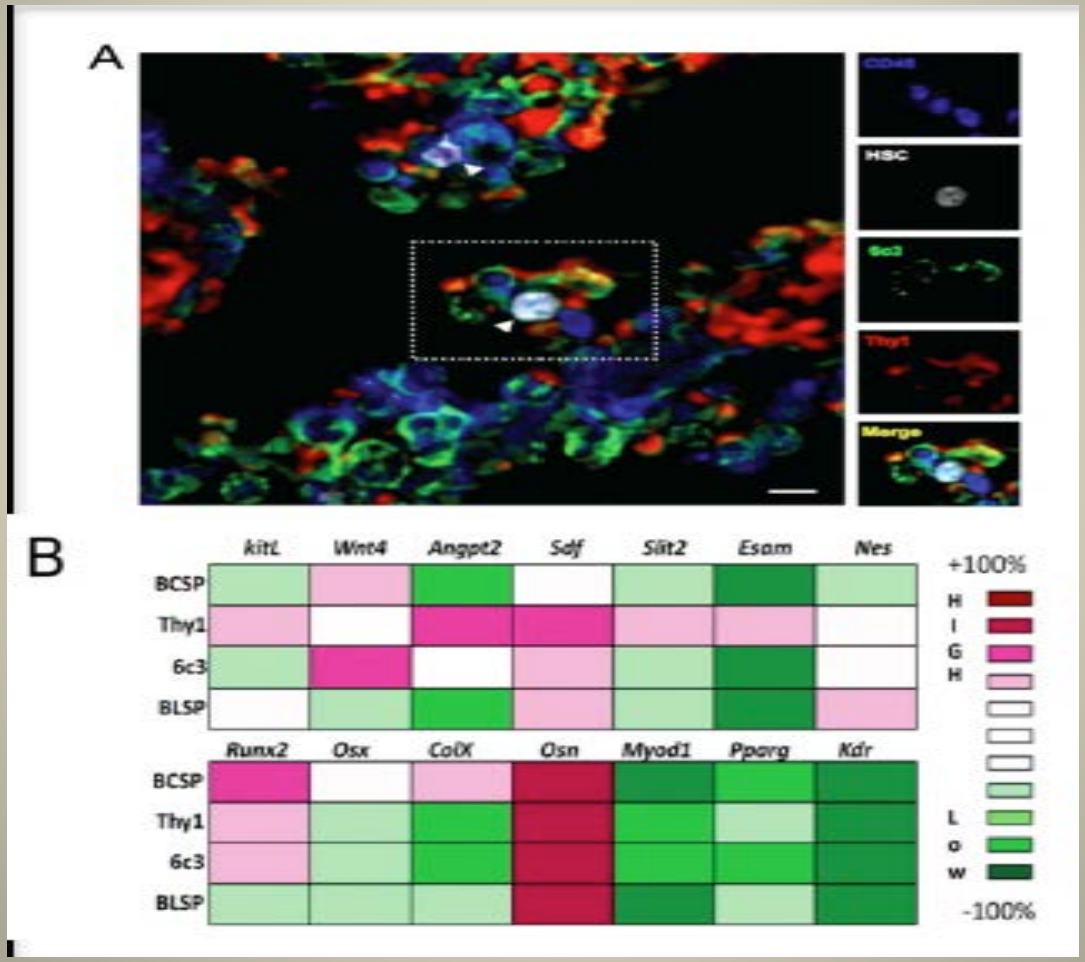


Figure 2. Identification and Characterization of the mouse skeletal stem cell (SSC)
(A) Diagram of the SSC demonstrating that it is self-renewing and multipotent. It

The HSC niche includes several distinct BCSC derived stromal cells



VSEL cells are the basis of a new company called Neostem

- The Vatican is a significant investor in Neostem
- Neostem purchased PCT, a major cell manufacturer of cell products, including those from ES or fetal sources
- Neostem and the Vatican co-sponsored a

Conference on stem cells in November, 2011 and another in April 2013

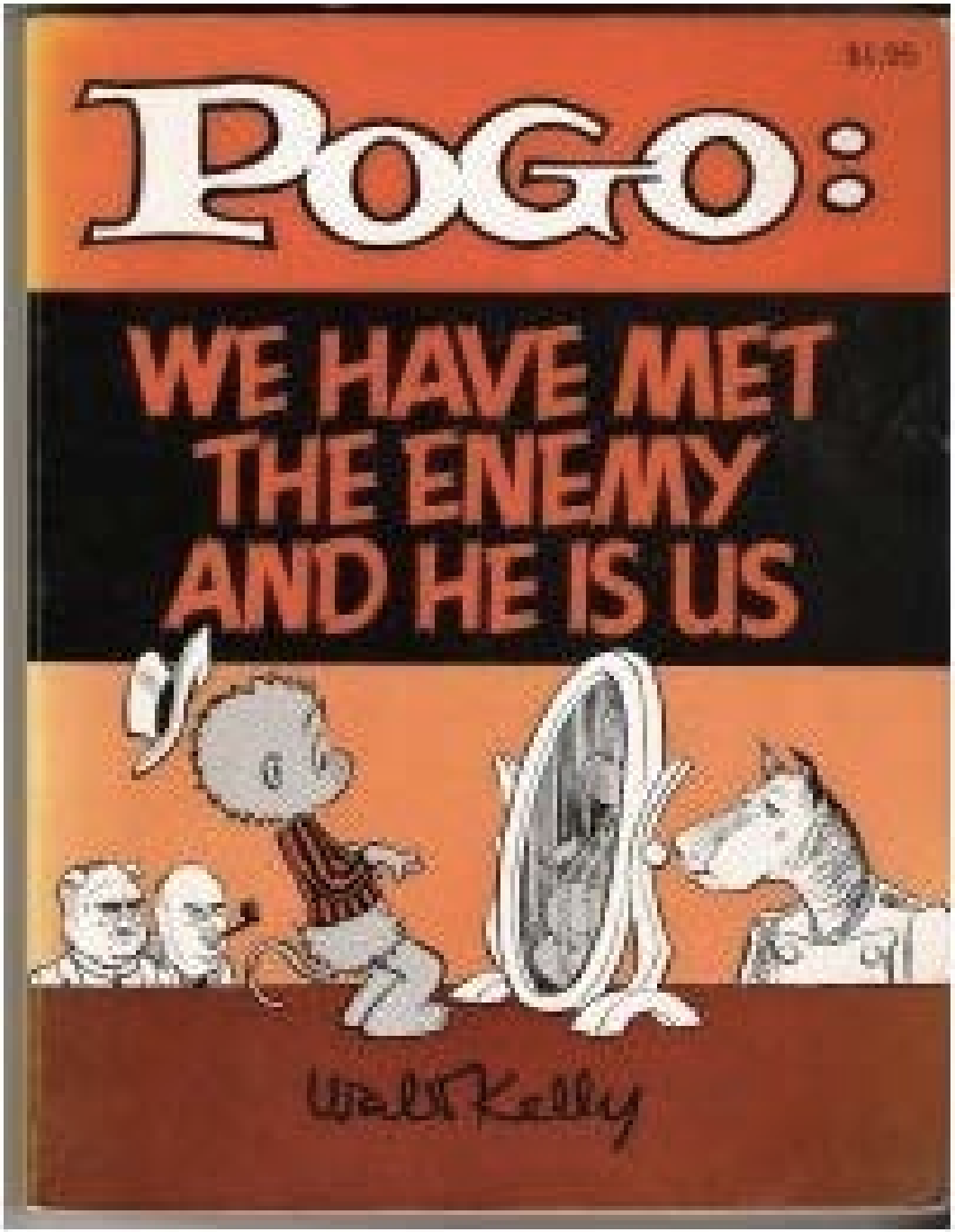
Toronto

Stem Cell Tourism

Stem cell treatments are being prematurely marketed around the world

At risk

- The health and safety of patients
- The public's trust and confidence
- Progress of legitimate stem cell clinical translation


$1.95
POGO:
WE HAVE MET THE ENEMY AND HE IS US
Walt Kelly

It’s the cells, stu…

Cell Stem Cell
Forum

Stem Cell Therapies Could Change Medicine… If They Get the Chance

Irving Weissman[1,*]
[1]Institute of Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA 94305 USA
*Correspondence: irv@stanford.edu
DOI 10.1016/j.stem.2012.05.014

Stem cell therapies have the potential to revolutionize the way we practice medicine. However, in the current climate several barriers and false assumptions stand in the way of achieving that goal.

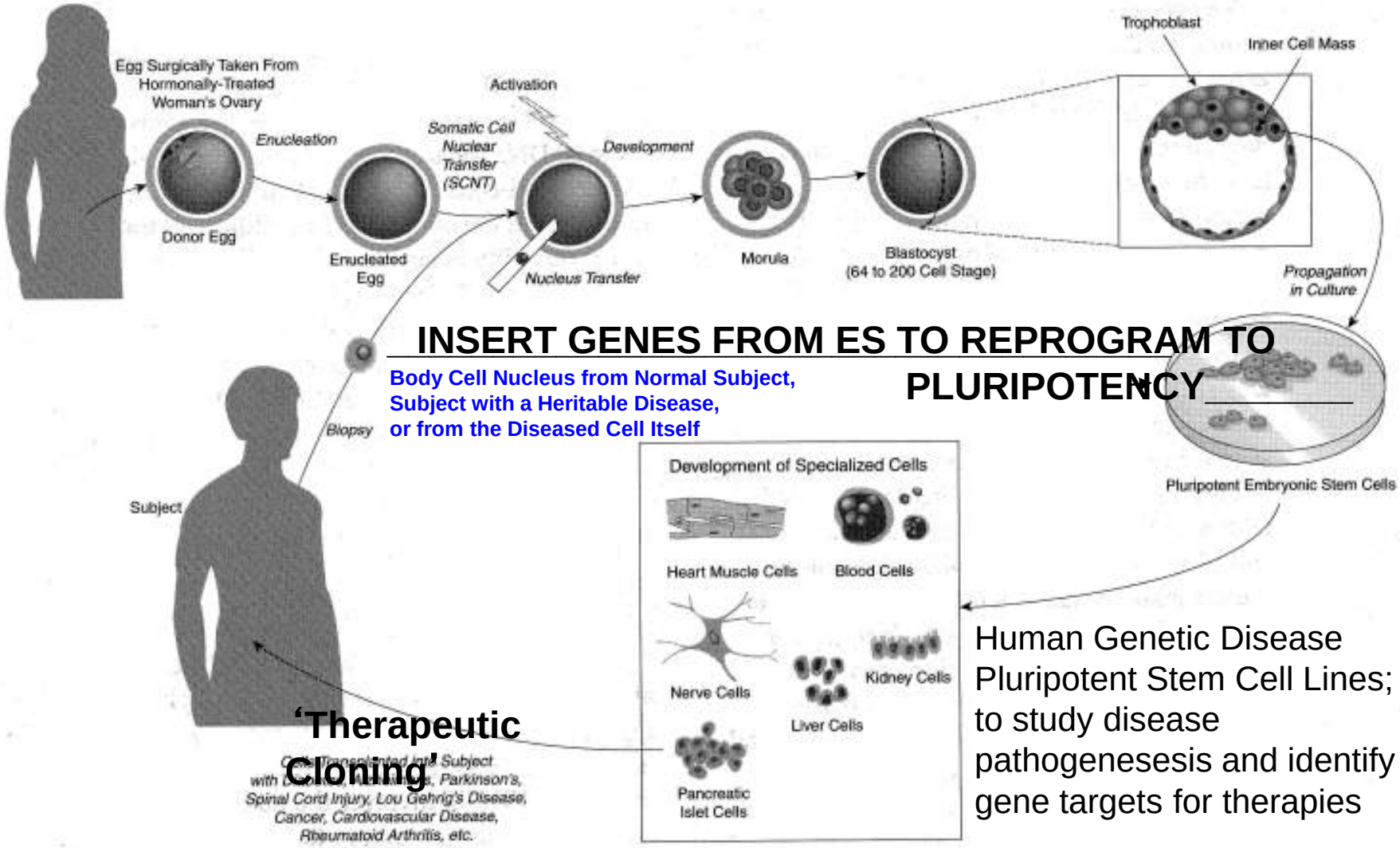
The Therapeutic Entity Is the Stem Cell Itself

Very few “adult” stem cells have been prospectively isolated, and only prospectively isolated blood-forming stem cells

out the pathways to achi
remains a critical roadbl
the field of regenerative

In Vivo Veritas

Nuclear Transplantation To Produce Stem Cells



Human Genetic Disease Pluripotent Stem Cell Lines; to study disease pathogenesesis and identify gene targets for therapies

Stem cell treatments are being prematurely marketed around the world

At risk

- The health and safety of patients
- The public's trust and confidence
- Progress of legitimate stem cell clinical translation

website:http://www.closerlookatstemcells.org//AM

Lessons learned to date

- Choice of animal models on target
- Animal models have been very good predictors; to date recapitulating in humans what we have seen in animal models
  - Safety profile show no concerns to date
  - Metric for calculating dose choices seem to be working
  - Small uncontrolled studies on patients
  - Preliminary evidence of HuCNS-SC biological activity in transplanted patients reflect what we have seen in animal models to date
- Accumulating long-term safety (1-5yrs), sustained biological effects

Reported non-embryonic multi-germ-layer stem cells

	Species	Sites	Potency	Hematopoiesis	Surface marker
Amniotic fluid stem cell (AFS)	Human	amniotic fluid	mesenchymal, neuronal, endothelial, hepatic	NE	CD105+CD90+CD73+CD44+ CD29+CD117+HLA-DR^{lo} CD133-CD45-CD34-
Multipotent adult stem cell (MASC)	Human	BM, heart, liver	pancreatic, neuronal, mesenchymal	NE	CD49b+CD90+CD13+CD105^{lo} CD73^{lo}CD44^{lo}HLA-ABC^{lo}CD133- CD45-CD34-CD14-HLA-DR-
Multipotent adult progenitor cell (MAPC)	Human Mouse	BM, muscle, brain	mesoderm, endoderm, ectoderm	Yes	CD9+CD13+CD31+CD117+ CD44-MHC-CD45-CD90-
Marrow isolated adult multilineage inducible cell (MIAMI)	Human	BM	mesoderm, endoderm, ectoderm	NE	CD164+CD122+CD81+SSEA-4+ CD45-CD34-CD117-
Unrestricted somatic stem cell (USSC)	Human	CB	mesoderm, endoderm, ectoderm	NE	CD10^{lo}Flk1^{lo}CD45-CD34-CD117-
Very small embryonic-like stem cell (VSEL)	Human Mouse	BM, CB, mobilized PB	mesoderm, endoderm, ectoderm	Yes	B220-TCR$\alpha\beta$-TCR$\gamma\delta$-Gr-1-CD11b- Ter119-7-AAD-CD45-Sca-1+

BM; bone marrow, CB; cord blood, PB; peripheral blood, NE; not examined

Hematopoietic potential of reported multi-germ-layer stem ce

【MAPC】

Generation: Required long-time culture (>4wks) with various growth factors

Hematopoietic potential: Less efficient (1000-fold more cells are required) compared to KLS **(Verfaillie CM, Nature 2002, Verfaillie CM, JEM 2007)**

【VSEL】

Generation: Directly isolated from BM

Hematopoietic potential: Comparable to HSC

(Ratajczak MZ, Leukemia 2006, Ratajczak MZ, Exp Hematol 2011)



Attractive source of regenerative medicine in hematopoietic system??

Vatican conference on stem cells in 2011

The First International Vatican Adult Stem Cell Conference: A Ground-Breaking Achievement

- November 16, 2011 at 1:27 PM
- Adult Stem Cells: Science and the Future of Man and Culture."

- Co-sponsor: Neostem Inc
- NeoStem, Inc. is engaged in the development and manufacturing of cell-based therapies in the U.S. Its January 2011 acquisition of Progenitor Cell Therapy, LLC ("PCT") is central to the Company's strategic mission of capturing the paradigm shift to cell therapy. The acquisition of PCT gives NeoStem not only access to a world class contract manufacturing cell therapy company but provides a platform and expertise around the evaluation, development and regulatory requirements to develop autologous, allogeneic, immunomodulatory and vaccine-based therapeutics. NeoStem also holds the worldwide exclusive license to VSEL(TM) Technology, which uses very small embryonic-like stem cells, shown to have several physical characteristics that are generally found in embryonic stem cells.

CLAIM: Umbilical cord blood cells are pluripotent

- Transplantation of UCB cells cures not only blood diseases, but a number of non-hematopoietic diseases such as cerebral palsy, heart septal defects, pulmonary fibrosis, etc.

Potential Uses for Allogeneic Hematopoietic Cell Transplantation

- Replacement of genetically defective blood-forming system, or ablated blood-forming system
- Induction of tolerance to transplanted solid organs
- Treatment of autoimmune diseases

Adult (tissue) stem cells are useful in tissue and organ regeneration:Examples

- Blood-forming stem cells to regenerate blood and immune systems.
- Brain-forming stem cells to regenerate neuroprotective potential in brain.
- Skin-forming stem cells/burns.
- Limbal cells: cornea
- Skeletal muscle stem cells [not myoblasts] for muscular dystrophies in mice.
- Lung?, liver?, kidney? Heart? Etc.

Sources of blood-forming stem cells

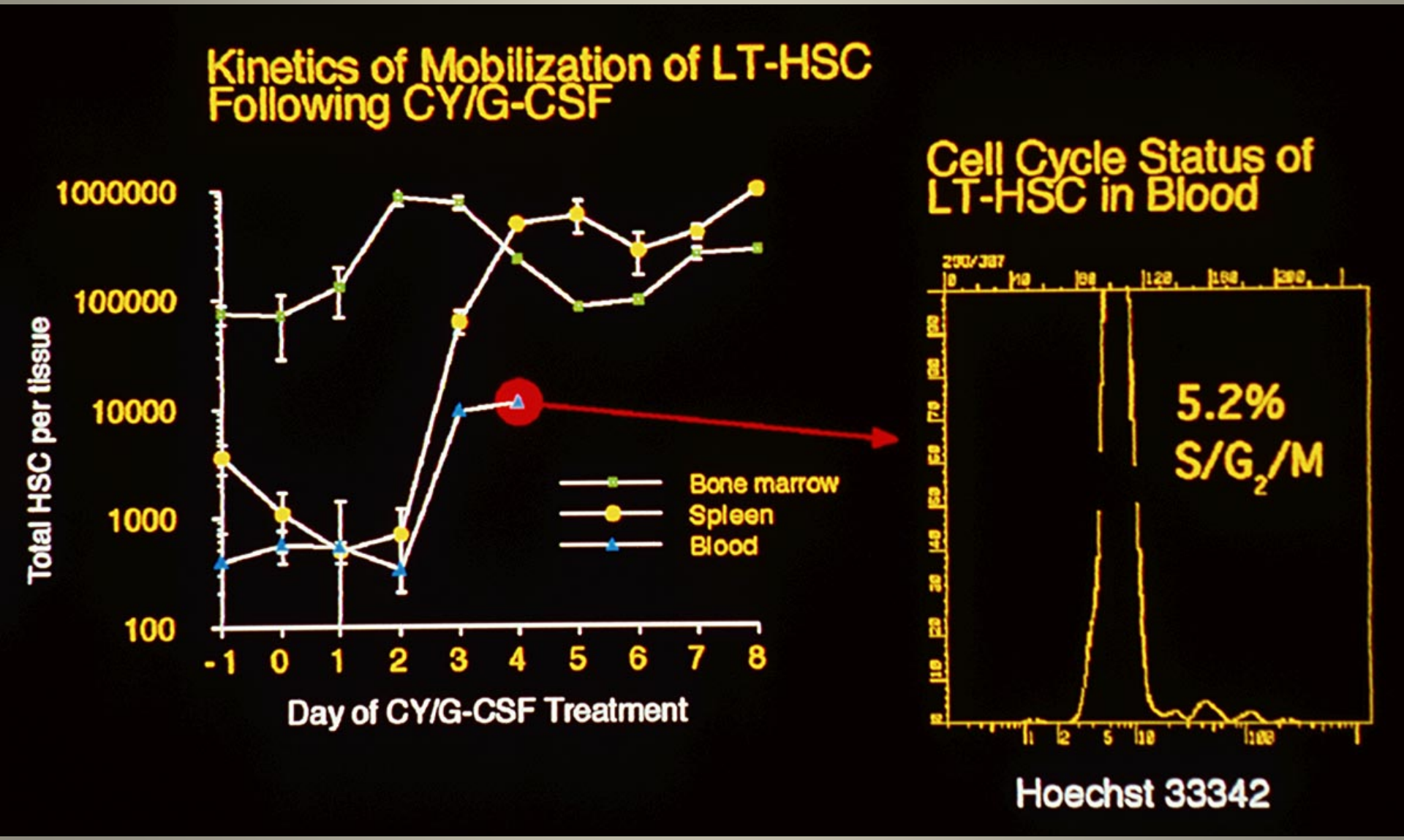
- Bone marrow
- Mobilized blood
- Umbilical cord

- All 3 stem cell populations only make blood, and are very rare cells (1 in 15,000)

Clonal assays for myeloerythroid, T, and B cells

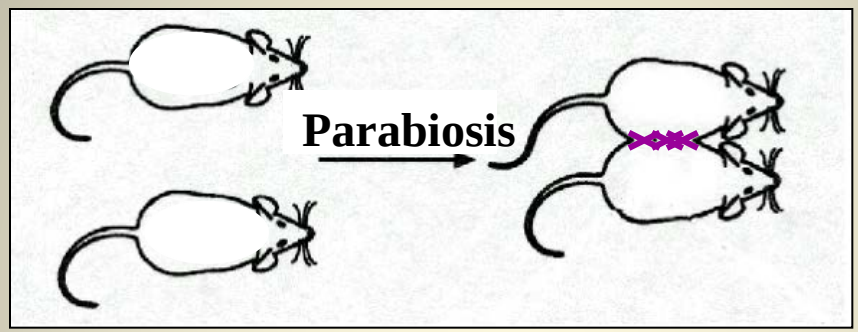
- Myeloerythroid spleen colonies: the Till-McCulloch cell
- Whitlock-Witte clonal B lineage colonies
- Thymic multi-T lineage colonies

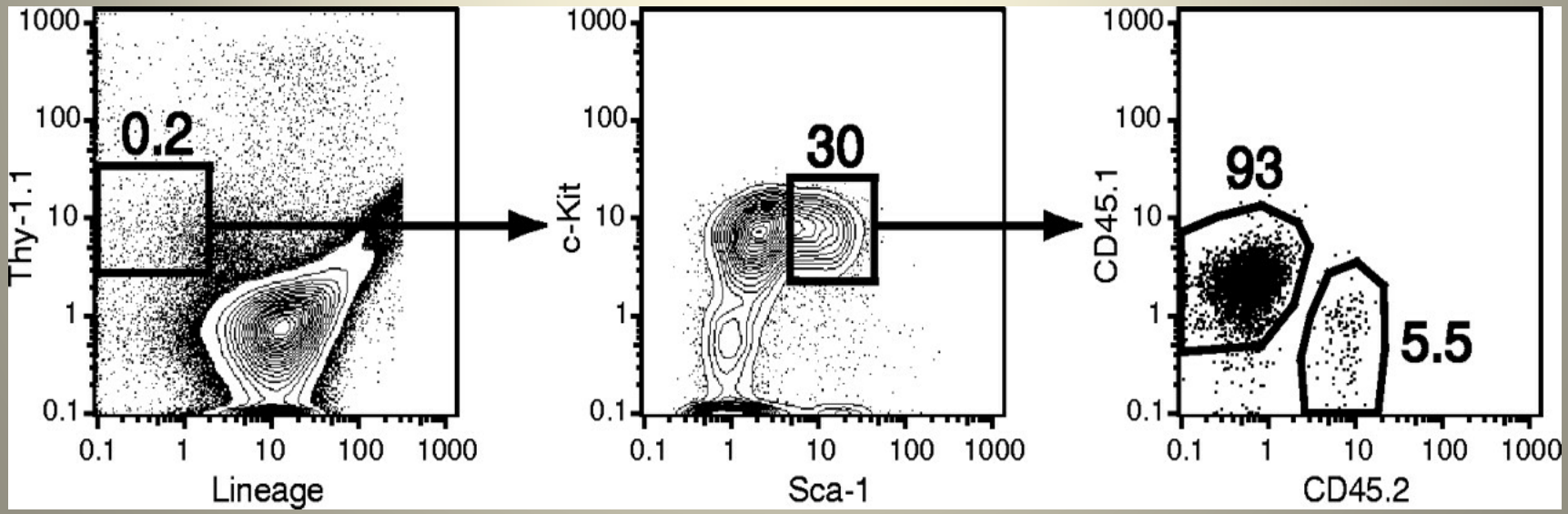
- Final assay: long-term multilineage engraftment with self-renewal


Kinetics of Mobilization of LT-HSC Following CY/G-CSF
Total HSC per tissue
1000000
100000
10000
1000
100
-1
0
1
2
3
4
5
6
7
8
Day of CY/G-CSF Treatment
Bone marrow
Spleen
Blood
Cell Cycle Status of LT-HSC in Blood
5.2%
S/G2/M
Hoechst 33342

Identification of cross-engrafting HSC by FACS

IN PARABIOSIS 7 WEEKS, OFF PARABIOSIS 7 WEEKS:ANALYZE MARROW





Wagers, Wright, Weissm

Properties of stem cells

- Self-renew to maintain tissues and tissue stem cells
- Migrate as stem cells or differentiating cells
- Find secondary niches and compete for occupation

Phase I/II Thoracic SCI Study

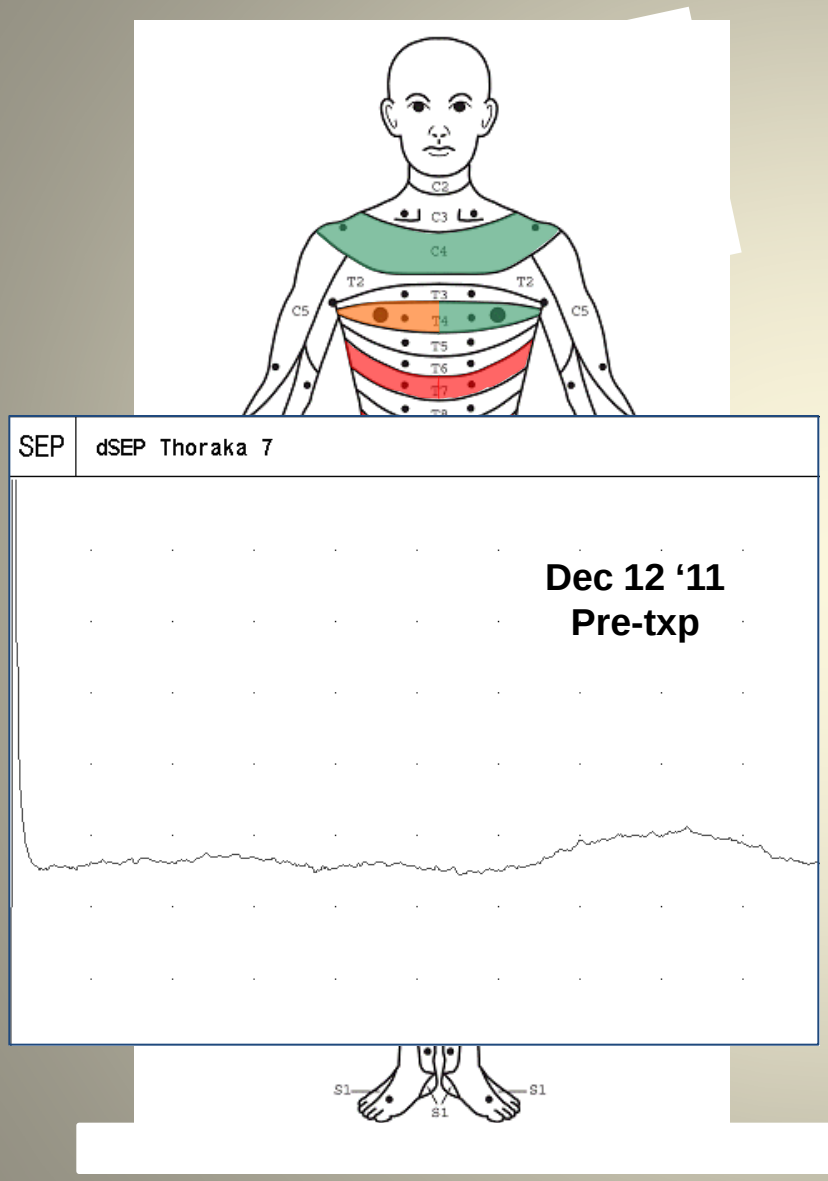
- Swissmedic authorized
- 12 patients with thoracic injury (tSCI)
- Injury level: T2-T11
- Chronic injury stage: 3-12 months post-injury
  - Broad range of injuries: AIS A, B, C
  - Significant cell dose: 20 million cells
- Safety and preliminary efficacy
  - Clinical
  - electrophysiological and
  - QoL endpoints

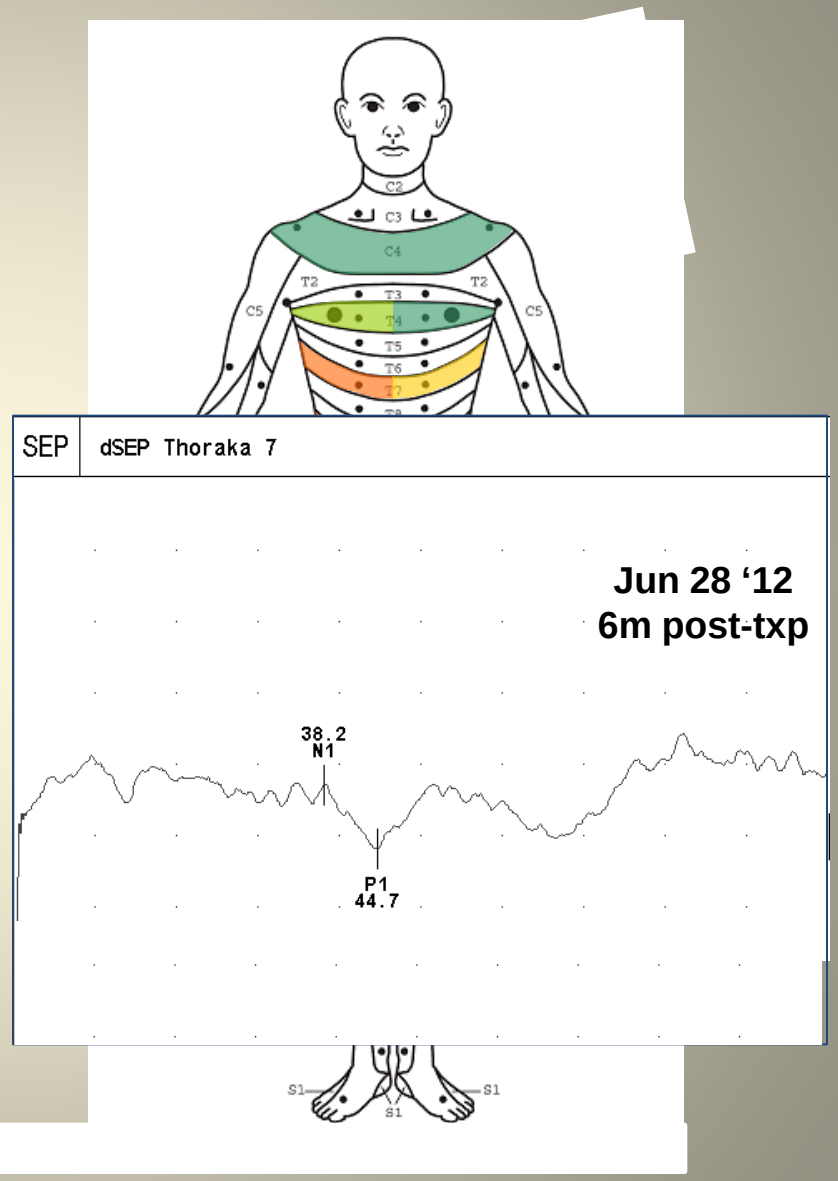


Universität Zürich[UZH]

uniklinik balgrist
EXPERTISE IN MOTION

Subject 3: EPT and dSSEP (T7 Left)



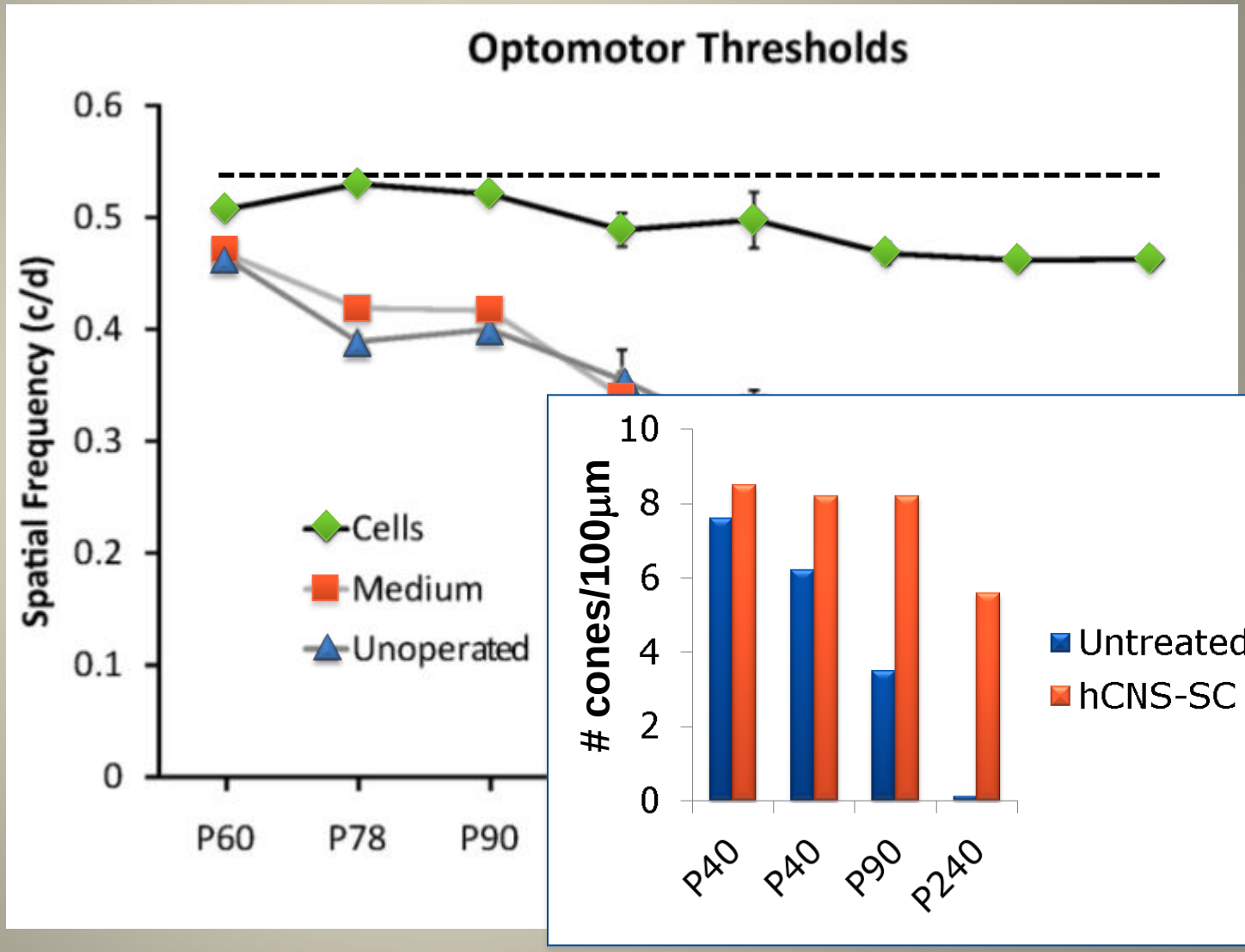


© StemCells, Inc. 2012

Preservation of visual acuity



McGill et al, 2012



Jamieson et al

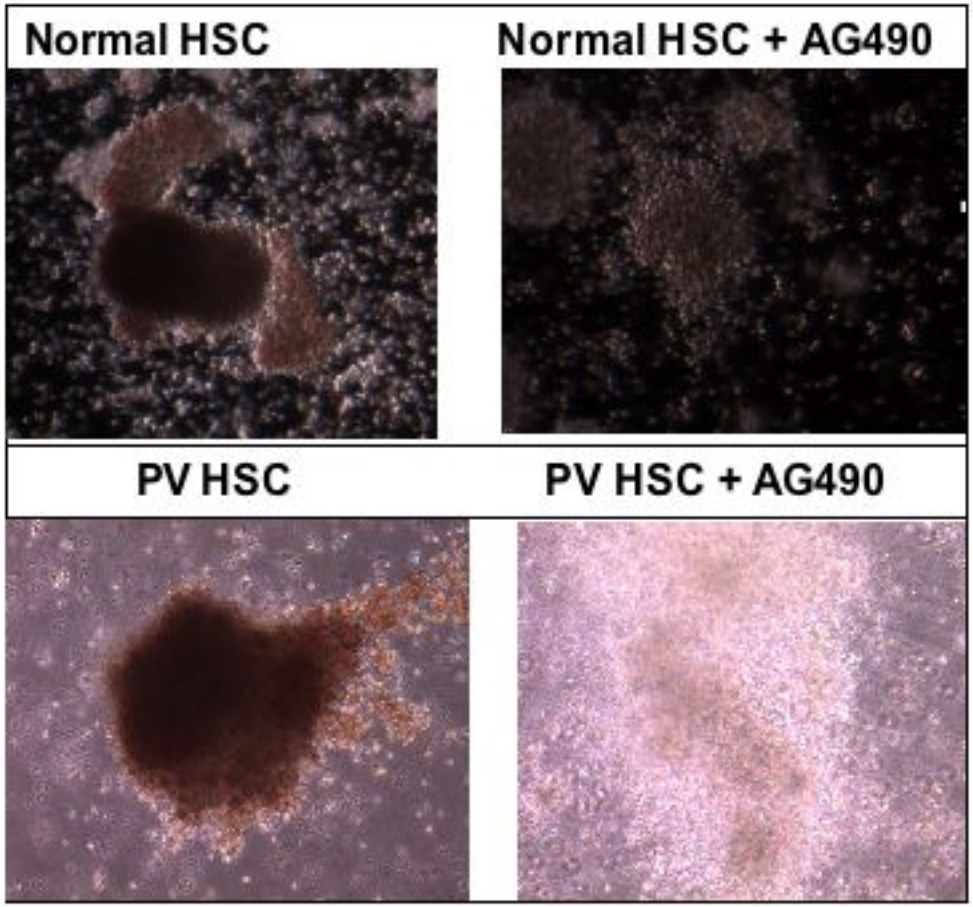


Figure 3C. Effect of JAK2 inhibition with AG490 on normal versus polycythemia vera (PV) hematopoietic stem cell (HSC) differentiation potential *in vitro*. Representative photomicrographs obtained with a Zeiss Axiovert microscope (10x objective) and SPOT software. Normal cord blood or PV peripheral blood HSCs were FACS sorted onto methylcellulose supplemented with or without AG490 in addition to cytokines.

Animal Studies: Production of Function Myelin

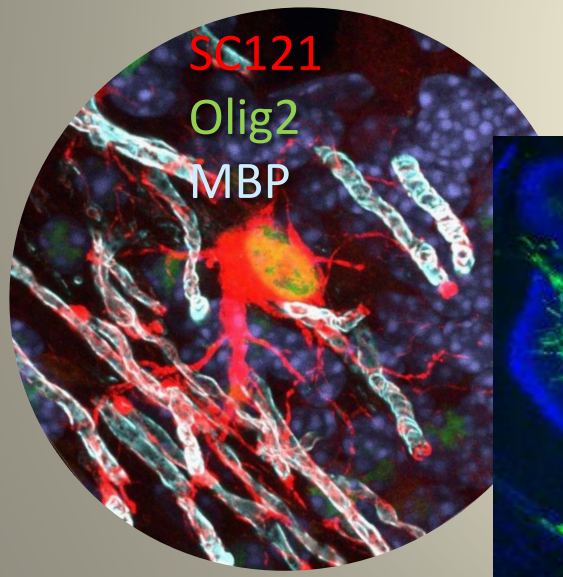
Uchida, et al. 2012

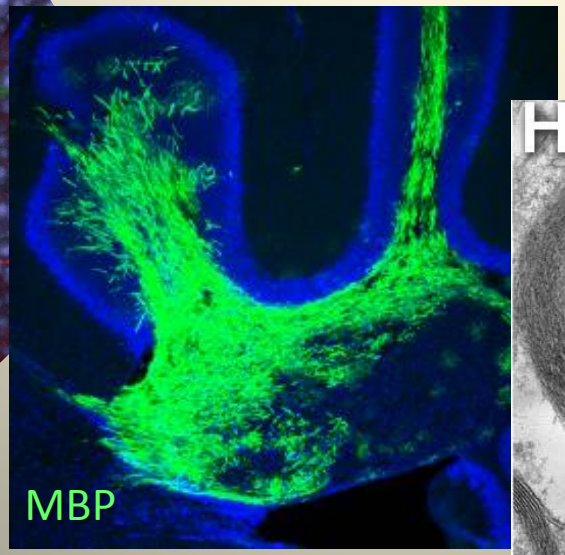
Science Translational Medicine
AAAS

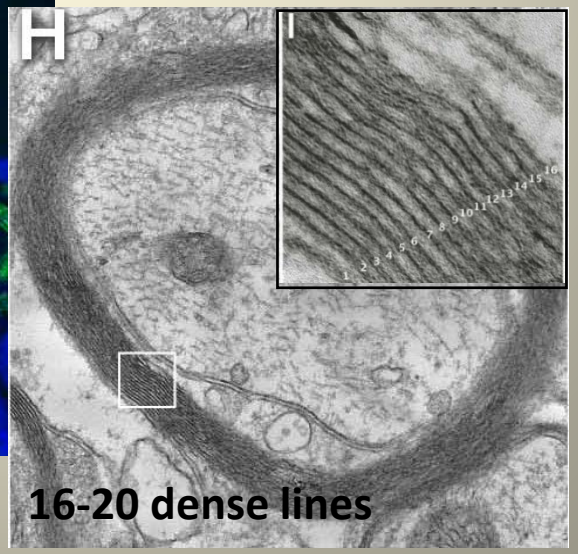
STEM CELLS

Human Neural Stem Cells Induce Functional Myelination in Mice with Severe Dysmyelination

- ✓ Oligodendrocyte differentiation – new functional myelin
- ✓ Myelin enhanced nerve conduction
- ✓ MRI used to detect human myelination in animals



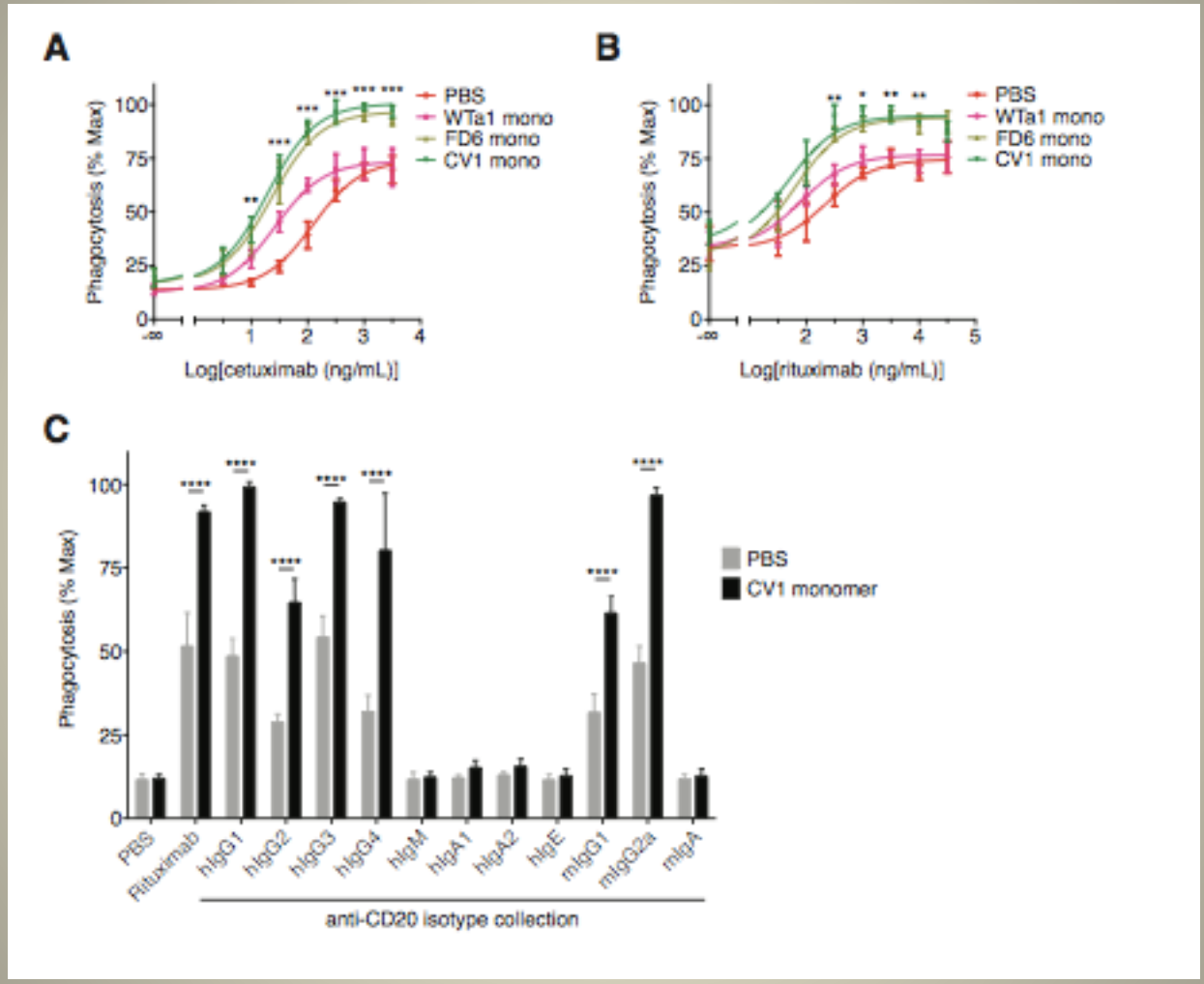




Clonal stem/progenitors in vivo shown by chimeras [tetrachimera and rainbow]

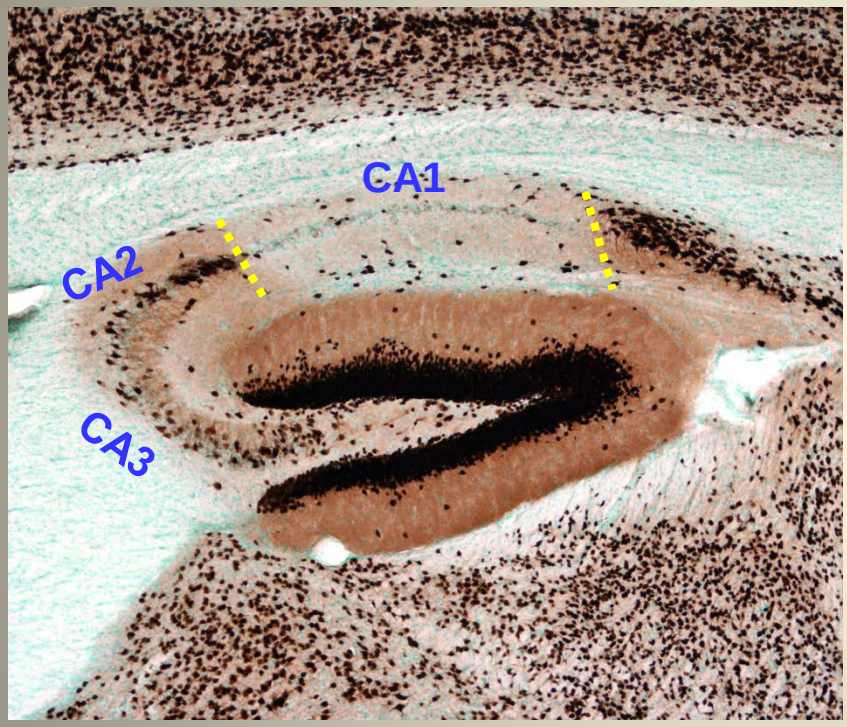
- Mouse digit tip regeneration involves no dedifferentiation, but endogenous lineage specific stem cells for skin, muscle, bone, etc
- Mouse thoracic and abdominal organ smooth muscle cells and fibroblasts derive from stem cells in the mesothelial covering
- Mouse dermal and mucosal fibroblasts are derived from distinct lineages [Eng or Wnt1] and have distinct healing patterns
- Mouse renal segments have separate stem/progenitors for ascending tubules, descending tubules, and collecting ducts [with Benny Dekel]

- Yuval Rinkevich et al

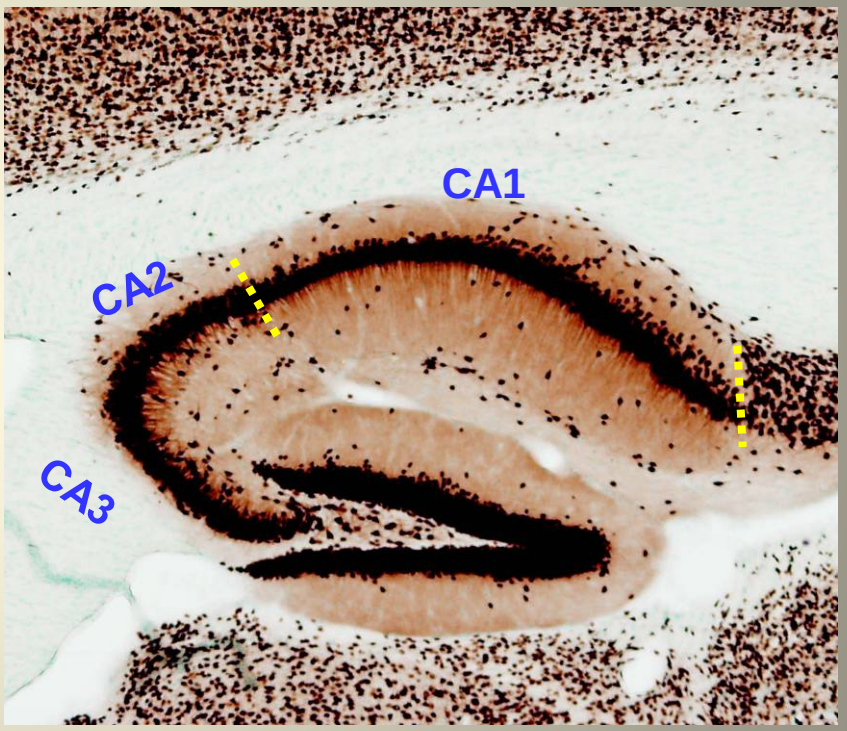

A
Phagocytosis (% Max)
Log[cetuximab (ng/mL)]
PBS
WTa1 mono
FD6 mono
CV1 mono
B
Phagocytosis (% Max)
Log[rituximab (ng/mL)]
PBS
WTa1 mono
FD6 mono
CV1 mono
C
Phagocytosis (% Max)
PBS
CV1 monomer
PBS
Rituximab
hIgG1
hIgG2
hIgG3
hIgG4
hIgM
hIgA1
hIgA2
hIgE
mIgG1
mIgG2a
mIgA
anti-CD20 isotype collection

Mouse Model that Mimics Human NCL

PPT 1-/-



Normal (NOD-scid)



NCL mouse brain: CA1 region only 8% of neurons were detected compare to normal

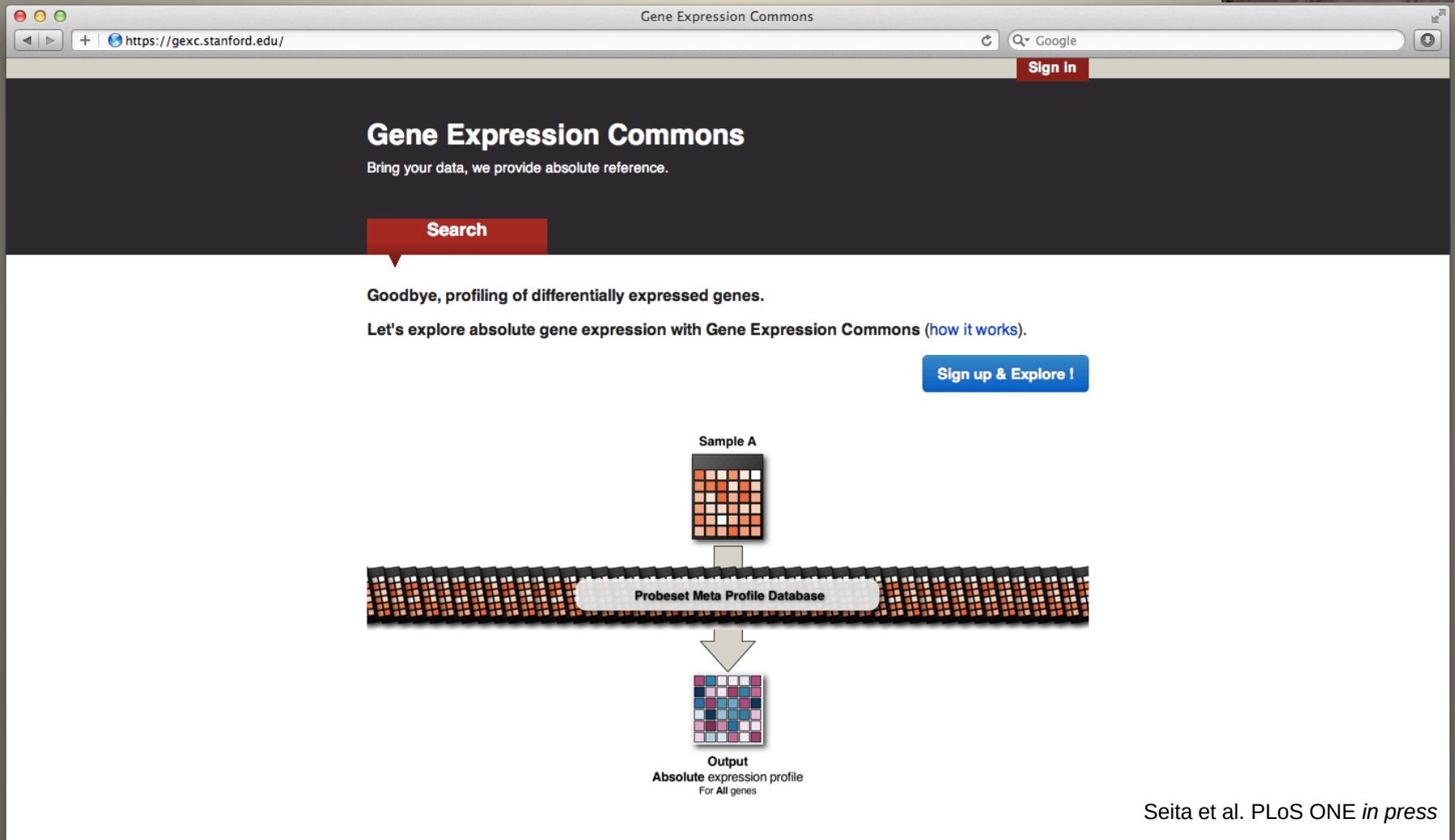
Poster F-2264

Gene Expression Commons

An Open Platform for Absolute Gene Expression Profiling

http://gexc.stanford.edu **Sign up Today !**





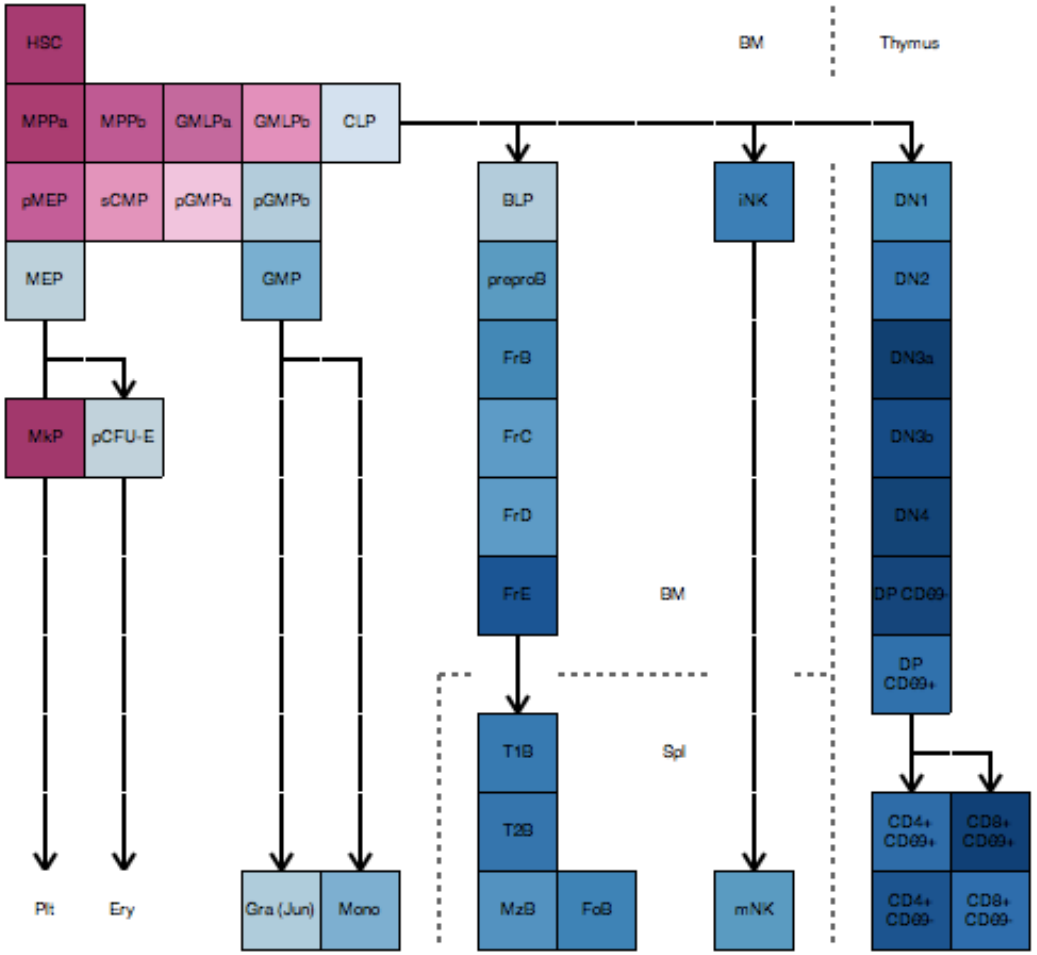
Seita et al. PLoS ONE *in press*

Mpl myeloproliferative leukemia virus oncogene

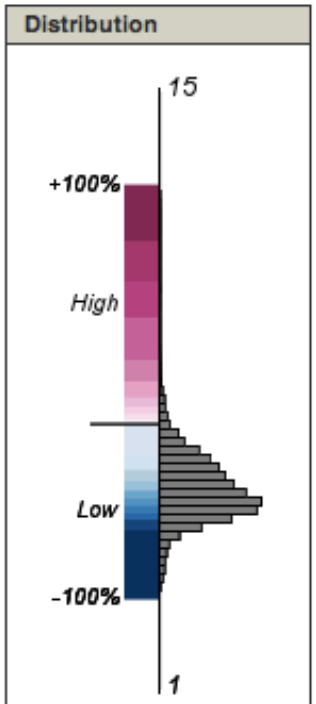
2 probesets were found.

Comment | Share | Save

Probeset: 1421461_at | Detail | Same Pattern



Probeset Meta-profile



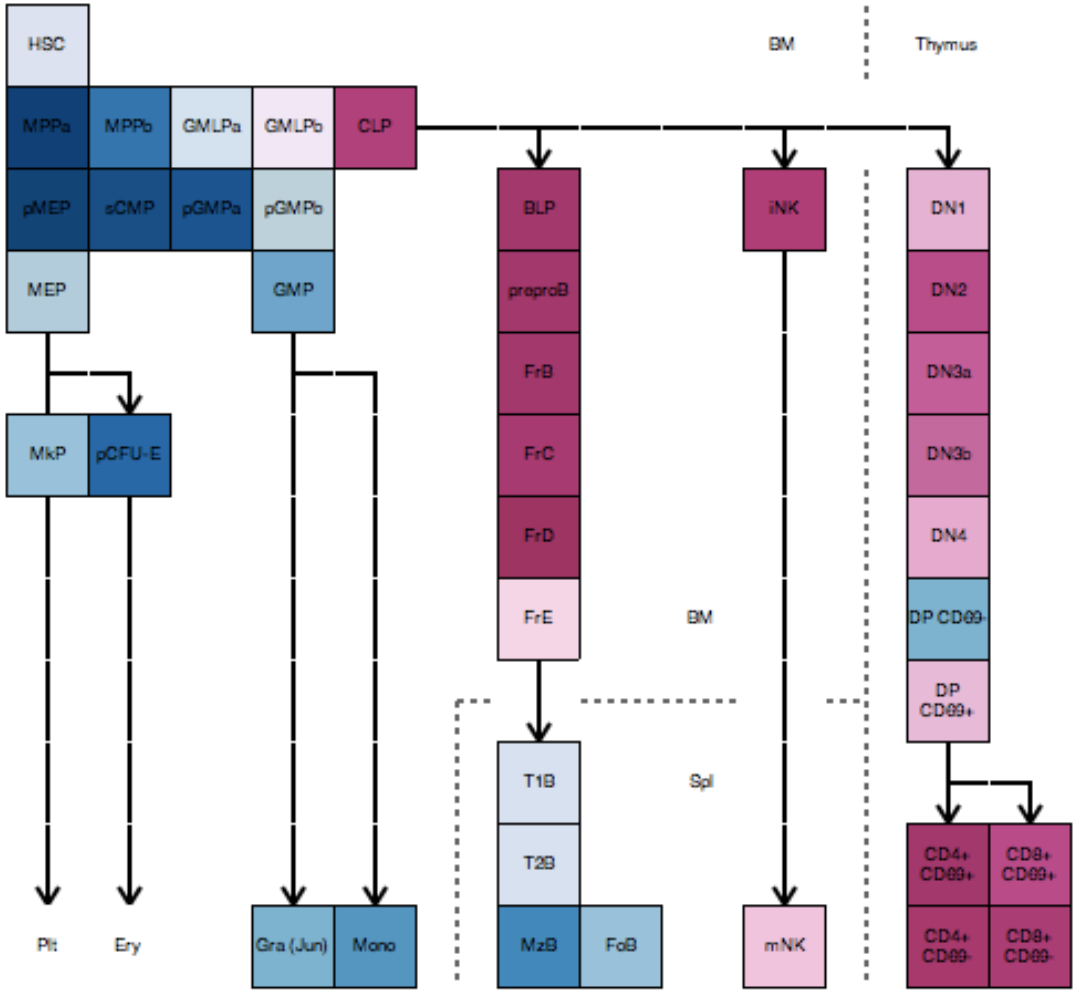
Dynamic-range (99%)
8.08703
Threshold
7.22122

Il7r interleukin 7 receptor

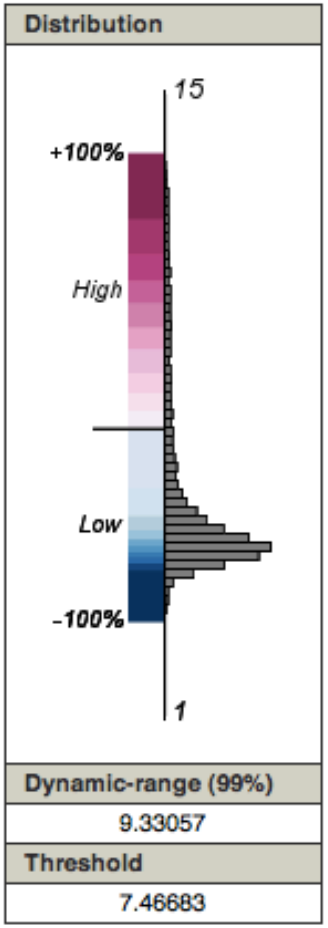
2 probesets were found.

Comment | Share | Save

Probeset: 1448575_at | Detail | Same Pattern



Probeset Meta-profile



Cebpa CCAAT/enhancer binding protein (C/EBP), alpha

Other Name: C/ebpalpha, CBF-A, and Cebp

NCBI Entrez Gene: 12606

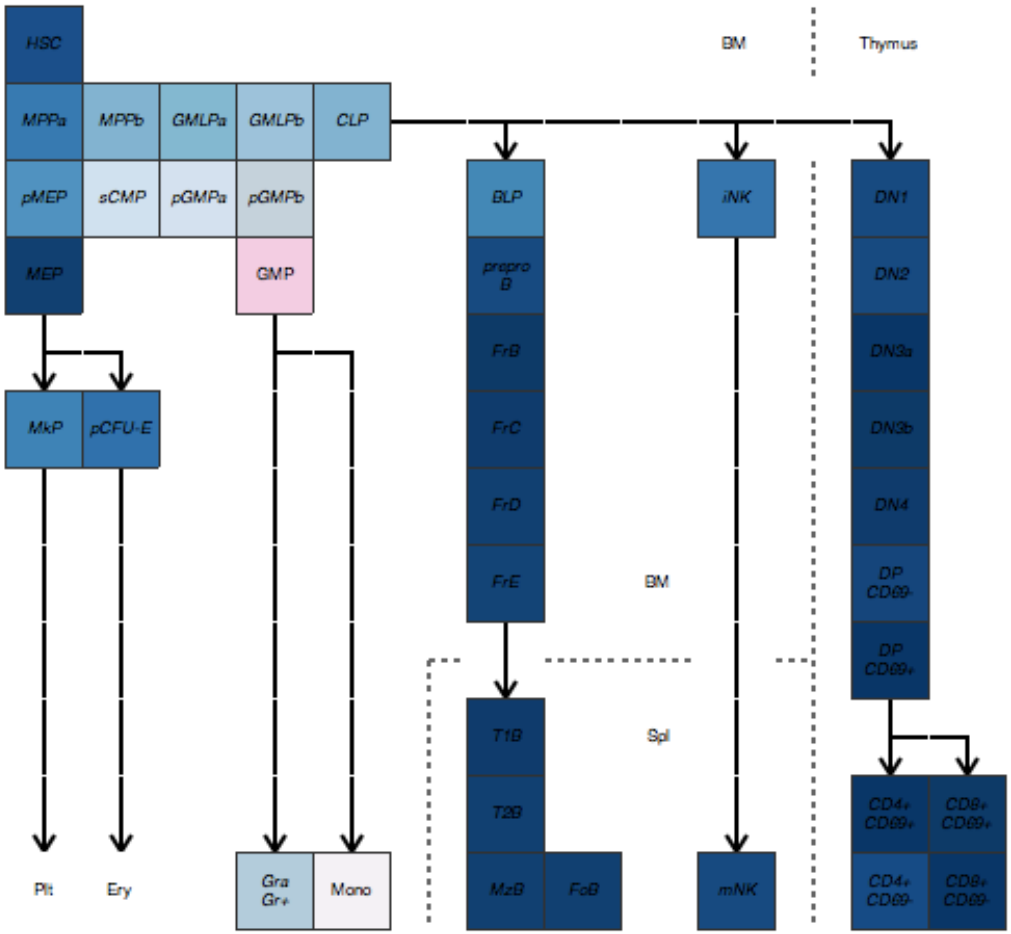
1 probeset on Affymetrix Mouse Genome 430 2.0 Array

Kondo/Weissman:1999.reprogram

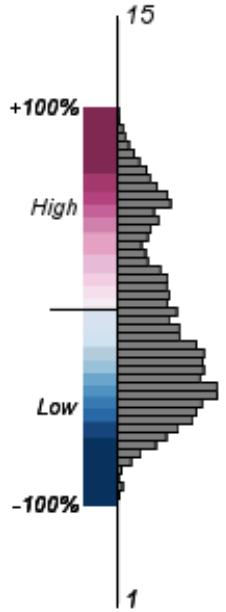
CLP to GMP

Probeset: 1418982_at for Cebpa

More detail | Download Figure (PDF) | Search Same Pattern



Probeset Meta Profile



Dynamic-range (99%)

8.10

Threshold

8.05

BCL2 blocks apoptosis, but not programmed cell removal of neutrophiles: Lagasse and Weissman JEM 1994

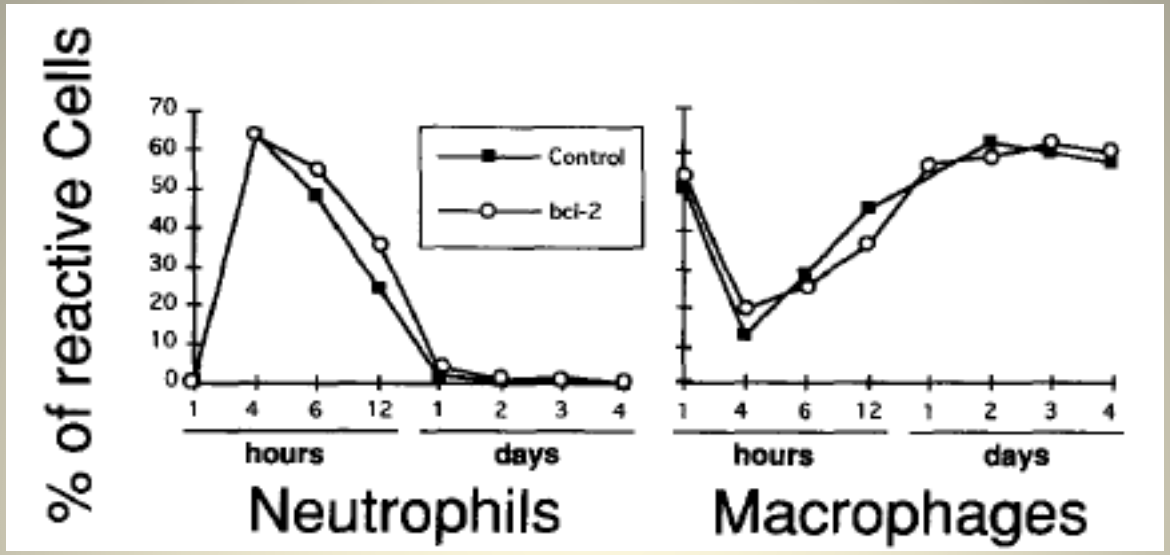
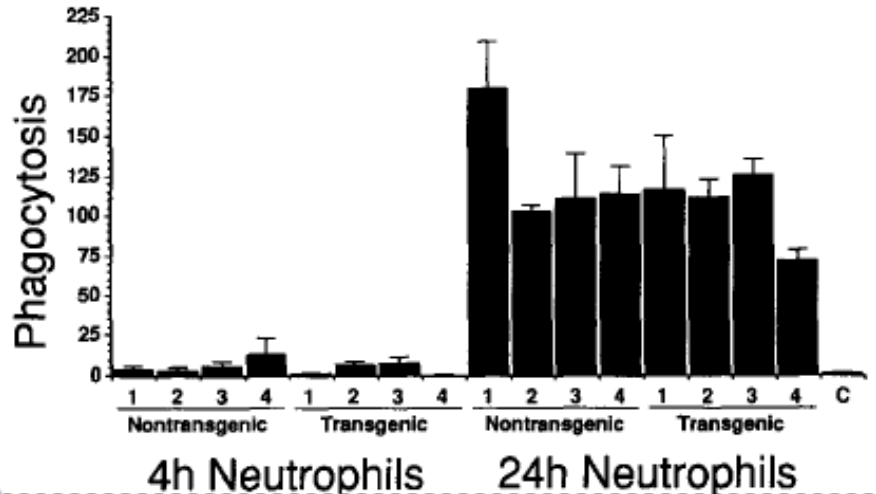


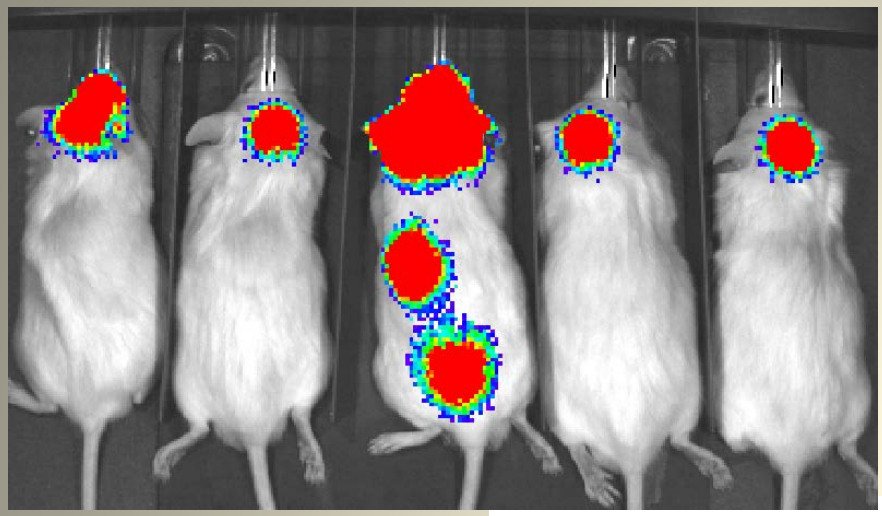
Table 1. *Neutrophil Content in the Bone Marrow, Blood and Spleen of Control and Transgenic Mice*

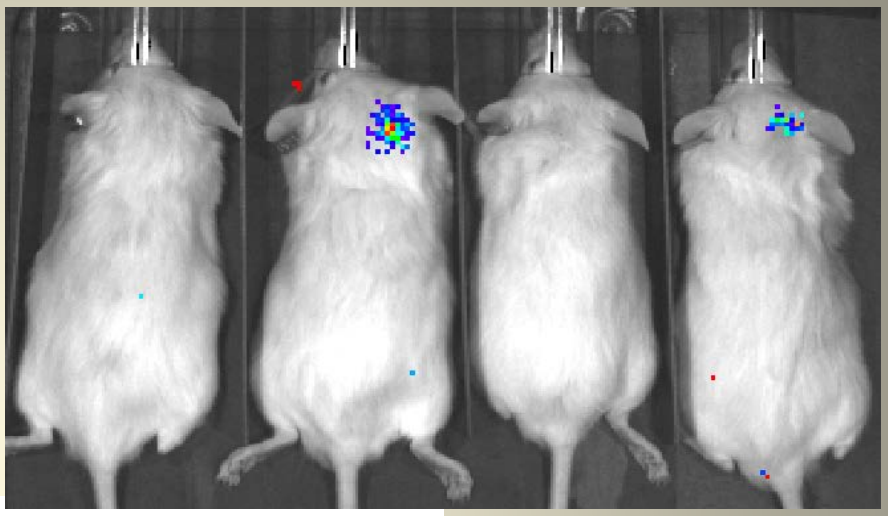
Mice	Bone marrow	Blood	Spleen
Nontransgenic	30.4 ± 4.0	5.8 ± 2.8	1.8 ± 0.4
Transgenic	30.3 ± 8.3	12.6 ± 3.8	2.2 ± 0.4

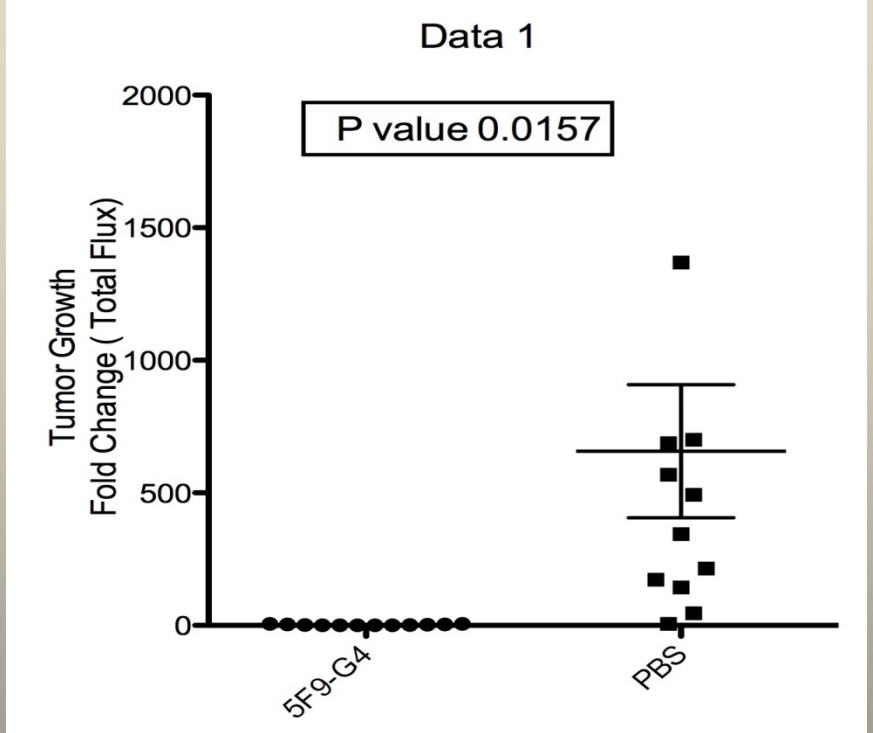
Neutrophils were counted by flow cytometric analysis of cells bearing Mac-1 and Gr-1 using two-color immunofluorescence. The results are expressed as arithmetric means (three mice) ± SD.



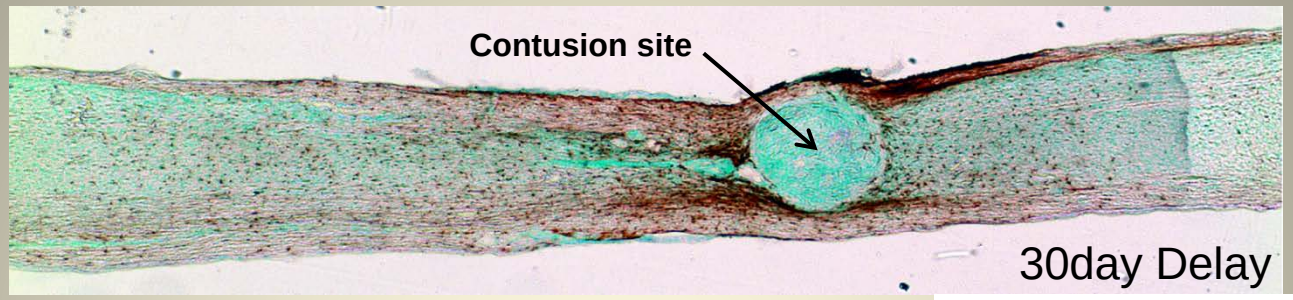
Hu 5F9G4 Inhibits human Myc-subgroup Medulloblastoma

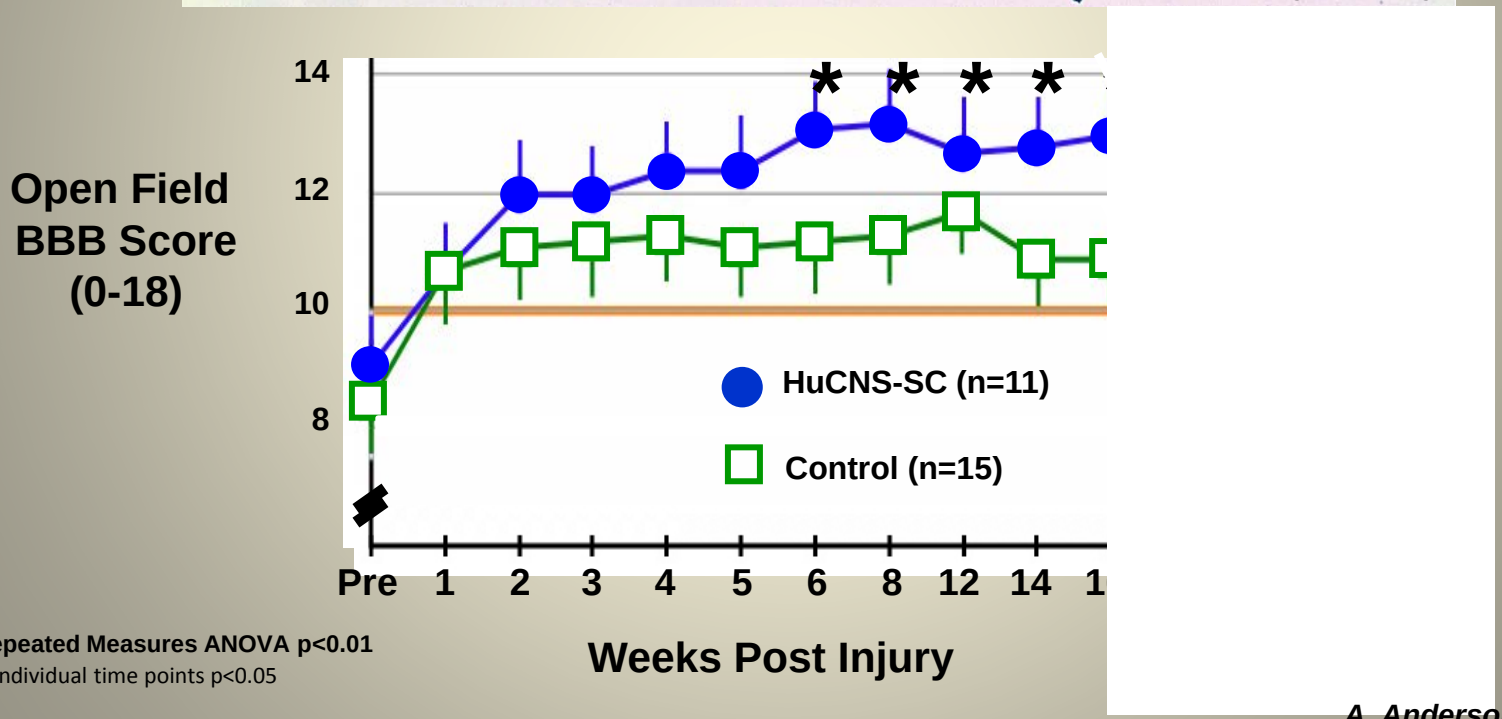






HuCNS-SC:
Spinal Cord Injury Preclinical Efficacy





Repeated Measures ANOVA p<0.01
* individual time points p<0.05

A. Anderson UC, Irvine

Gene expression profile of the BCSC derived stromal subsets

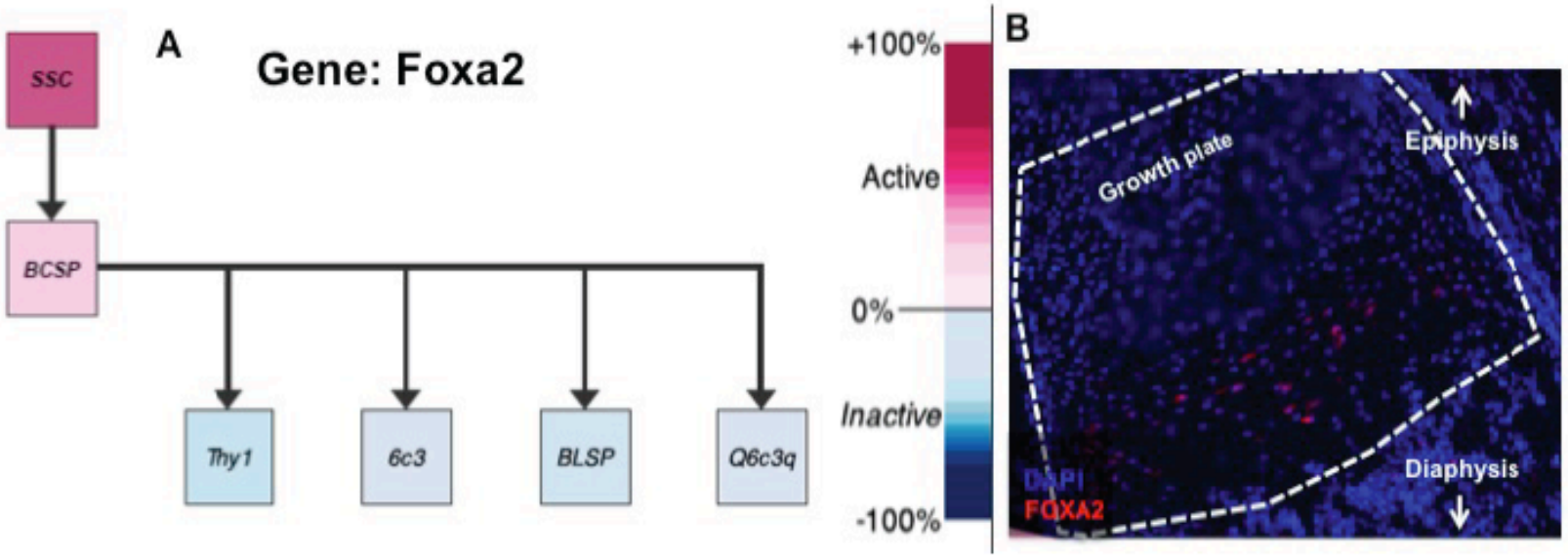


Figure 7. *In situ* localization of SSCs with "fingerprint" genes.
(A) Heat map depiction of microarray data demonstrates that *FOXA2* gene expression in mouse bone progenitors (SSC and BCSP), isolated from mice at post-natal day 3, highly express *FOXA2* gene.
(B) *In situ* immunofluorescent staining of a young adult mouse's hind limb localized FOXA2 to grow the plate.

PCD and PCR in cancers

- All cancers defeat programmed cell death: p53, bcl2, bax, etc
- All cancers defeat programmed cell removal: calreticulin and CD47
- Stimuli that induce PCD and/or PCR develop cell competition/selection that can result in cancer

Negligible phagocytosis is observed with control treatment

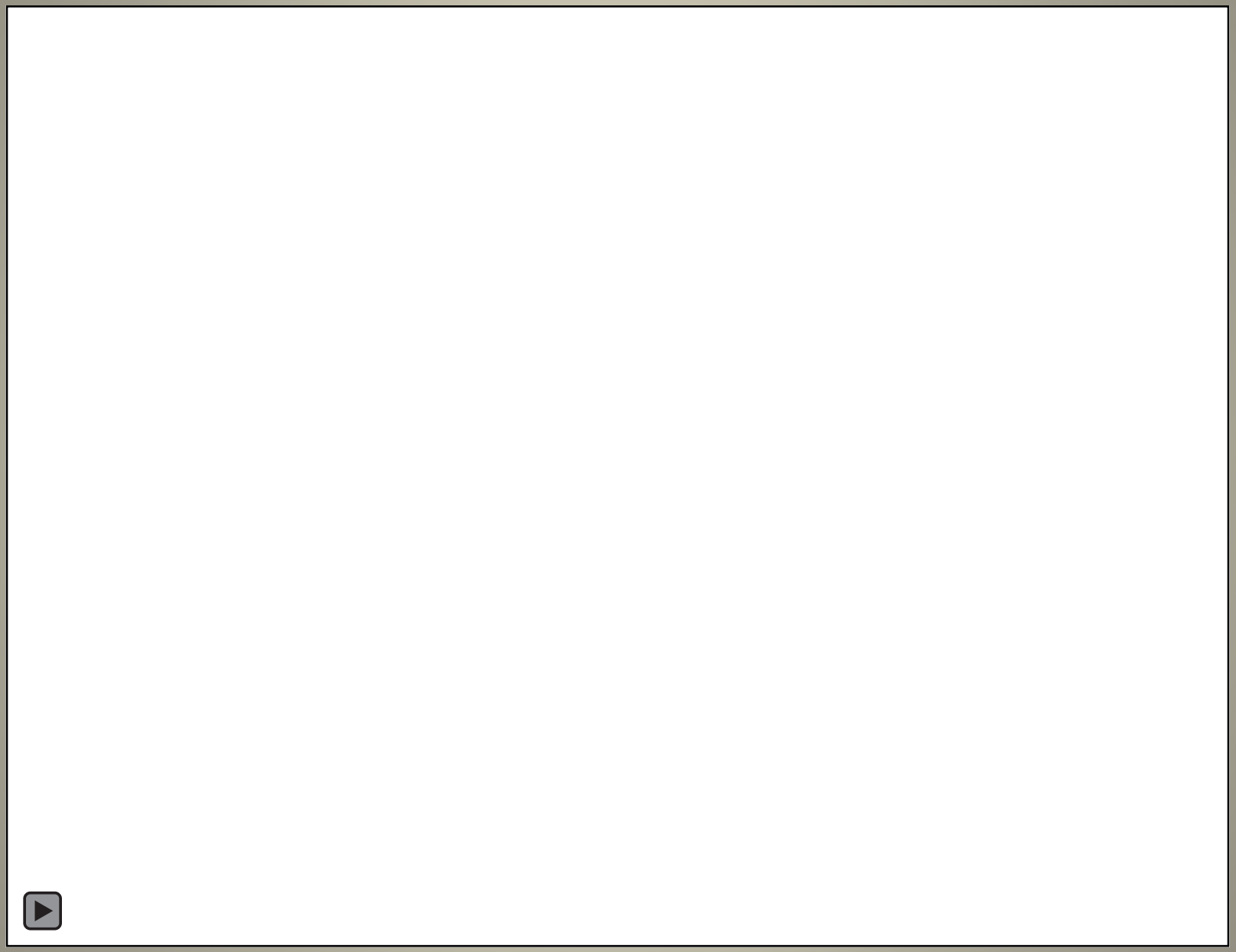
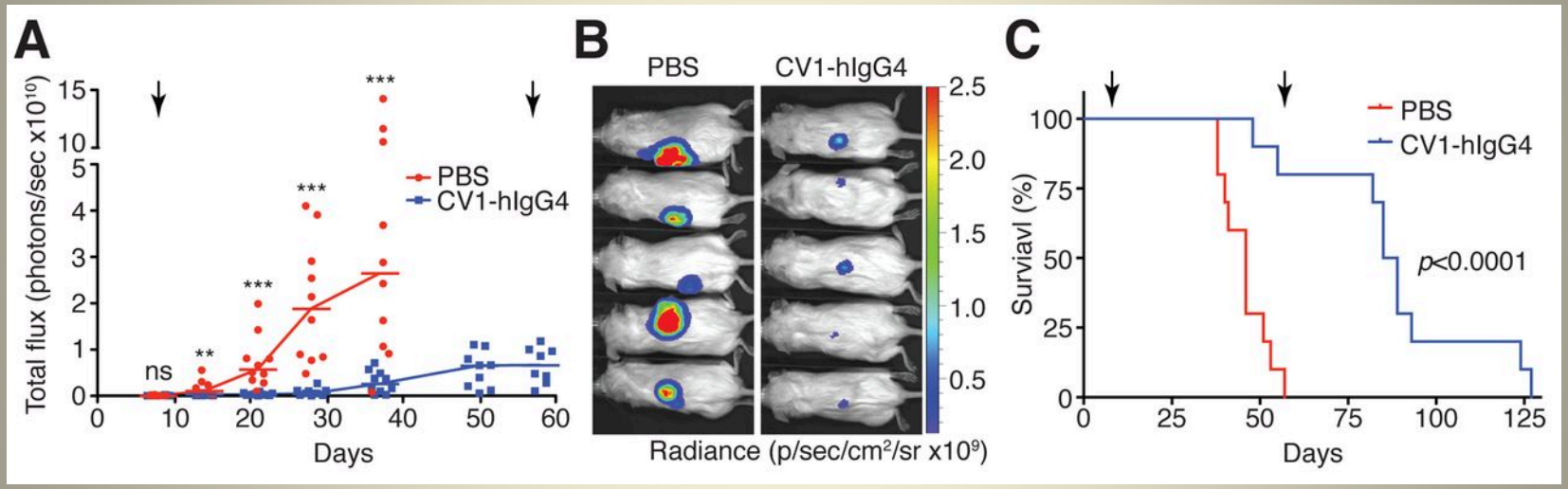
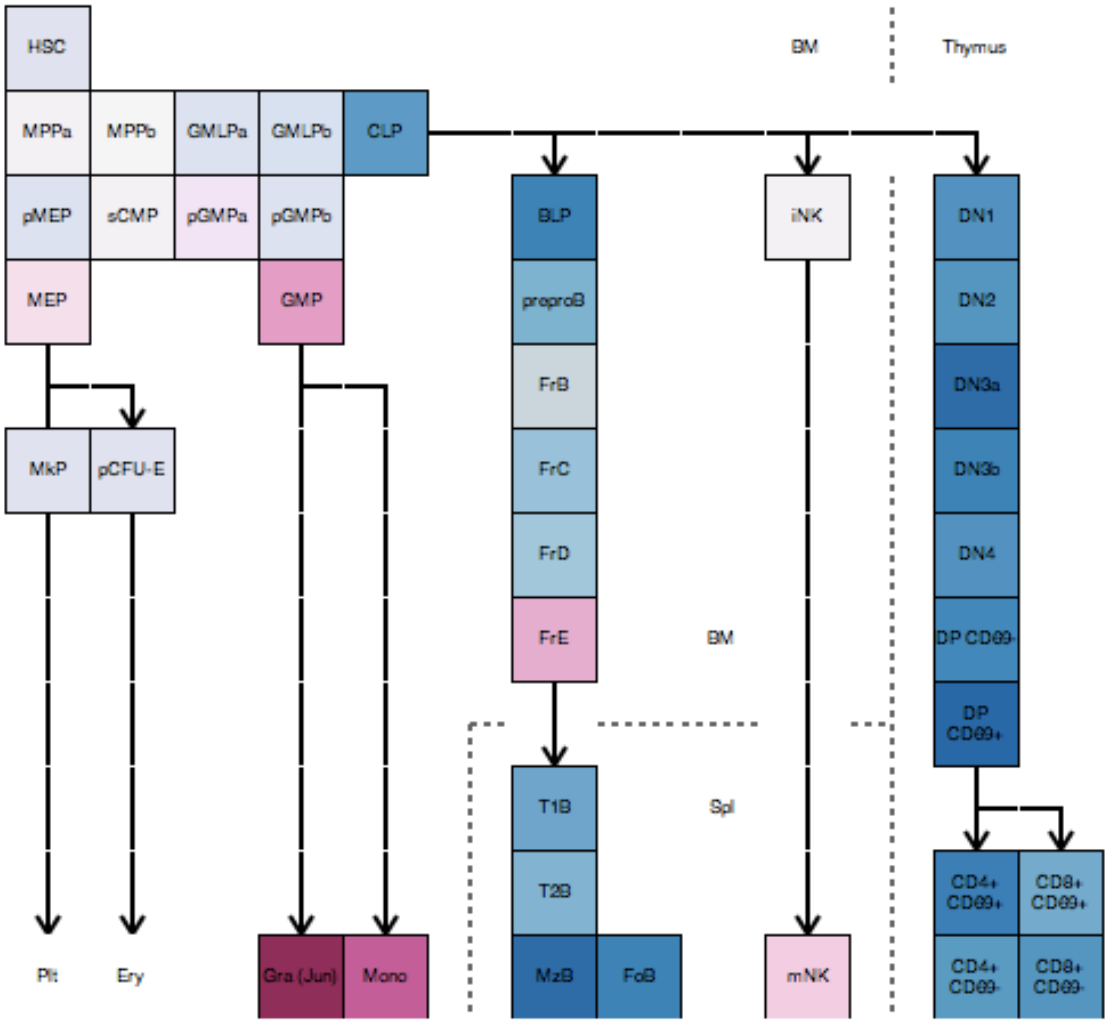


Fig. 3 High-affinity SIRPα-Fc fusion proteins are effective as single agents for cancer.(A) Growth of GFP-luciferase+ 639-V bladder cancer cells in the dorsal subcutaneous tissue of NSG mice upon daily treatment with vehicle control (PBS) or 200 μg high-affinity SIRPα-Fc (CV1-hIgG4) as evaluated by bioluminescence imaging.

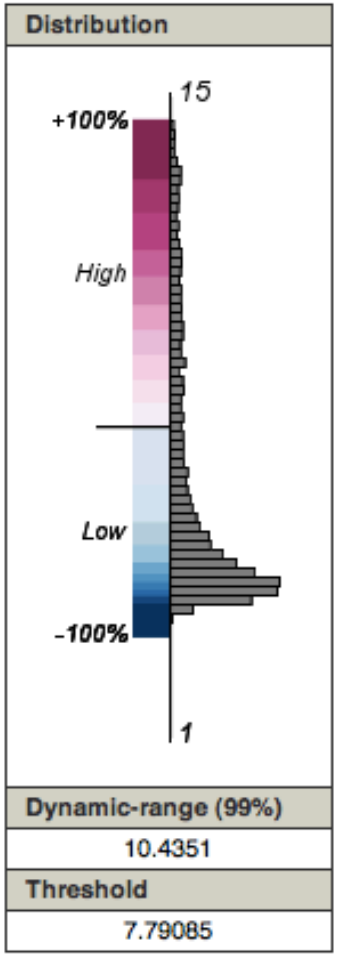


K Weiskopf et al. Science 2013;science.1238856

Published by AAAS

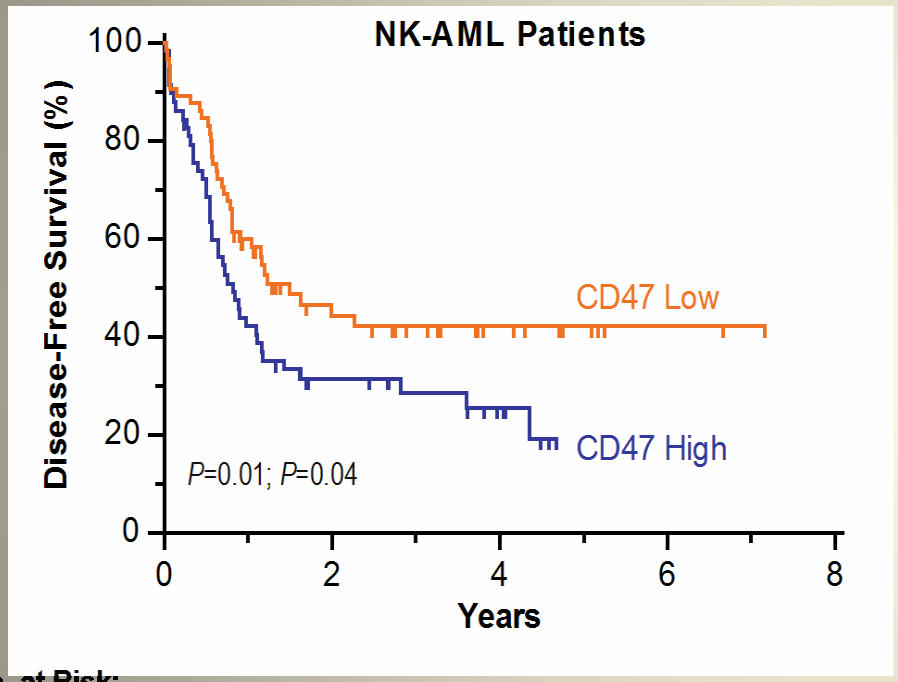


Probeset Meta-profile

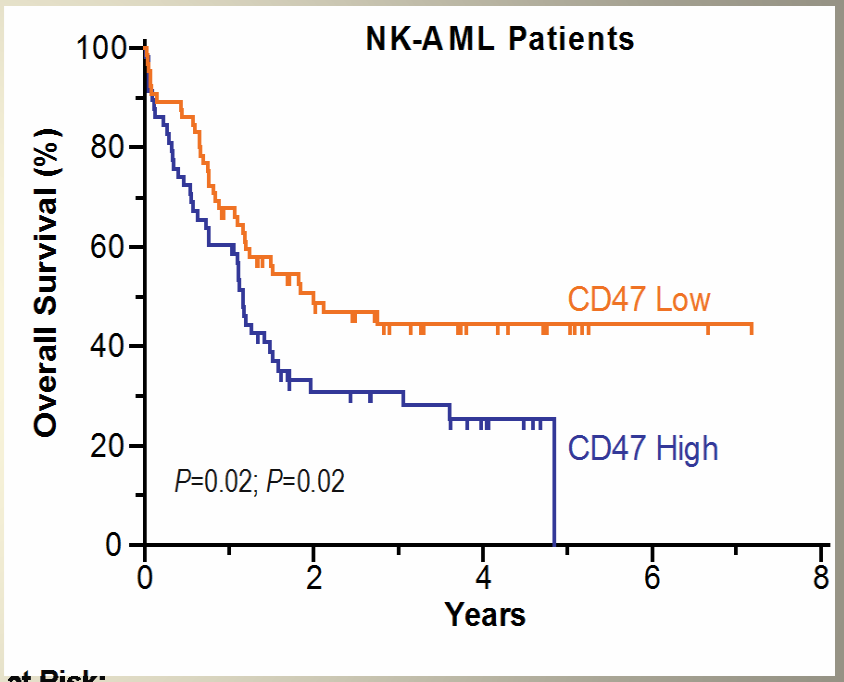


S100a8 S100 calcium binding protein A8 (calgranulin A)

Increased CD47 Expression is Associated with Worse Survival in Normal Karyotype (NK) AML Patients



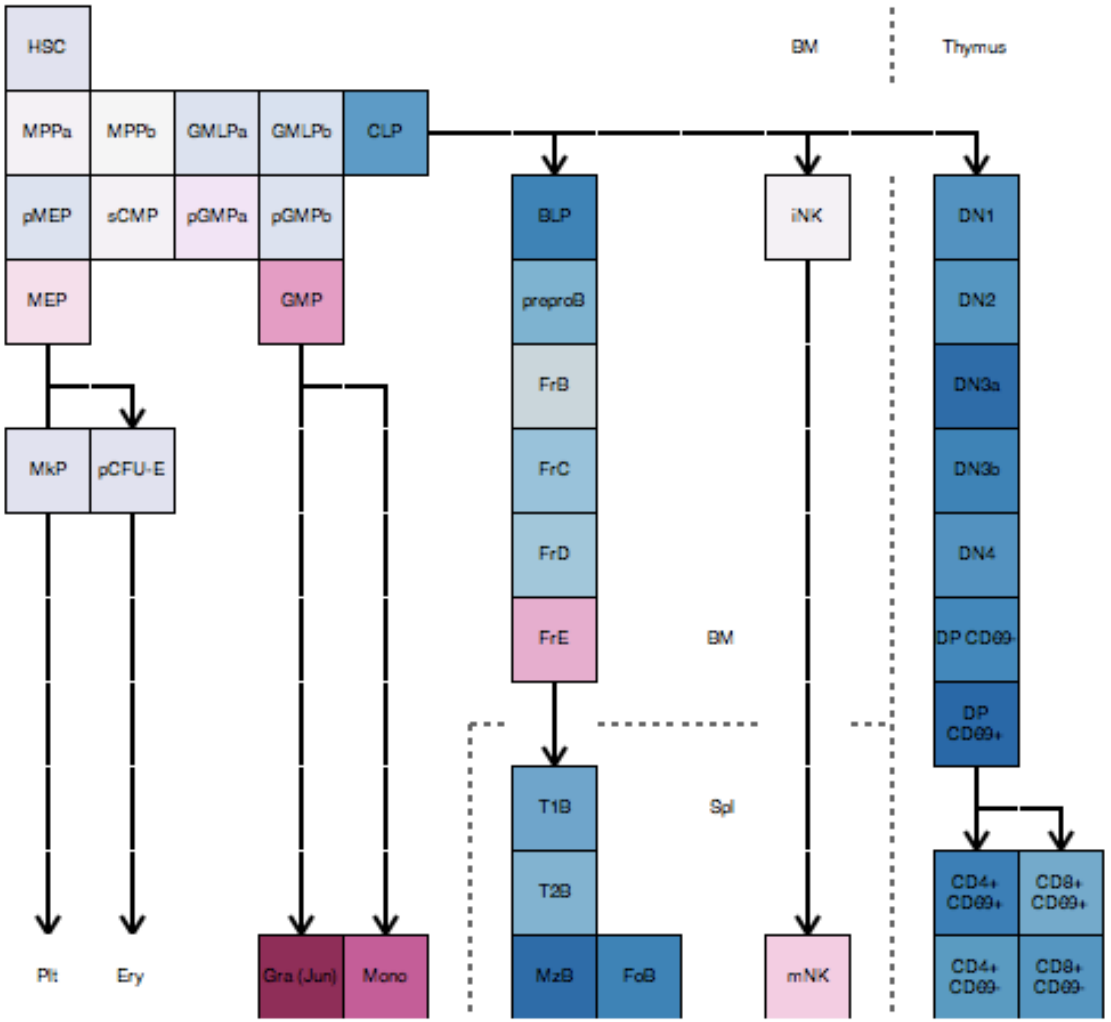
No. at Risk:					
CD47 Low:	65	20	9	2	1
CD47 High:	58	14	6	0	0



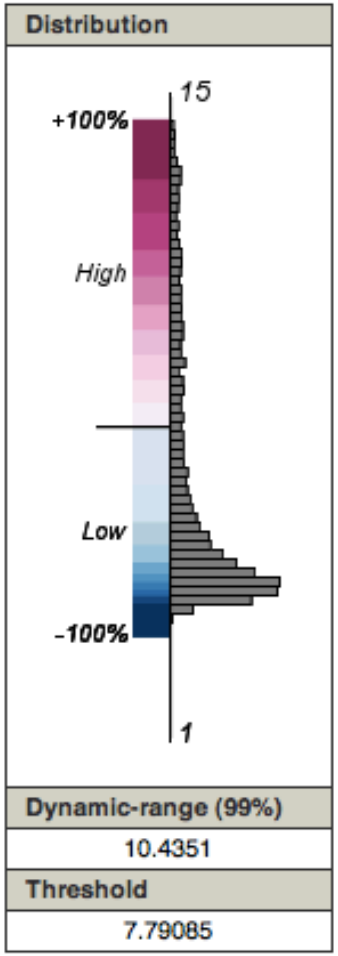
No. at Risk:					
CD47 Low:	65	26	10	2	1
CD47 High:	58	14	6	0	0

ASH ALIZADEH, RAVI MAJETI

Data analyzed from Bullinger L, et al. Blood (2008)



Probeset Meta-profile

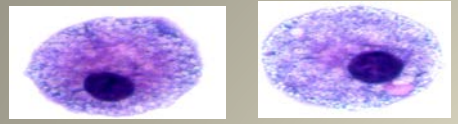


S100a8 S100 calcium binding protein A8 (calgranulin A)

Supplemental Figure 4

A

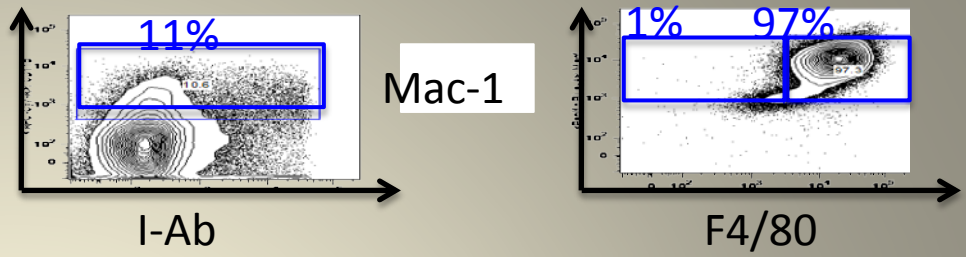
Macrophages



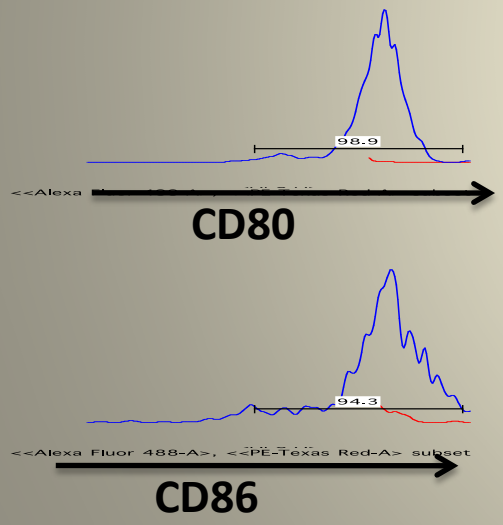
Dendritic cells (DCs)



B

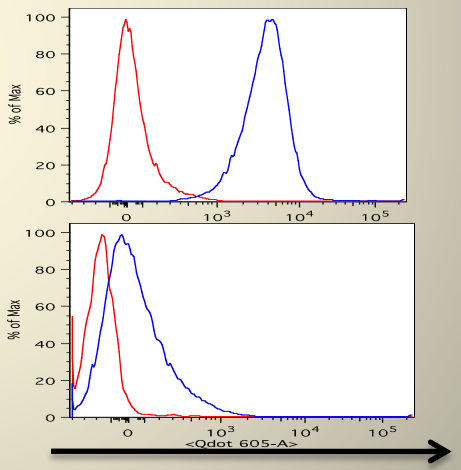


C



Macrophages
Dendritic Cells

D

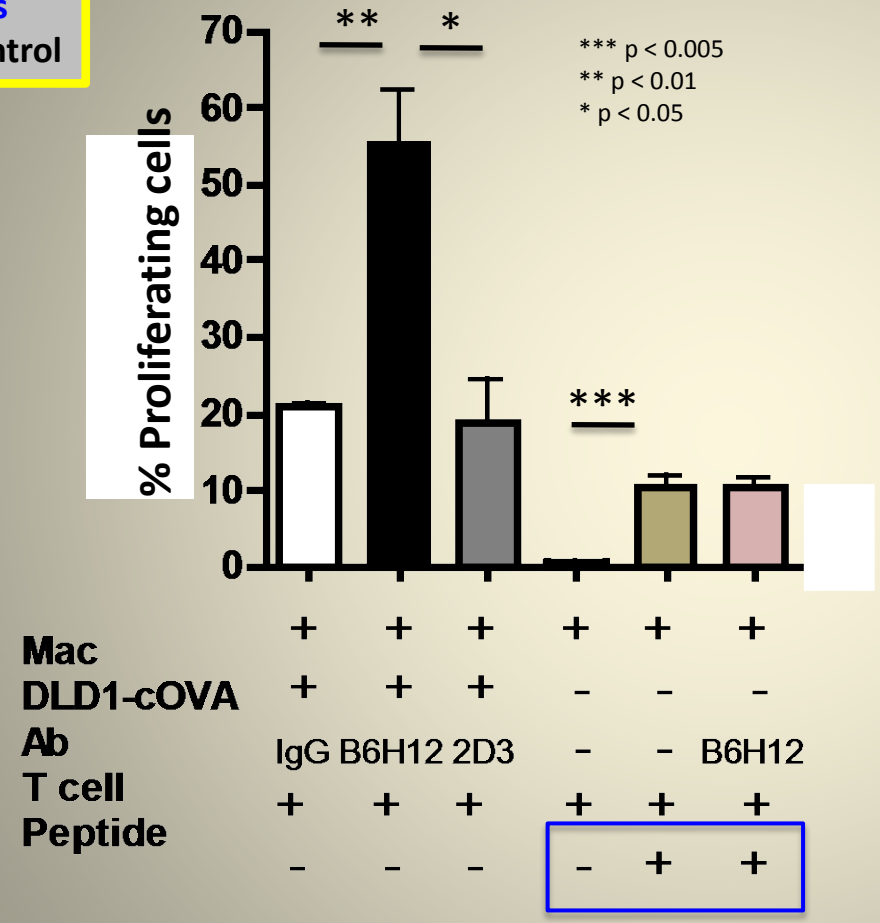


Unstained
SIRP-α stained

Diane Tseng, Jens Volkmer, and IW

Anti-CD47-mediated phagocytosis of tumors by macrophages leads to increased MHC I antigen presentation

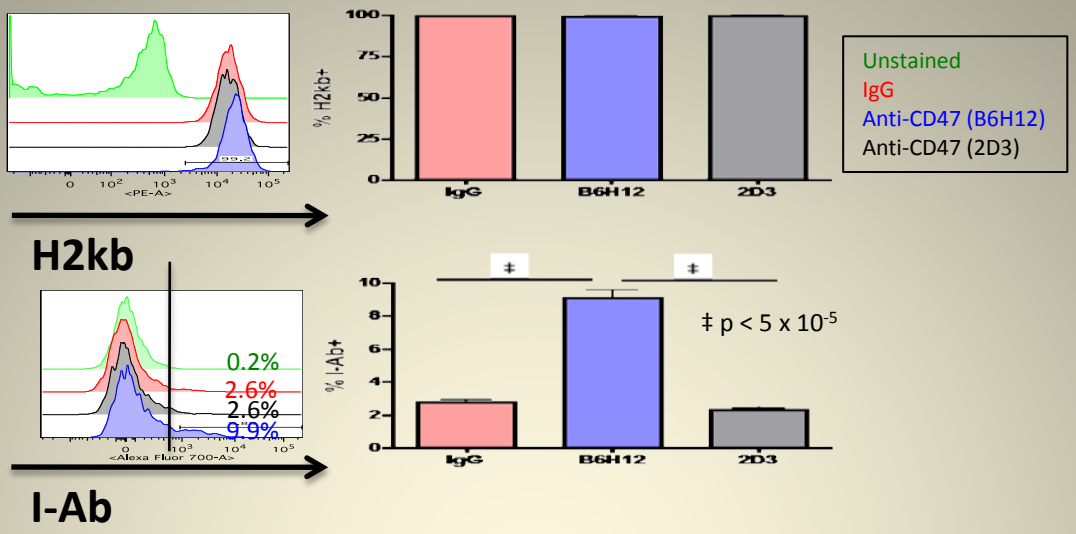
1) CD4 T cells
2) CD8 T cells
3) Tumor control



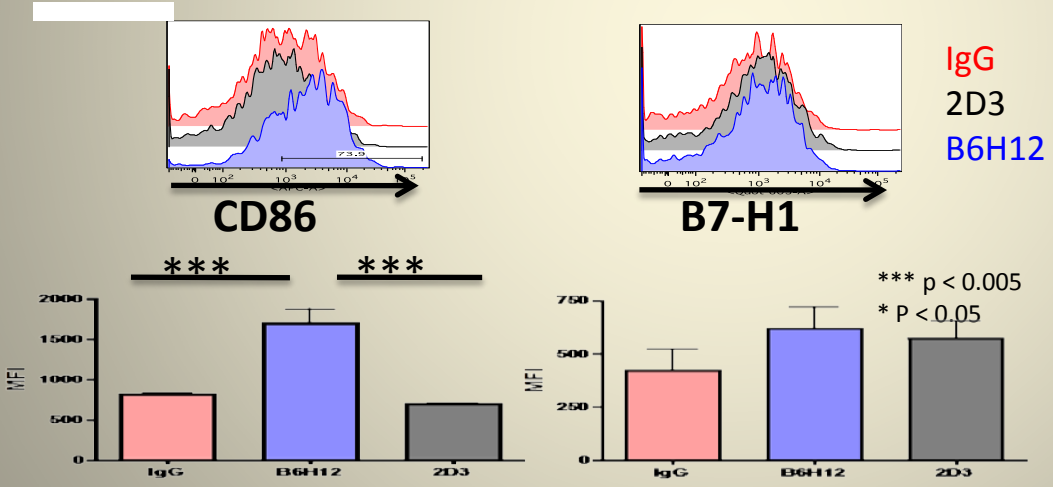
Diane Tseng

Supplemental Figure 5

A



B



Anti-CD47-mediated phagocytosis of cancer primes an effective anti-tumor T cell response

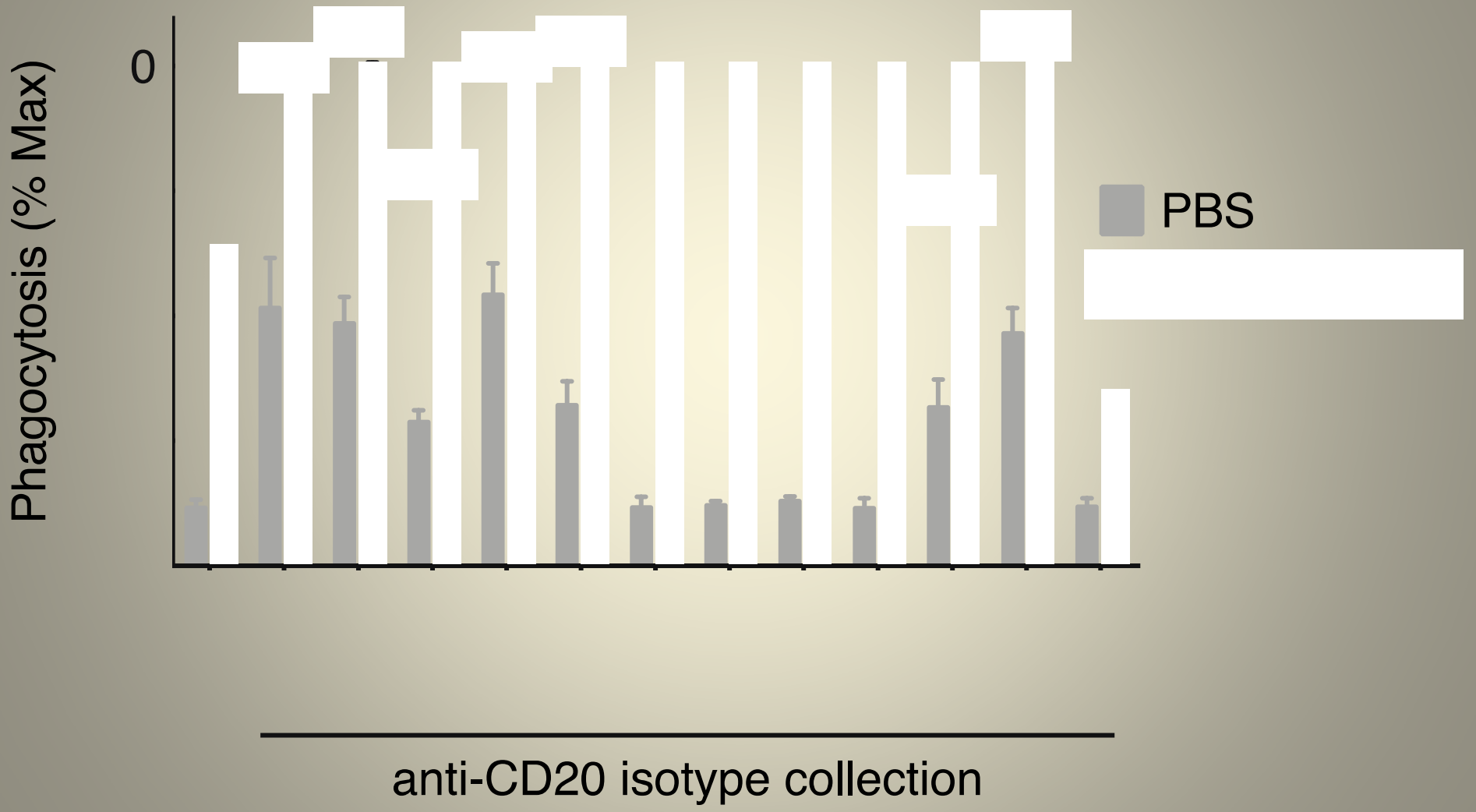
Diane Tseng and Jens Volkmer, MD



Stanford University School of Medicine
Laboratory of Dr. Irving Weissman

Jens photo

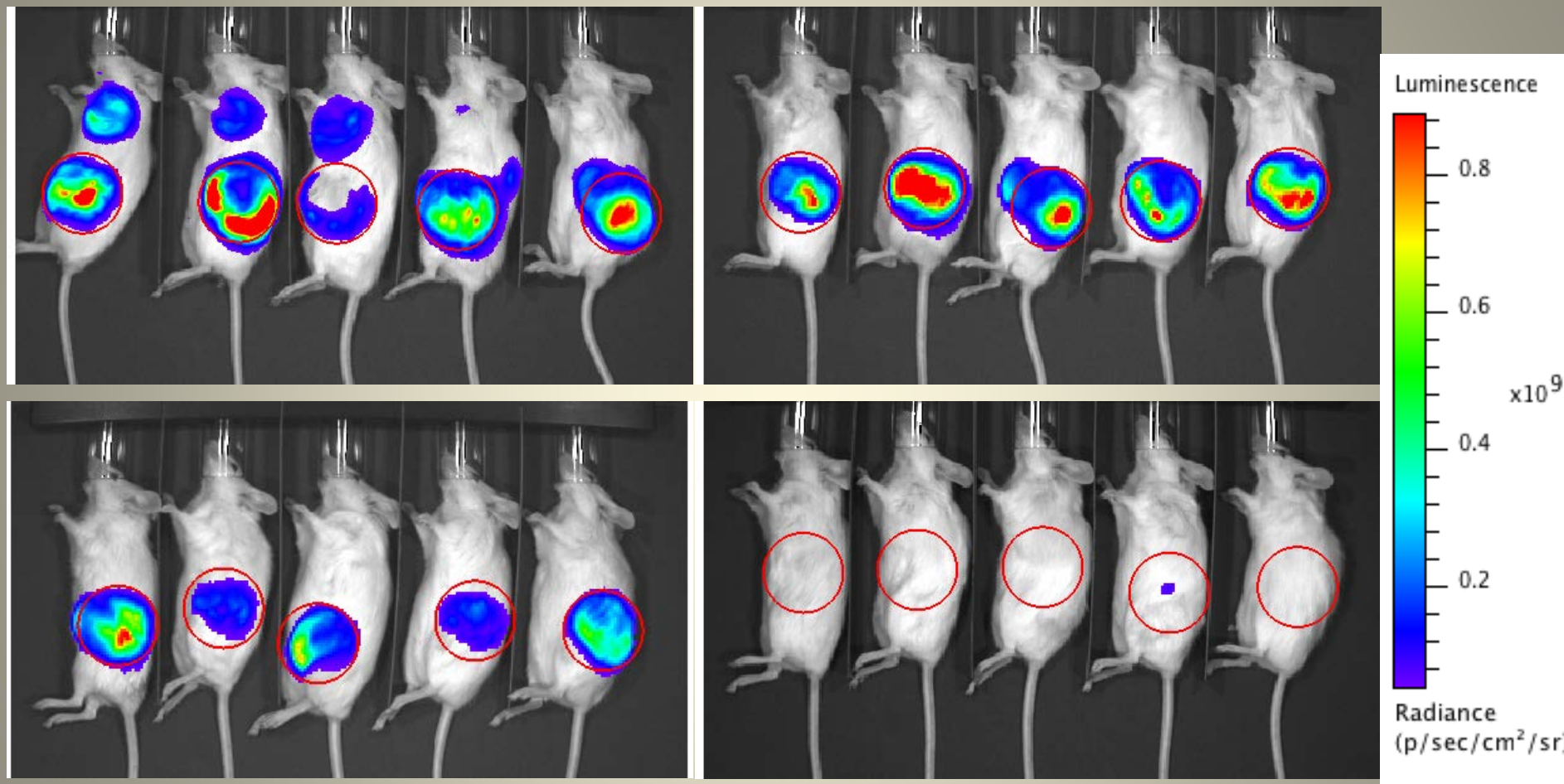
High-affinity SIRPα monomers enhance the efficacy of all human IgG subclasses



Bioluminescence imaging of lymphoma-bearing mice

PBS

CV1 monomer

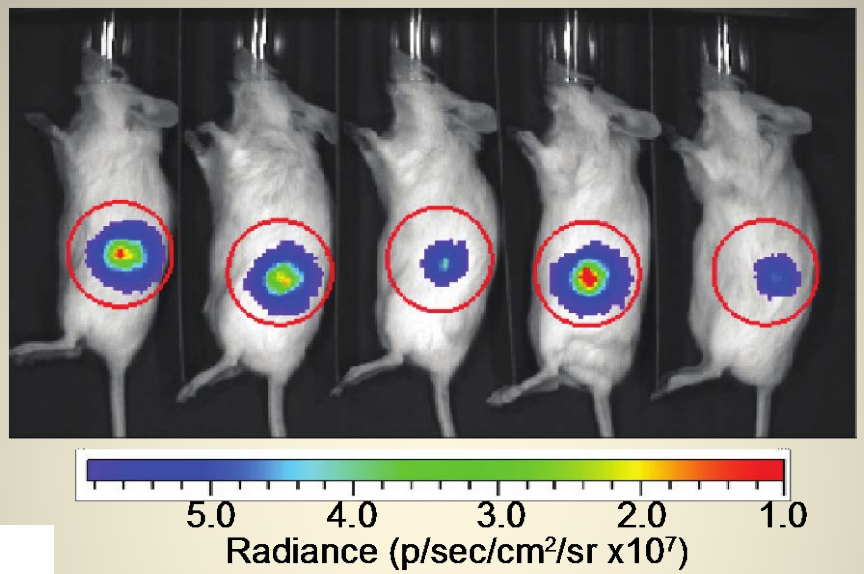


Rituximab

Rituximab+CV1

Rituximab/SIRPα monomer combo therapy produces long-term cures

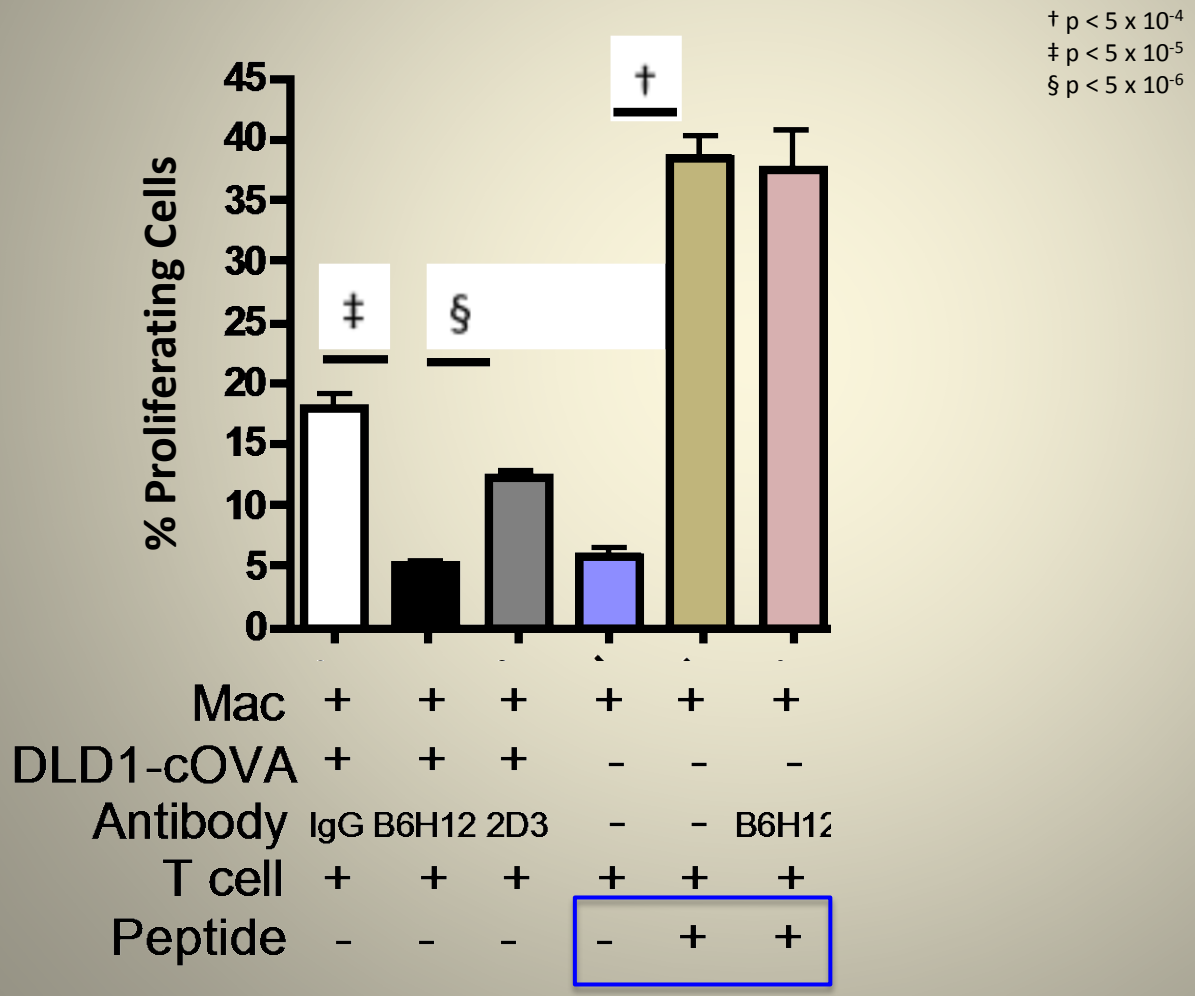
Pre-treatment



Day 209



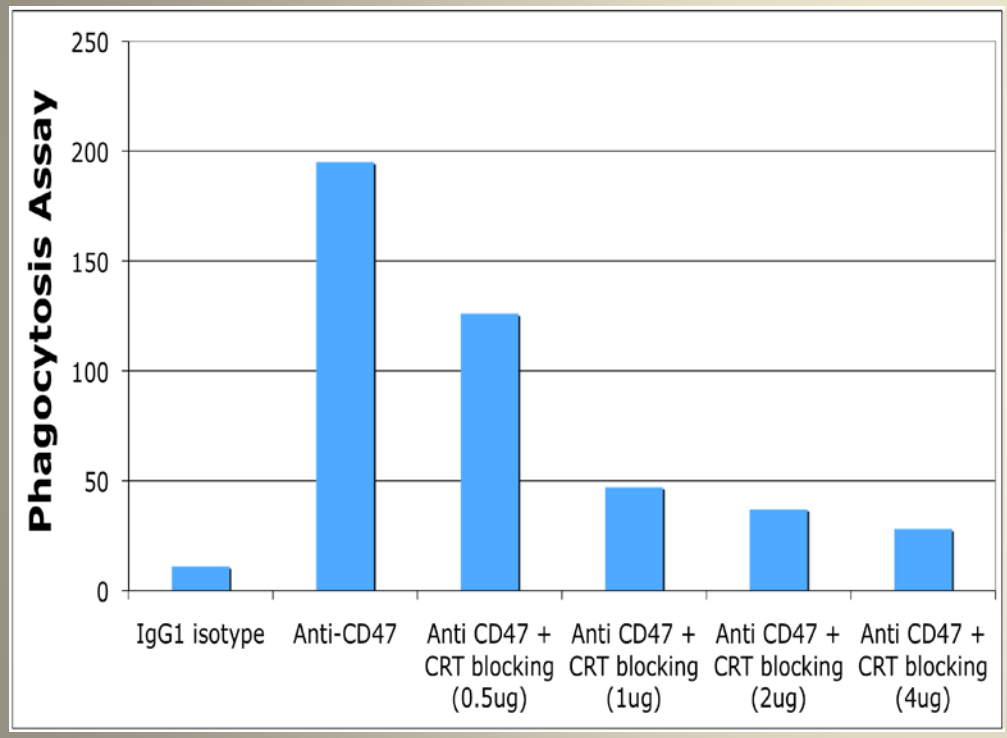
Anti-CD47-mediated phagocytosis of cancer by macrophages do not present antigen to OTII (CD4) T cells

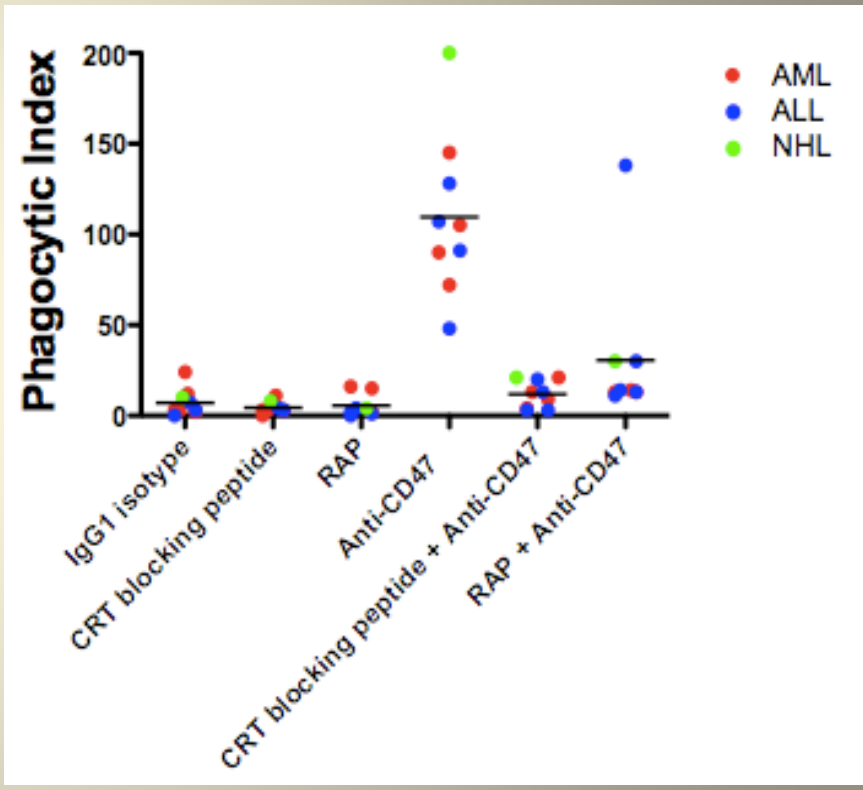


Diane Tseng

Blockade of calreticulin-LRP inhibits phagocytosis of cancer cells by anti-CD47 antibody

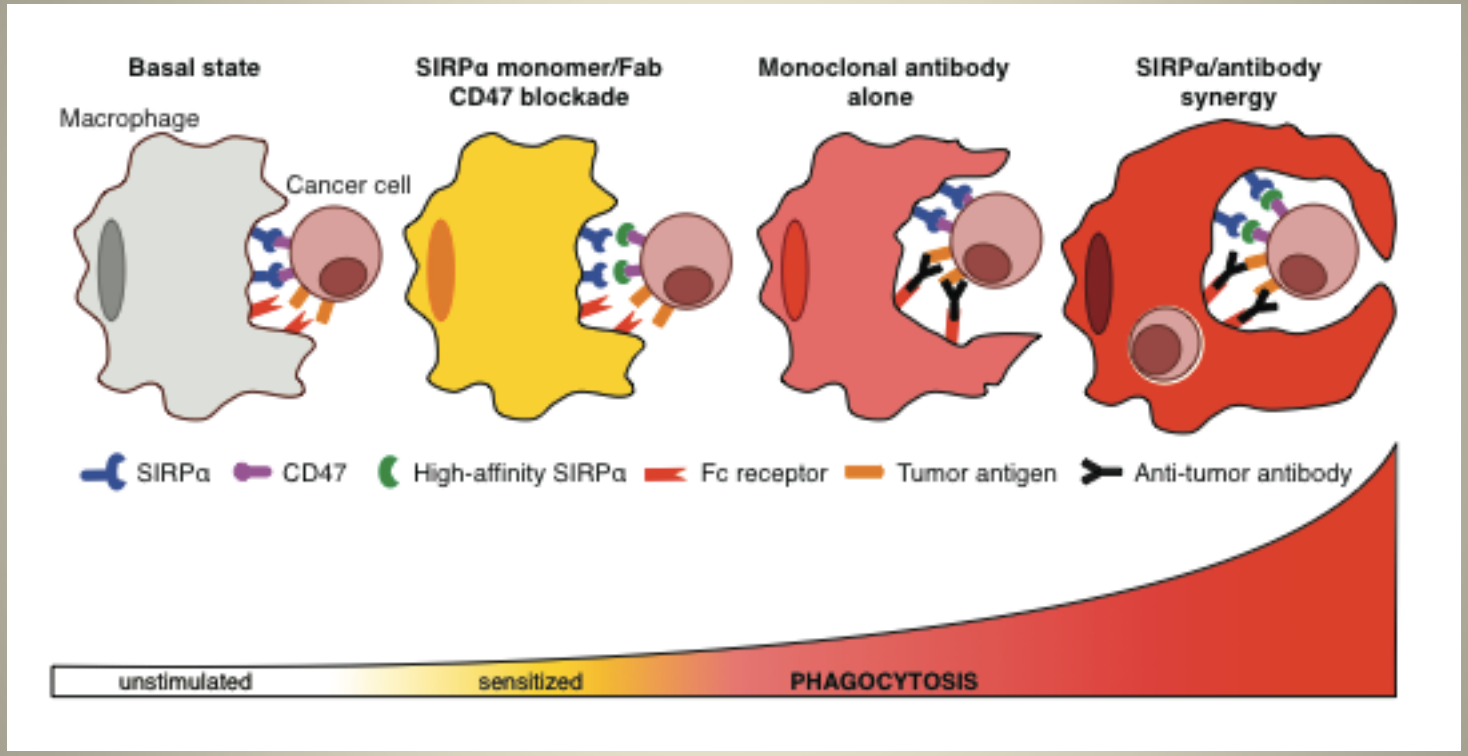
Jurkat cells

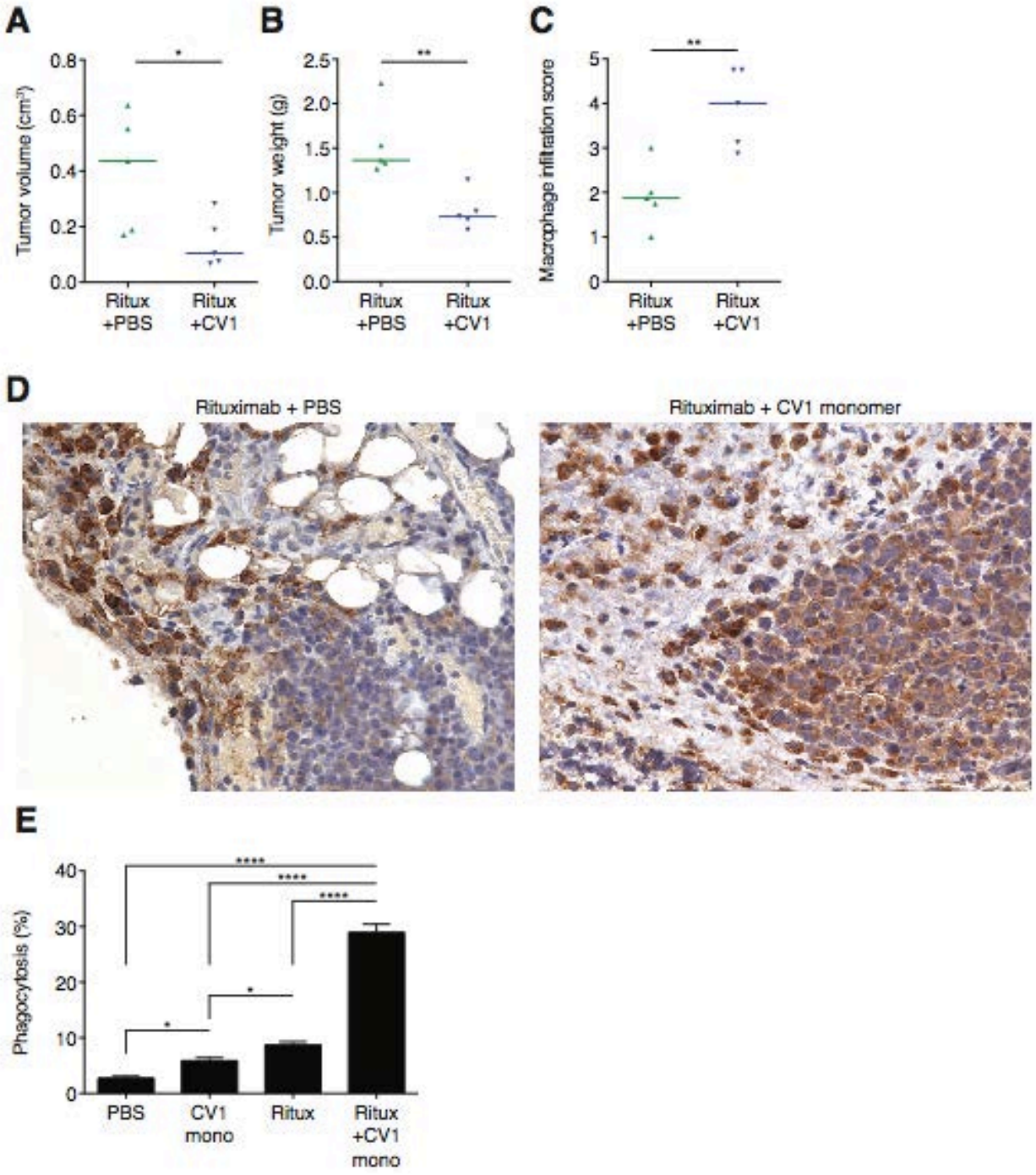


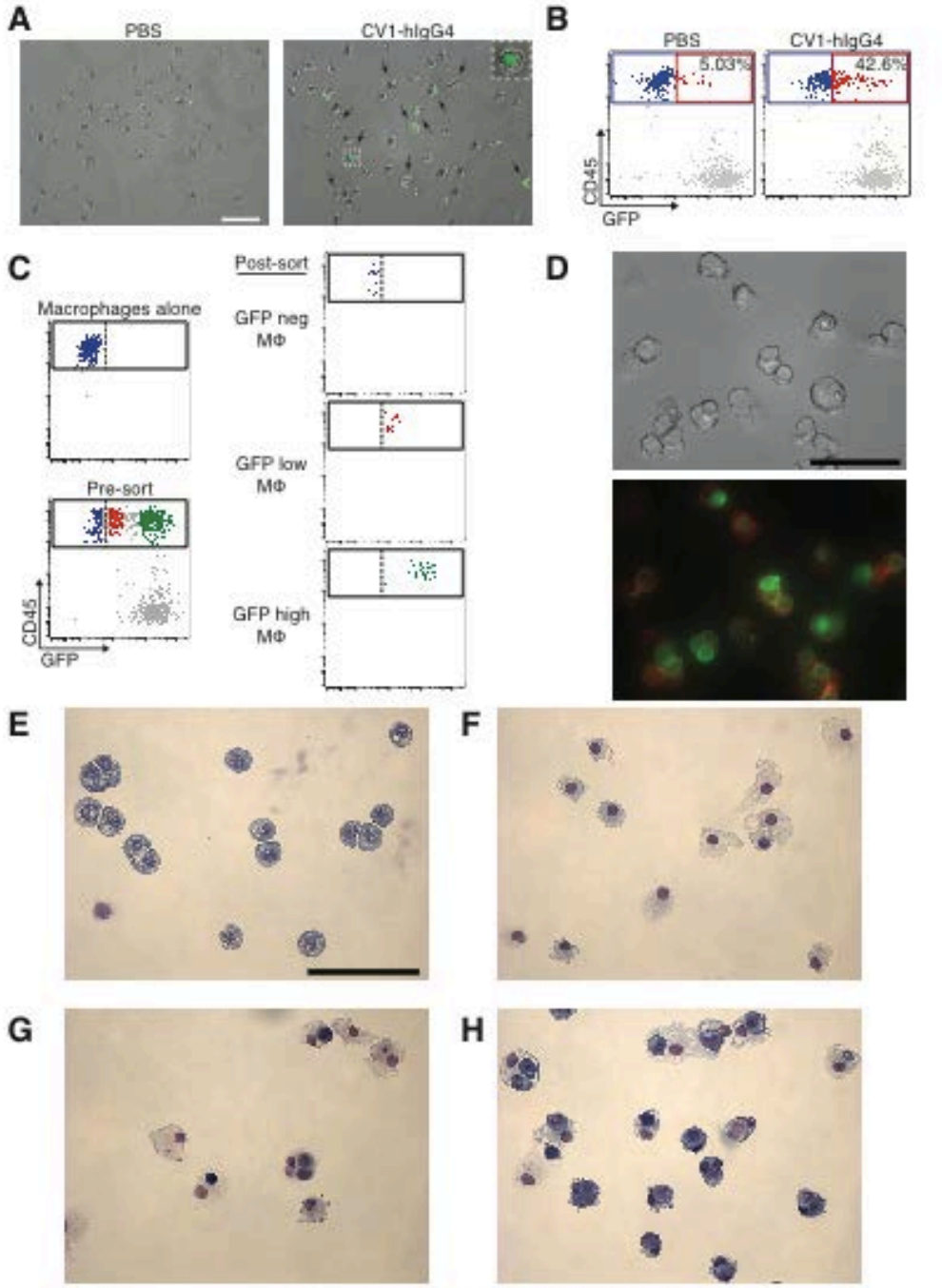


Mark Chao, Sidd Jaiswal, Rachel Weissman-Tsukamoto, and IW

Mark Chao


Basal state
Macrophage
Cancer cell
SIRPα monomer/Fab
CD47 blockade
Monoclonal antibody
alone
SIRPα/antibody
synergy
SIRPα
CD47
High-affinity SIRPα
Fc receptor
Tumor antigen
Anti-tumor antibody
unstimulated
sensitized
PHAGOCYTOSIS


A
Tumor volume (cm³)
0.8
0.6
0.4
0.2
0.0
*
Ritux +PBS
Ritux +CV1
B
Tumor weight (g)
2.5
2.0
1.5
1.0
0.5
0.0
**
Ritux +PBS
Ritux +CV1
C
Macrophage infiltration score
5
4
3
2
1
0
**
Ritux +PBS
Ritux +CV1
D
Rituximab + PBS
Rituximab + CV1 monomer
E
Phagocytosis (%)
40
30
20
10
0
****
****
****
*
*
PBS
CV1 mono
Ritux
Ritux +CV1 mono



Supplementary Figure 6

Supplemental Figure 6

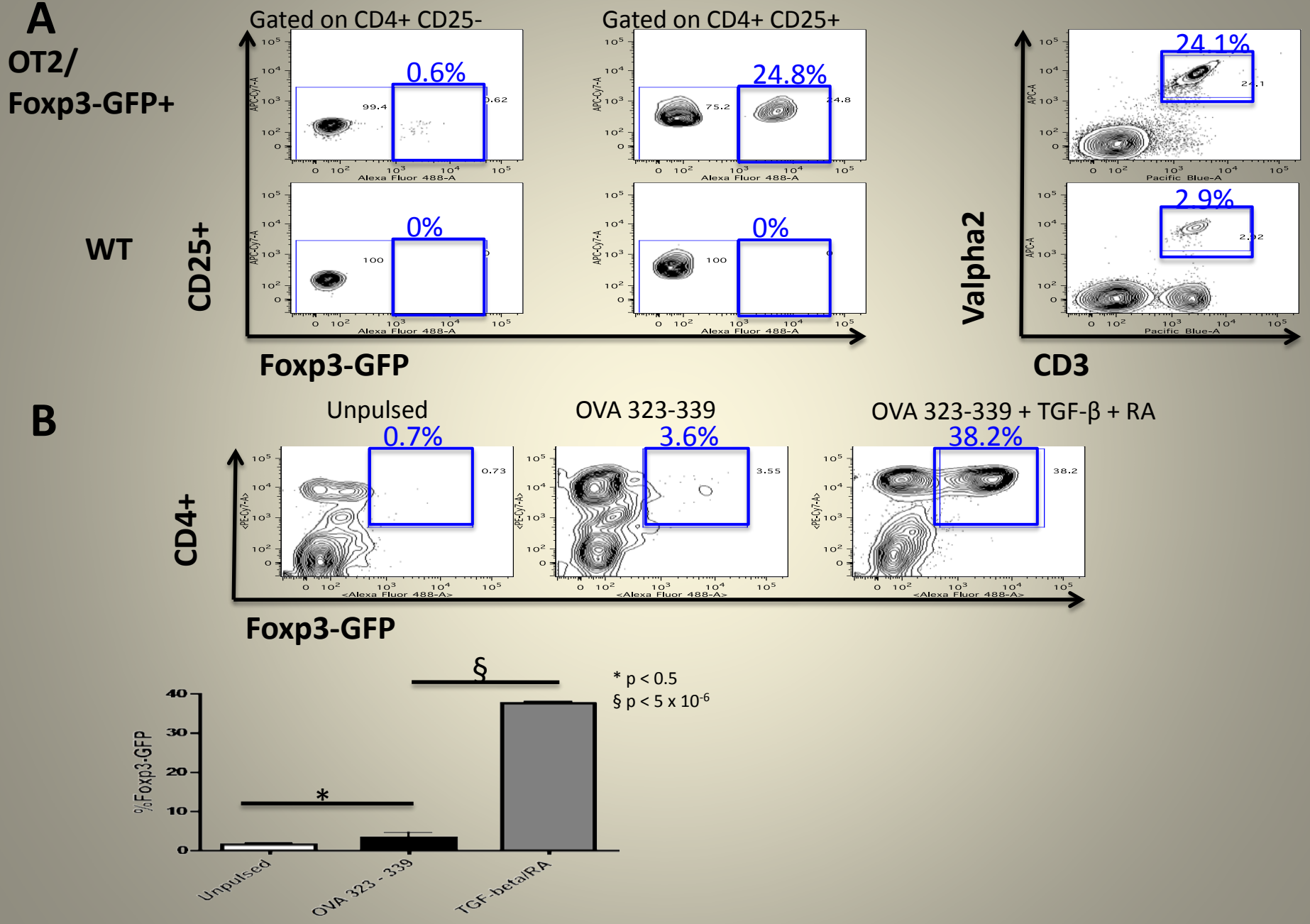
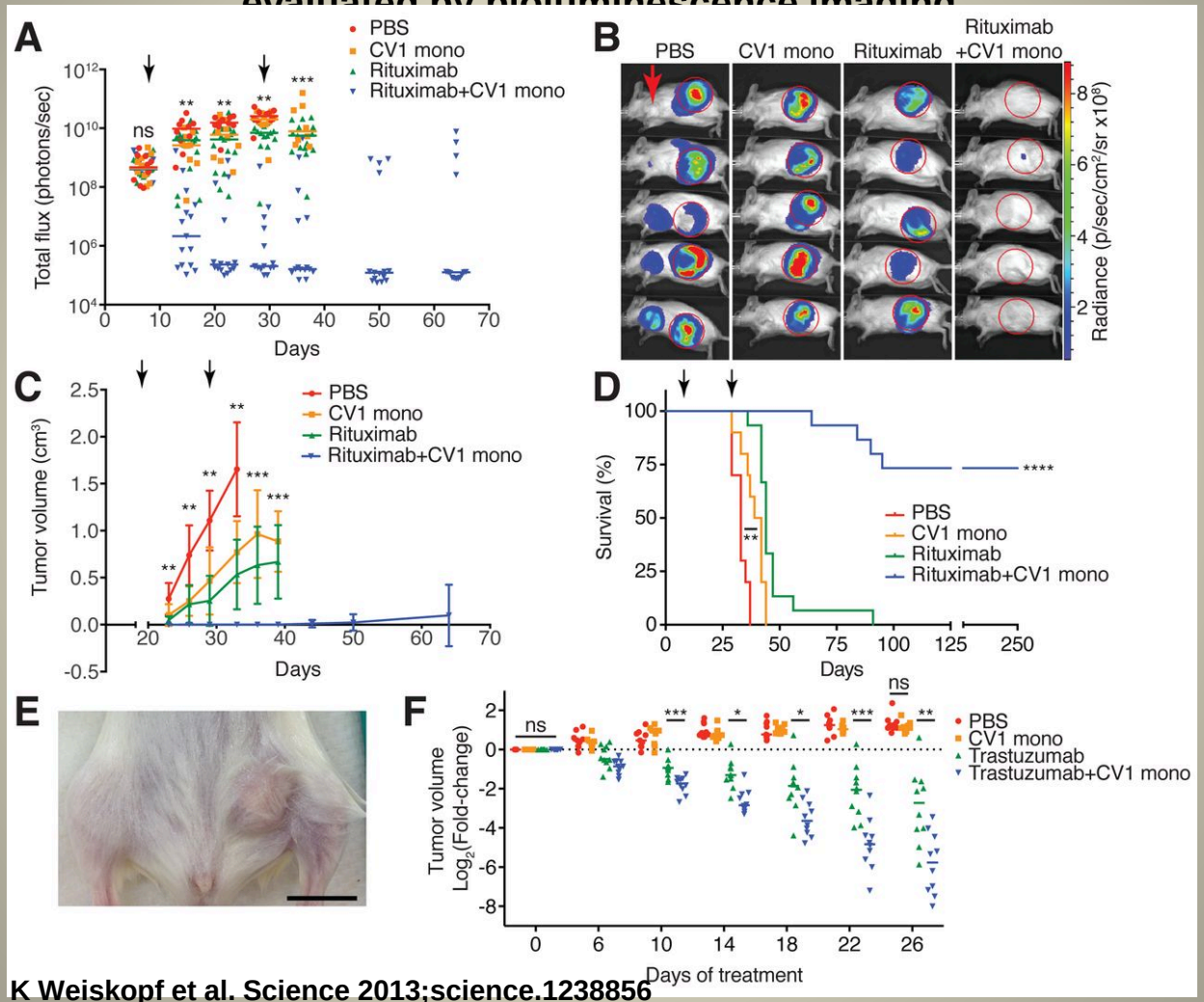
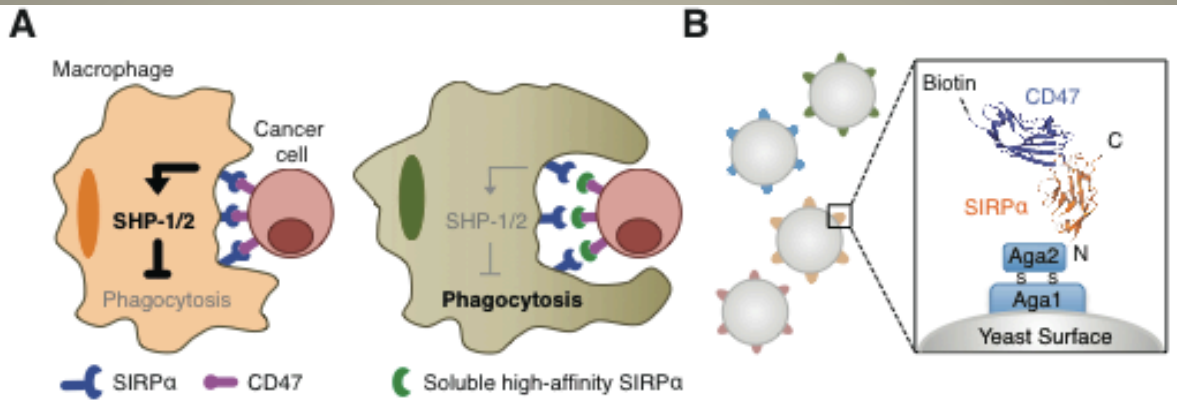


Fig. 4 High-affinity SIRPα monomers enhance the efficacy of therapeutic antibodies in vivo.(A) Growth of GFP-luciferase+ Raji lymphoma tumors upon daily treatment with PBS, 200 μg CV1 monomer, 200 μg rituximab, or rituximab plus CV1 monomer (200 μg each), as evaluated by bioluminescence imaging



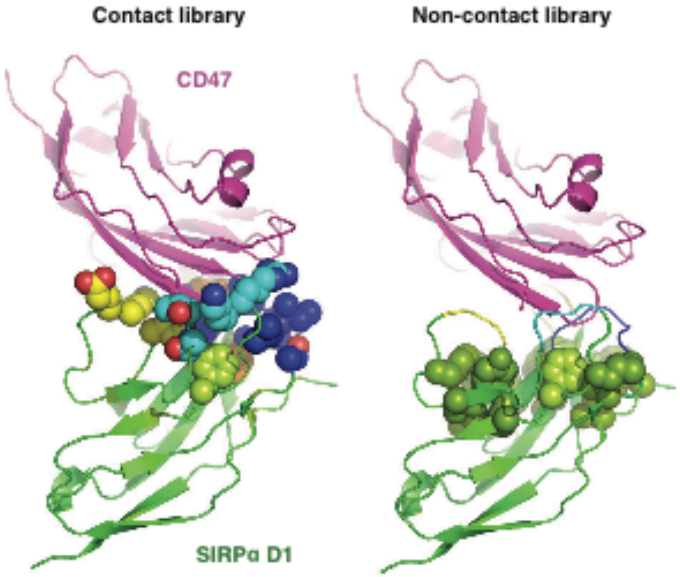
K Weiskopf et al. Science 2013;science.1238856

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C

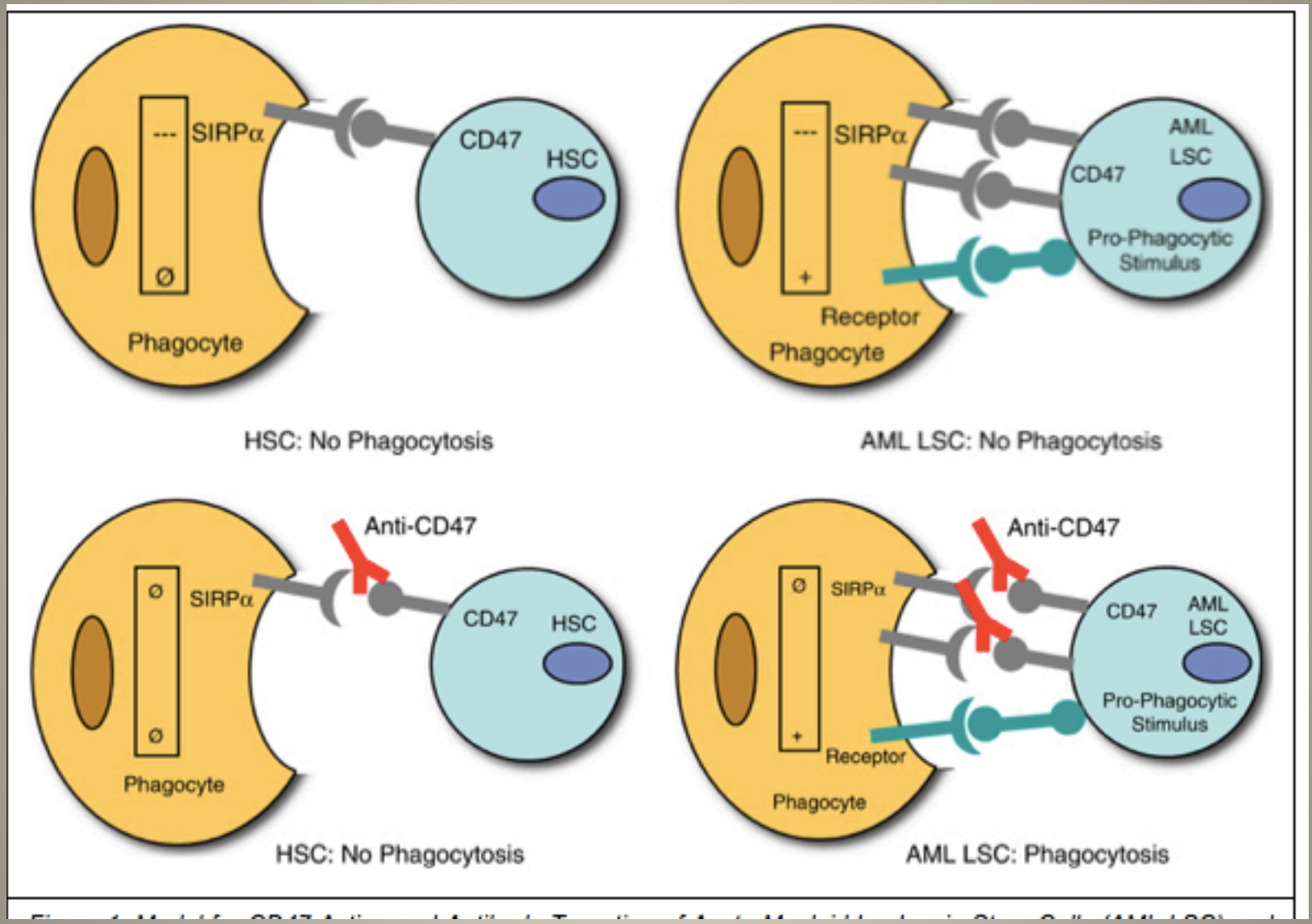
a.a.	Diversity
S29	S,T,G,A
L30	F,I,L,V
I31	F,I,L,V
P32	P,L,H,R
V33	F,I,L,V
G34	S,T,G,A
P35	P,L,H,R
Q52	Q,D,E,H
K53	K,R
E54	Q,D,E,H
S66	S,T,G,A
T67	S,T,G,A
K68	K,R
R69	K,R
F74	F,I,L,V
K93	K,R,M,T
K96	K,R,M,T
G97	S,T,G,A
S98	S,T,G,A
D100	D,E,N,K

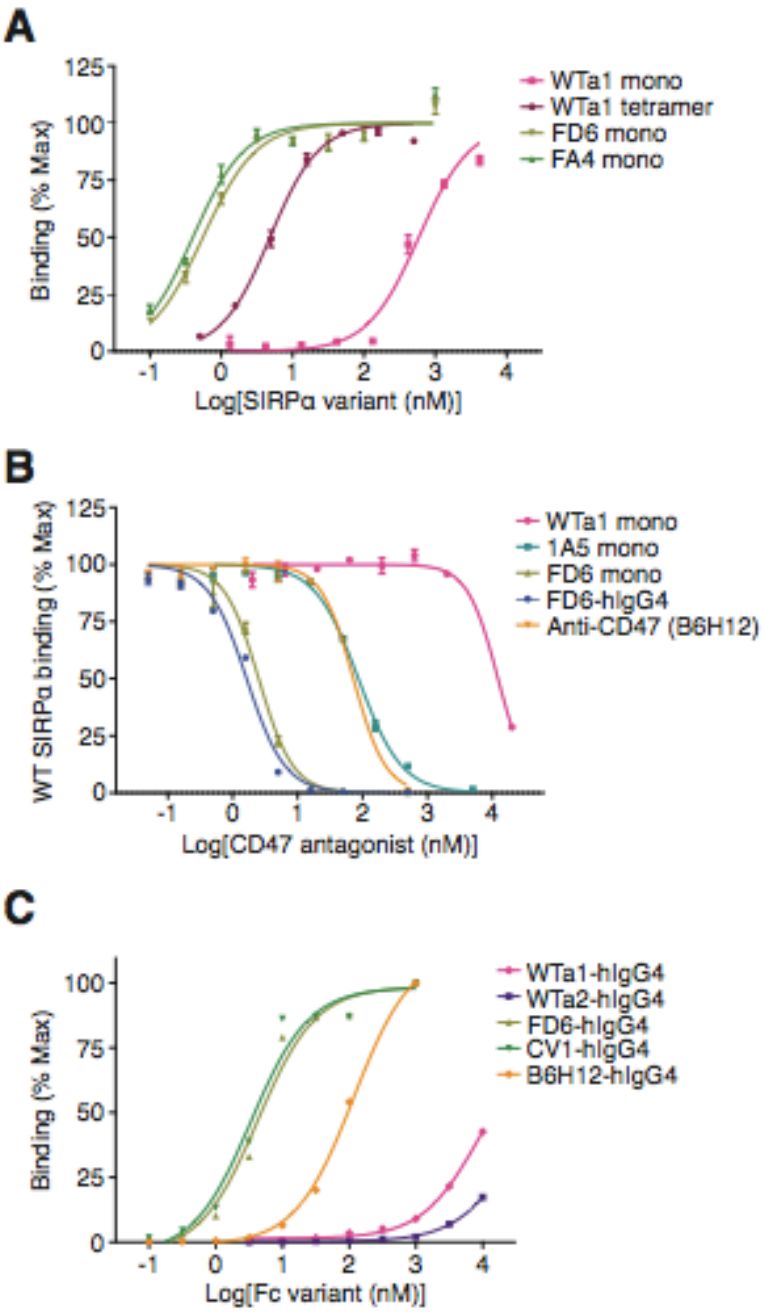


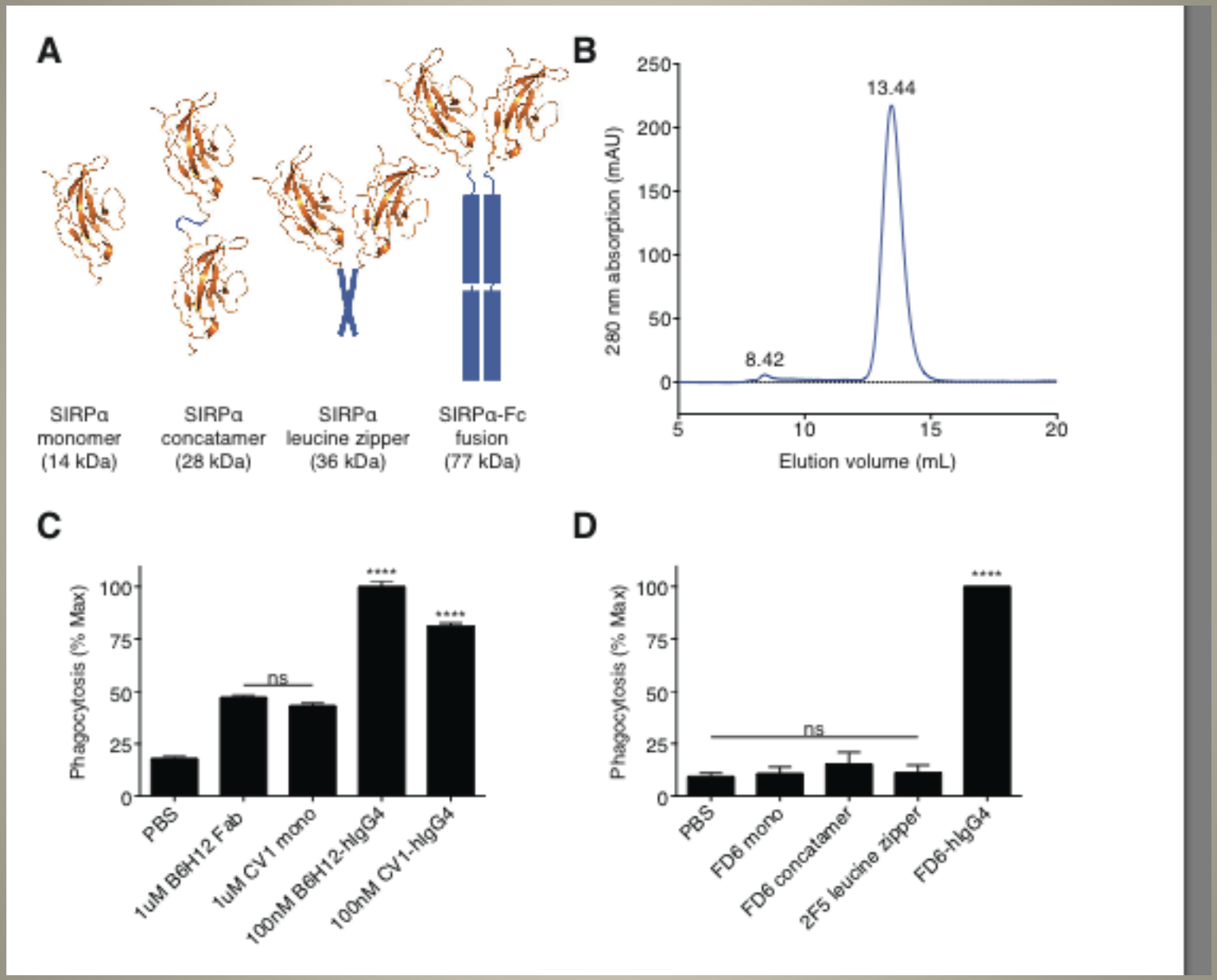
a.a.	Diversity
L4	F,I,L,V
V6	F,I,L,V
V27	F,I,L,V
I36	F,I,L,V
F39	F,I,L,V
L48	F,I,L,V
I49	F,I,L,V
Y50	F,I,L,V
F57	F,I,L,V
V60	F,I,L,V
M72	F,I,L,V
F74	F,I,L,V
I76	F,I,L,V
V92	F,I,L,V
F94	F,I,L,V
F103	F,I,L,V

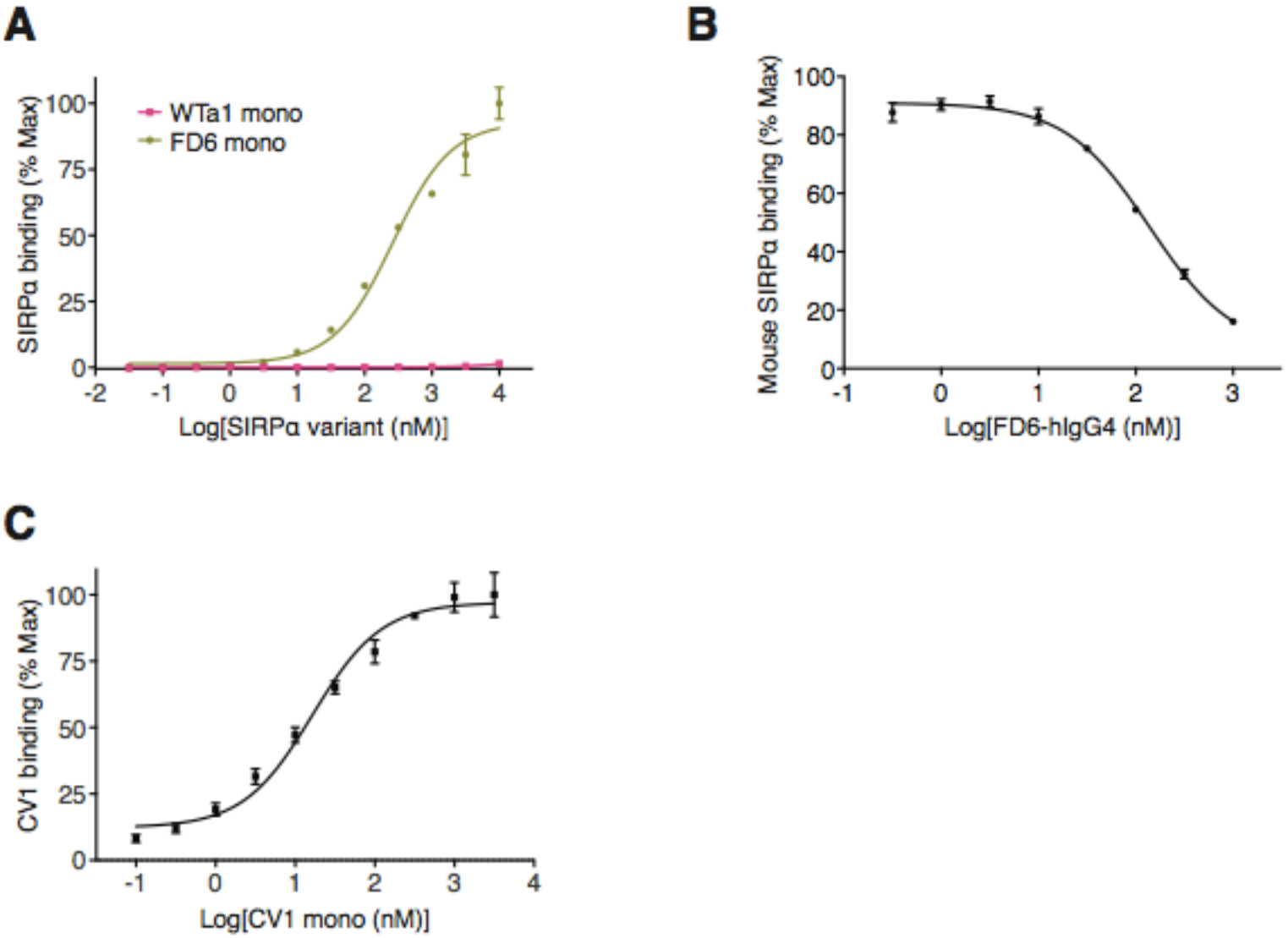
D

	4	6	27	31	*47*	53	54	*56*	66	68	92	94	103
WT SIRPα allele 1	L	V	V	I	*E*	K	E	*H*	S	K	V	F	F
A5	V		L		*V*	R	Q		G	R	I		
D1		L	I	T			D		G	R		L	
D4			I			R			T	R			V
E9				F		R				R			V
F6	V		I			R			T	R			V
H5	V		L			R	Q			R	I		
H10		I				R		*P*	A				I


SIRPα
CD47
HSC
Phagocyte
HSC: No Phagocytosis
SIRPα
AML
LSC
CD47
Pro-Phagocytic
Stimulus
Receptor
Phagocyte
AML LSC: No Phagocytosis
Anti-CD47
SIRPα
CD47
HSC
Phagocyte
HSC: No Phagocytosis
Anti-CD47
SIRPα
CD47
AML
LSC
Pro-Phagocytic
Stimulus
Receptor
Phagocyte
AML LSC: Phagocytosis


A
125
100
75
50
25
0
Binding (% Max)
-1
0
1
2
3
4
Log[SIRPα variant (nM)]
WTa1 mono
WTa1 tetramer
FD6 mono
FA4 mono
B
125
100
75
50
25
0
WT SIRPα binding (% Max)
-1
0
1
2
3
4
Log[CD47 antagonist (nM)]
WTa1 mono
1A5 mono
FD6 mono
FD6-hIgG4
Anti-CD47 (B6H12)
C
100
75
50
25
0
Binding (% Max)
-1
0
1
2
3
4
Log[Fc variant (nM)]
WTa1-hIgG4
WTa2-hIgG4
FD6-hIgG4
CV1-hIgG4
B6H12-hIgG4


A
SIRPα monomer (14 kDa)
SIRPα concatamer (28 kDa)
SIRPα leucine zipper (36 kDa)
SIRPα-Fc fusion (77 kDa)
B
280 nm absorption (mAU)
250
200
150
100
50
0
13.44
8.42
5
10
15
20
Elution volume (mL)
C
Phagocytosis (% Max)
100
75
50
25
0
****
****
ns
PBS
1uM B6H12 Fab
1uM CV1 mono
100nM B6H12-hIgG4
100nM CV1-hIgG4
D
Phagocytosis (% Max)
100
75
50
25
0
****
ns
PBS
FD6 mono
FD6 concatamer
2F5 leucine zipper
FD6-hIgG4


A
SIRPα binding (% Max)
100
75
50
25
0
-2
-1
0
1
2
3
4
Log[SIRPα variant (nM)]
WTa1 mono
FD6 mono
B
Mouse SIRPα binding (% Max)
100
80
60
40
20
0
-1
0
1
2
3
Log[FD6-hIgG4 (nM)]
C
CV1 binding (% Max)
100
75
50
25
0
-1
0
1
2
3
4
Log[CV1 mono (nM)]

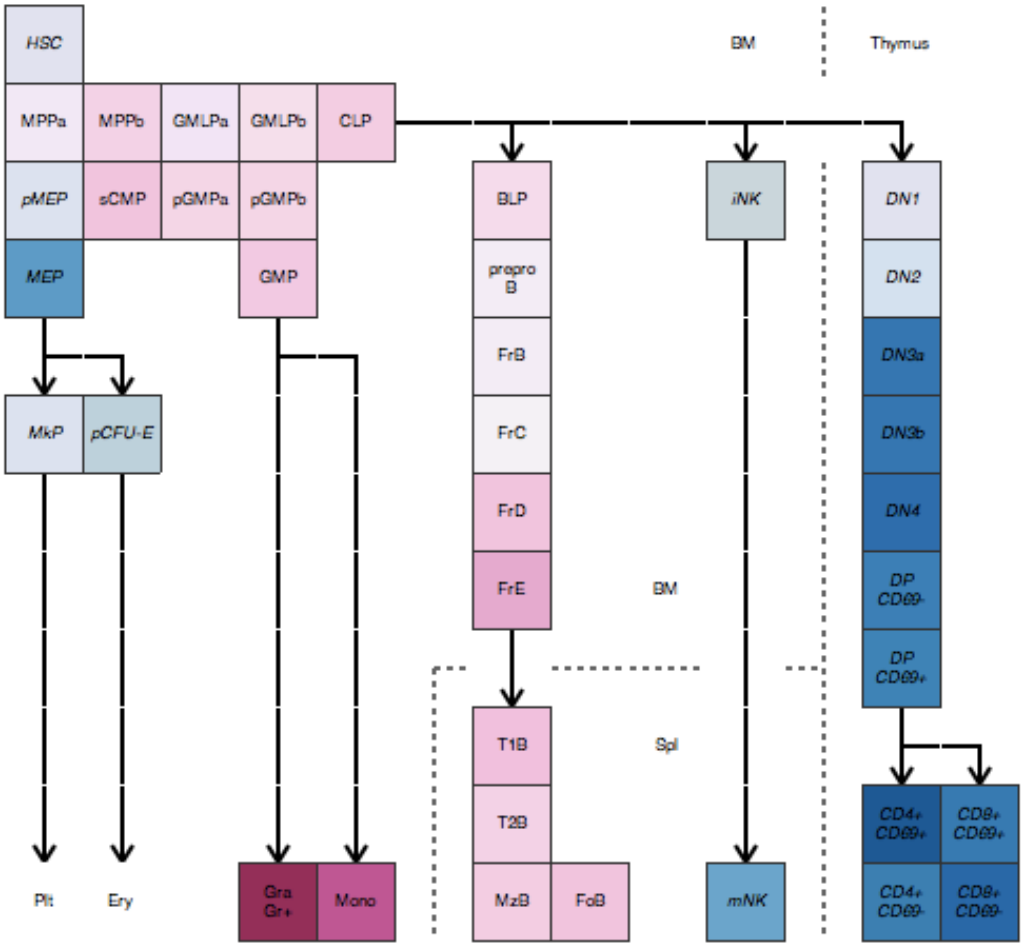
Sfpi1 SFFV proviral integration 1

Other Name: RP23-20F9.1, Dis-1, Dis1, PU.1, Sfpi-1, Spi-1, Spi1, Tcfpu1, and Tfpu.1
NCBI Entrez Gene: 20375

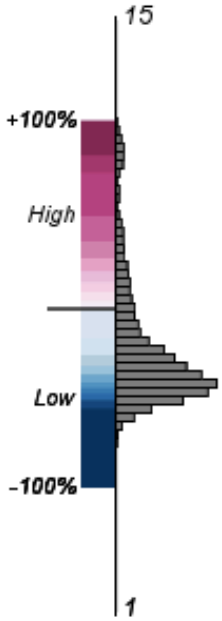
1 probeset on Affymetrix Mouse Genome 430 2.0 Array

Probeset: 1418747_at for Sfpi1

More detail | Download Figure (PDF) | Search Same Pattern



Probeset Meta Profile



Dynamic-range (99%)
6.96

Threshold
8.17

Reprinted from *Differentiation of Normal and Neoplastic Hematopoietic Cells*
© 1978 Cold Spring Harbor Laboratory

Fetal Hematopoietic Origins of the Adult Hematolymphoid System

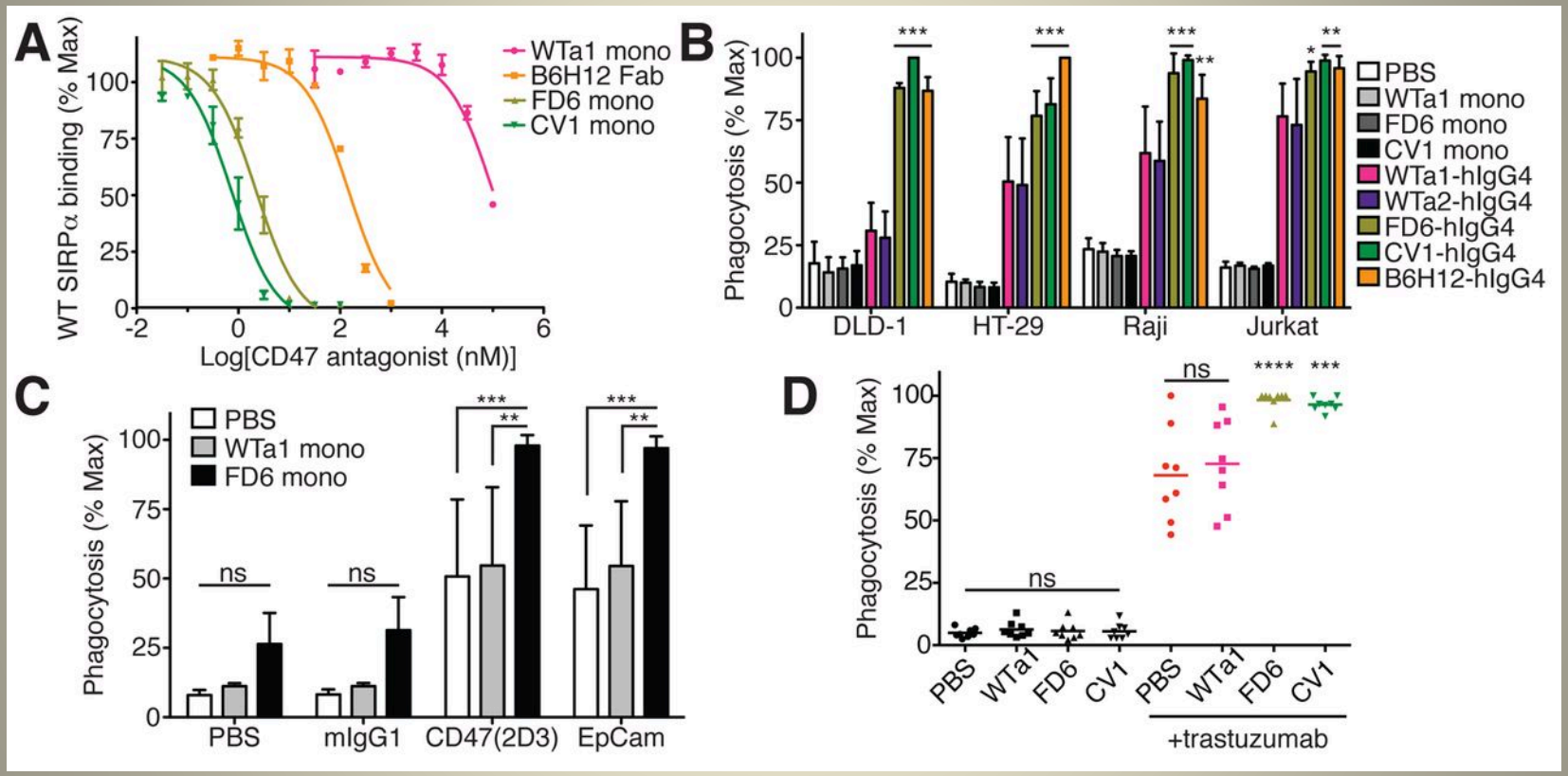
I. Weissman

Laboratory of Experimental Oncology, Department of Pathology
Stanford University Medical Center, Stanford, California 94305
and Medical Research Council Cellular Immunology Unit
Oxford OX1 3RE, United Kingdom

V. Papaioannou and R. Gardner

Department of Zoology
Medical Research Council Cellular Immunology Unit
Oxford OX1 3PS, United Kingdom

Fig. 2 High-affinity SIRPα variants lower the threshold for macrophage phagocytosis.(A) Dose-response curves of CD47 antagonism on Raji lymphoma cells with wild-type SIRPα allele 1 (WTa1), Fab fragments produced from the blocking anti-CD47 antibody clone B6H12, or two high-affinity SIRPα variants as monomers (FD6, CV1).

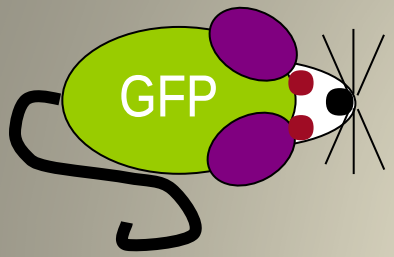


K Weiskopf et al. Science 2013;science.1238856

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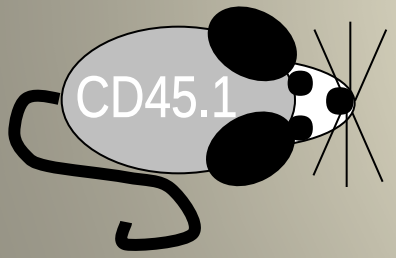
HSC vs HSC plus T cells in fighting infection

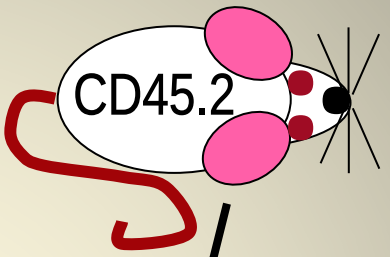
Hematopoietic stem cells [HSC]



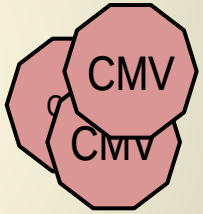
cKit^{+}
Thy1.1^{low}
Lin^{neg}
Sca-1^{high}

+





Day 0:
Lethal XRT / HCT



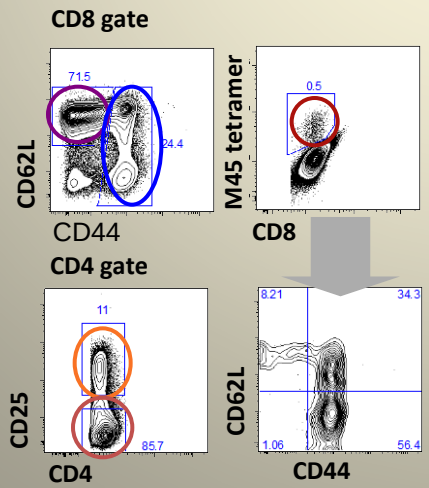
MCMV infection:

2 wks: early reactivation

8 wks: new infection



2 wks post-infection:
harvest of spleen



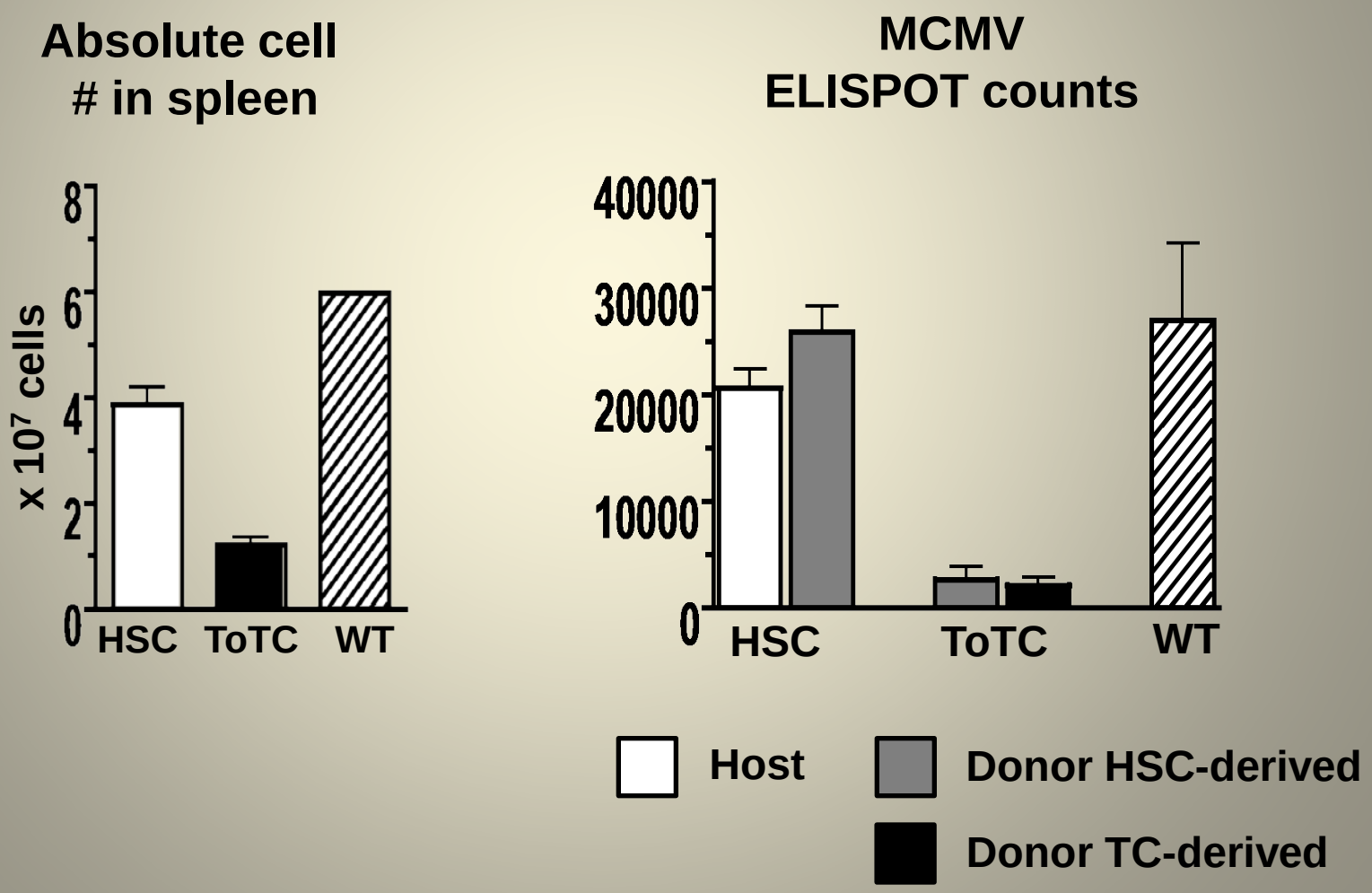
T lymphocytes

Total T cells (ToTC)
T cell subsets:
naïve CD8
memory CD8
conventional CD4
TREG

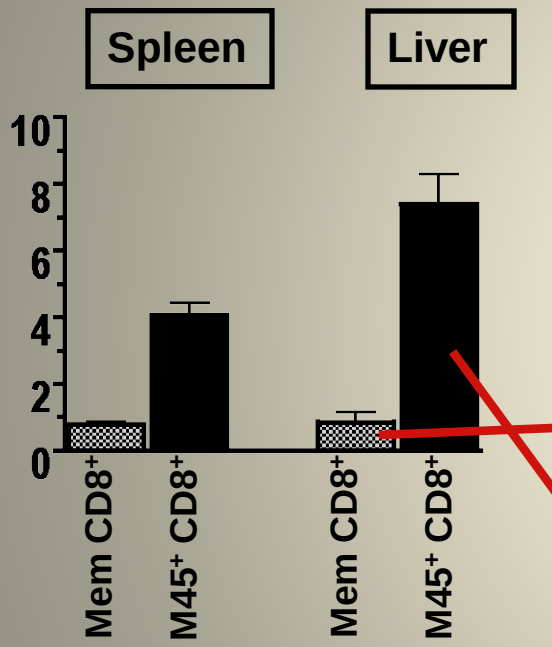
Immune response against MCMV:

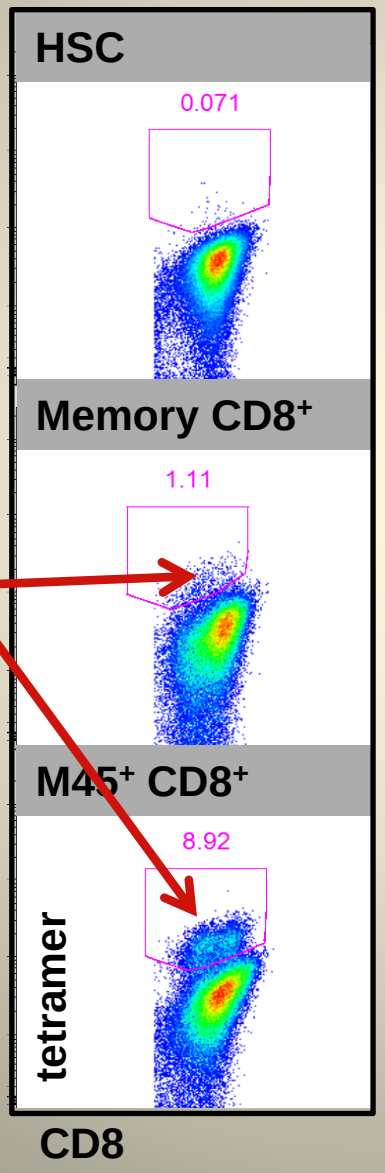
- ELISPOT
- Tetramers (M45)

Can pure HSC grafts regenerate the immune system as well as grafts that contain mature immune cells?

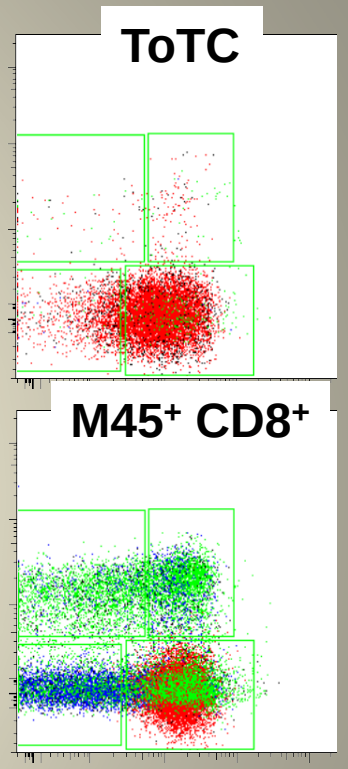


HSC transplants permit balanced T cell regeneration and the expansion of virus-specific cells





Host-derived
Do-HSC derived
Do-TC derived



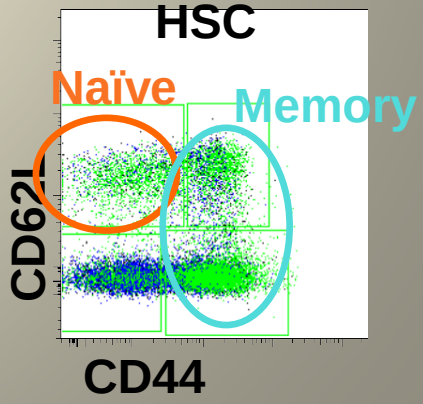


Figure 1

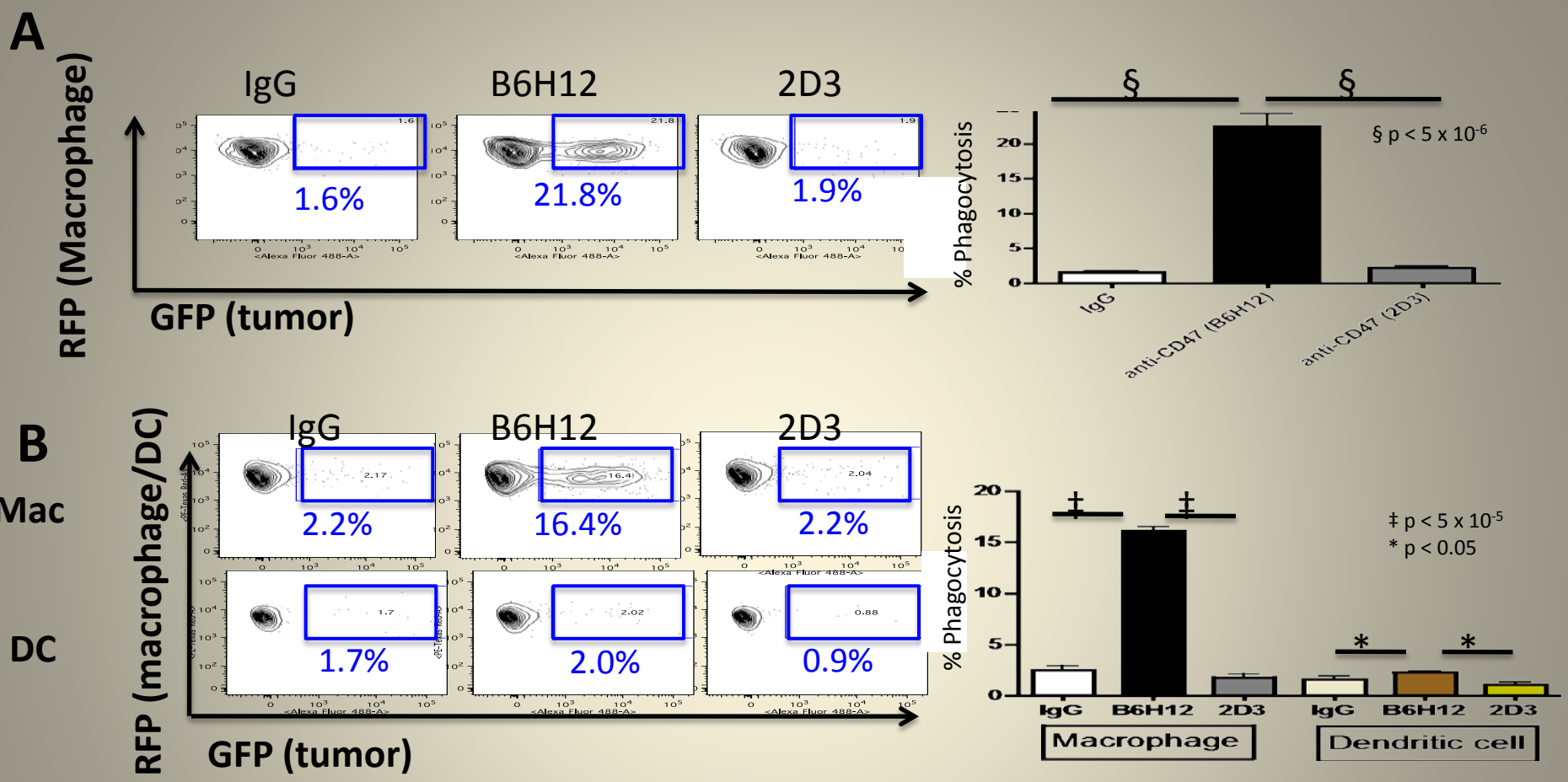

A
IgG
B6H12
2D3
1.6%
21.8%
1.9%
RFP (Macrophage)
GFP (tumor)
% Phagocytosis
§
§
§ p < 5 x 10-6
IgG
anti-CD47 (B6H12)
anti-CD47 (2D3)
B
Mac
DC
IgG
B6H12
2D3
2.2%
16.4%
2.2%
1.7%
2.0%
0.9%
RFP (macrophage/DC)
GFP (tumor)
% Phagocytosis
‡ p < 5 x 10-5
* p < 0.05
IgG
B6H12
2D3
IgG
B6H12
2D3
Macrophage
Dendritic cell

Figure 2

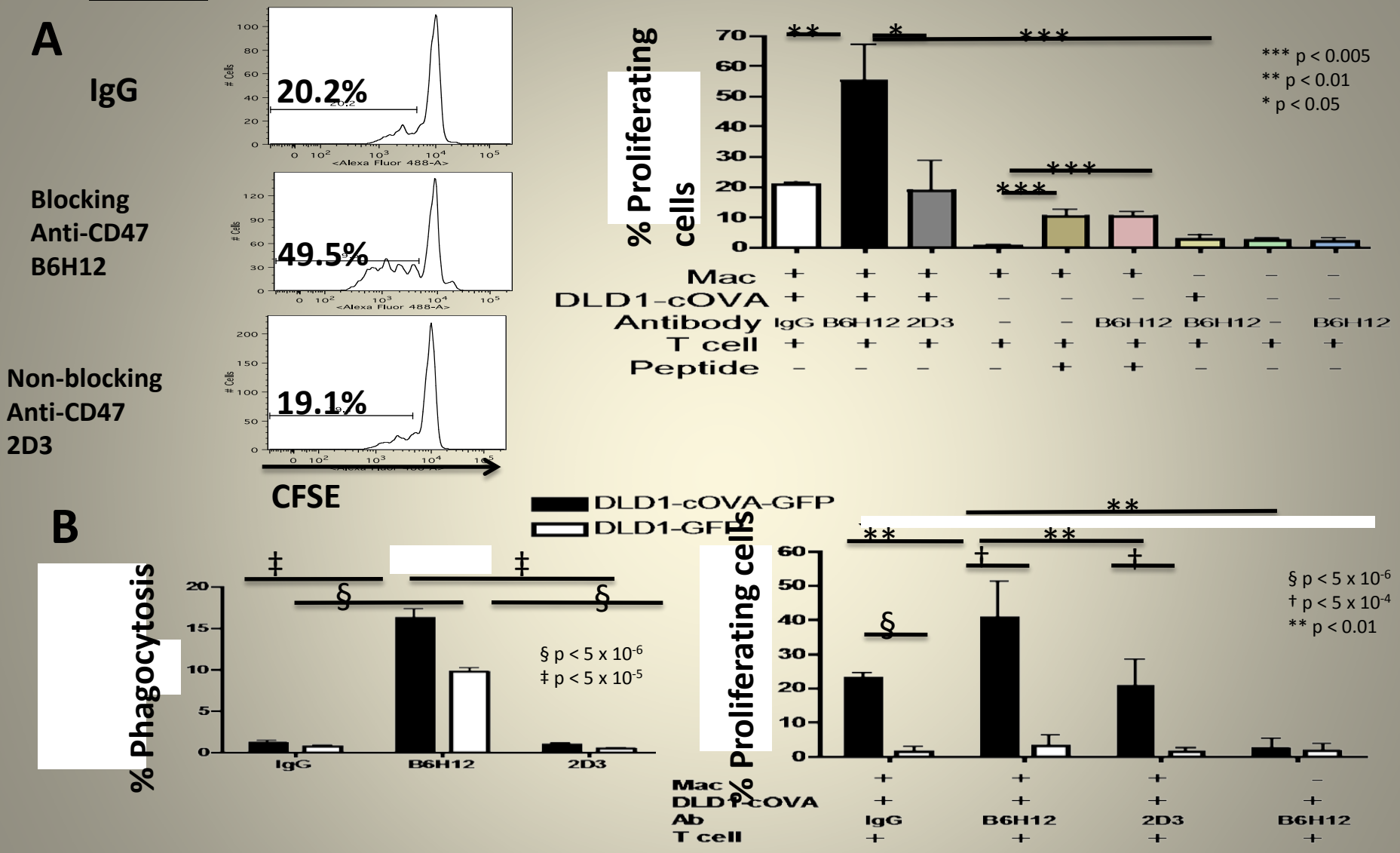

A
IgG
20.2%
Blocking
Anti-CD47
B6H12
49.5%
Non-blocking
Anti-CD47
2D3
19.1%
CFSE
Cells
<Alexa Fluor 488-A>
% Proliferating cells
*** p < 0.005
** p < 0.01
* p < 0.05
Mac
DLD1-cOVA
Antibody
IgG B6H12 2D3
T cell
Peptide
B
DLD1-cOVA-GFP
DLD1-GFP
% Phagocytosis
§ p < 5 x 10-6
‡ p < 5 x 10-5
IgG
B6H12
2D3
% Proliferating cells
§ p < 5 x 10-6
† p < 5 x 10-4
** p < 0.01
Mac
DLD1-cOVA
Ab
T cell

Figure 3

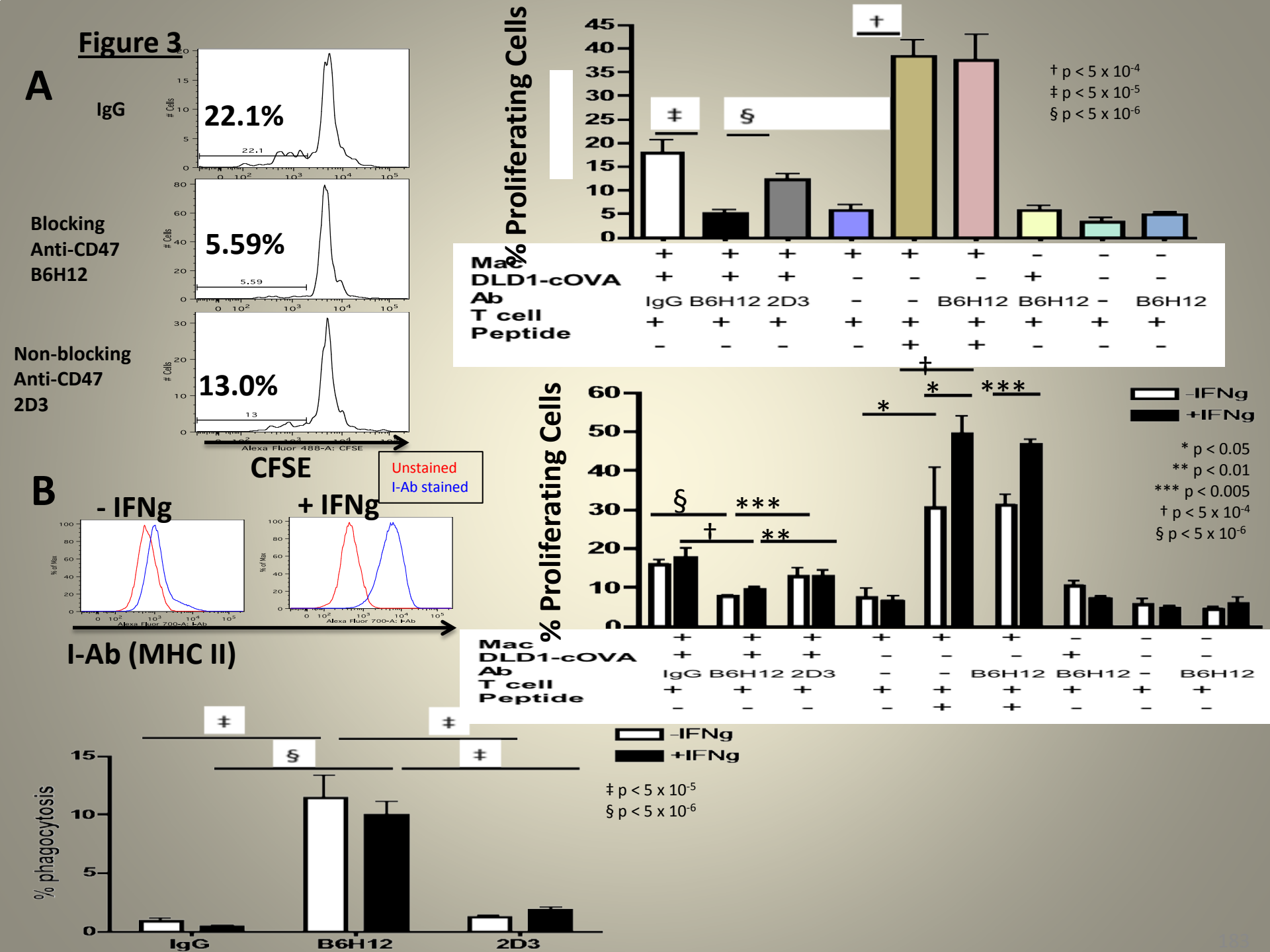
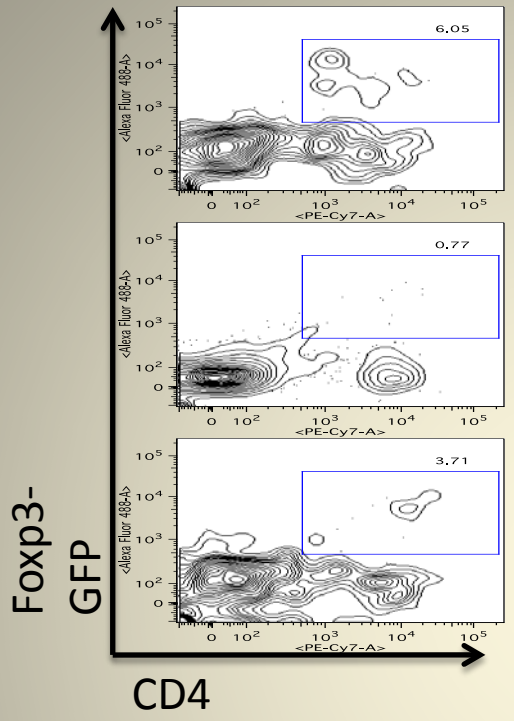

A
IgG
22.1%
Blocking
Anti-CD47
B6H12
5.59%
Non-blocking
Anti-CD47
2D3
13.0%
Cells
Alexa Fluor 488-A: CFSE
CFSE
Unstained
I-Ab stained
B
- IFNg
+ IFNg
Alexa Fluor 700-A: I-Ab
I-Ab (MHC II)
% Proliferating Cells
† p < 5 x 10-4
‡ p < 5 x 10-5
§ p < 5 x 10-6
Mac
DLD1-cOVA
Ab
T cell
Peptide
IgG B6H12 2D3
B6H12 B6H12
B6H12
-IFNg
+IFNg
* p < 0.05
** p < 0.01
*** p < 0.005
† p < 5 x 10-4
§ p < 5 x 10-6
% phagocytosis
IgG
B6H12
2D3
‡ p < 5 x 10-5
§ p < 5 x 10-6

Figure 4

IgG

Blocking
Anti-CD47
B6H12

Non-blocking
Anti-CD47
2D3



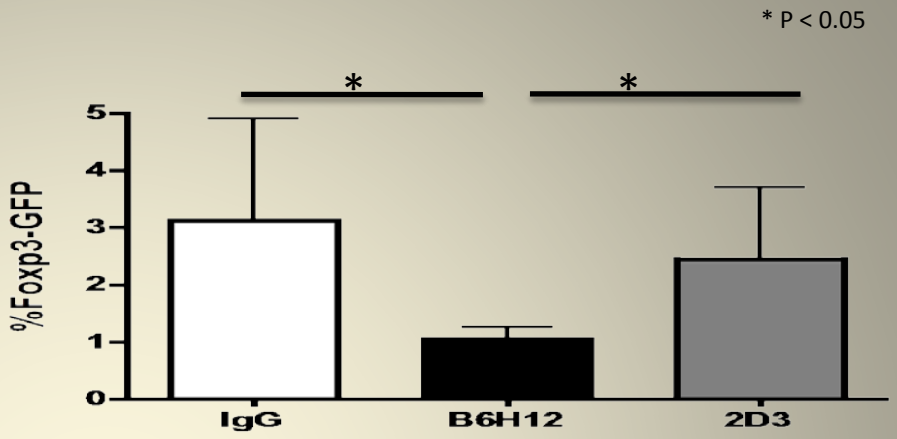


Figure 5

A

Experimental Setup

OT I mouse (CD45.2)

Purify CD8 T cells
CFSE label

Day 0

C57BL/6 (CD45.1)

Day 1: Injection footpad

Day 5: Popliteal Lymph node analysis

Mac-1 enrichment For macrophages

RFP mouse

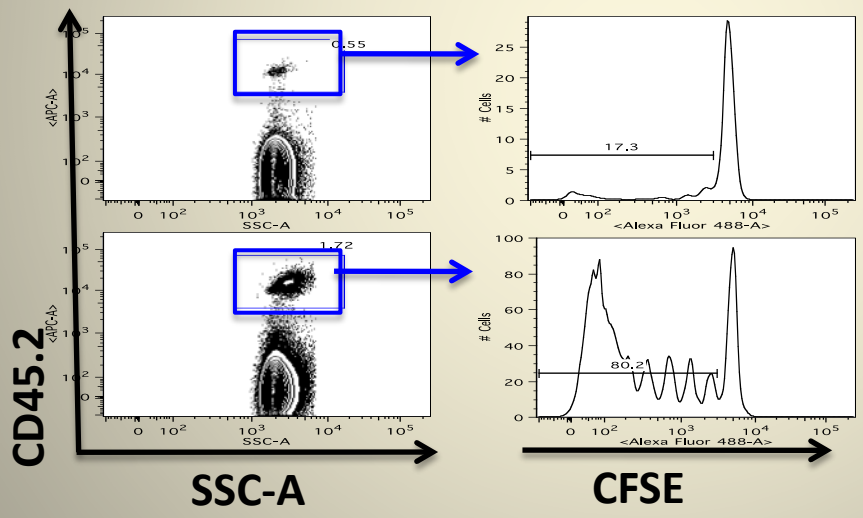
Bone-marrow derived Macrophages (7 days)

Co-culture with tumors 2 hr (IgG vs anti-CD47 B6H12)

B

IgG

Anti-CD47 B6H12



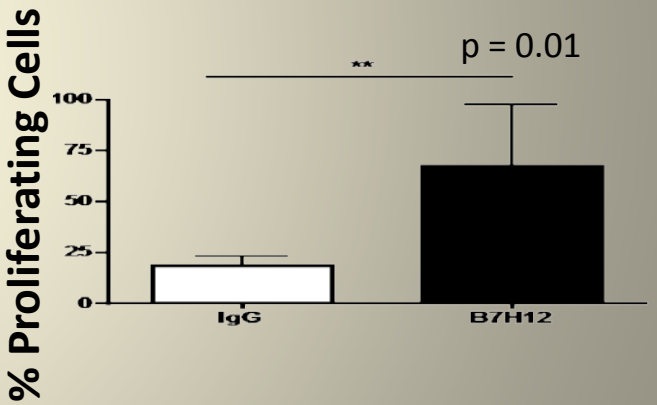
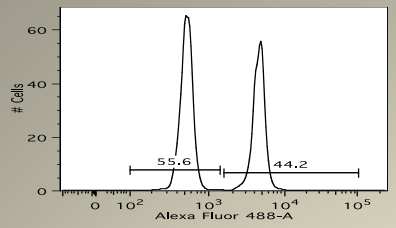


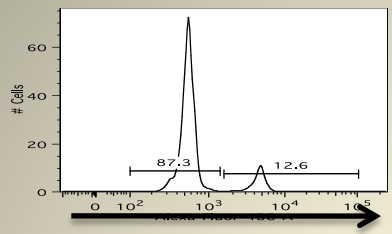
Figure 6

A

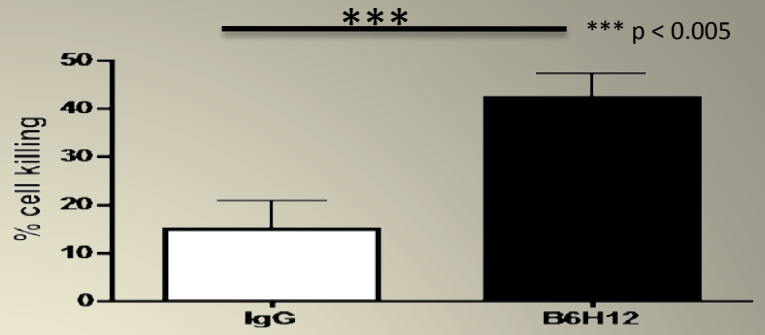
IgG



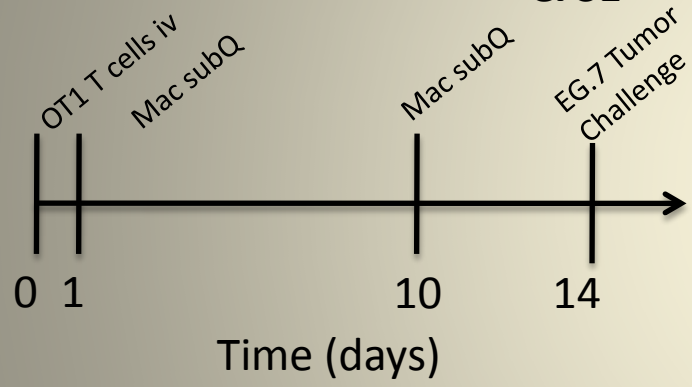
Anti-CD47 B6H12

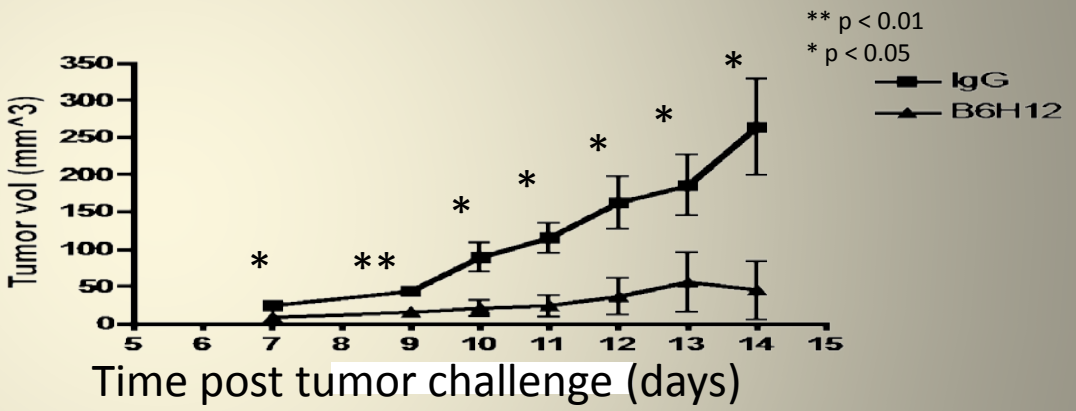


CFSE

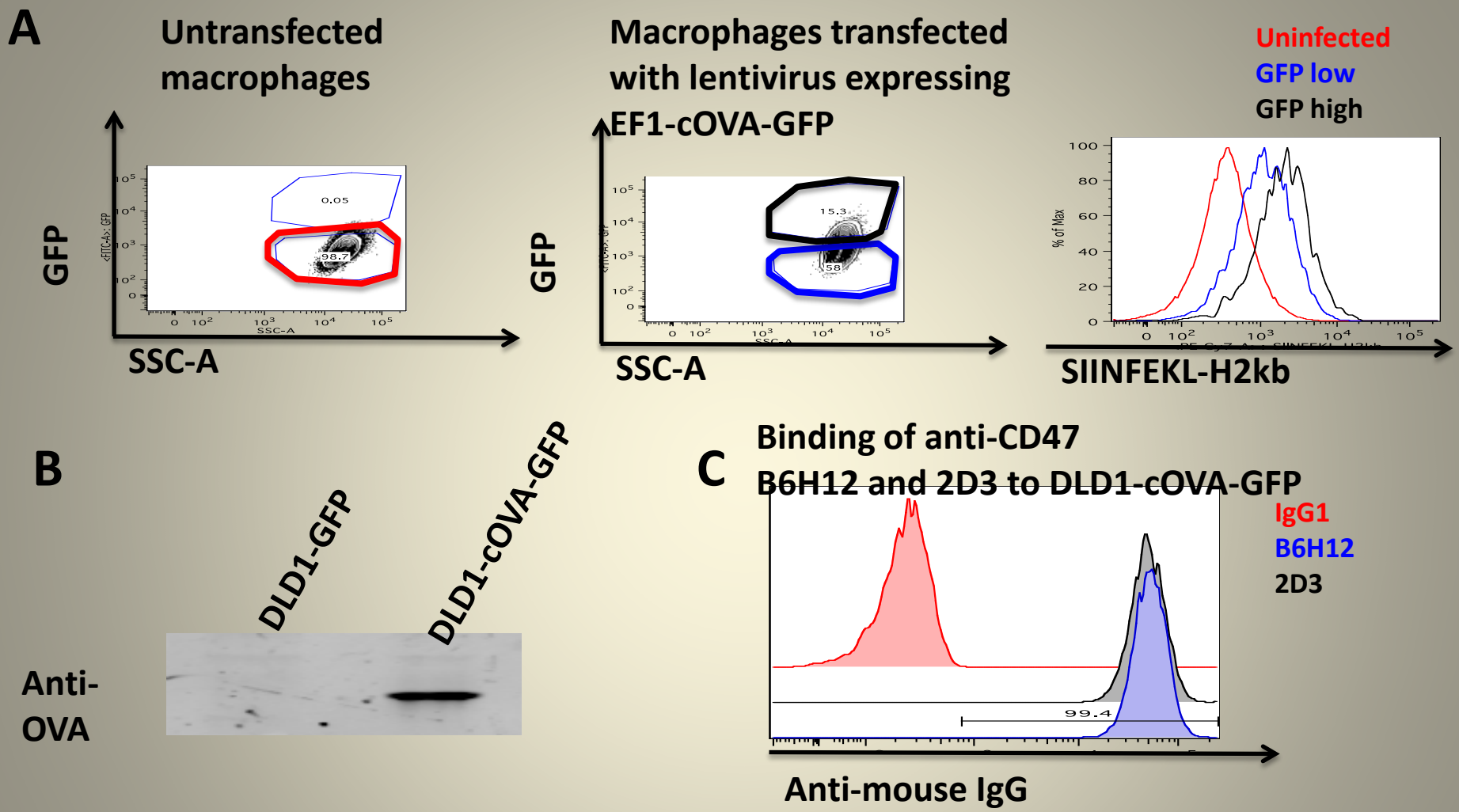


B



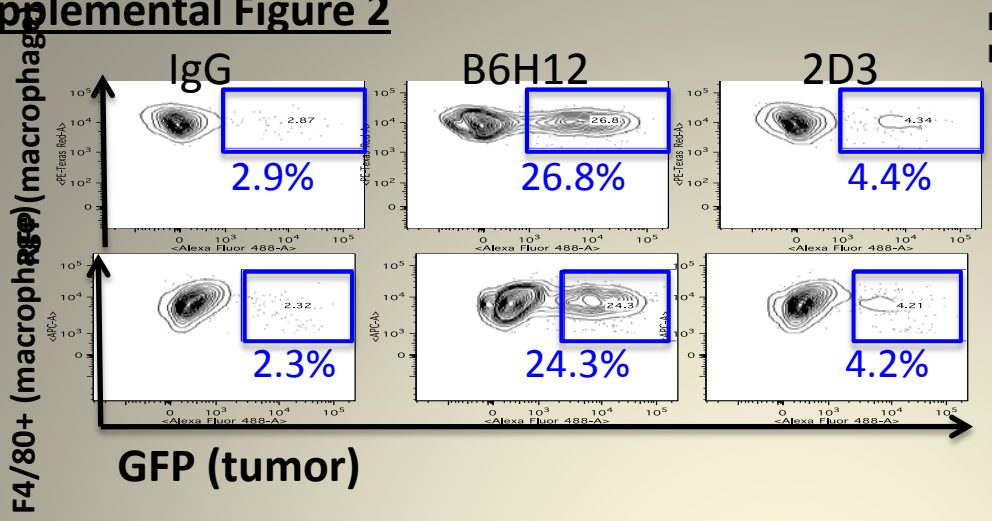


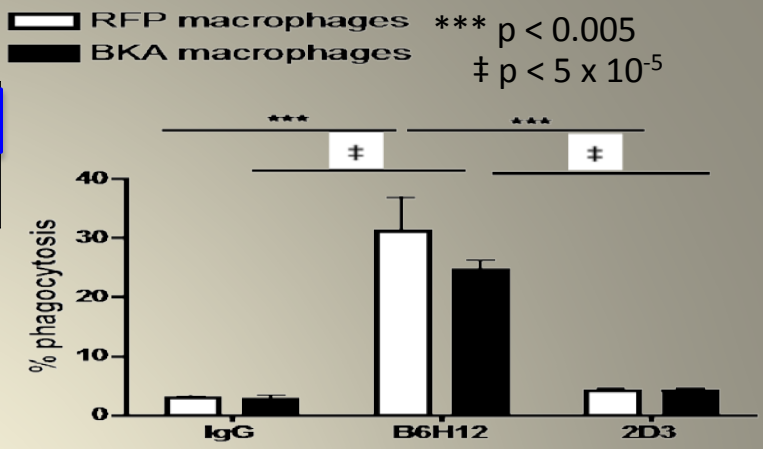
Supplemental Figure 1



Supplemental Figure 2

A

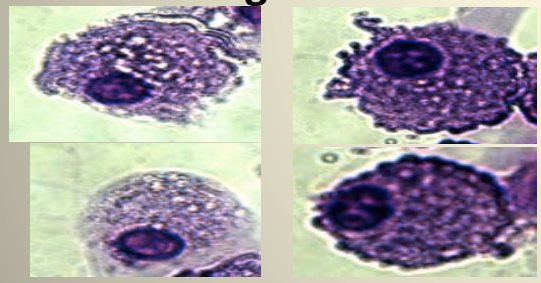




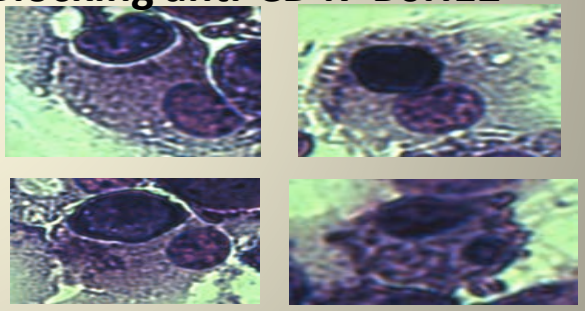
B

Macrophages, phagocytosis assay

IgG

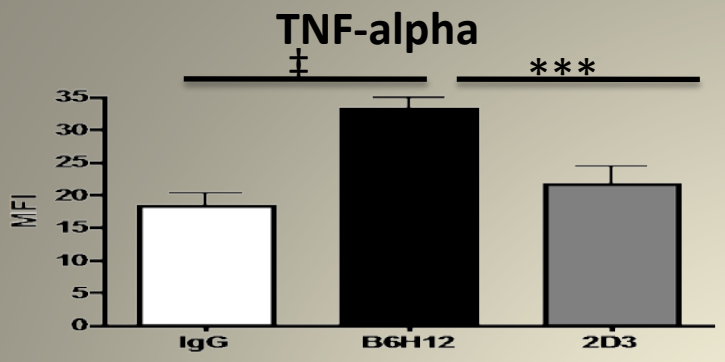


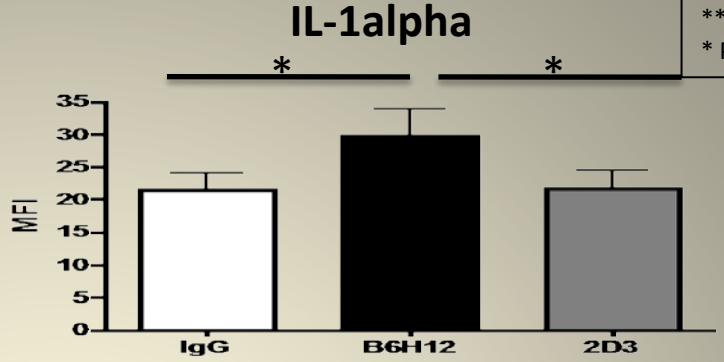
Blocking anti-CD47 B6H12

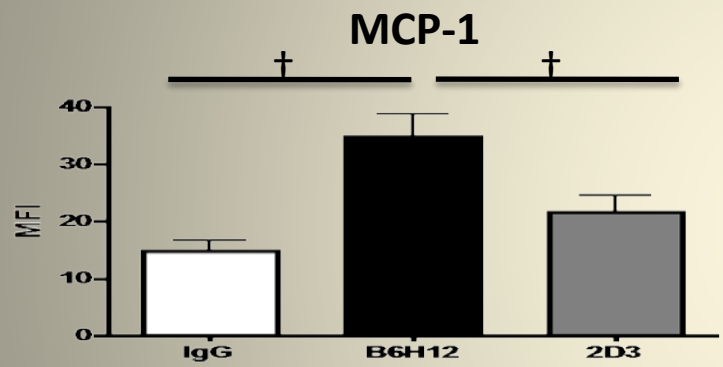


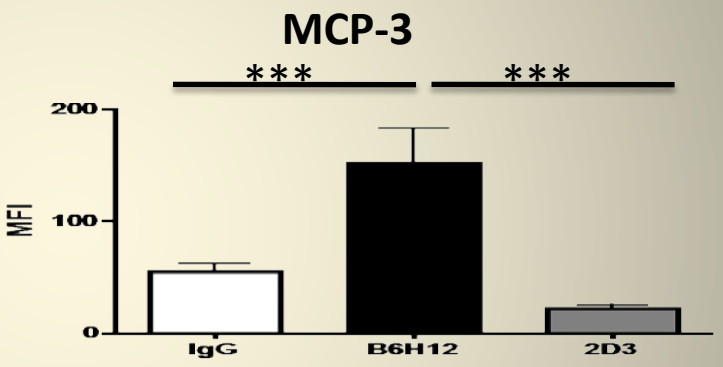
Supplemental Figure 3

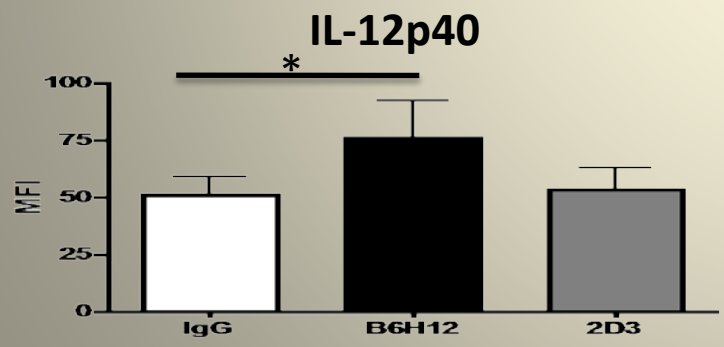
‡ $p < 5 \times 10^{-5}$
† $p < 5 \times 10^{-4}$
*** $p < 0.005$
* $P < 0.05$

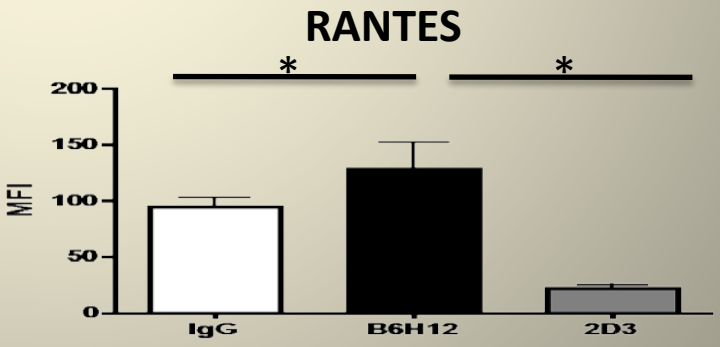




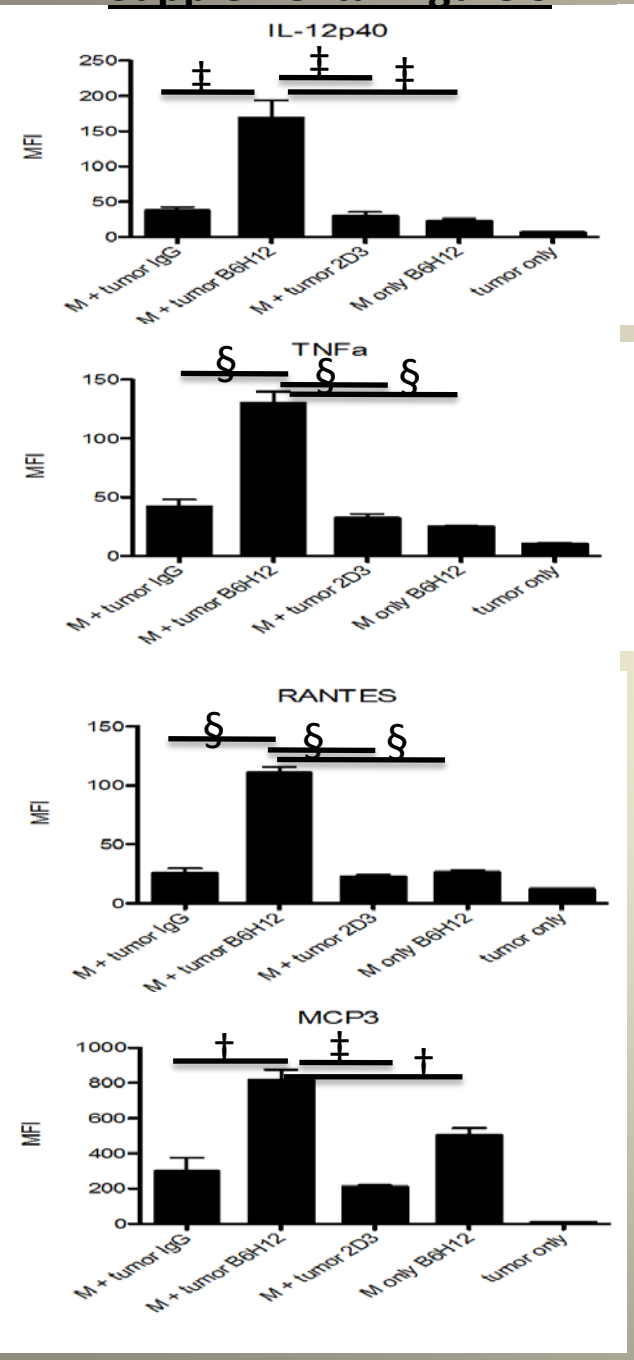


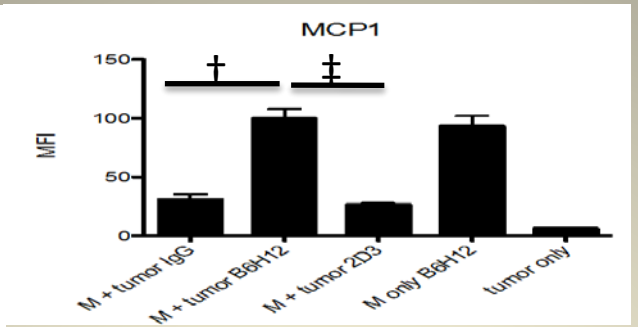


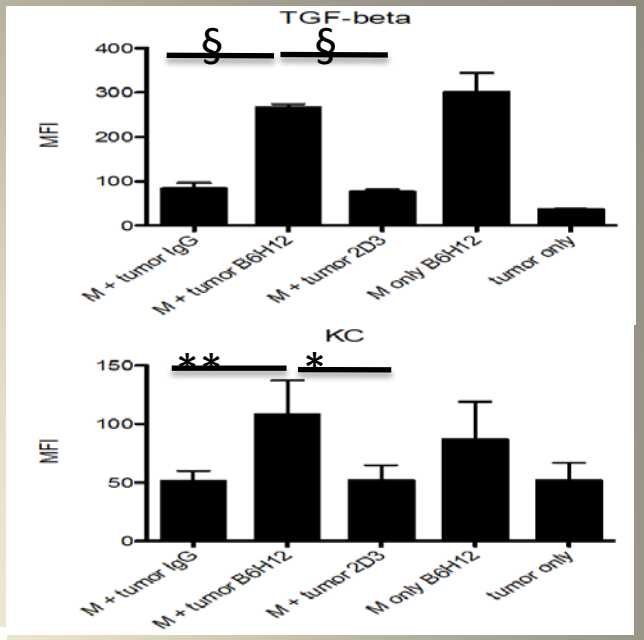


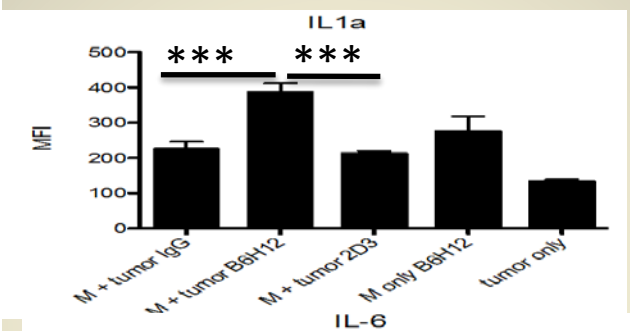


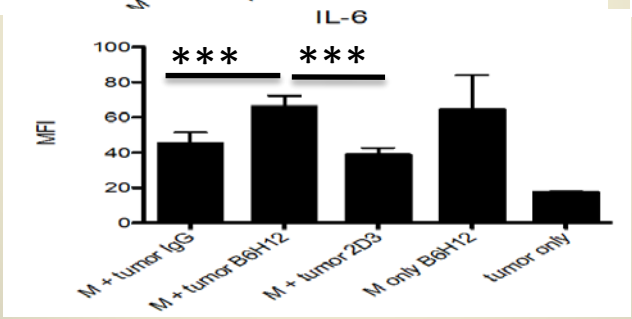
Supplemental Figure 3











§ $p < 5 \times 10^{-6}$
‡ $p < 5 \times 10^{-5}$
† $p < 5 \times 10^{-4}$
*** $p < 0.005$
** $p < 0.01$
* $P < 0.05$

Reconstitution of sublethally irradiated NSG pups transplanted with human CB mononuclear cells

16 weeks post-transplant	Number of CB samples	Median Cells Injected	Number of Mice Injected	% Mice with Human Engraftment	Average % Human Chimerism	Average % Myeloid Cells	Average % Lymphoid Cells
PB	10	10000000	10	100%	34.6%	3.4%	89.2%
BM	10	10000000	10	100%	39.8%	10.2%	88.3%