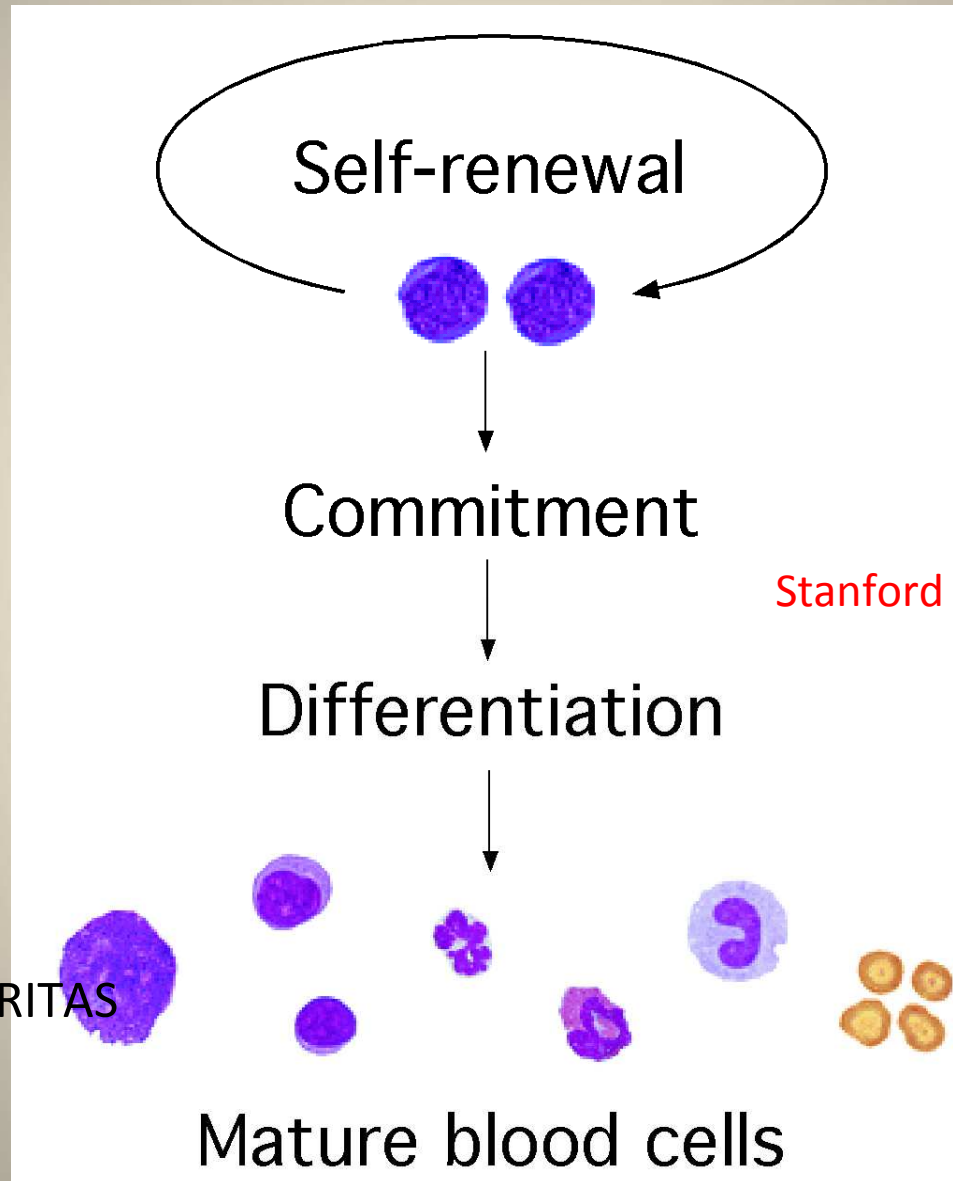


Blood-Forming Stem Cells



1 Development

2 Maintenance

3 Malignant transformation

Ludwig Center at

Stanford University

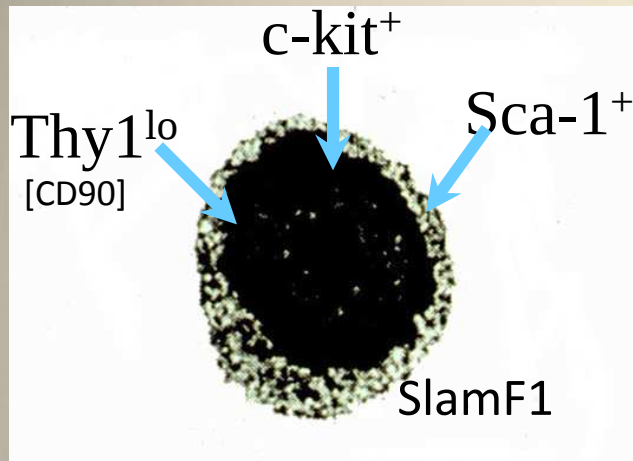
Blood-Forming Stem Cells

STANFORD

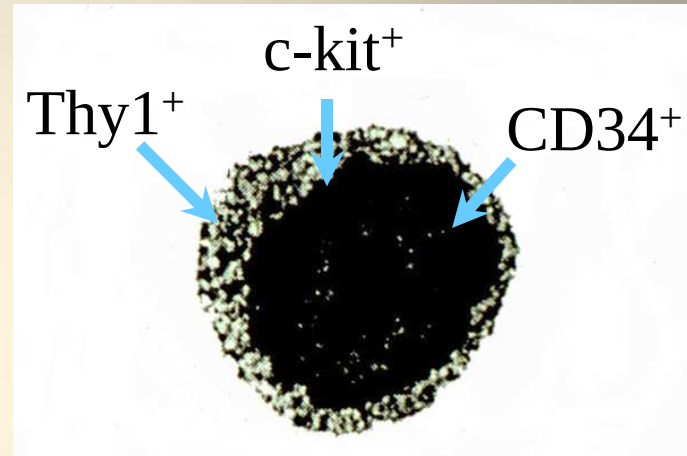
SYSTEMIX/NOVARTIS/CELLERANT

MOUSE

HUMAN



[CD90]



Stanford

Negative for: [morrison]

Negative for:

Novartis

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

CD10
CD14
CD15
CD16
CD19
CD20
CD 38

CD 3,4,8
Glycophorin A

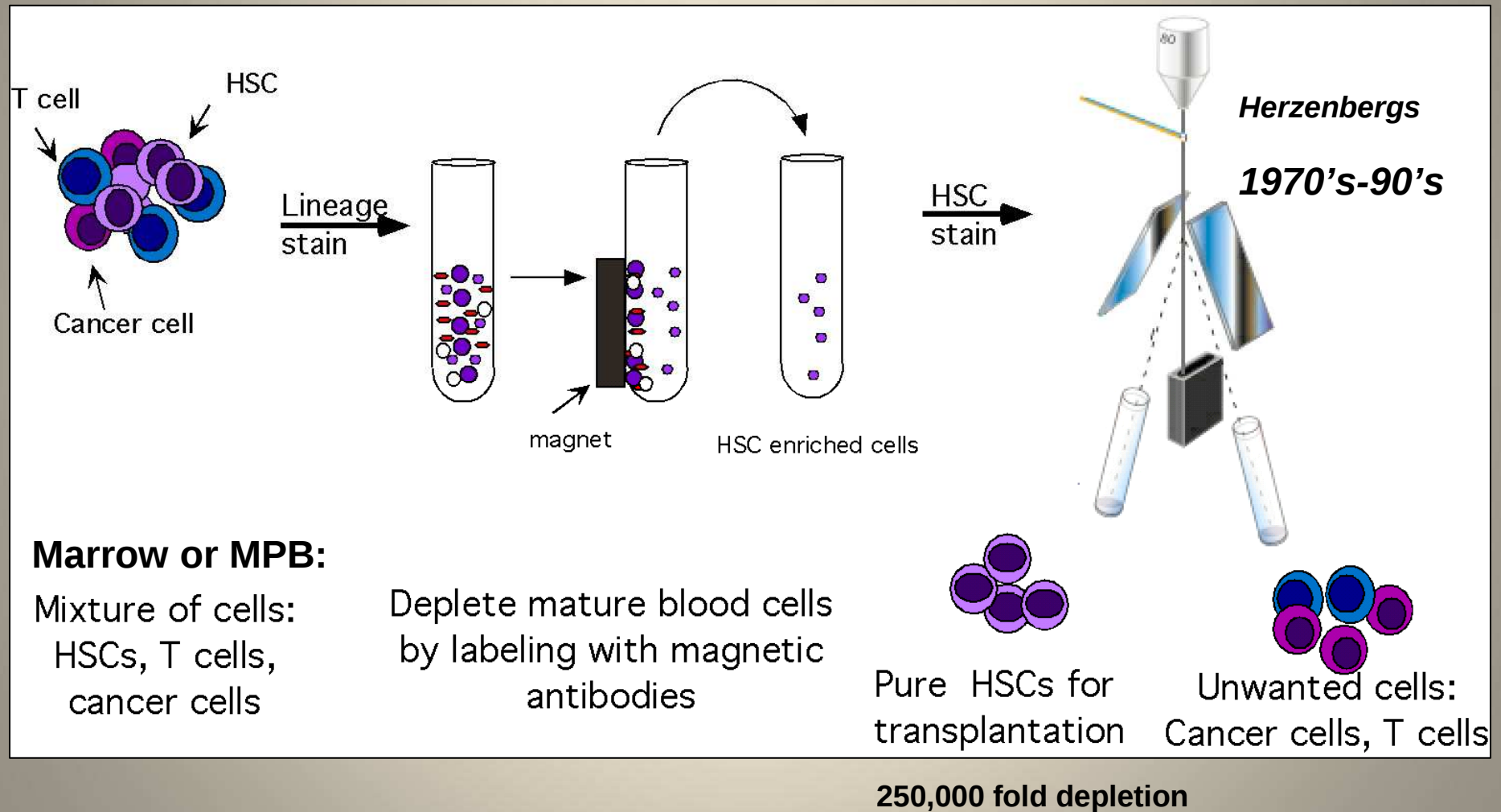
1988

Spangrude, Muller, Heimfeld, IW

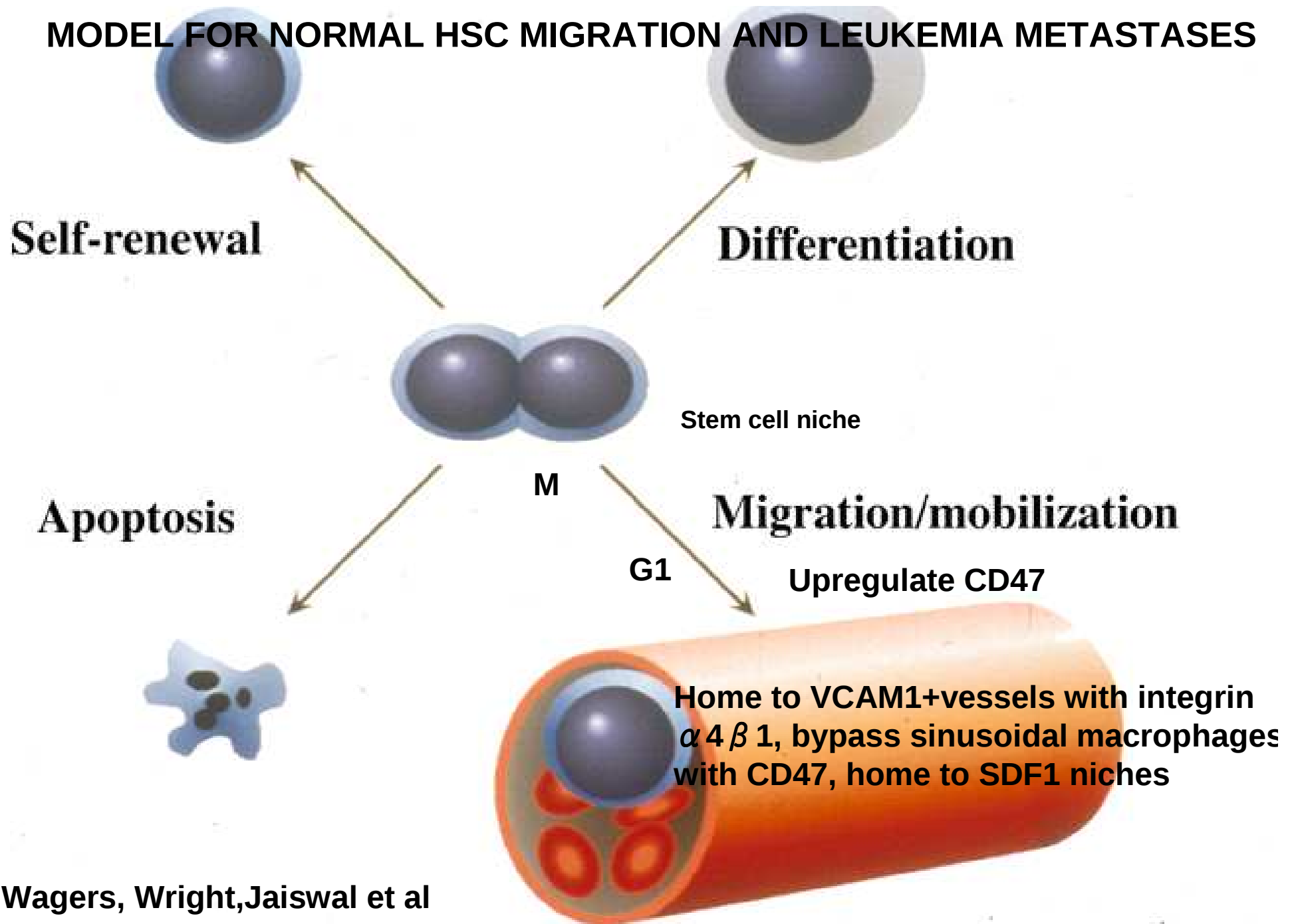
1992

Baum, Buckle, Peault, Tsukamoto, IW

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



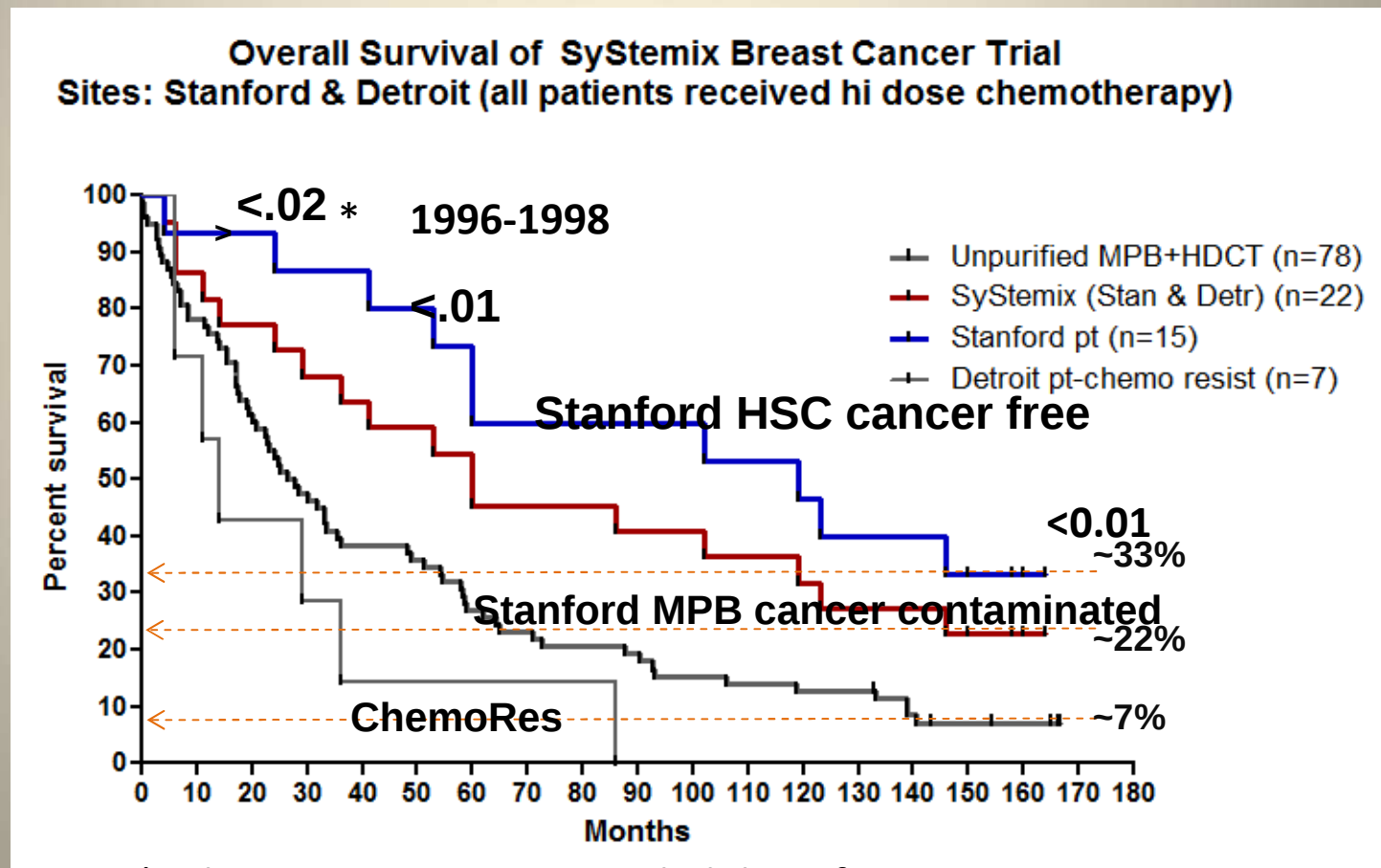
MODEL FOR NORMAL HSC MIGRATION AND LEUKEMIA METASTASES



Wagers, Wright, Jaiswal et al

Cancer Free Stem Cell Grafts Improves Survival

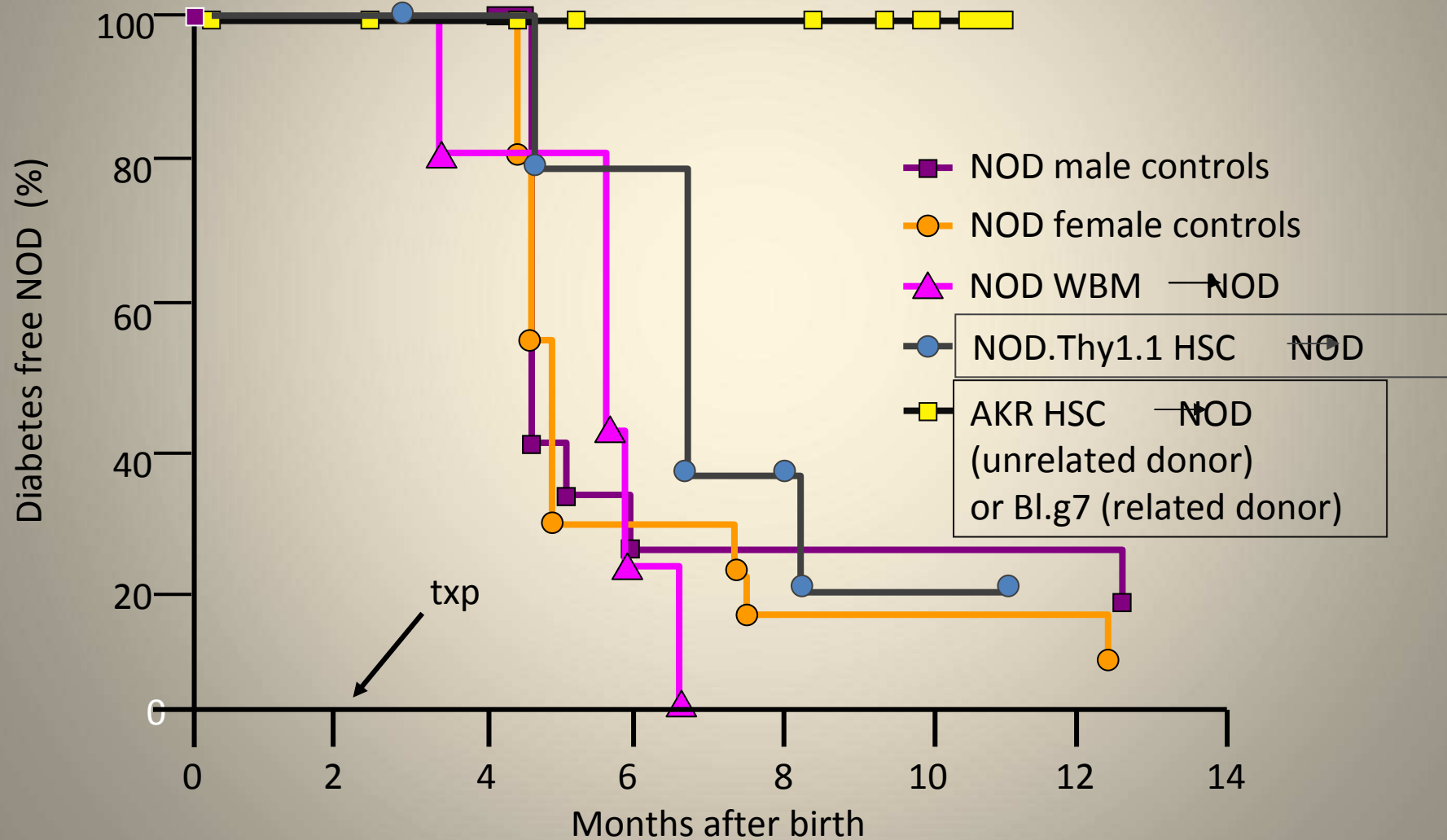
- Purified HSC show 3-fold higher survival vs non-purified MPB
- Stage 4 metastatic patients – failed all other therapies



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

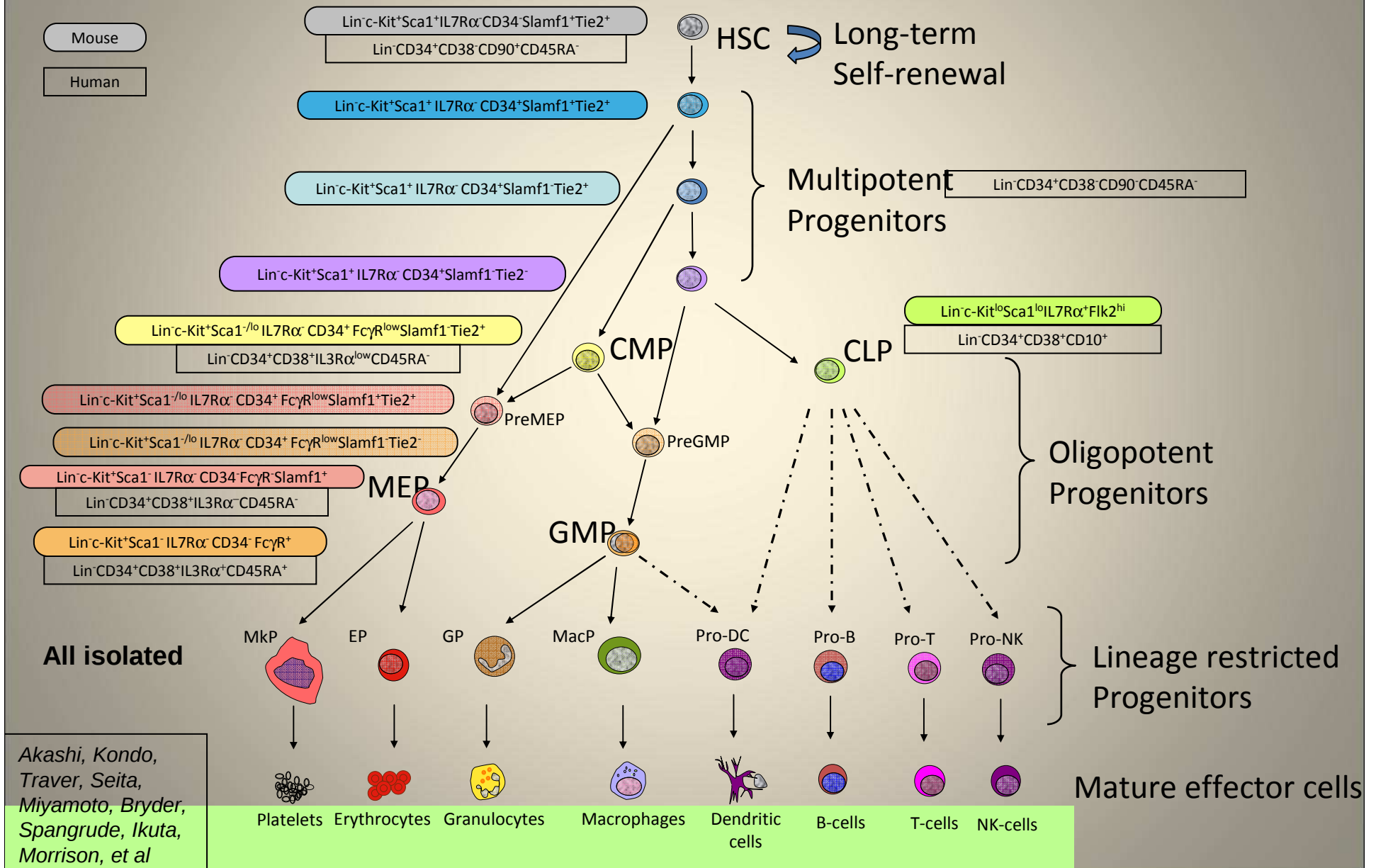
A. Mueller, I. Weissman, R. Negrin, and J. Shizuru, 2011

Rescue of Diabetic Mice with HSCs



Shizuru, Weissman, and Beilhack

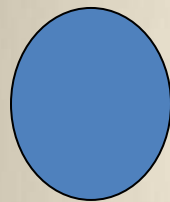
Hematopoietic Hierarchy



Leukemic cells in AML patients

LT STEM CELLS

CD34⁺CD38⁻Thy⁺Lin⁻



**5-40%
AML-1/ETO⁺**

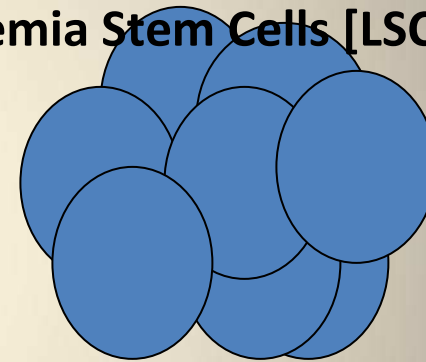


Normal colonies

MPP

CD34⁺CD38⁻Thy⁻Lin⁻

Leukemia Stem Cells [LSC]



Leukemic blast colonies

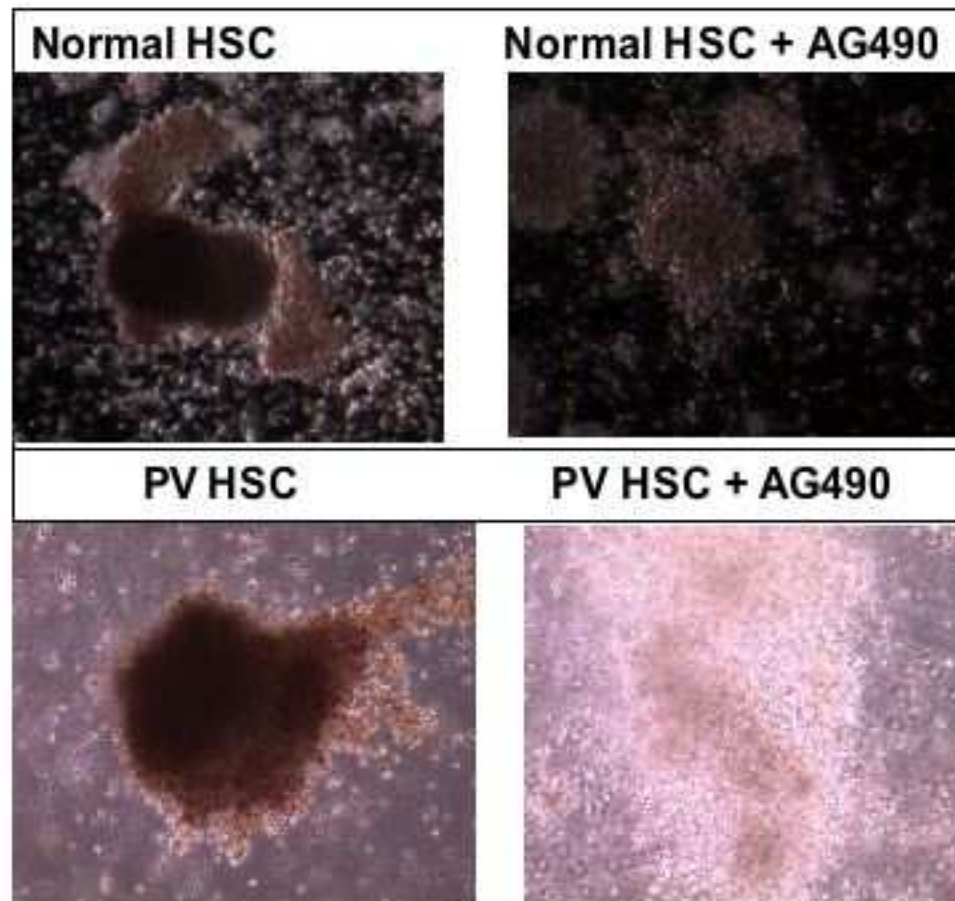
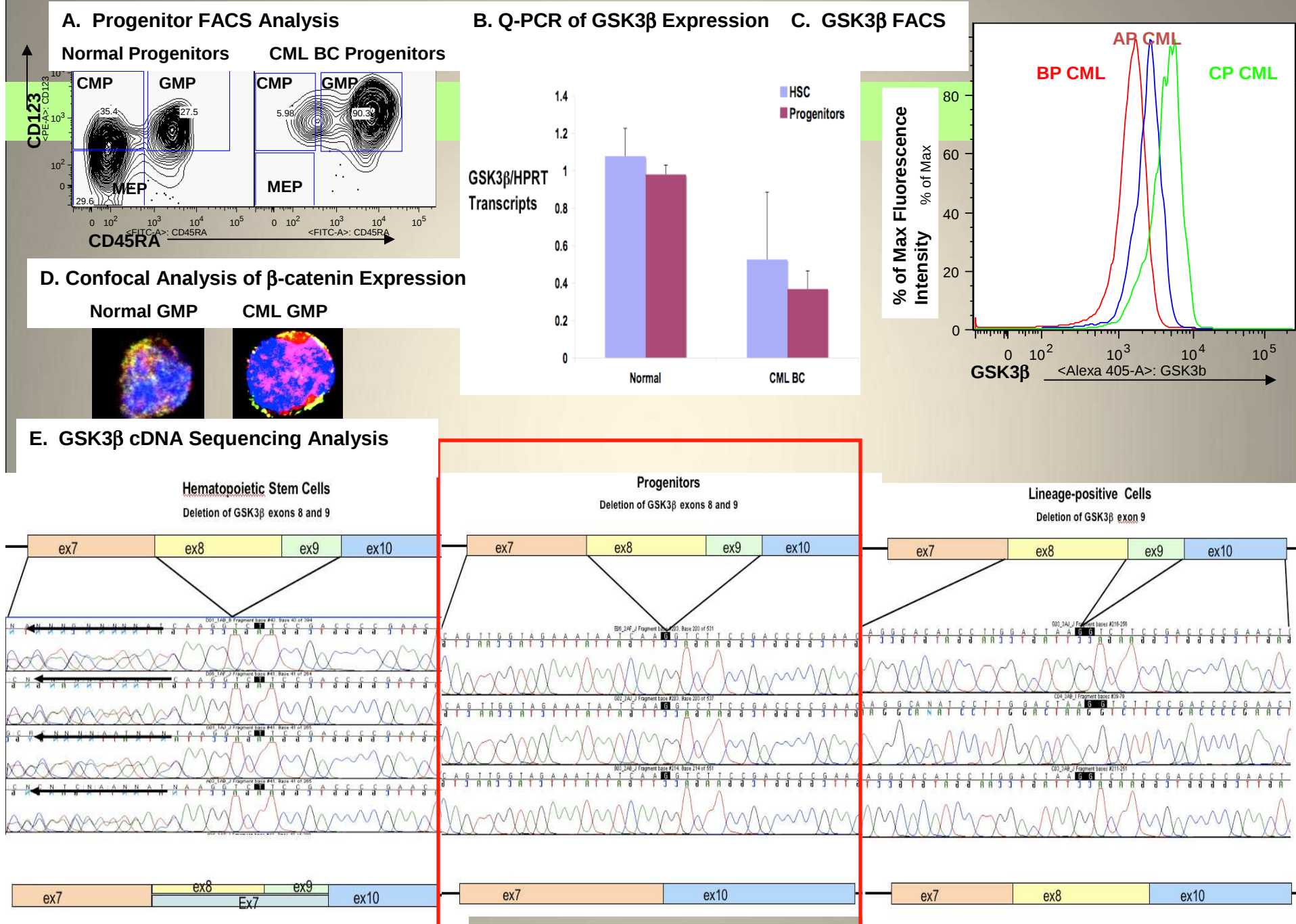


Figure 3C. Effect of JAK2 inhibition with AG490 on normal versus polycythemia vera (PV) hematopoietic stem cell (HSC) differentiation potential *in vitro*. Representative photomicrographs obtained with a Zeiss Axiovert microscope (10x objective) and SPOT software. Normal cord blood or PV peripheral blood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

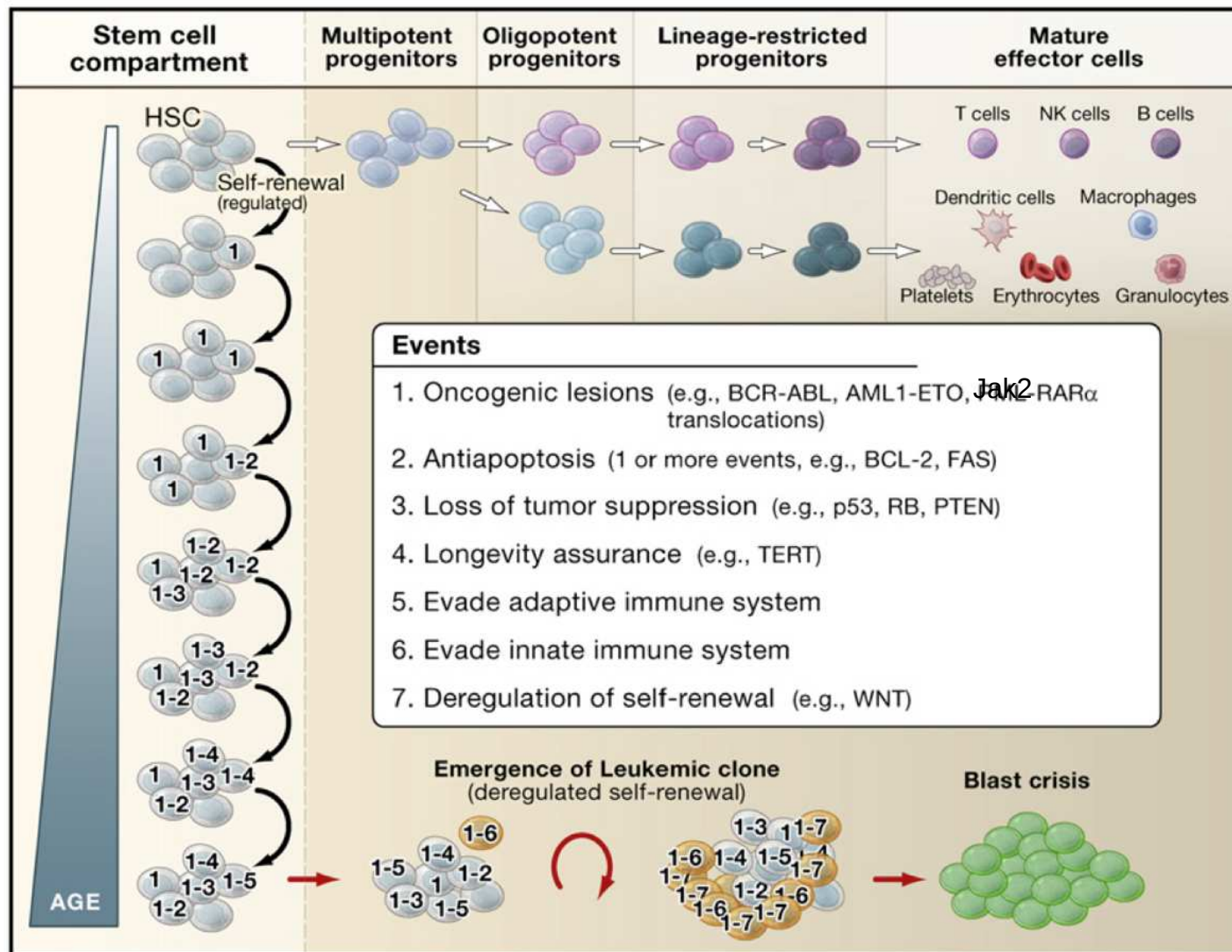
CML

- 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

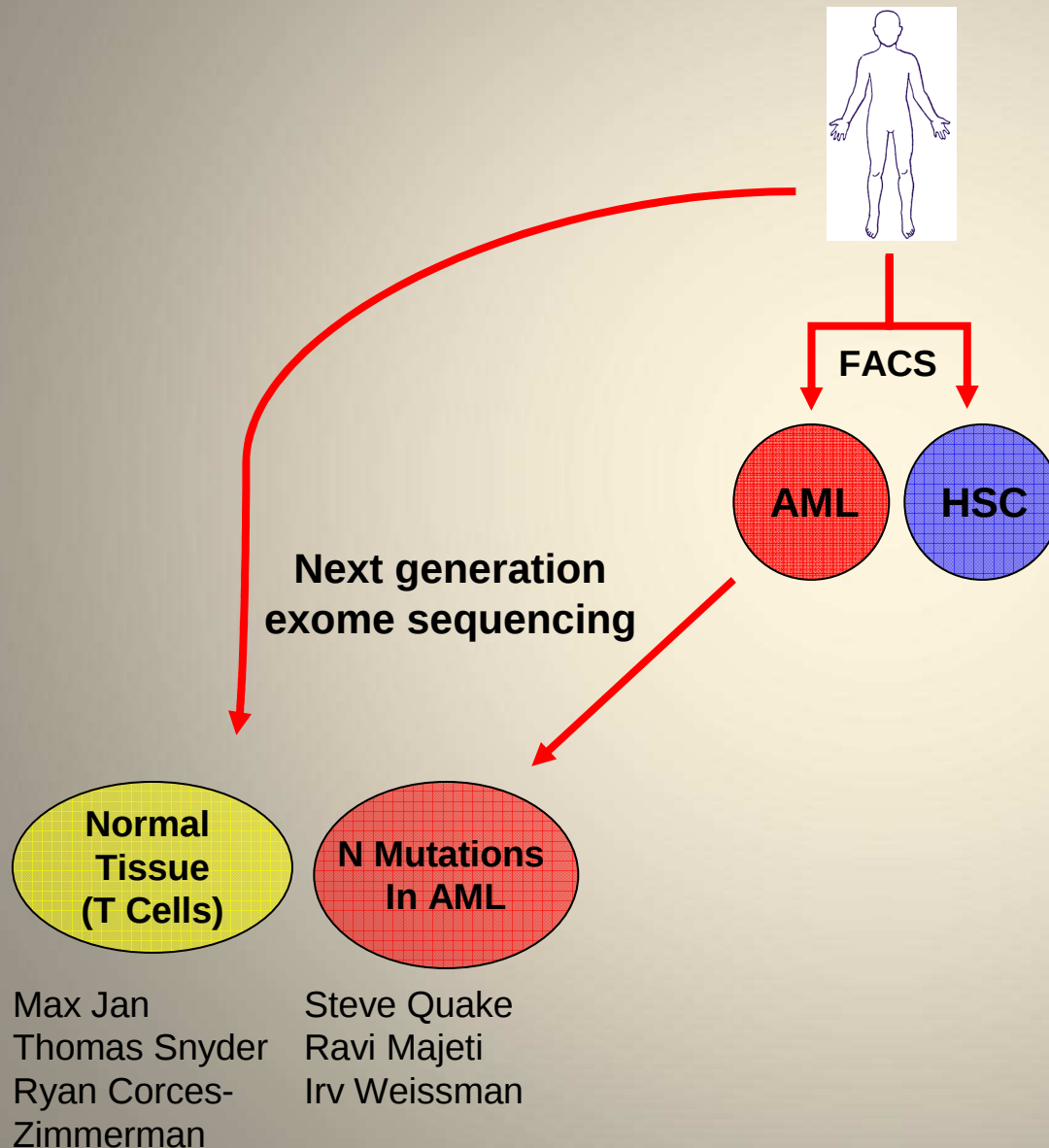
Figure 3. Aberrant GSK3 β Expression by Blast Crisis CML Progenitors



Cell of origin – progression to leukemia

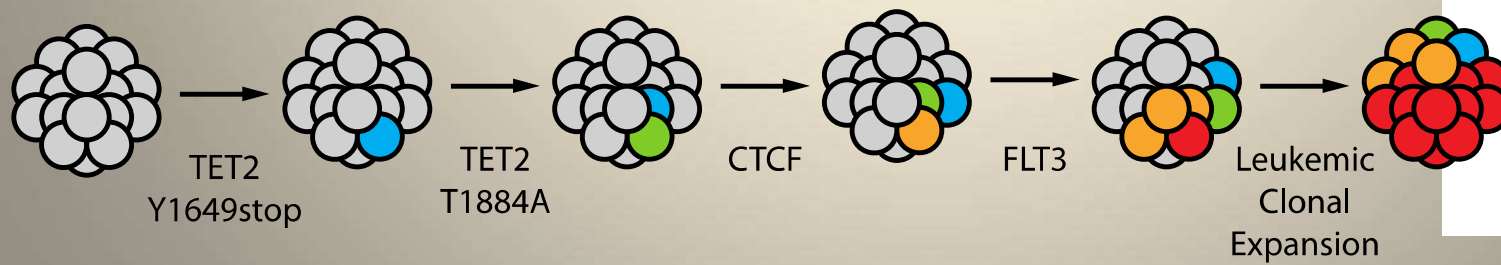
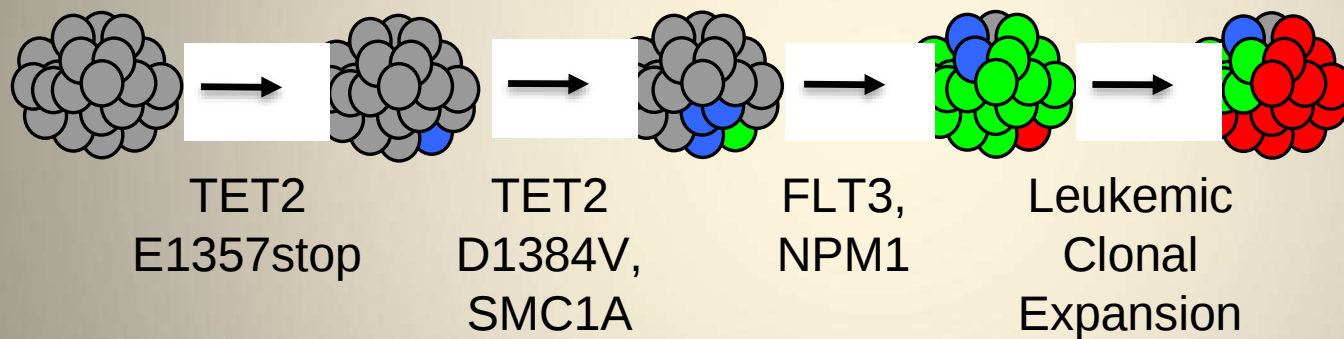
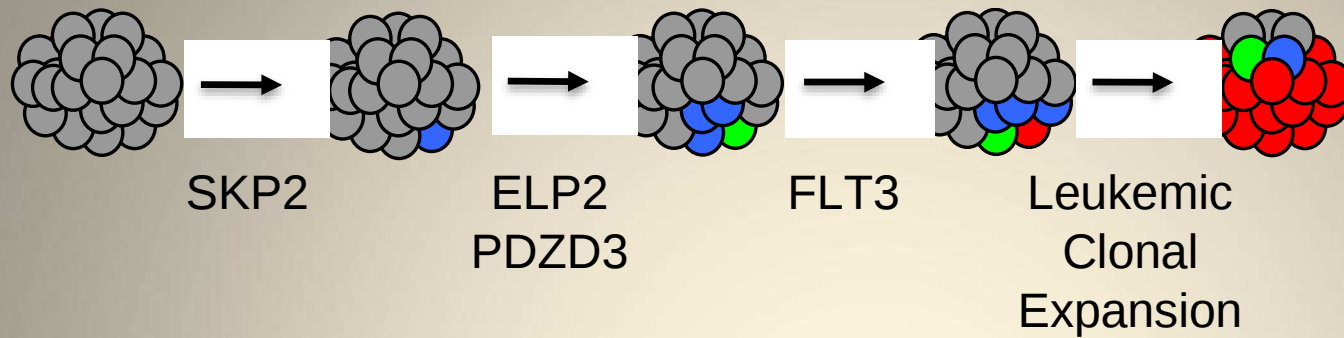


Identification of Somatic Mutations by Exome Sequencing



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

Analysis of Single HSC to Identify Pre-Leukemic Clones



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

Cell surface markers on LSC but not HSC

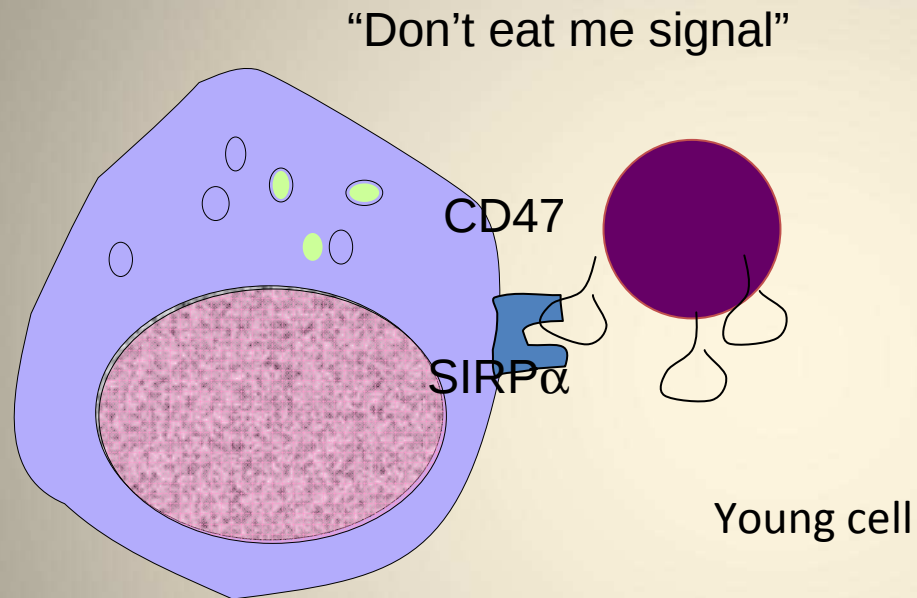
CD 96 > 40 fold in ~ 50% of AML samples

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 Weissman

Young cells overexpress CD47 to evade phagocytosis



Macrophage

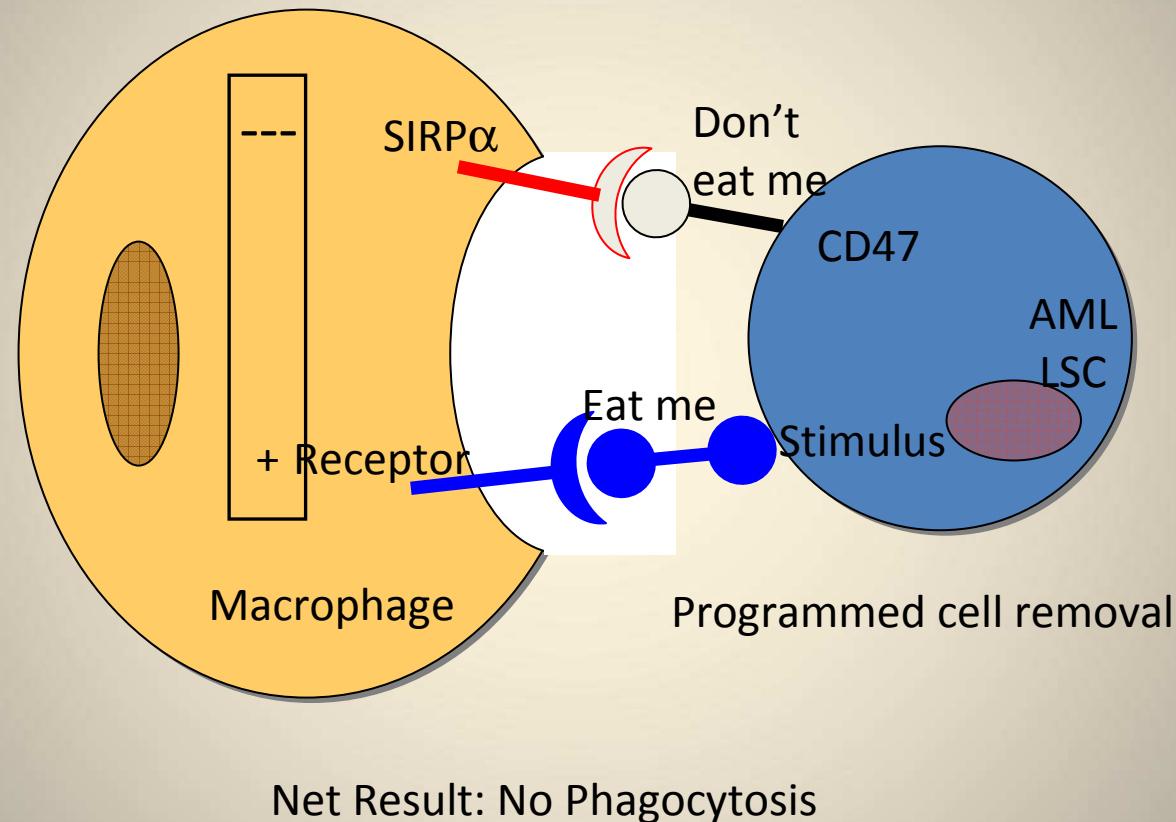
Oldenberg et al, 2000-2006

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

Jaiswal, Jamieson, Traver, and IW

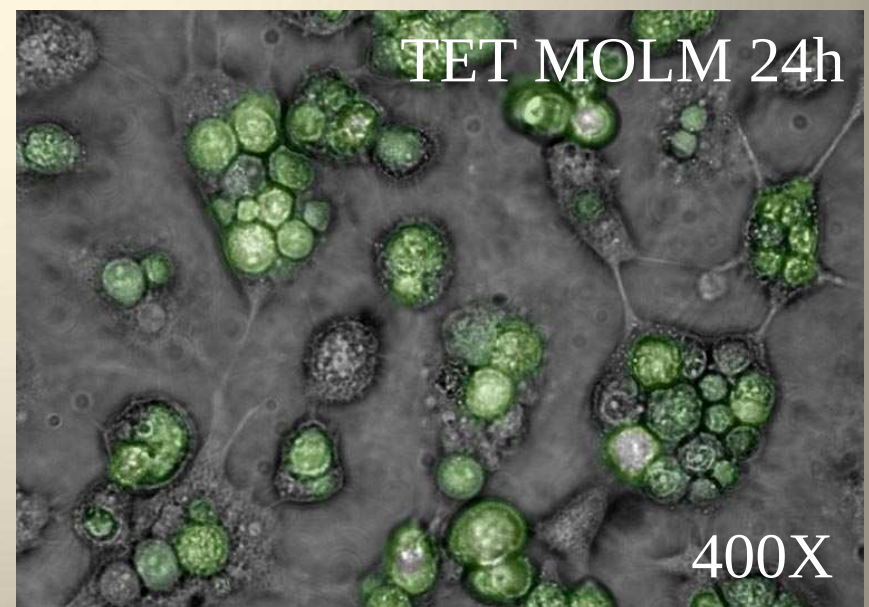
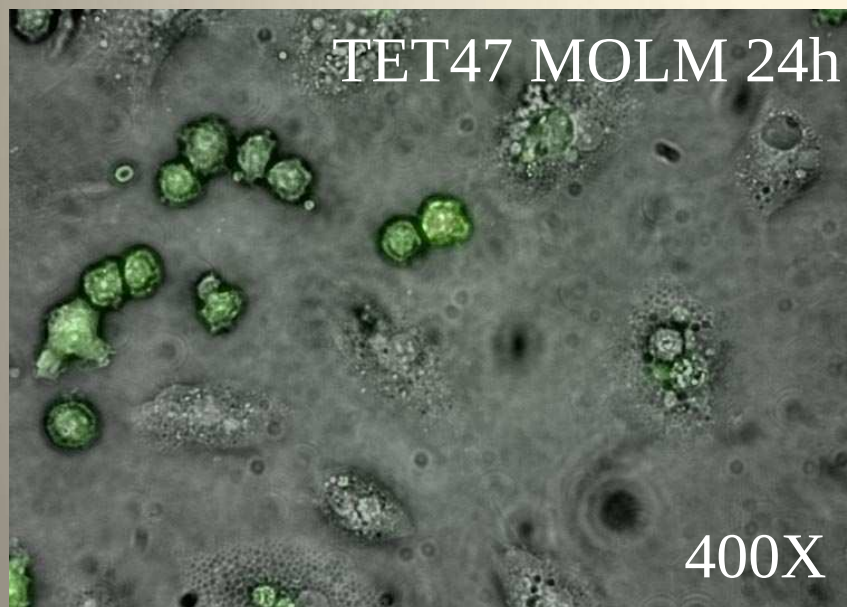
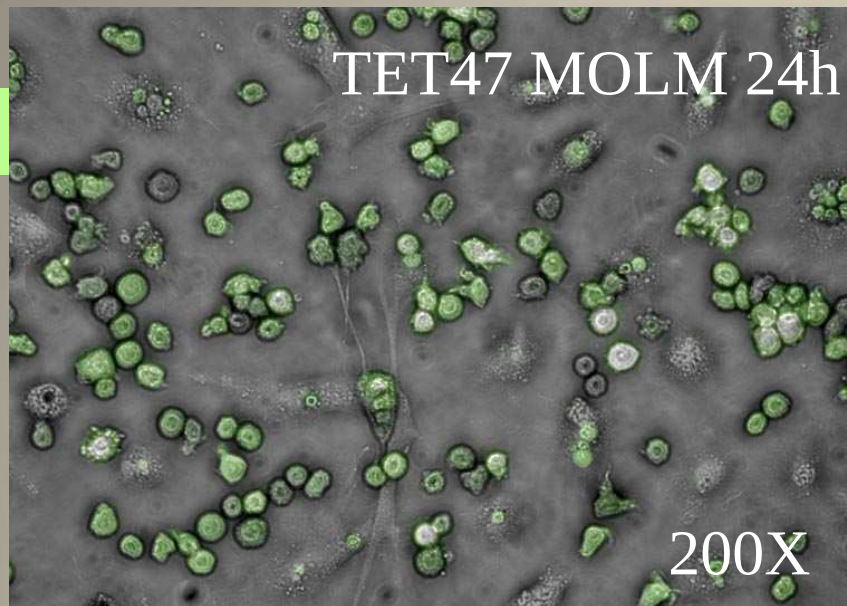
CD47 was discovered as a marker of aging RBC by Oldenborg. We found it on m/h AML LSC

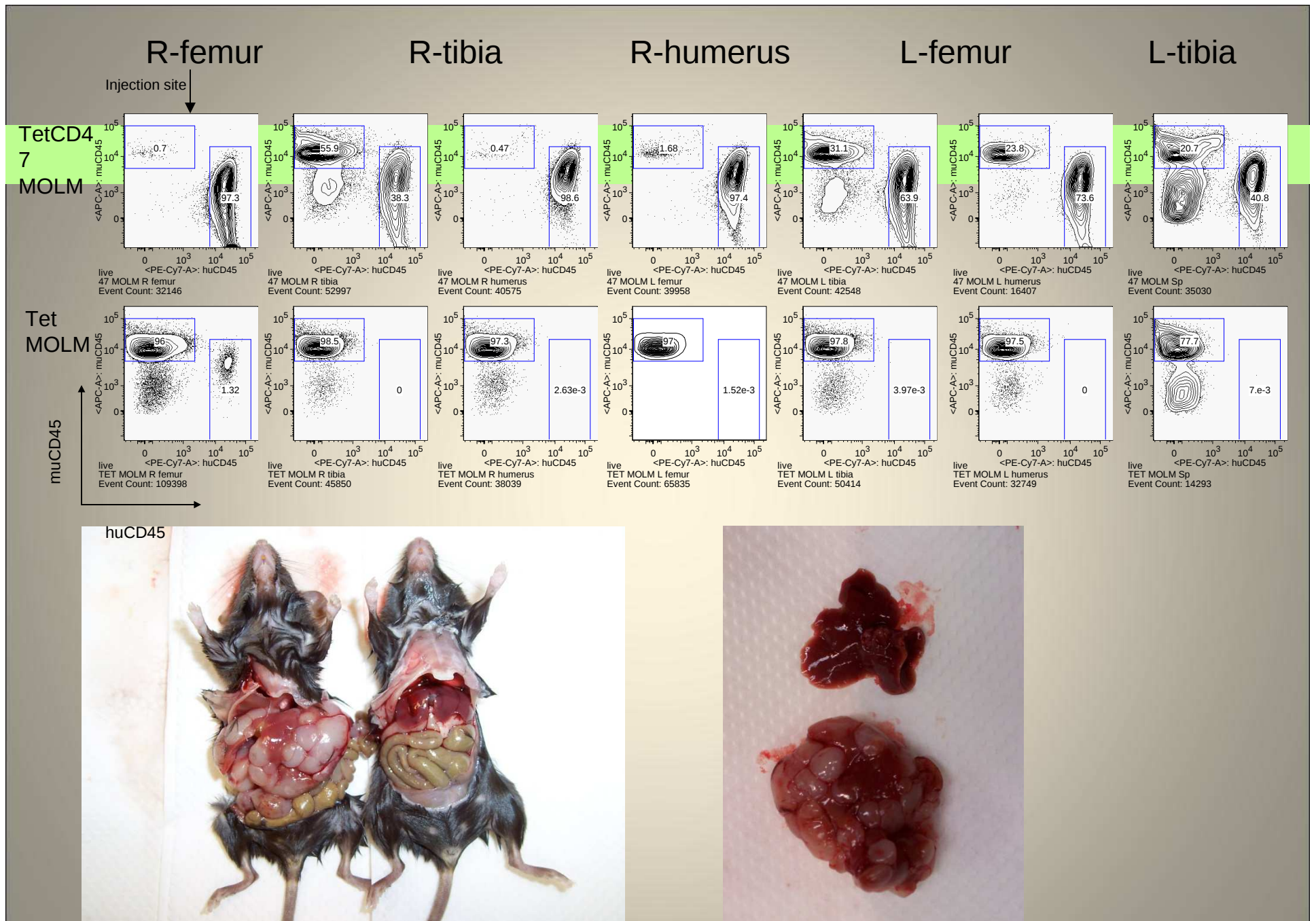
Hypothesis: Increased expression of CD47 on myeloid leukemia cells contributes to pathogenesis by facilitating evasion of phagocytosis



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

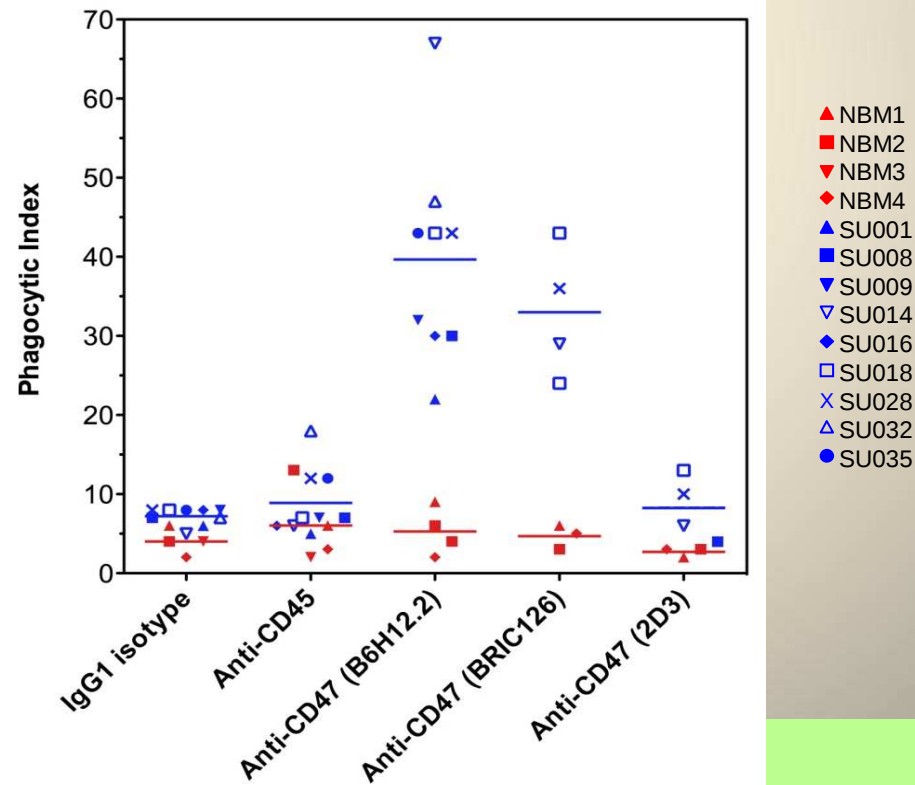
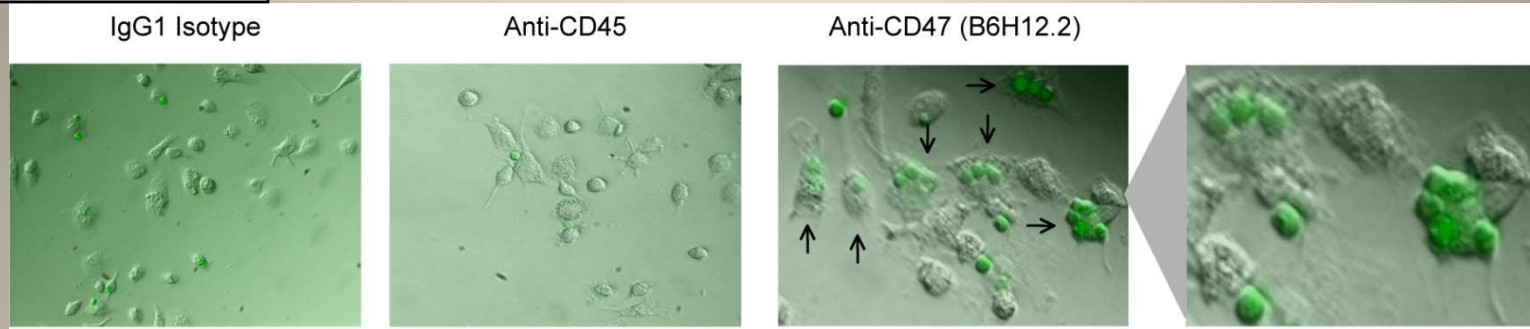
Traver and IW 1998; Jaiswal, Majeti, Chao, and IW 2008.





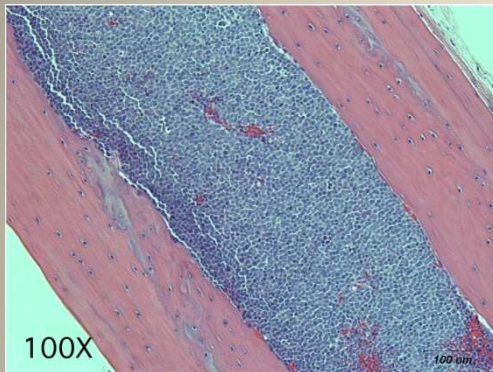
Anti-CD47 Antibodies Enable Phagocytosis of AML LSC

Human Macrophages

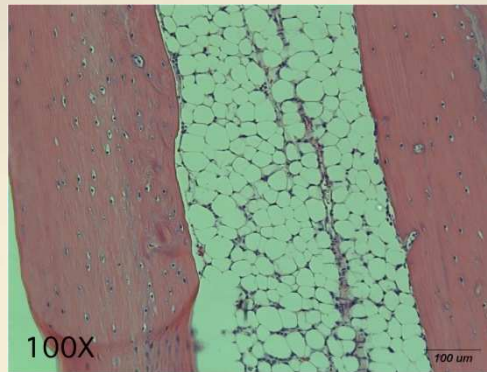


Anti-CD47 Antibody Depletes AML in the Bone Marrow

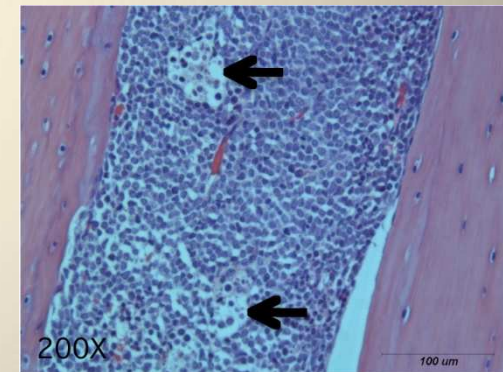
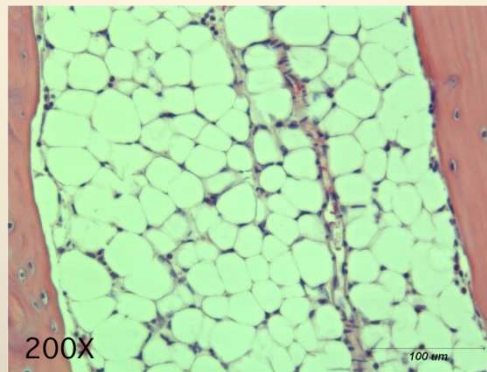
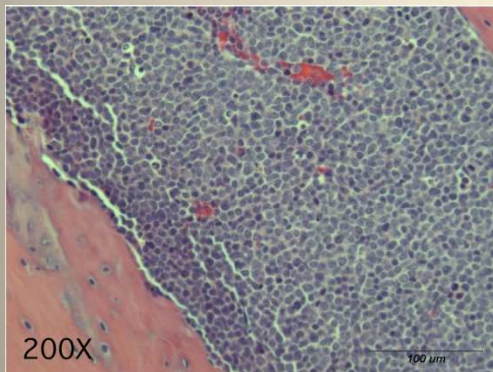
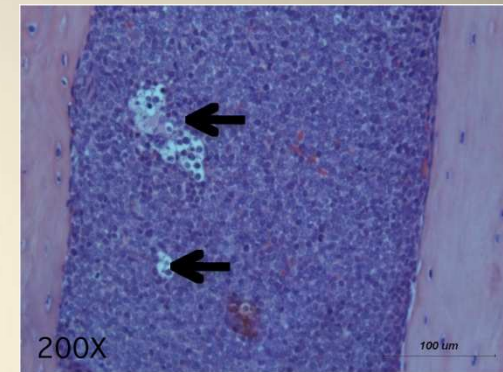
IgG Control



Anti-CD47

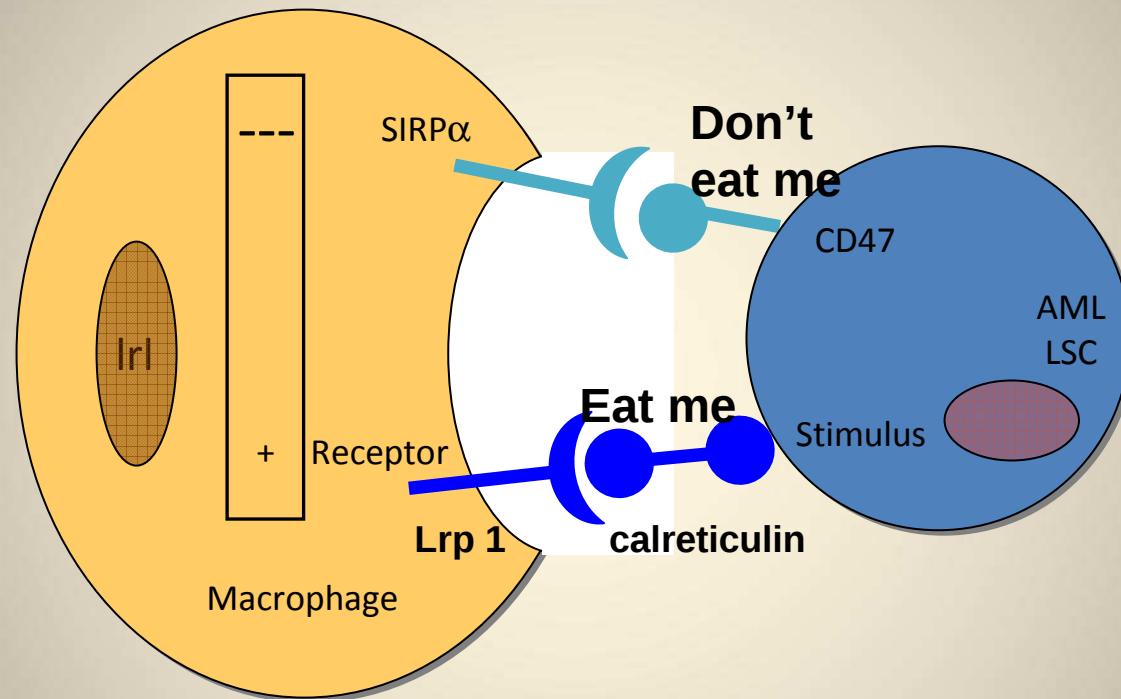


Anti-CD47



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

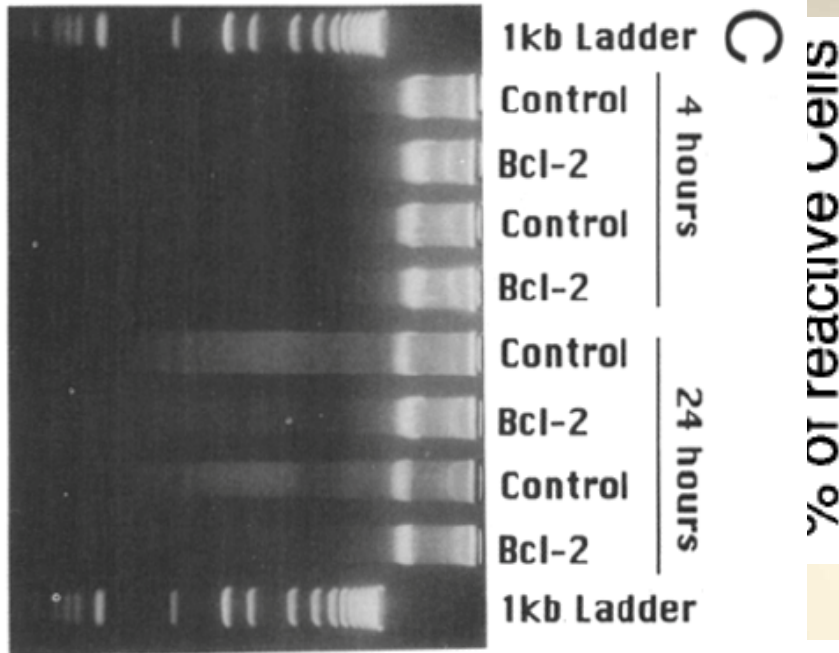
Increased expression of CD47 on myeloid leukemia cells contributes to pathogenesis by facilitating evasion of phagocytosis



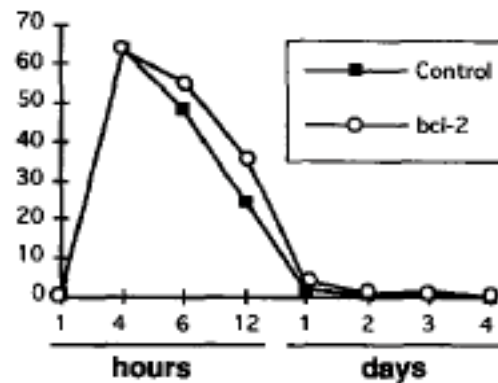
Net Result: No Phagocytosis

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

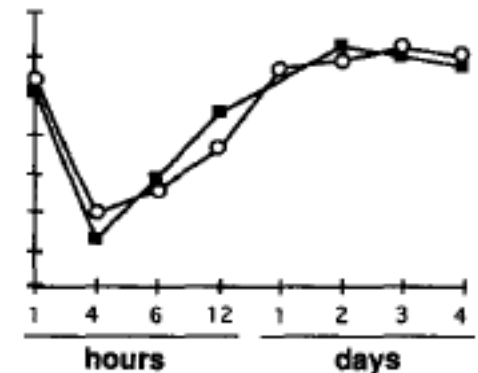
BCL2 blocks apoptosis, but not programmed cell removal of neutrophils: Lagasse and Weissman JEM 1994



% of reactive cells



Neutrophils

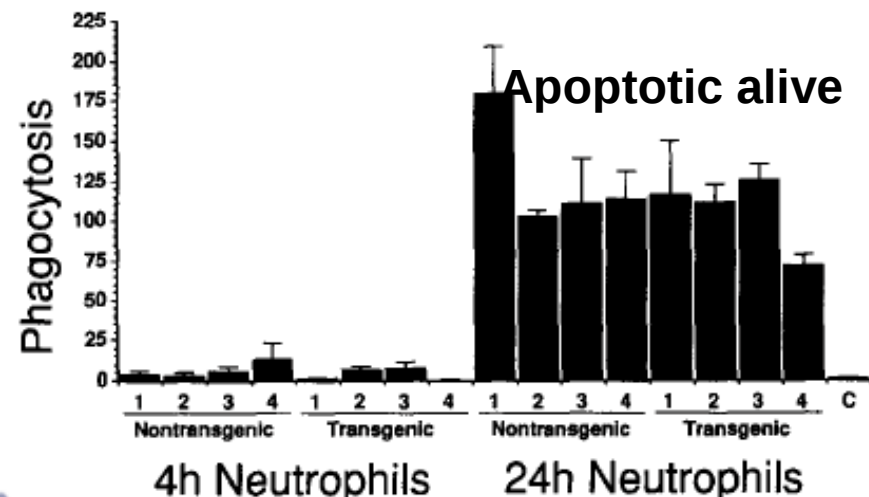


Macrophages

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.

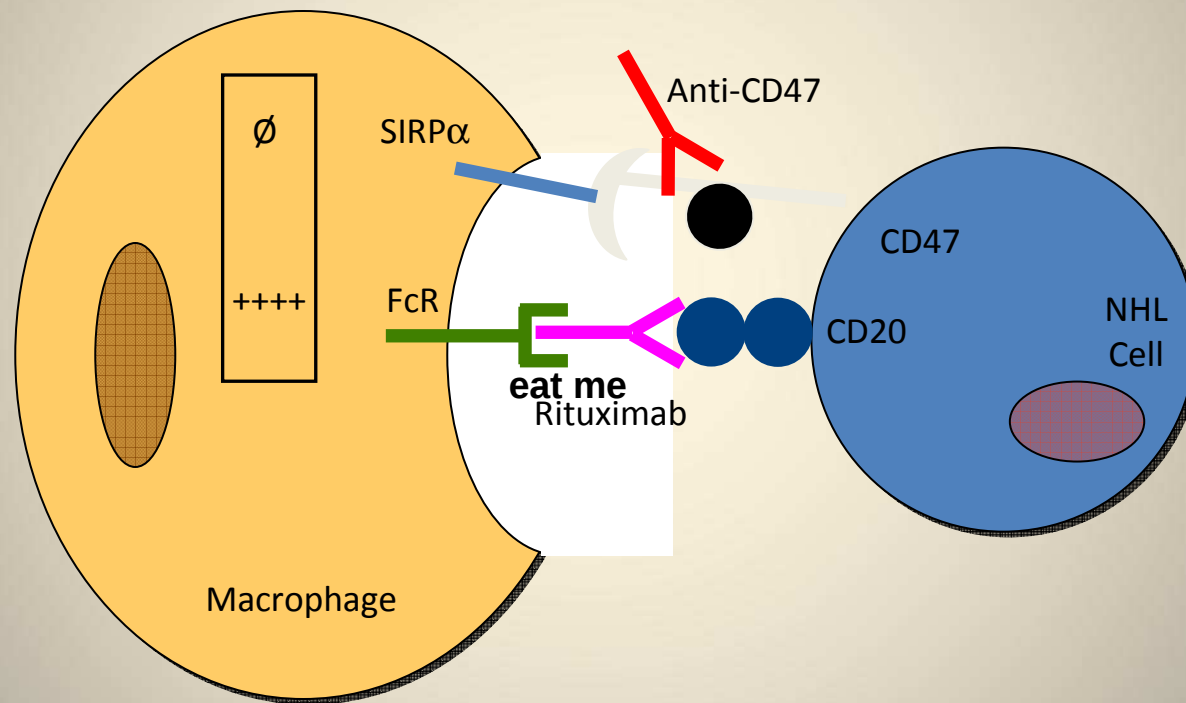


PCDeath and PCRemoval

- 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

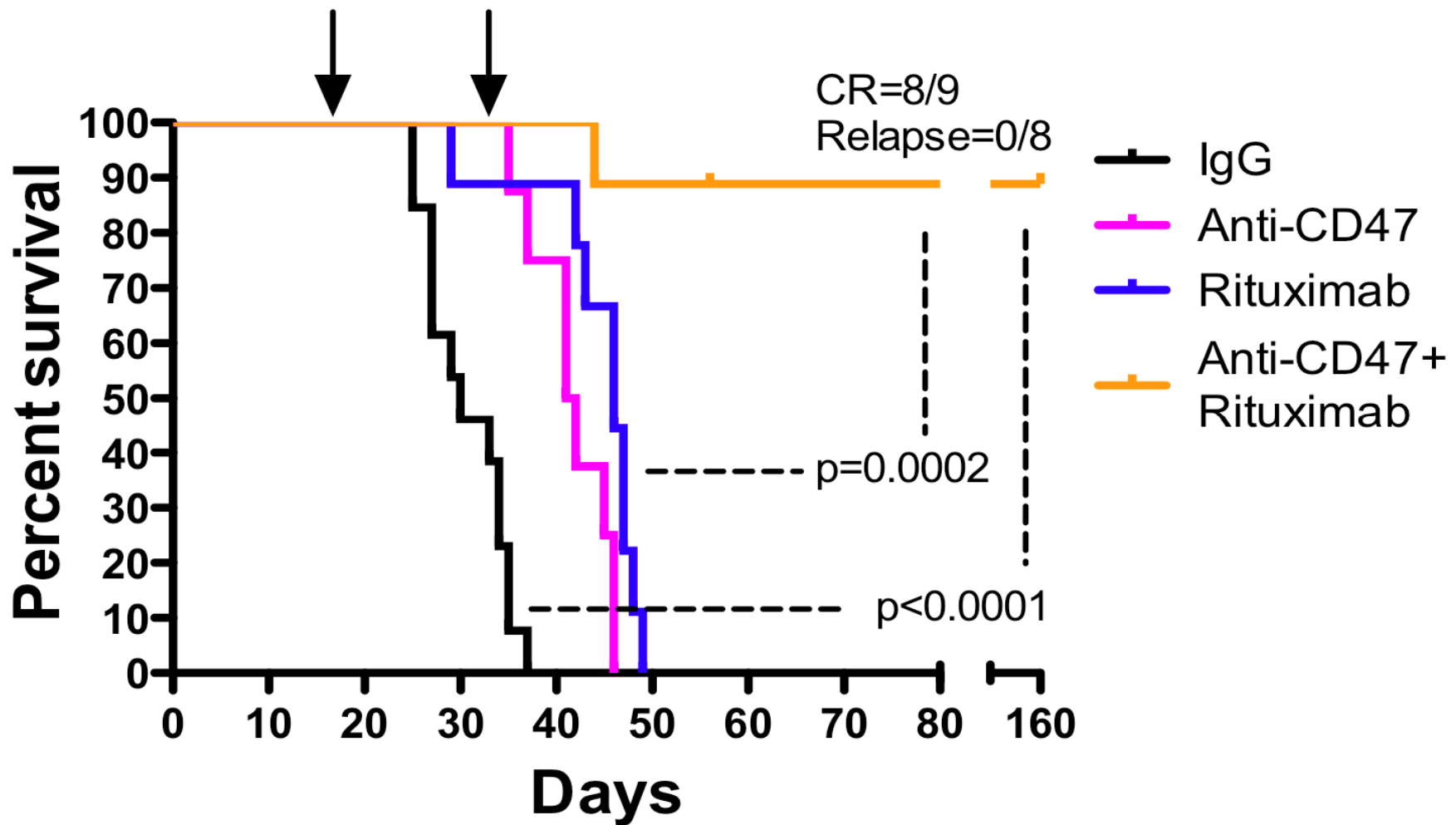
Model for Synergy of Anti-CD47 with Rituximab

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



Human primary solid

tumor xenografts

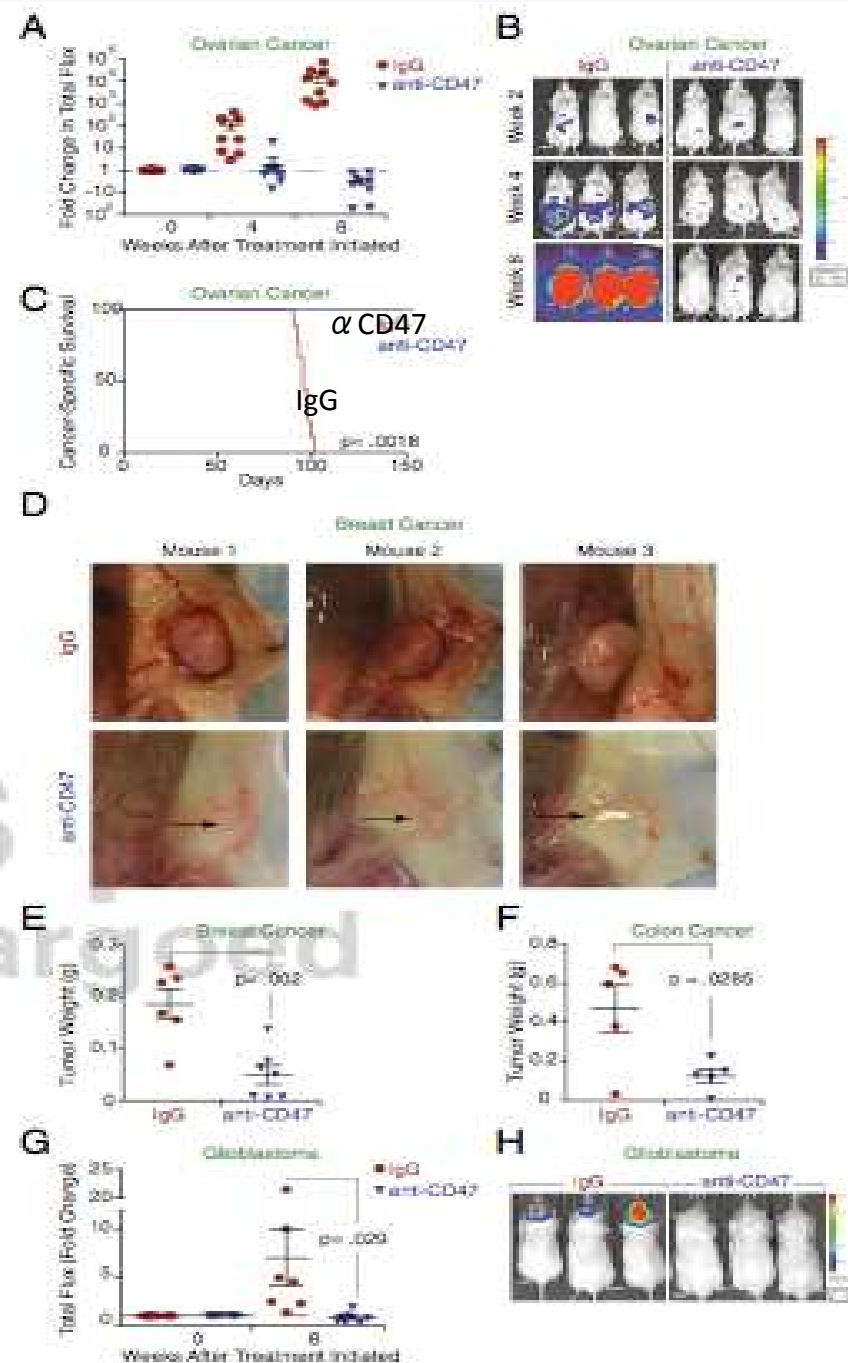
treated with anti-CD47

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



ANTI-CD47 ANTIBODIES CAN ELIMINATE ESTABLISHED METASTASIZED TUMORS

Inject LMS
Subcutaneous

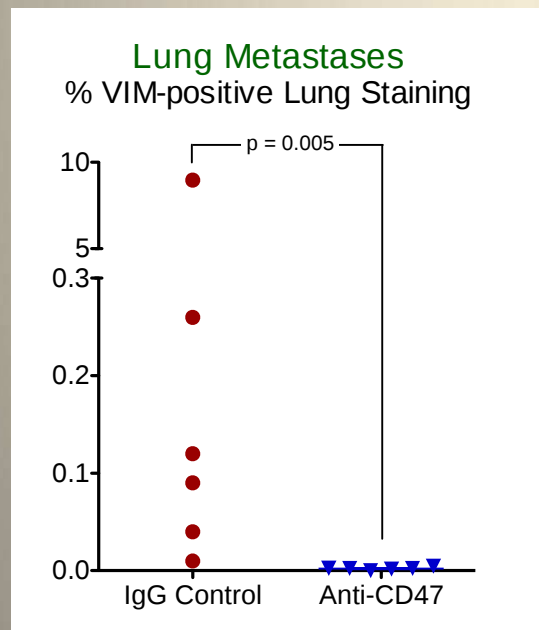
Resect Primary
Tumor

Anti-CD47 Therapy

Evaluate growth of
metastases

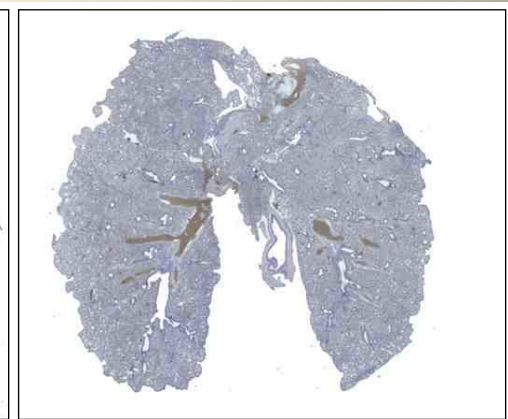
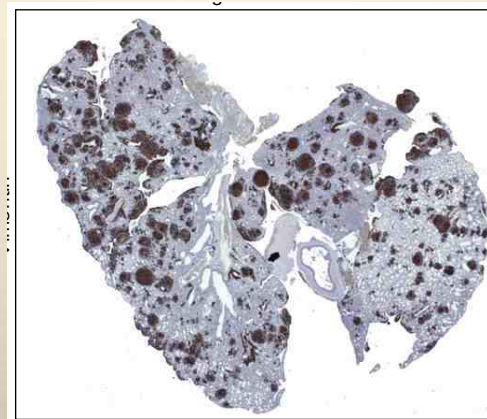
Week 7

Week 11



IgG Control

Anti-CD47



Vimentin

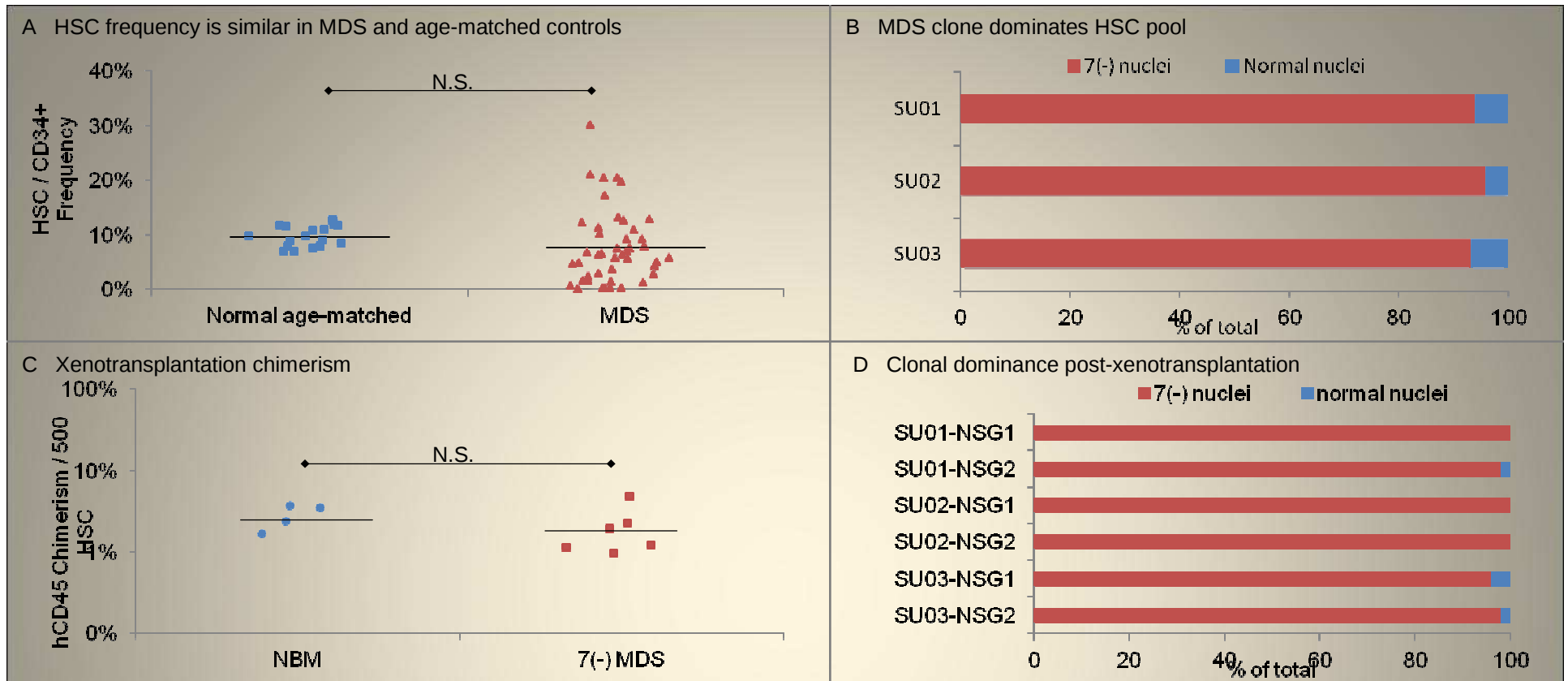
Investigation and Targeting of CD47 in Human Cancers

Breast
Ovarian
Bladder
Pancreatic
Colon
Prostate
Lung
Kidney
Leiomyosarcoma
Head & Neck
Melanoma

Glioblastoma
Medulloblastoma
Oligodendroglioma
Hepatocellular Carcinoma
Gastric Cancer
Multiple Myeloma
Chronic Myeloid Leukemia
Acute Myeloid Leukemia
Non-Hodgkin's Lymphoma
T-Acute Lymphoblastic Leukemia
B-Acute Lymphoblastic Leukemia

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

Patient Tumor Xenotransplantation Model
Treatment Data Available

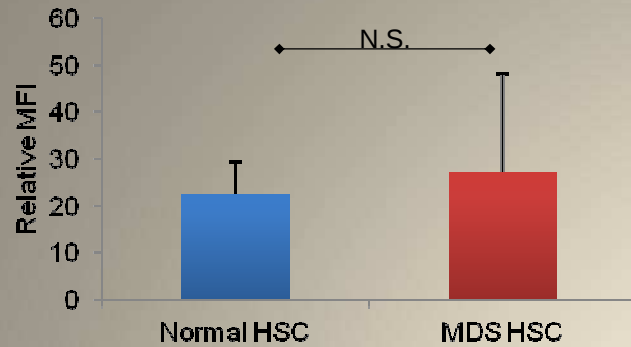


MDS is a pre-AML disease of older patients in which a cytopenia Precedes leukemia.

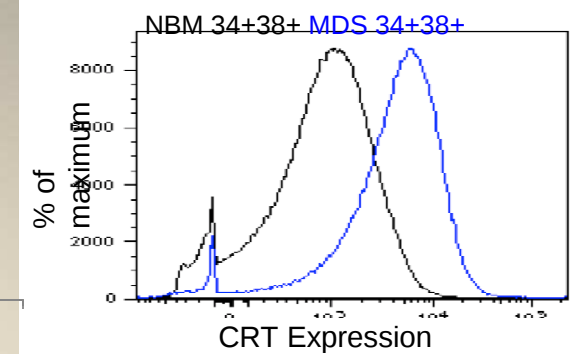
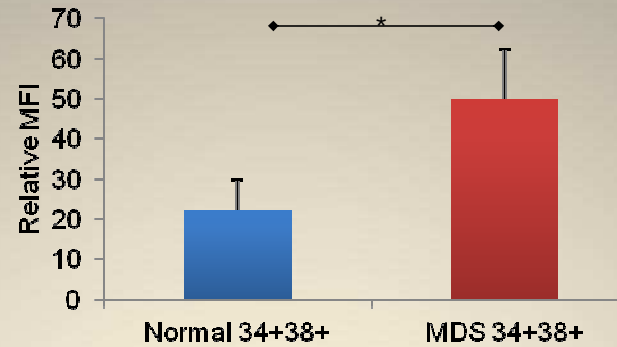
MDS HSC outcompete normal HSC in MDS patients and in transplanted NSG mice

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

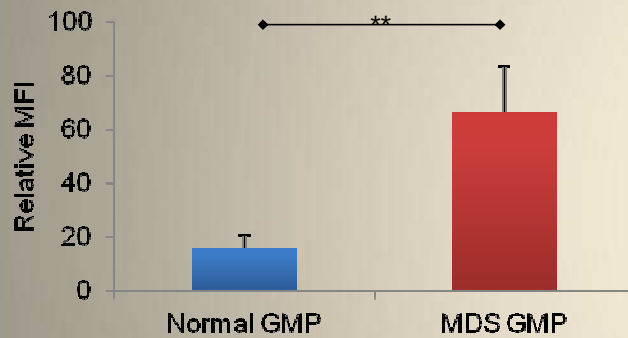
A CRT not increased on MDS HSC



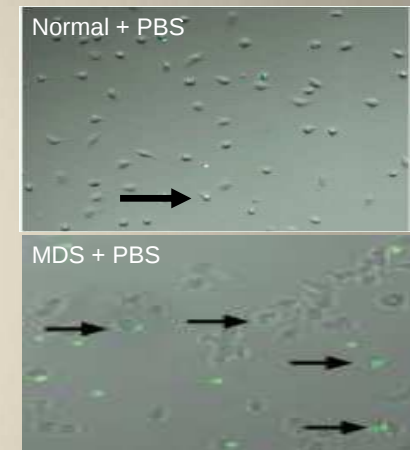
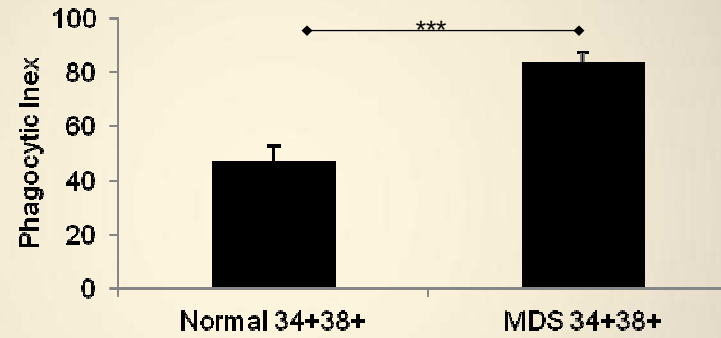
B Increased cell-surface CRT on MDS CD34+CD38+ cells



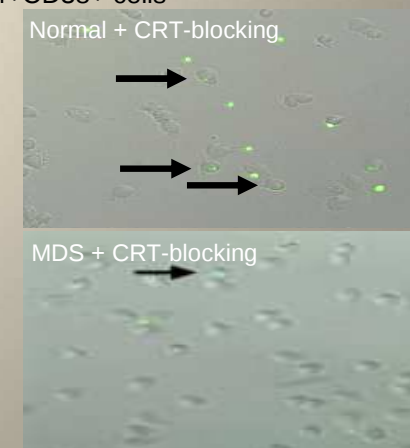
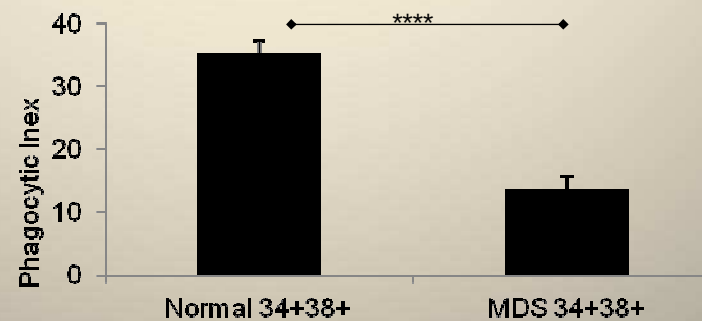
C Increased CRT on MDS GMP



D Increased phagocytosis of MDS CD34+CD38+ cells



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



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

**Wendy Pang, John Pluvinae,
Chris Park, IW, et al**

Conclusions

MDS HSC outcompete normal HSC in patient and xenotransplant

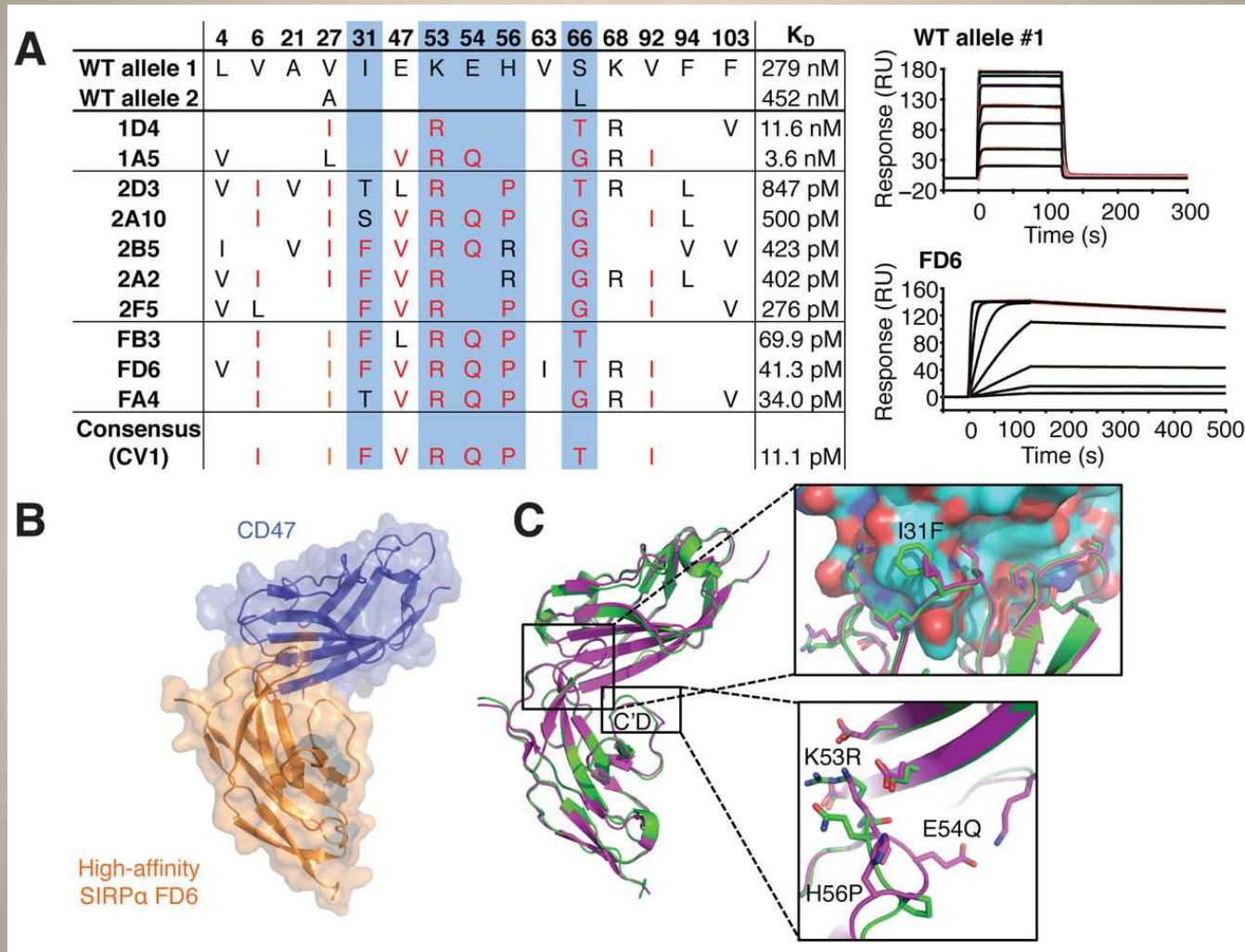
The MDS-initiating cell resides in HSC compartment

High CRT predisposes MDS myeloid progenitors for programmed cell removal

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

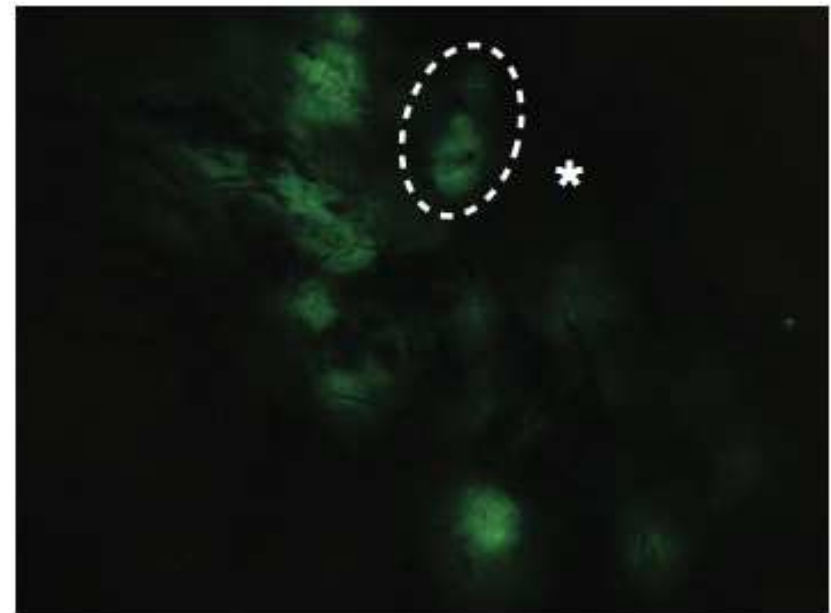
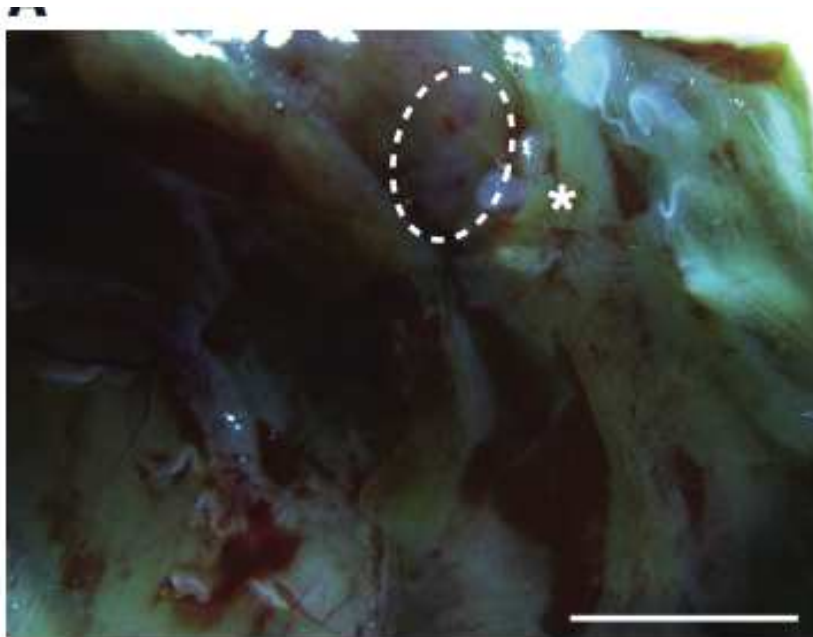
- Calreticulin is a potential therapeutic target

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

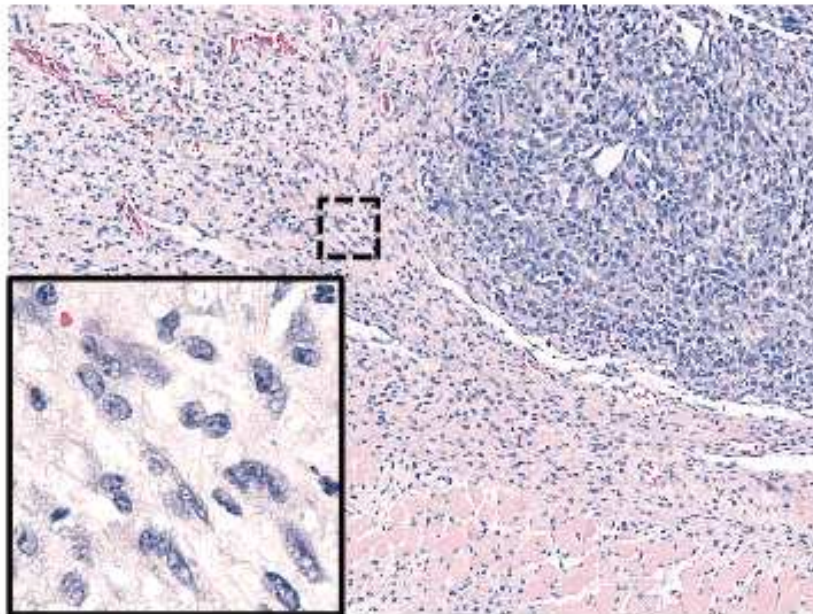


K Weiskopf et al. Science 2013;science.1238856

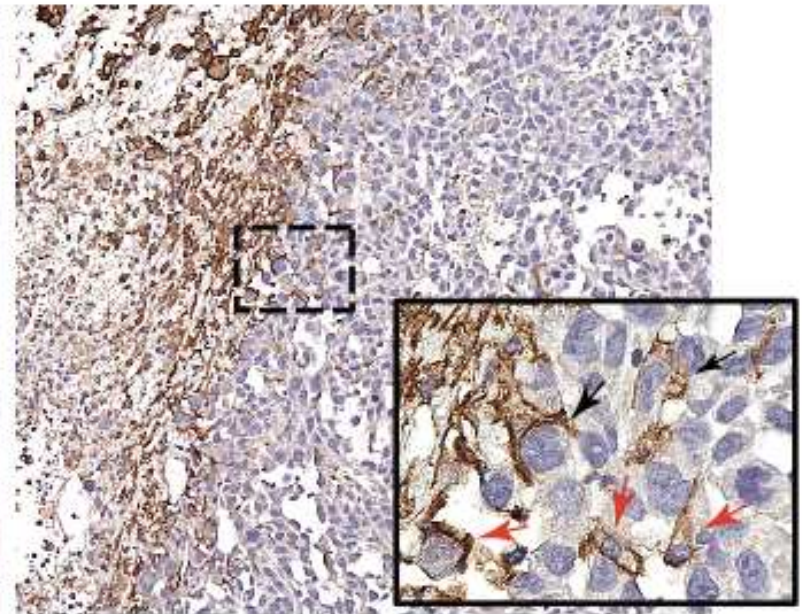
Weiskopf, Ring, Volkmer, IW and Garcia



B

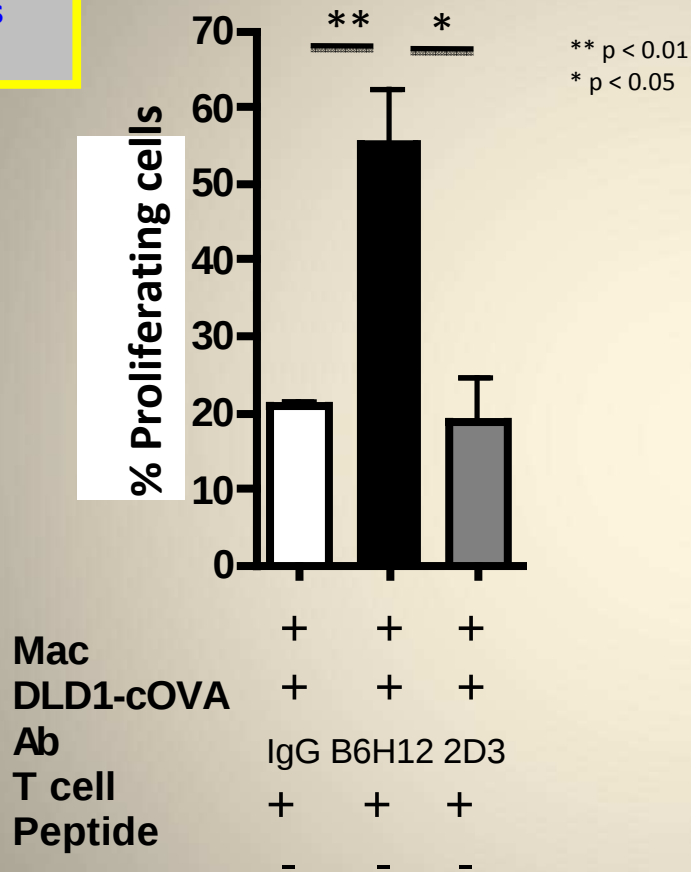


C

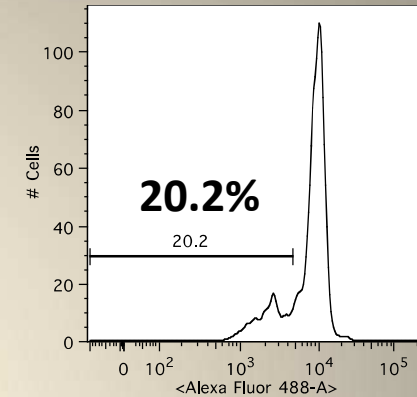


Anti-CD47-mediated phagocytosis of tumors by macrophages leads to increased MHC I antigen presentation

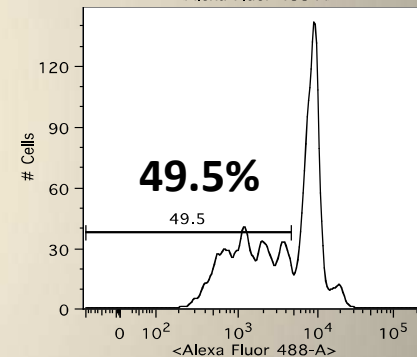
- 1) CD4 T cells
- 2) CD8 T cells
- 3) Tumor control



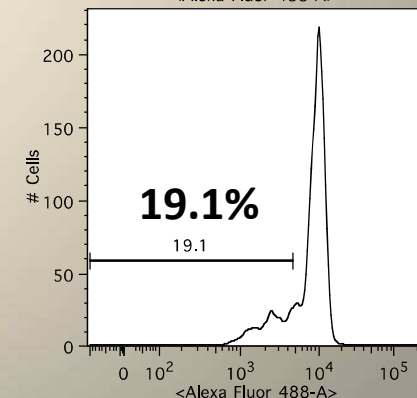
IgG



Anti-CD47
B6H12



Anti-CD47
2D3

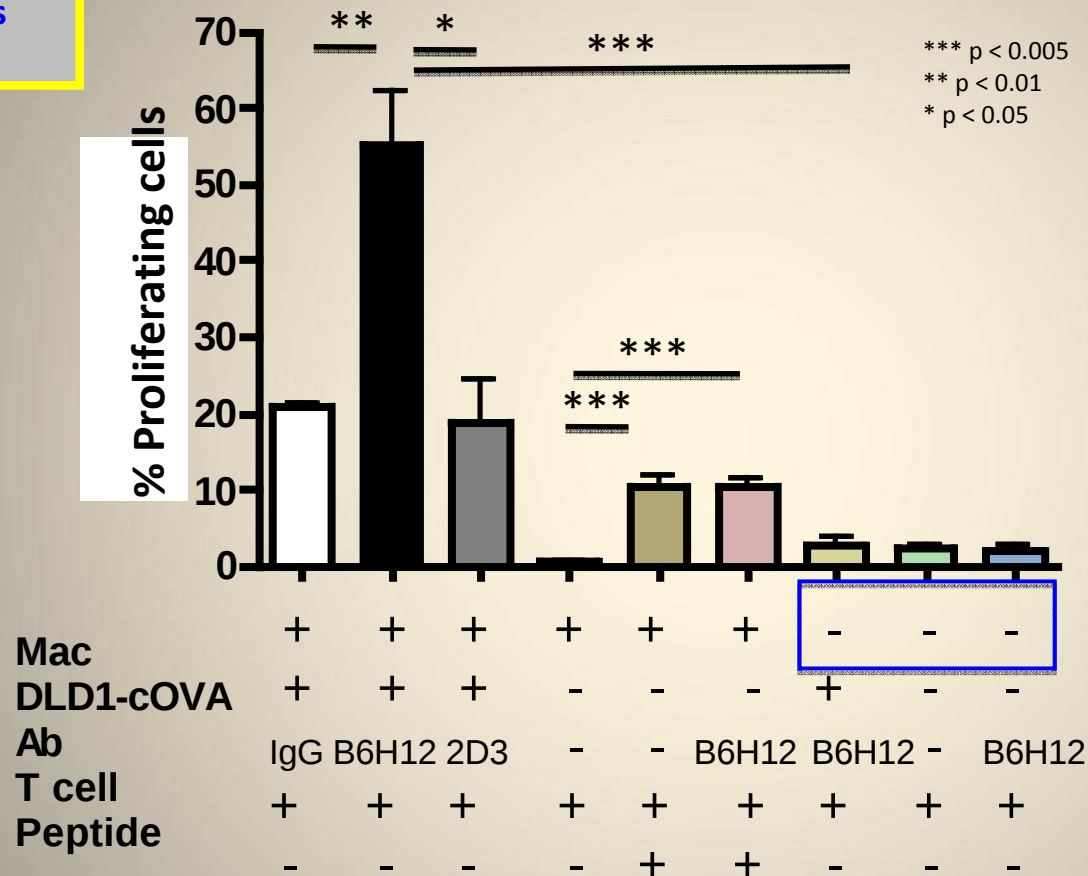


CFSE

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

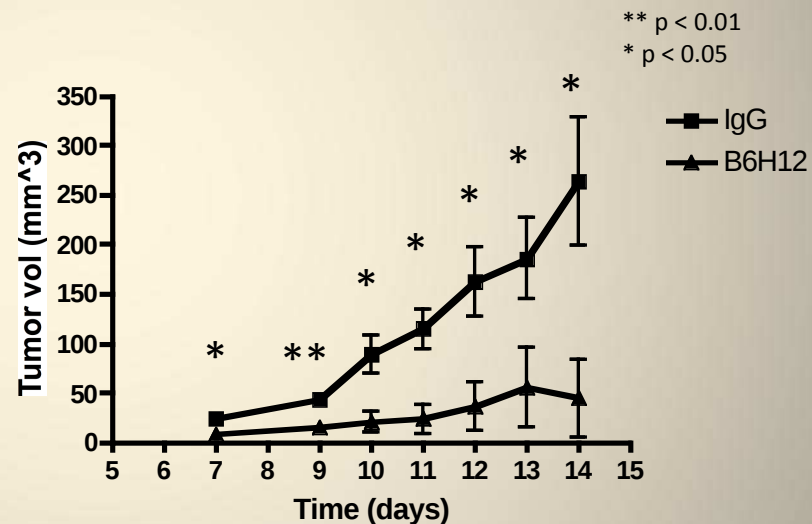
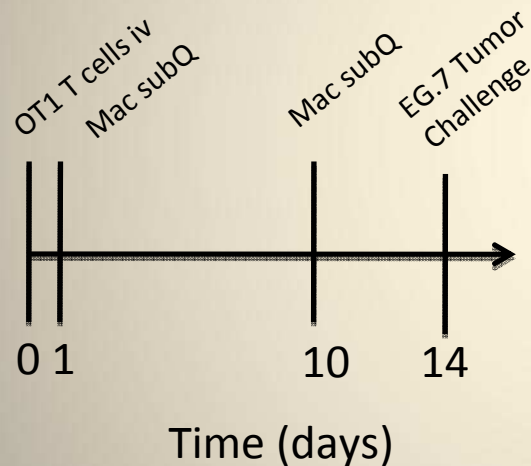


Conclusion: Following anti-CD47-mediated phagocytosis, macrophages activate the CD8+ T cell response

Diane Tseng

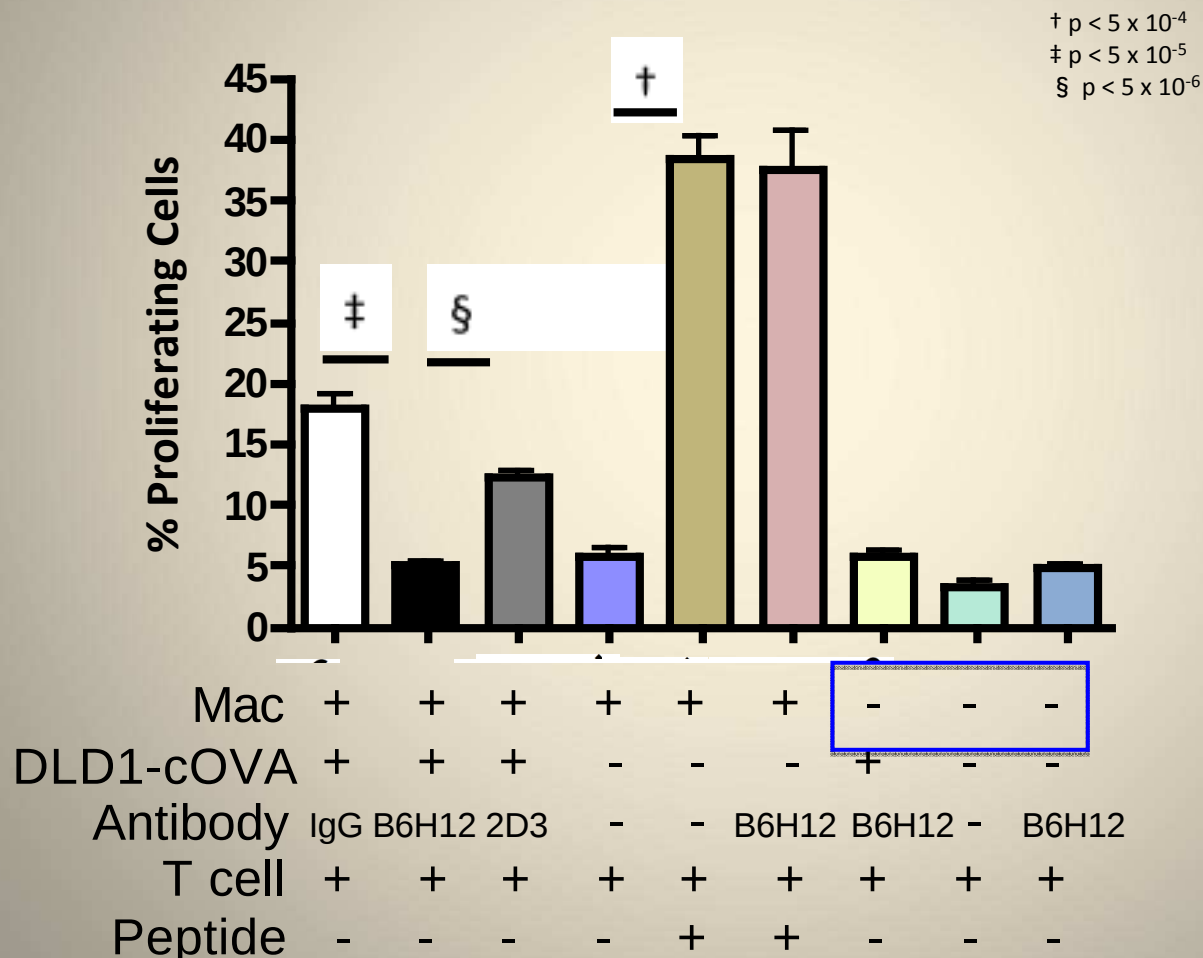
Following anti-CD47-mediated phagocytosis of cancer, macrophages prime an effective anti-tumor CD8 T cell response *in vivo*

- 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

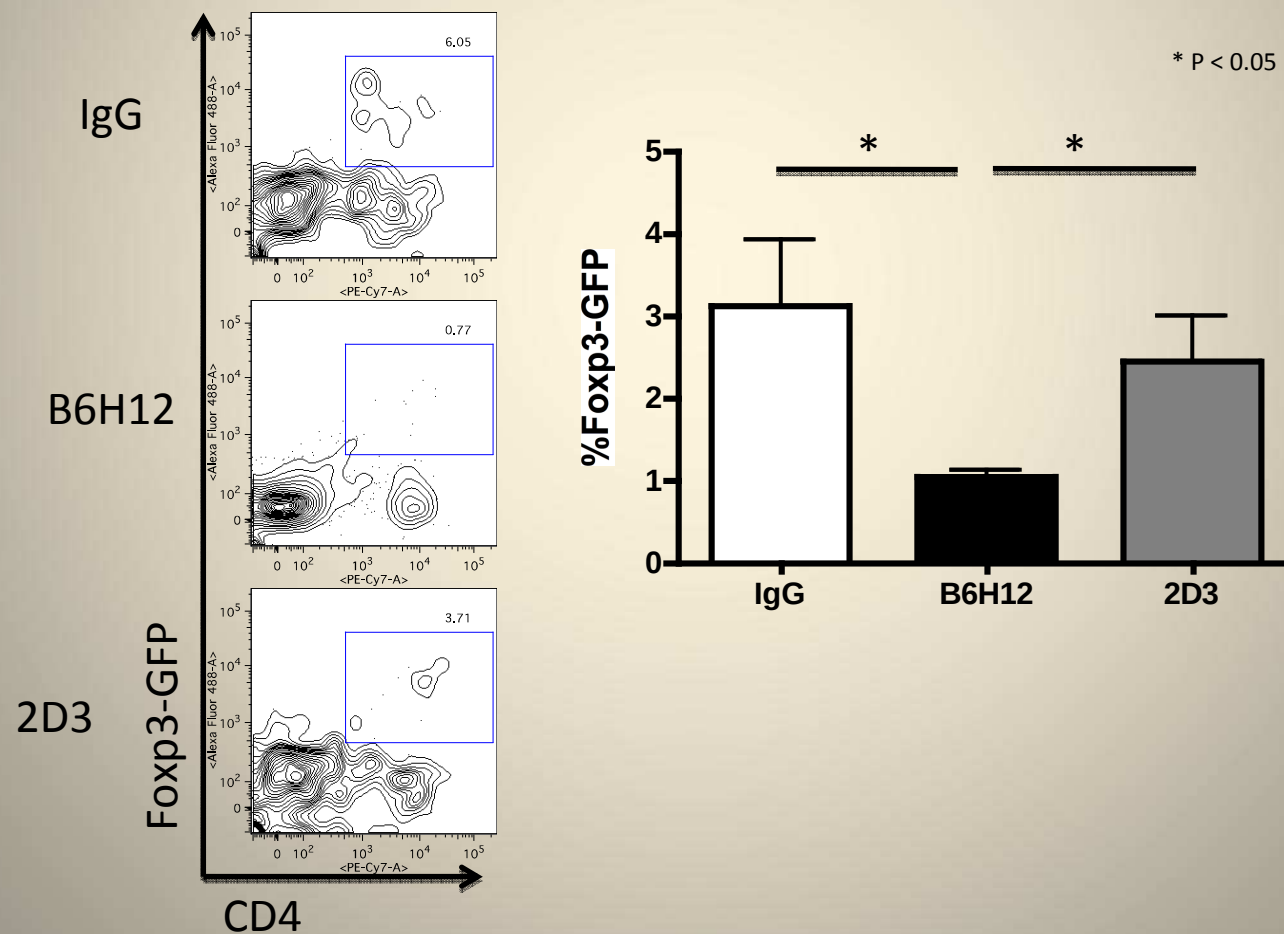
Anti-CD47-mediated phagocytosis of cancer by macrophages do not present antigen to OTII (CD4) T cells



Diane Tseng and
Jens Volkmer

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

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



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

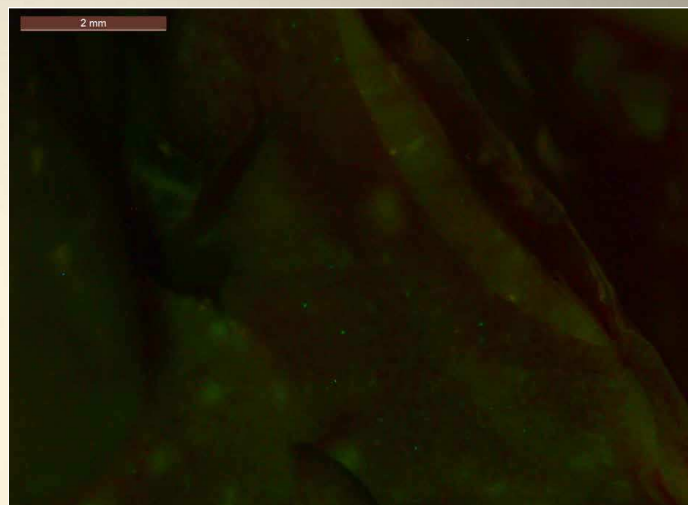
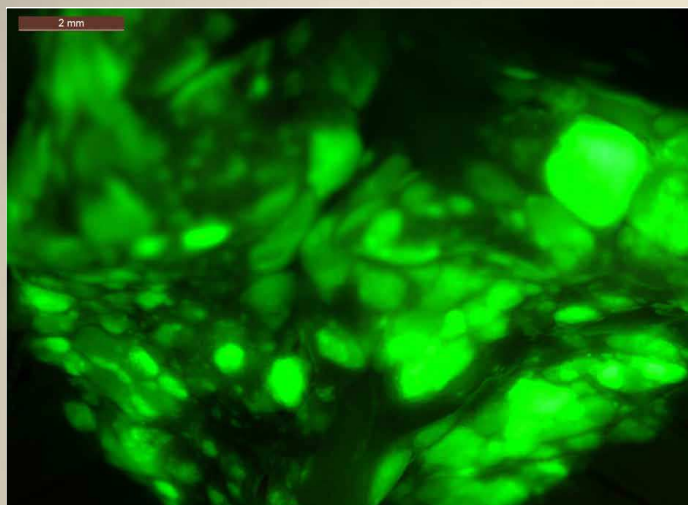
- Paresh Vyas and Alan Burnett have an MRC grant to do part of the trial: single payer.
- IW and Ravi Majeti have a California Institute of Regenerative Medicine grant of \$20 M to take the antibody through phase 1/2 trials. Disease Team of Maureen Howard, Susan Prohaska, Jens Volkmer, Jie Liu, students, MD/PhD trainees to do it.

Hu5F9-G4 inhibits Tumor Growth & eliminates Metastases

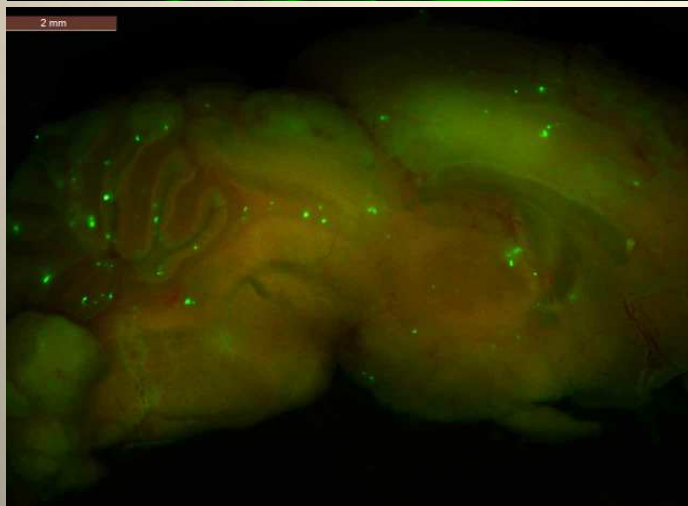
PBS

hu5F9-G4

Lung



Brain



Lokey Stem Cell Institute at Stanford



Institute for Stem Cell Biology and Regenerative Medicine



Ludwig Center at Stanford

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