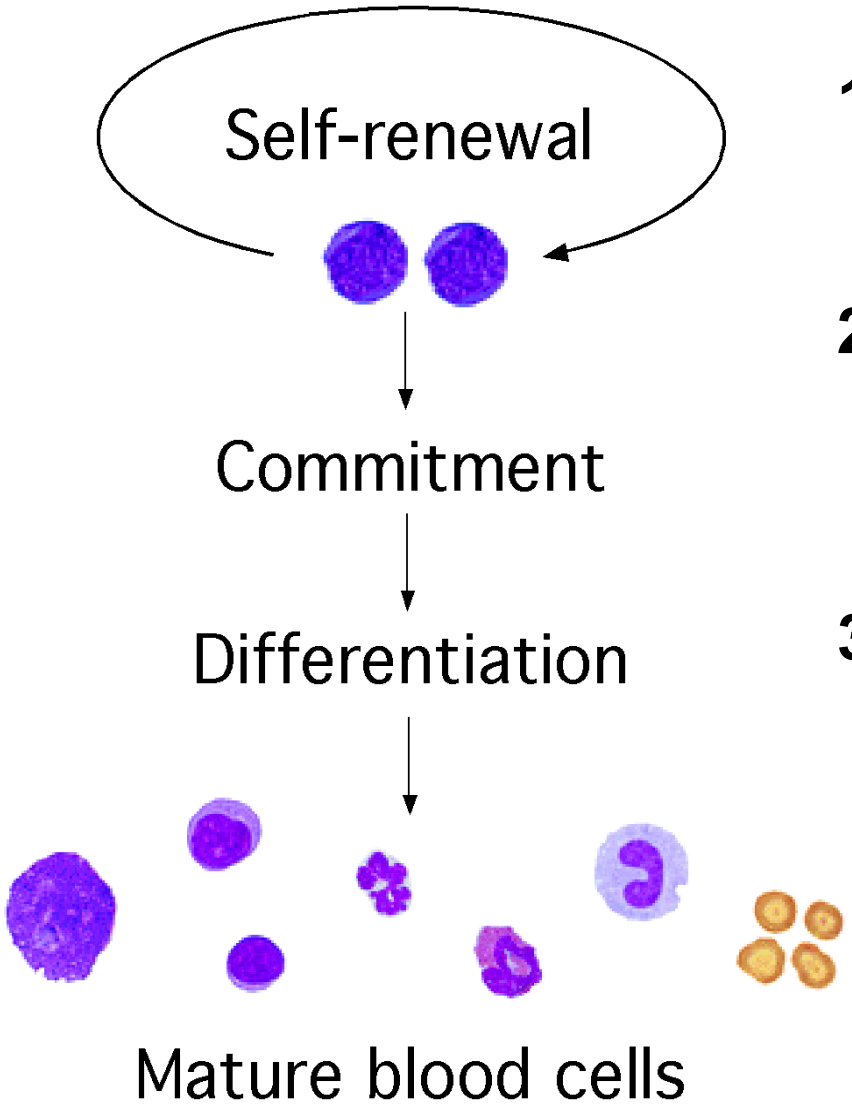
Blood-Forming Stem Cells



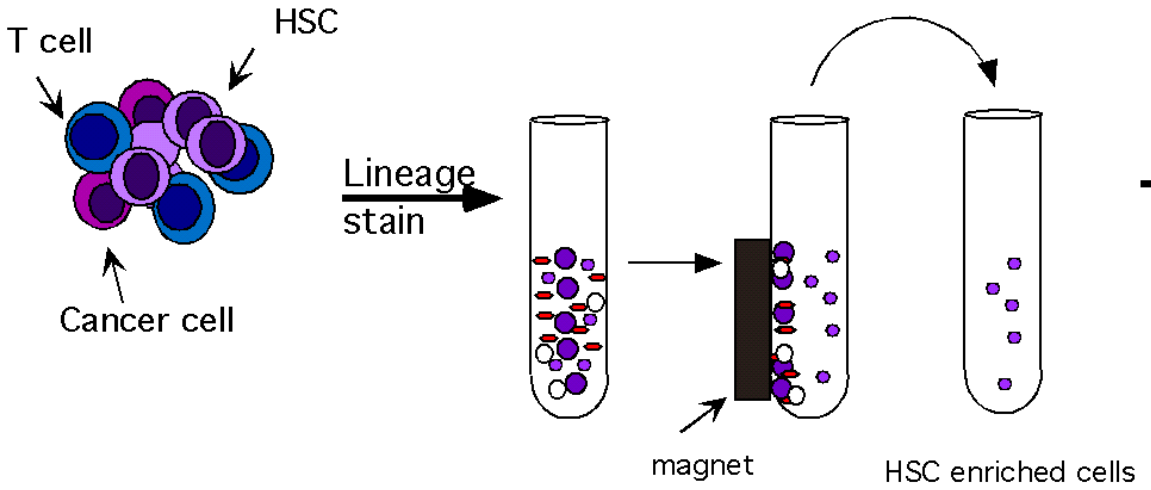
1. **Development**
2. **Maintenance**
3. **Malignant transformation and blood diseases**

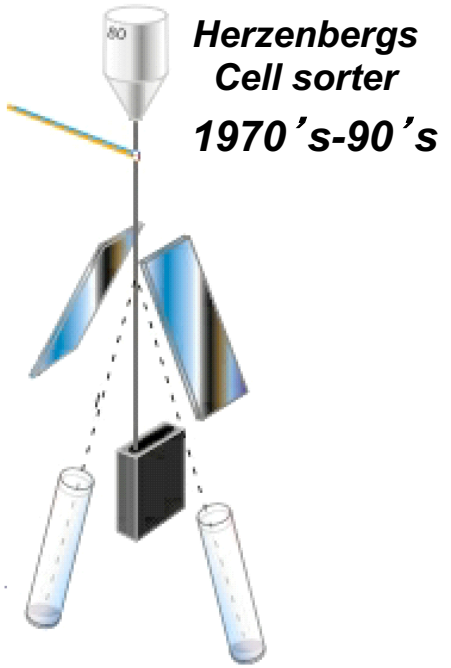
IN VIVO VERITAS

Blood forming stem cells make blood, and only blood

LUDWIG CANCER RESEARCH

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





Marrow or MPB:
Mixture of cells: HSCs, T cells, cancer cells

Deplete mature blood cells by labeling with magnetic antibodies

Pure HSCs for transplantation

Unwanted cells: Cancer cells, T cells

250,000 fold depletion
Cancer cells or T cells

Mouse HSC: ckit+sca1+150+34-flk2-48-Lin-

Human HSC: CD34+90+38loLin-45RA-

Both ~ 1 in 100,000 bone marrow cells

Using self cancer-free blood stem cell transplantation to treat women with metastatic breast cancer

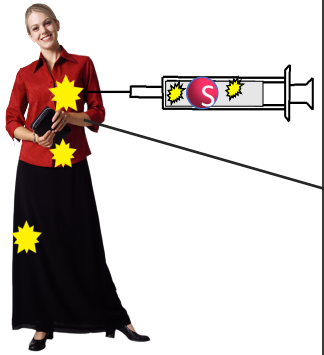
Muller, Kohrt, Shizuru, Negrin, Blume Weissman, et al. 1996-98, published BBMT 2012

ALL PATIENTS GIVEN VERY HIGH DOSE COMBINATION CHEMOTHERAPY TO KILL MORE CANCER

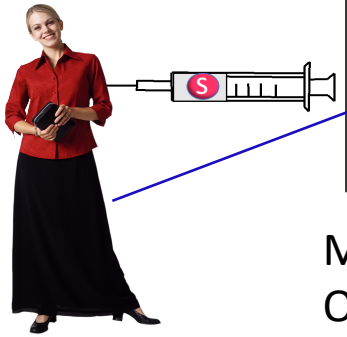
~160,000 women in US with metastatic breast cancer

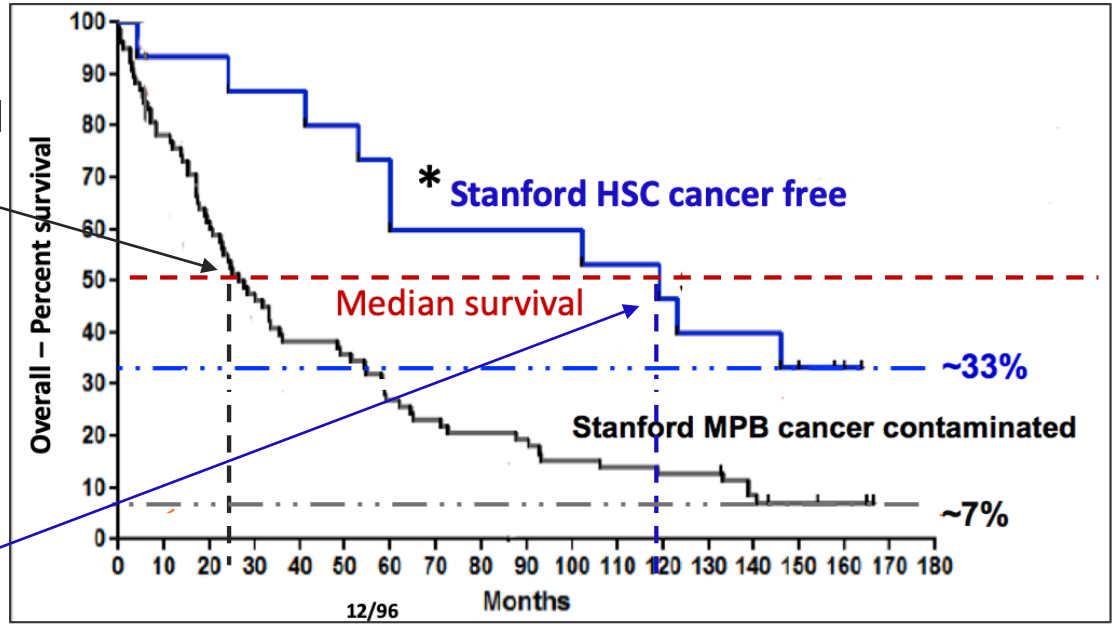
Cisplatin, cytoxan, carmustine

THE PROBLEM
Rescue blood system with unpurified graft; giving **cancer back**



THE SOLUTION
Rescue with **purified cancer free** stem cells





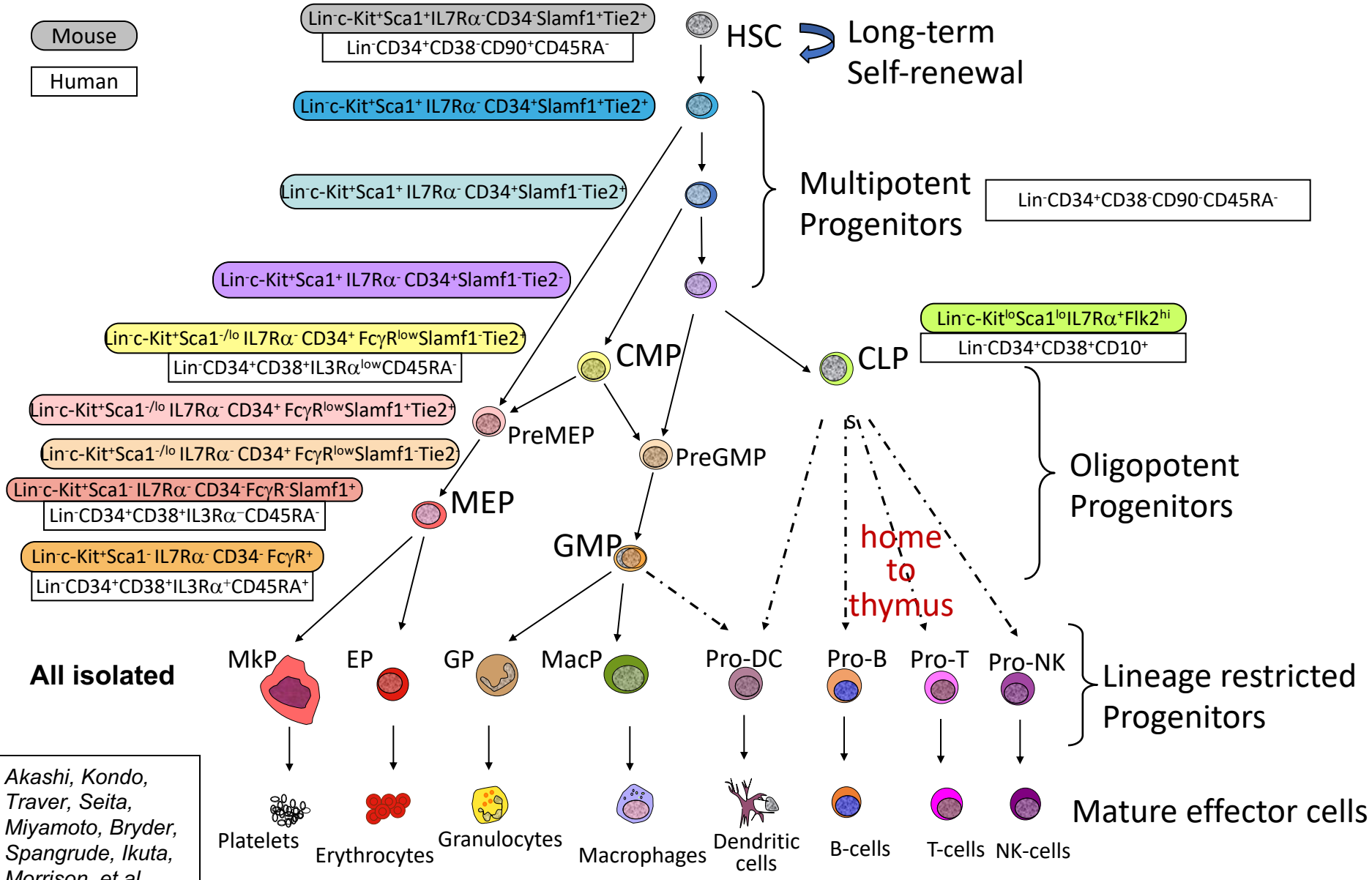
Median survival: **~2 yrs** vs. **10 yrs** with purified stem cells
Overall survival: **7%** vs **33%** with purified stem cells

Estimated MBC remaining after chemo ~ 100K; in models anti-CD47 clears these.

**SyStemix and all of its HSC therapies shut down by purchasing company*

Hematopoietic Hierarchy

Gene Expression Commons: Seita, Sahoo et al. http://gexc.riken.jp

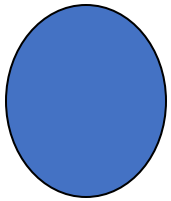


Akashi, Kondo, Traver, Seita, Miyamoto, Bryder, Spangrude, Ikuta, Morrison, et al

Leukemic cells in AML patients

LT STEM CELLS

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



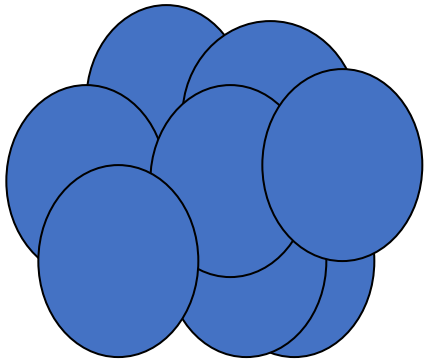
5-40%
AML-1/ETO$^+$

Normal colonies in vitro,
Normal blood
formation in vivo

STHSC:MPP=LSC

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

Leukemia Stem Cells [LSC ~5%]

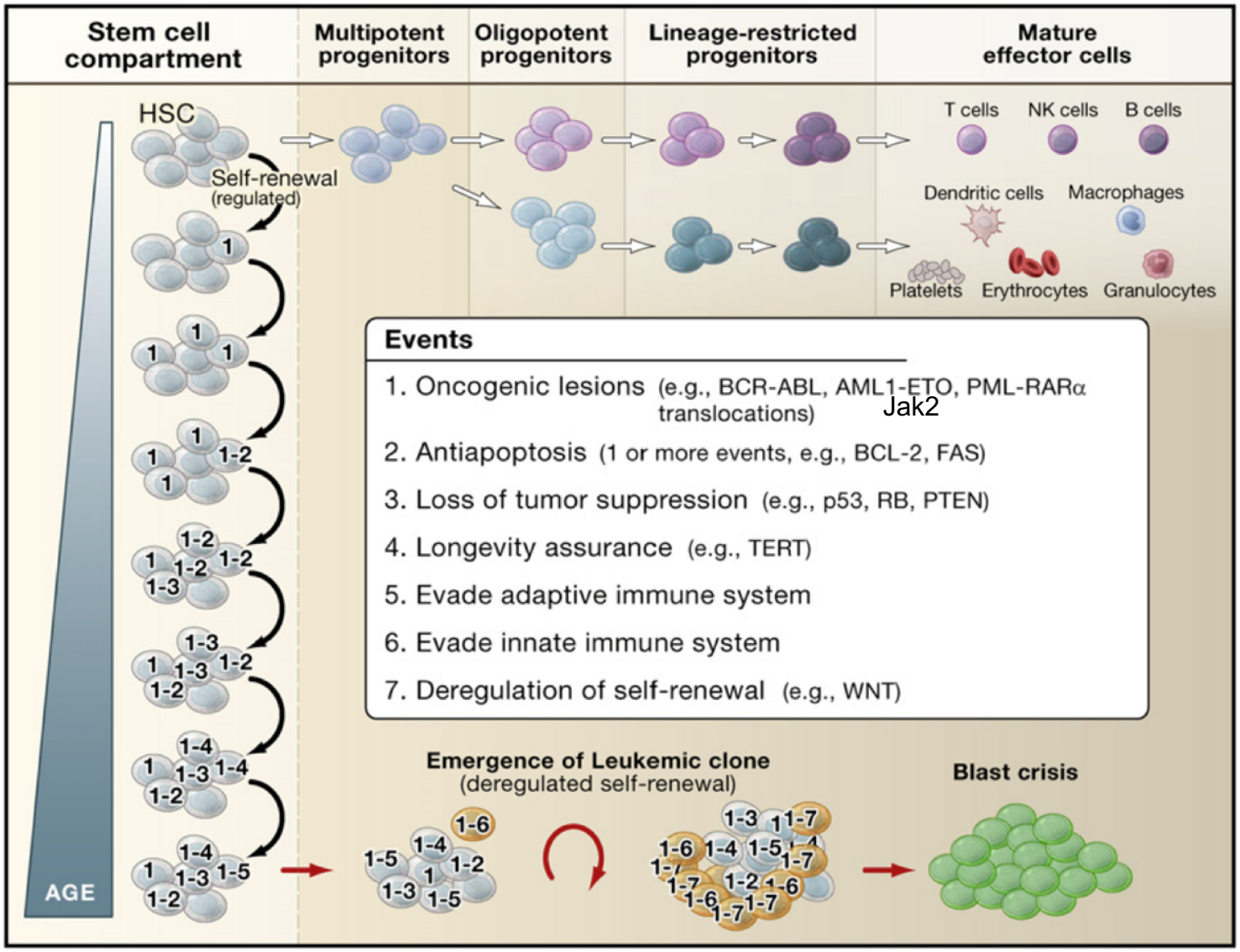


Leukemic blast colonies in vitro.
Leukemia in vivo: blasts cells not leukemia-initiating.

Miyamoto, Weissman, Akashi, PNAS 2000: 97: 6924
Majeti and Weissman 2009

Cell of origin – progression to leukemia

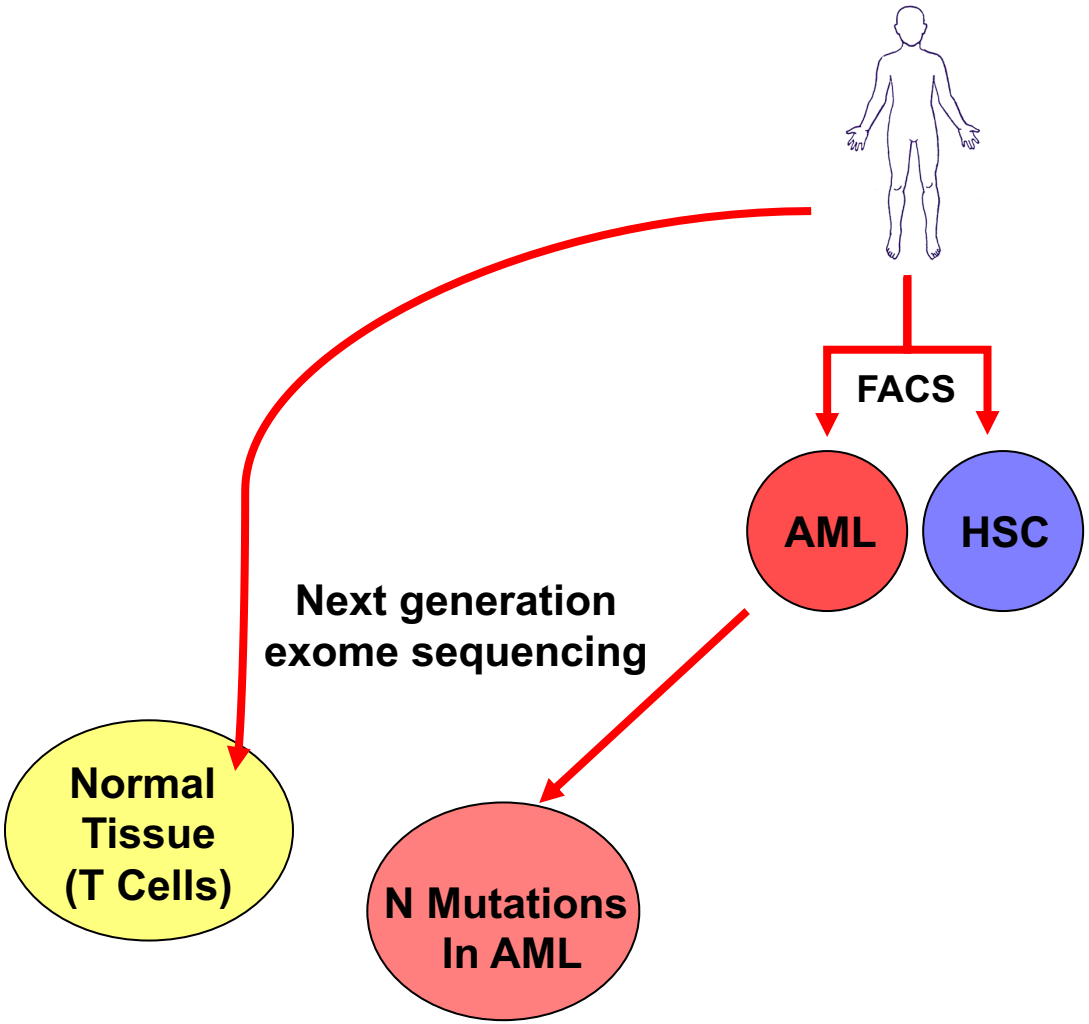
LSC: **MPP** IN AML & **GMP** IN CML Myeloid BLAST CRISIS



Competition!

Traver and IW, 1998, Reya, Morrison, Clarke, and IW, 2001

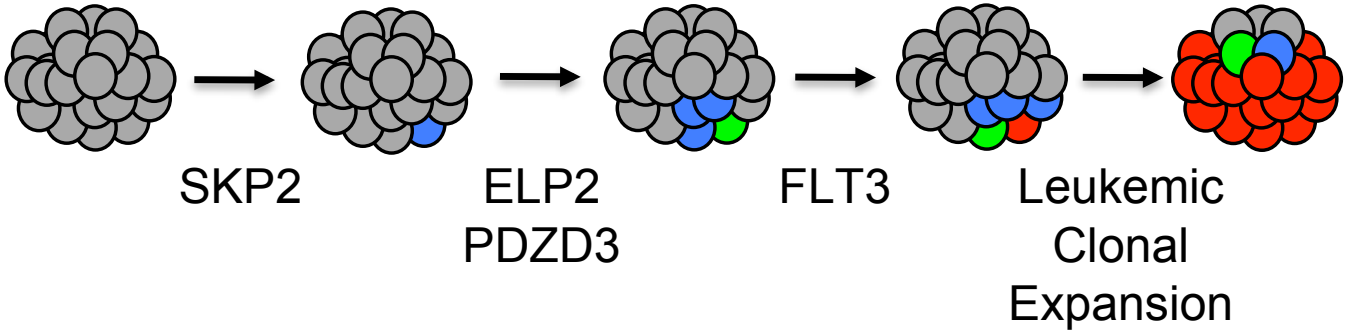
Identification of Somatic Mutations by Exome Sequencing



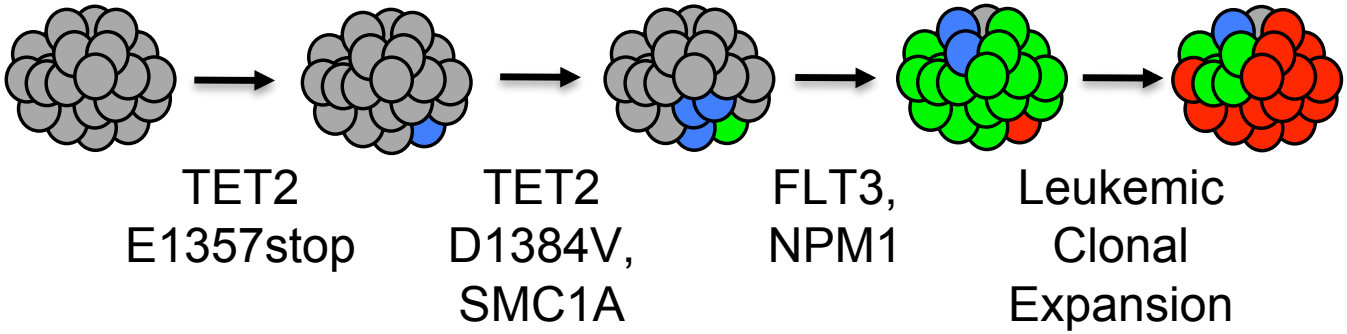
AML	Gene	Annotation
SU070	PXDN	V616I
	KALRN	S44P
	TET2	**Y1649stop**
	TET2	**T1884A**
	TMEM8B	nt G471A
	NCRNA00200	nt G354A
	TMEM20	A143T
	ZRANB1	nt G4659A
	SCN4B	H227N
	GABARAPL1	nt C1583T
	DOCK9	A1475V
	PLAG2G4D	P246A
	CACNA1H	R1069stop
	CTCF	**R339Q**
	GZF1	nt G3835C
	PRPF6	R527H
	CXorf36	I225L
	CXorf66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

Max Jan
Thomas Snyder
Ryan Corces-Zimmerman
Steve Quake
Ravi Majeti
Irv Weissman ***Science Translational Medicine 2012 4****(149): 149ra18.*

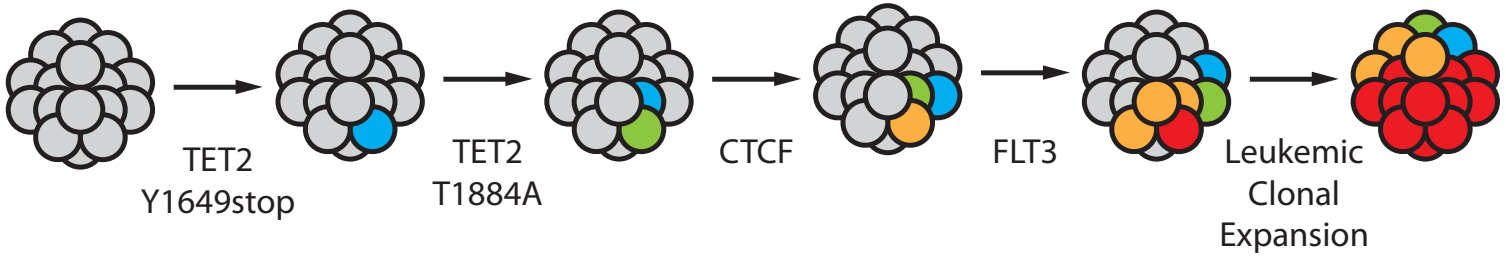
Analysis of Single HSC to Identify Pre-Leukemic Clones



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



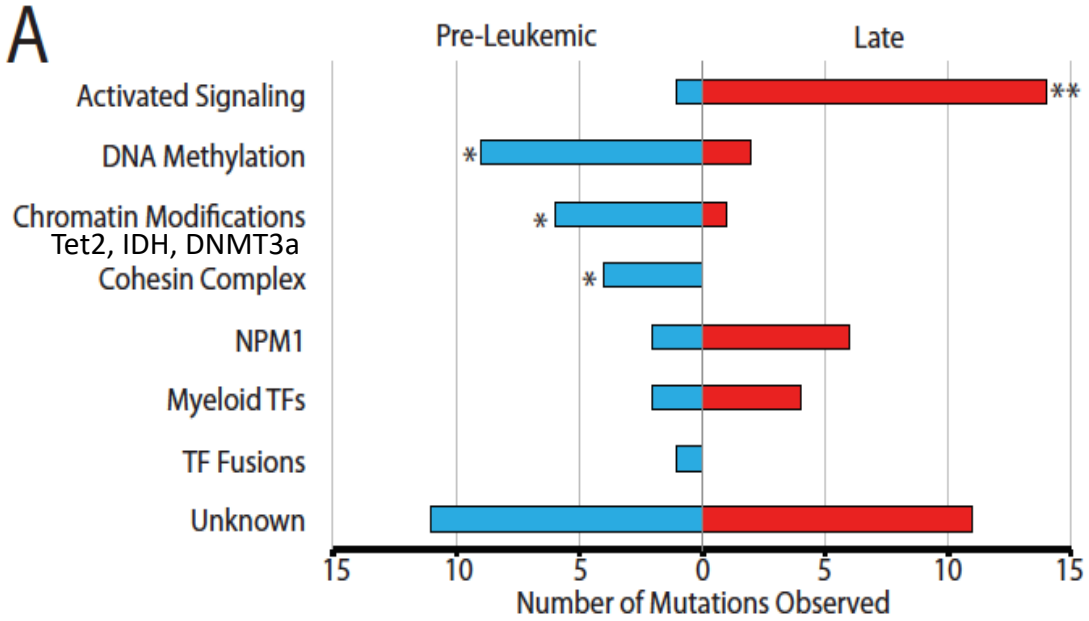
Tet2 LOF, DNMT3a LOF
IDH1/2 change of fxn,
CTCF change of DNAb
Are early HSC events that
Drive clonal expansion

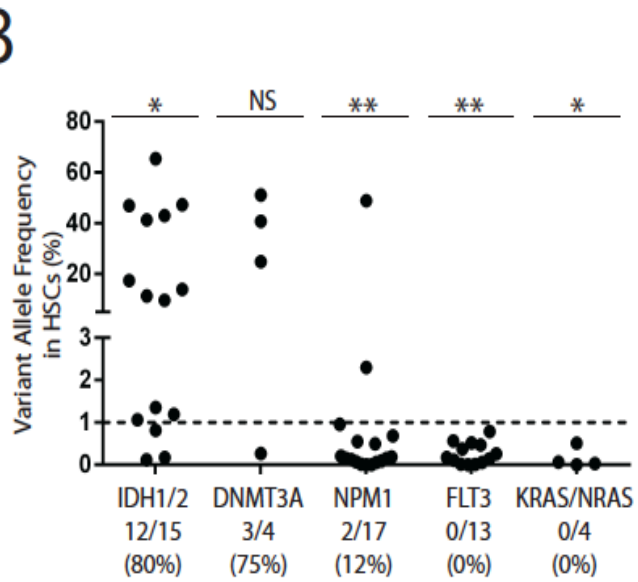


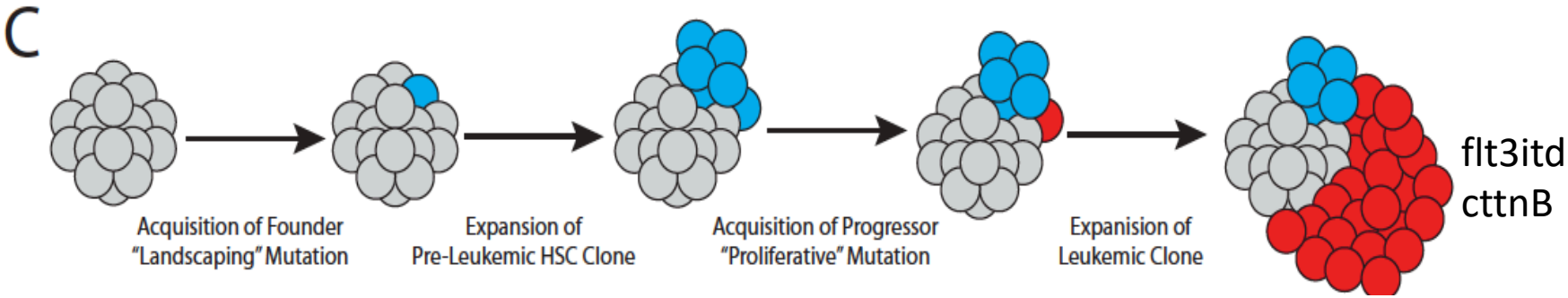
Flt3itd, Cttnb, or leuk ras mutations are at the MPP or GMP stage to give LSC

Normal HSC → **Stages of preleukemia progression in HSC clone** → **LSC at progenitor stage**

If you want to know which genes are likely oncogenes, ask the cancer.







Now data for 21 AMLs

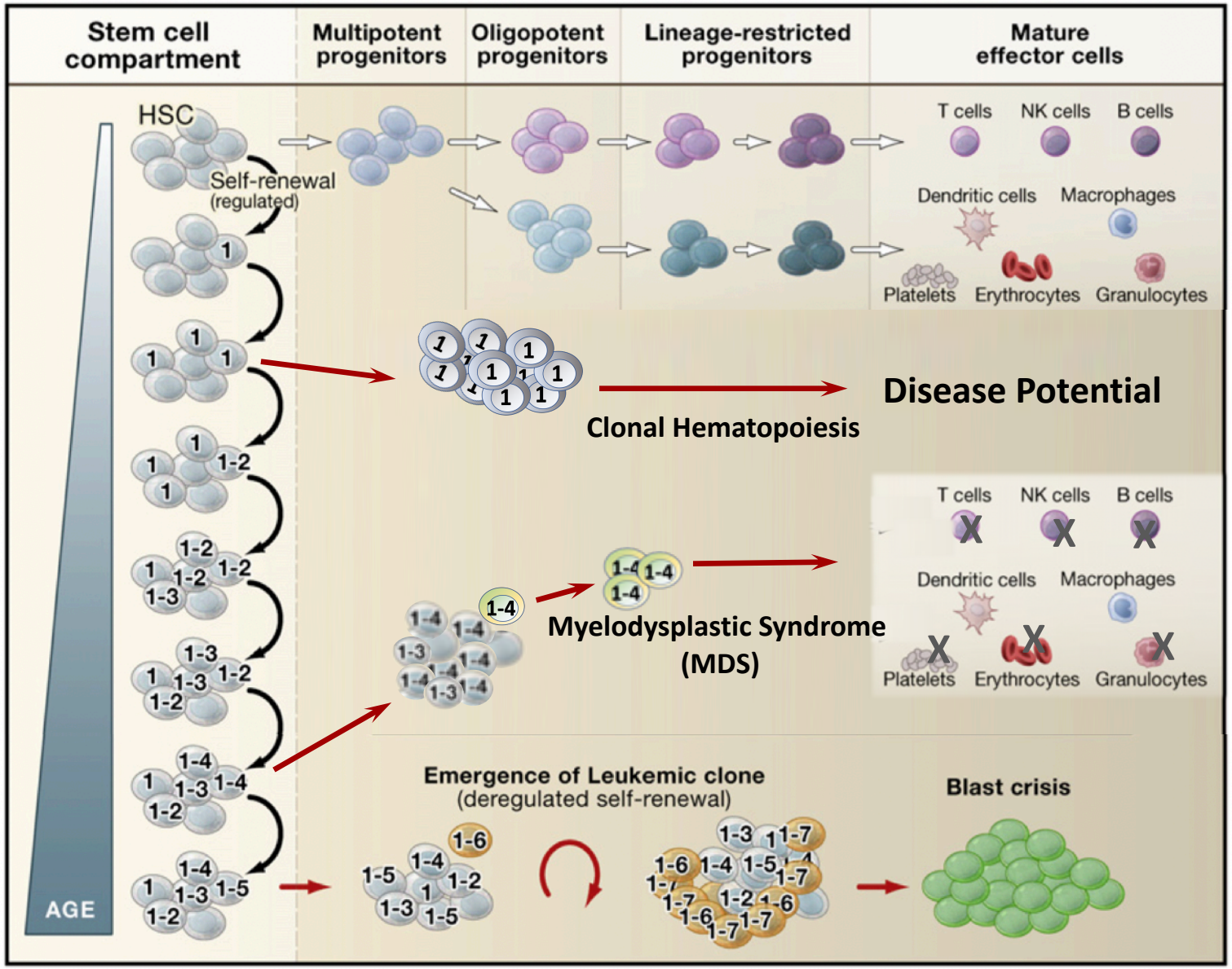
Ryan Corces, Ravi Majeti, and IW, PNAS 2014

Lessons for leukemia and perhaps all cancers

1. In AML, progression is in a blood stem cell clone, while the leukemia stem cells is at the progenitor stage: preleukemic clones of HSC ***compete*** with nl HS
2. There are no leukemias we have found that are leukemias of HSC
3. If this is true for leukemia, it is **probably true for all cancers in tissues that regenerate from tissue stem cells**
4. ***Clonal competition by precancerous stem cells can lead to diseases caused by the mutations/epigenetic changes in the dominant clone;***

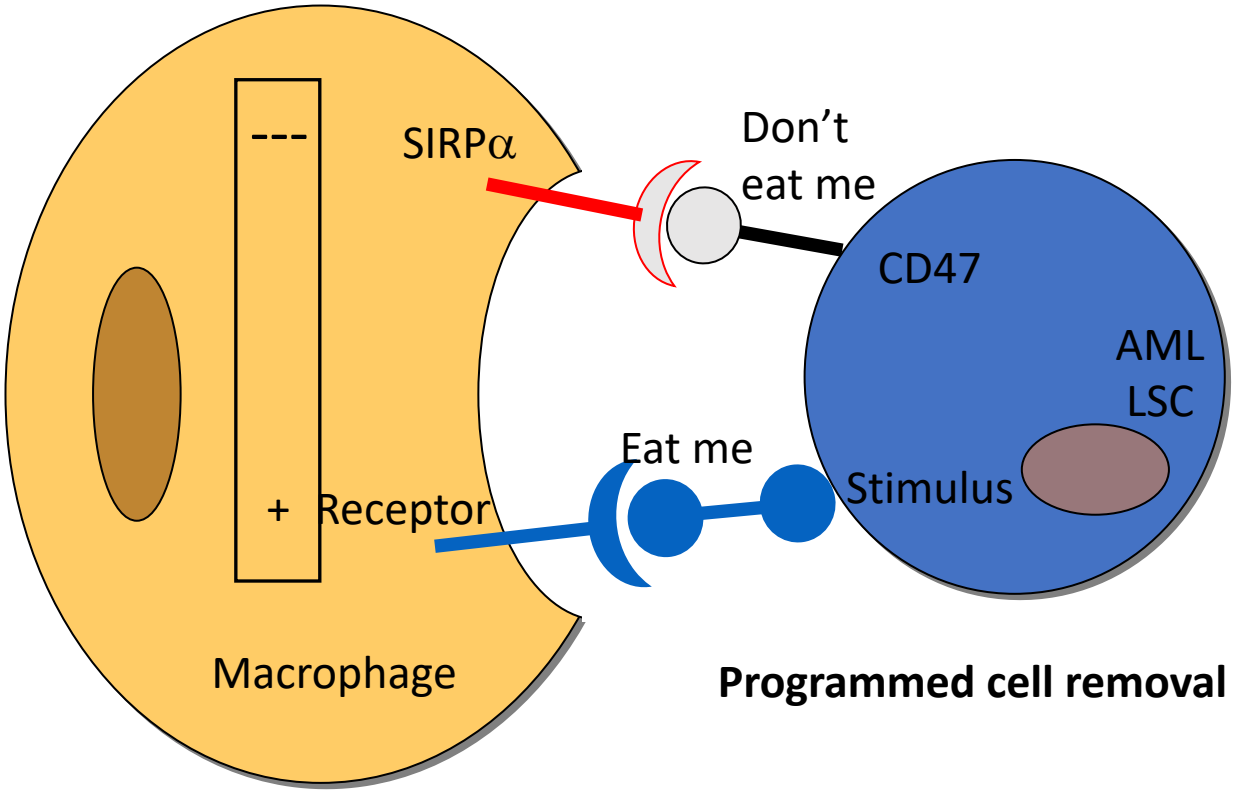
e.g., Pang, Pluvinage, Park IW: MDS;
Jaiswal and Ebert-clonal hematopoiesis of indeterminate potential.
?? Other tissue stem cells and adult onset diseases??

Acute Leukemia Progression Occurs in HSC to make a clone and to generate leukemic stem cells and blood diseases



CD47 was discovered as a marker of aging RBC by Oldenborg. We found it on mouse & human AML LSC

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

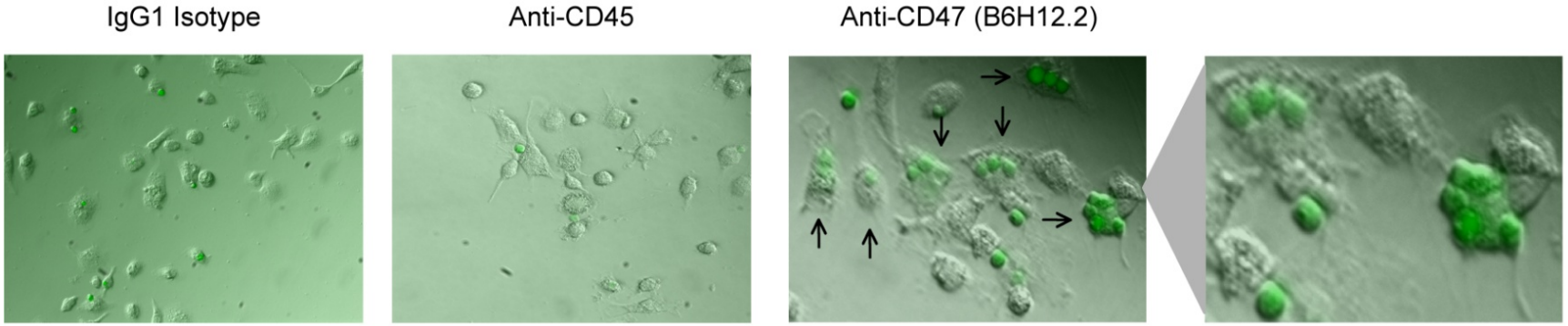


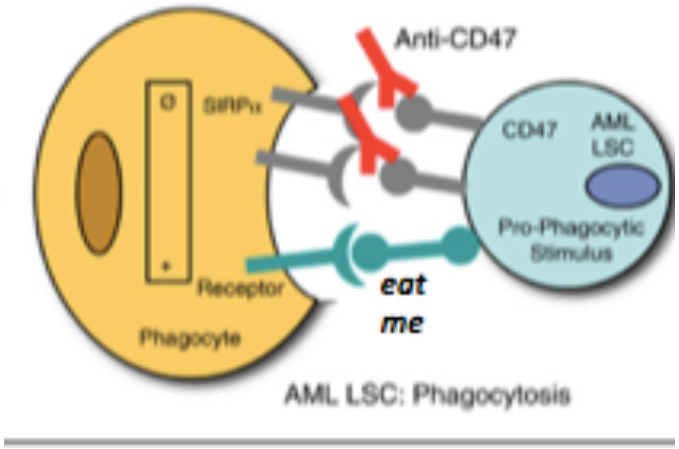
Net Result: No Phagocytosis

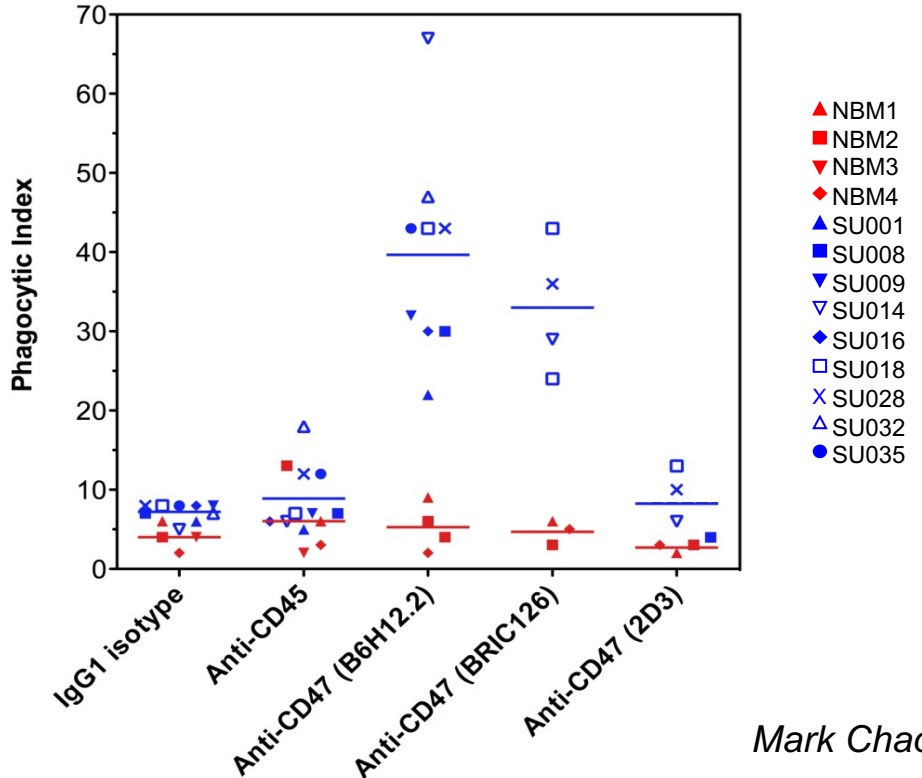
Traver and IW 1998; Jaiswal, Majeti, Chao,and IW 2008.

Anti-CD47 Antibodies Enable Phagocytosis of AML LSC

Human Macrophages

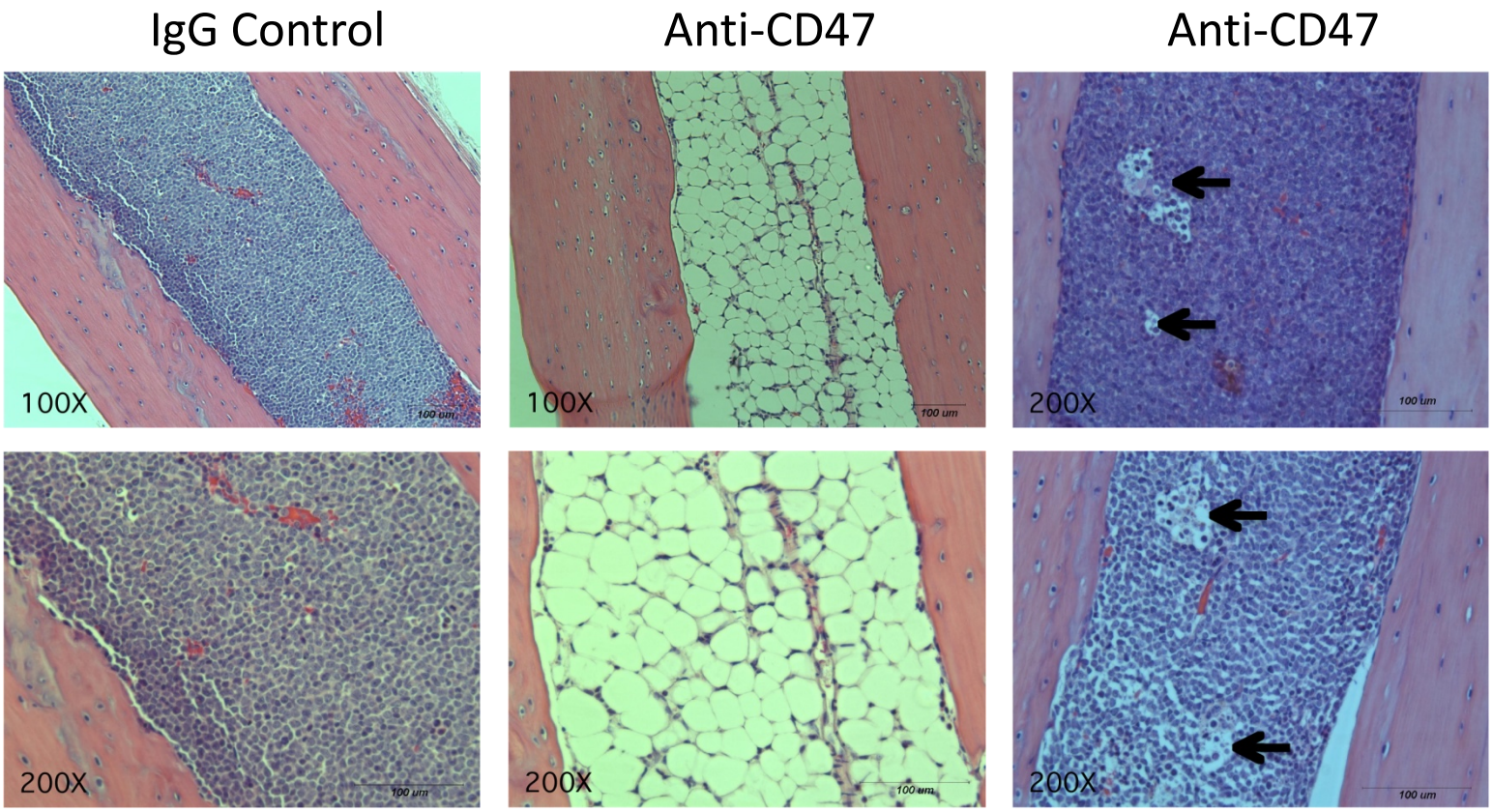






Mark Chao, Majeti et al

Anti-CD47 Antibody Depletes AML in the Bone Marrow

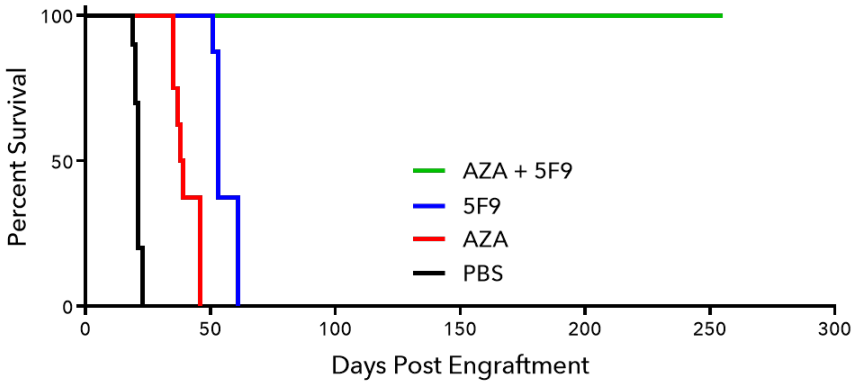


AML clinical trial with humanized anti-CD47 h5F9G4 led by Paresh Vyas at Oxford with the UK AML clinical trials group

Mark Chao, Majeti et al Cell 2009 Cell. **138**(2): 286-99

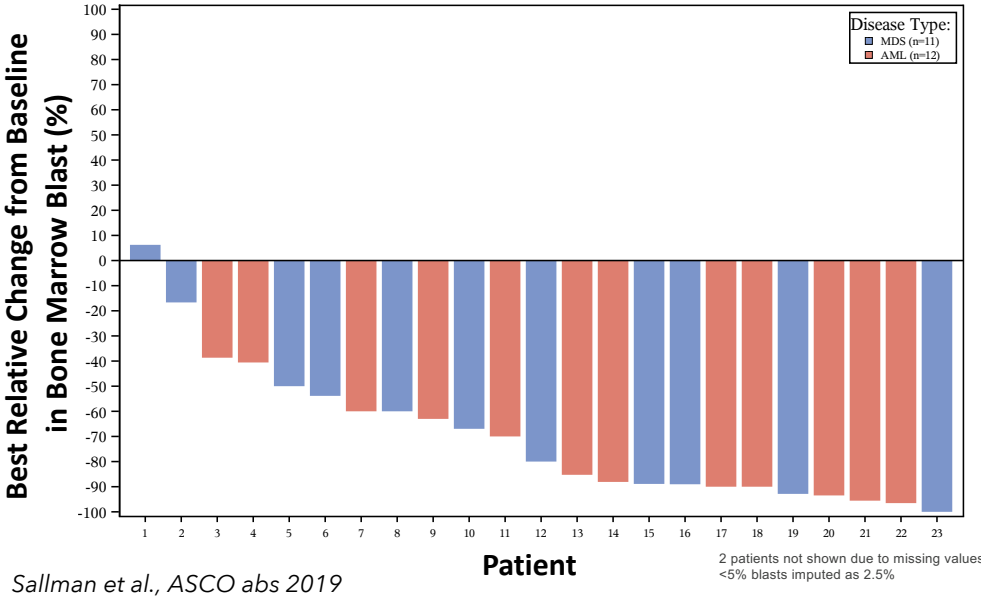
- Anti-Leukemic Activity is Observed with 5F9 Monotherapy and in Combination with Azacitidine in AML and MDS

5F9+Azacitidine in a Leukemic Xenograft Model



Feng et al., ASH abs 2018

5F9+Azacitidine in AML/MDS Patients



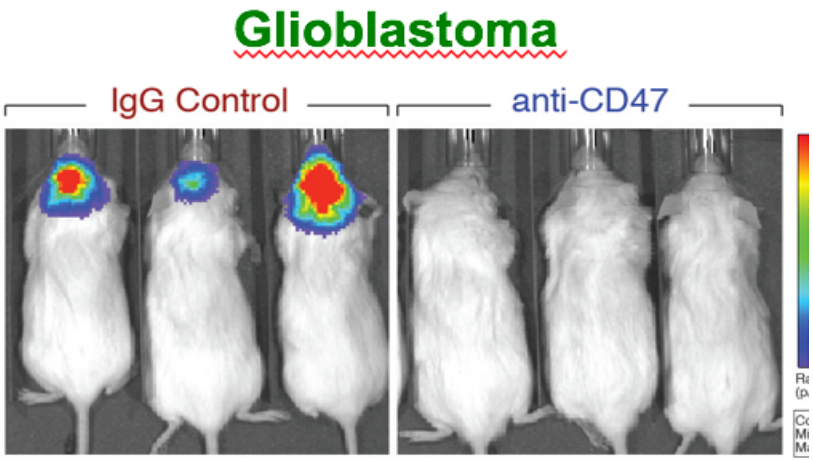
Sallman et al., ASCO abs 2019

- 5F9 synergizes with azacitidine to eliminate leukemic disease in pre-clinical models
- In a Phase 1b clinical trial, 5F9 + azacitidine has a response rate of 64% in untreated AML and 100% response rate in untreated MDS
- A registrational study of 5F9 + azacitidine in untreated MDS for FDA approval is in progress

Anti CD47 Antibody Treats Human Brain Cancers, *and all other patient xenograft cancers in NSG mice*

Stanford MEDICINE

We have tested these human cancers and have similar results



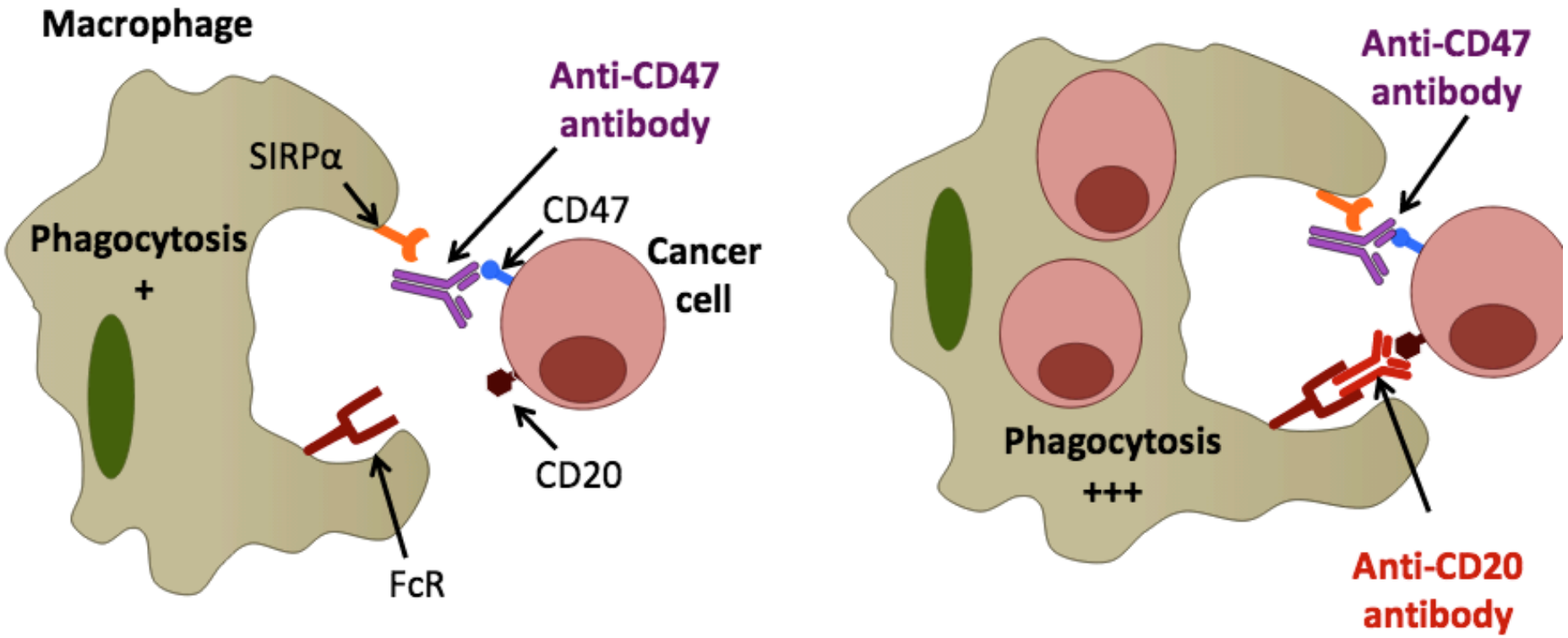
Breast	Glioblastoma
Ovarian	Medulloblastoma
Bladder	Oligodendroglioma
Pancreatic	Hepatocellular Carcinoma
Colon	Gastric Cancer
Prostate	Multiple Myeloma
Lung	Chronic Myeloid Leukemia
Kidney	Acute Myeloid Leukemia
Leiomyosarcoma	Non-Hodgkin's Lymphoma
Head & Neck	T-Acute Lymphoblastic Leukemia
Melanoma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

- For all tumors, small tumors and metastases are more easily cleared than large tumors.
- CD47 is the dominant don't eat me signal, but others exist

DISCLAIMER: ***November 30, 2015, Stanford licenses anti-CD47 IP to Forty Seven Inc; cofounders include I Weissman, R Majeti, J Volkmer, MP Chao***

Anti-CD47 Antibody Combination Therapy with Cancer-Targeting Monoclonal Antibodies

Improving the 'eat me' signal

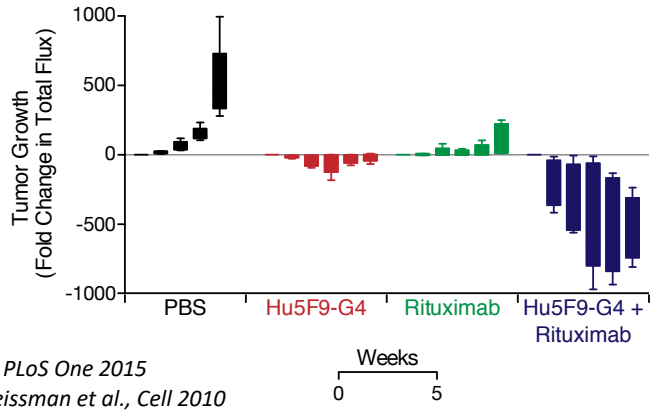


- **Combination with other cancer-targeting antibodies enhances phagocytosis of cancer cells and provide treatment to patients that are currently non eligible (e.g. rituximab - anti-CD20; cetuximab - anti-EGFR; trastuzumab - anti-HER2)**

Chao, M, Alizadeh, A, Kohrt, H, Majeti, R, and I. Weissman

Clinical Evidence of 5F9 + Rituximab Efficacy in Patients with Non-Hodgkin's Lymphoma

5F9+Rituximab in a Lymphoma Xenograft Model



Liu et al., PLoS One 2015
Chao, Weissman et al., Cell 2010

The NEW ENGLAND JOURNAL of MEDICINE

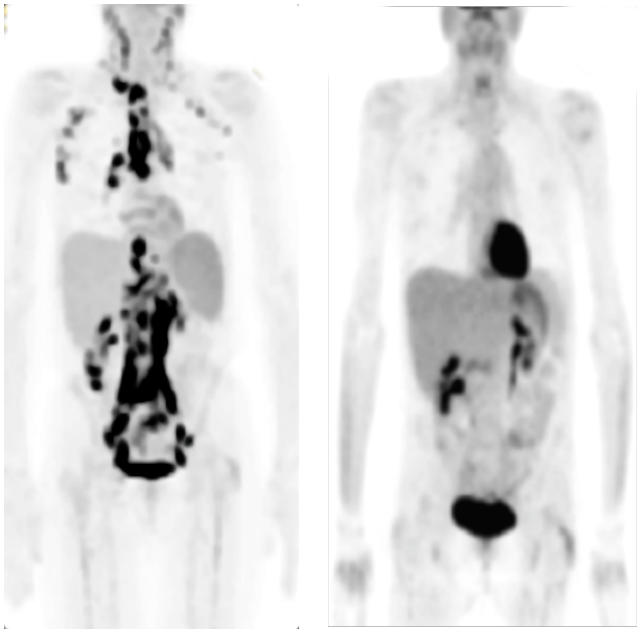
ORIGINAL ARTICLE

CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma

Ranjana Advani, M.D., Ian Flinn, M.D., Ph.D., Leslie Popplewell, M.D., Andres Forero, M.D., Nancy L. Bartlett, M.D., Nilanjan Ghosh, M.D., Ph.D., Justin Kline, M.D., Mark Roschewski, M.D., Ann LaCasce, M.D., Graham P. Collins, M.D., Thu Tran, B.S., Judith Lynn, M.B.A., James Y. Chen, M.D., Ph.D., Jens-Peter Volkmer, M.D., Balaji Agoram, Ph.D., Jie Huang, Sc.D., Ravindra Majeti, M.D., Ph.D., Irving L. Weissman, M.D., Chris H. Takimoto, M.D., Ph.D., Mark P. Chao, M.D., Ph.D., and Sonali M. Smith, M.D.

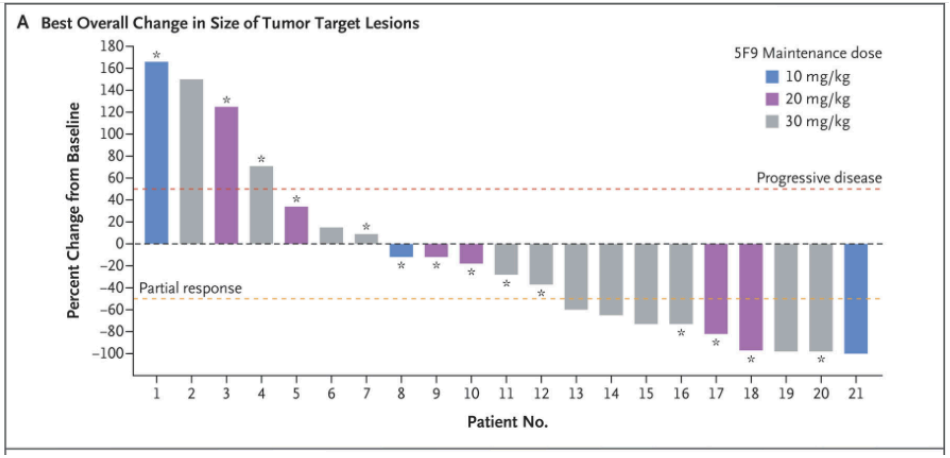
Follicular Lymphoma Patient (Complete Remission)

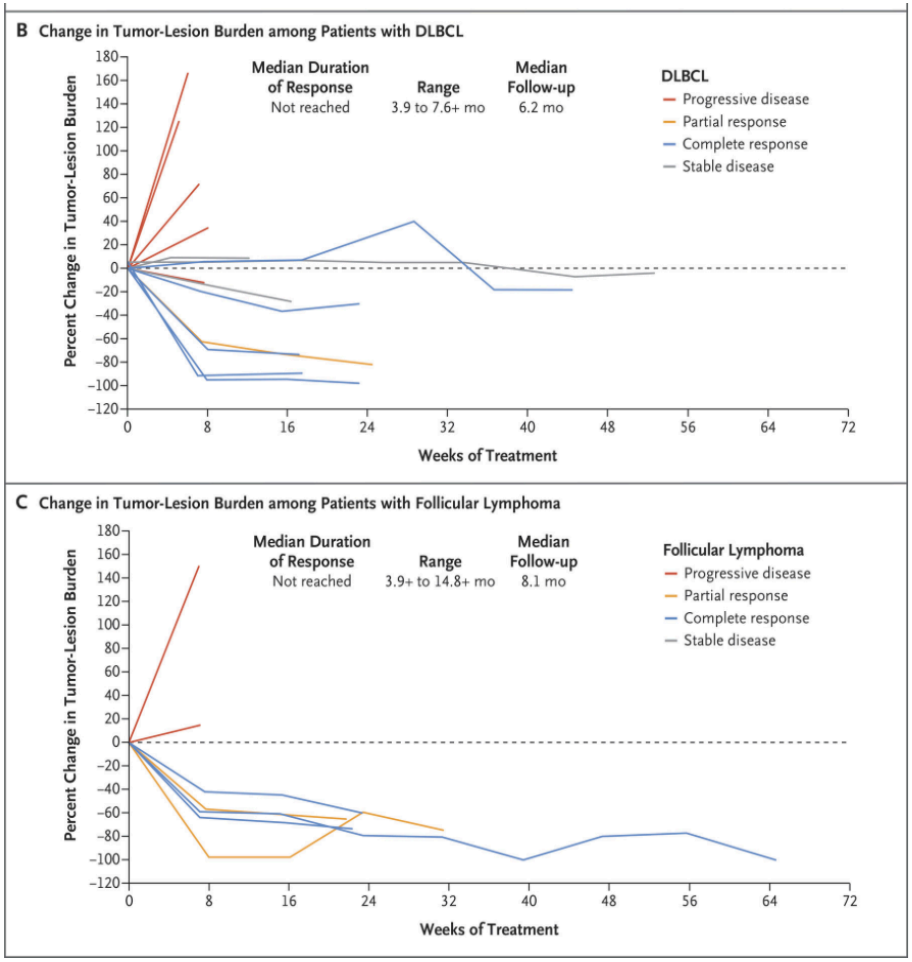
Baseline | Response at 8 wks



- 66F with follicular lymphoma
- Ten prior therapies, bulky disease
- Complete response at 8 weeks

Anti-CD47 synergy with Rituximab in refractory NHL:





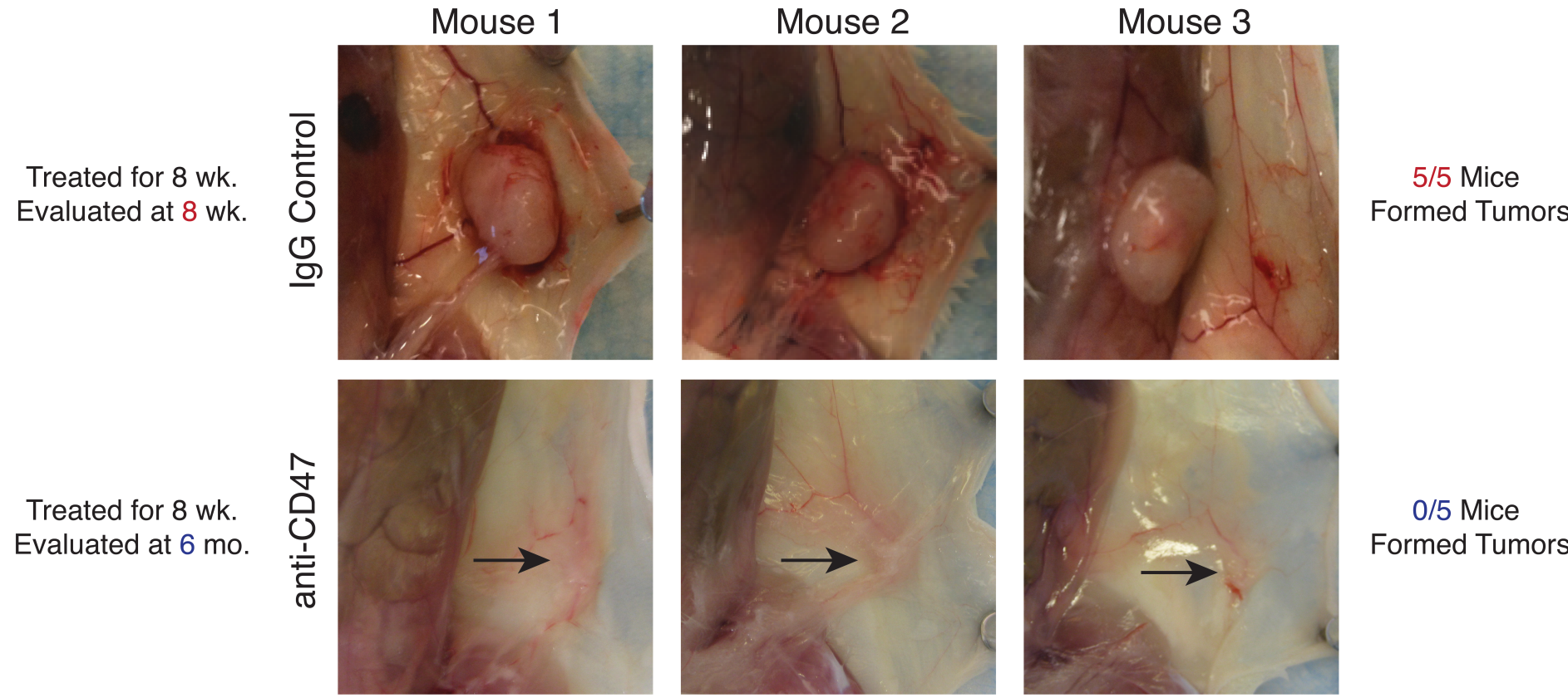
Now 3 more 'don't eat me Surface molecules and Macrophage receptors:

- PDL1:PD1 *Gordon/Maute/IW*
- MHC-b2m: LILRB1 *Barkal/Maute/IW*
- CD24: siglec 10; *Barkal/IW*

Advani et al, NEJM 2018

Anti-CD47 Antibodies Inhibit Growth of Xenotransplanted Patient Tumors

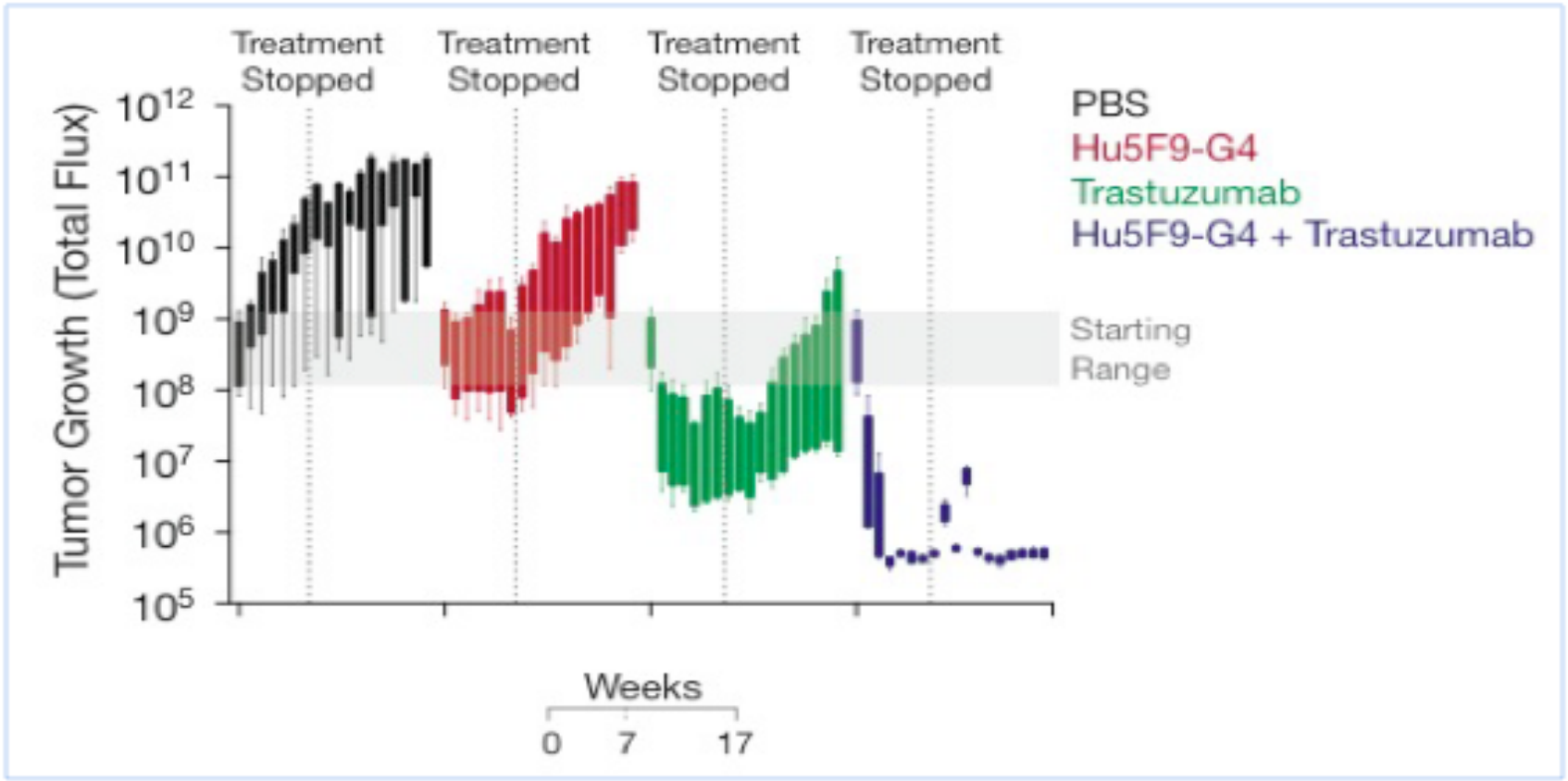
Breast Cancer



Think about cancer-free HSC: ?postransplant anti-CD47??

Willingham, Volkmer, Clarke, Scheeren, IW

Synergy between 5F9g4 and Trastuzimab for breast cancer



Can metastatic breast cancer be cured in most patients?

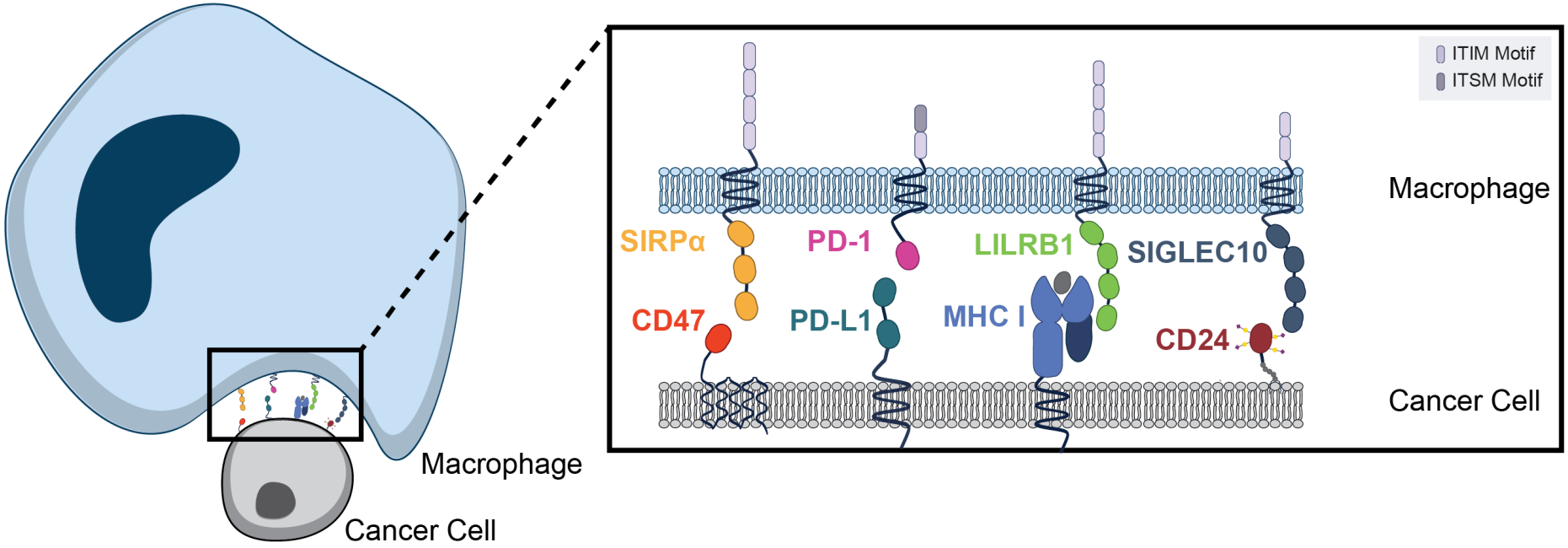
The bulk of breast cancer cells are removed by chemo+pure HSC;

Can this antibody alone or in combination change metastatic breast cancer outcomes?

Willingham, Volkmer, Jie, Majeti, Weissman et al

There are four innate immune checkpoints

Amira Barkal
MD/PhD Student



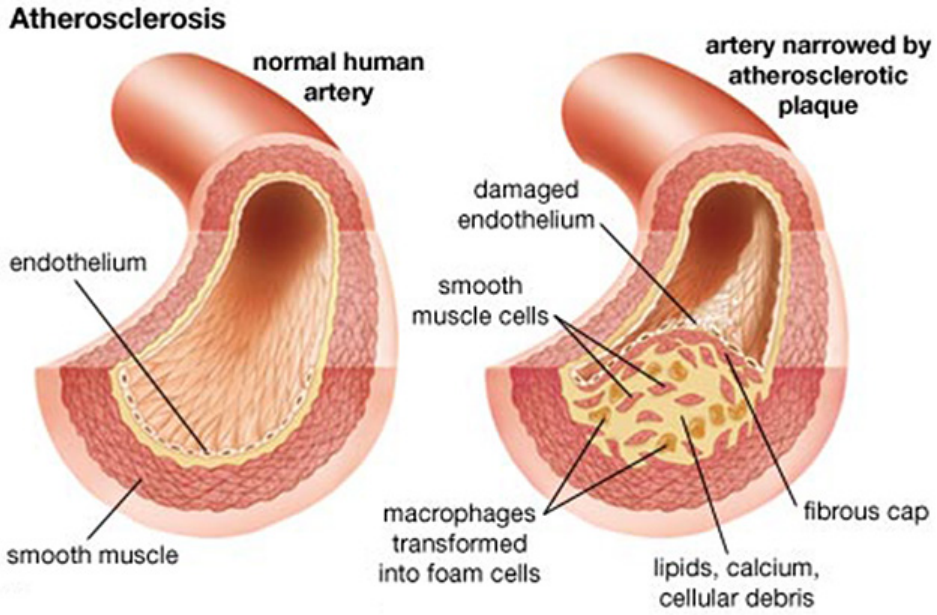
Barkal et al, Nature 2019

Other diseases in which CD47 and Calreticulin are involved

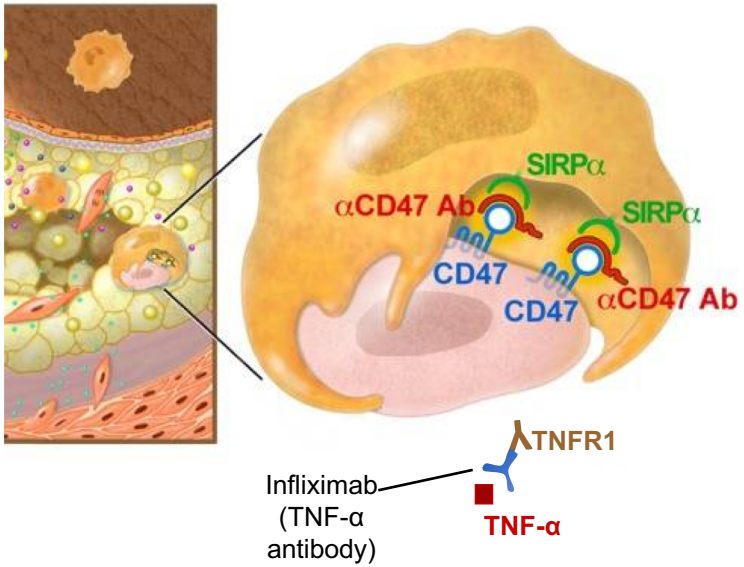
- *G Wernig and IW:* Mouse and human **fibrotic diseases** that include idiopathic pulmonary fibrosis, scleroderma, NASH, and renal fibrosis. Anti-CD47 is therapeutic in mouse model.PNAS 2017.
- *N Leeper, Y Kojima, and IW:* Mouse and human **atherosclerosis**, in which the oxidated LDL damages arterial smooth muscle cells, a subclone of which proliferates to narrow the blood vessel lumen, signal macrophage entry, but express CD47 to block the CalR eat me pathway. Anti CD47 is therapeutic. Nature 2016
- *J Tsai, Y Rinkevich, and IW:* Pathological surgery induced **peritoneal adhesions**. Anti-CD47 + anti MSLN is therapeutic. Science Trans Med.2018
- *LB Torrez-Dulgoff, S.Kumar [FDA]:* **Cerebral Malaria**; submitted
- *K. Hasenkrug, M. Tal, C. Stoddart, LBTorrez, M.McCune:* Cells **persistently infected** with pathogenic viruses induce CD47 expression
- *M.Tal, LB Torrez, P. Ying Yu:* Aspergillus, **Borrelia Bergdorfii**, and **Mycobacterium Tuberculosis** all make CD47 mimics

Clonal Expansion of Smooth Muscle Cells During Atherosclerosis Uses CD47 to Avoid Phagocytosis

http://anatomyandphysiologyi.com/atherosclerosis/



With anti-CD47 therapy

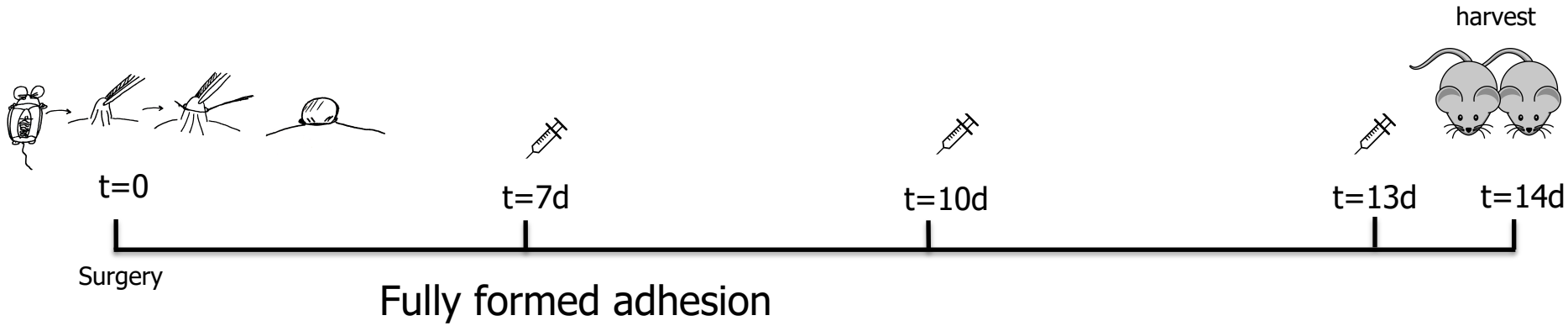


CD47–blocking antibodies restore phagocytosis and prevent atherosclerosis

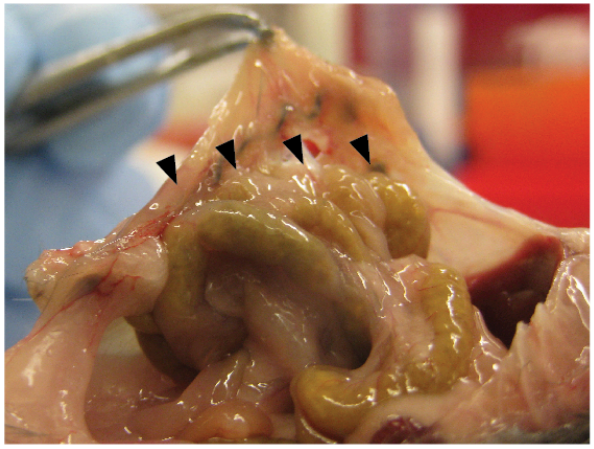
Yoko Kojima[1], Jens-Peter Volkmer[2], Kelly McKenna[2], Mete Civelek[3], Aldons Jake Lusis[3], Clint L. Miller[4], Daniel Direnzo[1], Vivek Nanda[1], Jianqin Ye[1], Andrew J. Connolly[5], Eric E. Schadt[6], Thomas Quertermous[4], Paola Betancur[2], Lars Maegdefessel[7], Ljubica Perisic Matic[8], Ulf Hedin[8], Irving L. Weissman[2] & Nicholas J. Leeper[1,4]

Kojima et al., Nature. 2017

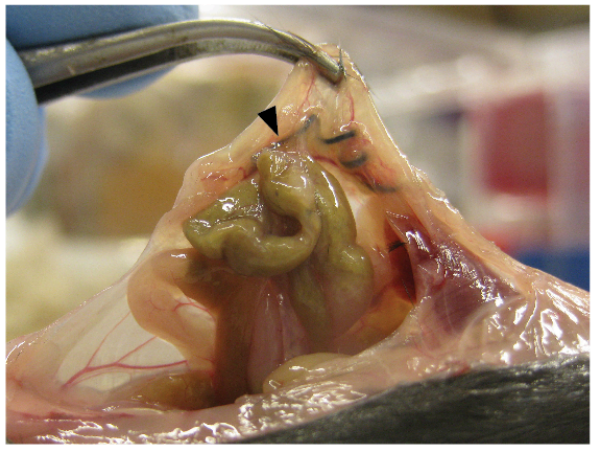
Anti-MSLN and anti-CD47 therapy diminishes preformed adhesions

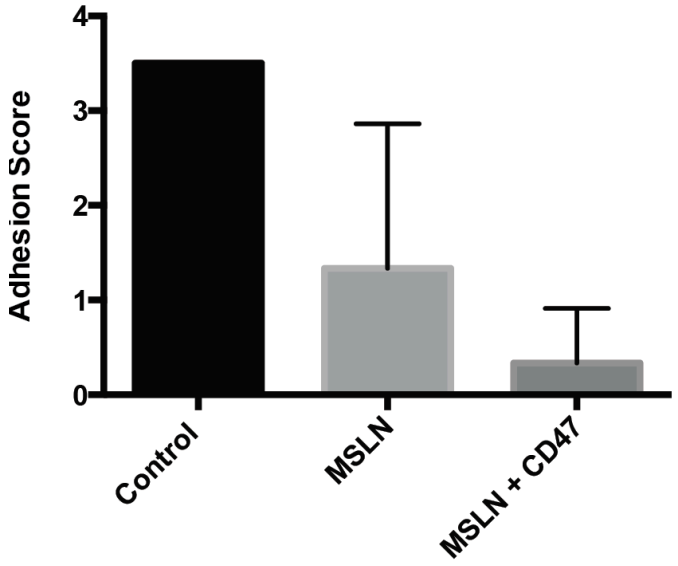


Control



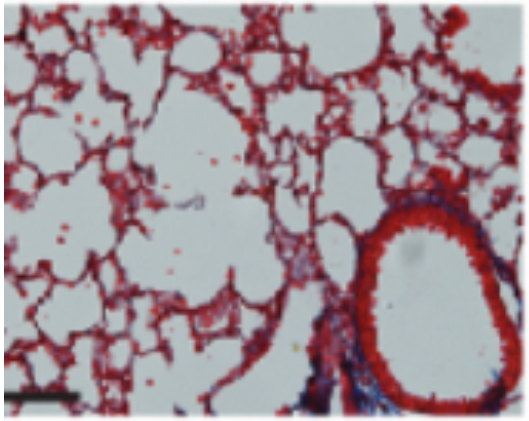
MSLN antibody



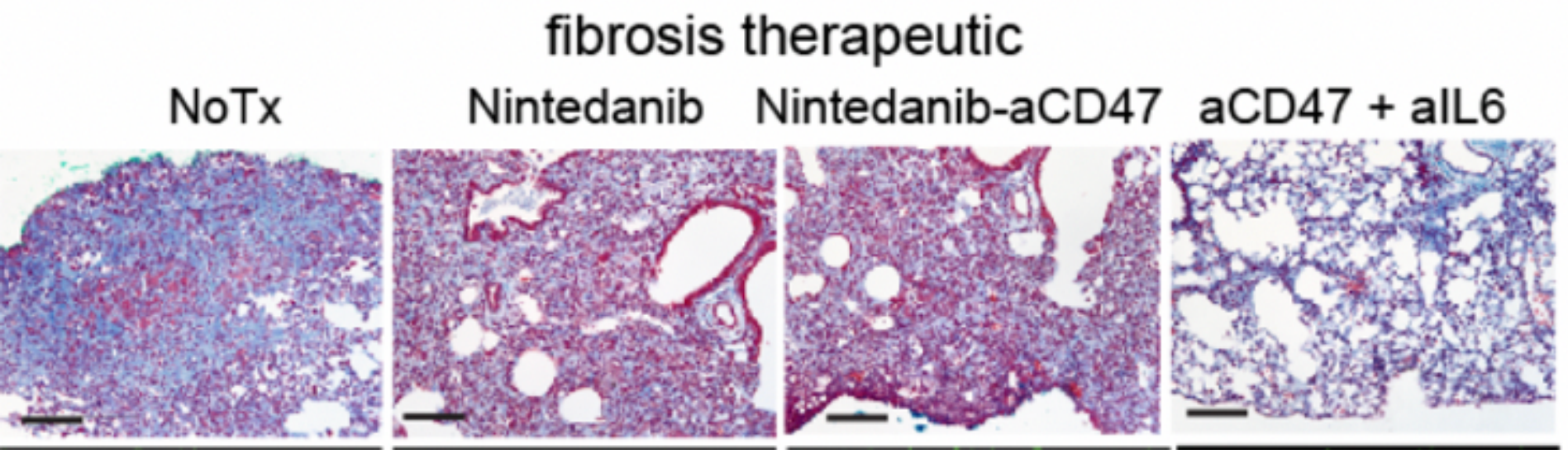


Pulmonary Fibrosis: Incurable ?

Normal lung

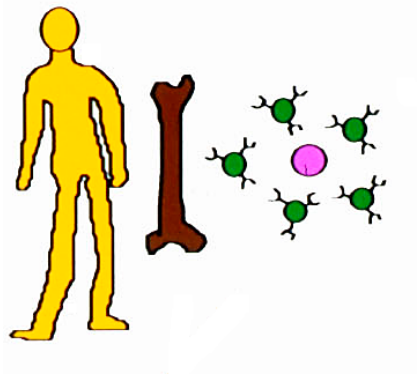


Idiopathic Pulmonary Fibrosis

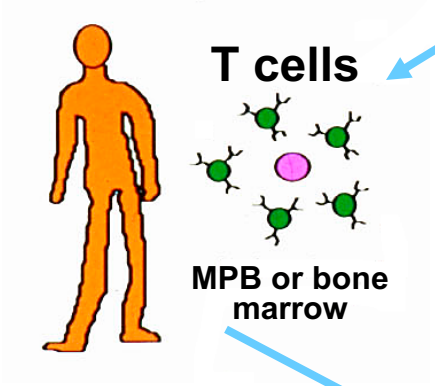


Gerlinde Wernig and team + IW

Healthy Donor Transplant: HSC are the platform for regenerative medicine

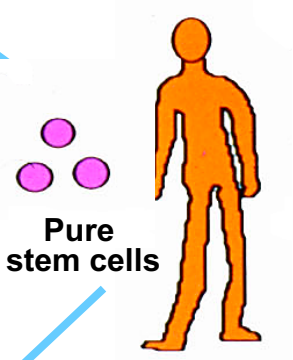


Recipient



RADIATION DAMAGE OR THERAPY

Recipient



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

Recipient

Graft vs Host Disease

Recipient

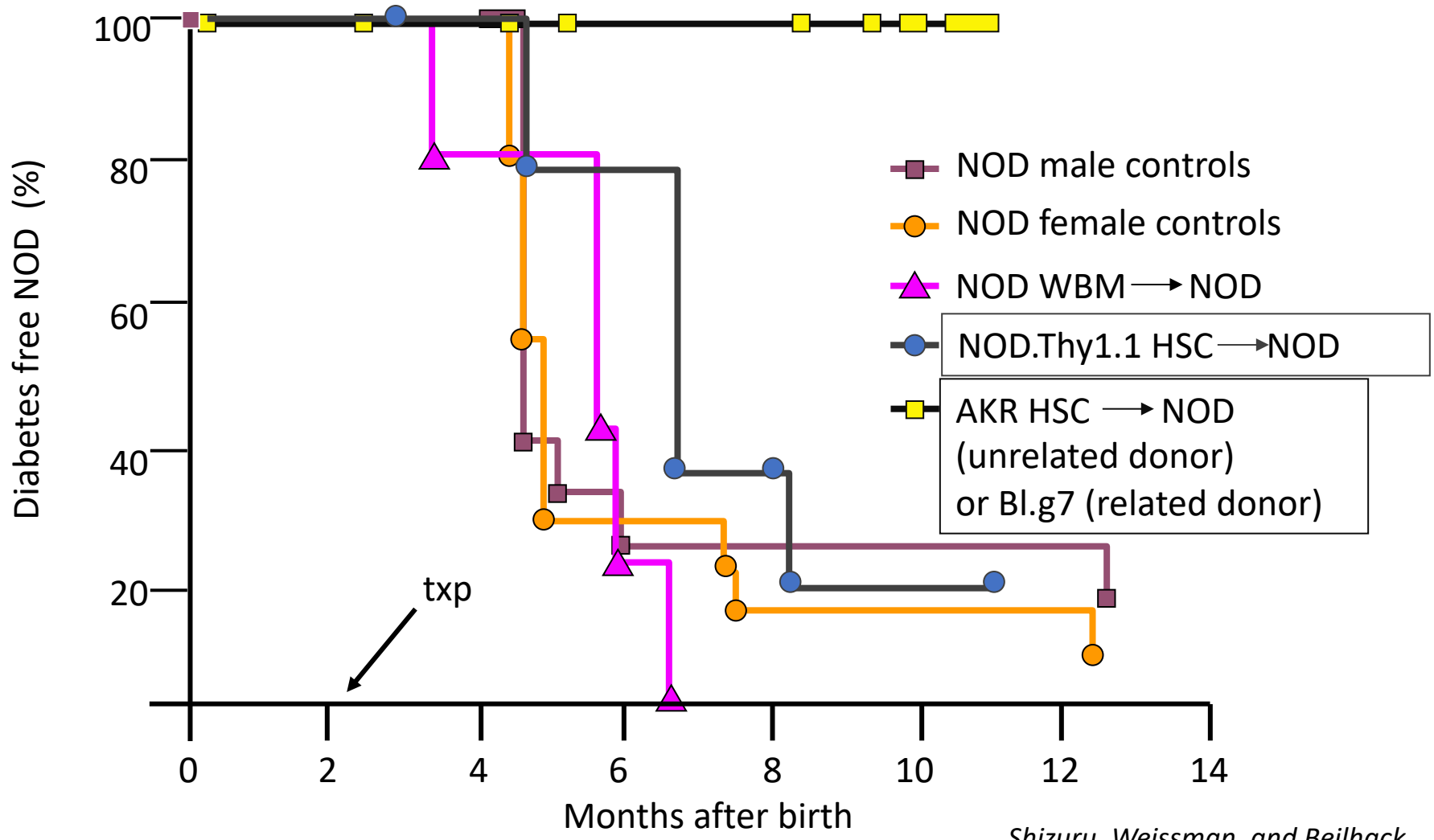
Healthy

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

Shizuru, Beilhack, Weissman

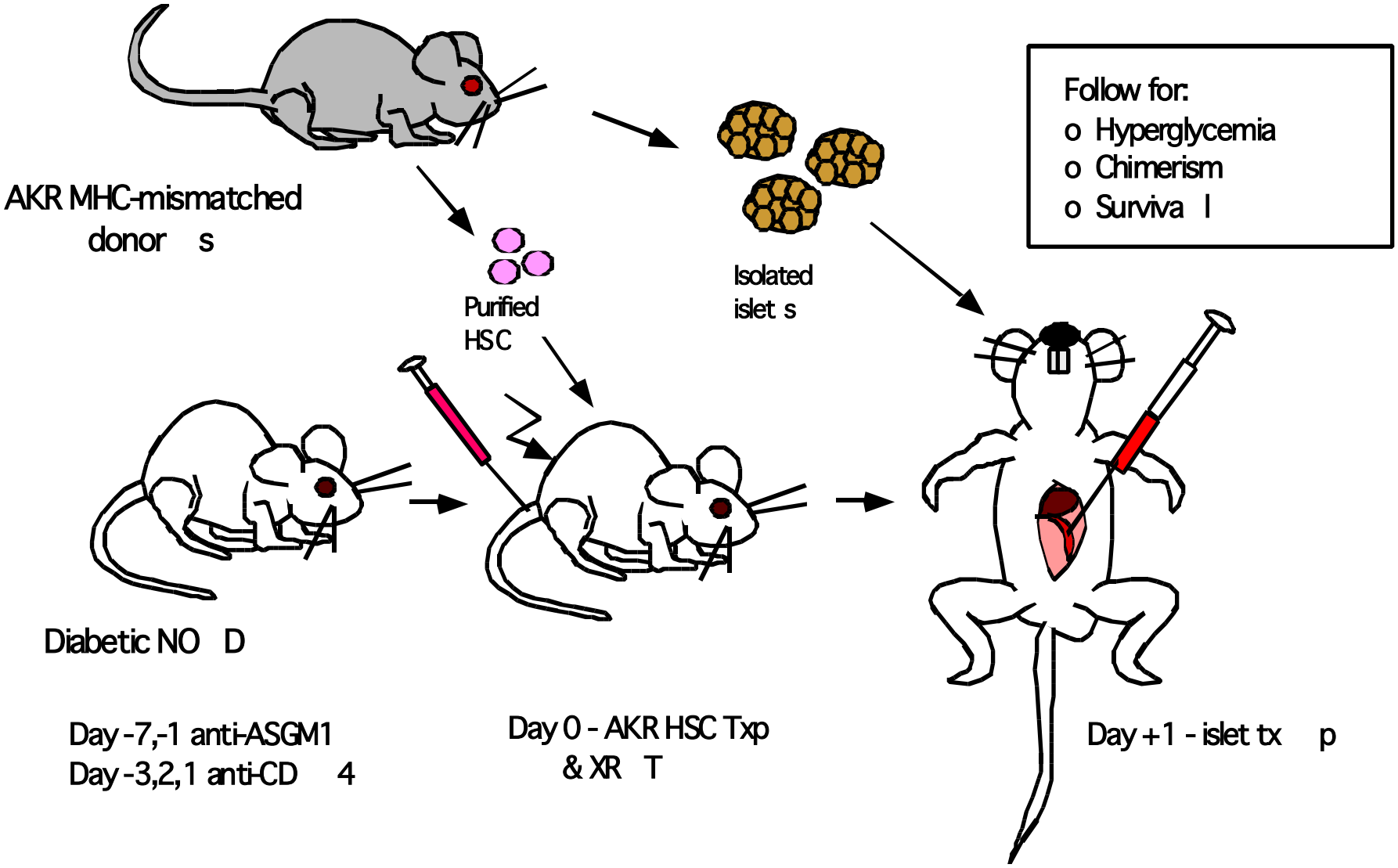
Rescue of Diabetic Mice with HSCs

CO-TRANSPLANT OF PURE HSC AND ISLETS CURATIVE AT ALL STAGES



Shizuru, Weissman, and Beilhack

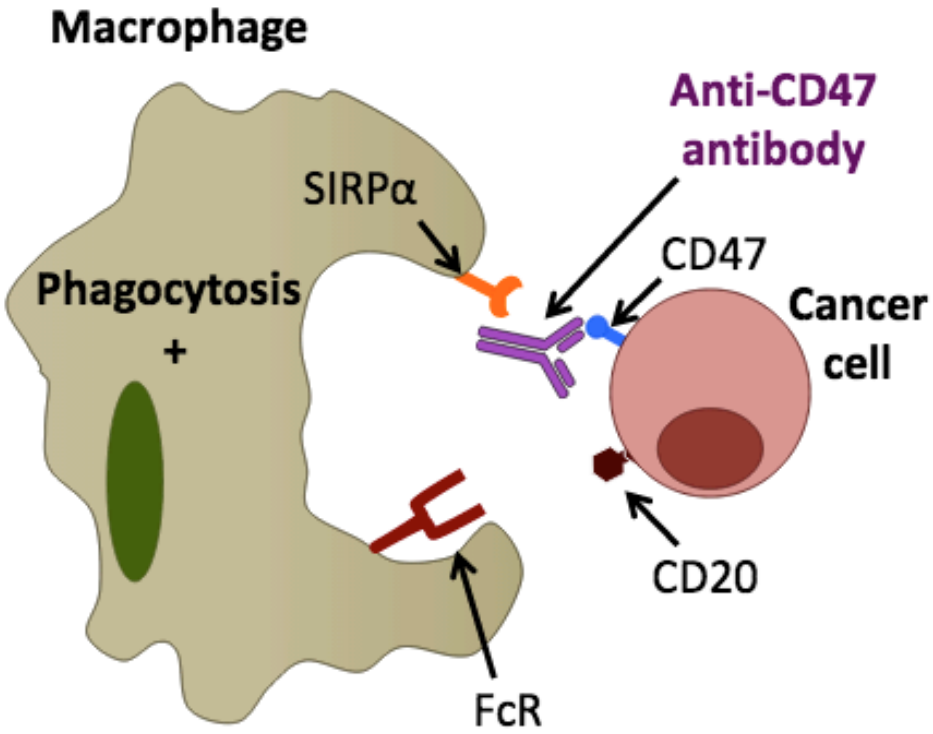
Combined HSC & islet transplantation

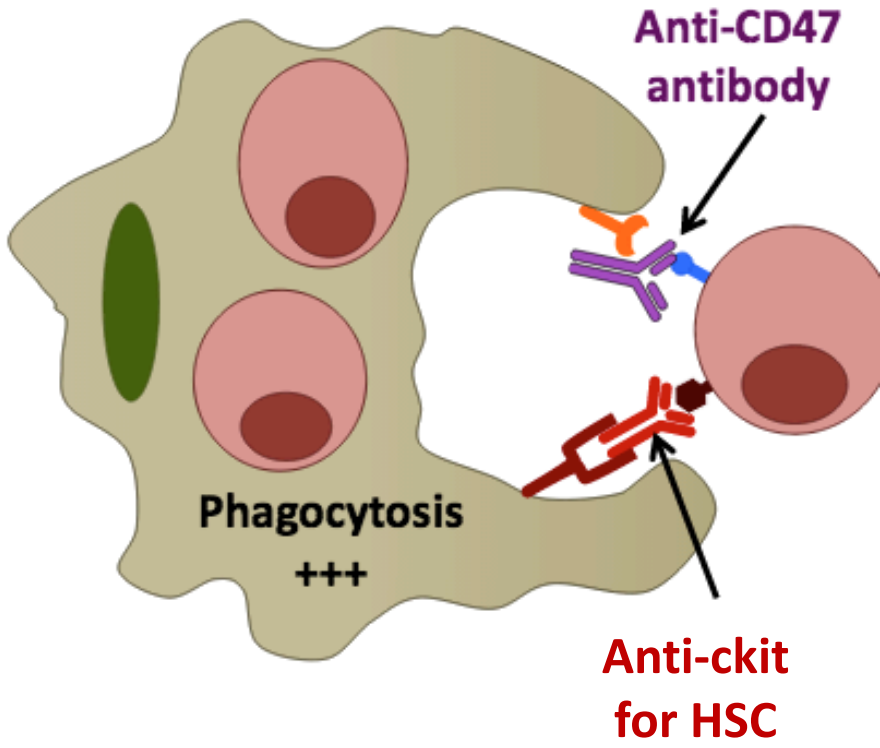


But radiation or chemotherapy is life-threatening and causes morbidity

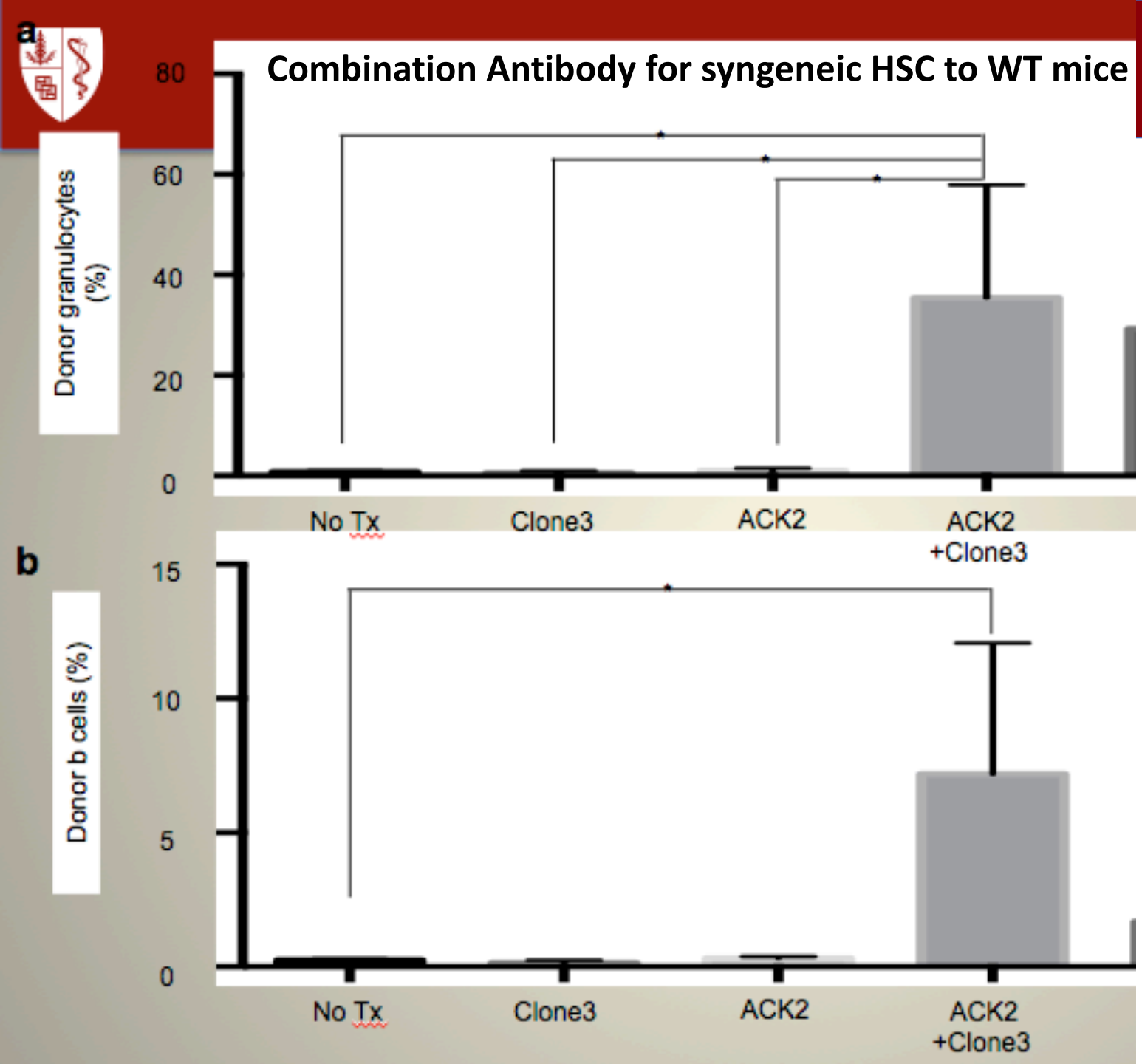
SHIZURU

Anti-CD47 Antibody Combination-Therapy With Cancer-Targeting Monoclonal Antibodies





- **Combination with other cancer-targeting antibodies enhances phagocytosis of cancer cells and provide treatment to patients that are currently non eligible (e.g. rituximab - anti-CD20; cetuximab - anti-EGFR; trastuzumab - anti-HER2)**

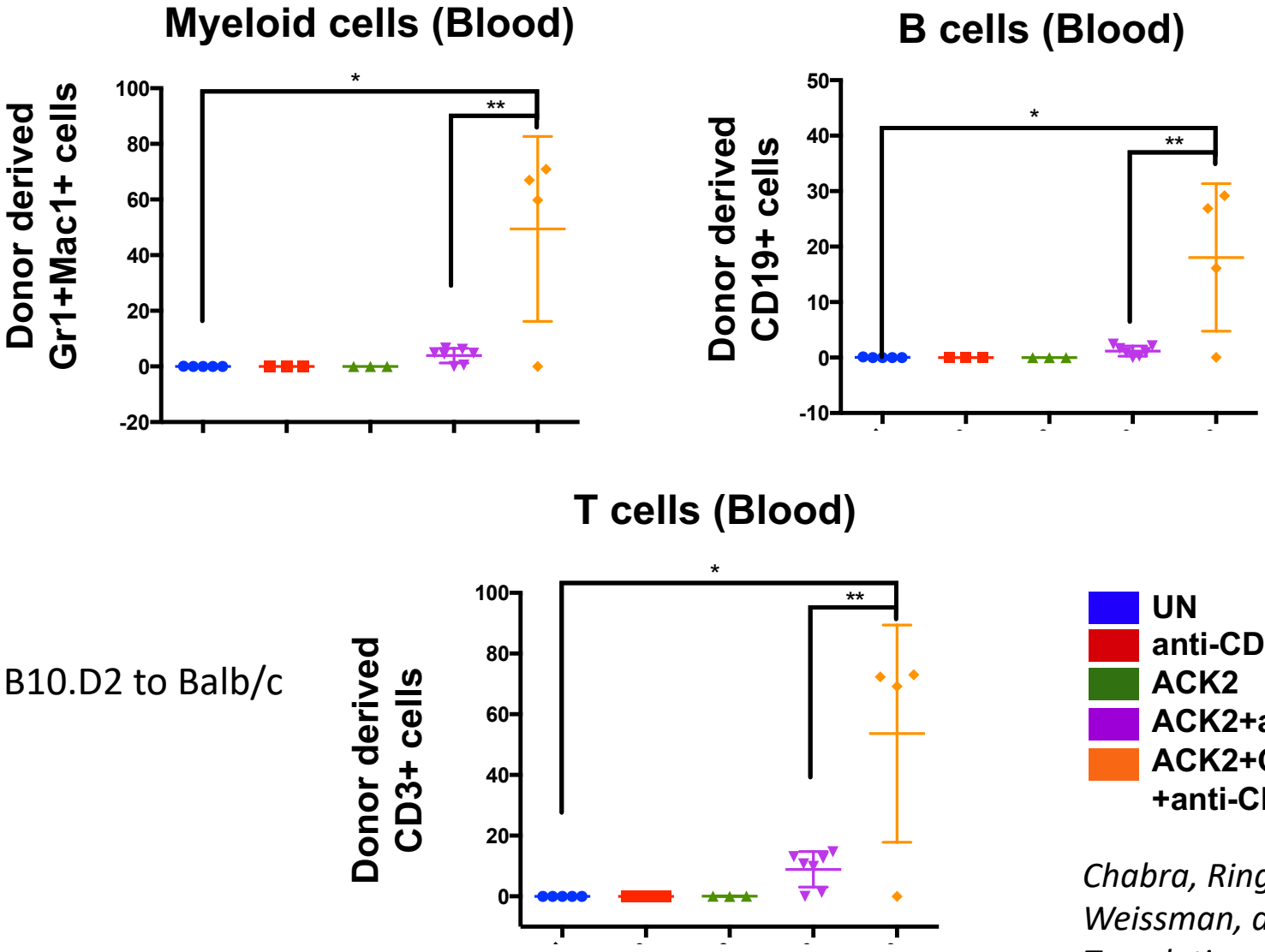


A.Chhabra
A.Ring
J. Volkmer
K.Weiskopf
I. Weissman
J. Shizuru

Clone 3=anti CD47

Successful multi-lineage chimerism in a MHC match/minor mismatch transplant model using ACK2 and Clone 3 Combo Therapy

ALL ANTIBODY CONDITIONING FOR MUD HSC TRANSPLANTS

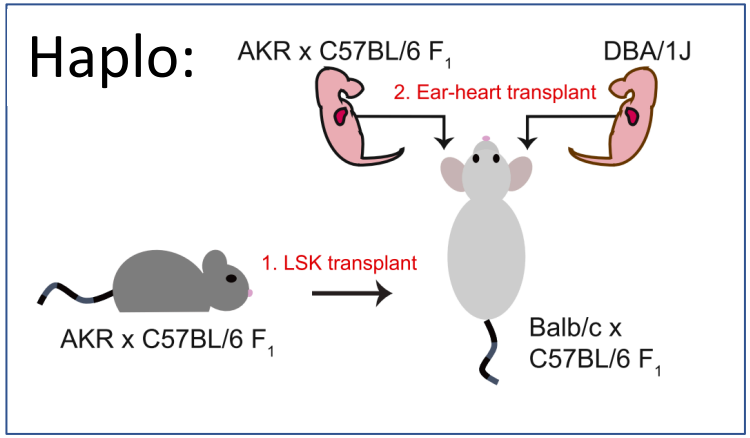


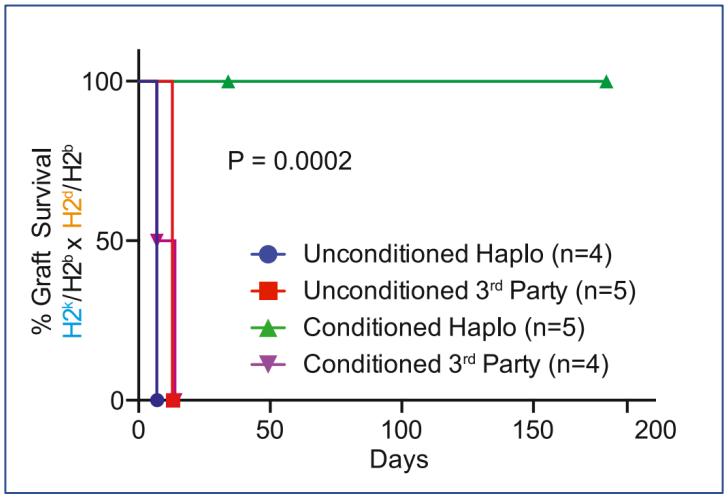
B10.D2 to Balb/c

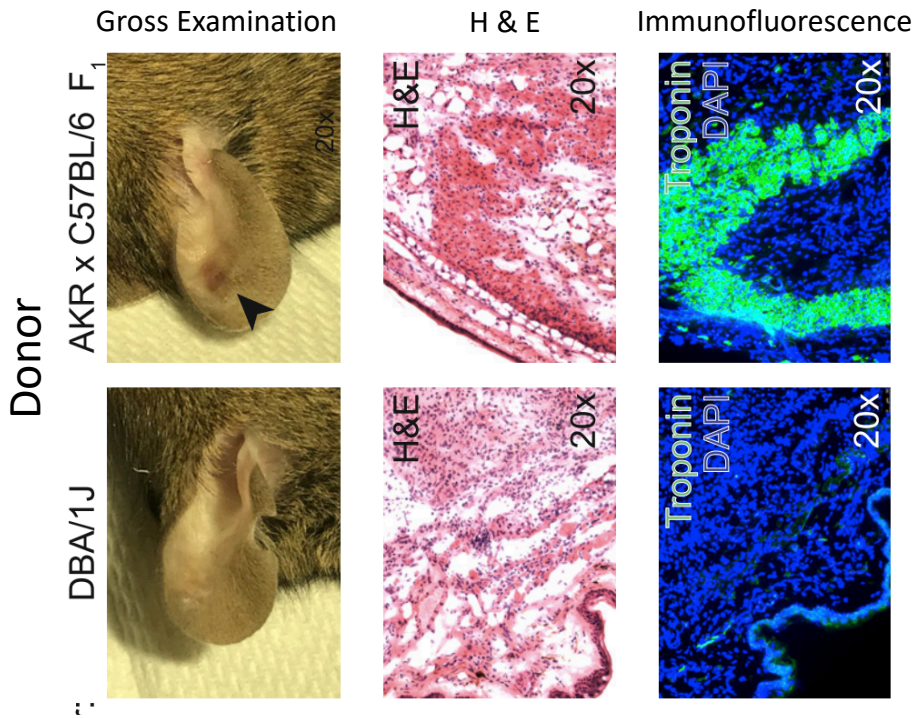
Chabra, Ring, Weiskopf, Volkmer, Weissman, and Shizuru. Science Translational Medicine, 2016

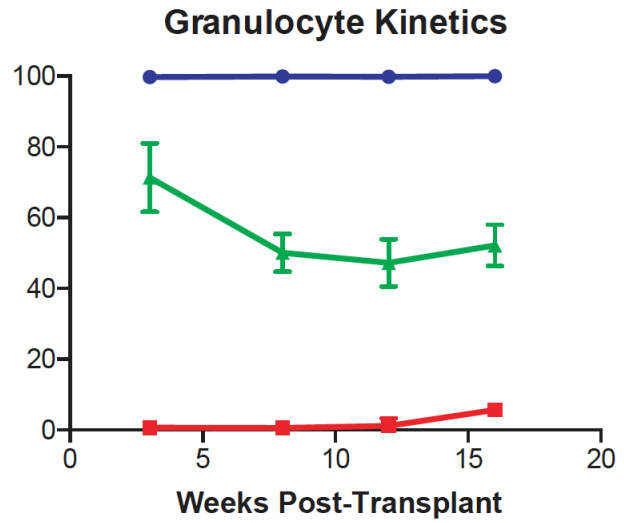
- But now few families have HLA matched sibs

Haplotransplants of HSC in Ab conditioned hosts induces cardiotransplant tolerance







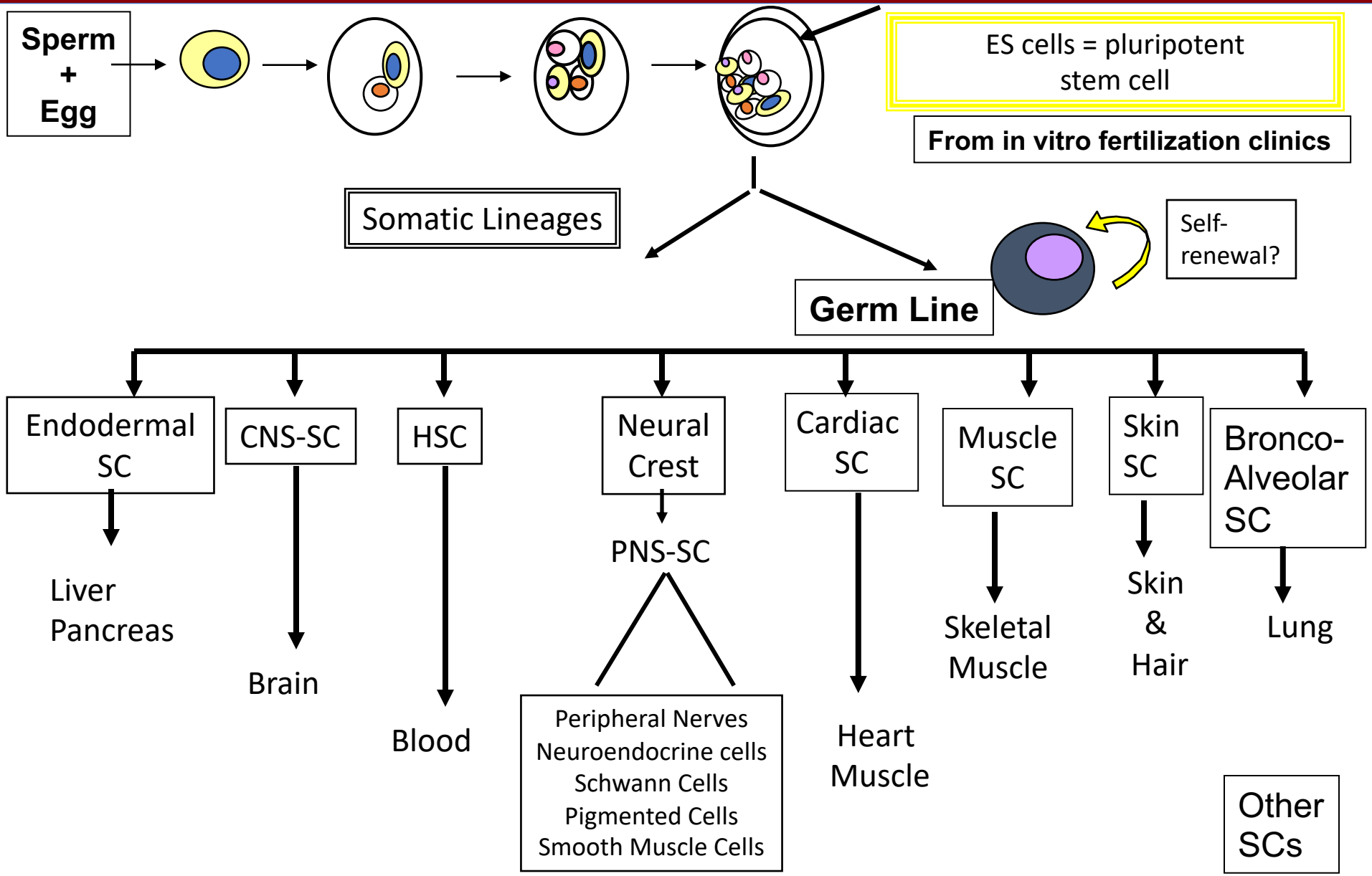


Benson George et al 2019 Cell Stem Cell

Blood forming stem cell (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 antibodies; All antibody conditioning!*
- Cotransplantation of tissue stem cell [CNS SC] and HSC from same donor or cell line
- In the far future [>5-10 years], ES or iPS derived HSC will be cotransplanted with other tissue stem cells from the same donor line.

Pluripotent Embryonic Stem Cells



SUMMARY

- STEM CELL BIOLOGY IS A WAY OF THINKING: THE CELL IS THE UNIT OF ORGANIZATION, NOT ANY SINGLE GENE, AND ONLY STEM CELLS SELF-RENEW.
- STEM CELLS ARE THEREFORE THE UNITS OF REGENERATIVE MEDICINE AS WELL, IF WE CAN FIND HOW TO ISOLATE AND TRANSPLANT THEM
- BLOOD FORMING STEM CELLS REPLACE THE BLOOD AND IMMUNE SYSTEM OF THE RECIPIENT, INDUCE TOLERANCE, AND BLOCK AUTOIMMUNE DISEASE
- CANCER STEM CELLS ARE THE UNITS OF CANCER SPREAD AND THEREFORE CANCER LETHALITY
- CANCER STEM CELLS ARE DERIVED FROM CLONES OF TISSUE STEM CELLS THAT UNDERGO CLONAL EXPANSION WITH EACH ADDED DRIVER MUTATION; THESE PRECANCER CLONES CAN TAKE OVER STEM CELL NICHES AND CAUSE DISEASE
- CANCER STEM CELLS HAVE UPREGULATED 'DON'T EAT ME' SIGNALS THAT PREVENT SCAVENGER MACROPHAGES FROM ELIMINATING THEM OR KEEPING THEM LOCAL
- BLOCKING THE DON'T EAT ME SIGNAL IS A NEW MACROPHAGE CHECKPOINT IMMUNOTHERAPY FOR CANCER
- OTHER PATHOGENIC CELLS IN ATHEROSCLEROSIS, FIBROSIS, AND OTHER SITUATIONS ALSO USE DON'T EAT ME SIGNALS TO AVOID ELIMINATION
- THIS I A MAJOR OPENING TO UNDERSTAND AND TREAT COMMON INCURABLE DISEASES; THIS IS NOT PHARMACEUTICAL SMALL MOLECULE THERAPY

In honor of Maurice Hilleman

- Hilleman proved that it was possible to make vaccines to protect people lifelong against the most deadly infections
- He led Merck to become the world leader; following his principles Merck made HPV vaccines that protect against a devastating venereal disease, and the cervical cancer it causes. No small molecules work.

Every year millions of people are protected against these diseases because of him, and the great university that brought his to his career [and mine]/

Lokey Stem Cell Institute at Stanford
Ludwig Center at Stanford;

Institute for Stem Cell Biology and Regenerative Medicine

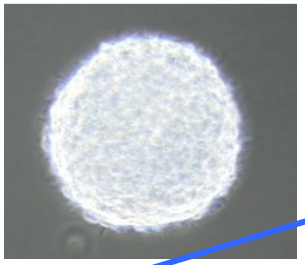
Siebel Stem Cell Institute of Stanford/UCB

Supported grants from Ludwig Cancer, NCI, NHLBI, Calif Inst Reg Medicine, Lacob Foundation, Siebel SCI,SU2C

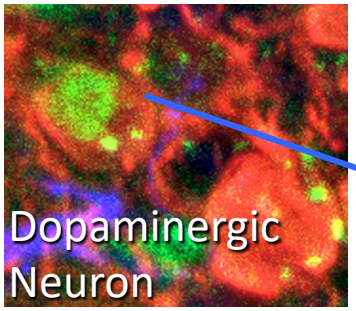
Human CNS-SC Neurosphere Cells Engrafted, Migrated & Differentiated Into Neurons, Astrocytes and Oligodendrocytes

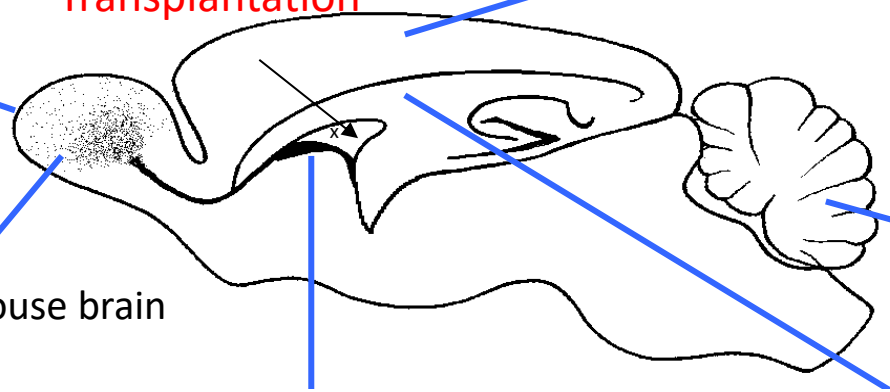
CD34-45-133+24-

In Vitro Expansion

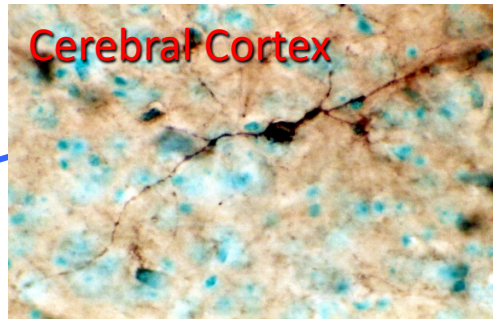


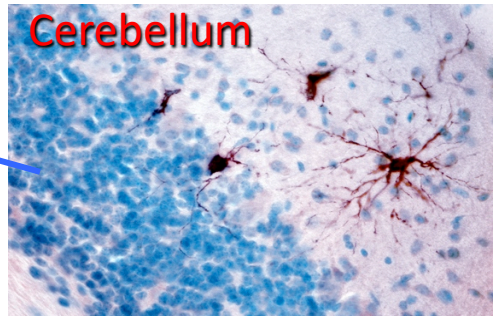
Transplantation



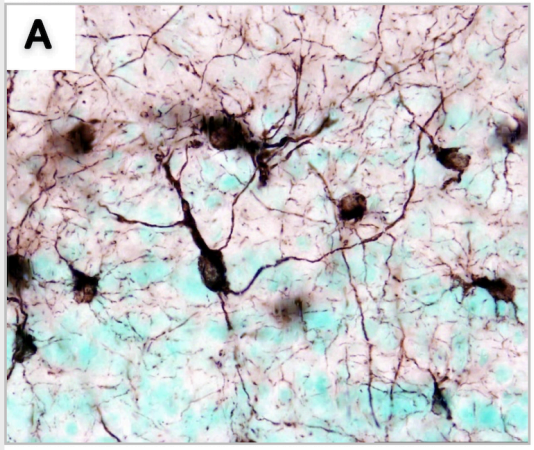


Immunodeficient mouse brain

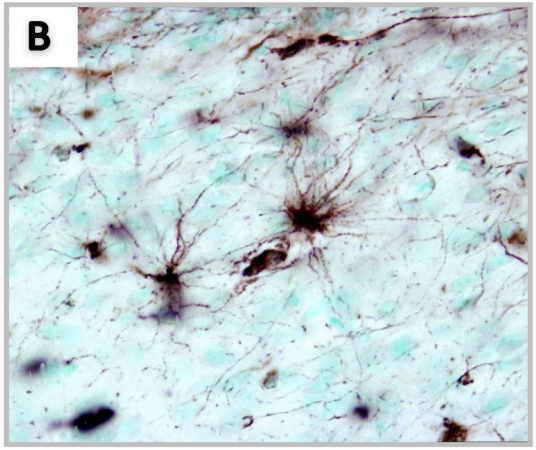




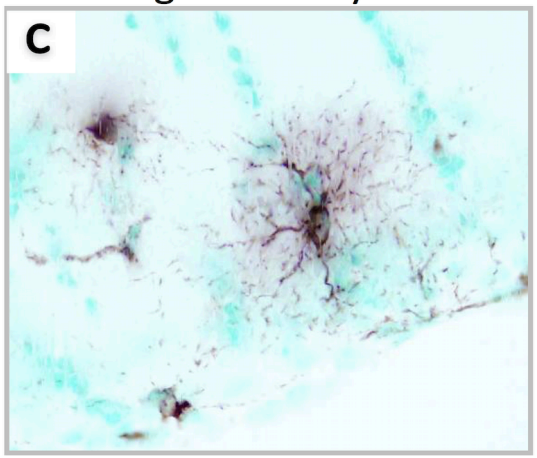
Neurons



Astrocytes



Oligodendrocytes

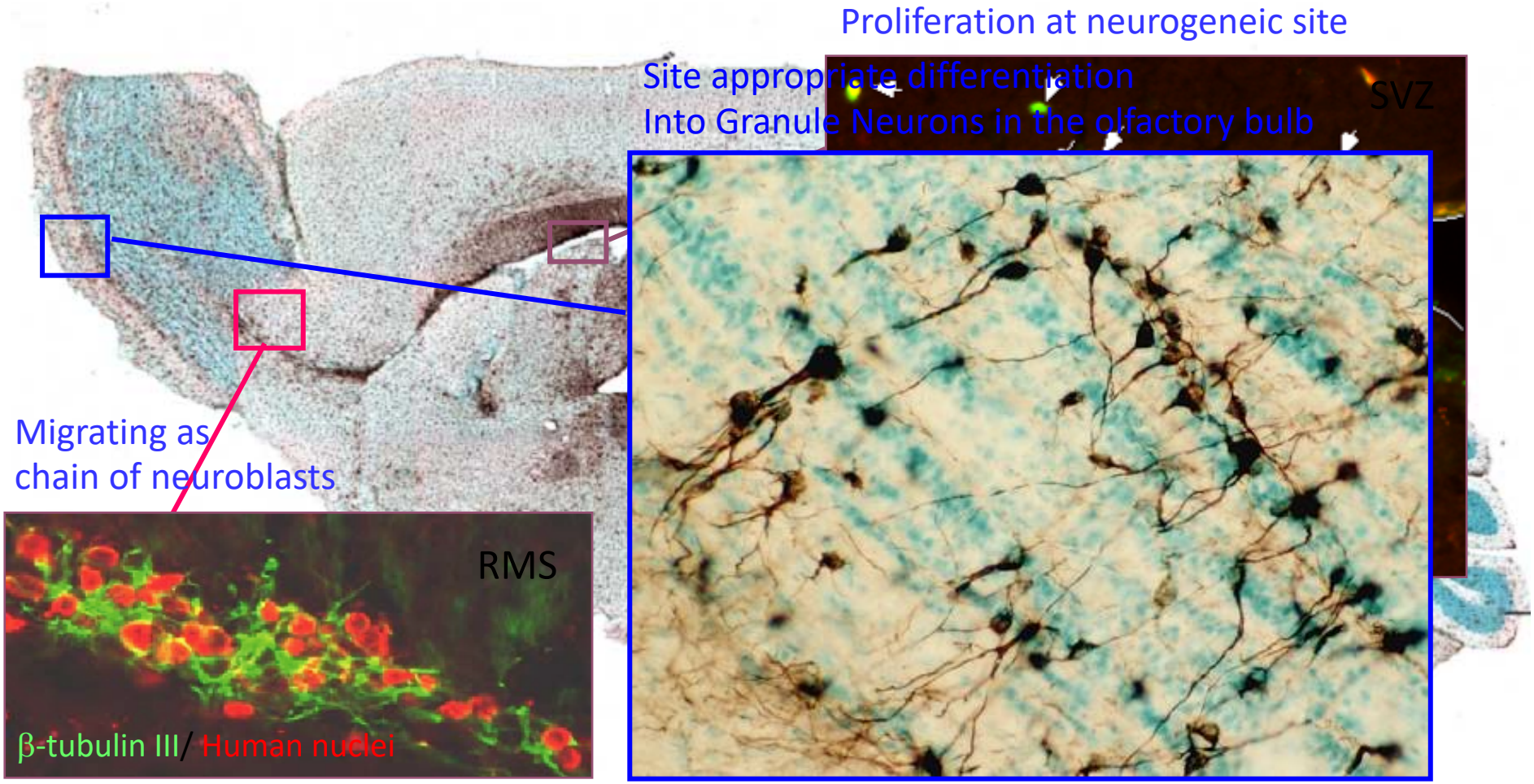


Uchida, Tsukamoto, Gage, Weissman 2000; Uchida and Porteus 2016, CrisPR modification

STEMCELLS

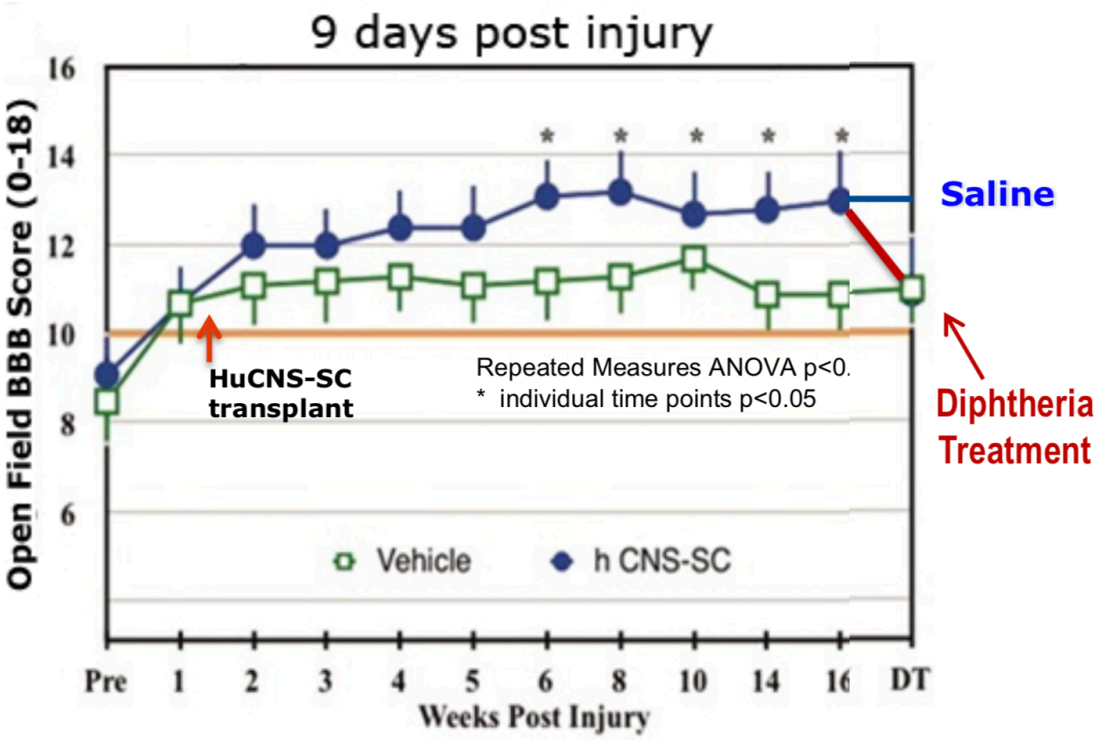
HuCNS-SC Engraft, Migrate & Mature

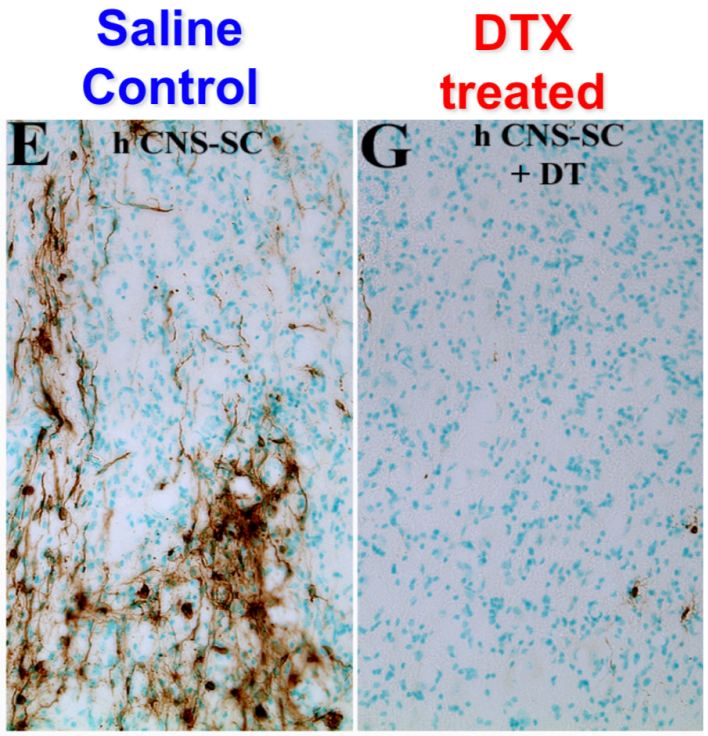
Human cells derived from HuCNS-SC transplant (brown)



Uchida, Tamaki, Tsukamoto-Weissman, IW

HuCNS-SC Restores Motor Function in Spinal Cord Injured Mice



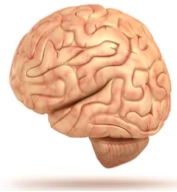


Cummings B J et al. PNAS 2005;102

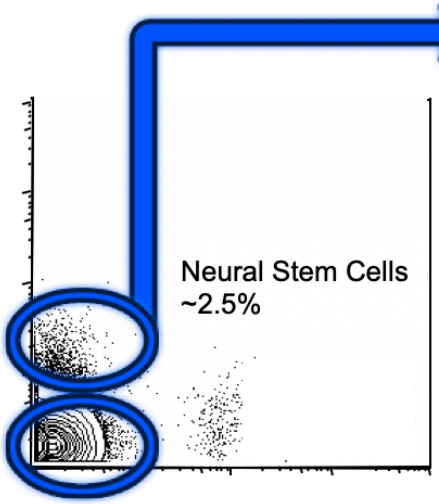
- Hind-limb motor function restored; lost when human cells ablated
- Continued presence of human cells required for maintenance of motor function
- What is the fate and potential mechanism of action of human cells?
- *Post-traumatice demyelination and neuronal loss*

Anderson, Cummings, Uchida, et al.

Human CNS SC repair mouse and human spinal cord injuries

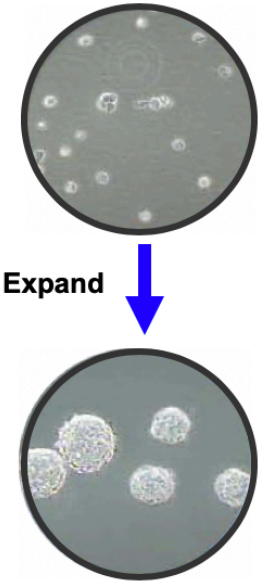


Isolation & Purification



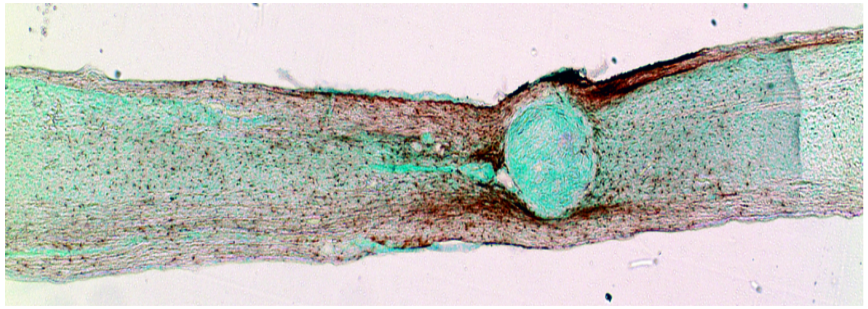
Human brain stem cells represent < 1/1000 cells

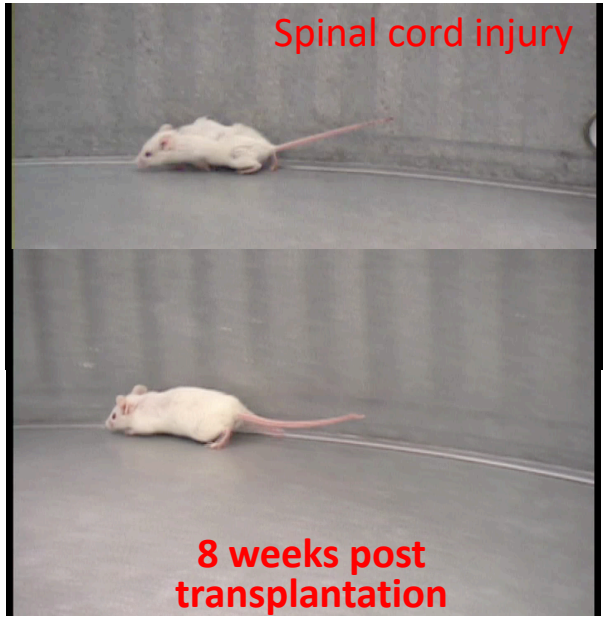
Expansion Process



Brain Stem cells grown in culture

Human stem cells <u>transplanted</u> into an injured mouse spinal cord





<u>Human clinical trials:</u>

Thoracic: 7/12 (58%) pts sensory gains

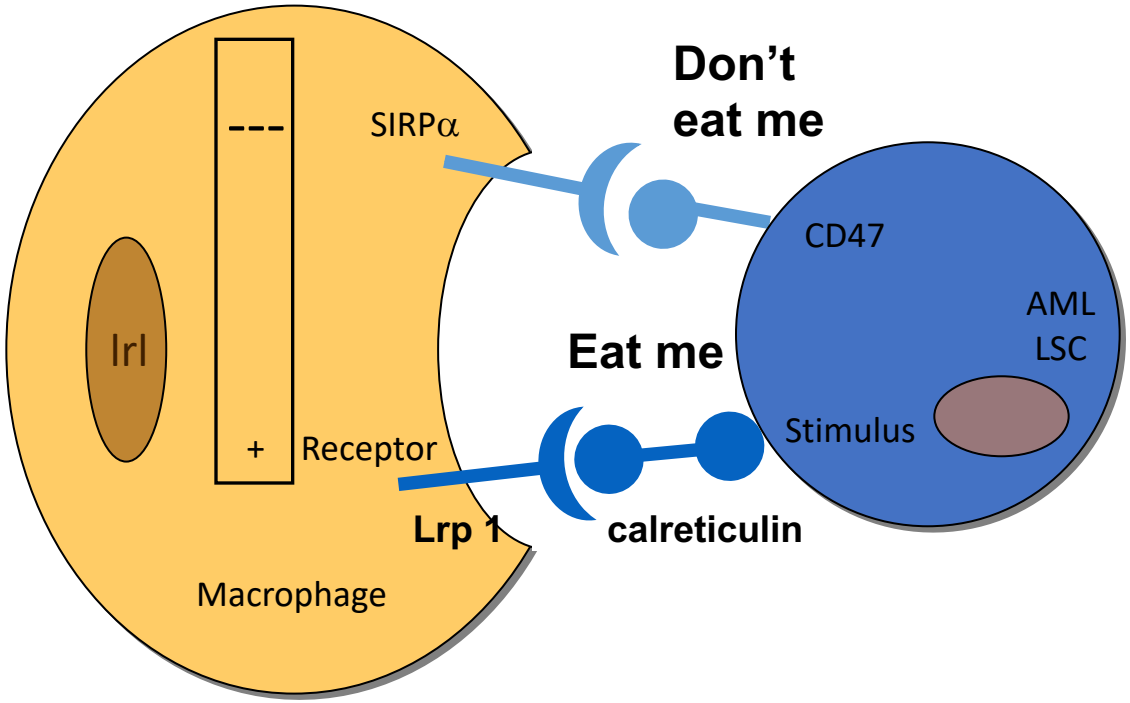
Cervical: 4/6 (67%) pts motor improvements

**Requires lifelong immune suppression*

Uchida, Cummings, Anderson, Tsukamoto-Weissman, IW

Pre-cancer cells express calreticulin for PrCR, and emergent cancer clones overcome this with CD47

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



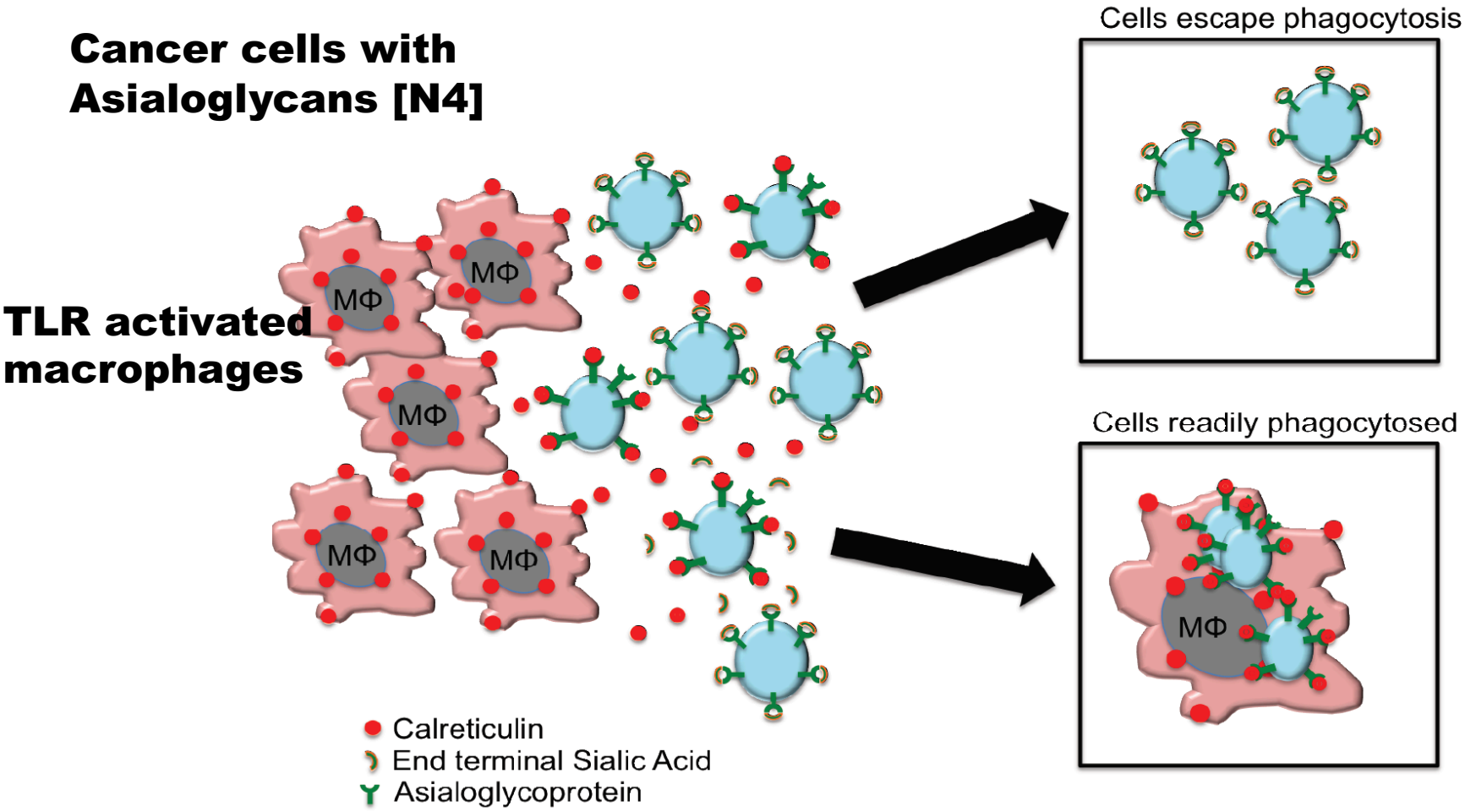
Calreticulin, an ER protein without a TM seq, is the dominant cell surface 'eat me' signal

What are the mechanisms of 'don't eat me' and 'eat me' signals?

Chao, Jaiswal, Tsukamoto-Weissman, Majeti, IW Science Translational Med 2010

PrCR involves activation and secretion of calR by macrophages

binding of calR on target cell asialoglycan, and phagocytic removal of target cancer and dying and pathogenic cells



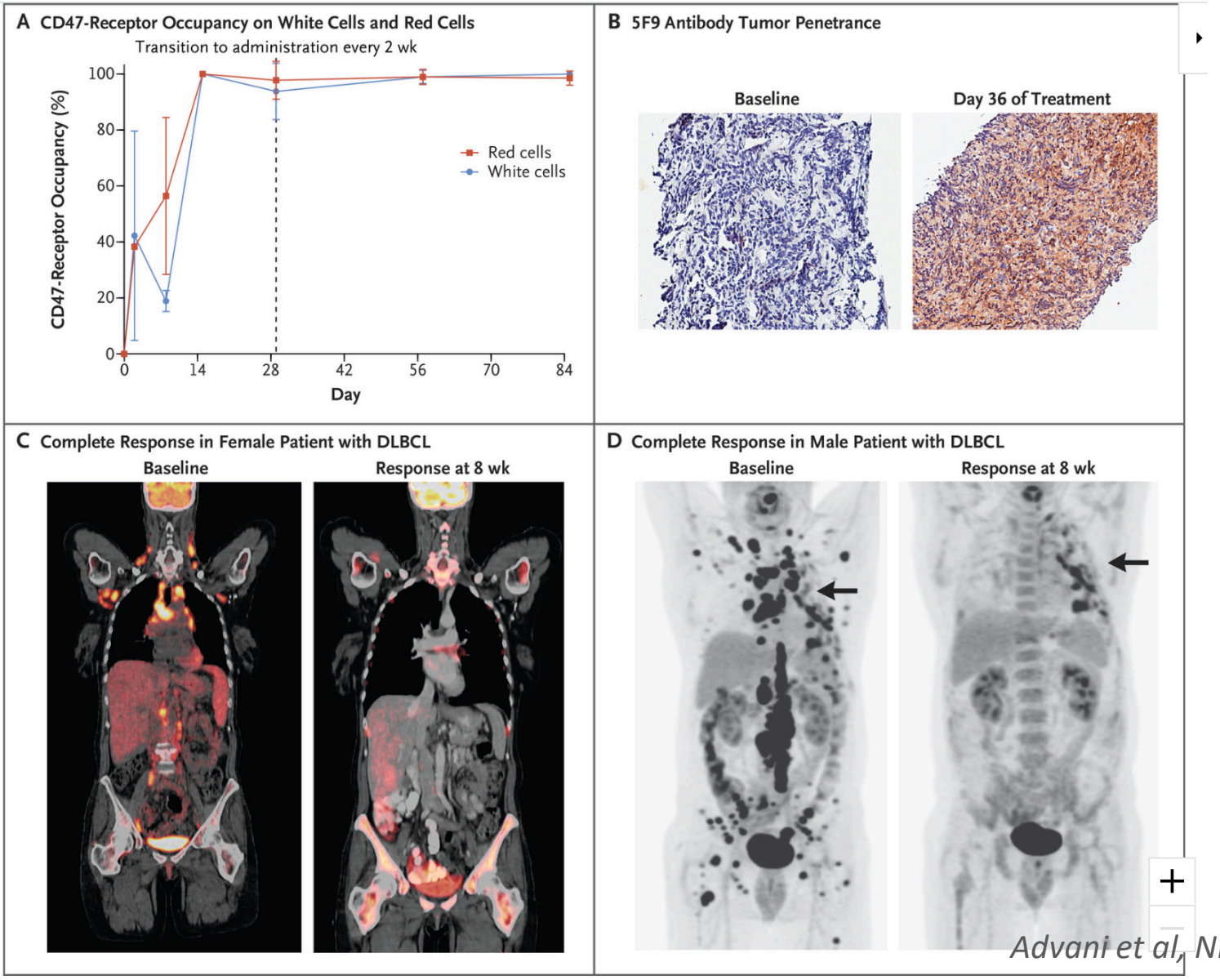
Kris Marjon, Mingye Feng, Denon Wang, Rachel W, and IW; Nature Communications 2018

Clonal HSC expansion in MDS: a single aberrant HSC can cause a blood disease

- MDS HSC *outcompete* normal HSC in patient and xenotransplants**:>98%** of pHSC are MDS;m7,5q-,*rbp* mutant ione HSC in the body outcompeted almost all normal HSC
- High **calR** predisposes MDS myeloid [GMP, MkP, EP] progenitors for programmed cell removal
- MDS HSC dominate niches but can't make blood
- Increased CD47 expression is a crucial step in the progression from MDS to AML
- Can single CNS SC cause a brain disease? A single fibroblast SC cause fibrotic disease? A single smooth muscle cause atherosclerosis?

Wendy Pang, John Pluvinage, Chris Park, IW, et al PNAS 2013 MDS
Wernig and IW PNAS 2017 Fibrotic disease
Kojima, IW, Leeper Nature 2016 Atherosclerosis

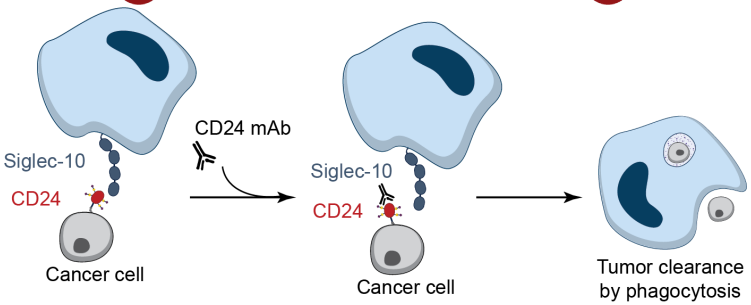
Anti-CD47 synergy with Rituximab in refractory NHL

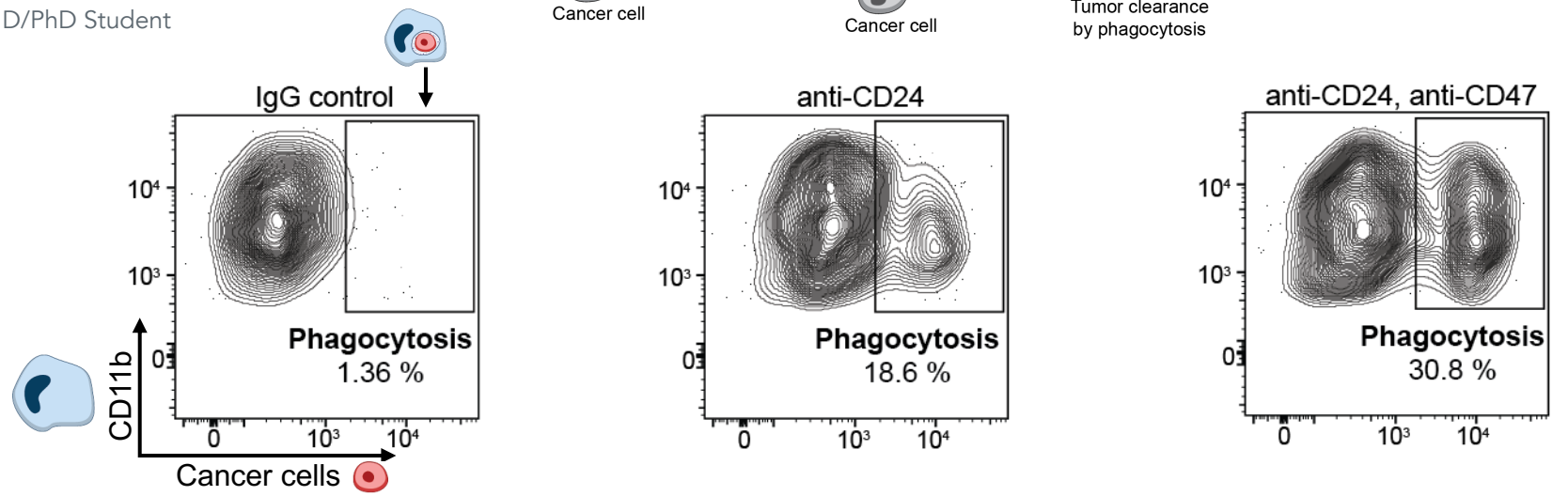


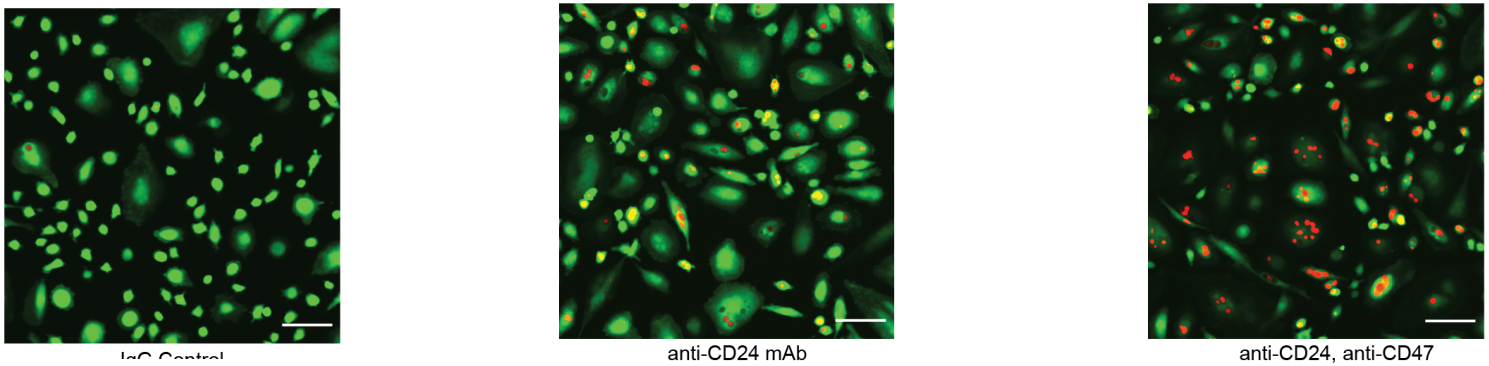
Advani et al, NEJM 2018

Treatment with anti-CD24 mAb promotes phagocytic clearance of human breast cancer

Amira Barkal
MD/PhD Student







anti-CD24 mAb

anti-CD24, anti-CD47

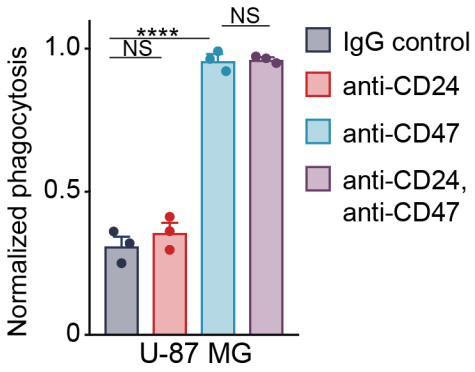
Macrophages
MCF-7

Scale bar is 100 μm

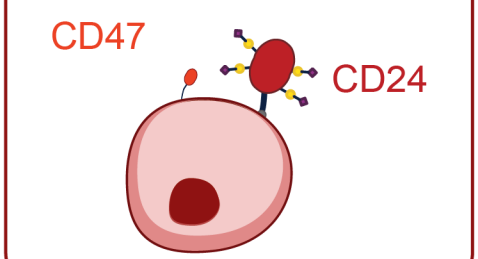
Barkal et al, Nature 2019

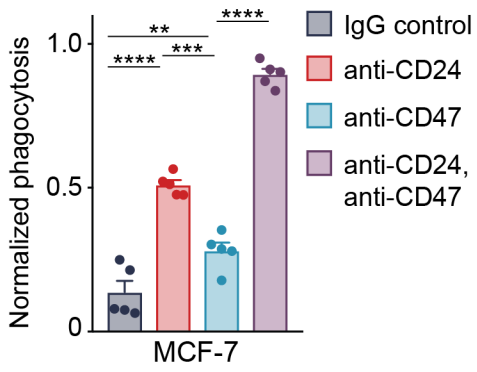
There are tumor-specific, dominant "don't eat me" signals

CD47 dominant

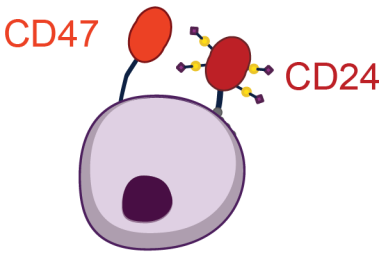


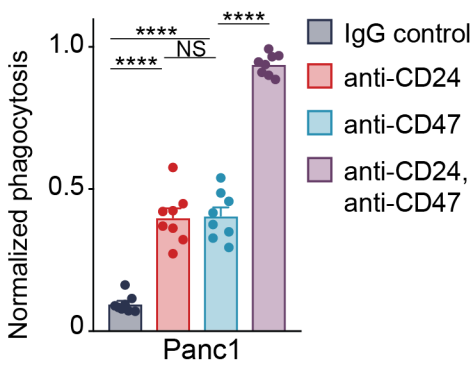
CD24 dominant





Co-dominant





Amira Barkal
MD/PhD Student

Goal: To profile expression of all known "don't eat me" signals on patient samples to guide therapy

Barkal et al, Nature 2019