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Macrophage Immune Checkpoint CD47 is a Novel Therapeutic Target

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HEALTH UNIVERSITY OF UTAH

No Disclosures

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Promise of Immune System Based Cancer Therapies

- **Use immune system to attack cancer**
- **Immune system can recognize self and destroy non self**
- **Cancers are caused by mutations and these mutations are potentially “non-self targets” to the immune system**

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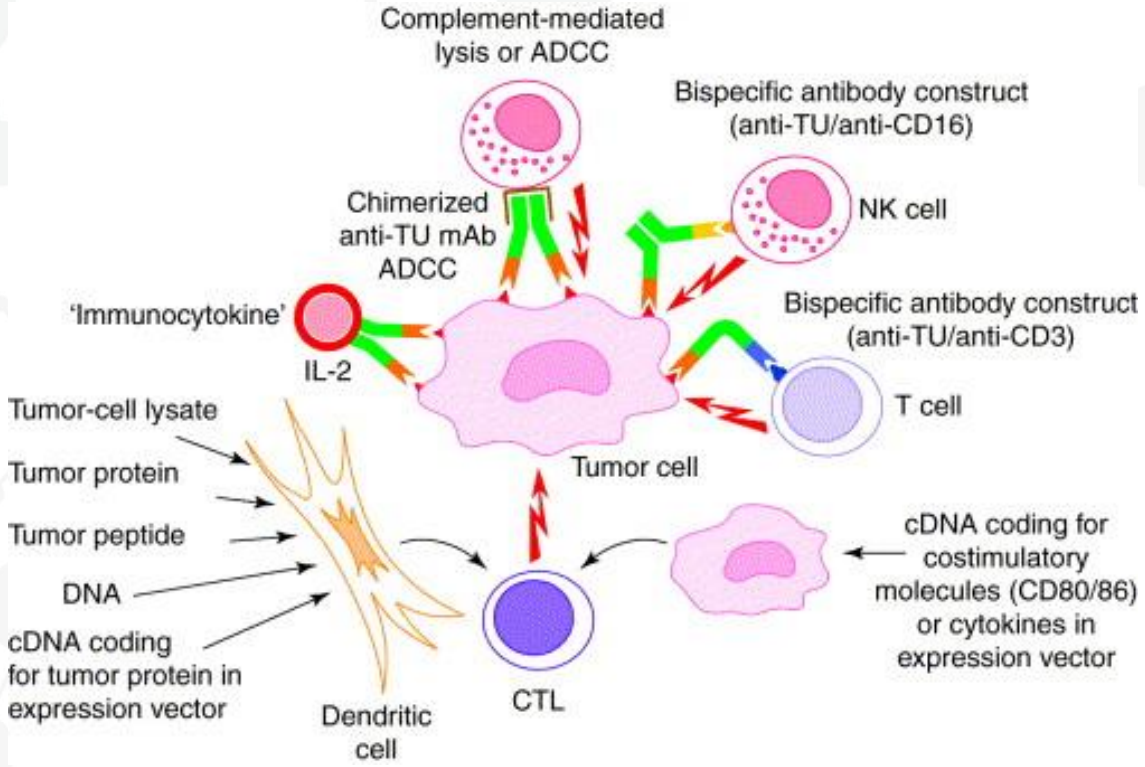
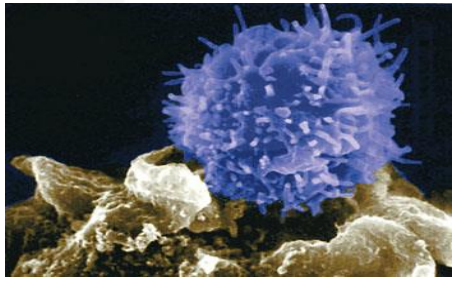
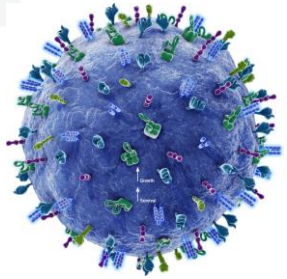
Cellular Mediators of Anti-Tumor Immunotherapies





T-Cells



B-Cells

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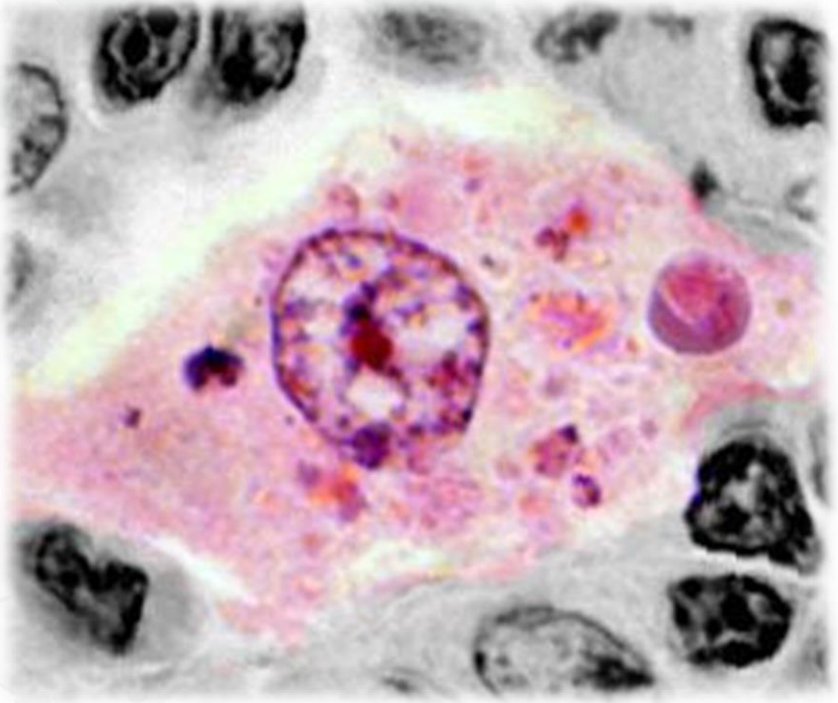
Macrophage: The Not So Boring Cell of the Immune System



Anti-CD47

Blocking Monoclonal Antibody to CD47 can stimulate macrophages into "eating" tumor cells

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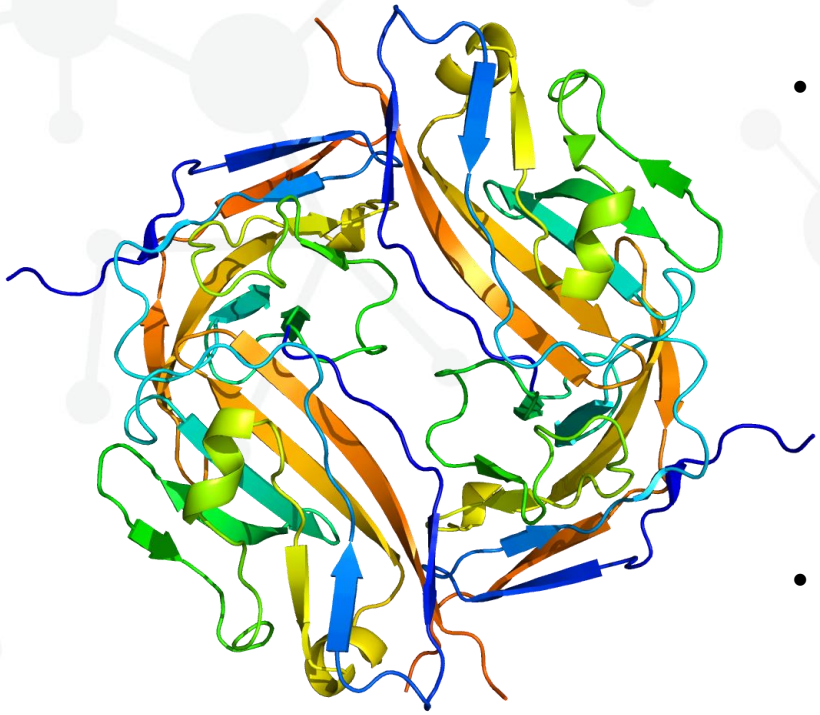
CD47



- CD47 is a 50 kDa transmembrane protein that belongs to the **immunoglobulin superfamily** and partners with membrane integrins and also binds the ligands thrombospondin-1 (TSP-1) and **signal-regulatory protein alpha (SIRPα).**

- CD47 is involved in a range of cellular processes, including apoptosis, proliferation, adhesion, and migration. Furthermore, it plays a key role in immune and angiogenic responses.

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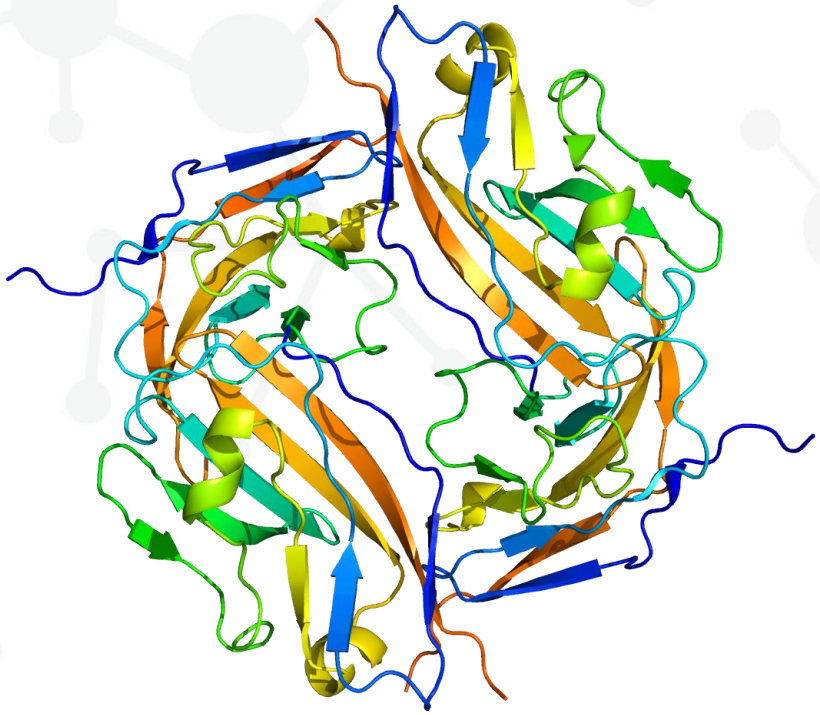
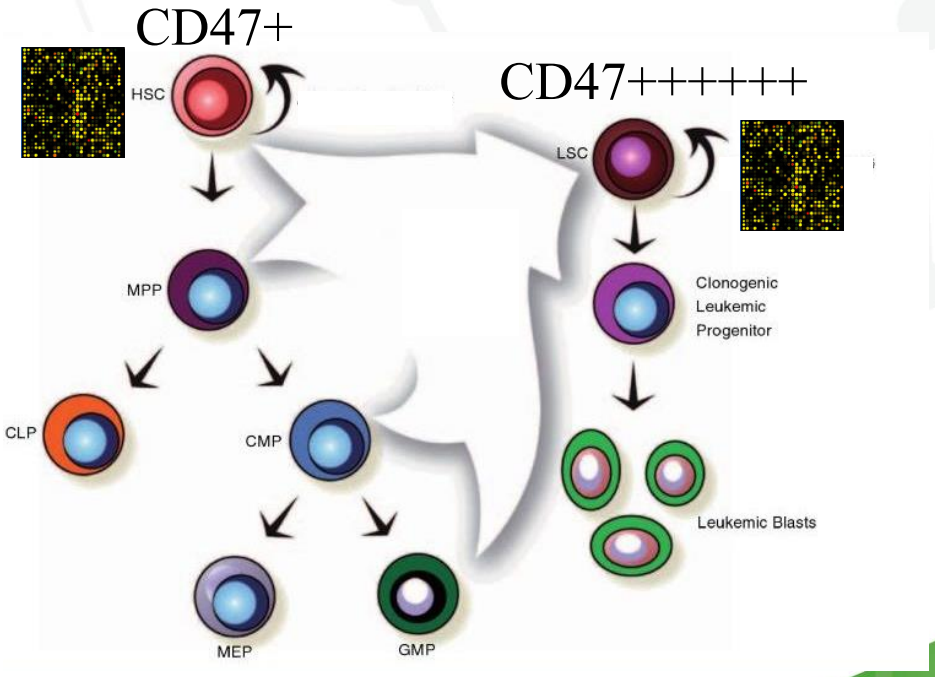
Discovery CD47 As Cancer Target: Leukemia



- Higher expression on Leukemia Stem Cells



- Increased expression of CD47 on cancer cells from most human solid tumors: lymphoma, breast cancer, ovarian cancer, colon cancer, bladder cancer, glioblastoma, and many others

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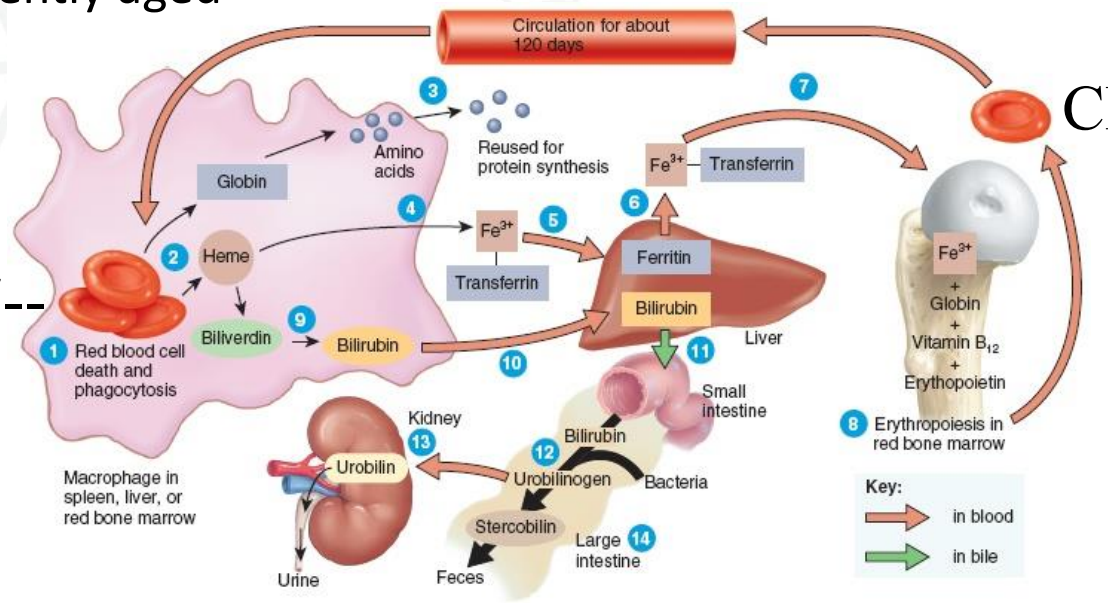
CD47 Inhibits Phagocytosis of RBC

- Expressed on red blood cells and prevents them from being eaten by macrophages in the spleen and liver until they are sufficiently aged

CD47--



CD47++

- Since it is expressed on cancers and CD47 degradation results in RBC phagocytosis,

does blocking CD47 interaction lead to tumor cell phagocytosis?

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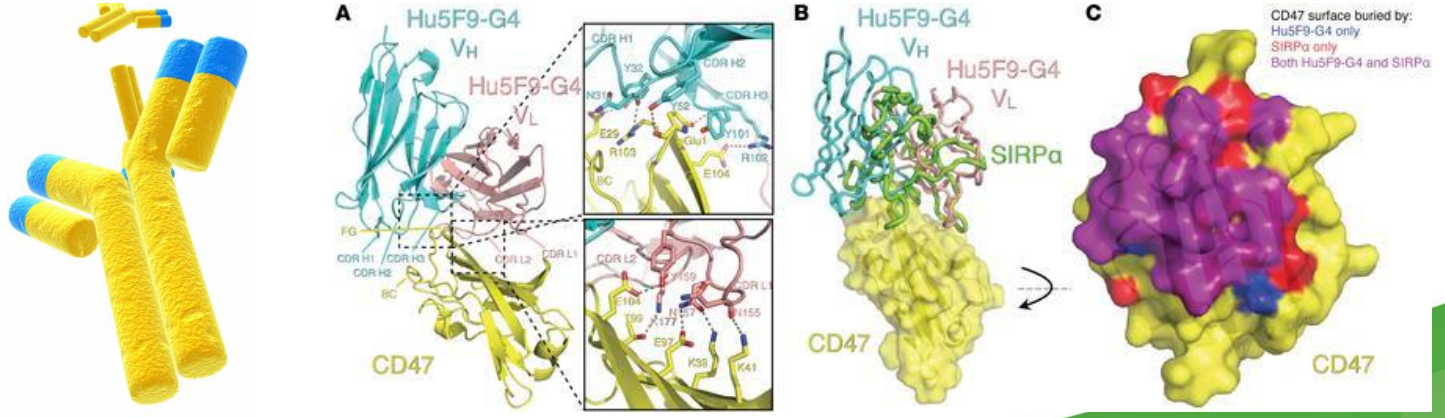
Humanized Anti-CD47 Antibody – Hu5F9-G4

- A humanized anti-CD47 antibody (Hu5F9-G4) has been developed
- Hu5F9-G4 was engineered as IgG4 variant to reduce Fc function
- Hu5F9-G4 exhibits potent anti cancer properties
- Now in Phase 2 Clinical Trials



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Wang, Majeti. PLOS1. 2015

Anti-CD47 Eliminates Cancer

Cell

CD47 Is an Adverse Prognostic Factor and Therapeutic Antibody Target on Human Acute Myeloid Leukemia Stem Cells

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PNAS PNAS

The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors

Stephen B. Willingham[a,1], Jens-Peter Volkmer[a,b,1], Andrew J. Gentles[c], Debashis Sahoo[a], Piero Dalerba[a,d], Siddhartha S. Mitra[a], Jian Wang[e,f], Humberto Contreras-Trujillo[a], Robin Martin[a], Justin D. Cohen[a], Patricia Lovelace[a], Ferenc A. Scheeren[a], Mark P. Chao[a], Kipp Weiskopf[a], Chad Tang[a], Anne Kathrin Volkmer[a], Tejaswitha J. Naik[a], Theresa A. Storm[a], Adriane R. Mosley[a], Badreddin Edris[a], Seraina M. Schmid[g], Chris K. Sun[h], Mei-Sze Chua[h], Oihana Murillo[a], Pradeep Rajendran[a], Adriel C. Cha[a], Robert K. Chin[a,i], Dongkyoon Kim[a], Maddalena Adorno[a], Tal Raveh[a], Diane Tseng[a], Siddhartha Jaiswal[a], Per Øyvind Enger[e,f], Gary K. Steinberg[j], Gordon Li[j], Samuel K. So[h], Ravindra Majeti[a,k], Griffith R. Harsh[i,l], Matt van de Rijn[m], Nelson N. H. Teng[n], John B. Sunwoo[a,l], Ash A. Alizadeh[a,k], Michael F. Clarke[a,d], and Irving L. Weissman[a,m,2]

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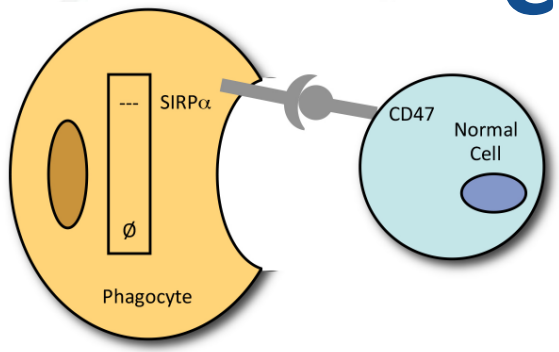
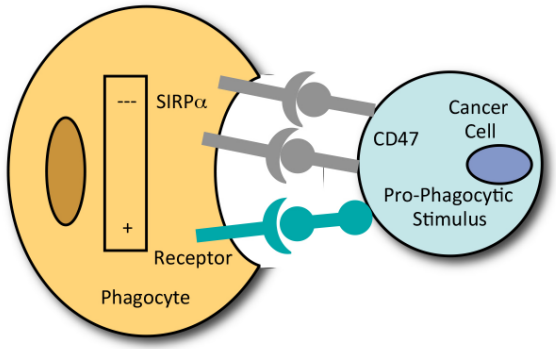
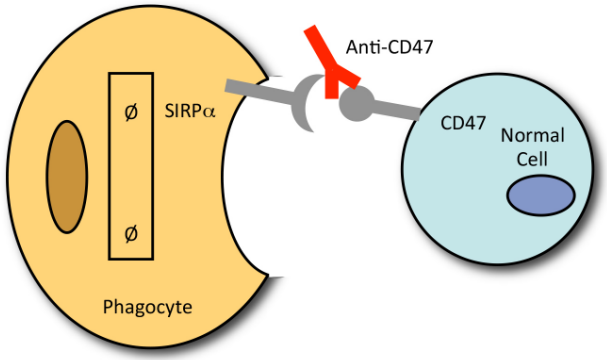
Model for Mechanism of Action in Targeting CD47



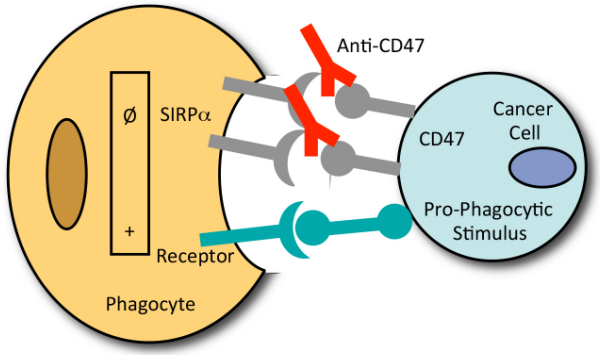
Normal Cell: No Phagocytosis



Cancer Cell: No Phagocytosis



Normal Cell: No Phagocytosis



Cancer Cell: Phagocytosis

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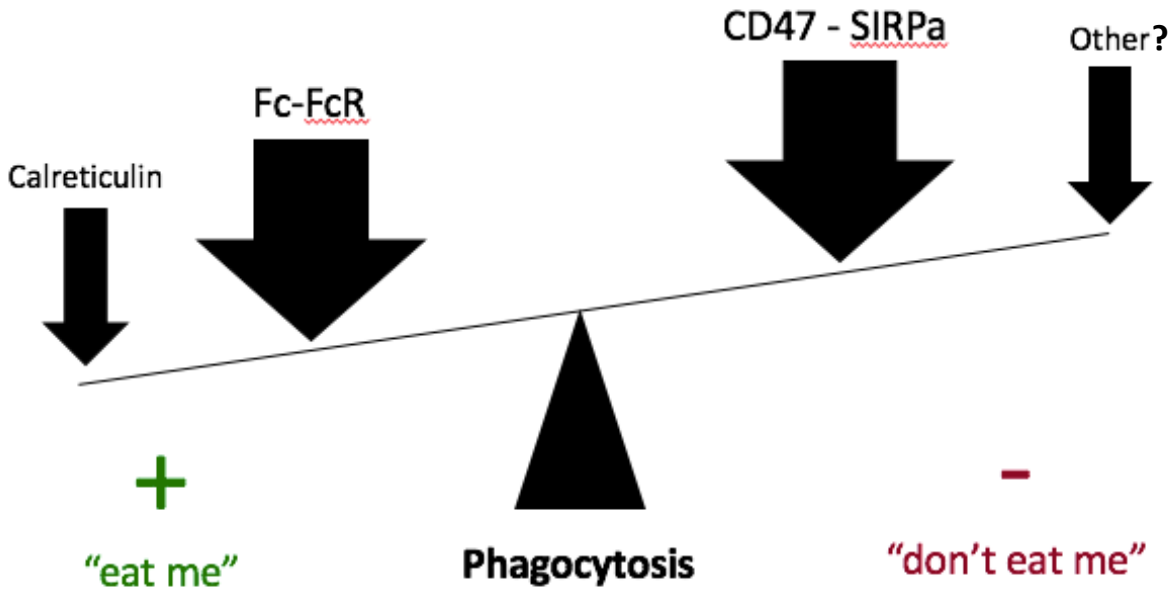
CD47 serves as a "Don't Eat Me Signal" that can be dominant over pro-phagocytic signals on cancer cells. Blocking antibodies against CD47 unmask the "Eat Me Signal" leading to phagocytic elimination of cancer cells.

Majeti. Oncogene. 2011

To Eat or Not to To Eat: The Balancing Act



- **Phagocytosis is the result of a dominance of stimulatory "eat me" signals over inhibitory " don't eat me signals"**
- **These signals can be cell intrinsic (CD47, calreticulin) or extrinsic (Fc molecules of cancer-targeting monoclonal antibodies)**

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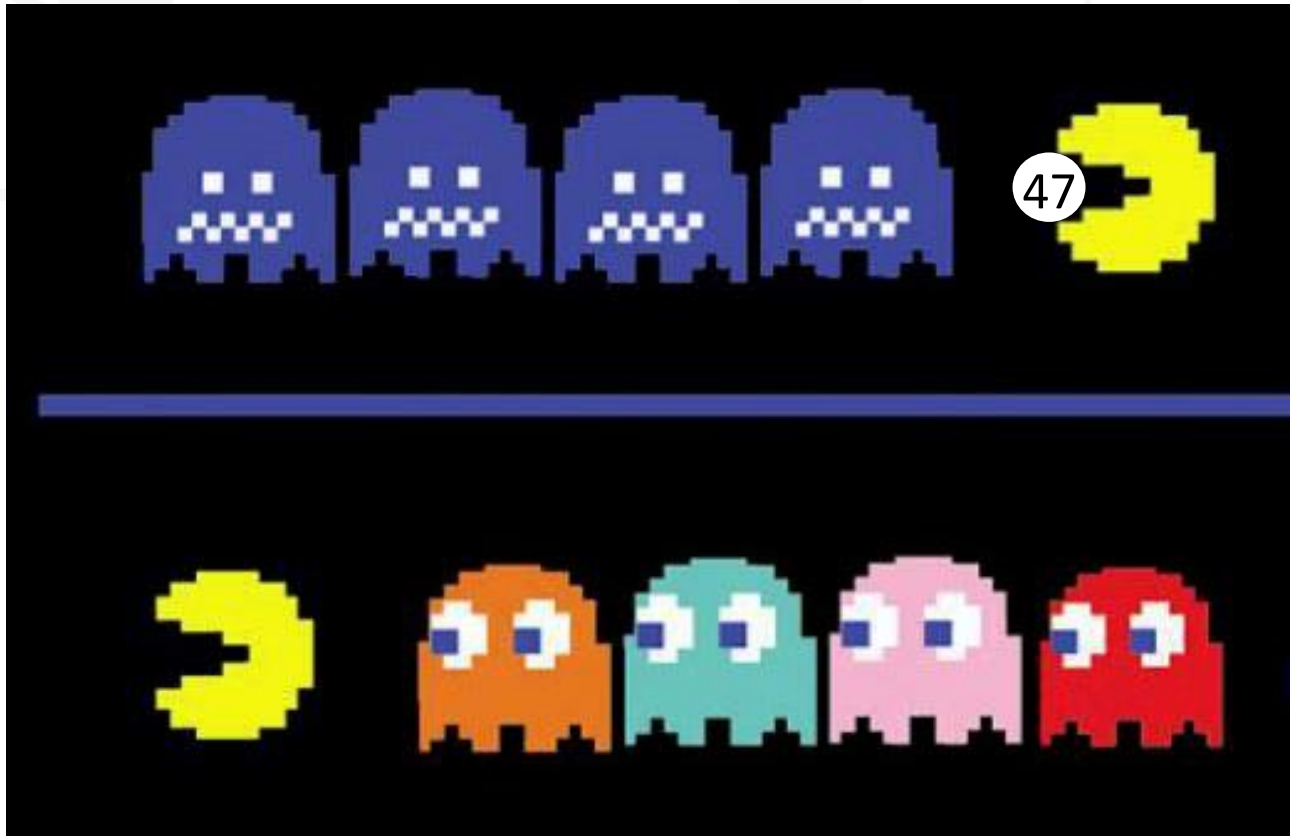
Simplified Model for Mechanism of Action in Targeting CD47



+ Anti-CD47

- Anti-CD47

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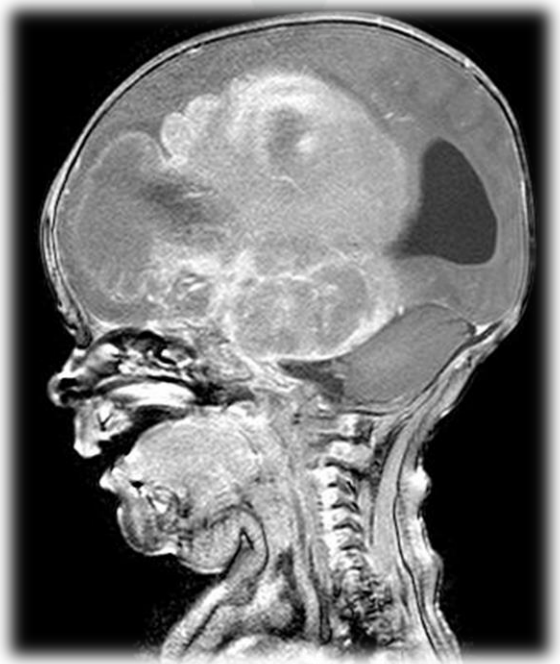
Pediatric Primary Malignant Brain Tumors

- # 1 Solid Tumor in Children
- #1 Cause of Cancer Death in Children
- Many are resistant to therapy
  - Inoperable
  - Resistant to all therapies
- Patients Pay the “Price of Treatment”
  - Diminished neurocognitive function
  - Hydrocephalus
  - Neurologic deficits
  - Organ damage
  - Secondary cancers
- Specific considerations of immune therapy in the brain



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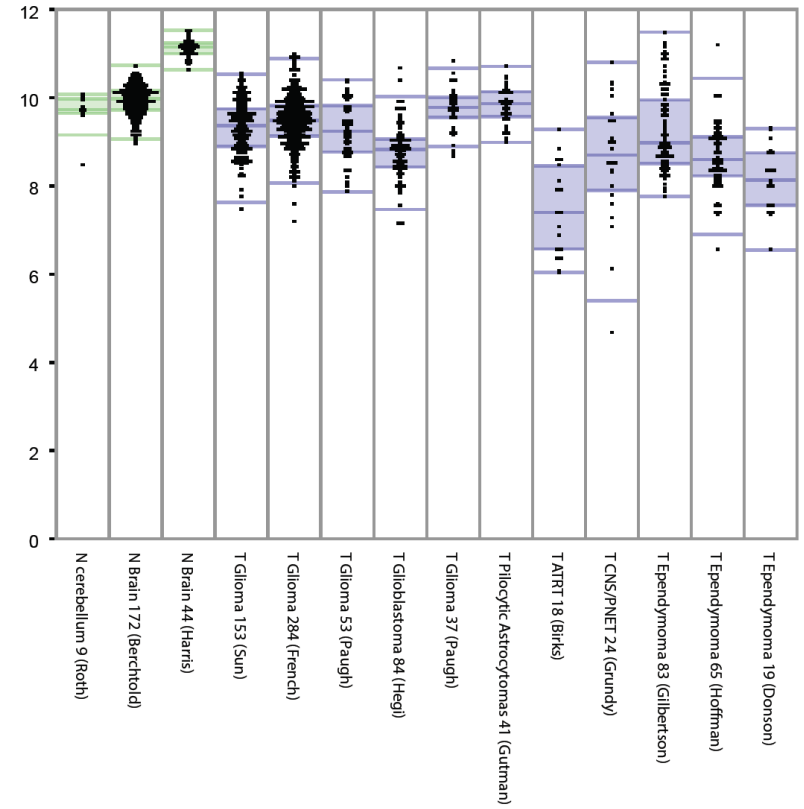
CD47 is Highly Expressed in Pediatric Brain Tumors

A CD47 Expression across Pediatric Brain Tumors



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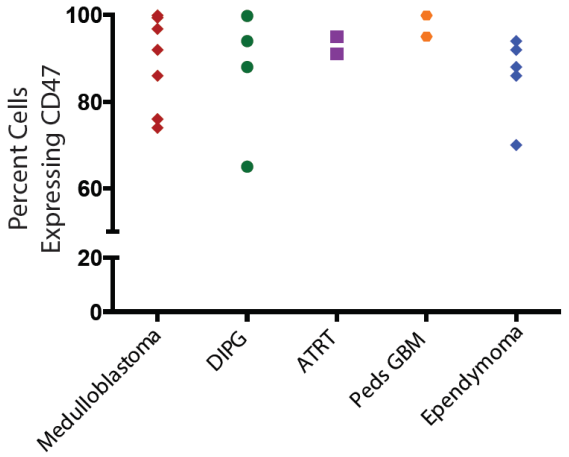

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Gholamin, Mitra, Cheshier. Science- Translational Medicine. March 2017

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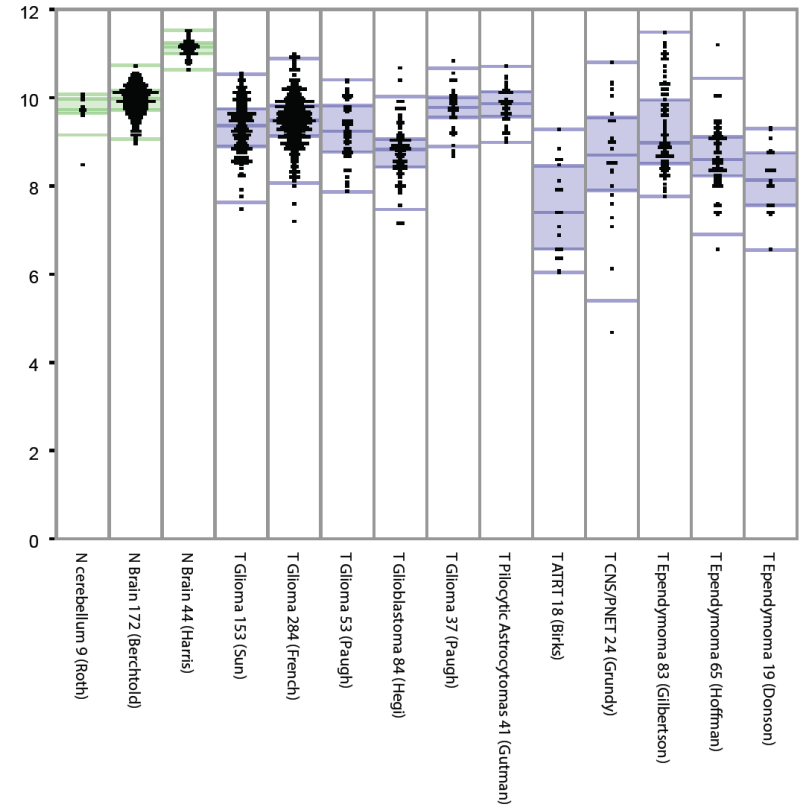

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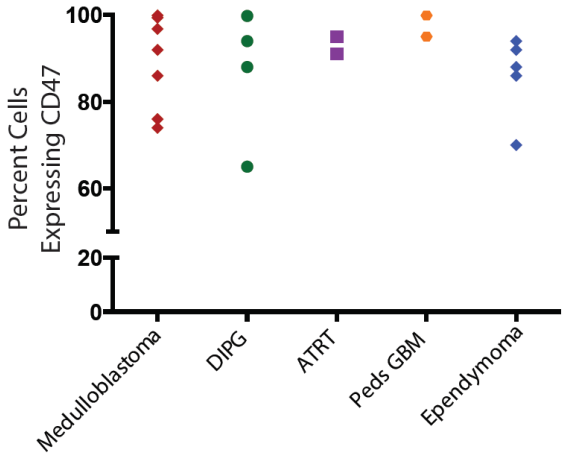

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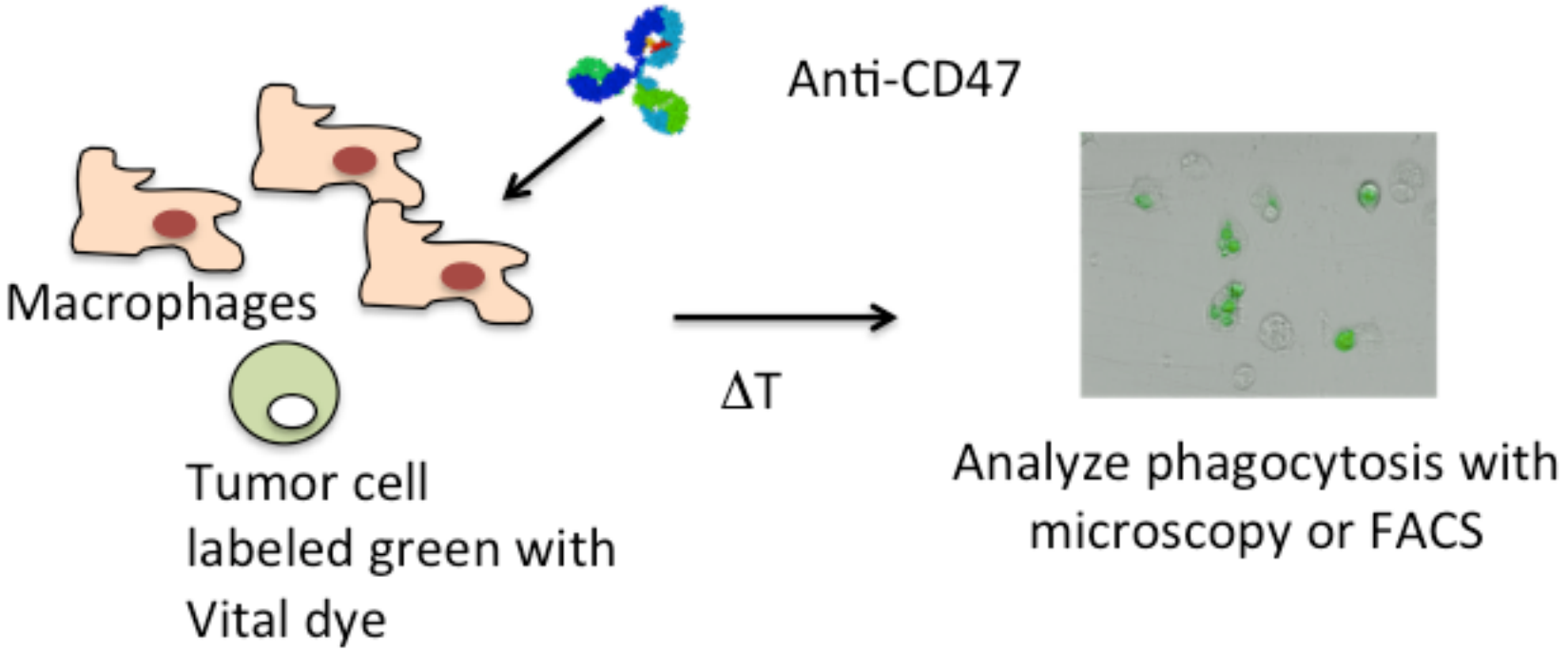
Gholamin, Mitra, Cheshier. Science- Translational Medicine. March 2017

In vitro Phagocytosis Assay



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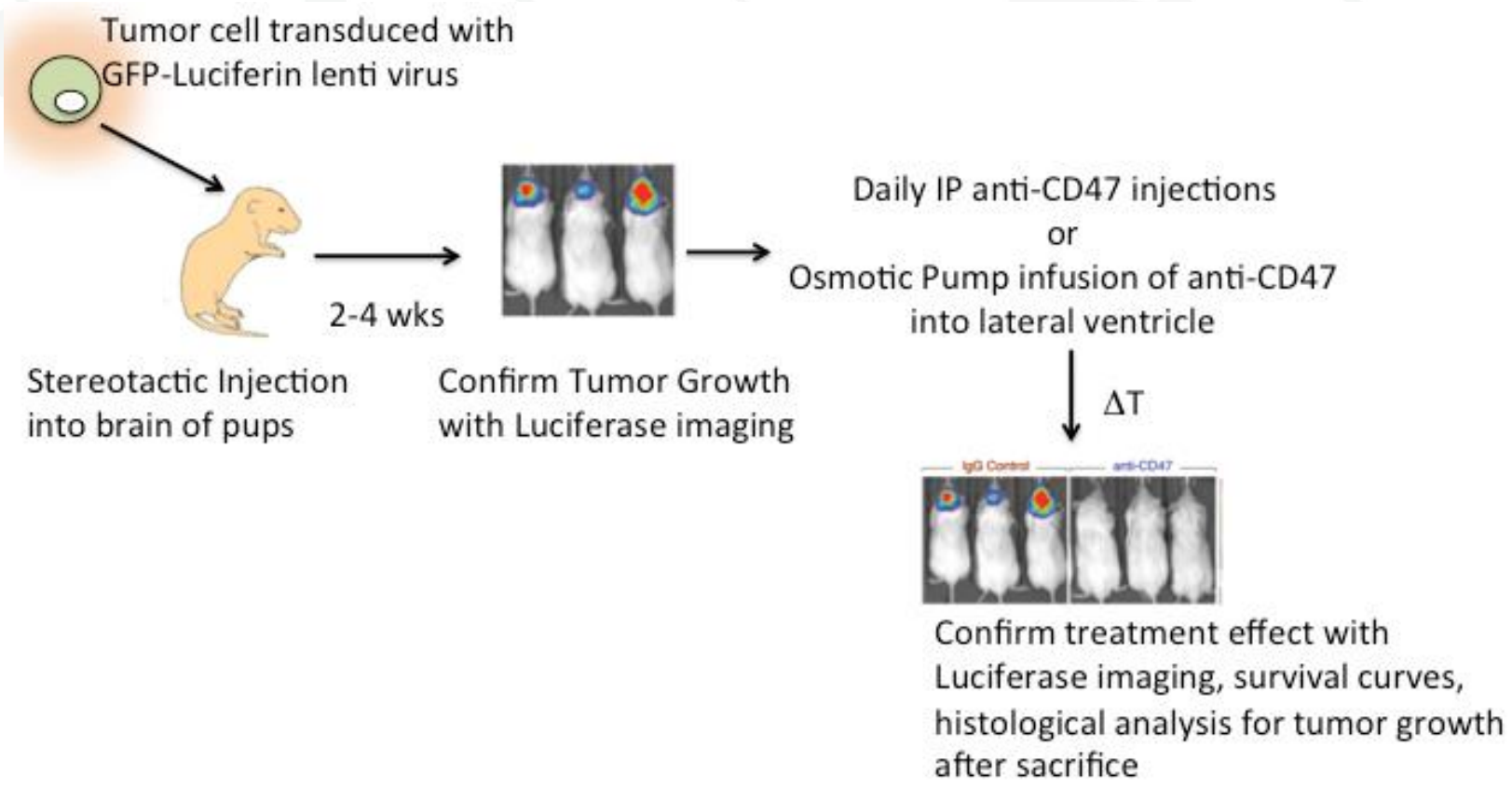
In vivo Effect of Anti-CD47 Treatment



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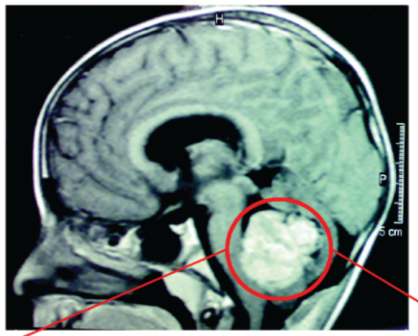
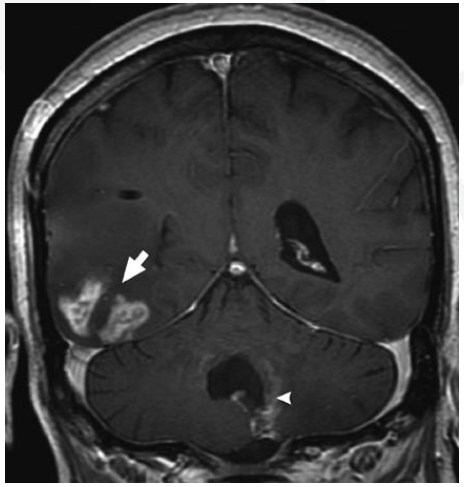
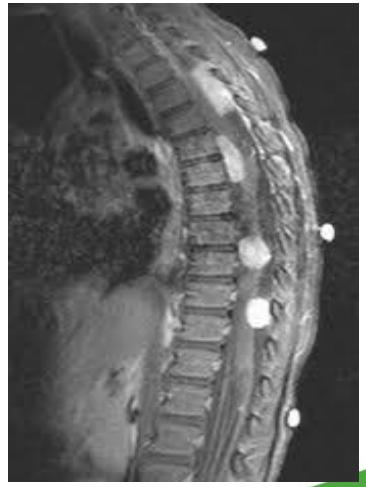
Medulloblastoma







WNT (10%)	SHH (30%)	Group 3 (25%)	Group 4 (35%)
CTNNB1 mutation Monosomy 6	*PTCH1/SMO/SUFU* mutation *MYCN* amplification	*MYC* amplification *PVT1-MYC* fusion	*CDK6* amplification Isochromosome 17q *SNCAIP* duplication
WNT signaling	SHH signaling PI3K signaling	Photoreceptor/GABAergic signaling TGF-ß signaling	Neuronal/Glutamatergic signaling NF-kB signaling
MYC +	*MYCN* +	*MYC* +++	Minimal *MYC/MYCN*
5y OS 94%	5y OS 87%	5y OS 32%	5y OS 76%
Rare M+	Uncommon M+	Very frequent M+	Frequent M+

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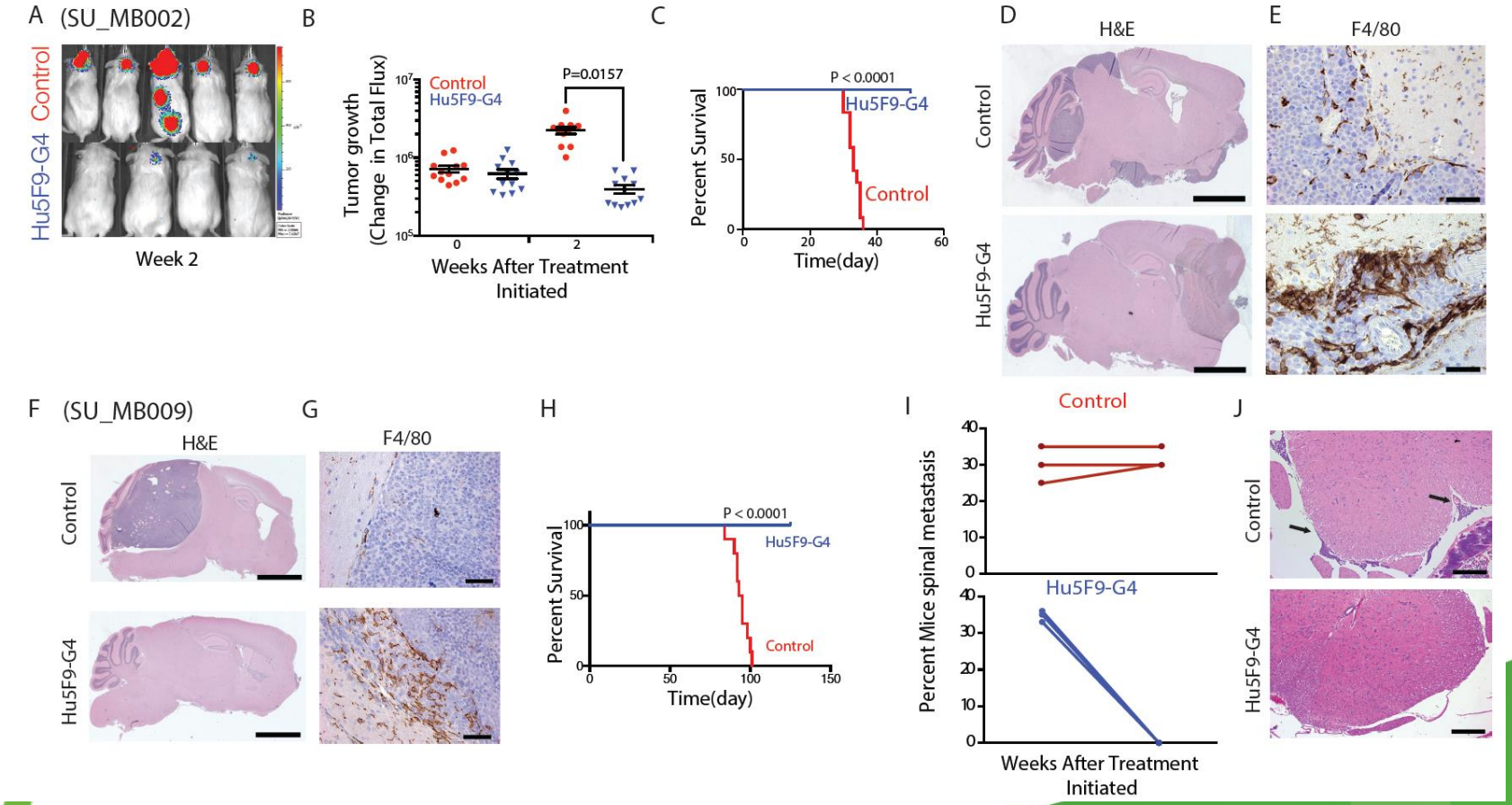
Hu5F9-G4 Inhibits Pediatric Brain Cancer Growth

C-Myc Medulloblastoma (Group 3)



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Gholamin, Mitra, Cheshier. Science- Translational Medicine. March 2017

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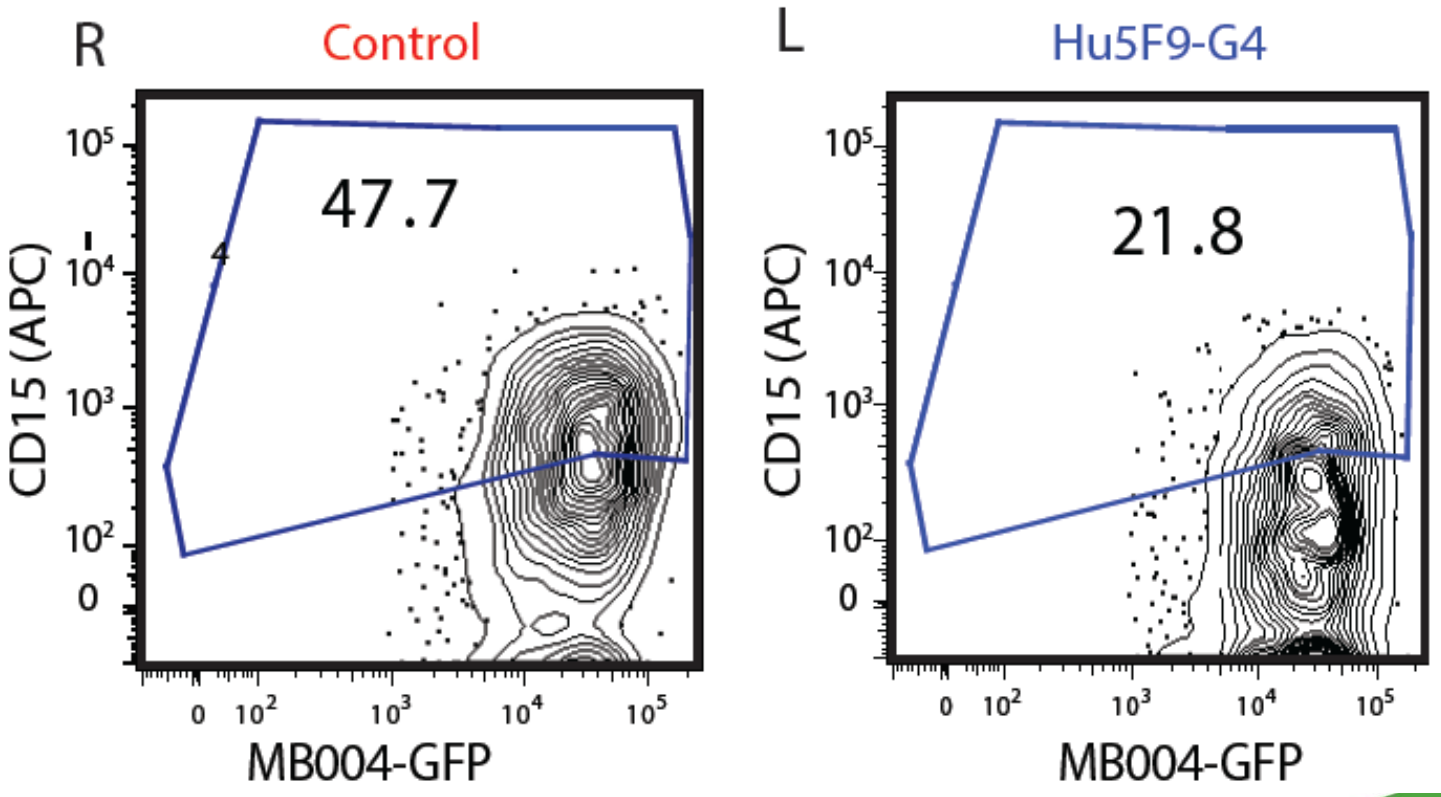

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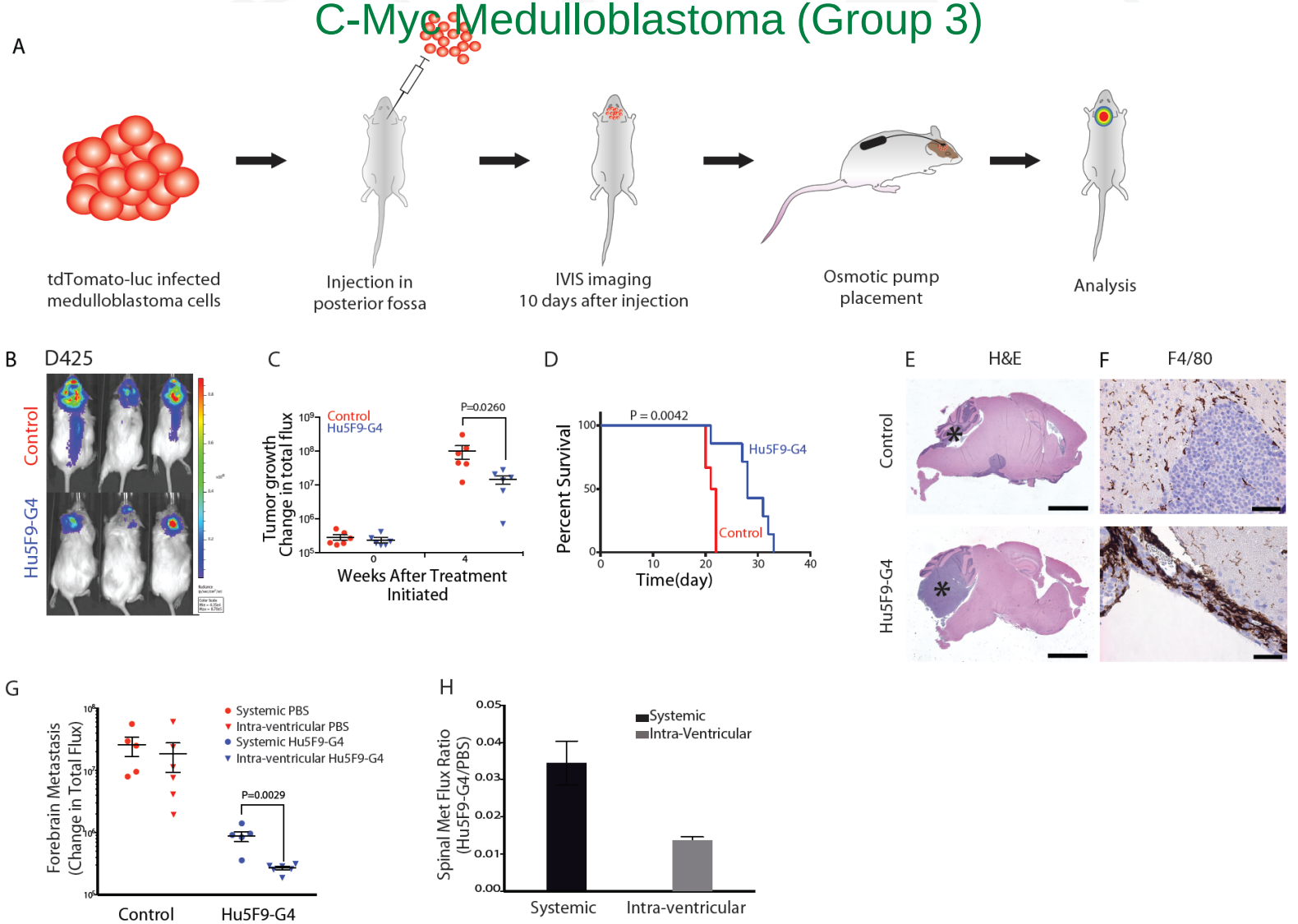
Gholamin, Mitra, Cheshier. Science- Translational Medicine. March 2017

Intraventricular infusion of Hu5F9-G4 accelerates Spinal and Leptomeningeal Metastasis Treatment

C-Myc Medulloblastoma (Group 3)



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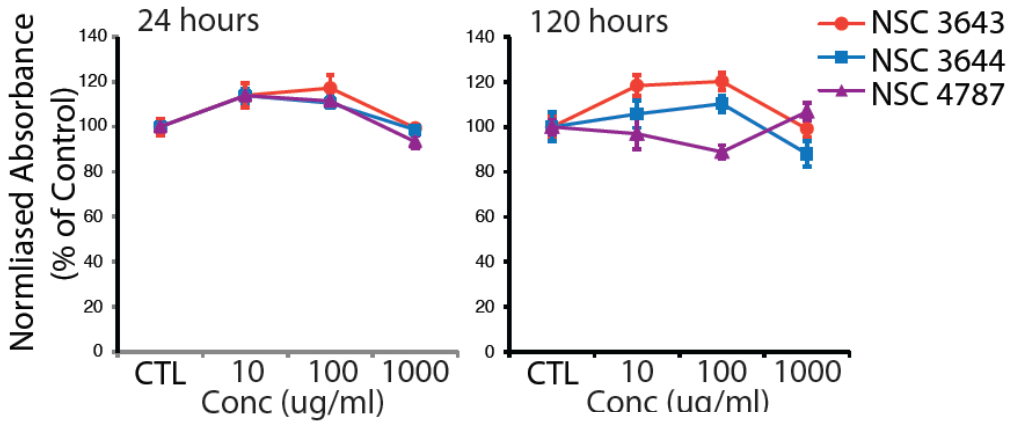
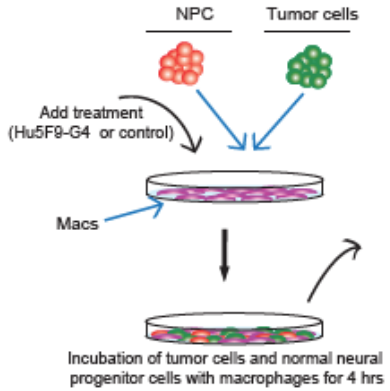
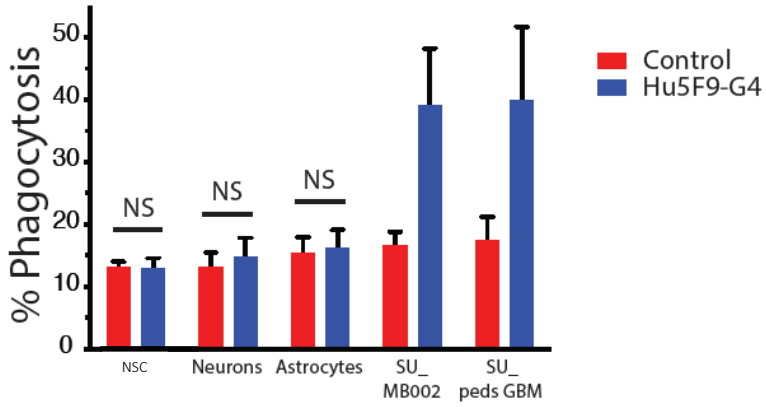
Gholamin, Mitra, Cheshier. Science- Translational Medicine. March 2017

Hu5F9-G4 Does Not Promote Phagocytosis of Normal Nerual Tissues Including NSC

C-Myc Medulloblastoma (Group 3)







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Hu5F9-G4 Does Not Promote Phagocytosis of Normal Neural Tissues Including NSC

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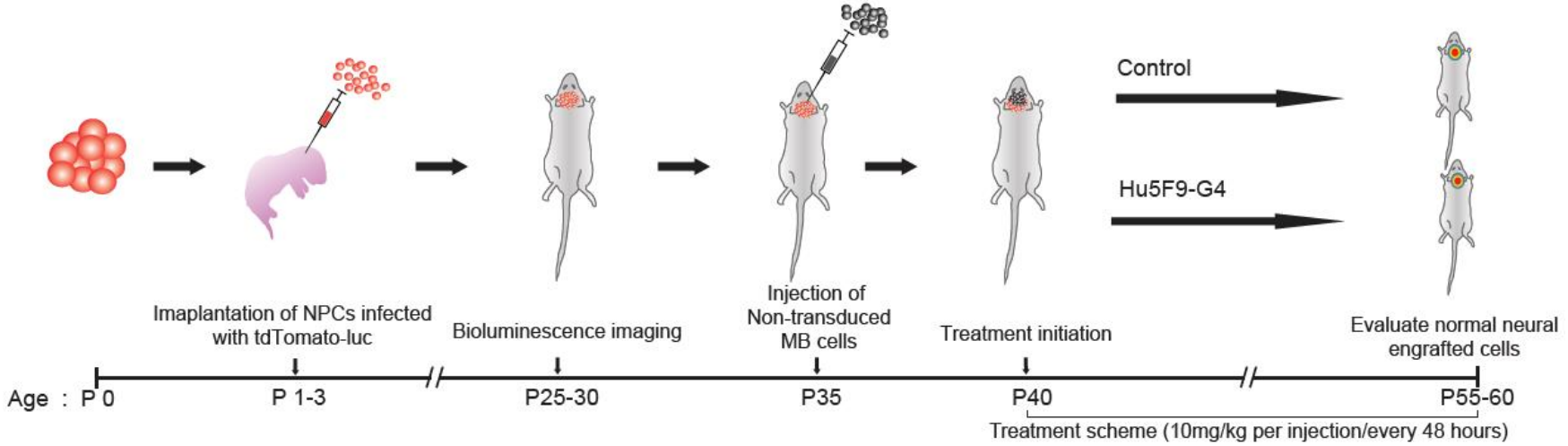

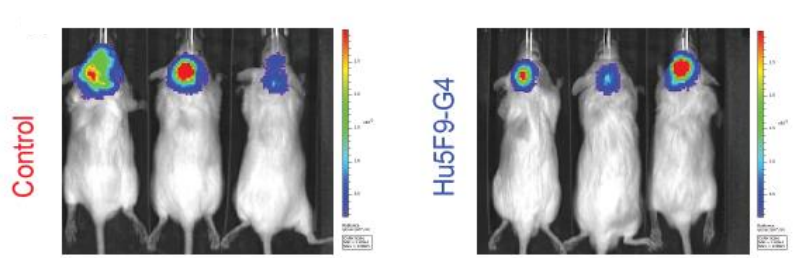

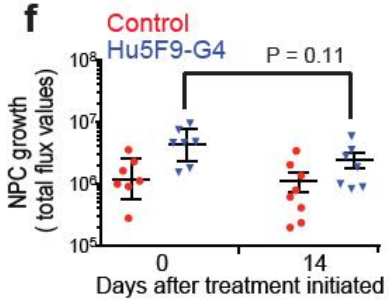

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Gholamin, Mitra, Cheshier. Science- Translational Medicine. March 2017

Summary

- *CD47 Expressed on all tumors*
- *Anti-CD47 highly efficacious in vitro and in vivo in all leukemias and solid tumors tested in the preclinical setting*
- *Anti-CD47 highly efficacious in vitro and in vivo in all brain cancers tested in the preclinical setting (Medulloblastoma, Pediatric Glioblastoma, Adult Glioblastoma, DIPG, ATRT, PNET)*
- *No toxicity to normal CNS tissue including progenitors*

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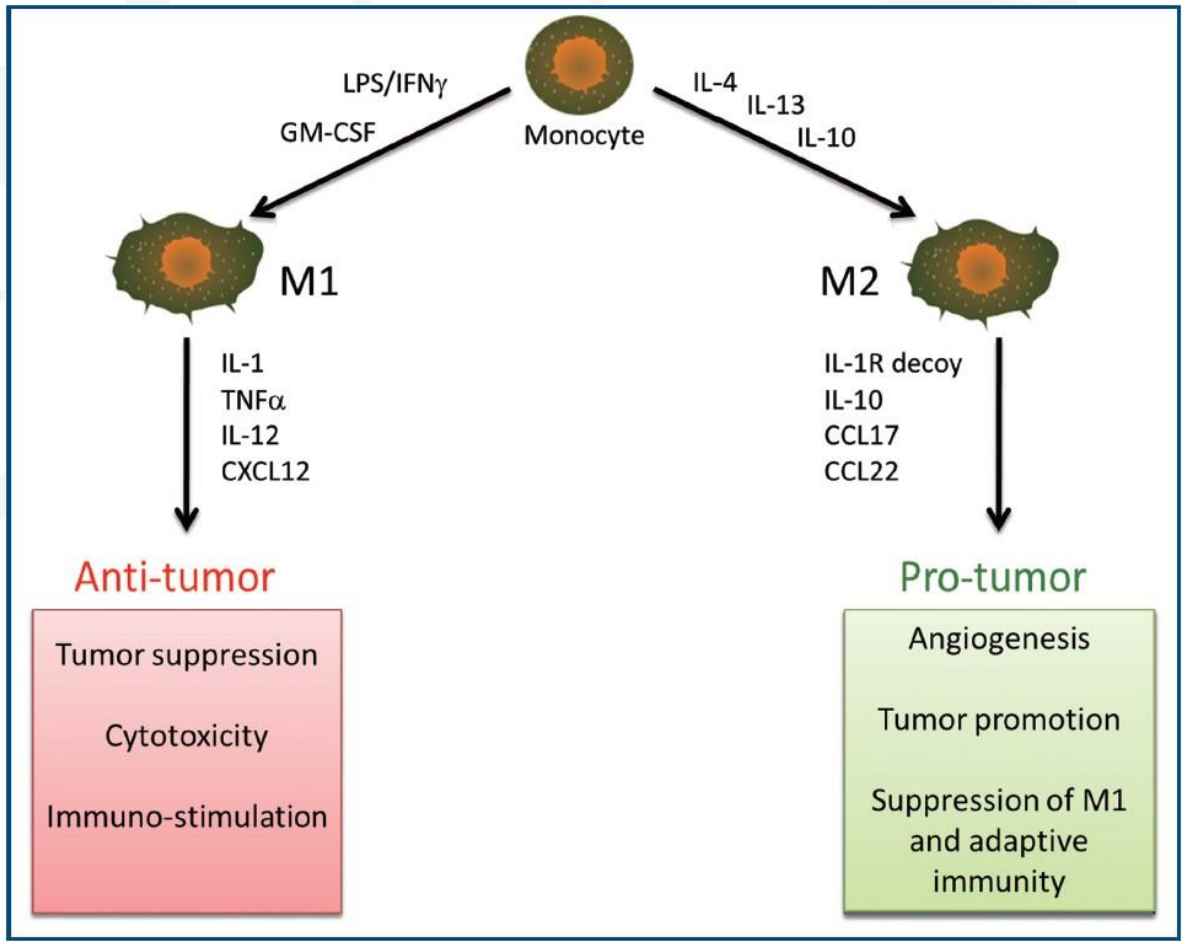
Macrophage Activation Subtypes



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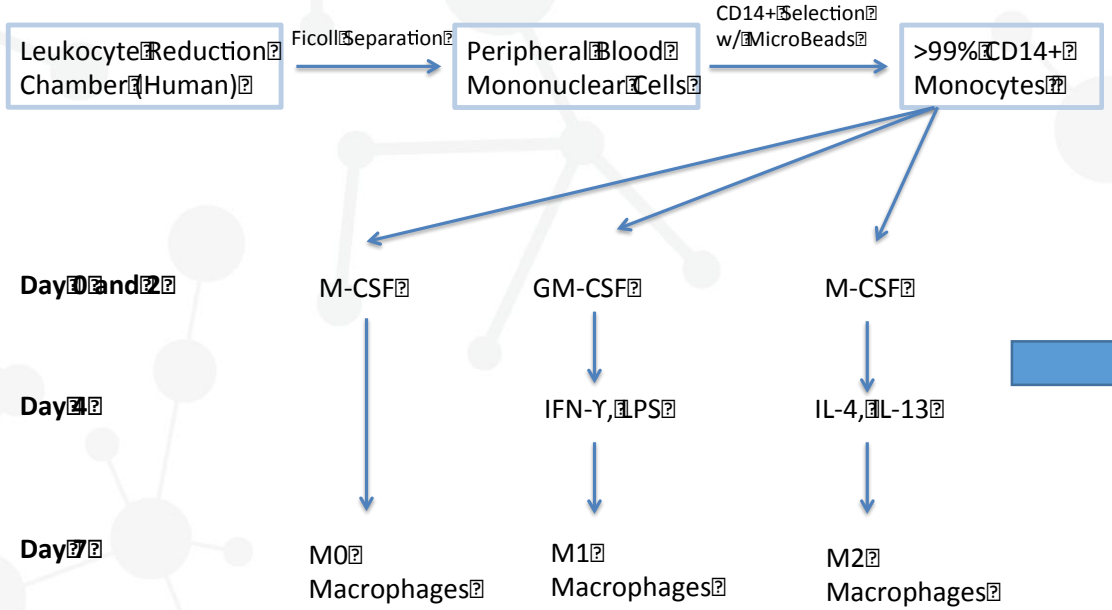
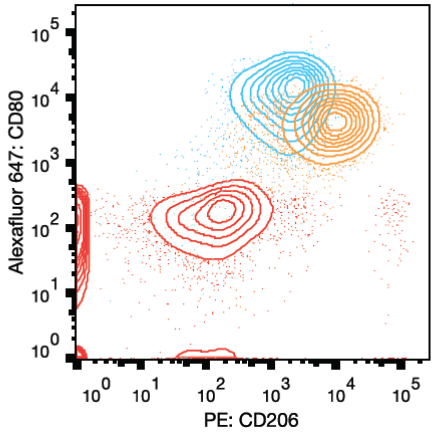
Enforced Macrophage Polarization to Generate M0, M1, and M2 Macrophages





	Subset Name
	M1 Macrophages
	M2 Macrophages
	GBM Cells

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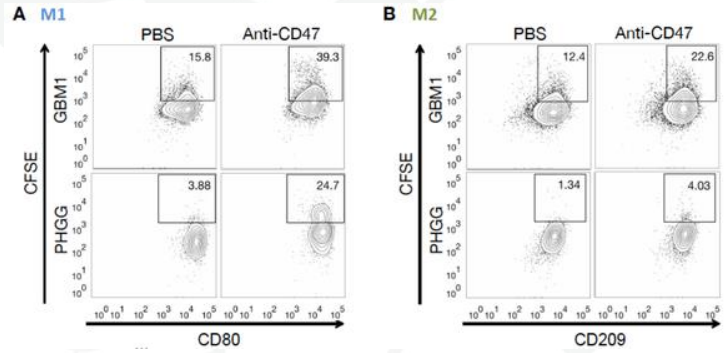
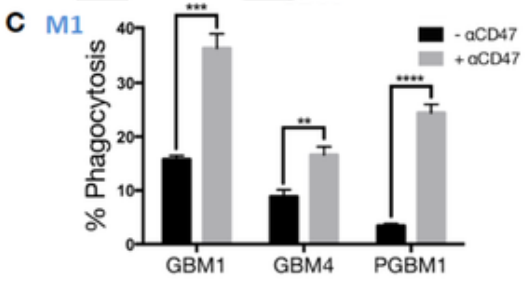
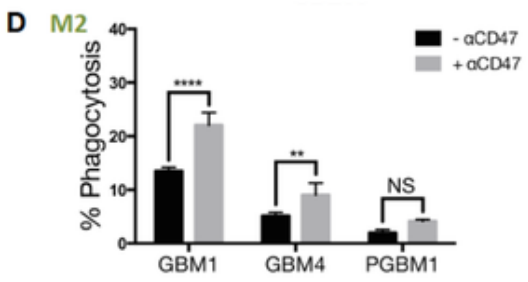
Zhang, Hutter, Mitra, Cheshier. PLOS-One. 2016

Anti-CD47 Promotes Tumor Phagocytosis by M1 and M2 Polarized Mouse and Human Macrophages

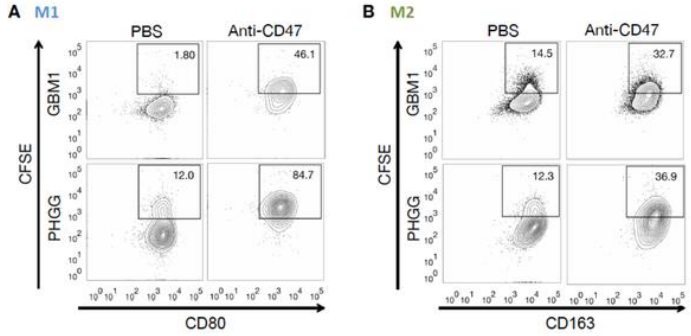
Mouse

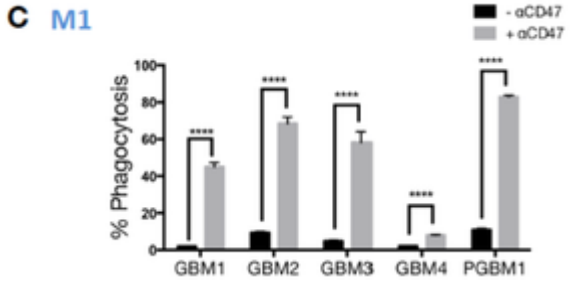


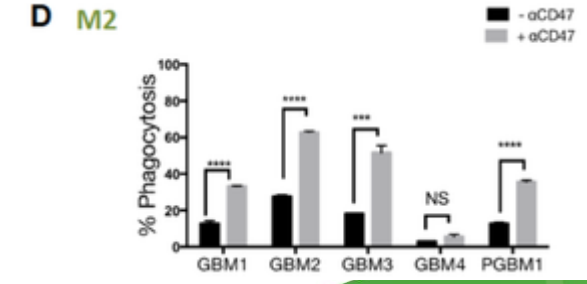




Human







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Zhang, Hutter, Mitra, Cheshier. PLOS-One. 2016

Anti-CD47 Promotes an M1 Phenotype of Macrophages within Brain Tumors In Vivo

Figure 5C

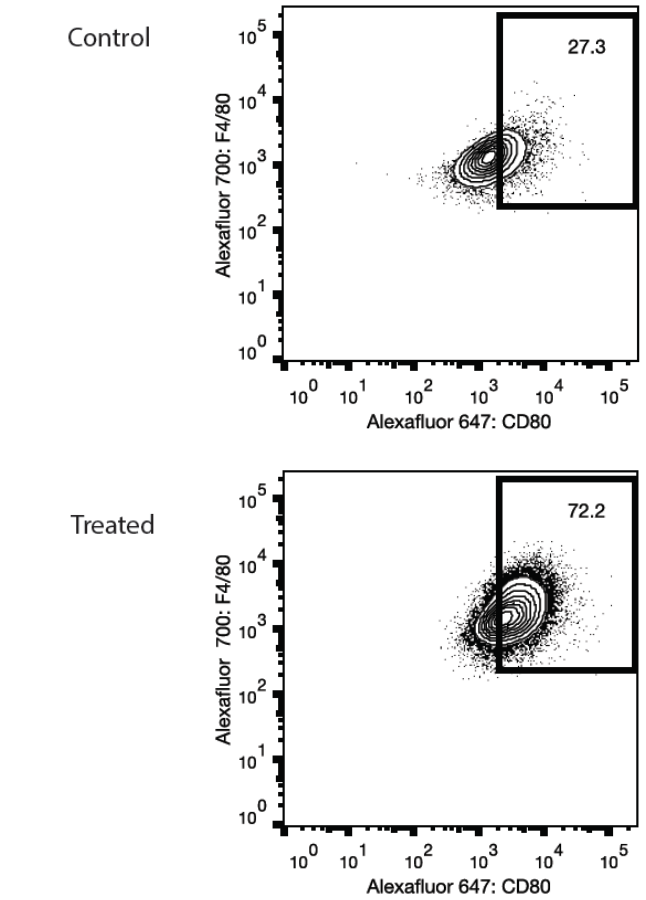
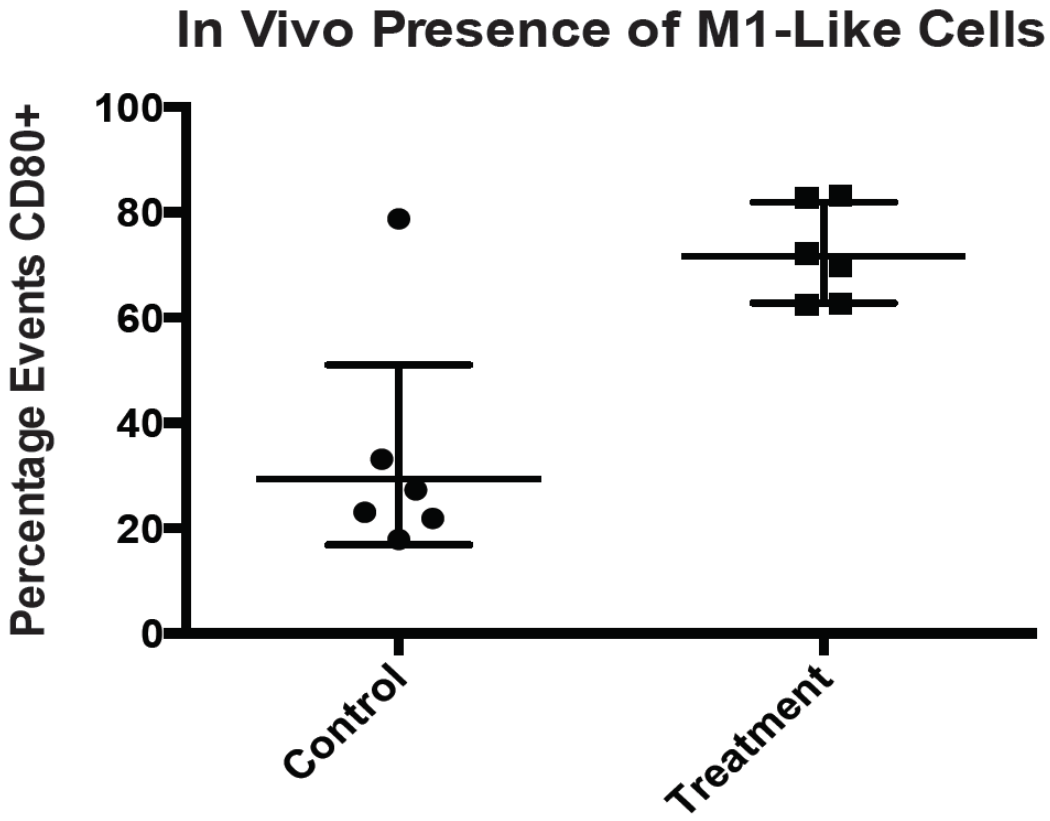


Figure 5D



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Zhang, Hutter, Mitra, Cheshier. PLOS-One. 2016

Summary

- *M1 and M2 polarized macrophages are capable of responding to anti-CD47 with increased tumor phagocytosis*

- *Tumor associated macrophages in the setting of in vivo anti-CD47 treatment possess the M1 phenotype*

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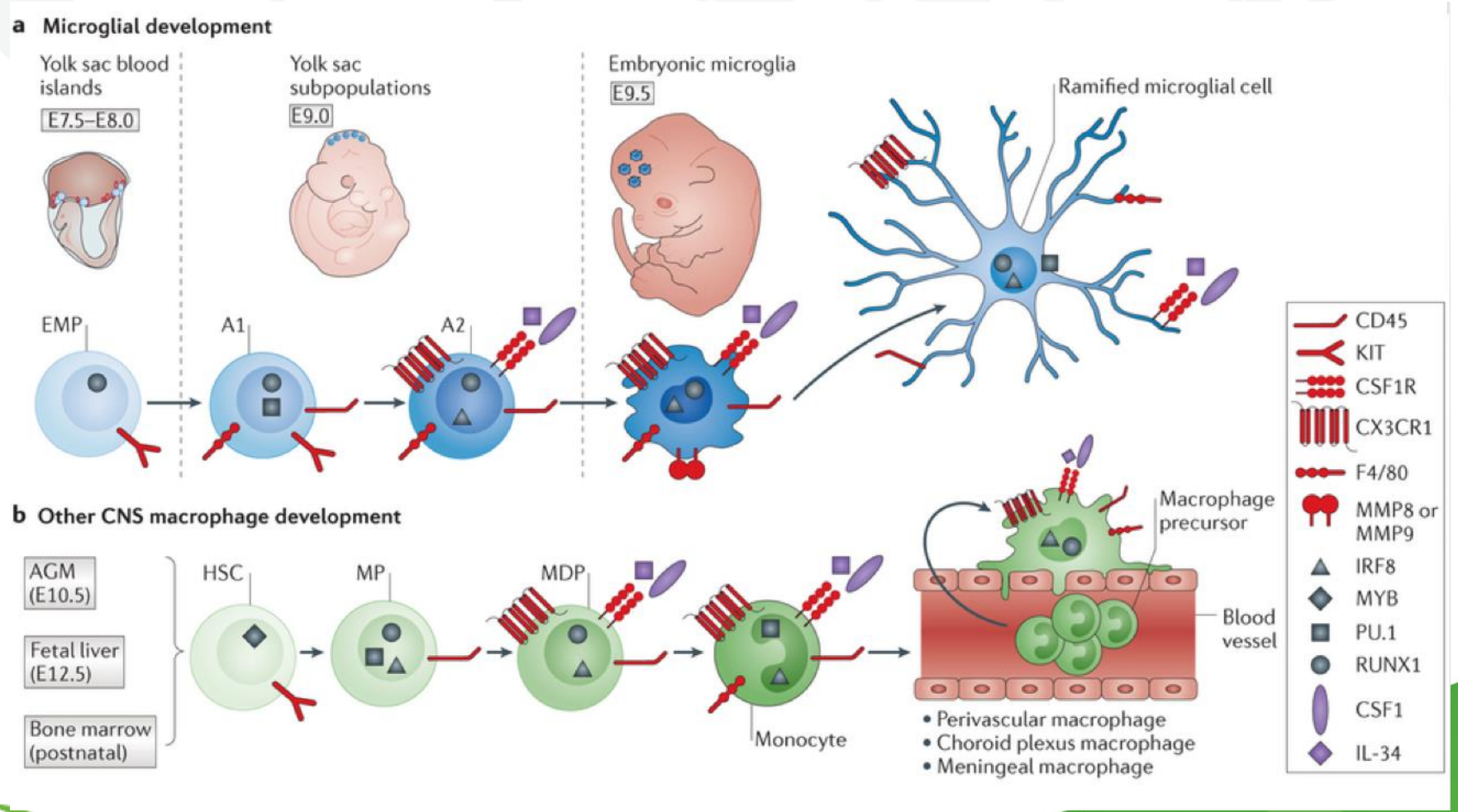
Macrophages vs. Microglia



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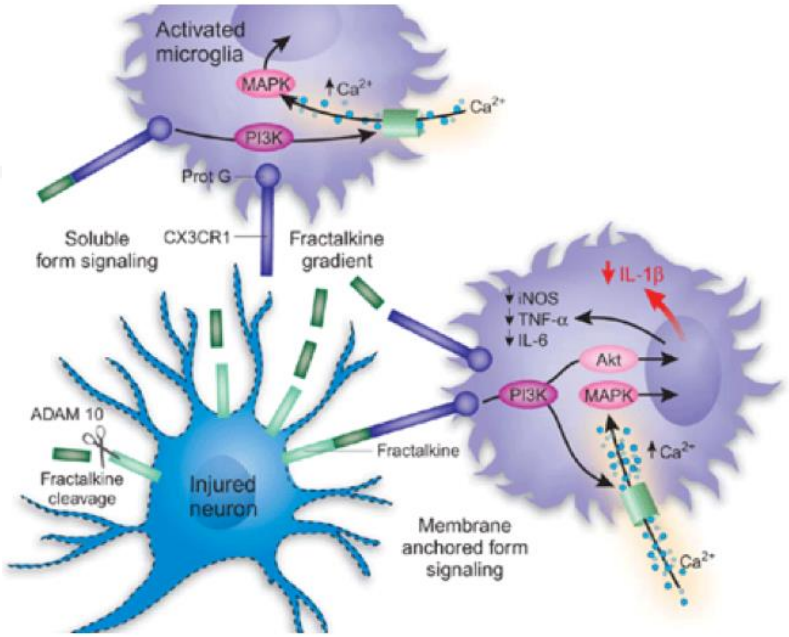
Prinz M, Priller J.Nat Rev Neurosci. 2014

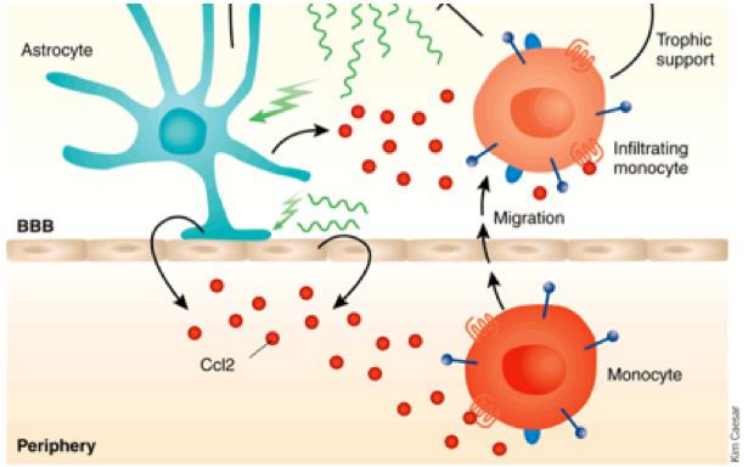
Microglia are Cx3cr1+
Macrophages are Ccr2+

Cx3cr1 (Fractalkine receptor)



Diane Bérangère Ré & Serge Przedborski
Nature Neuroscience **9**, 859 - 861 (2006)

Ccr2 (chemokine receptor 2)



Britschgi et al. Nat. Neuroscience

Expressed on microglia from embryonal stages through adulthood

CCR2 plays an important role in the extravasation and transmigration of **monocytes during inflammation**.

It controls the entry of bone-marrow-derived Ly6C^{hi} monocytes into the brain

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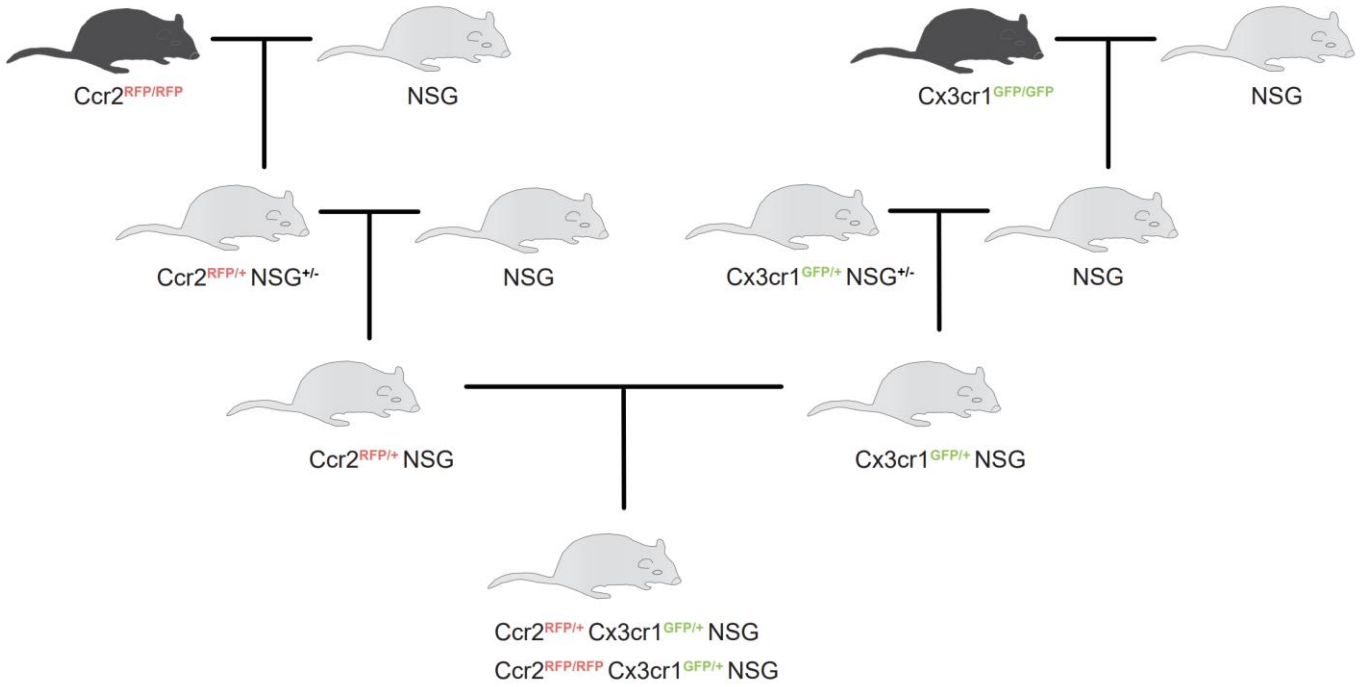
Generation of Mice to Distinguish Macrophages from Microglia



- Graft intracranially with human brain tumors
- Definitive separation of microglia from macrophages
- Direct invivo assessment of phagocytosis modulation

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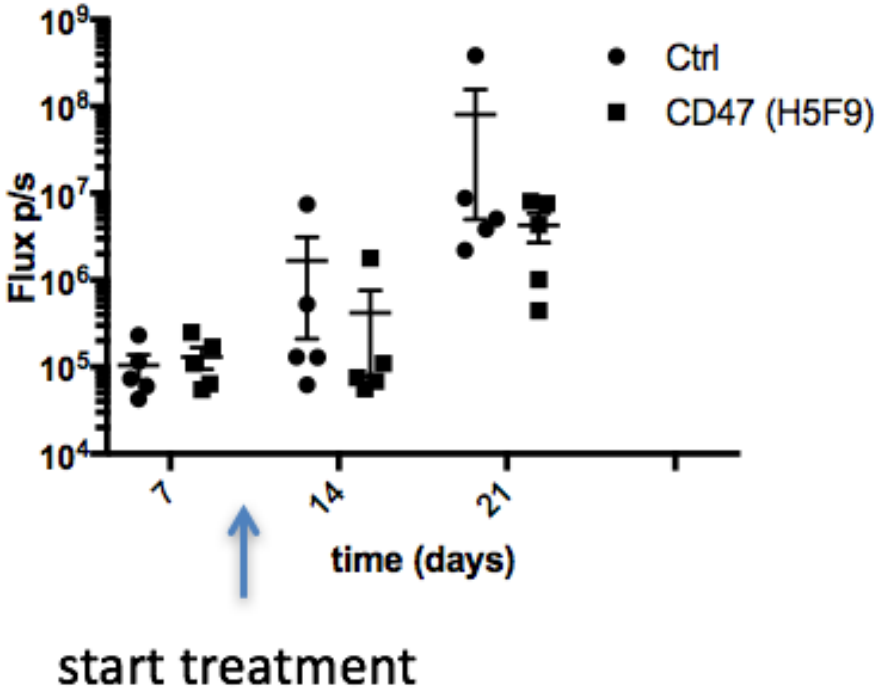
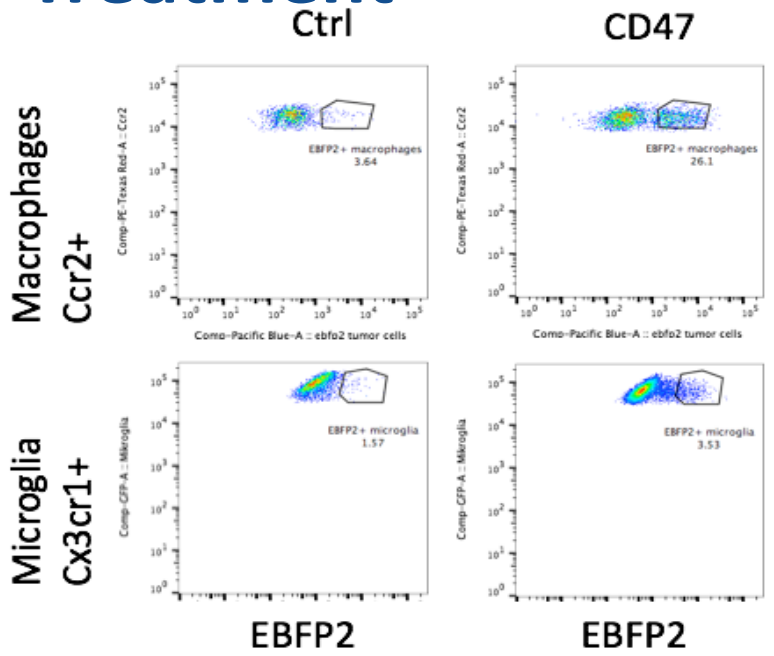
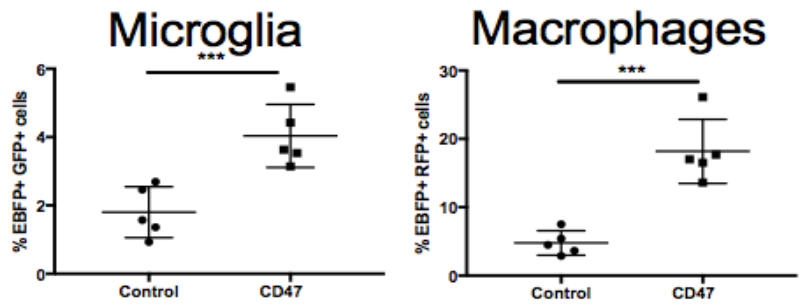
Hutter, Mitra, Cheshier. PNAS 2019

Macrophage and Microglia Within Implanted GBM Respond to Anti-CD47 Treatment







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Hutter, Mitra, Cheshier. PNAS 2019

RNAseq of Macrophage and Microgila in Response to Anti-CD47 Treatment

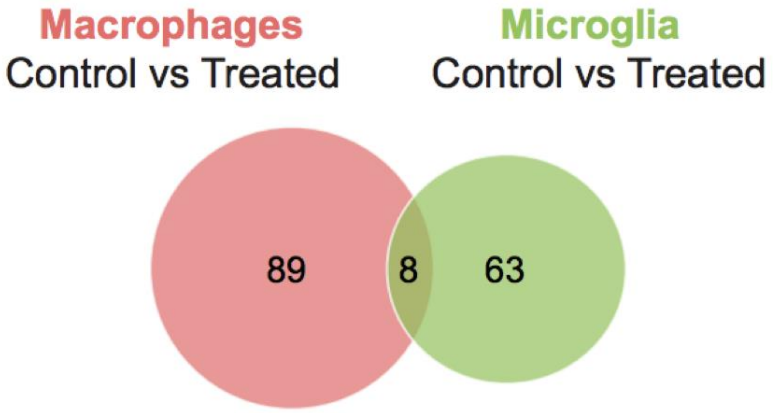
Iniate mesenchymal glioma (T387) in *NSG-Ccr2*$^{RFP/wt}$ *Cx3cr1*$^{GFP/wt}$ mice

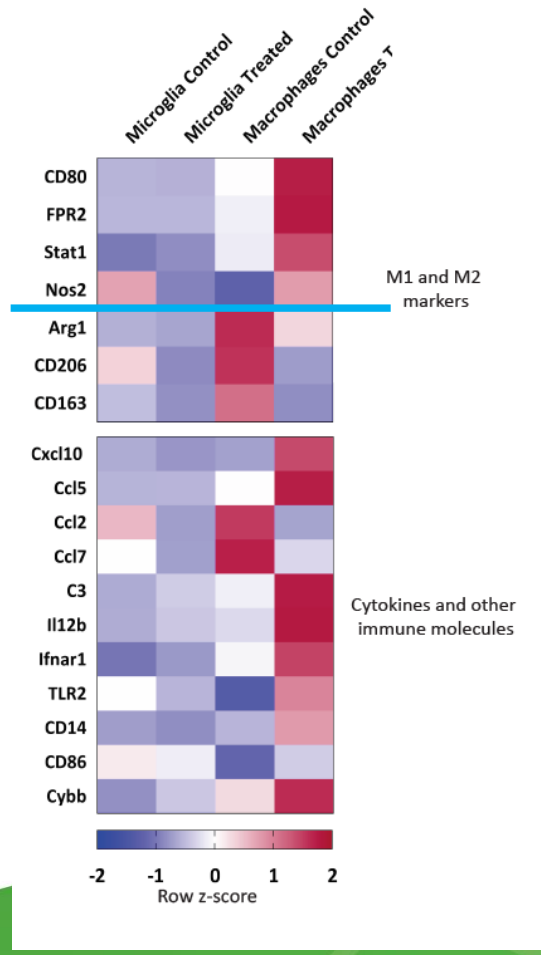
3 weeks of treatment

Isolation of TAMs

Macrophages: sorted for RFP bright GFP low

Microglia: sorted for GFP bright RFP low





Hutter, Mitra, Cheshier. PNAS 2019

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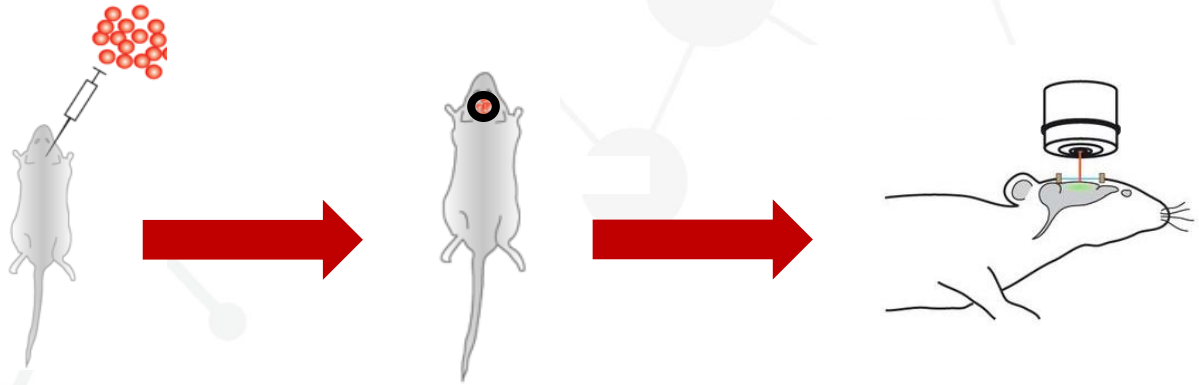
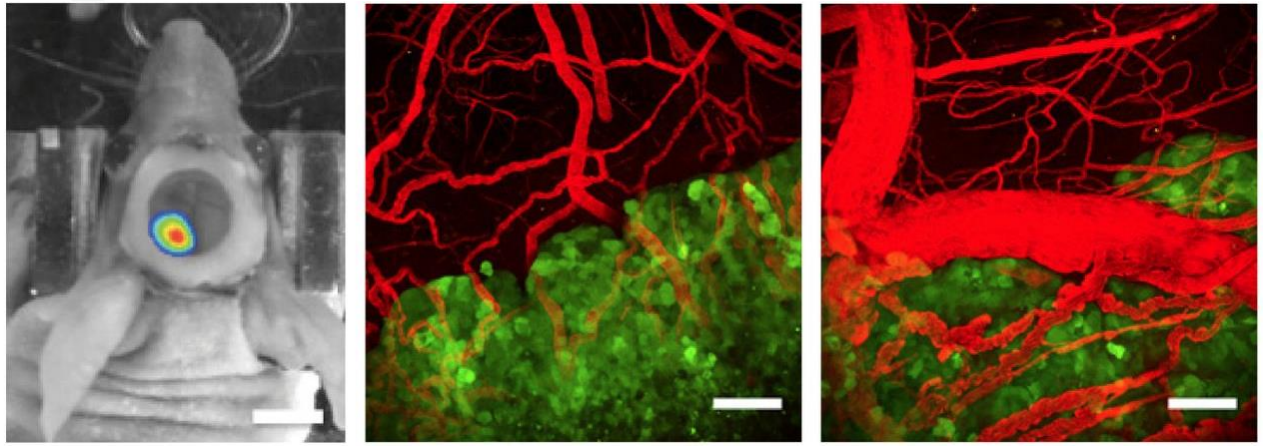
Cranial Windows Allow for Live Videos of Macrophages and Microglia within Implanted Human Malignant Glioma





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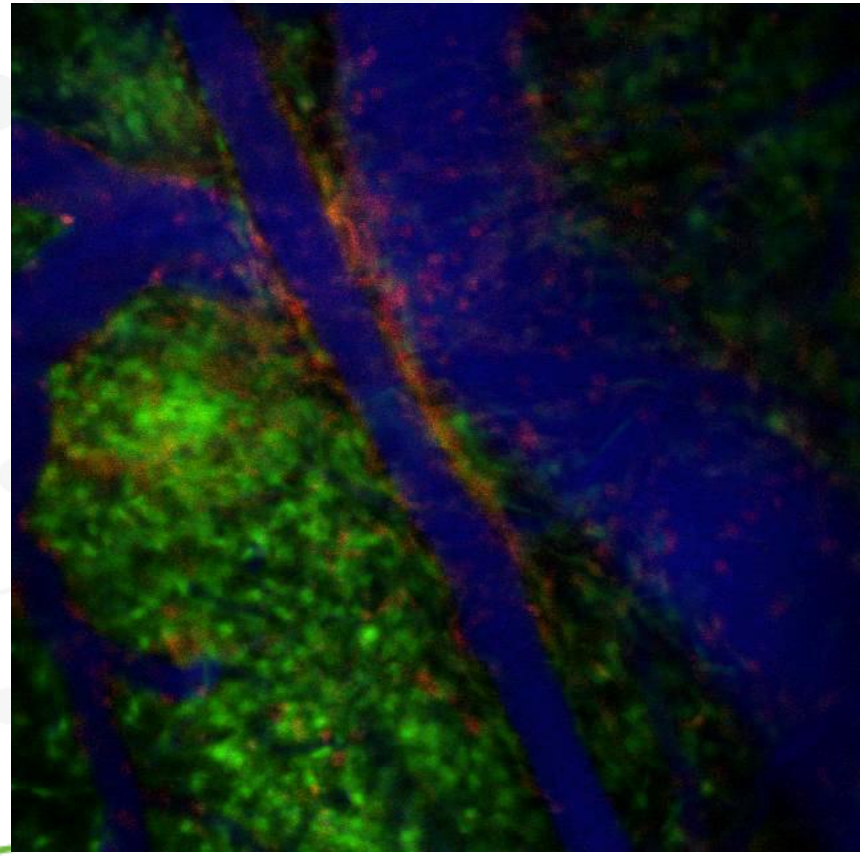
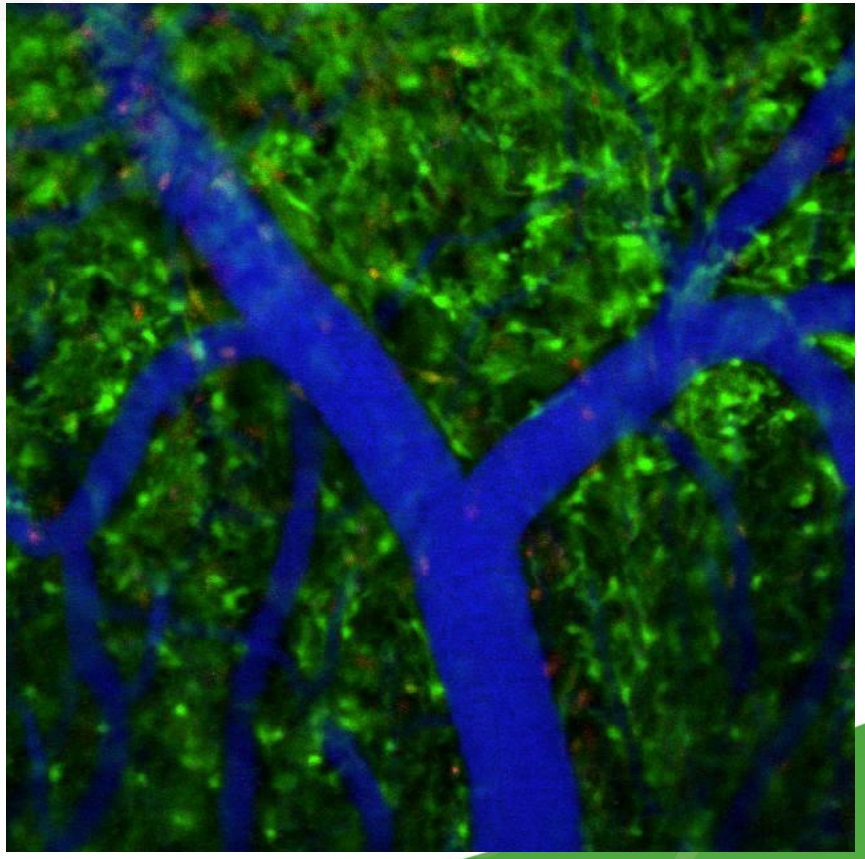
Hutter, Mitra, Cheshier. PNAS 2019

Live Videos of Macrophages in Human Malignant Glioma

+ Anti-CD47



- Anti-CD47



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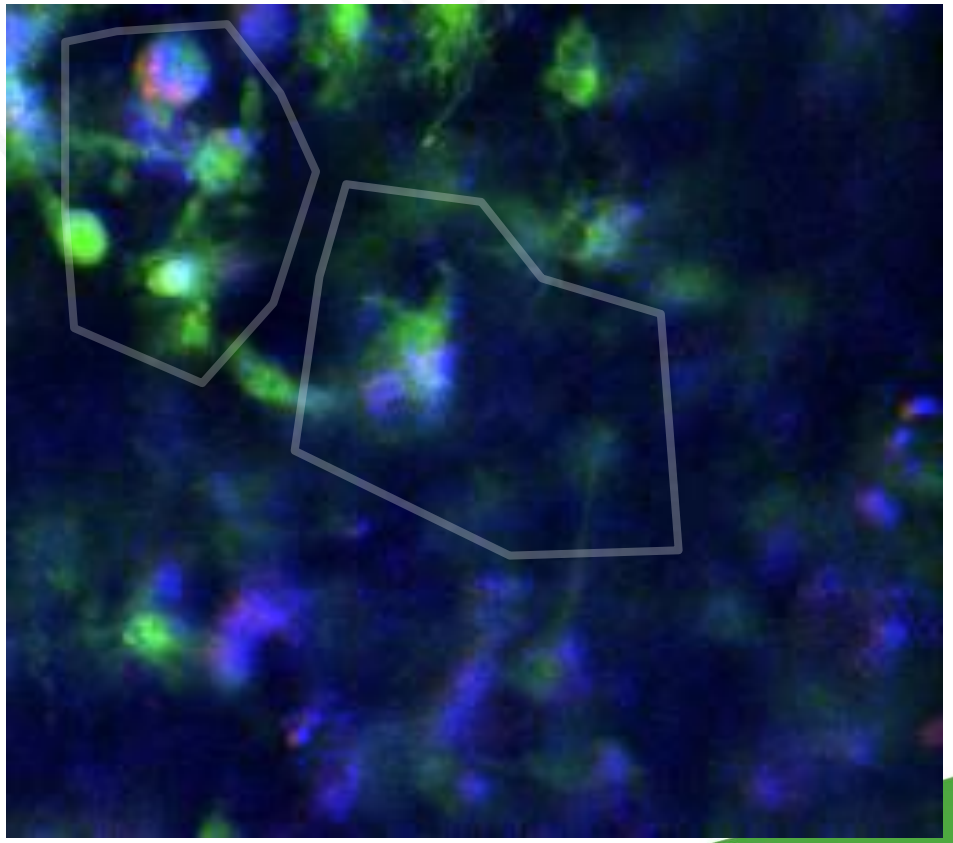
Hutter, Mitra, Cheshier. PNAS 2019

Live Videos of Microglia in Human Malignant Glioma

Microglia (Green) Eating Tumor (Blue)



+ Anti-CD47

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Hutter, Mitra, Cheshier. PNAS 2019

Summary

- *Macrophages and Microglia can be isolated from brain tumors of xenographted mice using the Cx3cr1 X CCL2 mouse model*
- *Macrophages and Microglia can respond to anti-CD47 treatment with increased tumor phagocytosis*
- *Macrophages and Microgila display very different gene expression changes in response to anti-CD47 with macrophages being more pro-inflammatory*

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CD47 Clinical Trials

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.

NEJM 2019

rapid communication

First-in-Human, First-in-Class Phase I Trial of the Anti-CD47 Antibody Hu5F9-G4 in Patients With Advanced Cancers

Branimir I. Sikic, MD[1]; Nehal Lakhani, MD, PhD[2]; Amita Patnaik, MD[3]; Sumit A. Shah, MD[1]; Sreenivasa R. Chandana, MD, PhD[2]; Drew Rasco, MD[3]; A. Dimitrios Colevas, MD[1]; Timothy O'Rourke, MD[2]; Sujata Narayanan, MD[1]; Kyriakos Papadopoulos, MD[3]; George A. Fisher, MD, PhD[1]; Victor Villalobos, MD, PhD[4]; Susan S. Prohaska, PhD[1]; Maureen Howard, PhD[1]; Muralidhar Beeram, MD[3]; Mark P. Chao, MD, PhD[2]; Balaji Agoram, PhD[5]; James Y. Chen, MD, PhD[5]; Jie Huang, ScD[5]; Matthew Axt[5]; Jie Liu, PhD, MD[5]; Jens-Peter Volkmer, MD[5]; Ravindra Majeti, MD, PhD[1,5]; Irving L. Weissman, MD[1]; Chris H. Takimoto, MD, PhD[3]; Dana Supan, RN[1]; Heather A. Wakelee, MD[1]; Rhonda Aoki[1]; Mark D. Pegram, MD[1]; and Sukhmani K. Padda, MD[1]

JCO 2019

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FortySeven

CD47

Pipeline

Publications

Posters and Presentations

Pipeline

Preventing Innate Immune Suppression

			Discovery	Pre-Clinical	Phase 1	Phase 2	Phase 3
5F9 Anti-CD47 Antibody	Monotherapy	Solid Tumor & Ovarian			PHASE 1		
		Acute Myeloid Leukemia & Azacitidine			PHASE 1		
	Combination Therapy: Tumor Targeting Antibodies	Non-Hodgkin Lymphoma: Rituximab				PHASE 2	
		Colorectal Cancer: Cetuximab				PHASE 2	
	Combination Therapy: T Cell Checkpoint Inhibitors	Ovarian: Avelumab			PHASE 1		
		Bladder: Atezolizumab			P1 PENDING		
		Acute Myeloid Leukemia: Atezolizumab			P1 PENDING		
FSI-189 Anti-SIRPα Antibody				PRE-CLINICAL			

NIH U.S. National Library of Medicine

ClinicalTrials.gov

19 Anti-CD47 Clinical Trials

Thank You

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