Society for Immunotherapy of Cancer (SITC)

Immunotherapy for Brain Malignancies

Amy B Heimberger
M.D. Anderson Cancer Center

Advances in Cancer Immunotherapy™ - Texas
June 19, 2015

Society for Immunotherapy of Cancer

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Pharmaceutical/Biomarker Financial Disclosures

- **Laboratory and Clinical Studies: Merck**
- **Paid Consultant: Celldex Therapeutics, Bristol Myers Squibb, Caris Life Sciences**
- **Stock/Equity: Celldex Therapeutics, Caris Life Sciences**
- **Licensing Fees: Celldex Therapeutics**
- **Strategic Partnerships: Immatics**
- **Patents: EGFRvIII peptide vaccine (CDX-110), WP1066, immune modulatory miRNA portfolio, LUNAR-301**

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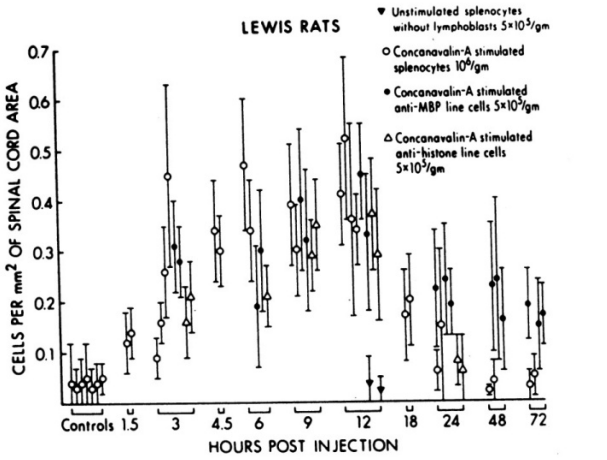
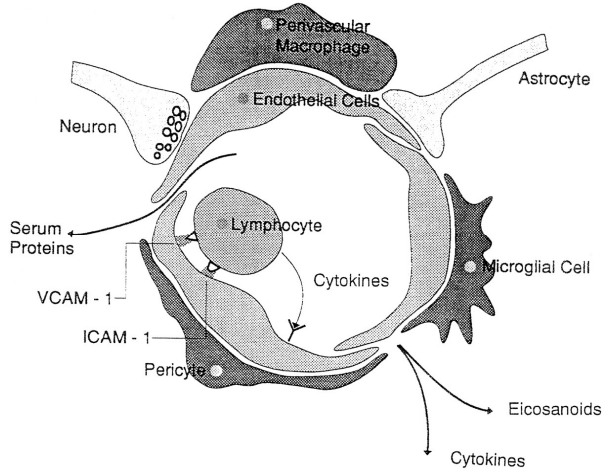
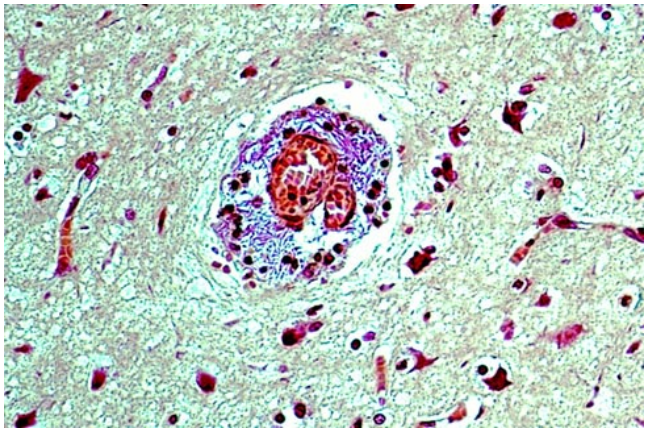
Myths of CNS Immunology

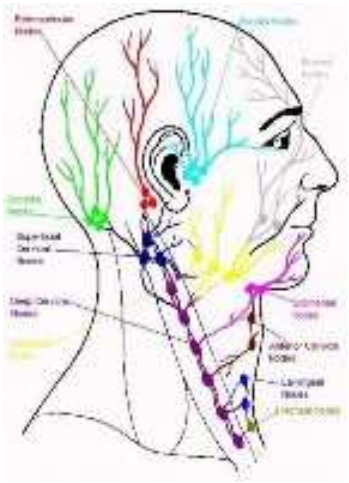
Immune cells do not gain access to the CNS secondary to the blood brain barrier





There is no lymphatic drainage from the brain





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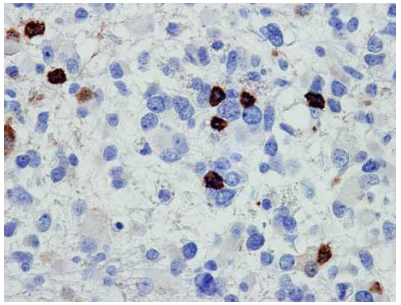
What is needed for an optimal anti-tumor immune therapeutic response?

- Immunological target (i.e. antigen) or a response that is not dependent on this (NK cells)
  - **EGFRvIII**, **IDH1 mutant, IMA-950**, **CMV pp65, HSP, tumor homogenates**
- Activate the T cell signal
  - 4-1BB antibodies/aptamers, OX40 antibodies, pro-inflammatory cytokines (**GM-CSF, IFN-γ**, IL-12, IL-15), **KLH, TLR agonists (CpG, poly IC**), **dendritic cells, viral therapy**, STING agonists
- Adequate trafficking to and infiltration of the tumor site
  - Chemokine (fractalkine receptor/CX3CR1)



- Maintenance of T cell effector function
  - **Inhibition of immune suppressive cytokines (TGF-β, IL-10),** STAT3 inhibitors, **checkpoint inhibitors**

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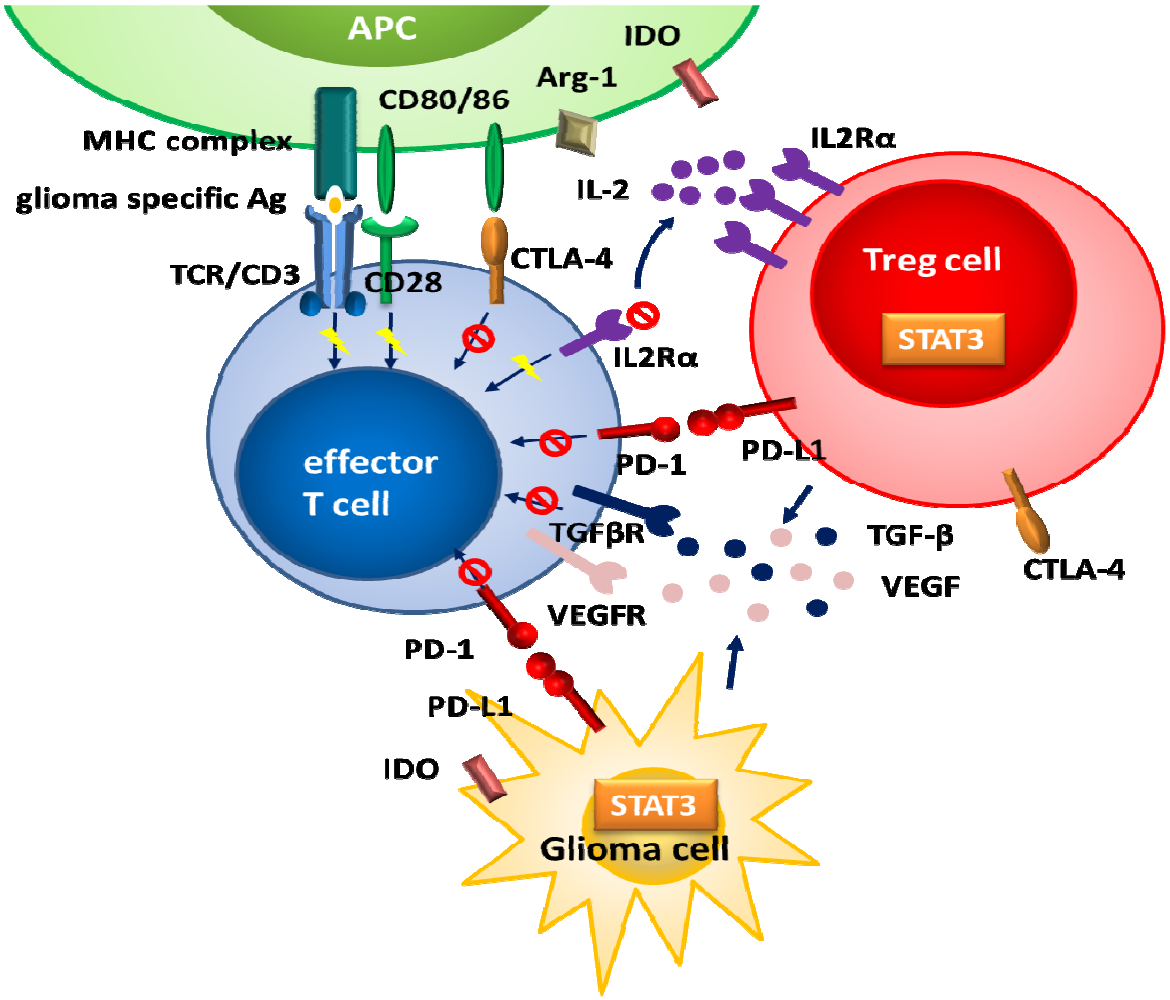
Mechanisms of Immune Activation and Inhibition



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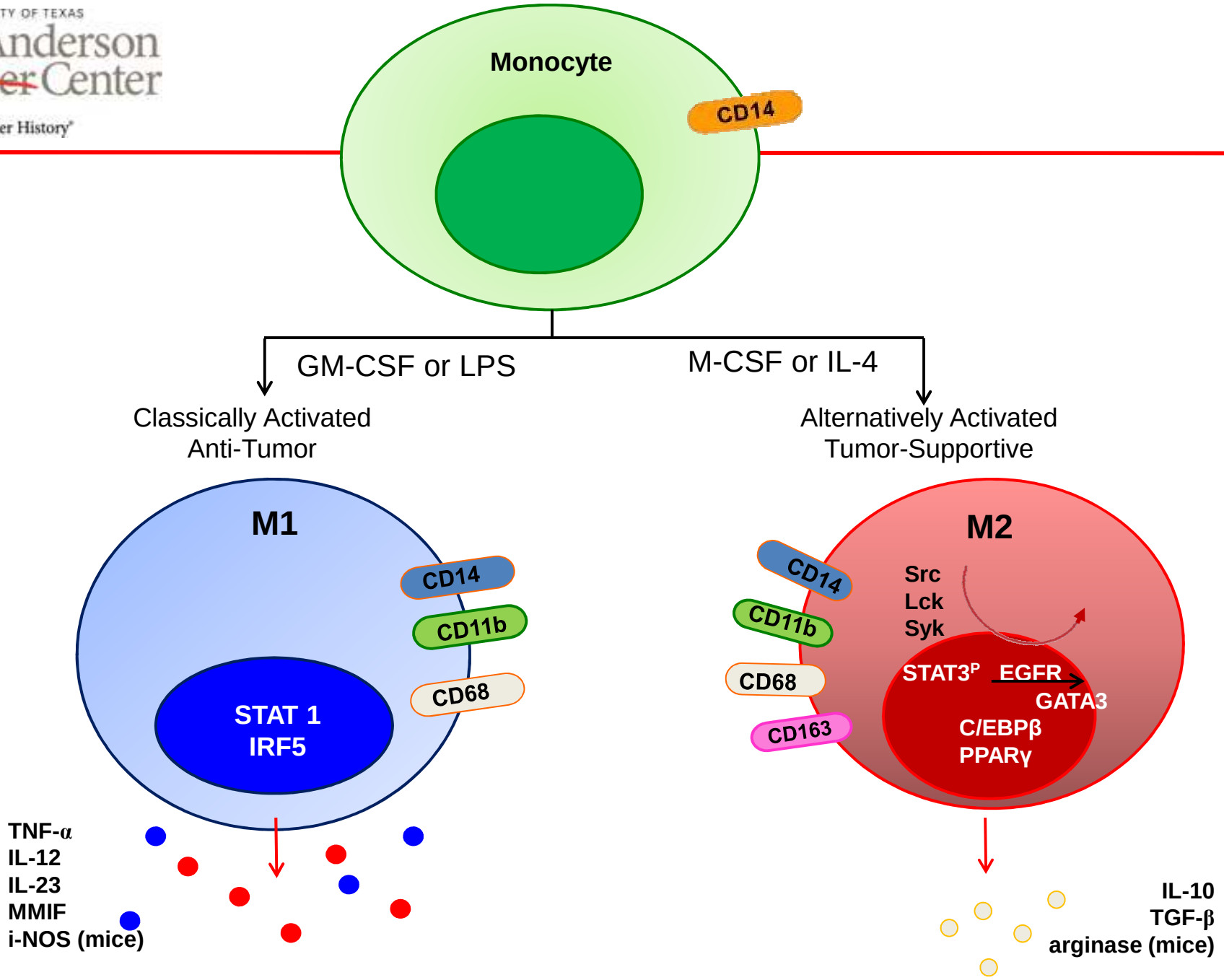

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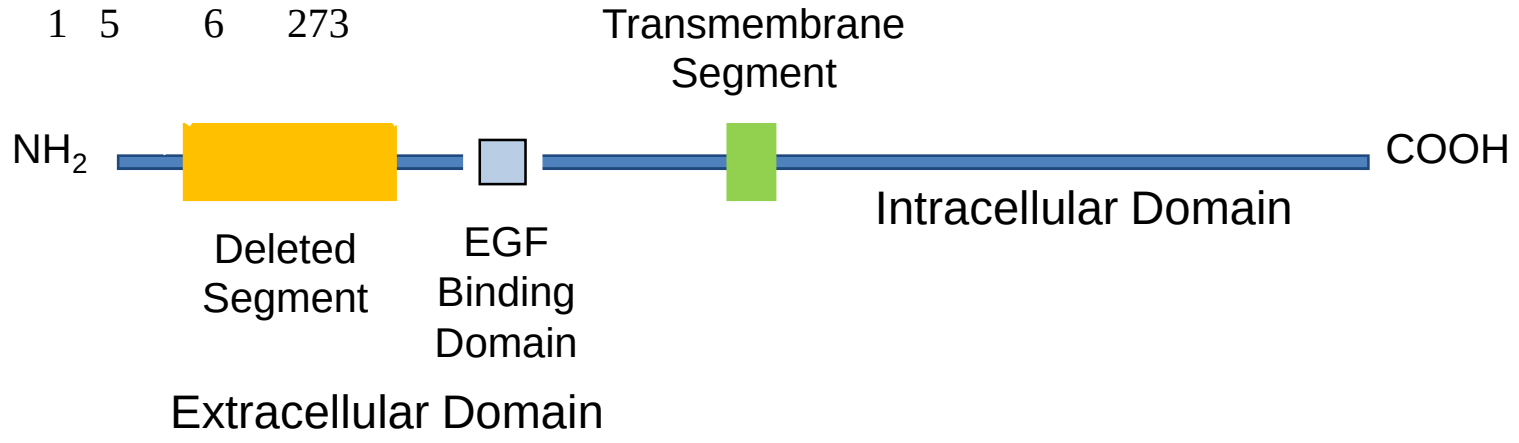
Epidermal Growth Factor Receptor Mutation



LEU-GLU-GLU-LYS-LYS-VAL-CYS-...-PRO-ARG-ASN-TYR-VAL-VAL-THR-ASP-HIS
Wild Type Amino Acid Sequence

CTG-GAG-GAA-AAG-AAA-GTT-TGC-...-CCC-CGT-AAT-TAT-GTG-GTG-ACA-GAT-CAC
Wild Type cDNA Sequence

CTG-GAG-GAA-AAG-AAA-GGT-AAT-TAT-GTG-GTG-ACA-GAT-CAC
Variant III cDNA Sequence

LEU-GLU-GLU-LYS-LYS-GLY-ASN-TYR-VAL-VAL-THR-ASP-HIS ← **PEP-3**
Variant III Amino Acid Sequence

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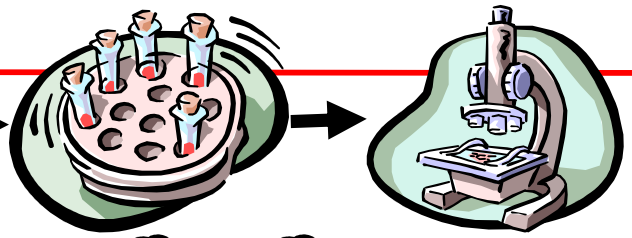
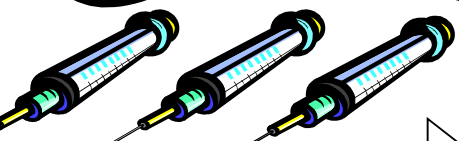
ACTIVATE Trial



Immunologic Monitoring

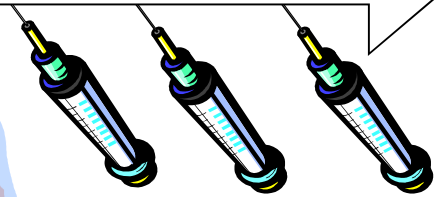
Leukapheresis



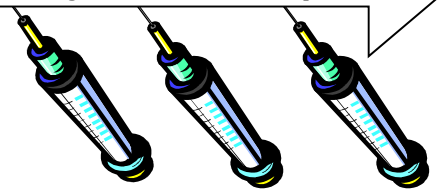
+ GM-CSF
(Every 2 weeks i.d.)

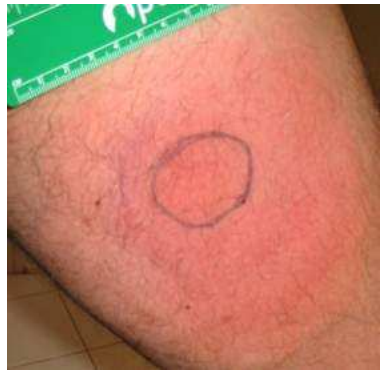
Randomize

PEPvIII-KLH + GM-CSF
(Every 2 weeks i.d.)

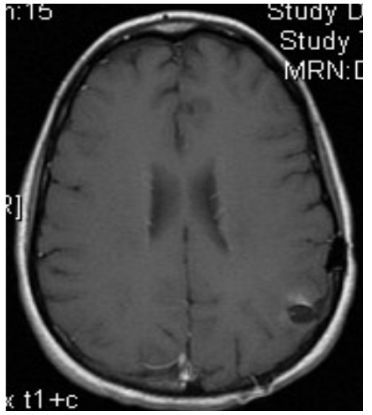


PEPvIII-KLH + GM-CSF
(Every 1 month i.d.)





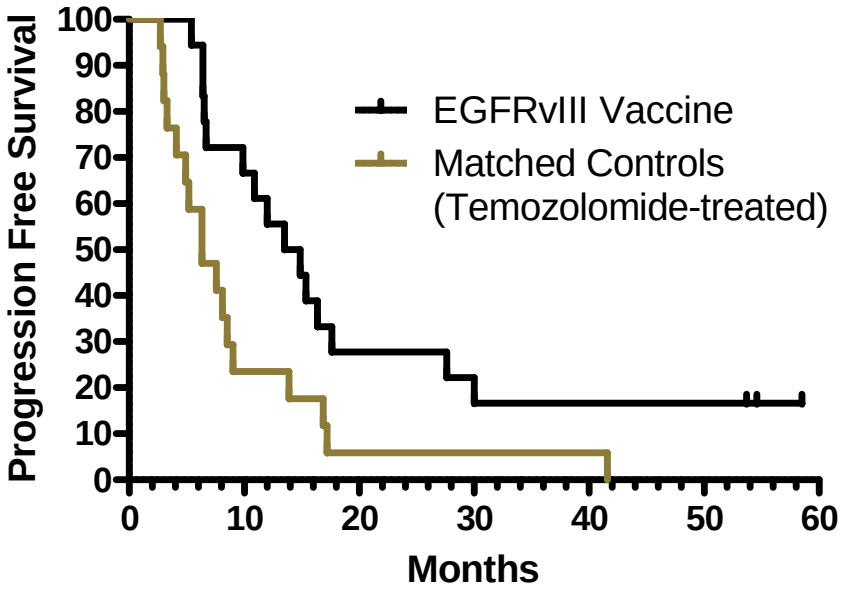
6000 cGy



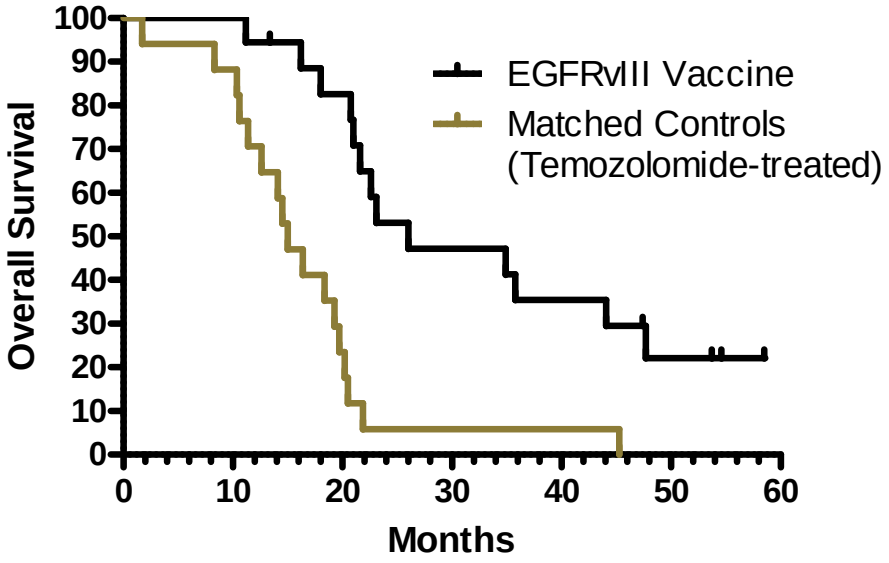
First patient treated in 7/04

Progression free survival and overall survival in ACTIVATE

PFS: 14.2 (CI95: 9.9, 17.6) months

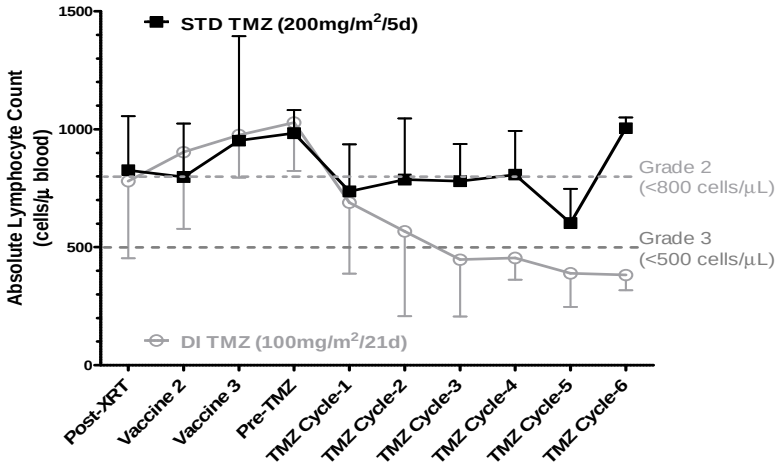


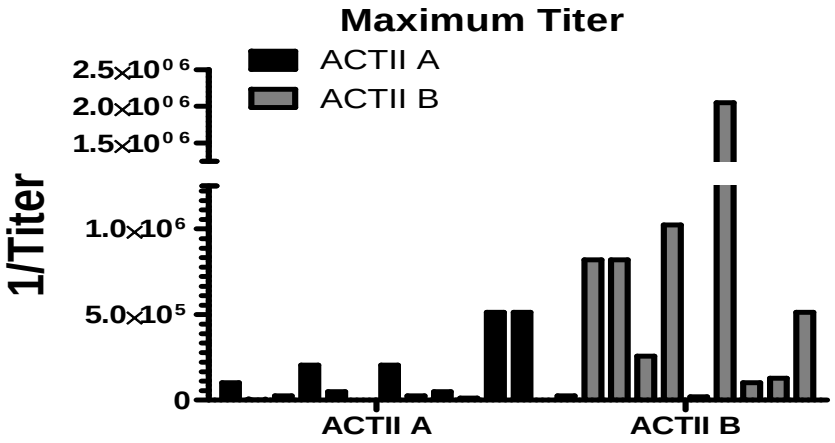
OS: 26.0 (CI95: 21.0, 47.7) months



Heimberger/Sampson, JCO, 2010

TMZ induced lymphopenia enhances immunological responses: ACT II





Sampson/Heimberger, Neuro-Oncology, 2011

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Summarized Clinical Efficacy of the PEP-3-KLH Vaccine in Glioblastoma Patients

	Clinical Sites	Median PFS from Diagnosis (months)	Median OS from Diagnosis (months)	OS at 24 Months
ACT III (n=65)	31	12.3	24.3*	50%*
ACT II (n=22)	2	15.3	24.4	50%
ACTIVATE (n=18)	2	14.2	24.6	50%
Matched historical control (n=17)[1]	1	6.4	15.2	6%
Standard of care radiation/TMZ (n=287)[2]	85	6.9	14.6	27%

- In all three rindopepimut trials, study treatment began ~3 months post-diagnosis
- Historical controls were treated at M.D. Anderson and matched for eligibility (EGFRvIII-positive, KPS ≥ 80%, complete resection, radiation/TMZ and without progression through ~3 months post-diagnosis)
- Confidence intervals for median PFS and OS for vaccinated patients do not overlap with those for historical control and standard of care
- Mature data for ACT II and ACTIVATE are presented

1. Sampson et al. J. Clin Oncol 2010 Nov 1 28(31), 4722-9
2. Stupp et al. N. Engl. J Med. 2005, 352, 987-96

* ACT III survival data not yet final

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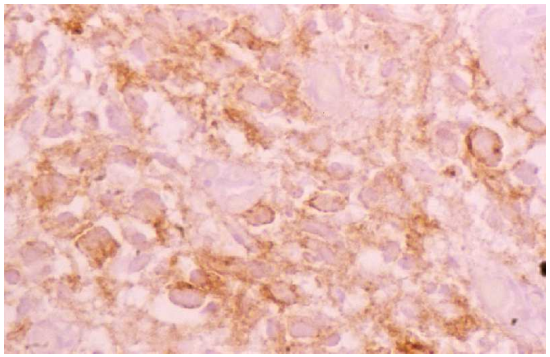
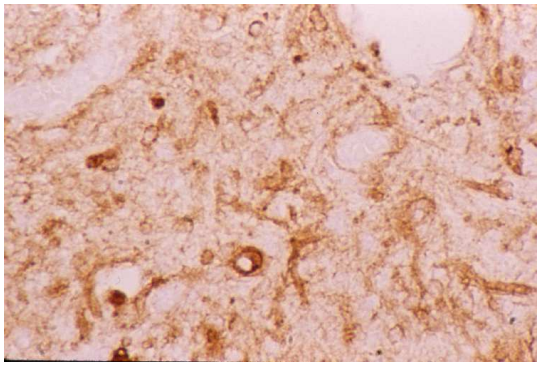
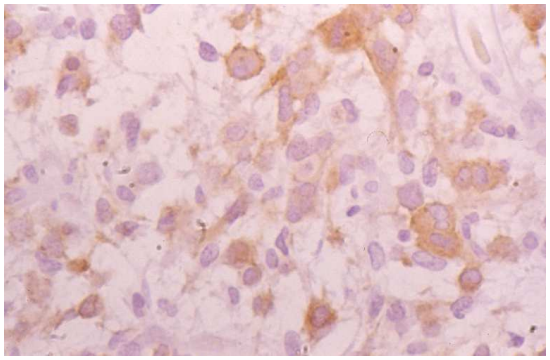
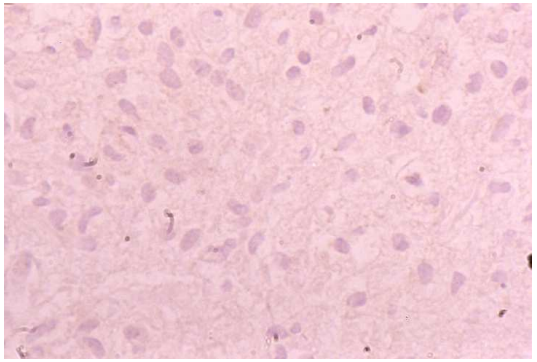
Mechanism of treatment failure: antigenic loss

	Pre-Vaccination	Post Vaccination Recurrence
EGFR		
EGFRvIII		

Magnification is 250X

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CMV Antigens are expressed in Glioblastoma Patient Tumors

NEURO-ONCOLOGY

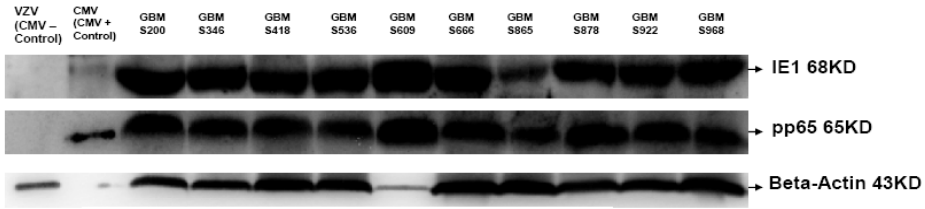
Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma

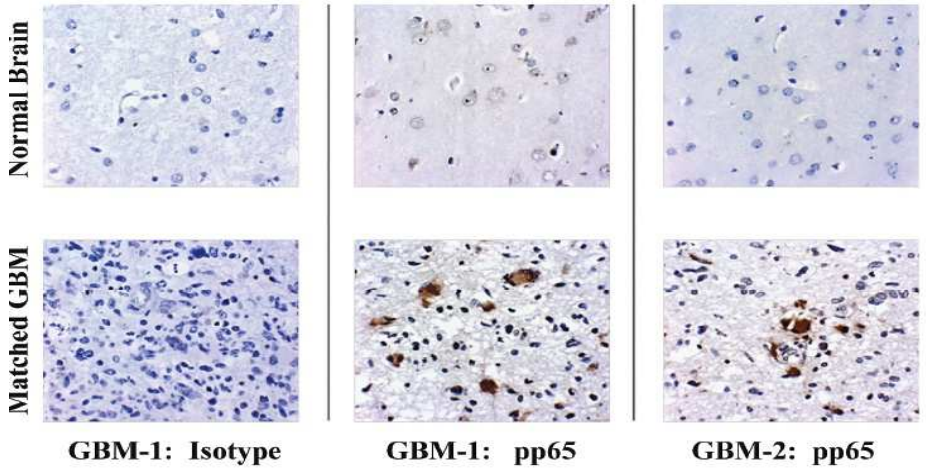
Duane A. Mitchell, Weihua Xie, Robert Schmittling, Chris Learn, Allan Friedman, Roger E. McLendon, and John H. Sampson

Division of Neurosurgery, Department of Surgery (D.A.M., W.X., R.S., C.L., A.F., J.H.S.), and Department of Pathology (R.E.M., J.H.S.), Preston Robert Tisch Brain Tumor Center at Duke; Duke University Medical Center, Durham, NC, USA

Table 1. Summary of HCMV detection in GBM specimens

HCMV	GBM Tissue Specimen	Primary GBM Cultures
IE1 IHC	42/45 (93%)[a]	4/4 (100%)
pp65 IHC	30/33 (91%)[a]	12/12 (100%)
HCMV DNA ISH	16/16 (selected cases)	not tested
gB PCR	21/34 (61.7%)[b]	13/17 (70.6%)
IE1 PCR	8/34 (24%)[b]	9/17 (53%)





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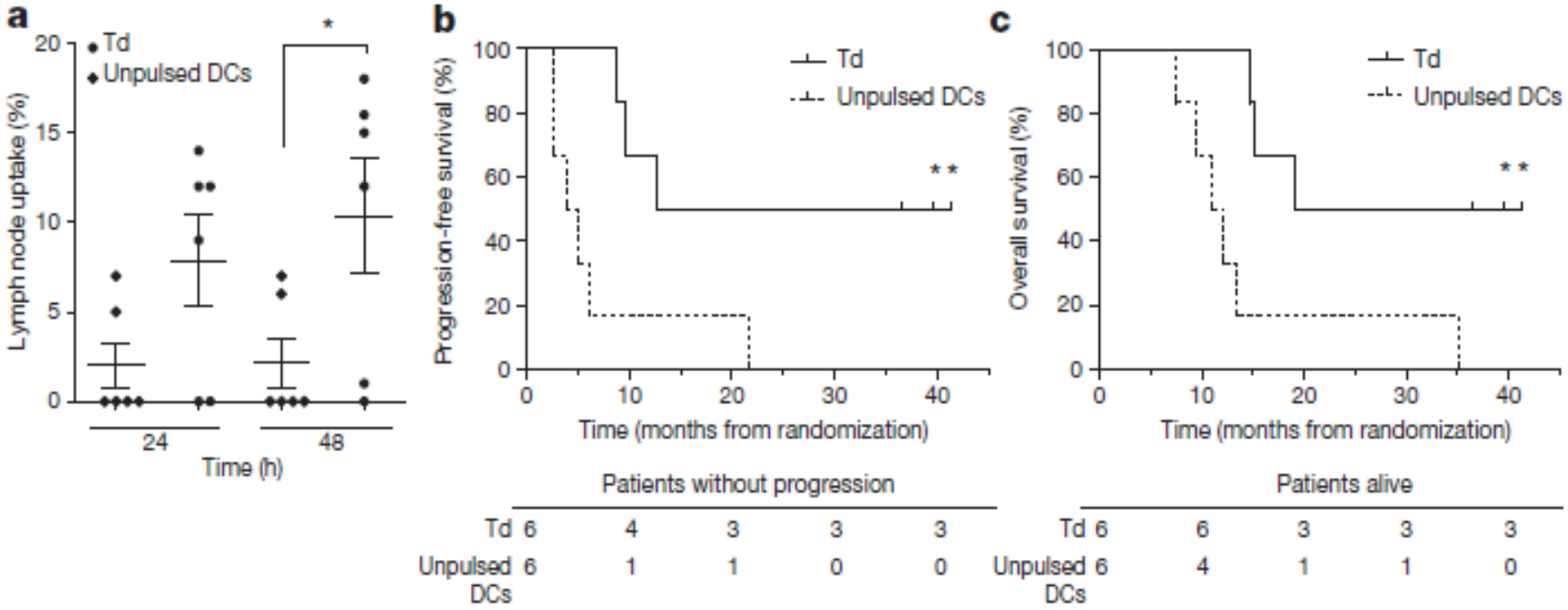
Immunotherapy targeting CMV pp65



Mitchell, *Nature*, 2015

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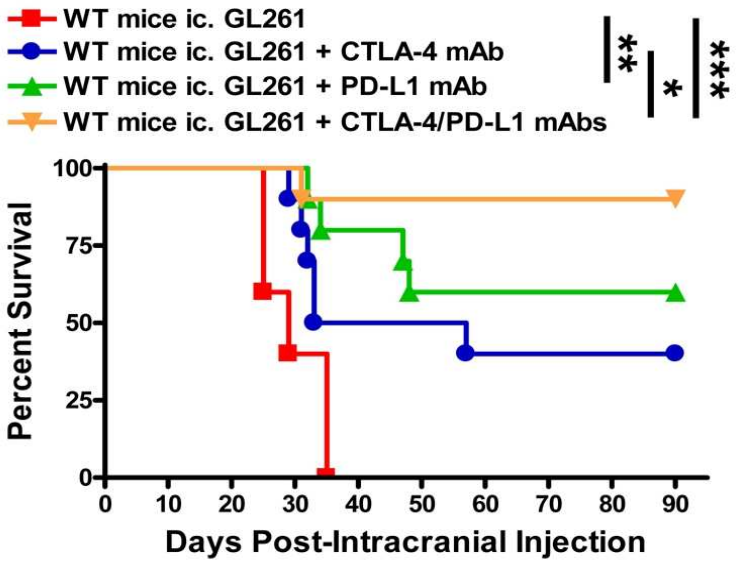
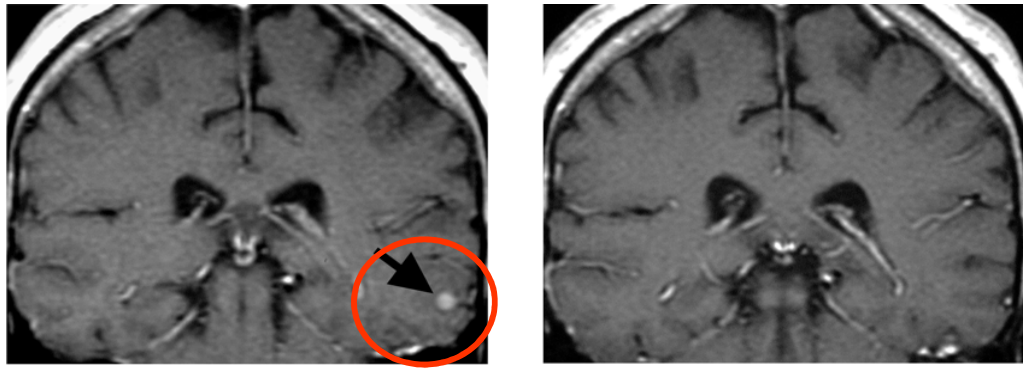
Rationale for Checkpoint Inhibition in Glioma Patients

Preclinical



Wainwright, CCR, 2014



ASCO 2013
NEJM 6/2/2013

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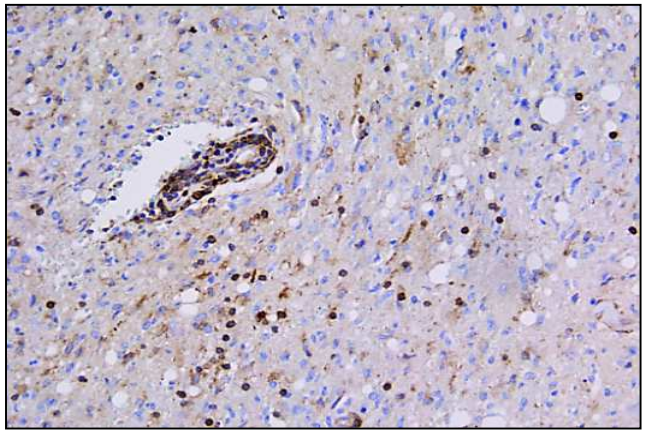
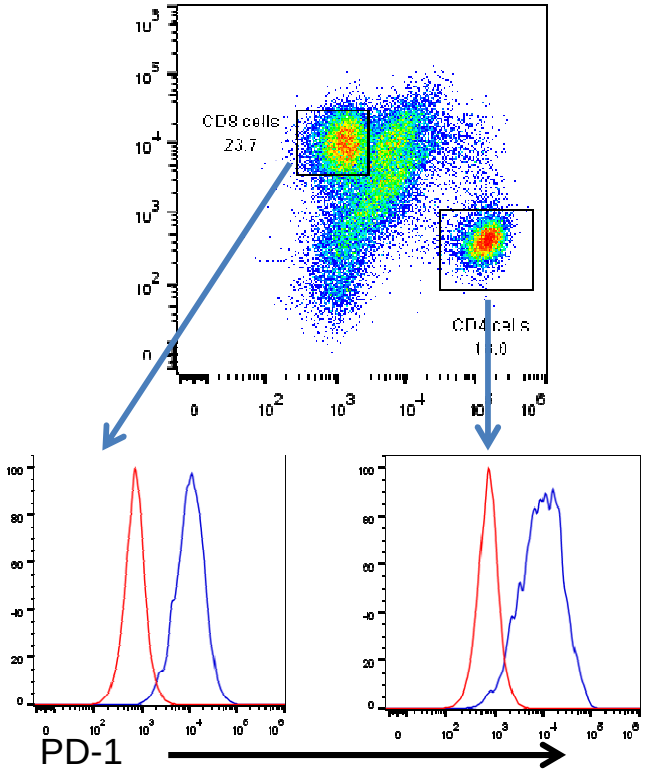
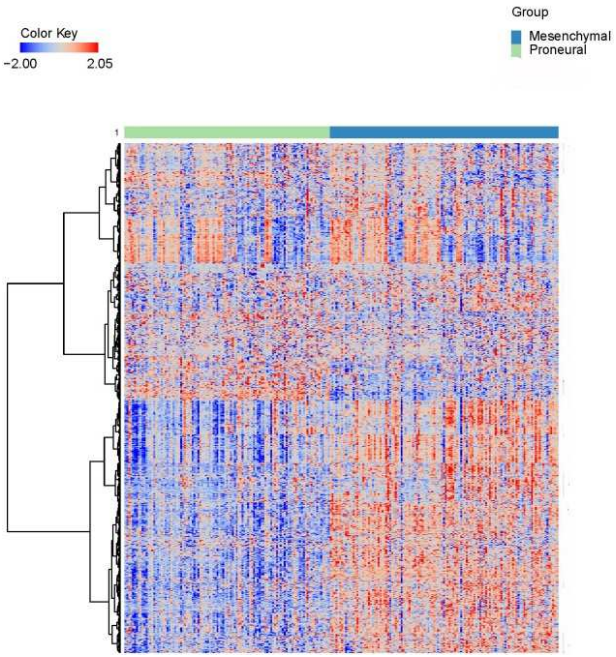
Immune checkpoints are operational within GBM



% of PD-L1 cells	% GBM patients (n=99)
>1	85.9
>5	35.9
>10	13.0
>25	1.1



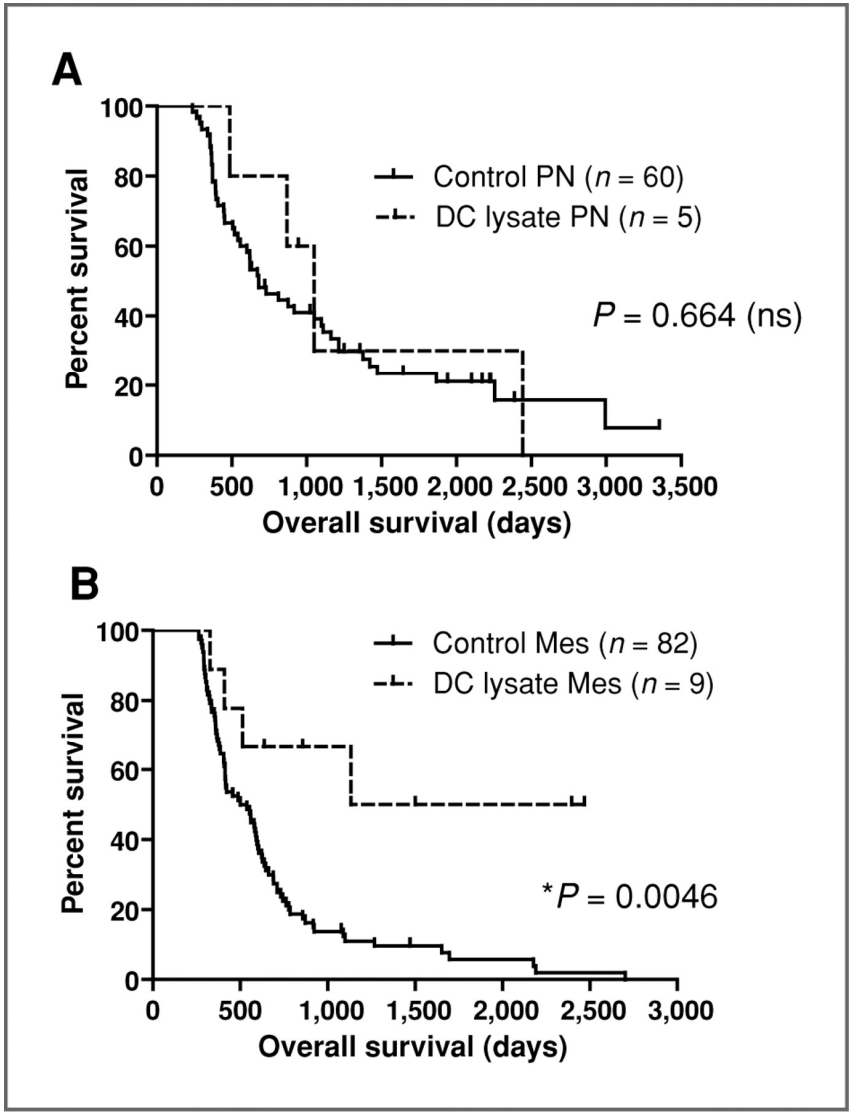
Immune suppression is enriched in the mesenchymal subset

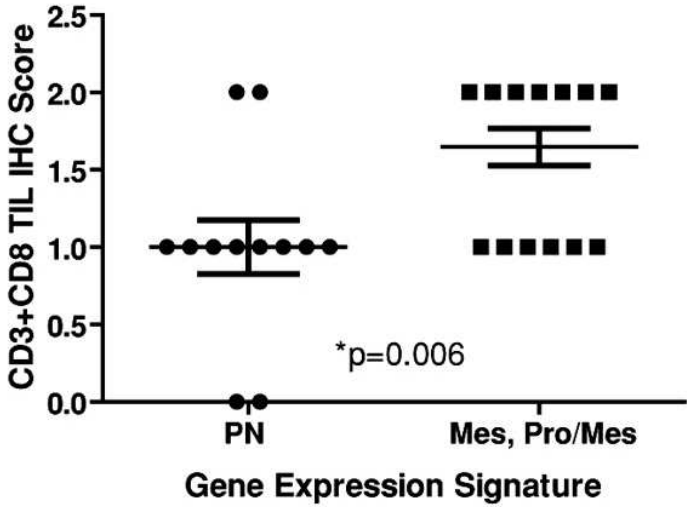


Immune suppressor/gene		Number of cases; % mRNA over expression			
		Proneural n=141	Mesenchymal n=160	Classical n=147	Neural n=96
Immune suppressive cytokines and checkpoints	Galectin-3/LGALS3	2; 1	**28; 18**	13; 9	6; 6
	VEGF/VEGFA	16; 11	26; 16	32; 22	3; 3
	IL-10/IL10	4; 3	**39; 24**	5; 3	13; 14
	IL-23/IL23A	4; 3	**21; 13**	12; 8	5; 5
	TGF-β/TGFB1	5; 4	**50; 31**	14; 10	2; 2
	PD-1/SPATA2	**28; 20**	14; 9	58; 39	27; 28
	PD-L1/PDL1	0; 0	**25; 16**	14; 10	5; 5
	CTLA-4/CTLA-4	12; 9	**30; 19**	8; 5	11; 11
Tumor-supportive macrophage chemotactic and skewing molecules	CSF-1/CSF	3; 2	**30; 19**	4, 3	1, 1
	CCL2/CCL2	5; 4	**53; 33**	9; 6	7; 7
	CCL-22/CCL22	10; 7	**33; 21**	17; 12	12; 13
	CD163/CD163	8; 6	**60; 38**	2; 1	11; 11
	CD204/MSR1	5; 4	**53; 33**	3; 2	8; 8
	MIC-1/GDF15	7; 5	**43; 27**	25; 17	14; 15
	Arginase/ARG1	9; 6	**23; 14**	16; 11	22; 23
	CD47/CD47	15; 11	**30; 19**	10; 7	19; 20
Immune suppressive signaling pathways	IL-6/IL6	32; 23	**83; 52**	16; 11	15; 16
	gp130/IL6ST	0; 0	**25; 16**	17; 12	8; 8
	Jak2	6; 4	**22; 14**	9; 6	11; 11
	STAT3/STAT3	8; 6	**31; 19**	26; 18	0; 0
	Pim-1/PIM1	4; 3	**44; 28**	13; 9	6; 6
	SOCS3/SOCS3	5; 4	**36; 23**	10; 7	3; 3
	STAT5A/STAT5A	4; 3	**48; 30**	10; 7	2; 2
Markers of Tregs	CD4/CD4	5; 4	**57; 36**	0; 0	9; 9
	CD278/ICOS	8; 6	**23; 14**	9; 6	9; 9
	IDO/IDO1	6; 4	**25; 16**	14; 10	4; 4

Doucette, CIR, 2013

Distinct glioblastoma subtypes may benefit from immunotherapy





Prins, Clinical Cancer Research 2011

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Key Biomarker Issues for Immune Therapeutic Clinical Trials

- There is no universally accepted peripheral blood immune assays that correlates with clinical efficacy/polyvalent immune responses.
- Monitoring will need to be tailored to the select agents
- Peripheral blood monitoring does not take into account effector function at the tumor site.
- No vetted imaging approach for determining the presence or absence of an immune response in the tumor microenvironment.
- Assumption of generalized immune assays for monitoring responses (harmonization)
- Immunotoxicity
- Radiographic monitoring of immune responses

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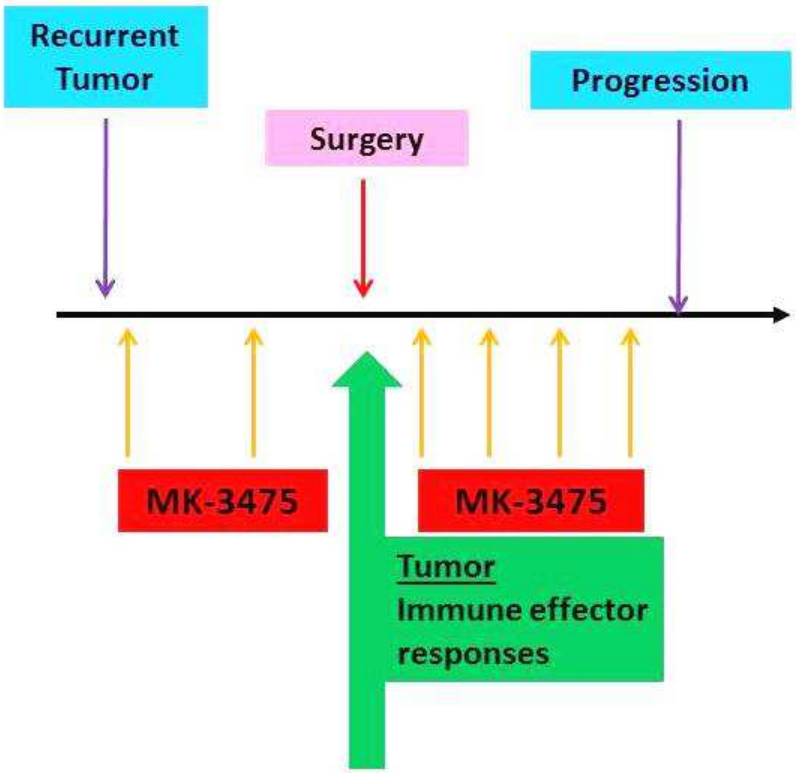
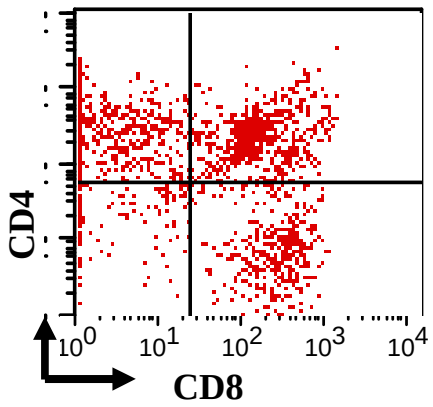
Immune Checkpoint Clinical Trials in GBM

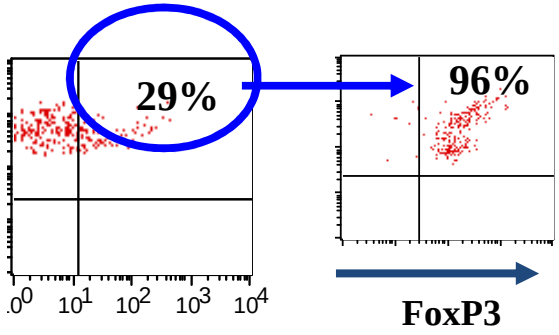


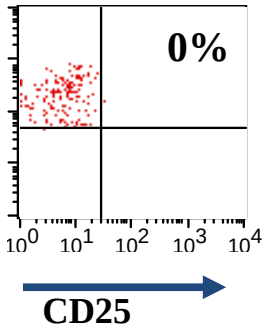
MERCK

- Is there an increase in the frequency of the T cells?
- Is there modulation of checkpoint expression in tumor infiltrating immune cells?
- Are there increased functional T cell responses (IL-2R, IFN-γ, TNF-α, granzyme B, perforin)?

Activated CD4+ T cells are predominantly Tregs







CD8+ T cells are not activated

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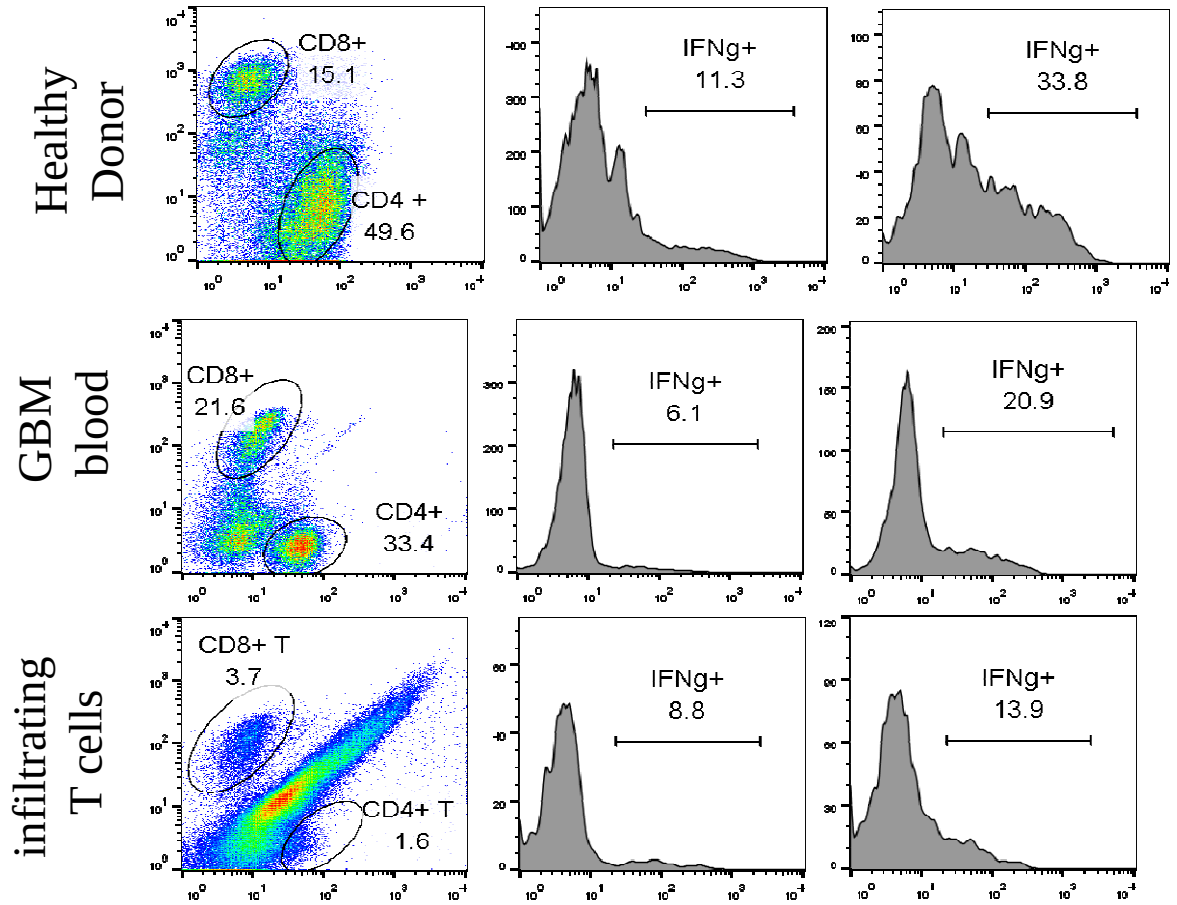
Monitoring the GBM-infiltrating immune responses



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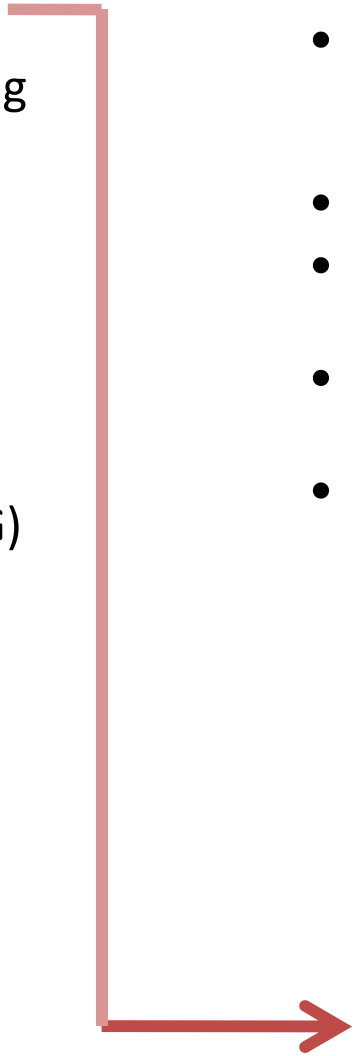
Immune suppression in Malignant Glioma Patients

Mechanisms

- Cytokines – IL-10, TGF, PGE2
- Lack of functional antigen presenting cells i.e. immunosuppressive microglia/macrophages (microglia, paucity of myeloid dendritic cells)
- Induction of T cell apoptosis (FasL; Galectin-3)
- Treg recruitment to the tumor
- Increase expression of immune regulatory molecules (B7-H1, HLA-G)
- Loss of antigen
- Decreased B2 microglobulin and/or HLA
- Induction of inappropriate T-helper function (skewing to Th2)
- Cancer stem cells/initiating cells
- Tumor hypoxia/HIF-1α

Manifestations

- Decreased delayed type hypersensitivity responses to recall antigens
- Diminished antibody responses
- Impaired T cell proliferation and responses to IL-2
- Impaired cytotoxic/effector T cell responses
- T cell anergy/unresponsiveness



Is there a common pathway?

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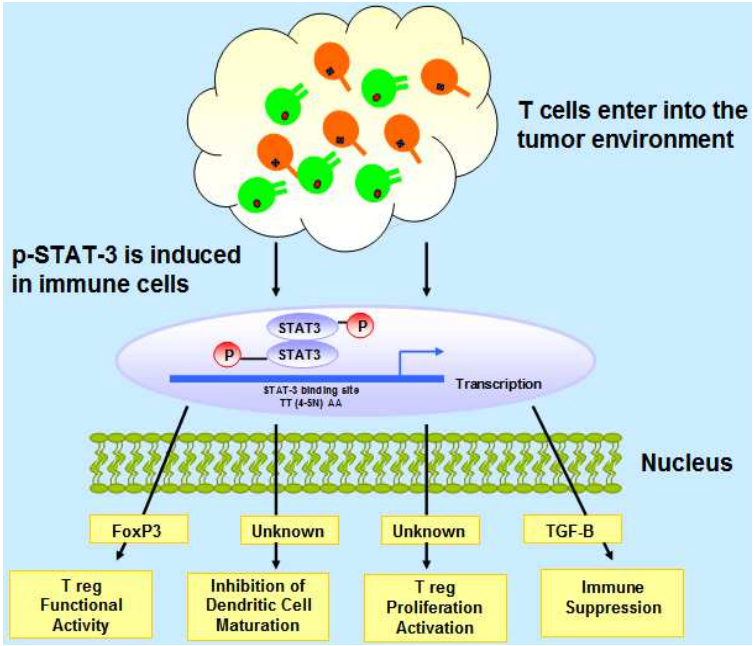
The STAT3 pathway is a key regulatory pathway in global immune suppression



- pSTAT3 becomes active in immune cells in the presence of malignancy (Yu, Nature Rev Immunology, 2007).
- Induces the expression of immune suppressive cytokines
- STAT3 activity turns off antigen presenting cells like dendritic cells.
- STAT3 suppresses macrophage/microglia activation and function; induces M2 macrophages.
- STAT3 is a transcriptional regulator of FoxP3 in Tregs (Zorn, Blood, 2008).
- Ablating STAT3 in hematopoietic cells in mice resulted in marked enhancement of immune responses and marked anti-tumor activity (Kortylewski , Nat Med, 2005).
- STAT3 blockade in the immune cells from glioma patients can restore T cell proliferation and responses.
- Can be found in the peripheral blood of malignant glioma.

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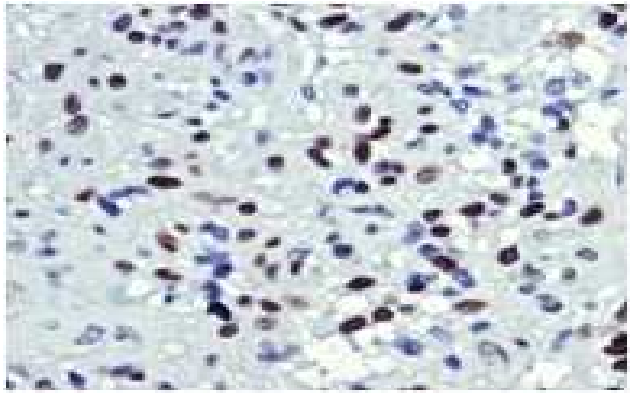
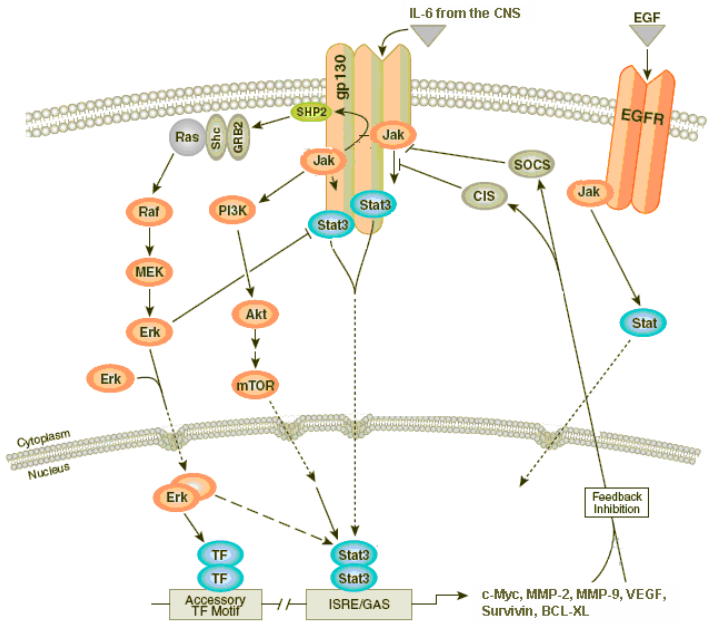
The STAT3 pathway is active in many cancers and especially within malignant gliomas





- Constitutive activation is observed in majority of many malignancies or can be induced by EGF, PDGF, IL-6, IFN, CMV, TLR-9 (among others).
- Upon phosphorylation of tyrosine^{705} (p-STAT3), dimerization occurs and subsequent nuclear translocation.
- The p-STAT3 is a potent transcriptional factor that regulates key factors that mediate tumor proliferation and survival (e.g., cyclin D1, p53, BCL-XL), migration and invasion (e.g., MMP-2, MMP-9), and angiogenesis by VEGF, basic fibroblast growth factor, and HIF-1α.
- Is a negative prognostic factor for survival.
- Shown to mediate the proneural to mesenchymal transition .
- Maintains “stemness” (Sherry, Stem Cells, 2009).

STAT3 positively and significantly correlates with glioma grade

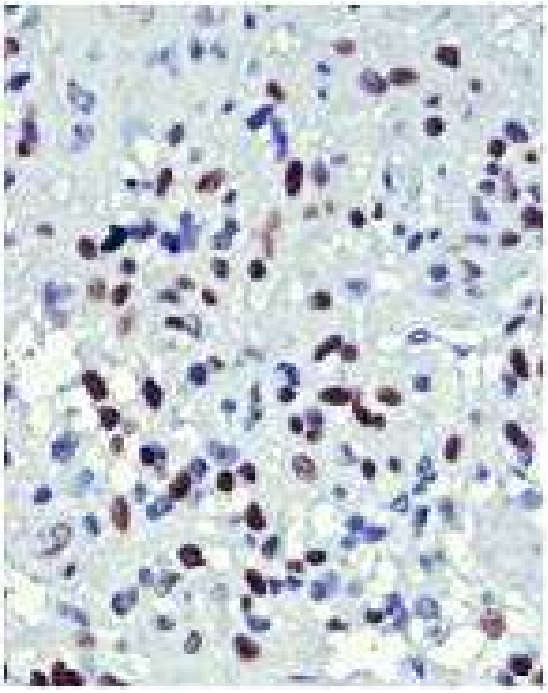


Table 3. Proportion of p-STAT3–positive cases according to pathology and WHO tumor grade.

Pathology	p-STAT3 >0/Total (%)	*Mean (SD)	(Min, Max)
O	6/16 (38%)	4.3 (7.7)	(0.0, 23.5)
MOA	6/6 (100%)	67.9 (55.2)	(9.0, 136.0)
AO	6/15 (40%)	17.5 (41.0)	(0.0,153.0)
AMOA	7/12 (58%)	13.3 (27.0)	(0.0, 85.5)
LGA	0/3 (0%)	0	0
AA	9/17 (53%)	5.6 (7.6)	(0.0, 23.0)
GS	5/7 (71%)	23.1 (34.1)	(0.0, 91.0)
GBM	27/53 (51%)	11.7 (24.4)	(0.0, 133.5)

Abbreviations: AA, anaplastic astrocytoma; AMOA, anaplastic mixed oligoastrocytoma; AO, anaplastic oligodendroglioma; GS, gliosarcoma; LGA, low-grade astrocytoma; O, oligodendroglioma; MOA, mixed oligoastrocytoma.

*The mean calculation was based on the mean p-STAT3 value.

Abou-Ghazal, Clin Cancer Res. 2008

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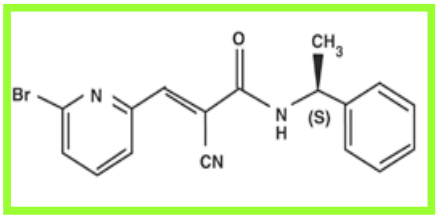
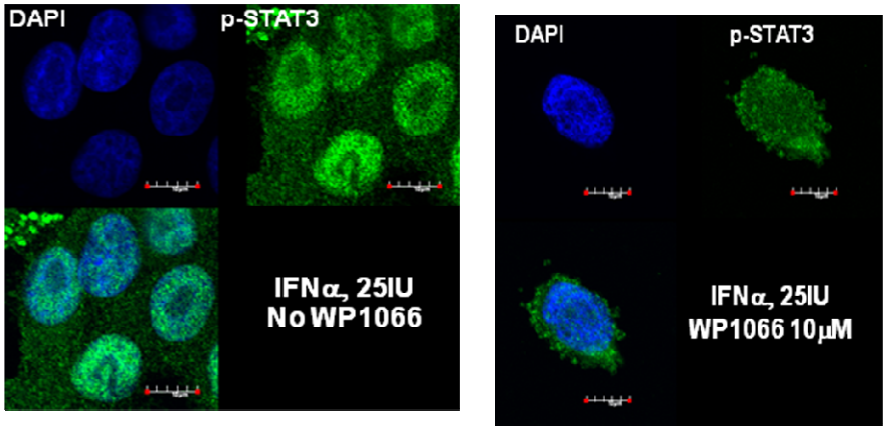
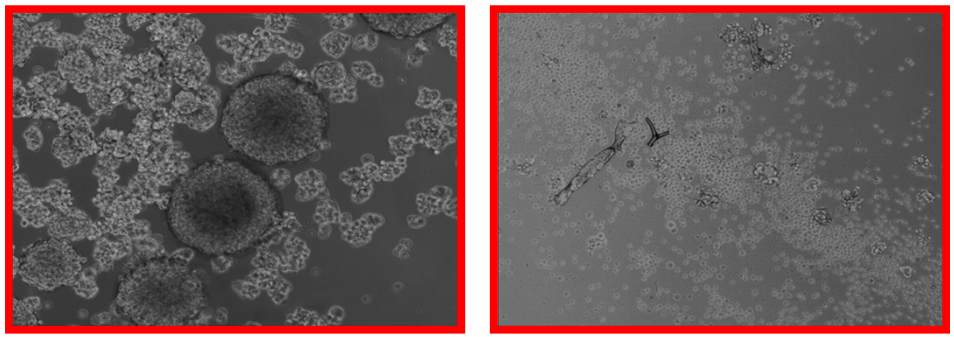
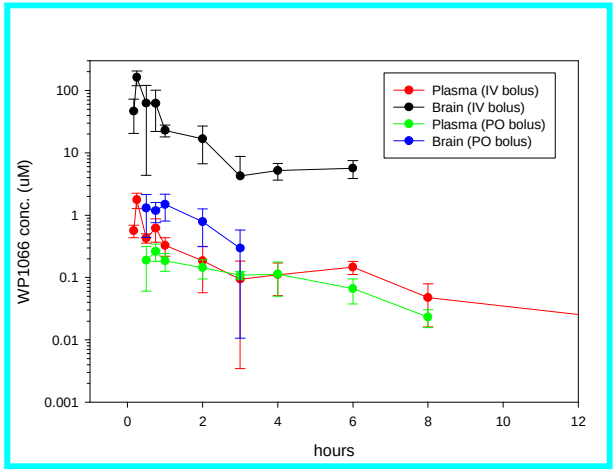
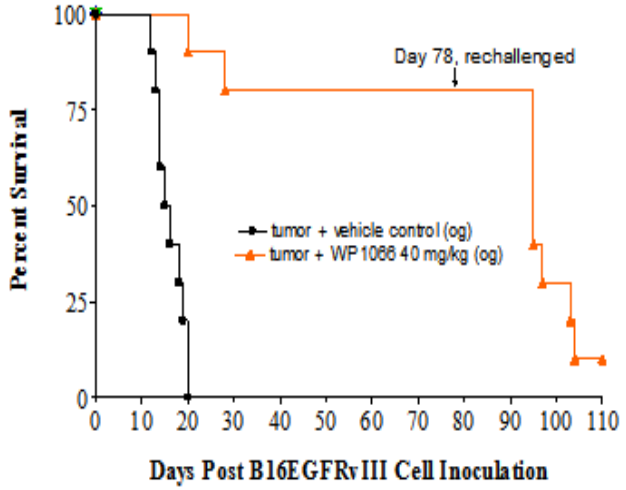
WP1066: Small molecule inhibitor of STAT-3











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WP1066 demonstrates minimal in vivo toxicity

Table 1 Systemic histopathological effects of WP1066 in C57BL/6 mice

Drug	Adminis-tration Route	Total (mg/kg)	Pathology of Systemic Organs							
			Spleen	Thymus	Lung	Heart	Kidney	Brain	Liver	GI
Vehicle	i.p.	N/A	1/10[b], 2/10[a]	0/8	0/9	0/10	0/10	0/10	1/10 [b]	4/10[f]
WP1066	i.p.	20	4/5[a]	0/7	1/7[b]	1/7[d]	2/7[b]	0/7	4/7 [b]	1/6 [b], 2/6 [f]
WP1066	i.p.	10	4/10[a]	0/8	1/10[ae]	1/10[b]	3/10[b]	0/10	1/10[c], 2/10 [b]	1/9 [b], 1/9 [f]
Vehicle	o.g.	N/A	3/10[a]	1/8[b]	0/10	0/10	0/10	0/10	0/10	1/10 [b]
WP1066	o.g.	40	0/5	0/5	0/5	0/4	1/5	0/5	1/5[g], 2/5 [b]	0/4

a hemosiderin staining within macrophages
b autolysis
c chronic inflammatory infiltrate in connective tissue adjacent to the liver
d likely post-mortem bacterial endocarditis
e pulmonary congestion
f reactive lymphoid follicles with germinal center
g chronic inflammation

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A Phase I Trial of WP1066: NCT01904123

Dose Escalation Schedule	
Dose Level	Dose of WP1066*
Level 1	0.08
Level 2	0.15
Level 3	0.3
Level 4	0.6
Level 5	1.15
Level 6	2.3
* Doses are stated as exact dose (e.g., mg/kg)	

Toxicity observed in 1 patient	Change to dose escalation schema
Grade 1 adverse event	No change; enroll next patient in next higher dose level at double the previous dose.
First grade 2 adverse event	No change; enroll next patient in next higher dose level at double the previous dose.
Second grade 2 adverse event	Expand current cohort to enroll a total of 3 patients; future dose-escalation will occur in 33% increments.
First grade 3 non-DLT toxicity	Expand current cohort to enroll a total of 3 patients; future dose-escalation will occur in 33% increments
First DLT	Expand current cohort to enroll a total of 3 patients; if no further DLT is noted among these three patients, 3 more patients will be added at this dose level; if only one of the 6 patients experiences a DLT, the dose may be escalated by 25% in the next cohort of 6 patients.

Key Features:

Dose escalation proceeds according to an accelerated titration design

Biomarker endpoints include pharmacokinetic bioavailability of WP1066, PBMC p-STAT3 levels, peripheral blood Tregs frequency, and T cell effector responses

Retrospective analysis of GBM subtype (mesenchymal, proneural, classical, neural) with treatment response to WP1066

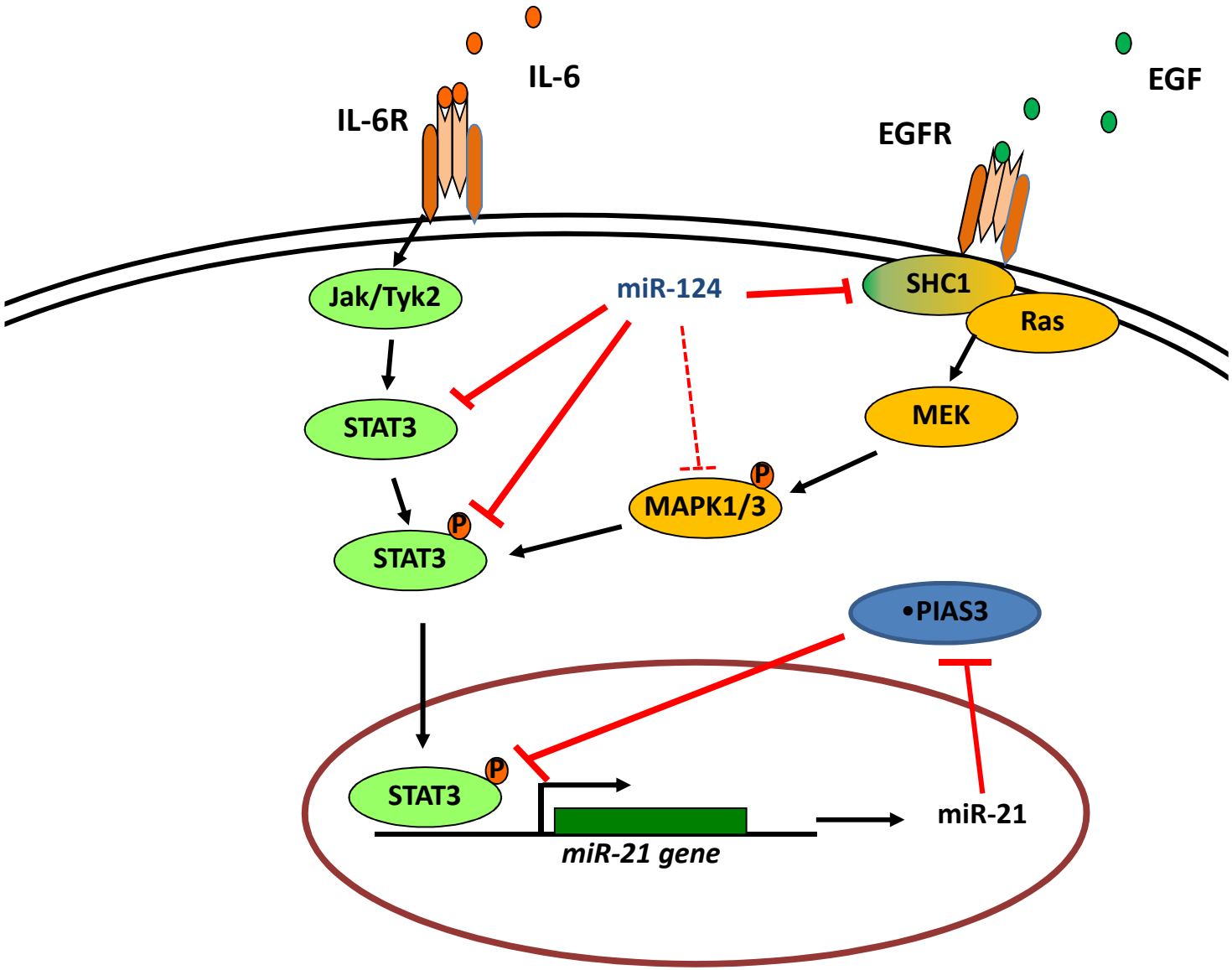
Measurement of effect includes radiographic responses using $RECIST_{immune}$ criterion

The primary objective is to determine MTD, safety and tolerability

Secondary objectives include: pharmacokinetic, immune, radiographic, PFS and OS parameters

Total number of patients: 21

miR-124 modulates multiple nodes in the STAT3 signaling pathway



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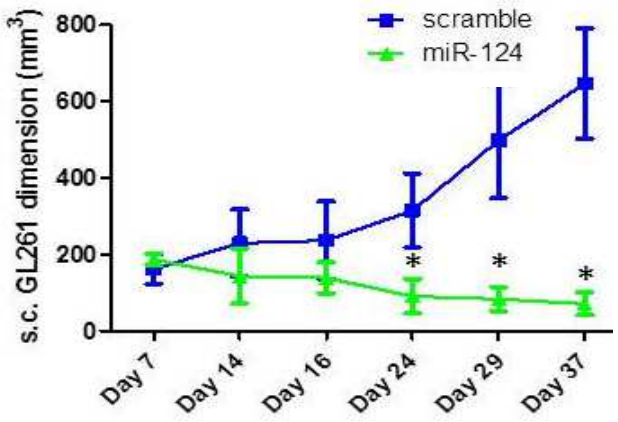
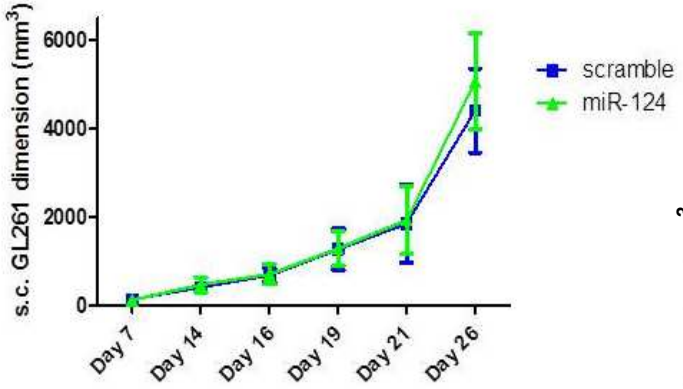
miR-124 blocks glioma growth via an immunological process

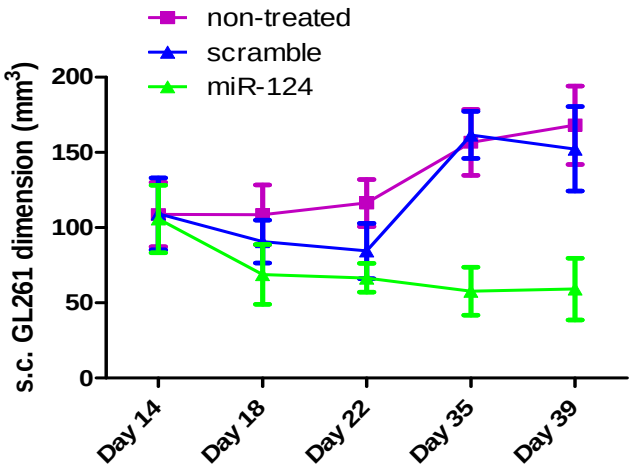
C57/BL6 Mice



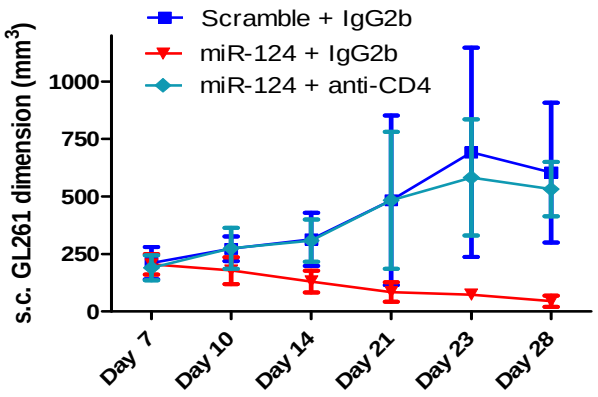
Nude Mice



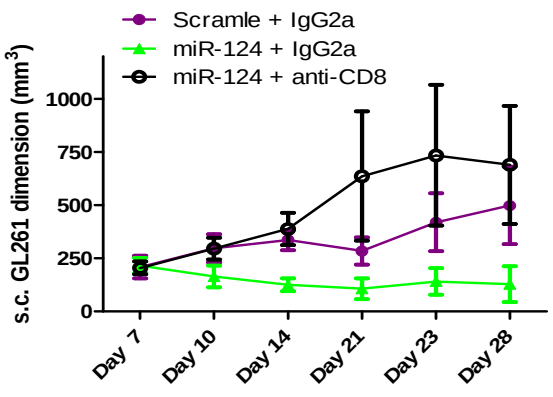
C57/BL6 Mice with addition of treated CD3+ cells at day 14



CD4+ T Cell Depletion



CD8+ T Cell Depletion



Wei, Cancer Research, 2013

Screening approach for checkpoint inhibition

Candidate miR	RNA22	microRNA.org	RNA22	microRNA.org
	PD-1	PD-1	CTLA-4	CTLA-4
138	A: 4 binding sites; B: 1 binding site; C: 3 binding sites	1 binding site	B: 3 binding sites; C: 1 binding site	None
370	A: 1 binding site; C: 1 binding site	3 binding sites	A: 1 binding site B: 1 binding site C: 1 binding site	1 binding site
211	A: 2 binding sites C: 2 binding sites	1 binding site	A: 1 binding site B: 1 binding site C: 1 binding site	None
16	A: 1 binding site C: 1 binding site	2 binding sites	A: 1 site C: 1 site	None
410	None	1 binding site	None	1 binding site
134	None	1 binding site	C: 1 binding site	None
374b	None	2 binding sites	None	None
200a	None	1 binding site	None	None
874	A: 2 binding sites C: 2 binding sites	2 binding sites	None	None
195	A: 2 binding sites C: 2 binding sites	2 binding sites	None	None
424	None	3 binding sites	None	None
328	A: 3 binding sites B: 2 binding sites C: 3 binding sites	2 binding sites	None	None
15b	A: 2 binding sites C: 2 binding sites	2 binding sites	None	None
374a	None	1 binding site	None	None

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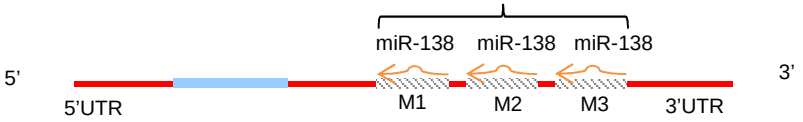
miR-138 inhibits both CTLA-4 and PD-1

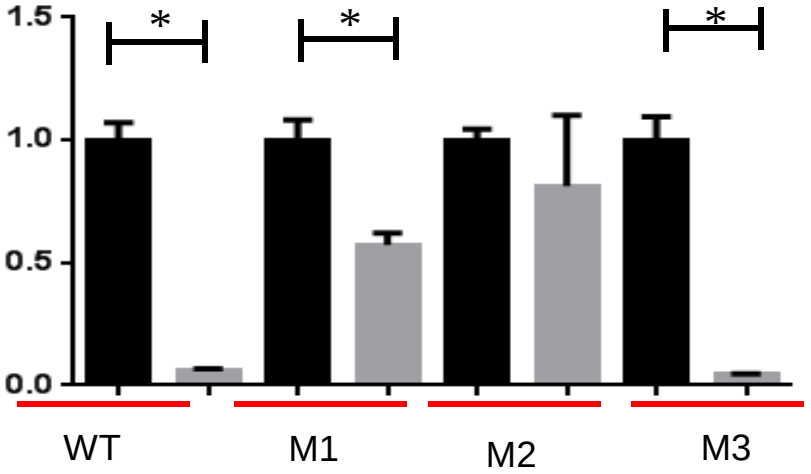


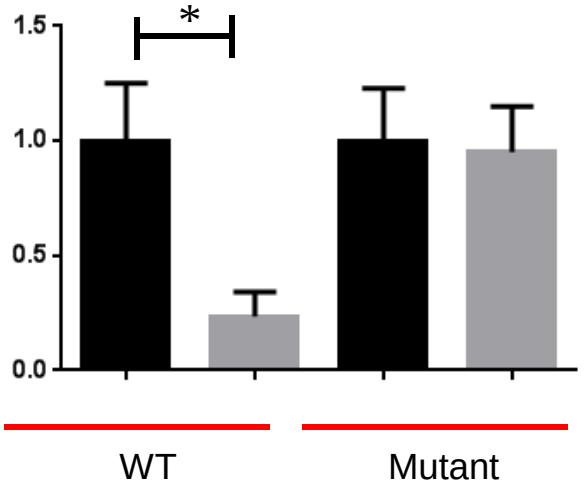

```
        3'UTR 5' 1020:-GGGAUGUUUCUGUCACAUCAGCU 3'
                       ||  || |||    ||| |||||
mir138      3'      GCCGGACUAAGUGU-UGUGGUCGA 5'
                       ||  || |||    |      ||
 CTLA-4 M1   5' 1020:-GGGAUGUUUCUGUCAGUUGUCCU 3

CTLA-4 3'UTR 5' 1550:ACAAGGUC--AUUUGCUA-ACUAGCU 3'
                          || |  |||  |   || ||||
 mir138      3'      G---CCGGACUAAGUGUUGUGGUCGA 5'
                          || |  |||  |         |
 CTLA-4 M2   5' 1550:ACAAGGUC--AUUUGCUA-UGUUCGU 3'

 CTLA-43'UTR 5' 1640:--GCUUUGGGGCUUUUACACCAGUU 3'
                       || | |   |    ||||||| |
 mir138      3'      GCCGGA-CUAAGUGU-UGUGGUCGA 5'
                       || | |   |    |       |
 CTLA-4 M3   5' 1640:--GCUUUGGGGCUUUUACUGGUCUU 3'
```


```
PD1 3'UTR         5' 1986: ACUCCUGGGGCAGGGCCACCAGCA 3'
                           ||||   ||    |||||||
miR138            3'       GCCGGACUAAGUGUU-GUGGUCGA 5'
                           ||||   ||    |     |
PD1 3'UTR-Mutant 5' 1986: ACUCCUGGGGCAGGGCCUGGUCCA 3'
```





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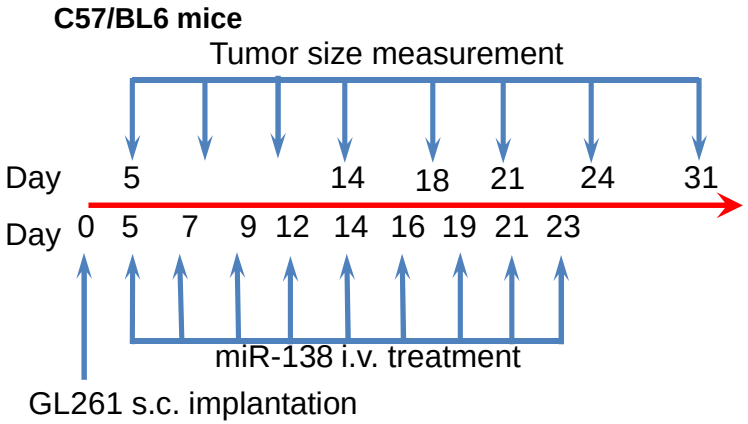
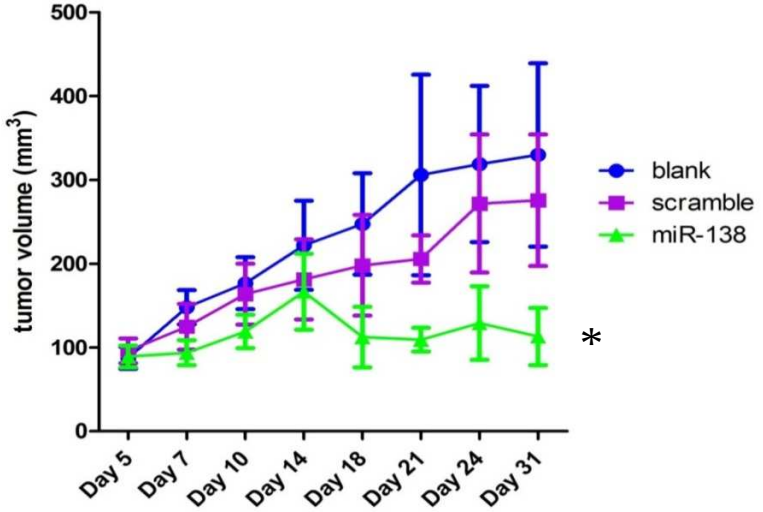
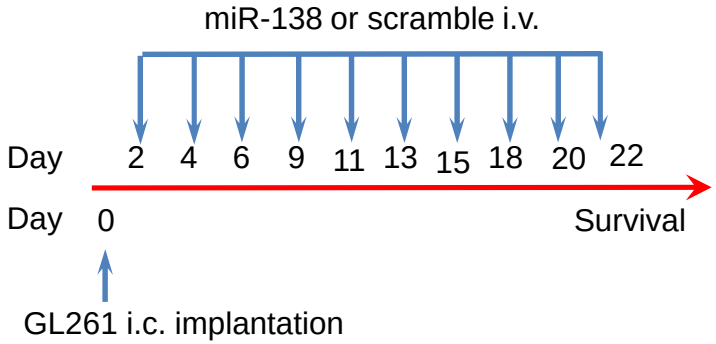
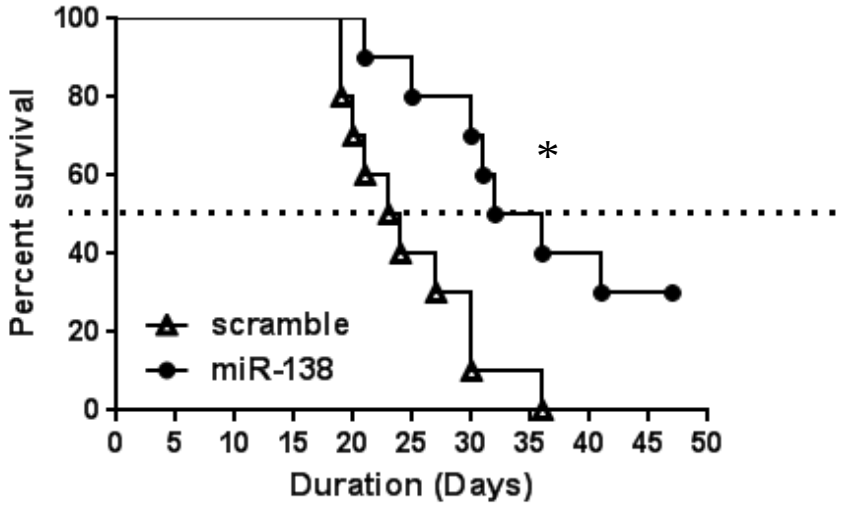
In vivo activity of miR-138









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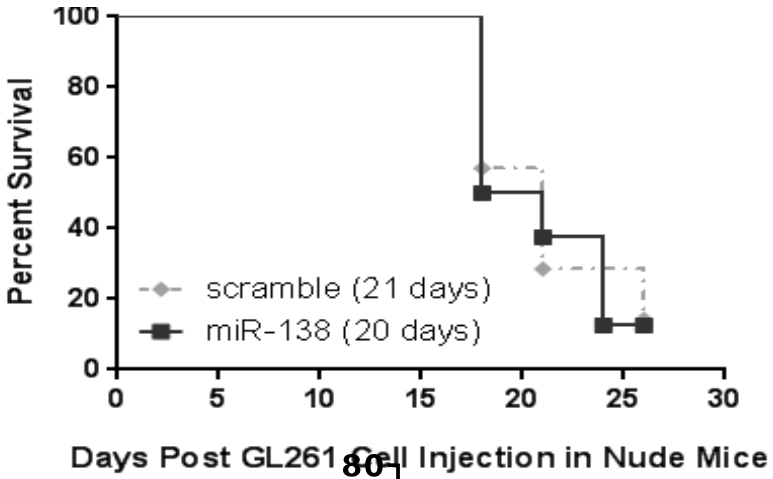
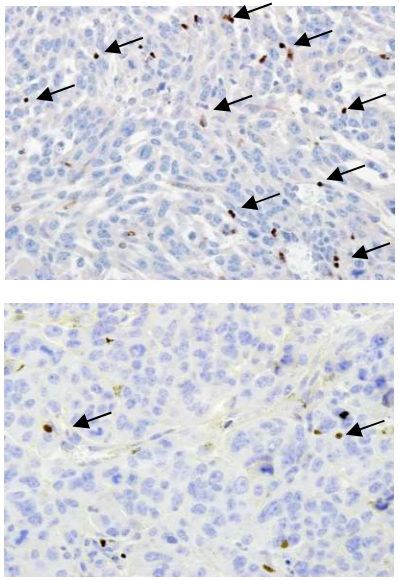
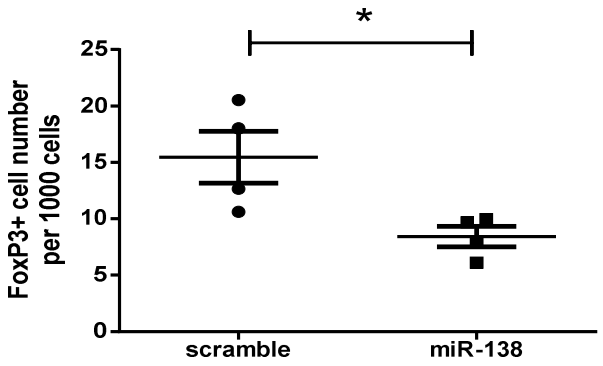
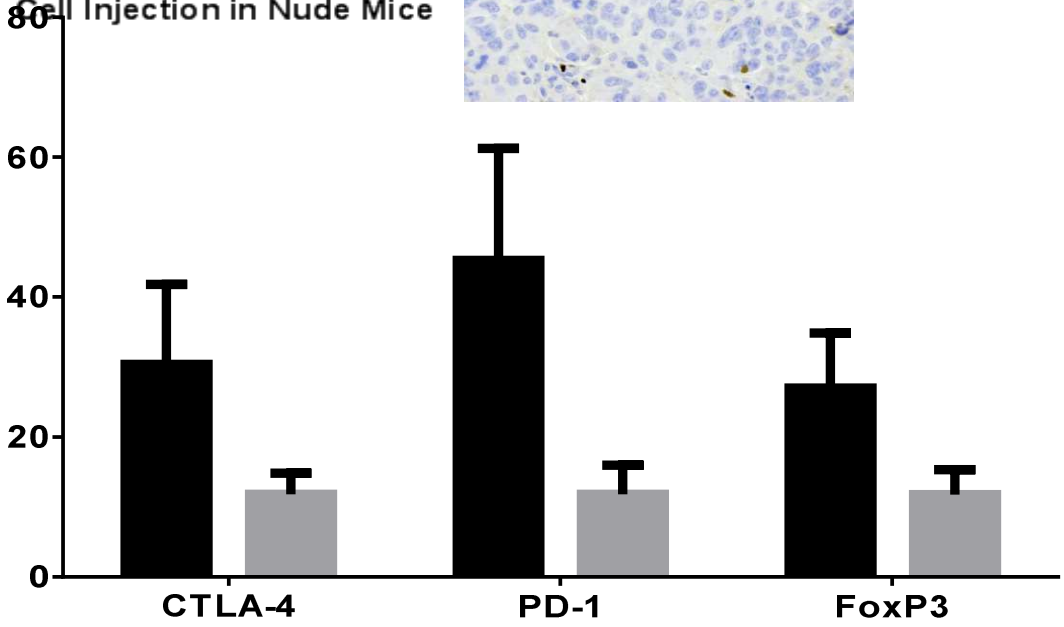
Immune modulatory properties of miR-138









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Challenges/Opportunities for Immunotherapy

- Does the immune system really prevent the development of gliomas? If so, what is the trigger.
- Uncharacterized immune suppressive mechanisms (exosome)
- Immune suppressive features of low grade glioma
- Cost and production ease of GMP cellular products
- Target only membrane expressed targets
- Redundancy of immune suppression
- Assumption of generalized immune assays for monitoring responses (harmonization)
- Polyvalent immune response
- Immunotoxicity
- Synergy with conventional chemotherapeutics/radiation/steroids
- Radiographic monitoring of immune responses