Emerging immune therapeutics targeting glioma-mediated immune suppression

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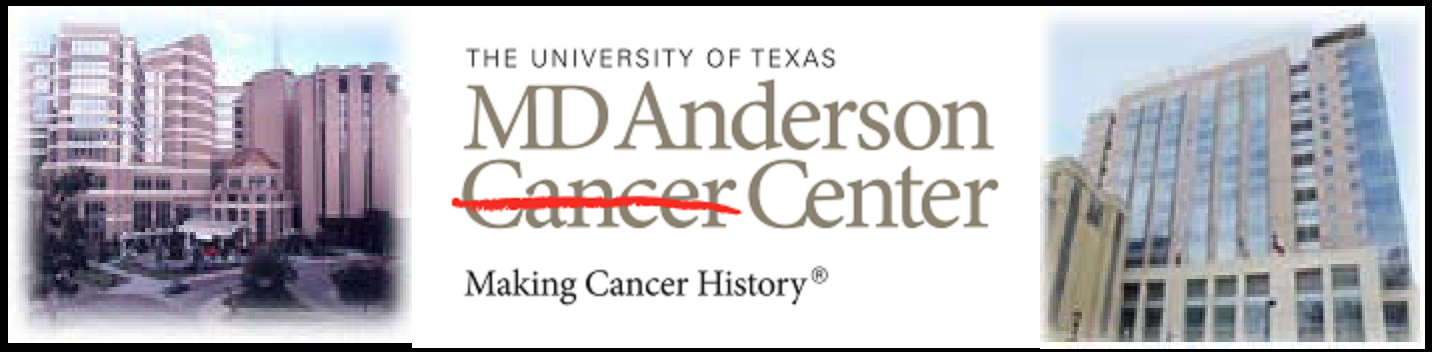
September 9, 2012
Neurological Surgery



Financial Disclosure

- Laboratory and Clinical Studies: National Institute of Health, American Association of Neurological Surgeons, American Brain Tumor Foundation, National Brain Tumor Foundation, Dr Marnie Rose Foundation, Bullock Research Fund, AstraZeneca
- Paid Consultant: Celldex Therapeutics, Bristol Meyers Squibb
- Stock/Equity: Celldex Therapeutics
- Licensing Fees: Celldex Therapeutics/Pfizer
- Patents: EGFRvIII peptide vaccine (CDX-110), WP1066

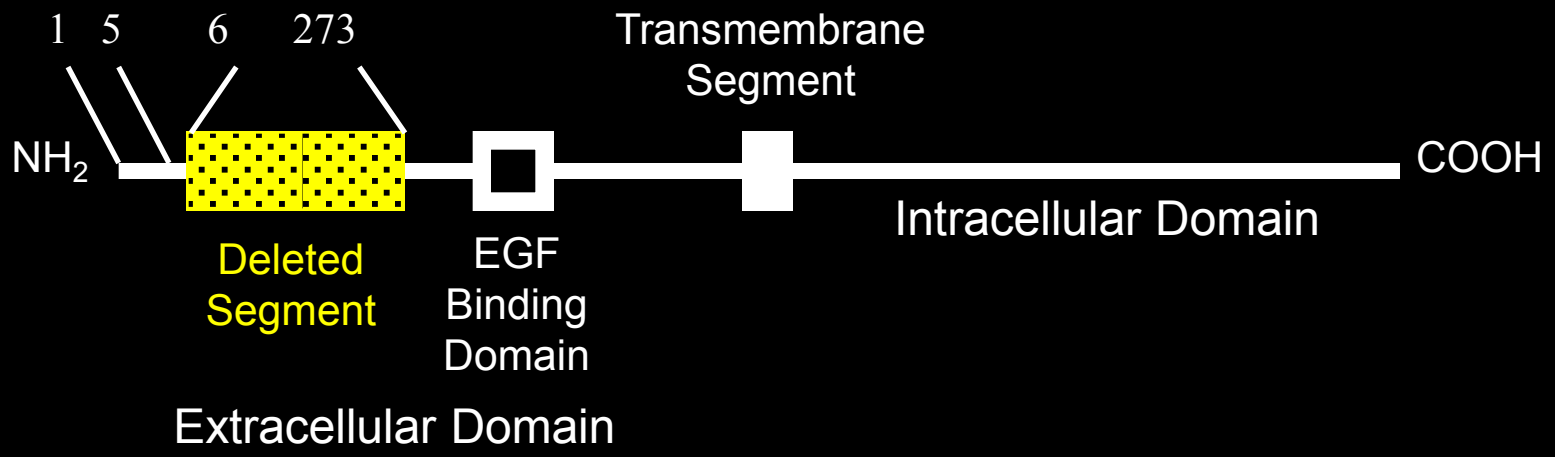
Companies that are in Phase II/III vaccine/immunotherapy development

- Advaxis
- AEterna Zentaris/Keryx
- Alfacell
- Anosys
- Antigenics
- Aphton
- Argos Therapeutics
- Avantogen
- AVAX Technologies
- AVI BioPharma
- Biomira
- BioVex
- Bristol Meyers Squibb
- CancerVax
- CancerVac (Prima BioMed)
- Celldex Therapeutics
- Cell Genesys
- Cytos Biotechnology
- Dendreon
- Favrille
- Genitope
- Genzyme
- Geron
- GlaxoSmithKline
- IDM Pharma
- Immutep
- ImmunoCellular Therapeutics
- Introgen Therapeutics
- LipoNova
- Ludwig Institute for Cancer Research/CSL
- Medarex
- Merck and Co
- Northwest Biotherapeutics
- NovaRx
- Onyvax
- Oxford BioMEdica
- Pharmexa
- Pfizer
- Progenics
- Sanofi-aventis
- Stressgen Biotechnologies
- Therion Biologics
- The Vaccine Company
- Transgene
- Tvax Biomedical
- Vical
- Xenova (Celtic Pharma)
- YM BioSciences

Clinical Efficacy Data from Recent Representative Immunotherapy Clinical Trials in Malignant Glioma Patients

Agent delivered/Site	Sponsor or Centers Involved	Results
PEP-3-KLH + GM-CSF (ACTIVATE)/ Systemic	The Univ. of Texas M. D. Anderson Cancer Center/Duke University Medical Center	Median survival = 2.3 years Newly diagnosed; n=18
PEP-3-KLH + GM-CSF with temozolomide (ACT II)/Systemic	The Univ. of Texas M. D. Anderson Cancer/Duke University Medical Center	Median survival = 2.3 years Newly diagnosed; n=22
Dendritic cells + PEP-3-KLH/Systemic	Duke University Medical Center	Median survival = 1.8 years Newly diagnosed; n=14
Dendritic cells + autologous tumor lysates/	University of Leuven and Wurzburg	Median survival from relapse = 0.8 years Recurrent GBM; n=56
Dendritic cells + tumor homogenate/ Systemic	Cedars Sinai Medical Center	Median survival = 1.8 years for immune responders vs 1.2 for non newly diagnosed GBM; n=11 Median survival = 1.6 years for immune responders vs 1.1 for non recurrent GBM; n=21
Dendritic cells + acid eluted tumor peptides/Systemic	UCLA	Median survival = 2.0 years Newly diagnosed and recurrent GBM patients; n=12

Epidermal Growth Factor Receptor Mutation

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     1                5    6                273
    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
Variant III Amino Acid Sequence
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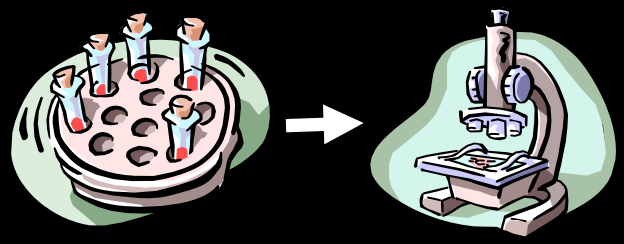
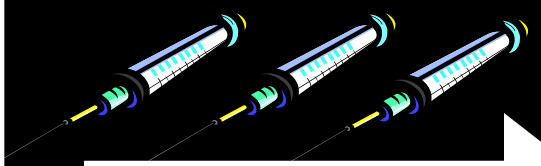
PEP-3

ACTIVATE Trial



Immunologic Monitoring

Leukapheresis

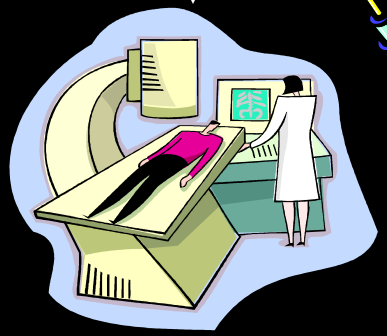


Saline + GM-CSF
(Every 2 weeks i.d.)

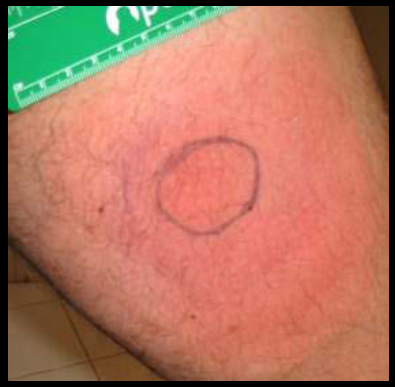
Randomize

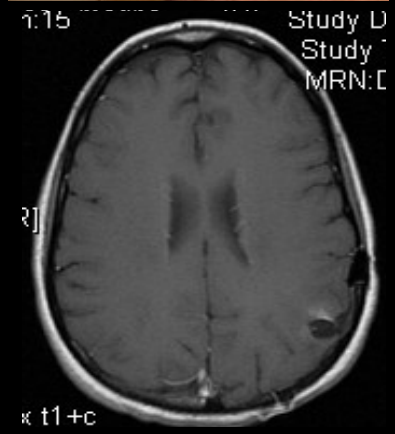
PEPvIII-KLH + GM-CSF
(Every 2 weeks i.d.)

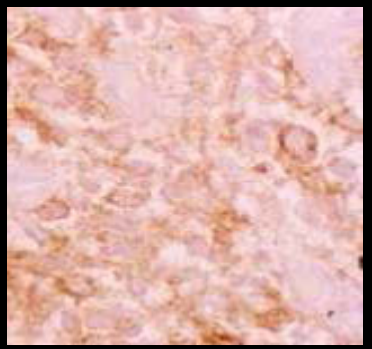
PEPvIII-KLH + GM-CSF
(Every 1 month i.d.)



6000 cGy

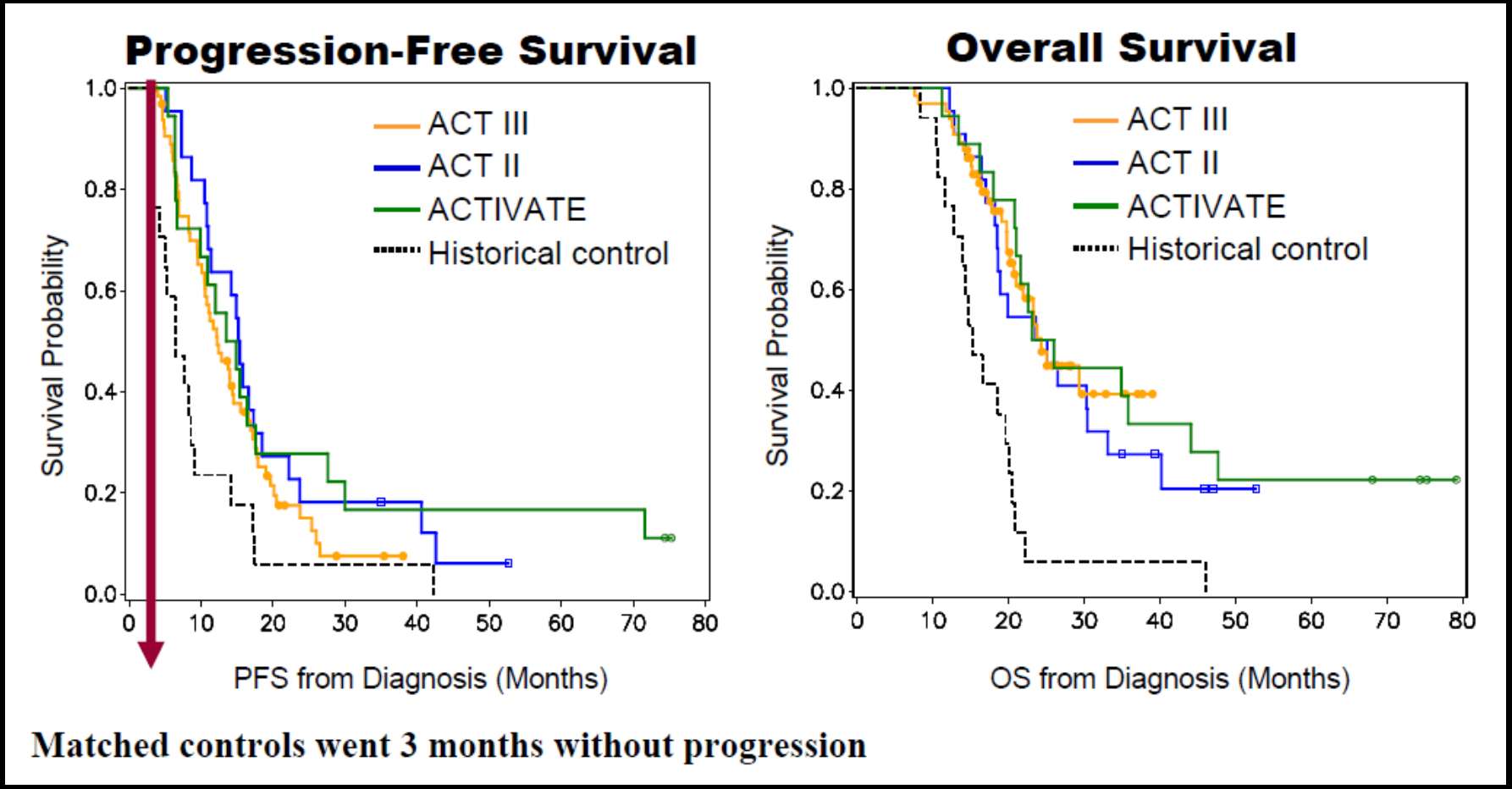






First patient treated in 7/04

Efficacy of PEP-3-KLH vaccine



Matched controls went 3 months without progression

Efficacy of PEP-3-KLH vaccine

	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%

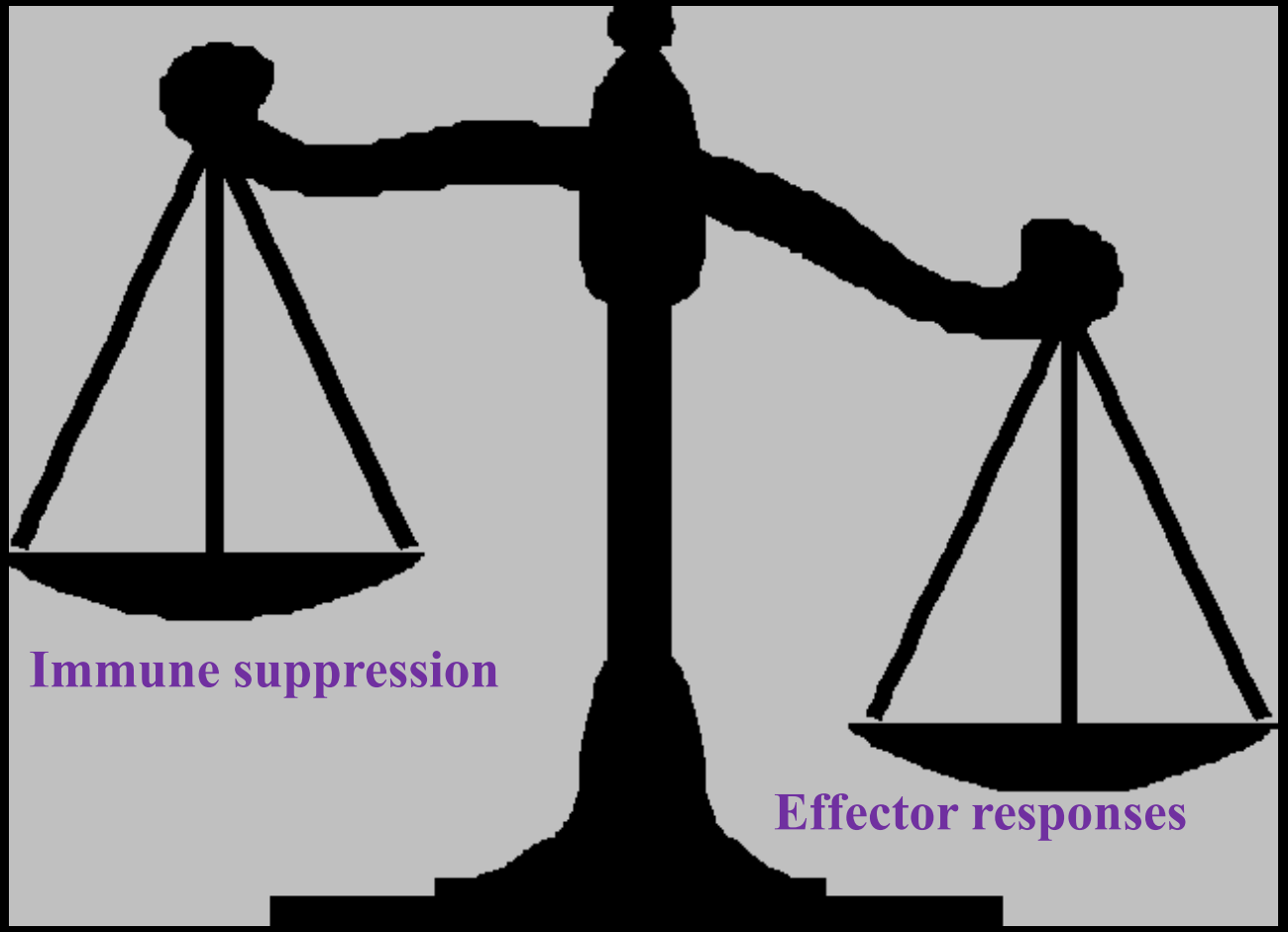
- In all three rindopepimut trials, study treatment began ~3 months post-diagnosis
- Historical controls were treated at M.D. Anderson and matched for eligibility (EGFRvIII-positive, KPS ≥ 80%, complete resection, radiation/TMZ and without progression through ~3 months post-diagnosis)
- Confidence intervals 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

Shifting the paradigm of the immune therapeutics for targeting malignancy

Tumors can be immunologically recognized/ eliminated if global mediators of immune suppression are targeted



Sufficiently potent immune responses need to be generated to overcome profound immune suppression and/or the immune suppression has to be negated/minimized (GTR)

Immunosuppression 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
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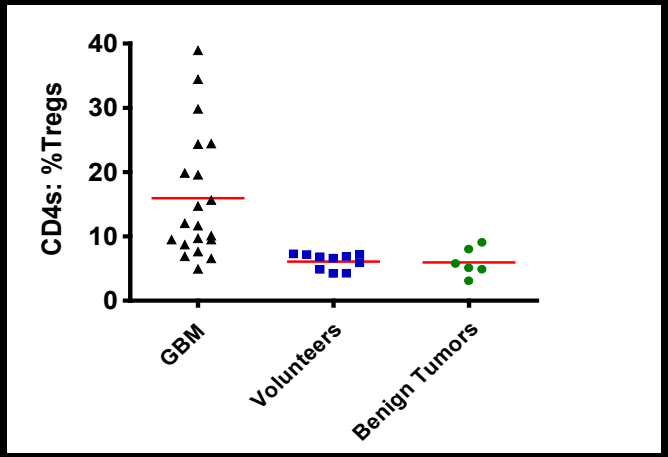
- Tumor hypoxia/HIF-1α

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

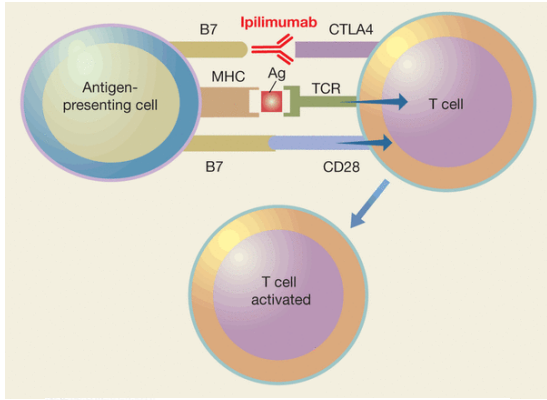


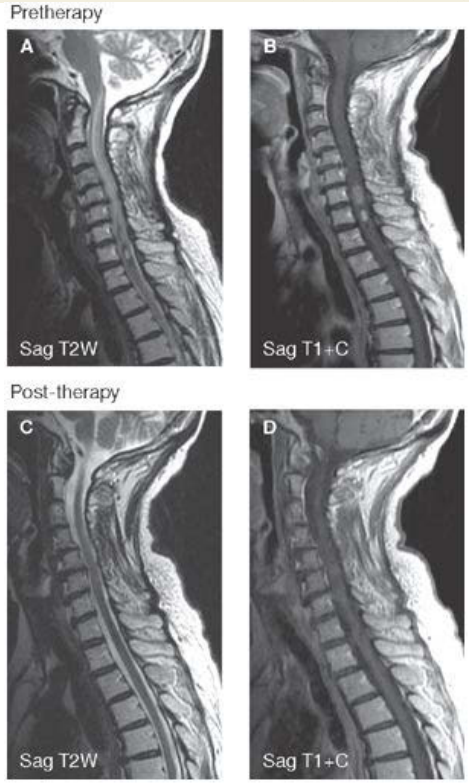
Enrichment of immune response in the mesenchymal subtype

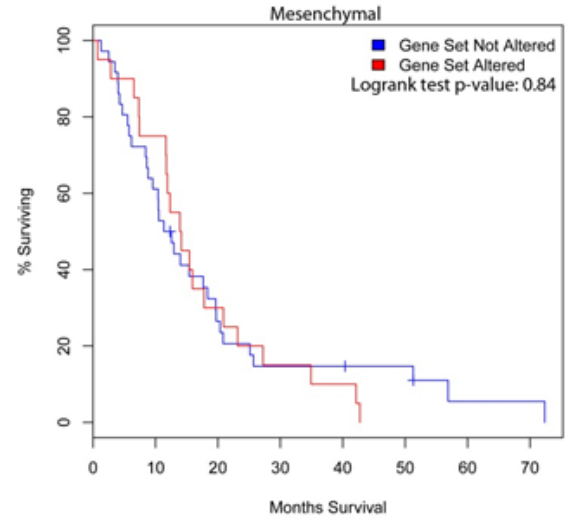
Immune suppressive gene	% mRNA overexpression			
	Proneural	Mesenchymal	Classical	Neural
Galectin-3	0	9	6	0
VEGF	9	23	22	0
IL-10	4	34	7	21
IL-23	5	18	20	7
TGF-β	2	54	19	3
PD-1	13	7	41	31
PD-L1	0	21	11	7
CTLA-4	20	36	13	17
CSF-1	2	34	6	0
CCL2	4	43	7	7
CCL-22	13	29	20	3
CD163	5	32	0	7
CD204	4	46	4	10
MIC-1	11	38	31	17
Arginase	4	14	11	17
CD47	13	13	6	28
IL-6	11	32	13	24
gp130	0	13	6	7
Jak2	5	11	6	10
STAT3	5	23	19	0
Pim-1	5	41	13	7
SOCS3	7	43	24	7
STAT5A	4	41	9	0
STAT5B	18	9	11	0
CD4	5	48	0	14
ICOS	5	13	4	0
IDO	18	16	20	7

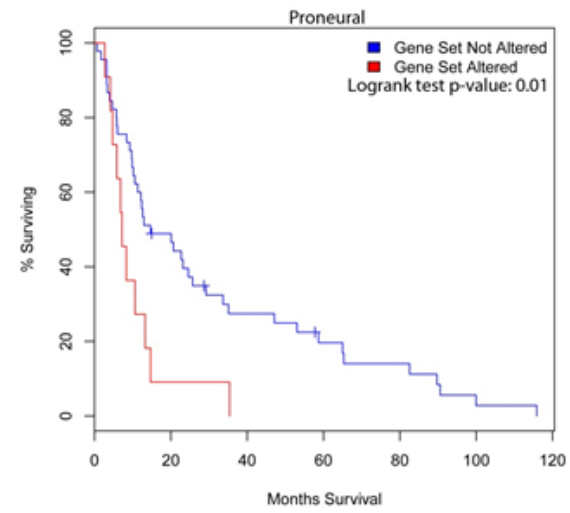
Immune effector gene	% mRNA overexpression			
	Proneural	Mesenchymal	Classical	Neural
IFN-γ	11	20	13	7
IL-1	13	16	6	14
IL-2	0	0	6	3
IL-4	0	0	7	7
IL-7	7	29	9	14
IL-12	9	18	20	10
IL-15	11	27	6	17
TNF-α	20	21	6	24
CD3	13	30	6	7
CD8α	16	25	7	14
CD8β	4	25	13	14
CD80	5	25	2	3
CD86	4	43	0	21
CD40	11	30	6	14
HLA-A	0	0	13	21
HLA-B	4	18	6	10
HLA-C	2	5	6	10
HLA-DRA	7	29	6	14
HLA-DQA1	16	38	19	24
HLA-DPB1	9	38	6	17

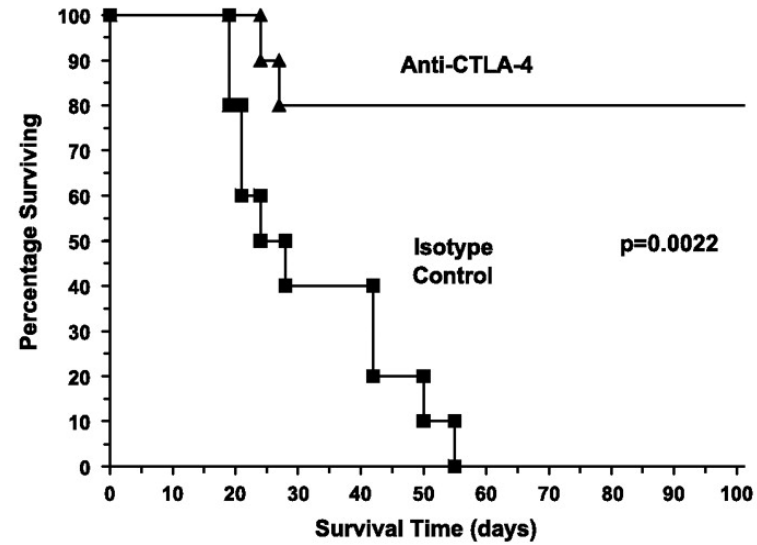
Rationale for anti-CTLA-4 in GBM









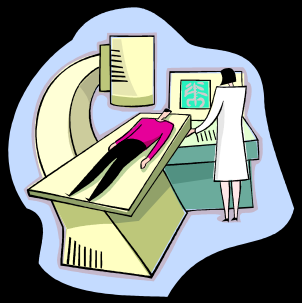


Fecci, CCR, 2007

RTOG 1125 Trial: Phase II/III

Sample size: 815; 190 for the phase II component

Molecular profile



6000 cGy + TMZ

Randomize based on molecular profile

TMZ d 1-5 of 28-d cycle +
Ipi iv q 4 wks
6 cycle maximum

Ipi IVq 3 months x 6 cycles

2:1

TMZ d 1-5 of 28-d cycle +
Placebo iv q 4 wks
6 cycle maximum

placebo IV q 3 months x 6 cycles

Primary Endpoints

Phase II

Progression-free survival

Phase III

Overall survival

Secondary Endpoints

Phase II

Treatment-related toxicities

Phase III

Progression-free survival

Treatment-related toxicities

Net Clinical Benefits (NCB): Symptom burden measured by the MDASI-BT;

HRQOL measured by the EORTC-QLQ30/BN20 instrument;

and neurocognitive function measured the Hopkins Verbal Learning Test

Molecular and immunological predictors of response to immunotherapy

Immune monitoring components:correlation with clinical responses

Baseline immune competency

ALC recovery kinetics

Serum chemokine and cytokine profiles (Multiplex and Meso scale)

T cell subset and phenotypic analysis

T cell immune responses (EPIMAX)

TCR repertoire (NanoString)

HLA

Intratumoral immune response

Immunosuppression 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)
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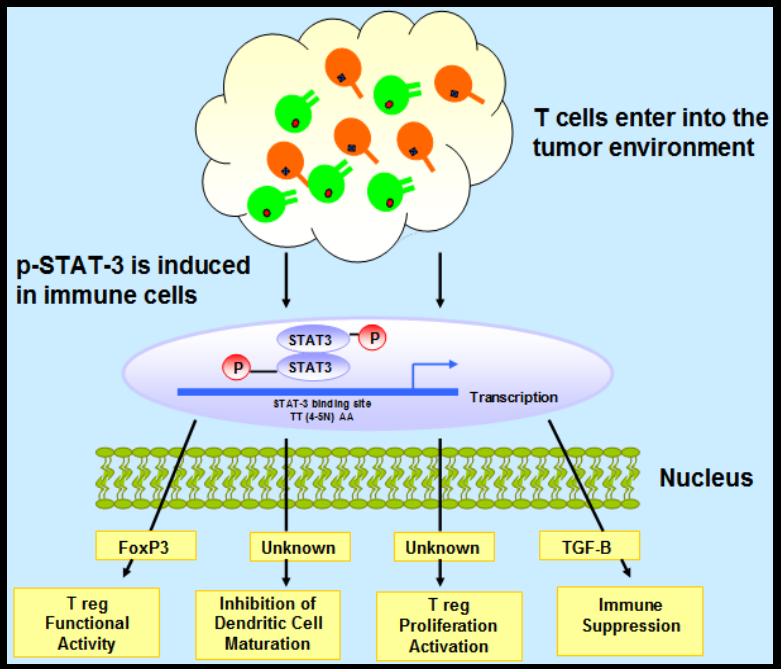
Manifestations

- 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

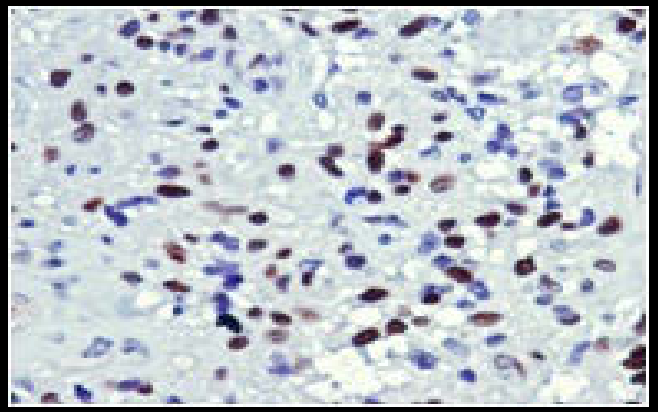
Is there a common pathway?

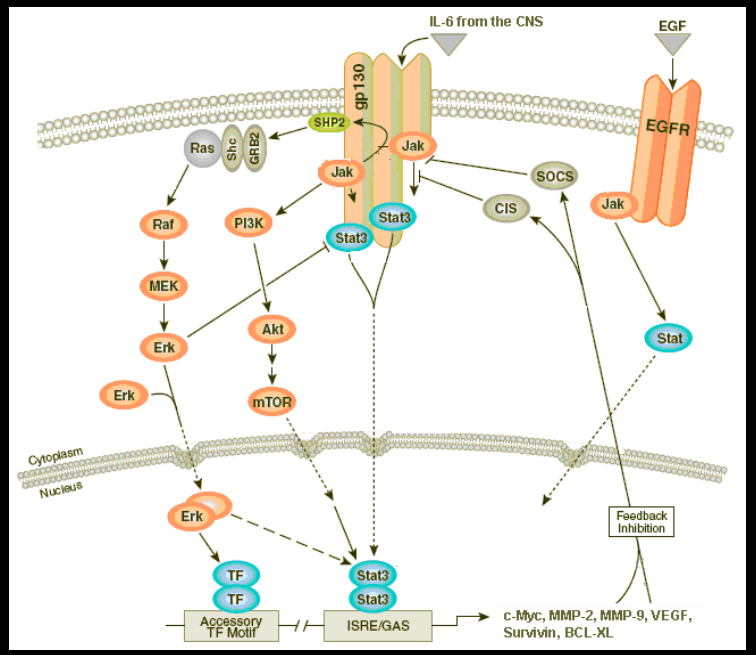
The STAT3 pathway is a key regulatory pathway in global immune suppression



- pSTAT3 becomes active in immune cells in the presence of malignancy.
- 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.
- Ablating STAT3 in hematopoietic cells in mice resulted in marked enhancement of immune responses and marked anti-tumor activity.
- 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.

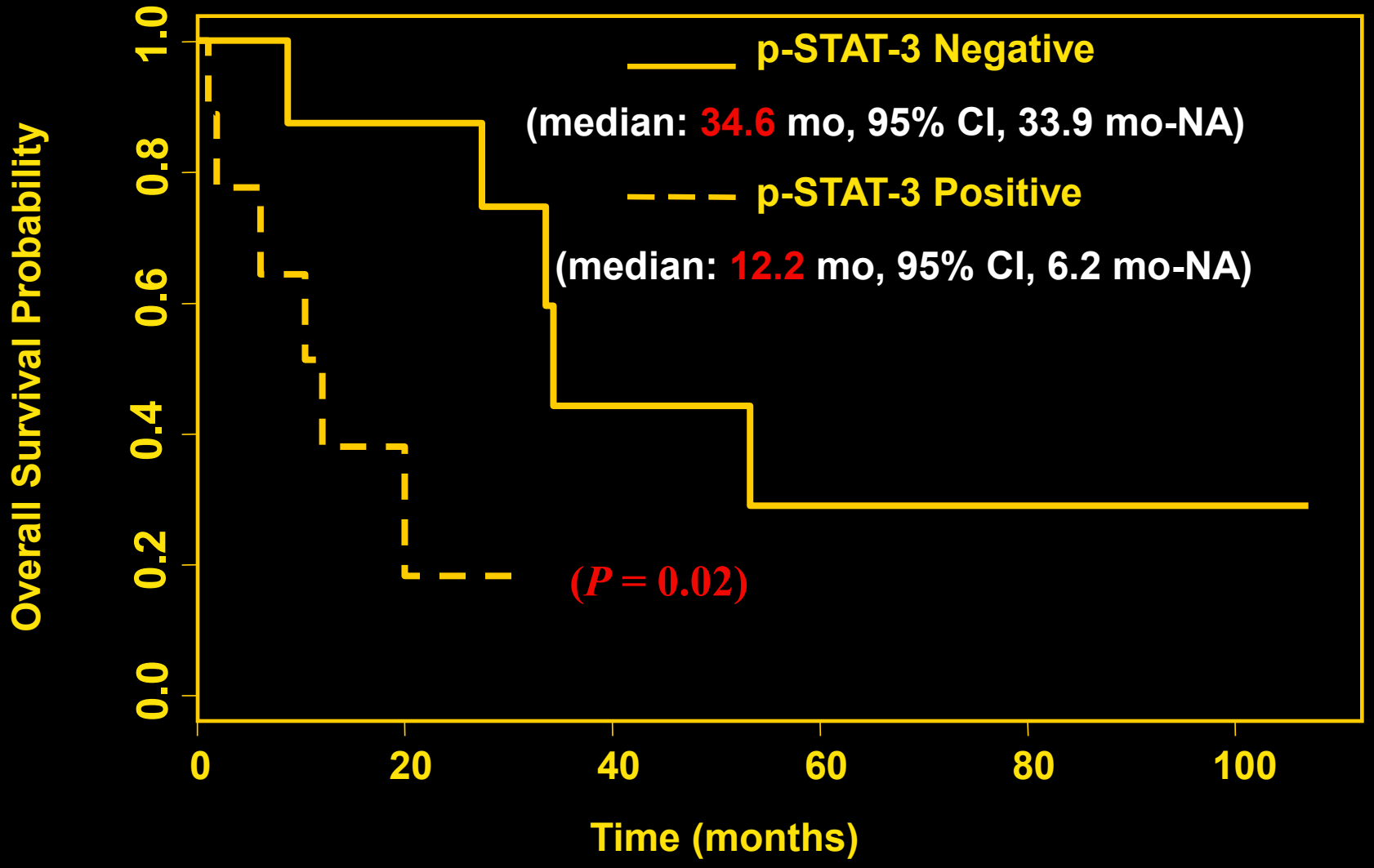
The STAT3 pathway is active in many cancers and especially within malignant gliomas





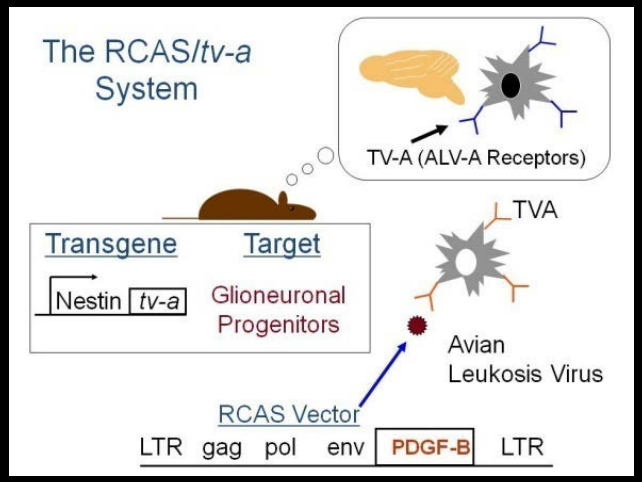
- Constitutive activation is observed in majority of many malignancies or can be induced by EGF, PDGF, IL-6, CMV.
- Upon phosphorylation of tyrosine^{705} (p-STAT3), dimerization occurs and subsequent nuclear translocation.
- The p-STAT3 is a potent transcriptional factor that regulates key factors that 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”.

p-STAT3 expression within anaplastic astrocytomas was a negative prognostic factor

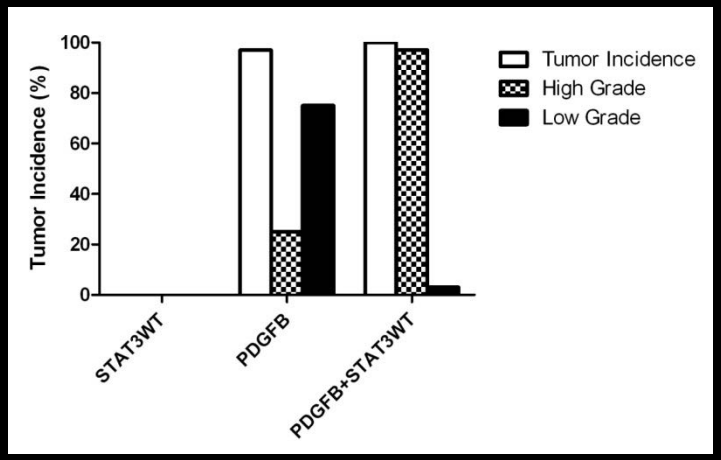


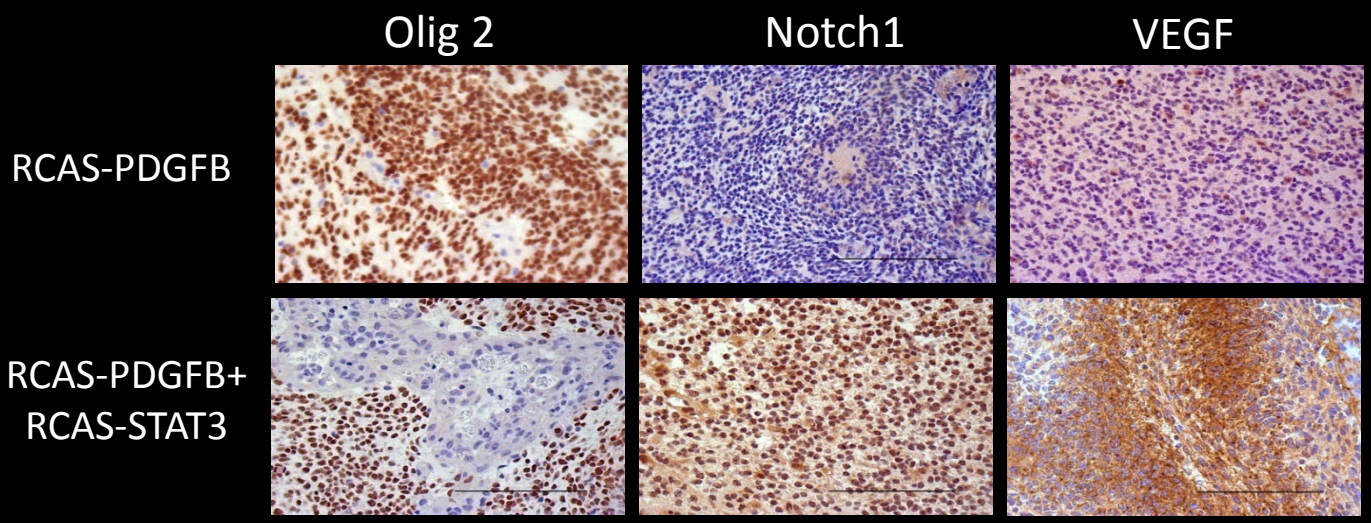
Abou-Ghazal, CCR, 2008

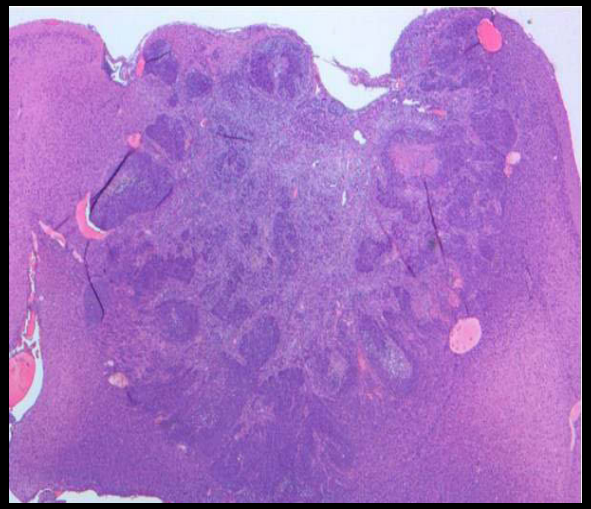
Induction of high-grade malignant gliomas in immune competent mice (*Ntv-a* model)



n=29 n=38



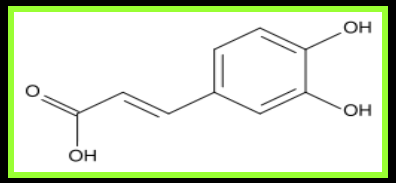




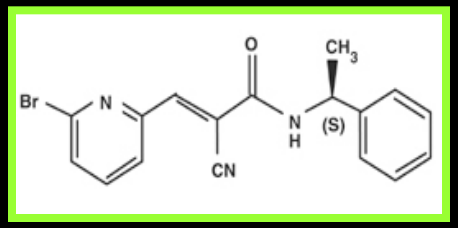
Doucette, Neuro-Oncology,

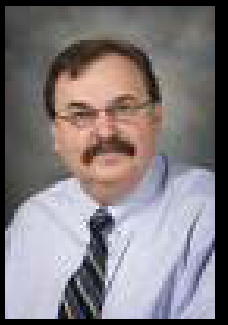
WP1066: Blocks nuclear translocation of dimerized p-STAT3

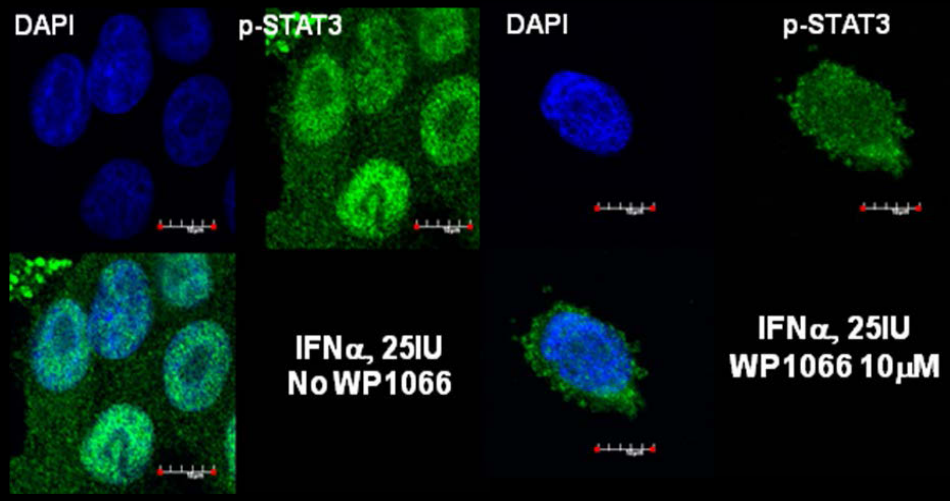
Caffeic Acid



WP1066

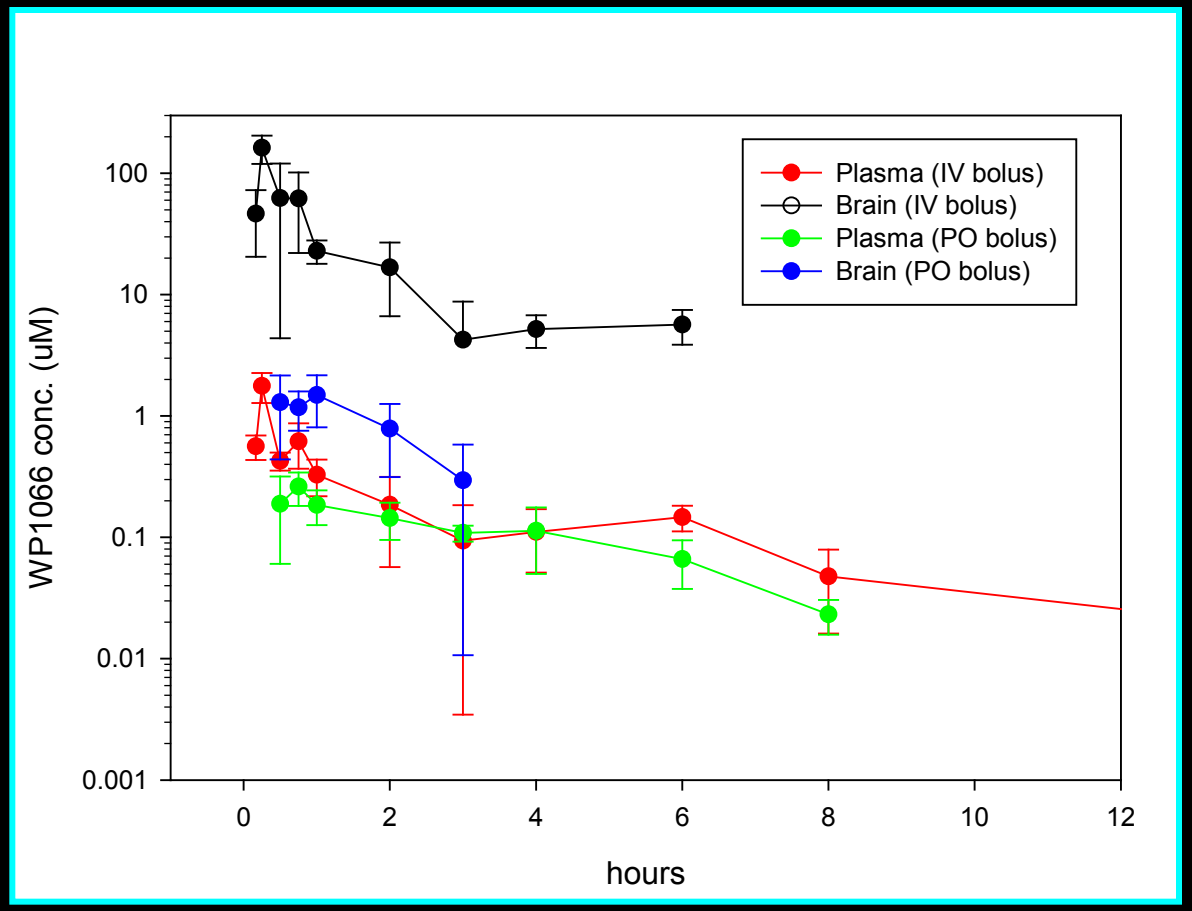






Courtesy of W Priebe

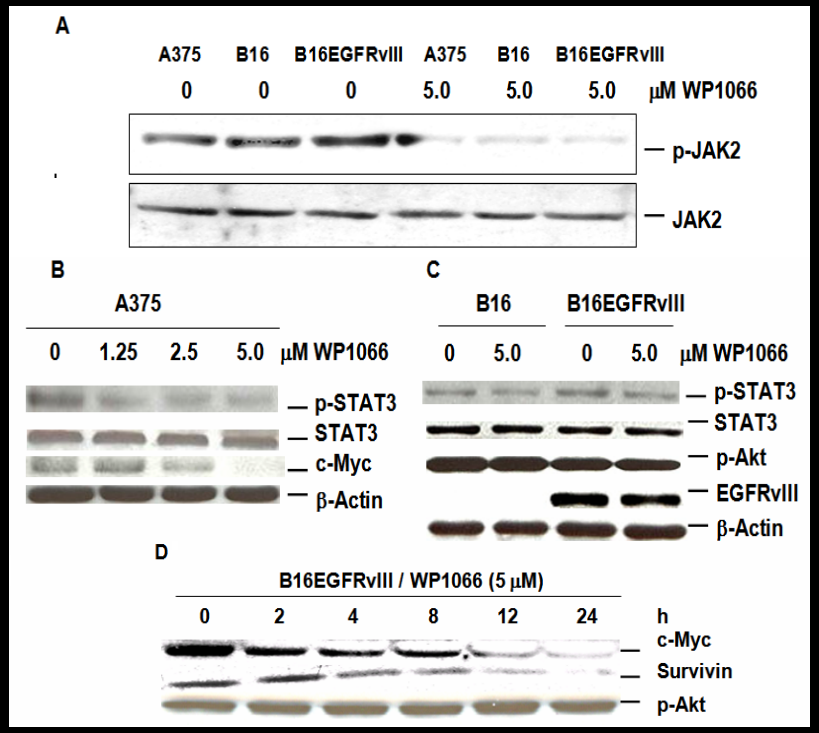
WP1066: Achieves preferential deposition in the CNS



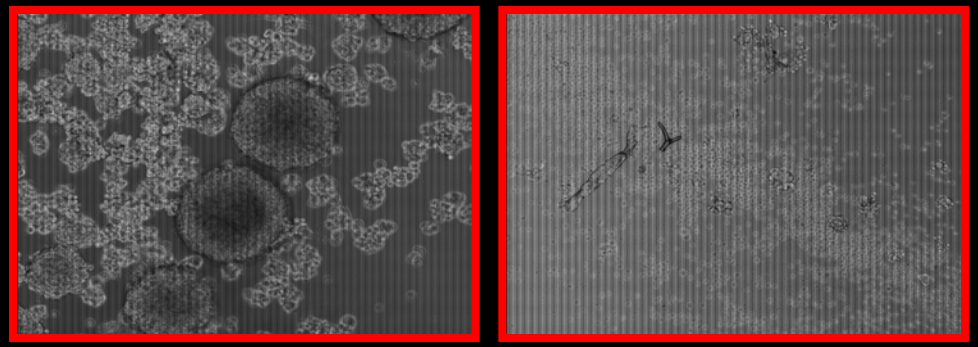
In collaboration with W. Priebe and T. Madden

Key Findings of *in vitro* WP1066

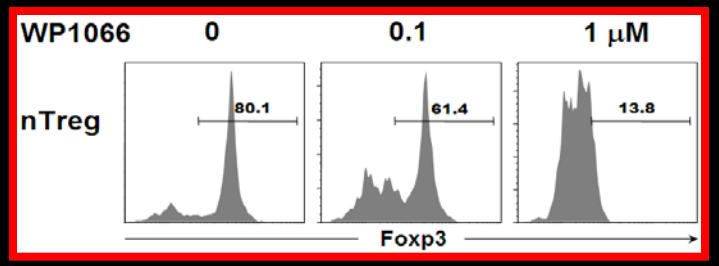
Potent inhibitor of STAT3



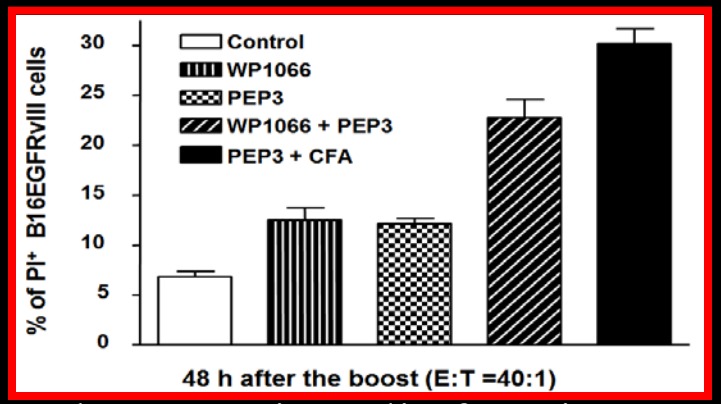
Inhibits cancer stem cells



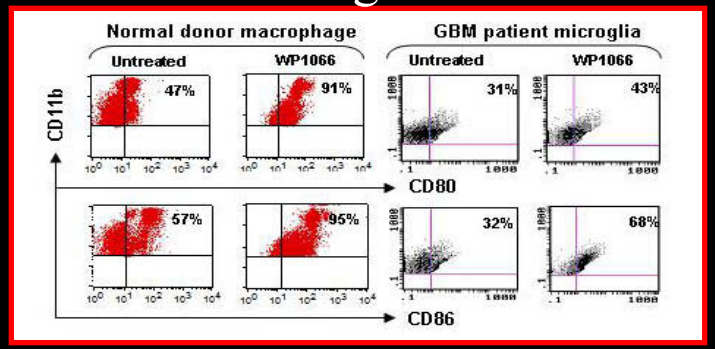
Inhibits Tregs



Enhances tumor cytotoxcity



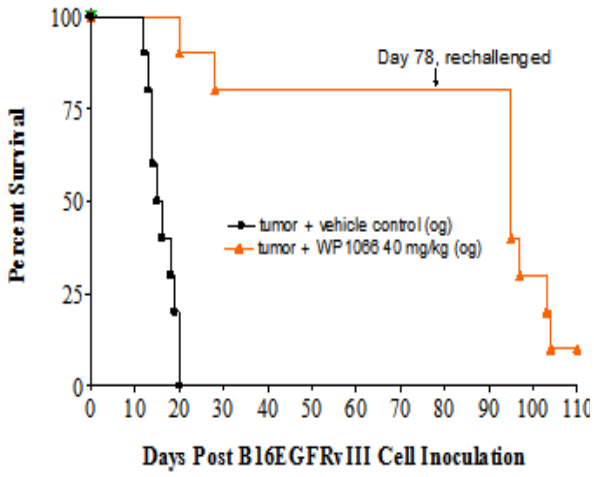
Enhances microglia function



Hussain, CR, 2007; Kong, CCR, 2008

WP1066 exerts potent in vivo efficacy against intracerebral melanoma and gliomas

A



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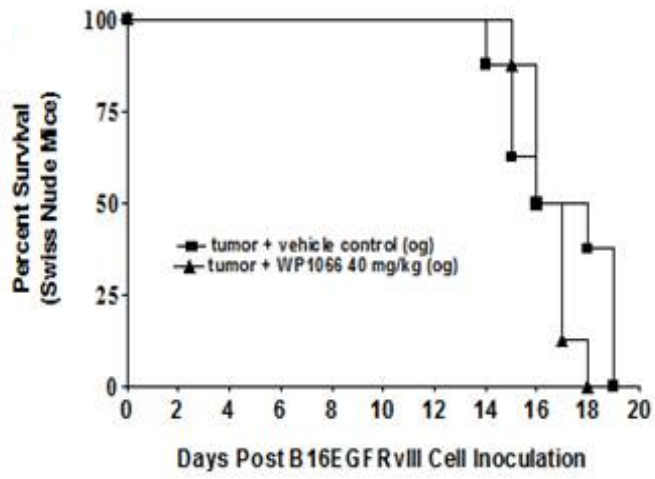

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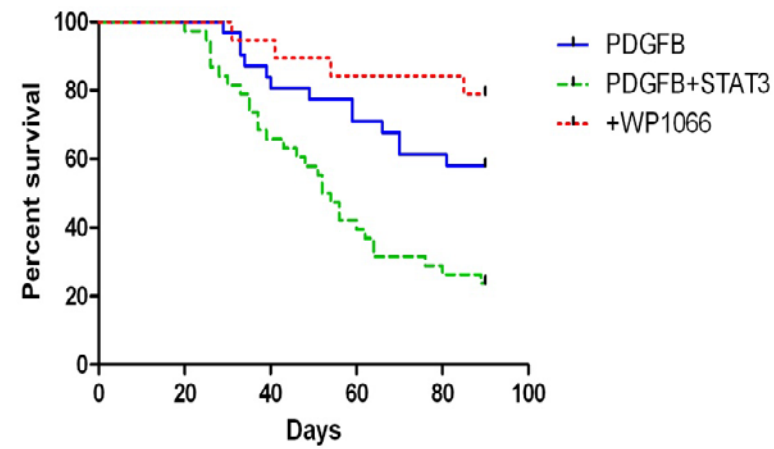
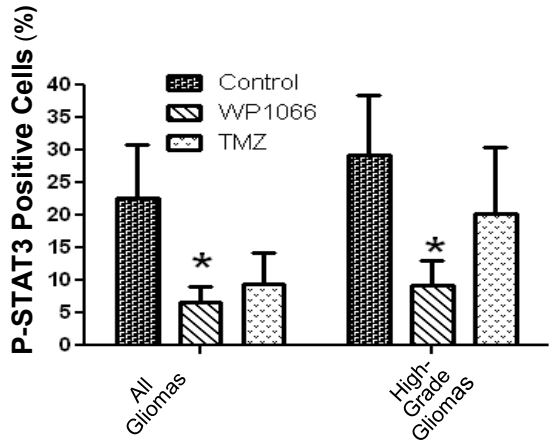
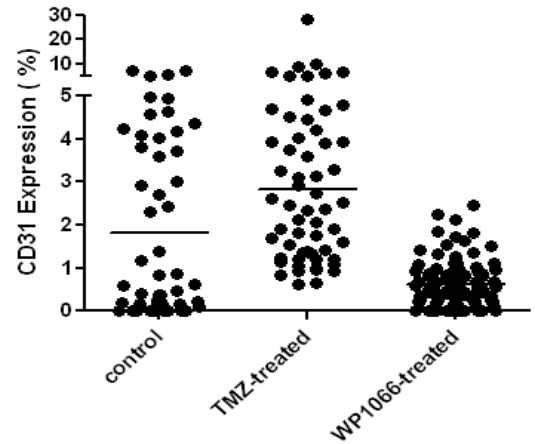
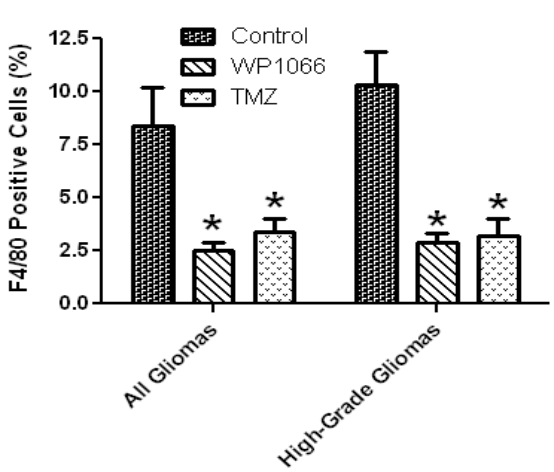


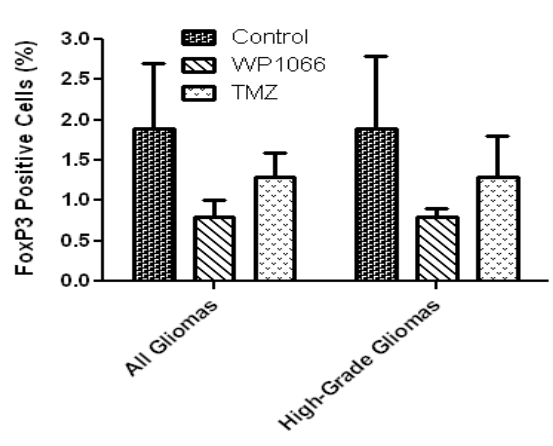
Kong, CCR, 2008; Kong, CCR 2010; Doucette, NO 2012

WP1066 modulates the tumor microenvironment and immune response



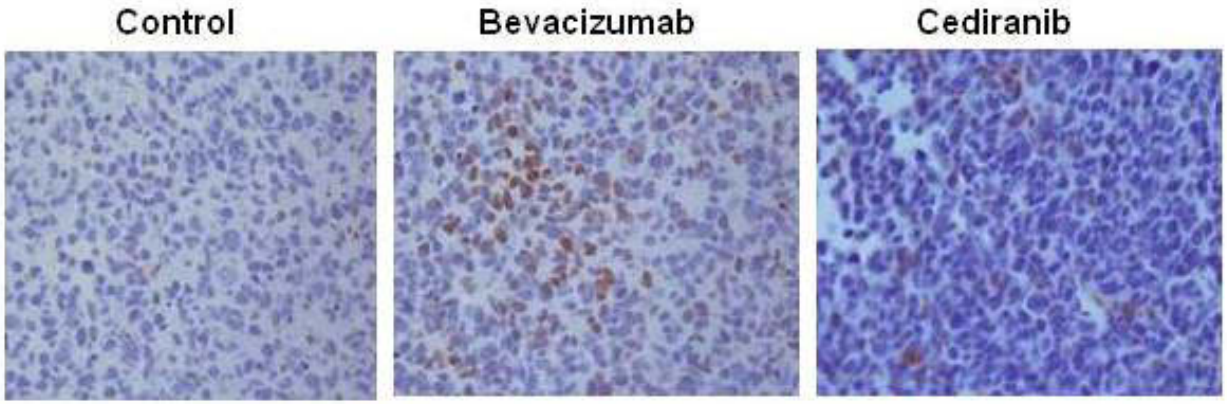




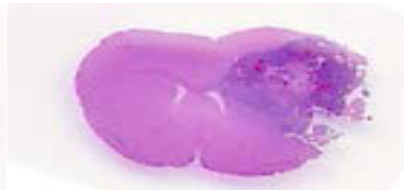


Doucette, Neuro-Oncology, 2012

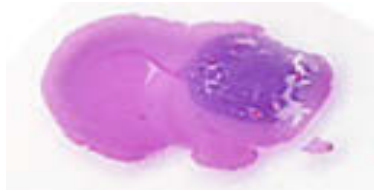
The STAT3 pathway and therapeutic treatment failure



Vehicle

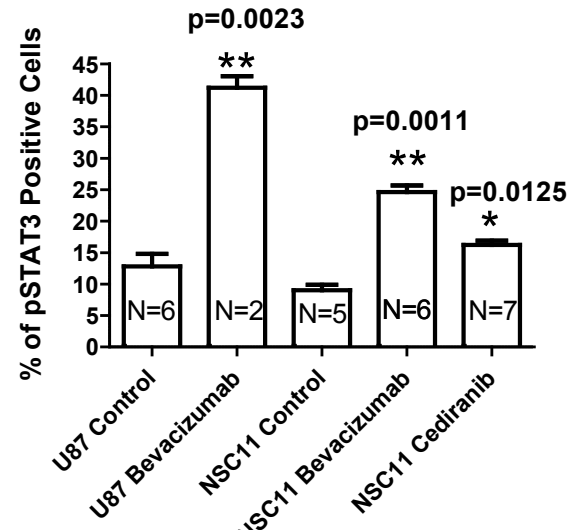


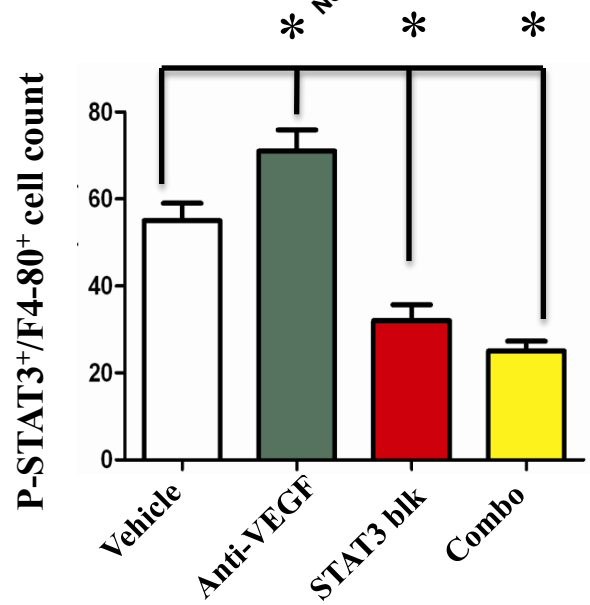
Anti-VEGF



STAT3 blockade

Combo





In collaboration with J. de Groot

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

Investigational New Drug (IND) application

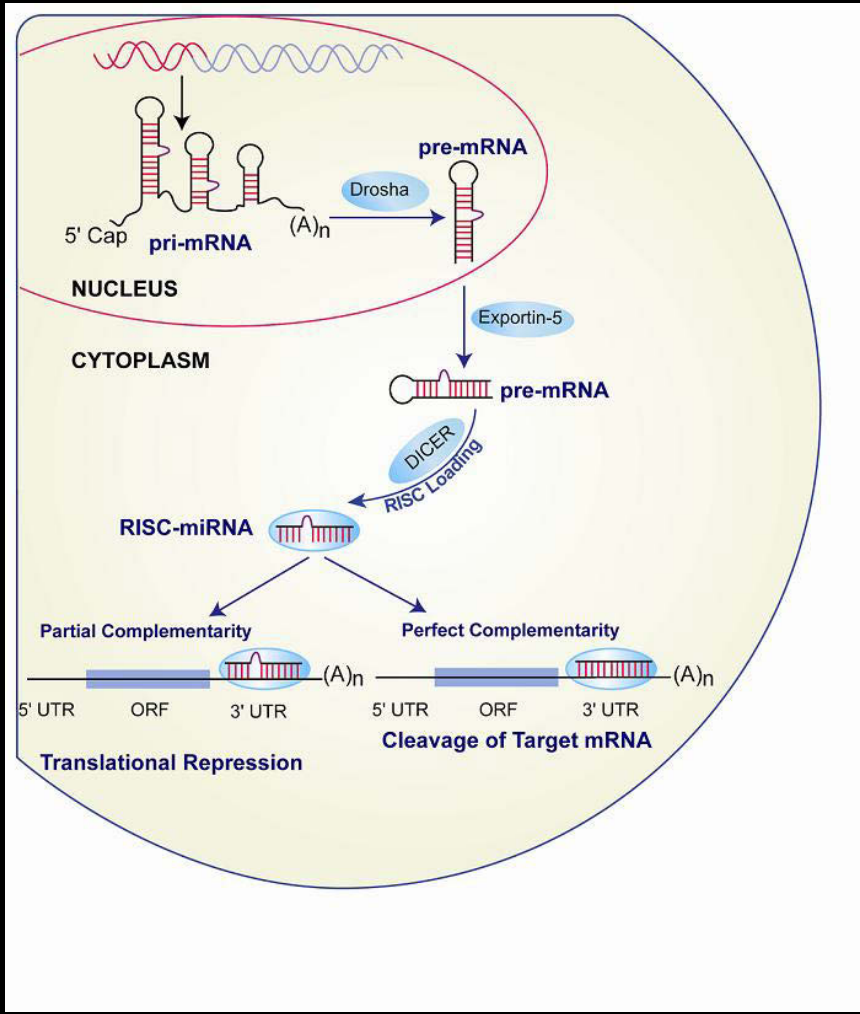
- Initial IND has been submitted (11-21-2011) with funding support from the SBIR I and II mechanisms and philanthropy

- Review call with the FDA has been completed (12-21-2011)
  - FDA is requiring second species toxicity and PK studies
  - FDA is requiring more detail on formulation and release criteria

Drug (API) is being made and formulated (CMC)

Anticipated (soon) submission of Phase I clinical trial to MDACC IRB

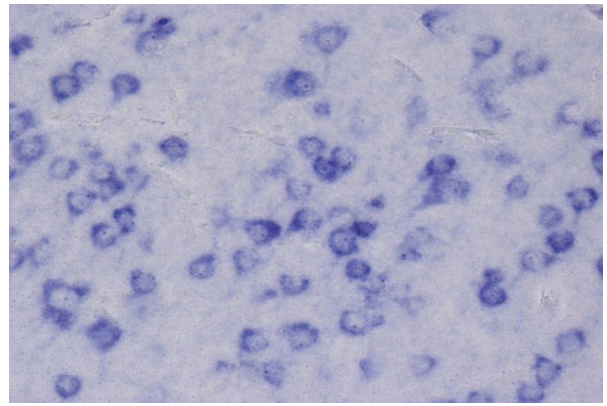
Key miRNAs down modulated in gliomas



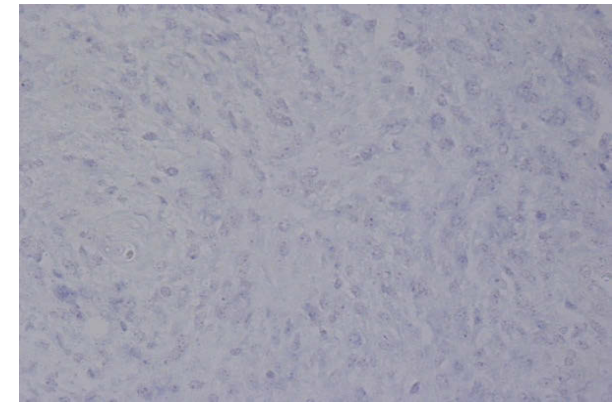
miRNA	relative down regulation
miR-124	24.6
miR-3172	13.8
miR-138	13.4
miR-3196	8.5
let-7b	7.3
let-7e	6.9
miR-1826	5.9
miR-1228*	5.8
miR-4284	5.6
let-7d	5.6
miR-3162	5.4
miR-874	5.2
let-7c	5.2
miR-103	5
miR-128	4.9
let-7a	4.7
miR-26a	4.5
miR-762	4.5
miR-7	4.2

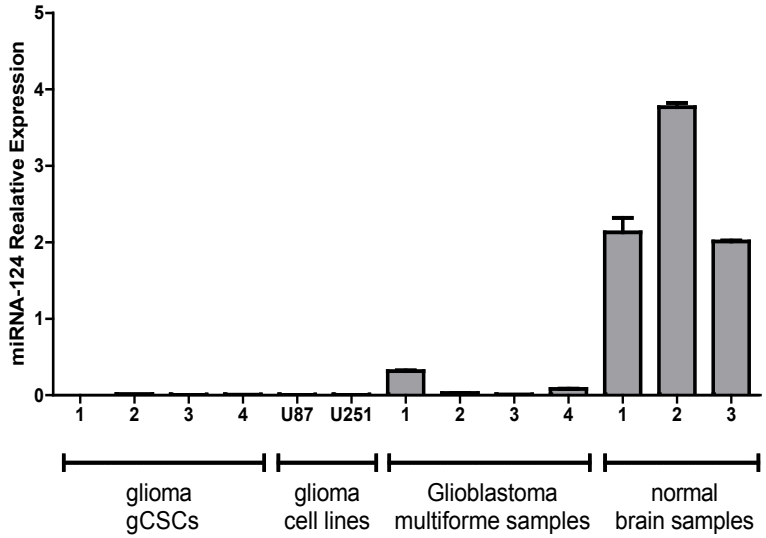
miR-124 expression is lost in all gliomas

Normal Brain



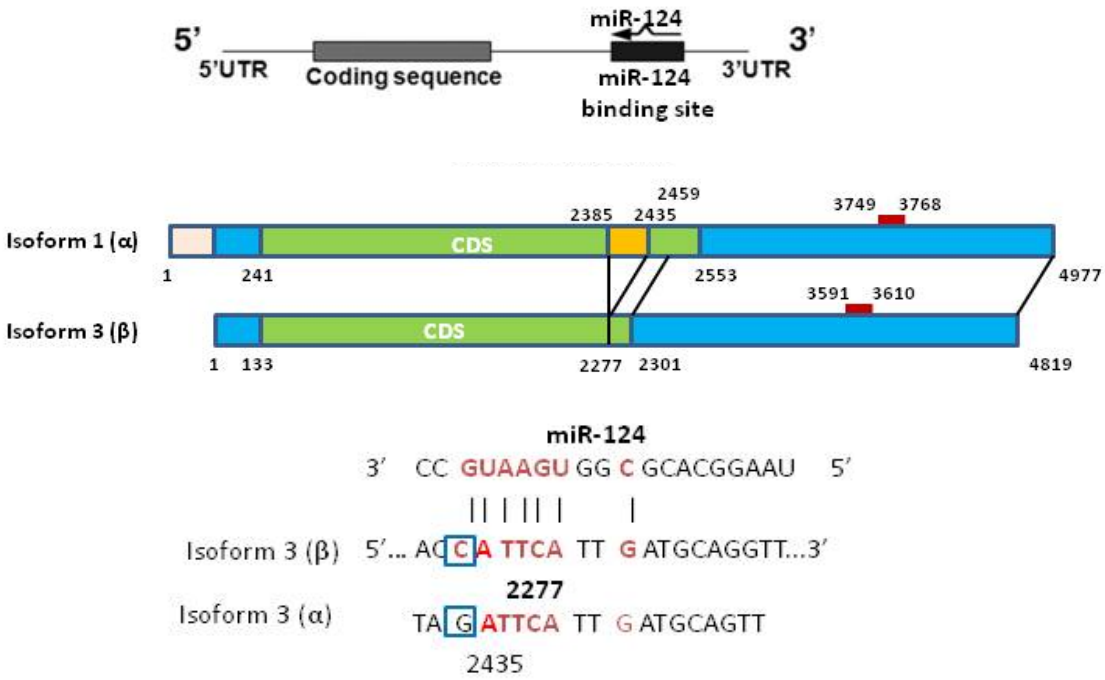
Glioblastoma

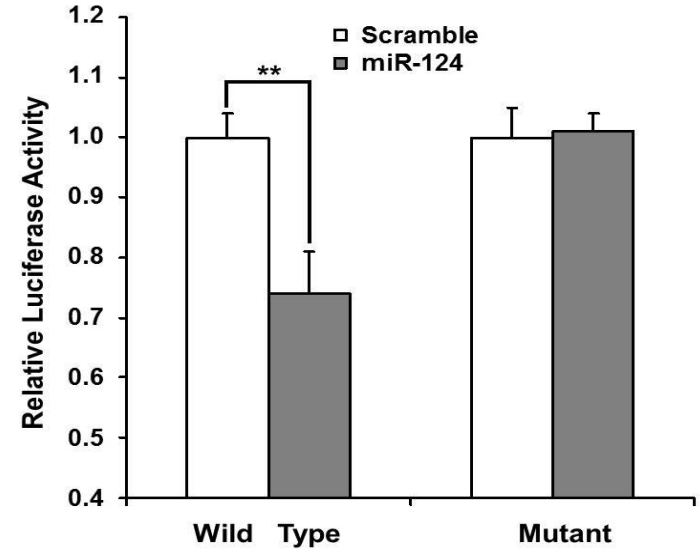




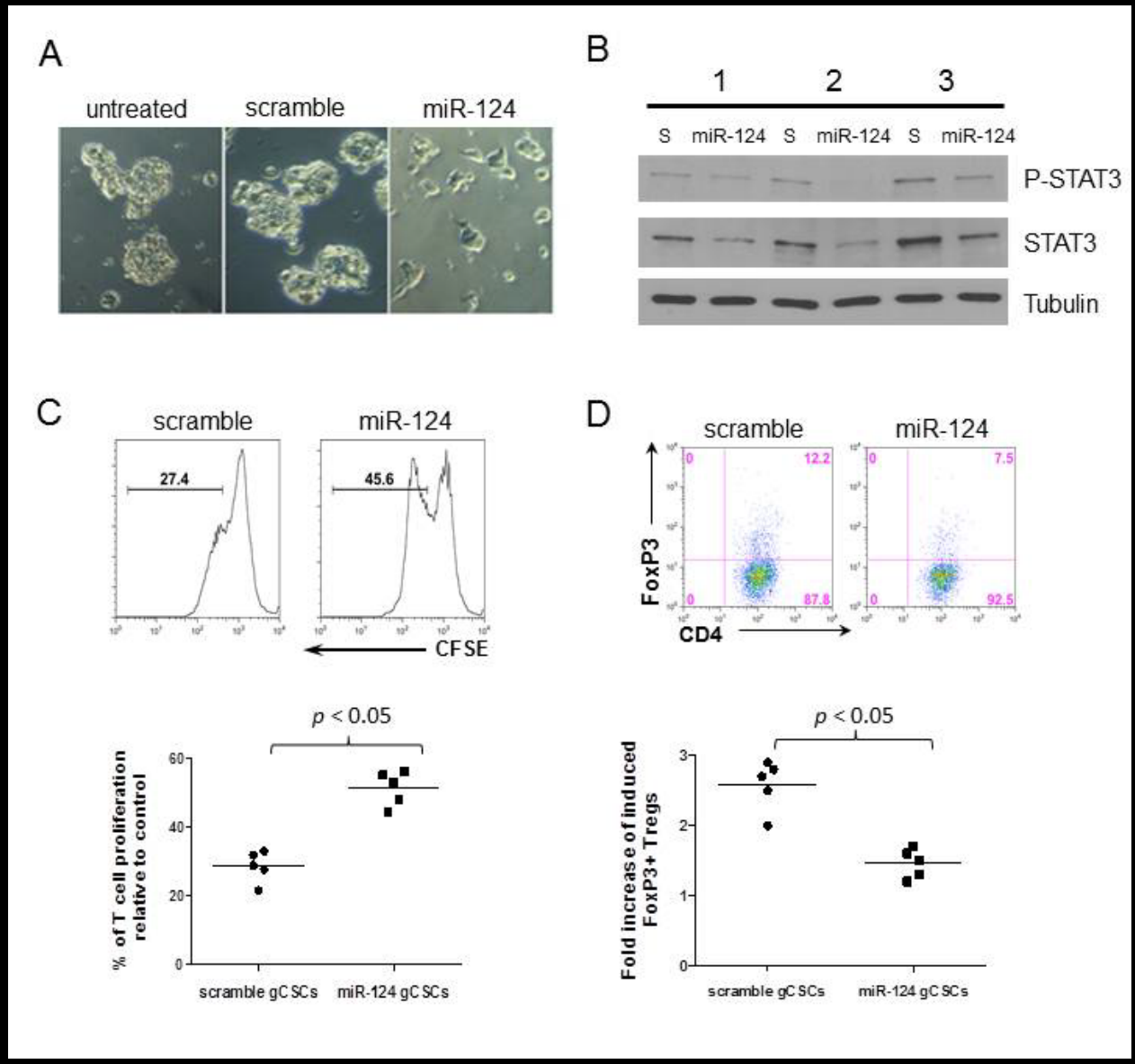
Tumor Pathology	miR-124 positive samples
Glioblastoma	0/150
Anaplastic astrocytoma	0/24
Low Grade Astrocytoma	0/1
Subependymoma	0/2
Gliosarcoma	0/6
Oligodendroglioma	0/24
Mixed Oligoastrocytoma	0/5
Anaplastic Oligodendroglioma	0/16
Anaplastic Mixed Oligodendroglioma	0/9

miR-124 targets STAT3

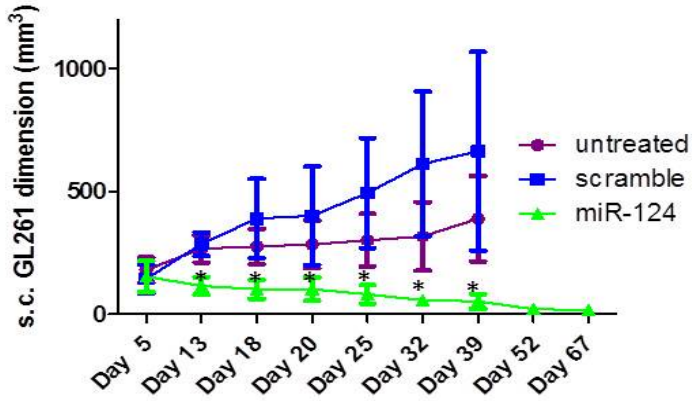


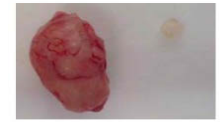


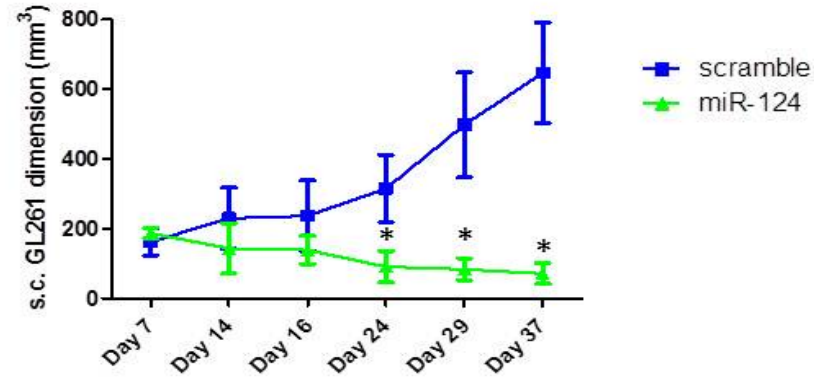
MiR-124 influences the immune biology of cancer stem cells

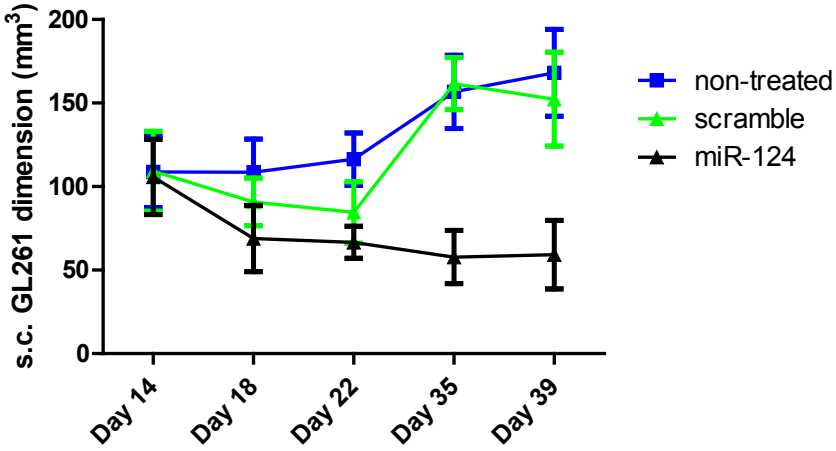


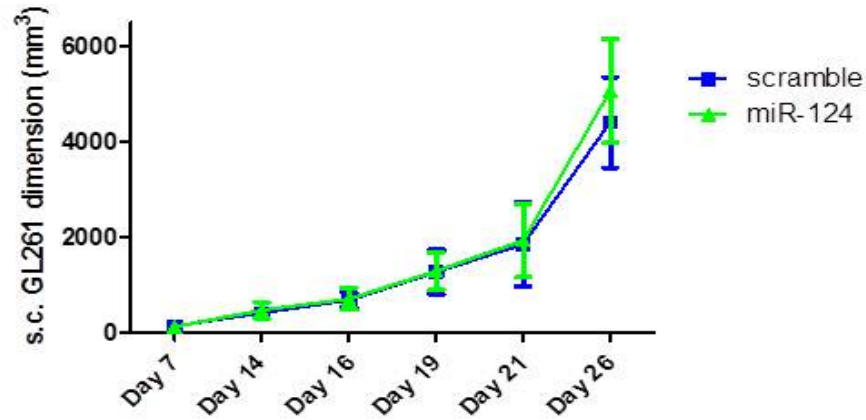
miR-124 blocks glioma growth

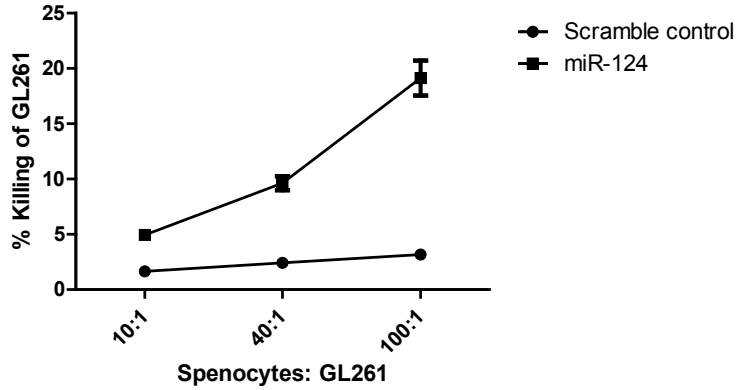


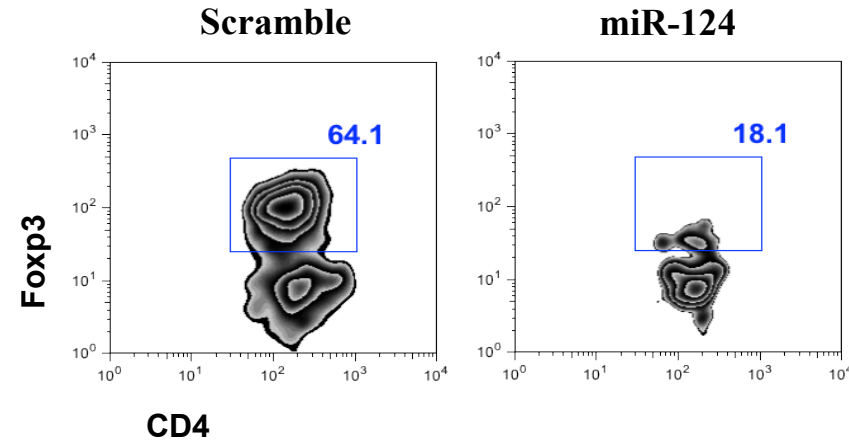


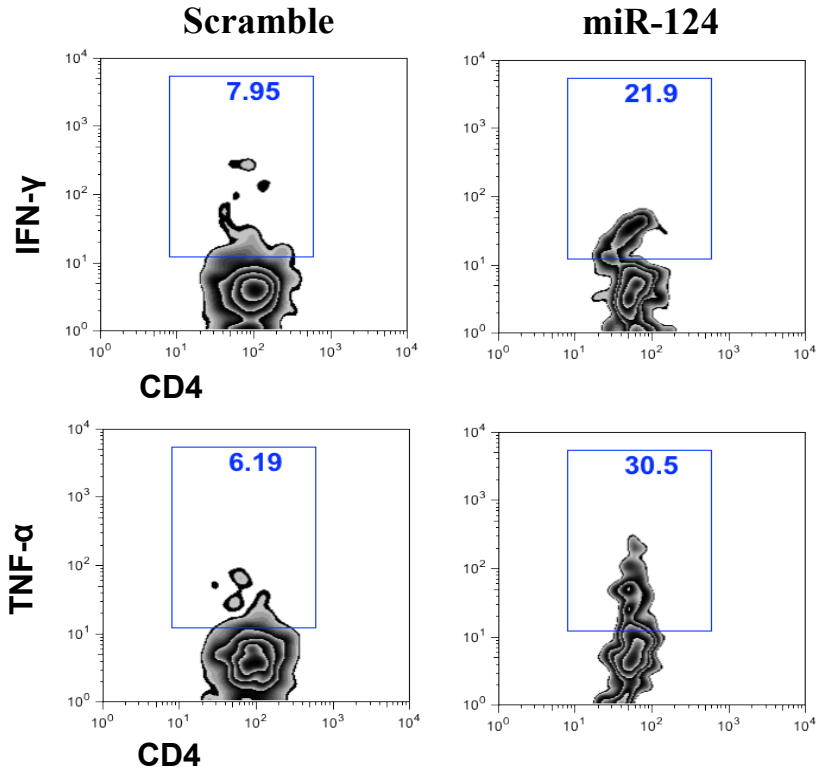


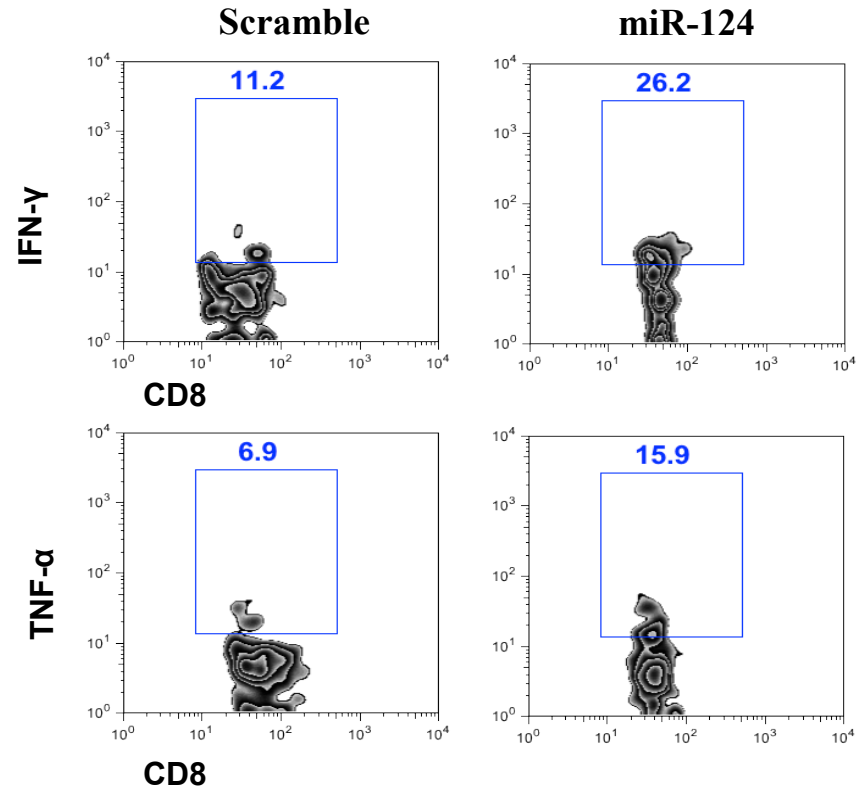




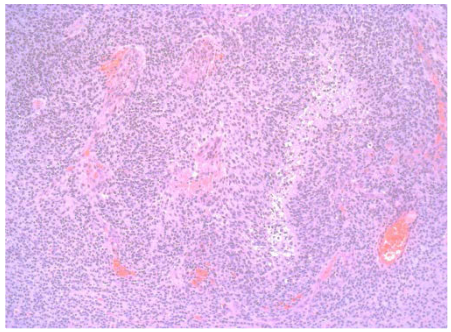

% Killing of GL261
25
20
15
10
5
0
10:1
40:1
100:1
Spenocytes: GL261
Scramble control
miR-124

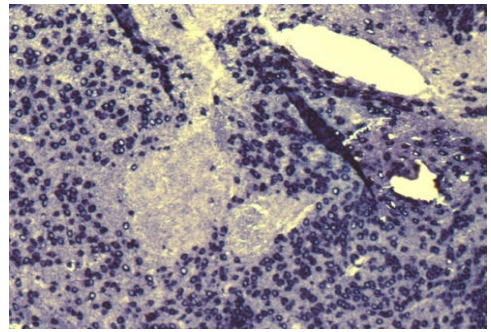

Scramble
miR-124
Foxp3
CD4
64.1
18.1

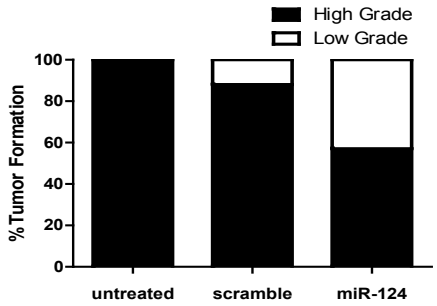

Scramble
miR-124
IFN-γ
CD4
7.95
21.9
TNF-α
CD4
6.19
30.5

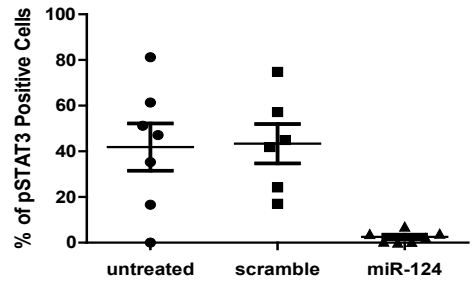

Scramble
miR-124
IFN-γ
CD8
11.2
26.2
TNF-α
CD8
6.9
15.9

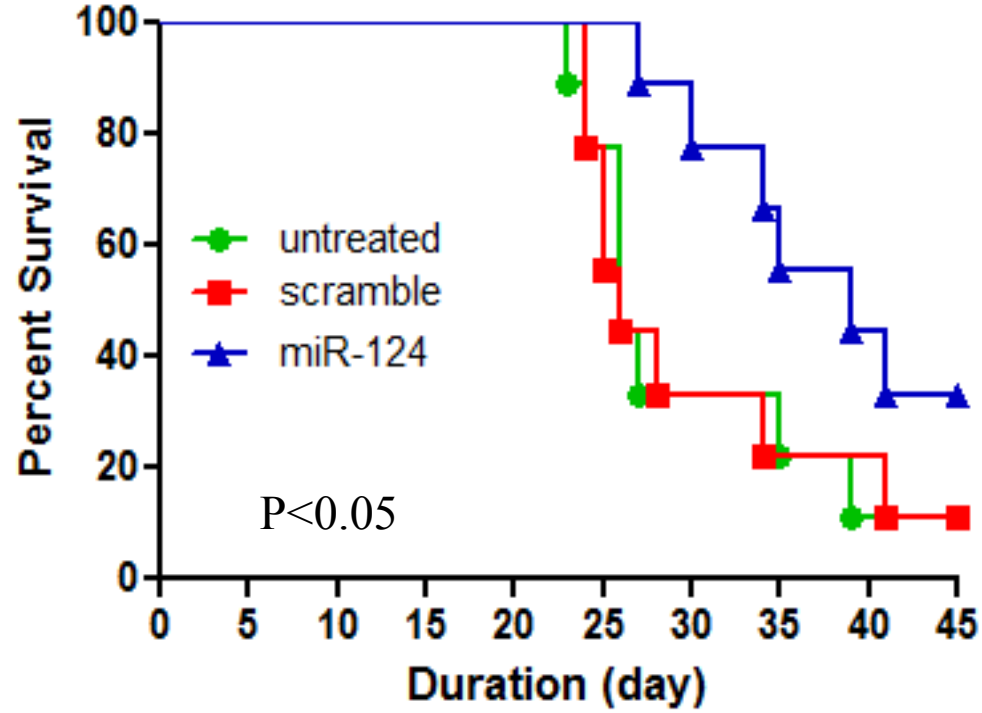
miR-124 exerts a therapeutic effect against heterogeneous gliomas



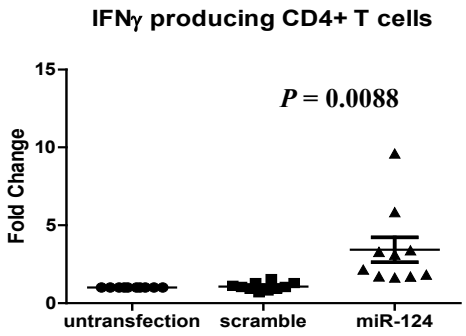


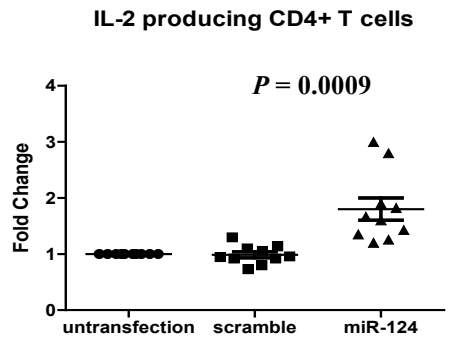


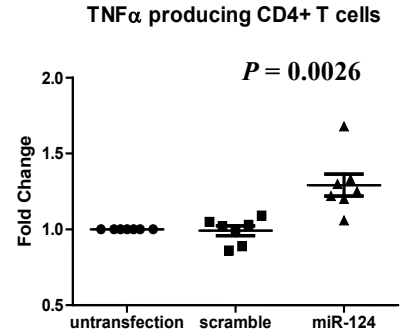


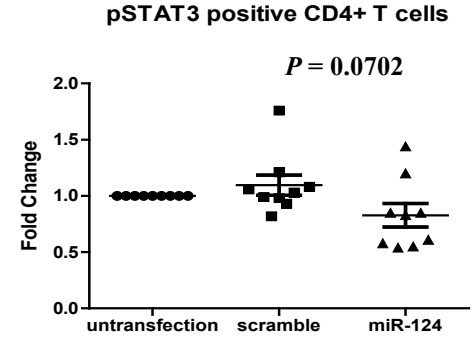


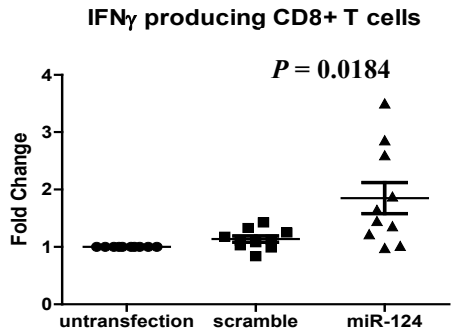
MiR-124 induces immune effector responses from GBM patients

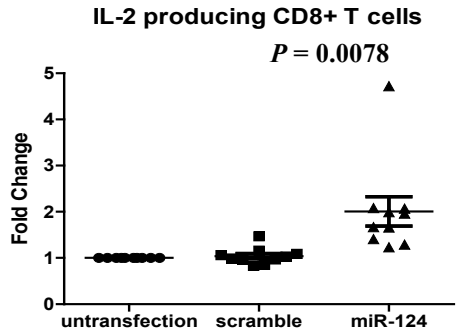


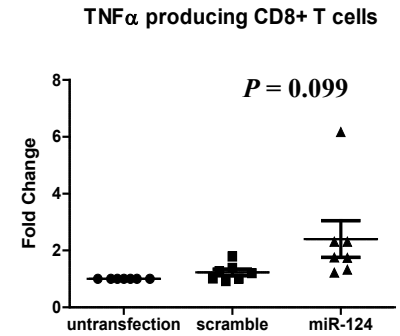


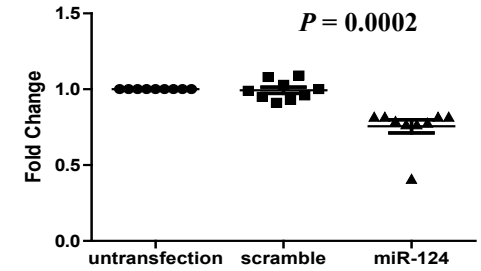












Key Considerations for Immune Therapeutic Clinical Trials

- Sufficiently potent immune responses need to be generated to overcome profound immune suppression and/or the immune suppression has to be negated/minimized (GTR)
- Agents that are targeted to a single immune suppressive mechanism are unlikely to have durable efficacy and will likely only treat a select subset of patients
- Targeting "drivers" of malignancy are more likely to be efficacious
- Immune suppression is heterogeneous and needs to be considered in patient stratification - patient specific tailored immune therapeutics based on tumor characteristics is a future goal

Acknowledgments

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