Induction of CD8+ T Cell Responses Against Novel Glioma-Associated Antigen Peptides and Clinical Activity by Vaccinations with α-Type-1-Polarized Dendritic Cells and Poly-ICLC in Patients with Recurrent Malignant Glioma

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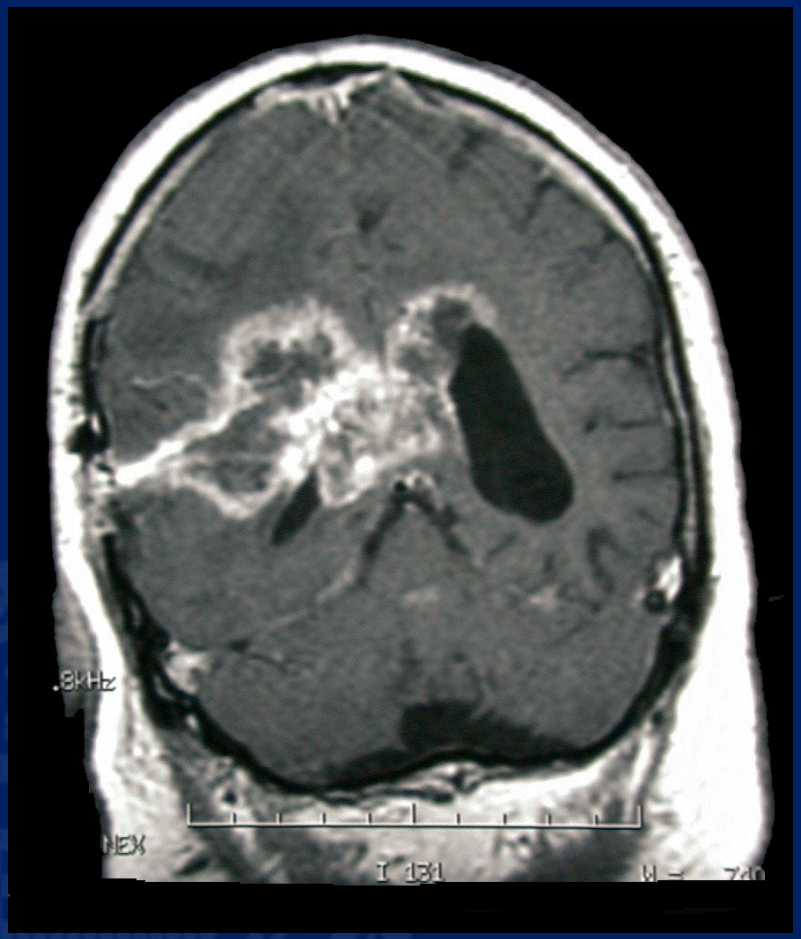
Conflicts of Interests (COI)

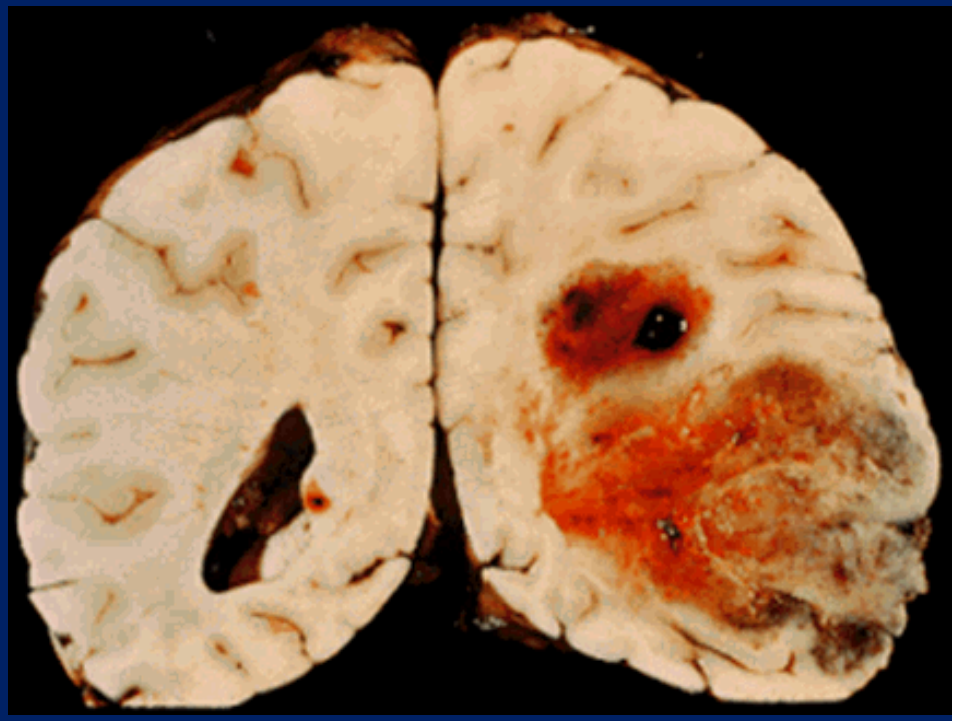
Hideho Okada is one of the inventors of the IL-13Rα2 (345-353:1A9V) and EphA2 (883-891) peptides, for which an exclusive licensing agreement has been executed with Stemline, Inc.

Per the University of Pittsburgh COI policy, interpretation of presented data was not performed solely by Hideho Okada, but by the investigator team.

Malignant Gliomas

- WHO grade 3 anaplastic glioma
- WHO grade 4 glioblastoma multiforme (GBM)



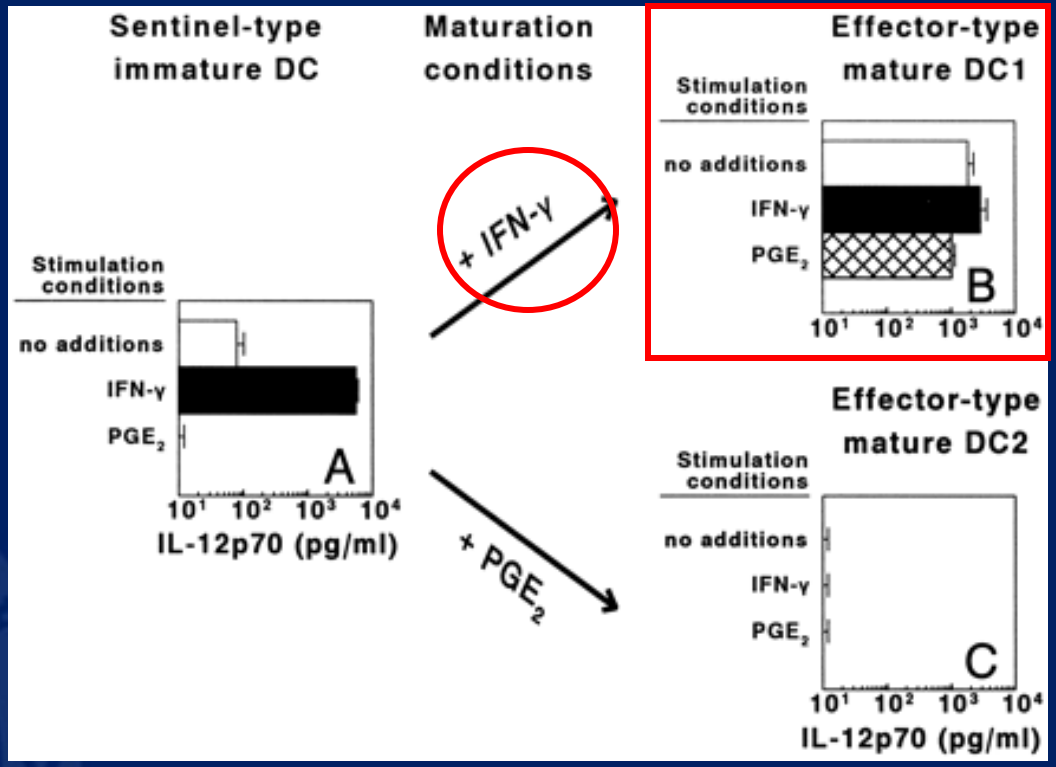


Critical Aspects/Factors for Potent Dendritic Cell Vaccines (05-115)

- Type of DCs
  - Type-1 DCs (alphaDC1)
- Target Antigens
  - Multiple CTL epitopes from 4 GAAs
- Administration Route
  - Intranodal administration (superior to s.c)
- Adjuvant
  - Poly-ICLC as a ligand for intracellular dsRNA receptors

Background
Glioma Vaccines with Type 1 DCs

Cytokines modulate the IL-12 production in DC and promote the development of stable, polarized DC (DC1).



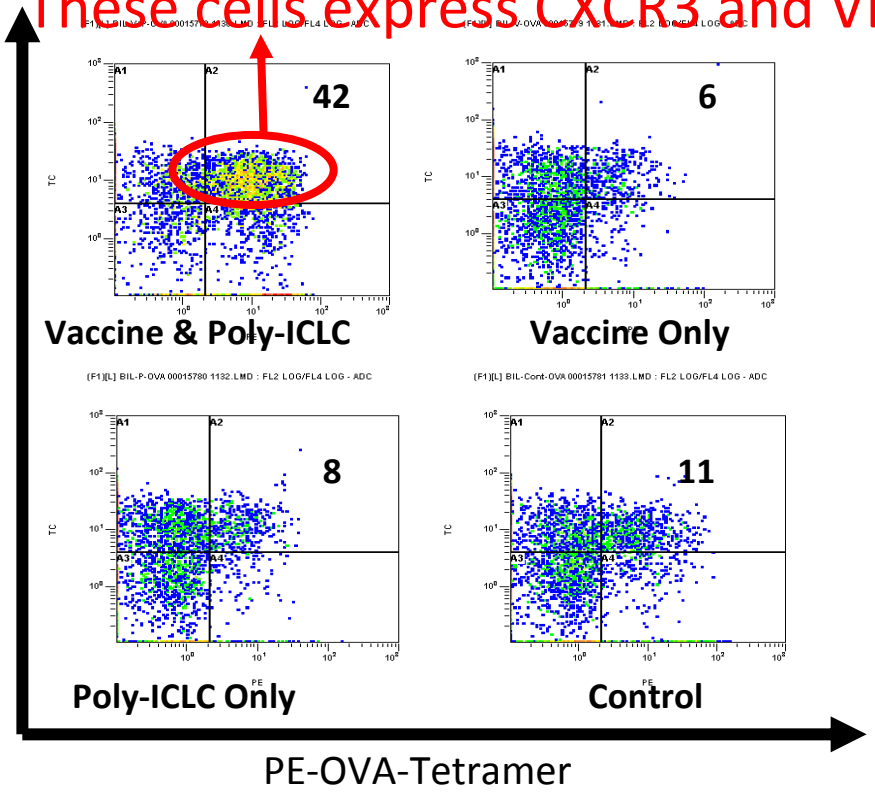
Type-1 "Polarizing" cocktails
IFN-γ
IFN-α
TNF-α
IL-1β
Poly-IC

Kalinski P *et al.* The J. Immunol. 2000, Cancer Res 2004
Kalinski P and Okada H. Seminars in Immunology 2010

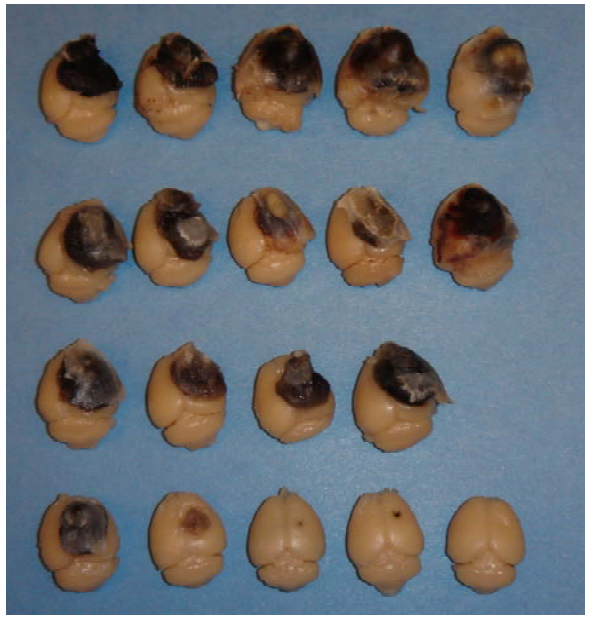
Poly-ICLC administration enhances the infiltration of OVA-Specific T cells and therapeutic efficacy

Brain-Infiltrating T cells

These cells express CXCR3 and VLA-4



Anti-Tumor Effect



Zhu X. *et al.*

Objectives (UPCI 05-115)

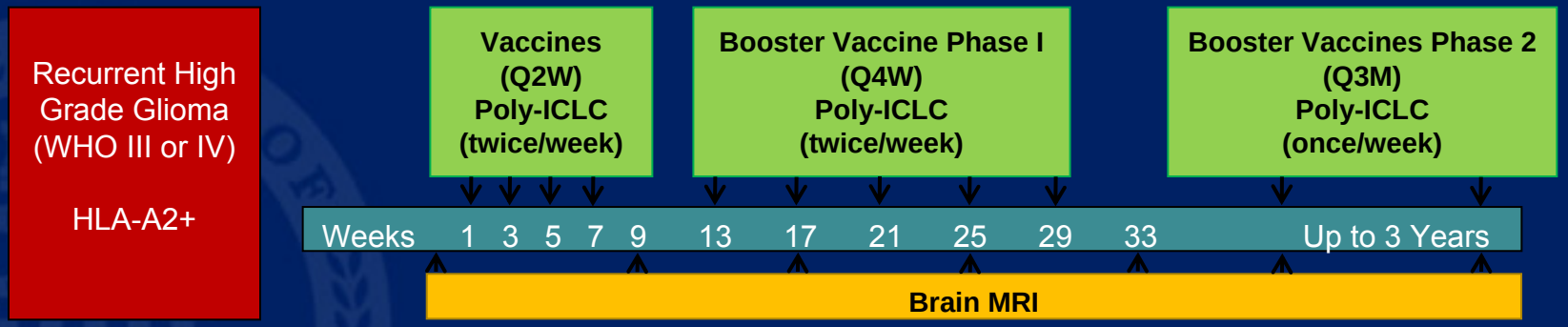
- Primary Objective
  - Safety - to determine the maximal tolerated dose of DC1 and evaluation of toxicities
- Secondary Objectives
  - Assess immunological response against GAAs using ELISPOT and tetramer assays
  - Assess the preliminary anti-tumor clinical activity of the vaccines as measured by radiological response (MRI), overall survival as well as 6 month-progression free survival (PFS).

Eligibility

- Adult patients with recurrent GBM or WHO grade 3 AG
- HLA-A2+ based on flow-cytometry
- Minimum corticosteroid (4 mg/day or less for Dexamethasone)
- Maximum 2 previous recurrences

Treatment

- Ultrasound-guided intranodal injections of type-1 DC1 (1 or 3 x 10^7 /injection with dose escalation) loaded with 4 glioma-associated antigen (GAA)-derived HLA-A2-restricted CTL epitopes (IL-13Ra2$_{345-353:1A9V}$, gp100$_{209-217:2M}$, EphA2$_{883-891}$ and YKL-40$_{201-210}$)
- Intramuscular injections of poly-ICLC (20 mcg/kg; Twice/week)

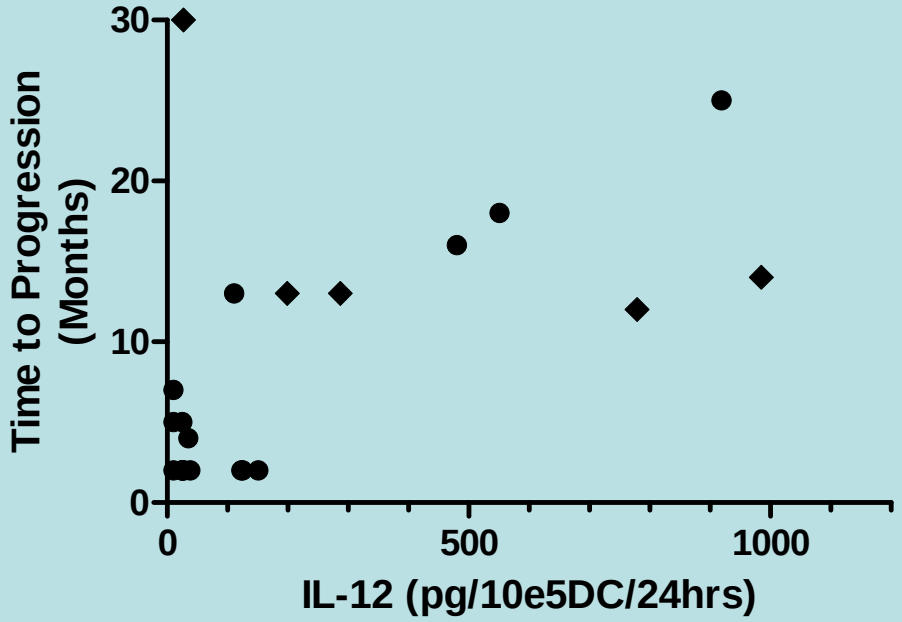


SL-701 Adverse Events

No grade 3 or 4 toxicities observed

	Grade 1		Grade 2	
Adverse Event	No.	%	No.	%
Blood/Bone Marrow				
Leukocytopenia			1	5
Injection site reactions				
Redness, induration, pruritis, pain	17	77	1	5
Constitutional symptoms				
Fatigue (lethargy, malaise, asthenia)	16	73		
Fever	5	23		
Chills/Rigors	4	18		
Nausea	7	32		
Vomiting	1	5		
Headache	5	23	2	9
Insomnia	1	5		
Light headed/dizziness	2	9		
Myalgia	7	32		
Body ache	6	27		
Dermatological				
Skin rash	3	14		
Dry skin	1	5		
Bruising	2	9		
Urticaria	1	5		
Pulmonary/Upper Respiratory				
Rhinitis/Runny nose	1	5		

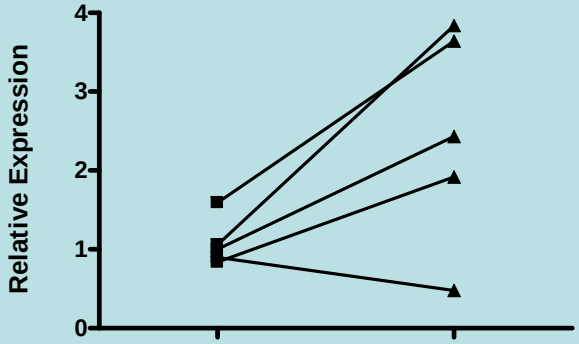
IL-12 production levels positively correlated with PFS (*p*=0.0255 based on Cox regression test)



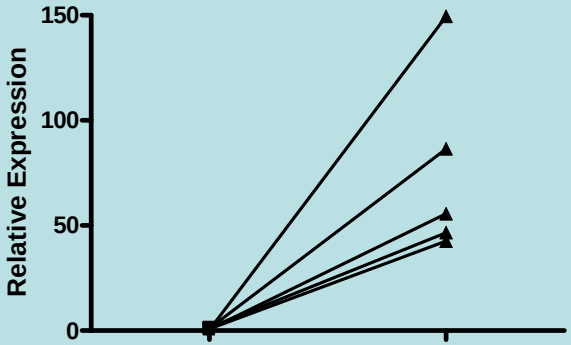
Closed circles indicate patients who have already progressed, whereas closed diamonds represent patients who have not recurred to date.

Promotion of type-1 responses detected in post-vaccine PBMCs by RT-PCR

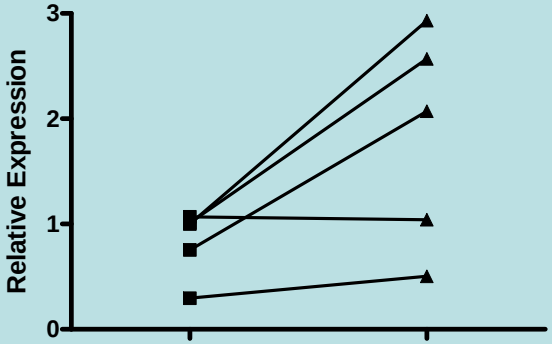
IFNα1 (p=0.0306)



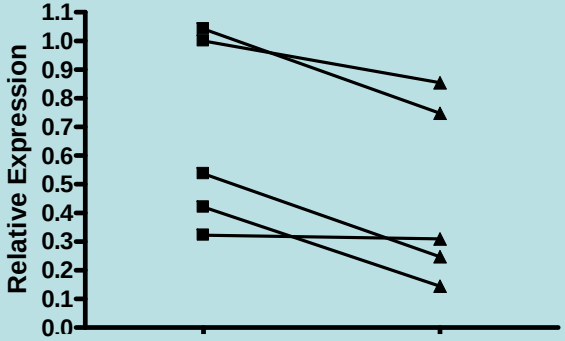
CXCL10 (p=0.0098)



TLR3 (p=0.0303)



CCL22 (p=0.0105)

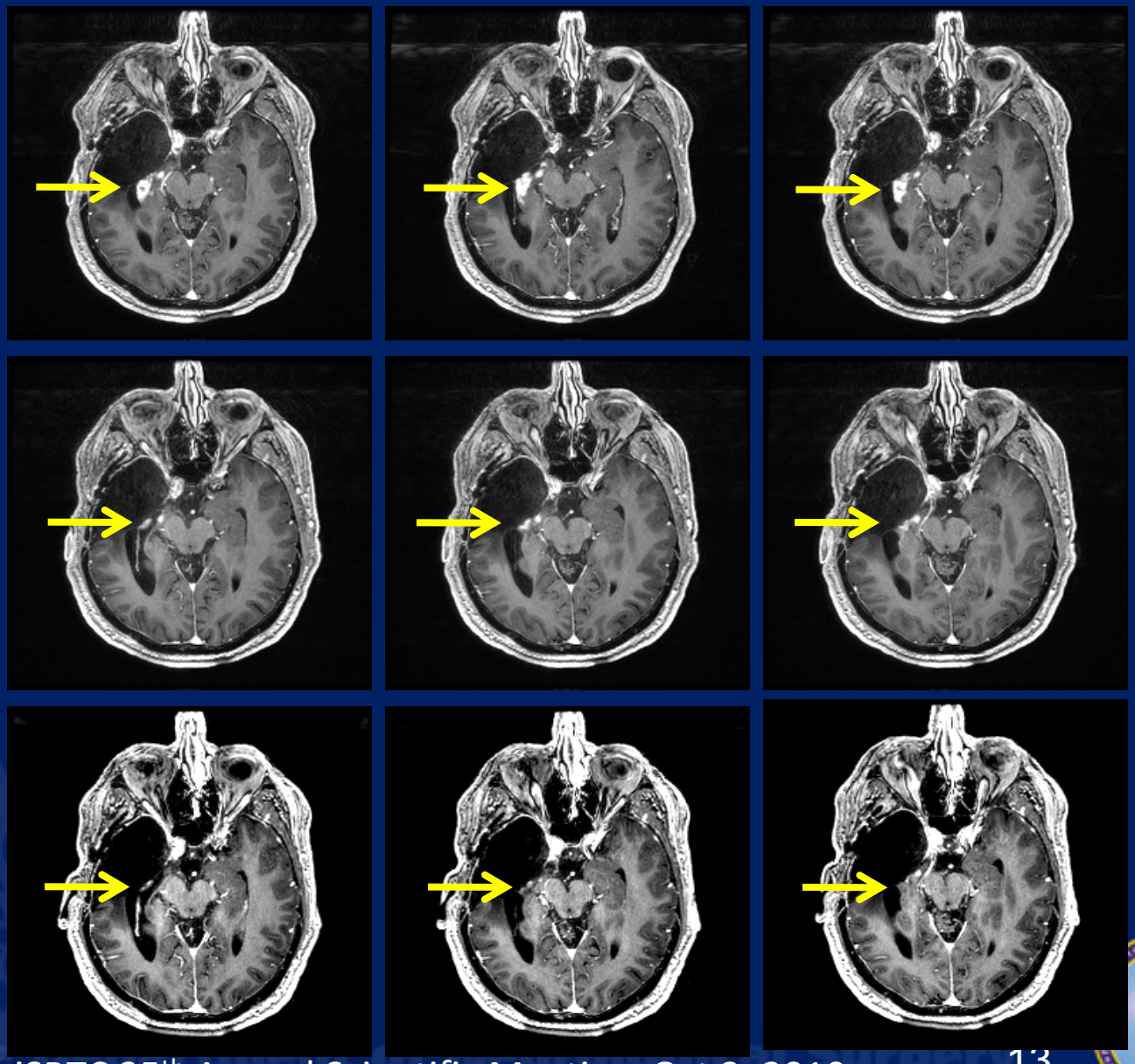


Complete Radiological Response in A Patient with Recurrent GBM (Pt #20)

Pre-Vaccine
Week 0

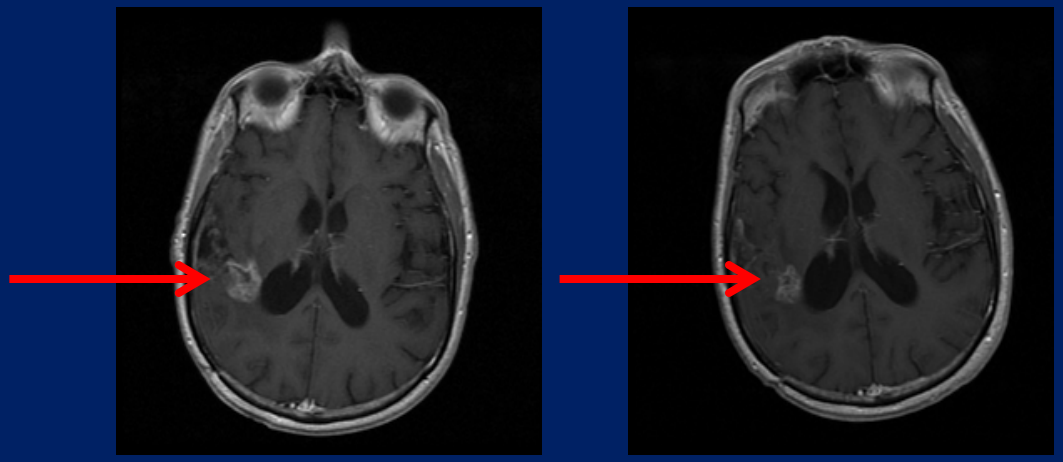
Post-Vaccine
Week 9

Post-Vaccine
Week 17

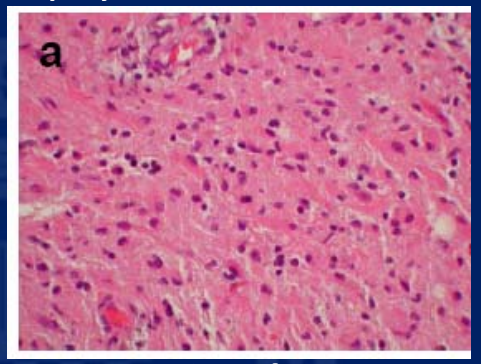


PR Patient with subsequent pseudo-tumor progression

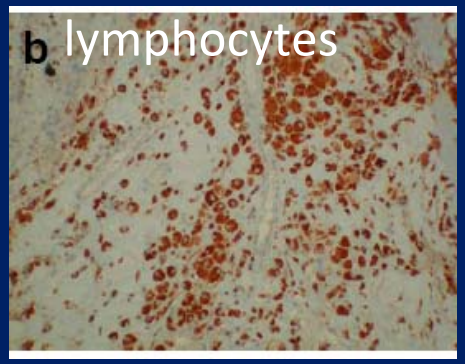
Pre-Vaccine **Post-Vaccine (wk 9)**



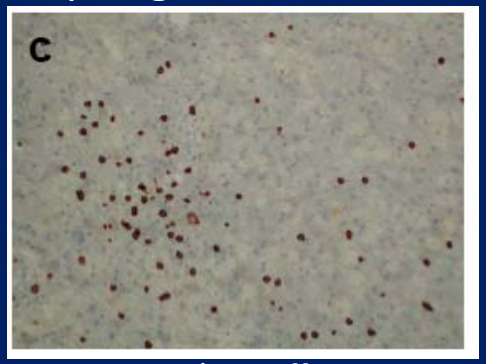
Biopsy demonstrates intratumoral infiltration of macrophages and $CD8^+$ T lymphocytes



Reactive gliosis, possible residual glioma



Numerous $CD68^+$ macrophages



$CD8^+$ T cells

Summary 05-115; Phase I/II Vaccine Study in Adult Recurrent Malignant Glioma (JCO *In Press*)

- The regimen was well tolerated in 22 patients.
- Immune responses against at least one of the vaccination-targeted GAAs were detected in post-vaccine PBMC in 11 of 19 patients.
- Analyses of PBMC demonstrated significant up-regulation of type-1 cytokines and chemokines, including IFN-α and CXCL10.
- Nine (4 GBM, 2 AA, 2 AO and 1 AOA) achieved progression free status lasting at least 12 months. One patient with recurrent GBM demonstrated sustained complete response.
- IL-12 production levels by aDC1 positively correlated with progression-free survival.

Primary vs. Secondary GBM

p53 (17p13) mutation (>65%)

PDGF, FGF2
overexpression (~60%)

Low grade astrocytoma

CDK4 (12q13) amplification
RB (13q13) alteration (~25%)
LOH 19q (~50%)

Anaplastic astrocytoma

PTEN (10q23) loss (~4%)
PDGFR-α amplification (<10%)

Secondary glioblastoma
mean = 45 yrs

EGFR (7p12)
amplification (~40%)
overexpression (~60%)

MDM2 (12q14)
amplification (~8%)
overexpression (~50%)

CDKN2A (9p21) loss (~50%)

PTEN (10q23) loss (~70%)

Primary glioblastoma
de novo
mean = 55 yrs

tif

Contributors

Co-Investigators

Pawel Kalinski – development of alphaDC1

Ryo Ueda, Aki Hoji and Gary Kohanbash – cytokine and tetramer assays

Frank S. Lieberman and Teresa E. Donegan – patient management

Arlan H. Mintz, Johnathan A. Engh, David L. Bartlett, Charles K. Brown, Herbert Zeh, Matthew P. Holtzman and Ian F. Pollack– surgical aspects

Todd A. Reinhart – *in situ* hybridization

Theresa L. Whiteside and Lisa H. Butterfield – immuno-monitoring

Ronald L. Hamilton – neuro-pathology

Douglas M. Potter – biostatistics

Andres M. Salazar - provision of poly-ICLC

Brain Tumor Program

Clinical Research Services

IMCPL of the UPCI

Funding Sources

NIH/NCI and NIH/NINDS

Musella Foundation

Pittsburgh Foundation

The Brain Tumor Society

Participants and their families

I am stopping my talk

- though our work will never stop at any time!
THANK YOU!

A Bi-Institutional Pilot Study of Vaccinations with GAA-peptides in Adult Patients with High-Risk LGG – University of Pittsburgh and Wake Forest University

Primary Objectives – To determine safety and glioma-associated antigen (GAA)-specific immune responses of the regimen.

Rationale –

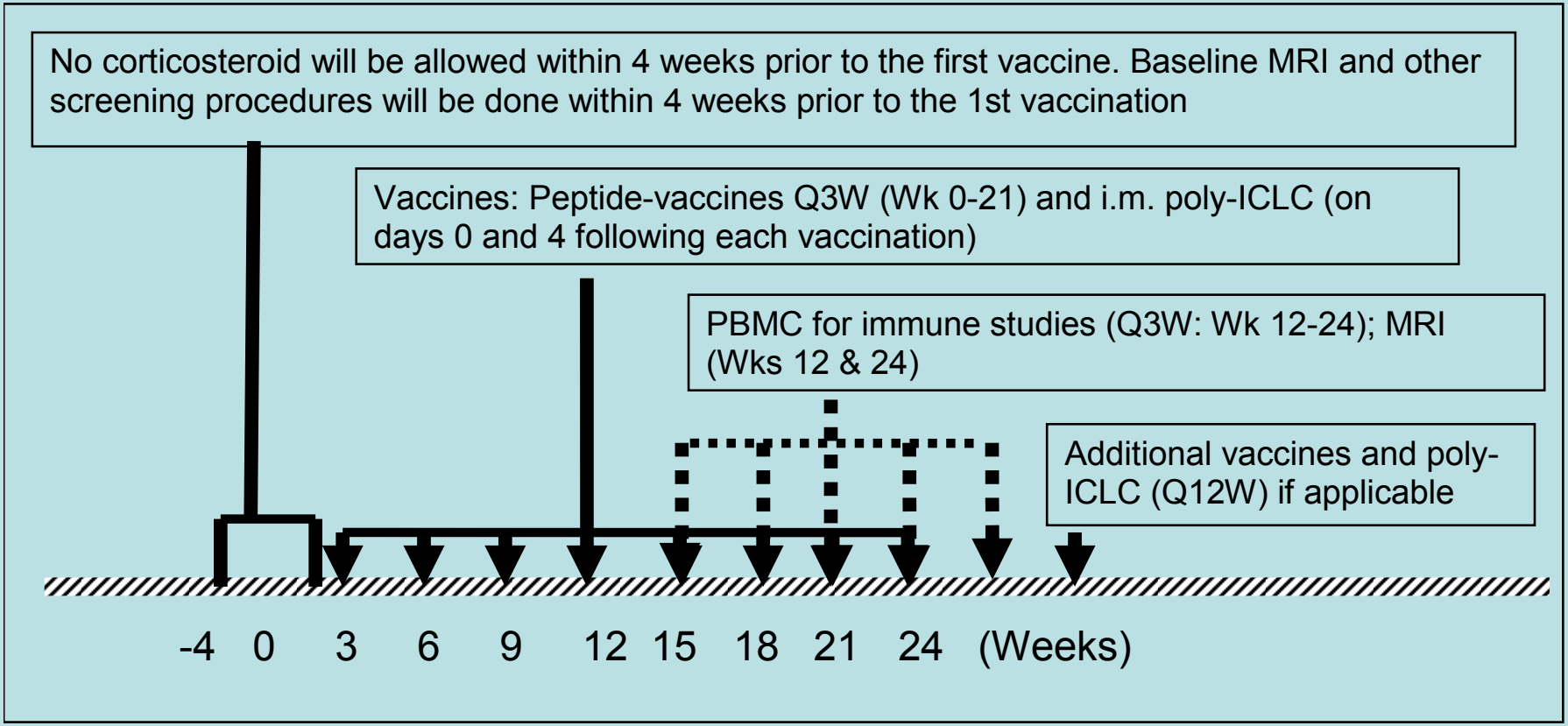
1) The slow growth rate of LGG should allow sufficient time to repeat multiple immunizations, and the induction of high levels of GAA-specific immunity

2) minimal toxicity allows for maintenance of high quality of life

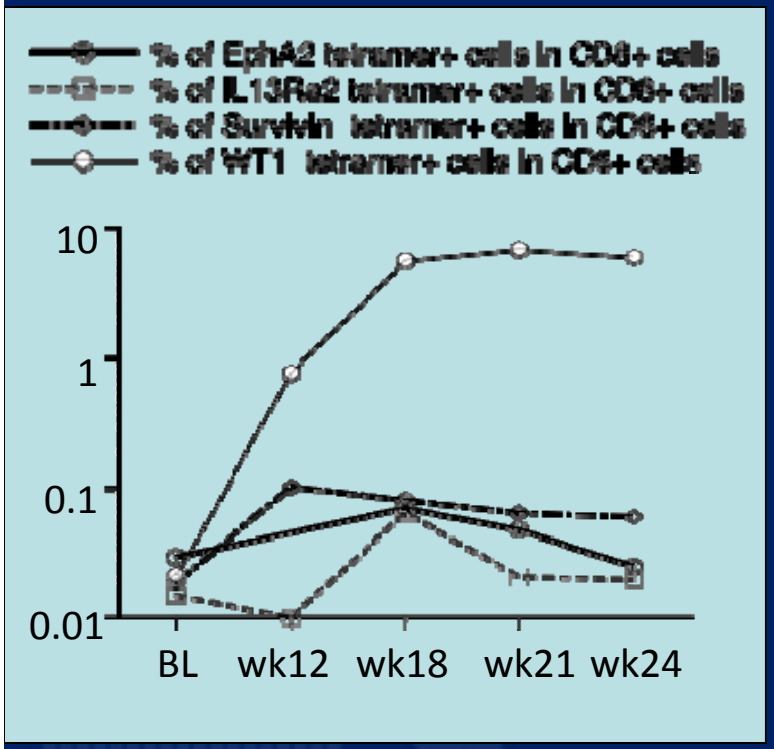
3) SL-701 could delay the use of RT in this patient population

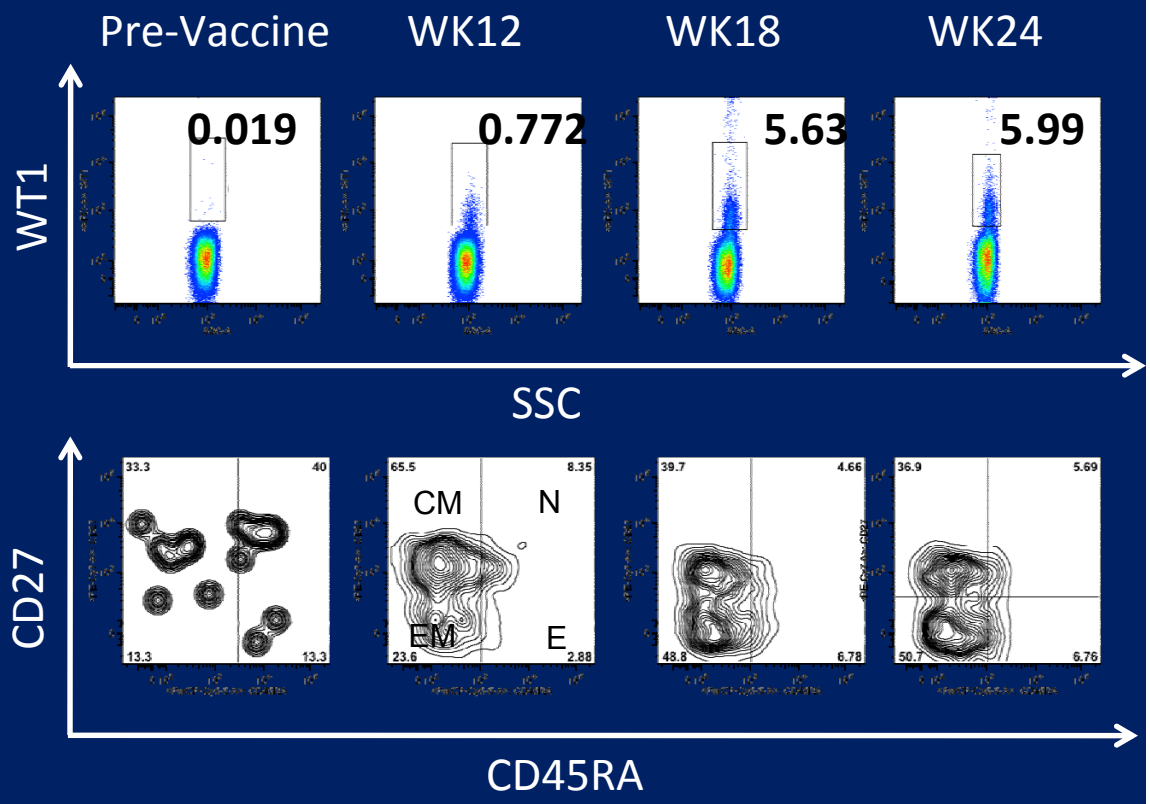
Eligibility - HLA-A2+ adult patients WHO grade II astrocytoma or oligoastrocytoma with "high-risk" factors - defined as at least one of the following conditions: 1) age ≥ 40 with any extent resection
2) age 18-39 with incomplete resection (post-op MRI showing >1cm residual disease) or
3) the tumor size is ≥ 4 cm

Treatment Schema

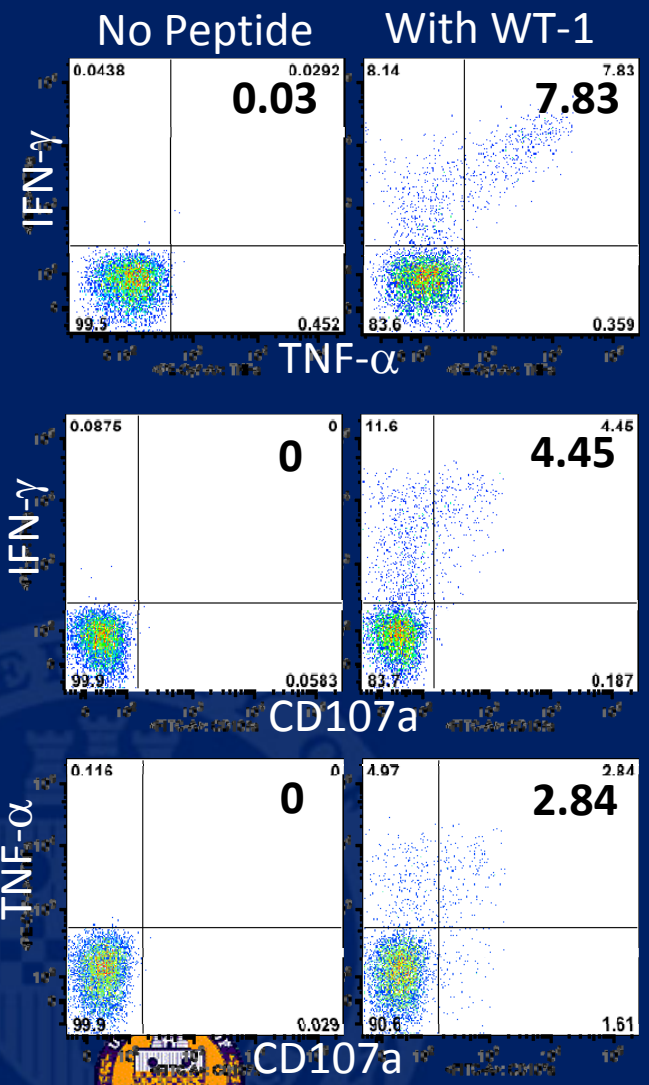


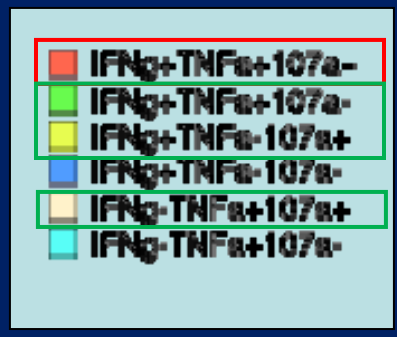
Robust Induction of GAA–Specific CD8+ cell Response in a Subject with WHO Grade 2 Low Grade Glioma





Type-1 effector function of CD8+ T cells in response to brief *ex vivo* stimulation with the WT-1 peptide





Acknowledgement

Co-Investigators

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Key External Collaborators

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John H. Ohlfest (U. Minnesota)
Andres M. Salazar (Oncovir, Inc)

Brain Tumor Program
Clinical Research Services
IMCPL
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The Brain Tumor Society

Participants and their families

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Ana Taffel, BS
Nikki Urban, RN

Streets around Univ. Pittsburgh

University of Pittsburgh Medical Center
University of Pittsburgh Cancer Institute

Central Nervous System (CNS) immunology

<u>Concept of CNS as immunologically privileged</u>

1. Blood Brain Barrier/No lymphatic system
2. No dendritic cell (DC) distribution
3. Roles of microglia/brain macrophages as antigen-presenting cells (APC) are not clear

Central Nervous System (CNS) immunology
- "Privileged" status has been revised -

- Autoimmune diseases in the CNS

  (Experimental Autoimmune Encephalitis , Multiple Sclerosis, Paraneoplastic Cerebellar Degeneration [PCD] against cdr2)
- A-beta1-40/42 formulated in QS-21 as a vaccine for Alzheimer's disease- a few treated patients developed signs of aseptic encephalitis/meningitis following the second administration of the vaccine.

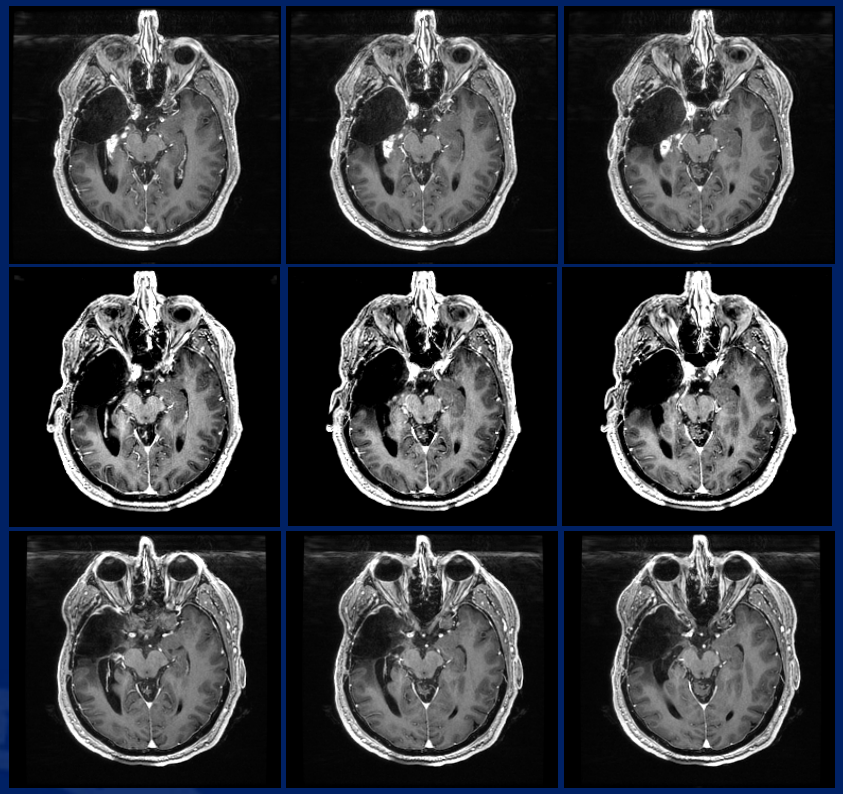
Figure 4

A

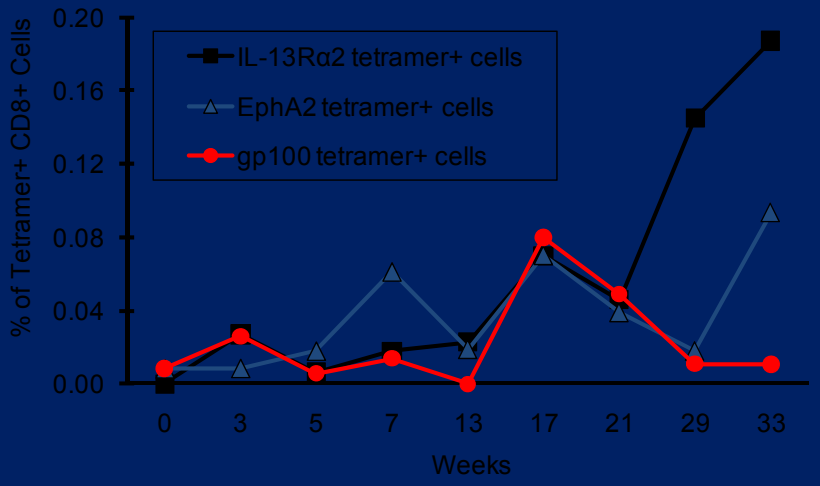
Week 0

Week 17

Week 33



B



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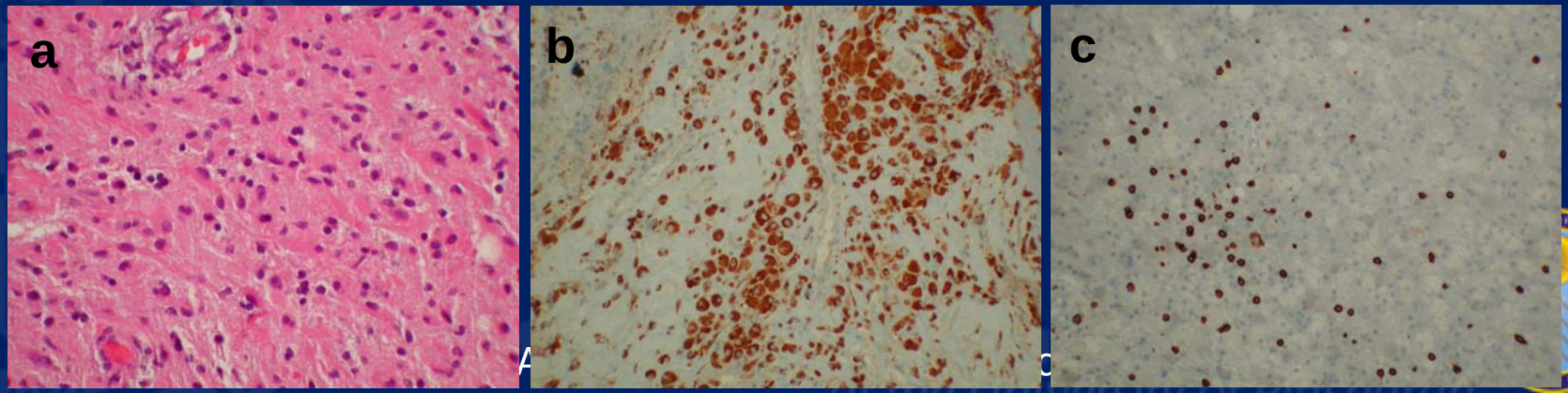
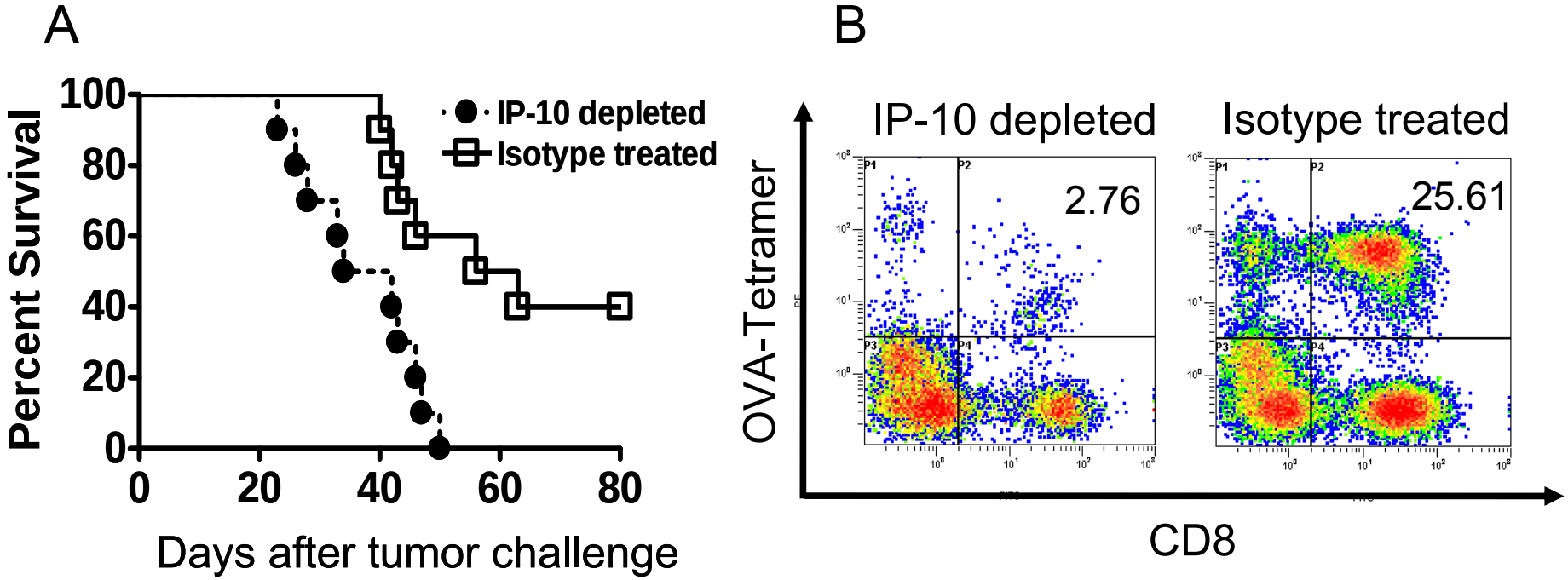


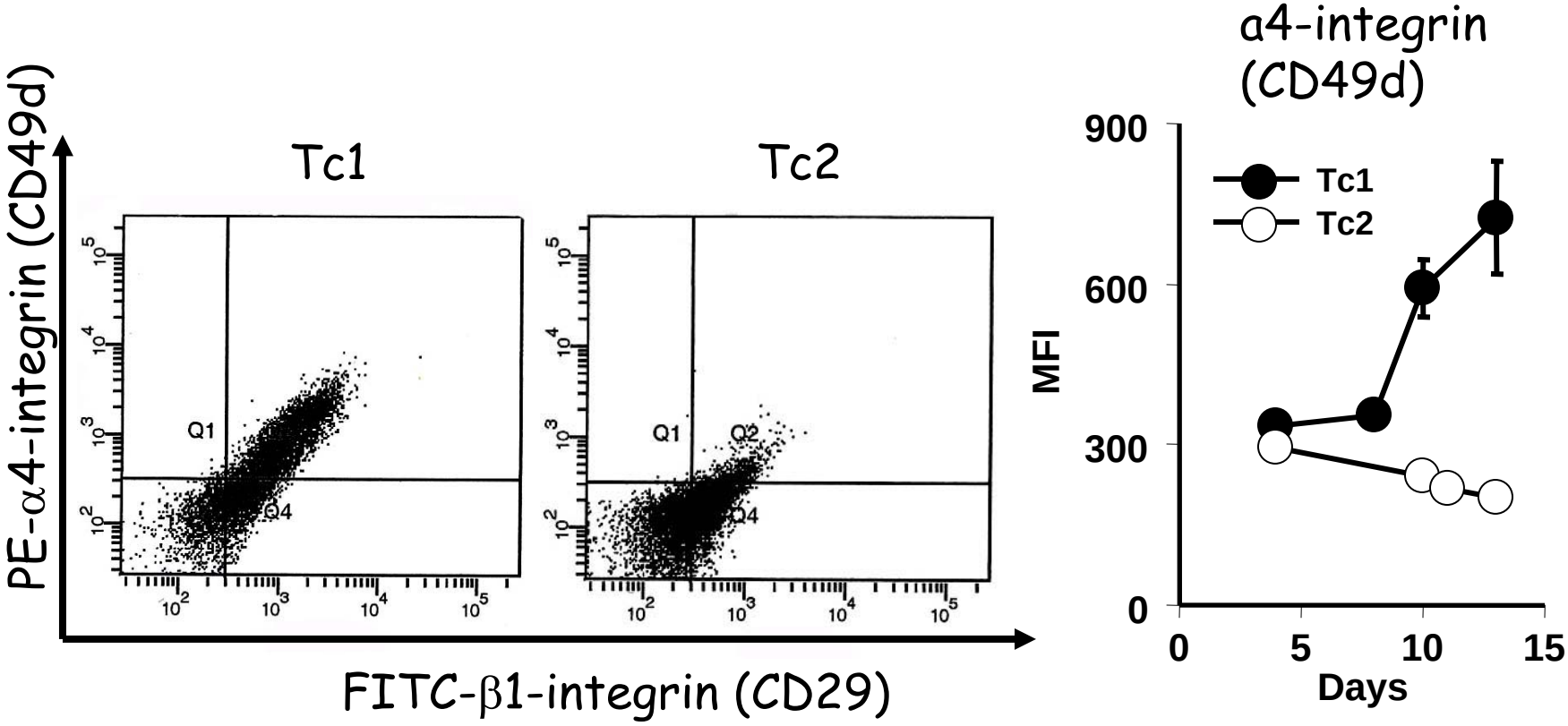
IFN-inducible protein (IP)-10/CXCL10 plays a critical role in the recruitment of Tc1 cells to the CNS tumor site



Nishimura F. *et al.* Cancer Res. 2006 66(8):4478-87

IP-10 deficient mice bearing day 7 i.c. M05 received i.v. adoptive transfer of 3 x 10^6 Tc1 cells and anti-IP-10 i.p. (250 μ g on day 6 and 100 μ g on days 7, 8, and 9). On the same day (day 7), 50 μ g anti-IP-10 was i.t. co-injected with 1 x 10^5 DC-IFN-α. BILs were harvested 3 days after adoptive transfer

High level expression of VLA-4 (heterodimer of α4-integrin [CD49d] and β1-integrin [CD29]) on Tc1



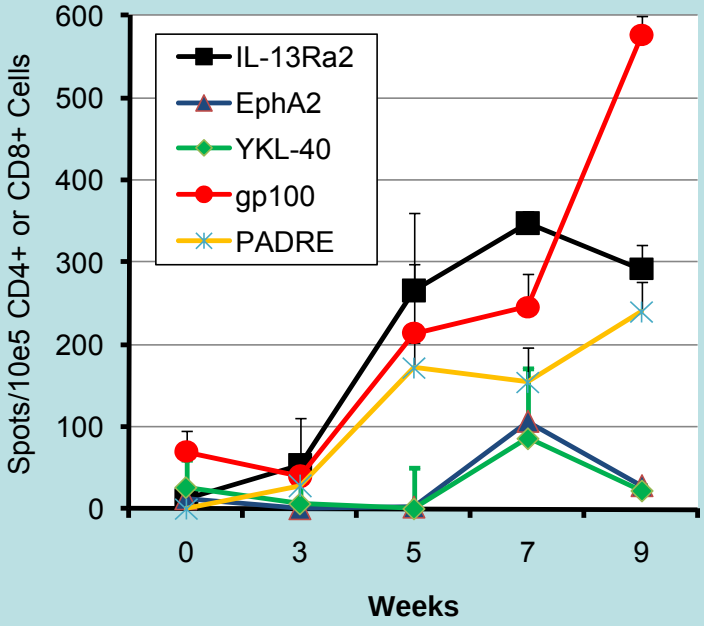
Sasaki et al. Cancer Res, *2007*

Pittsburgh Night View

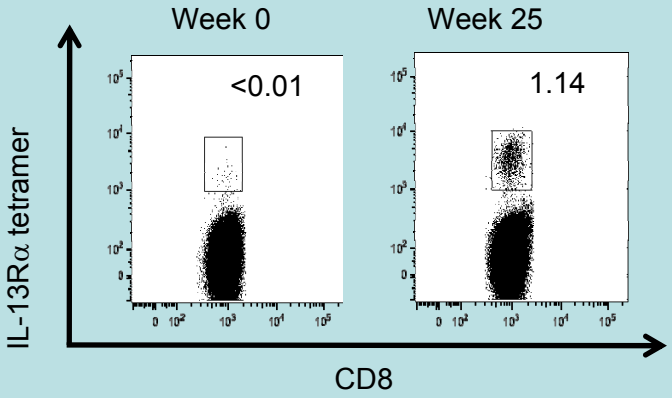
Pittsburgh from Mt. Washiongton

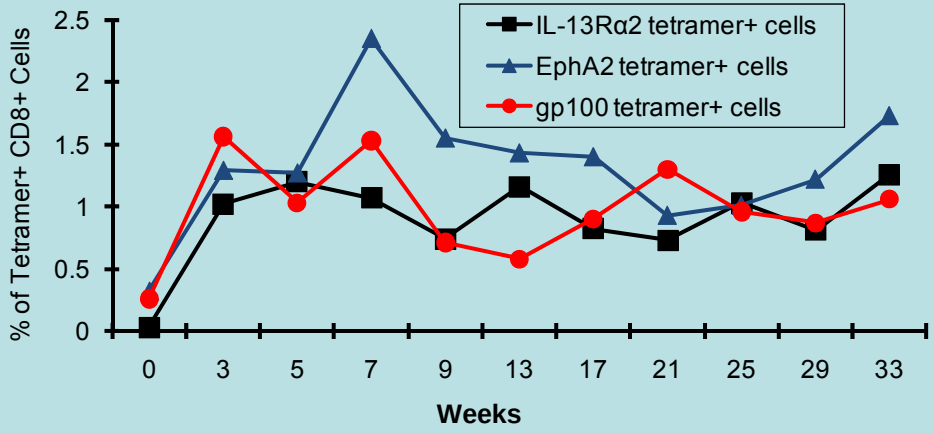
Representative ELISPOT and Tetramer Responders

Patient 10 (GBM)

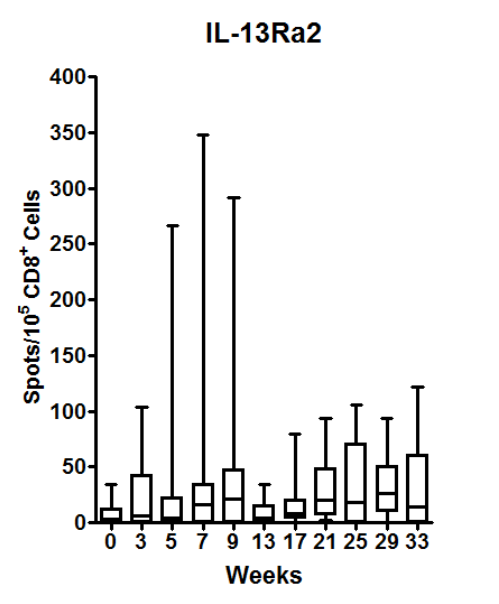


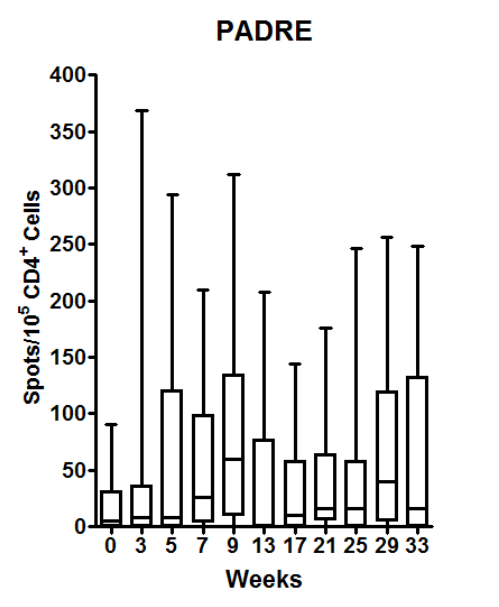
Patient 6 (AOA)

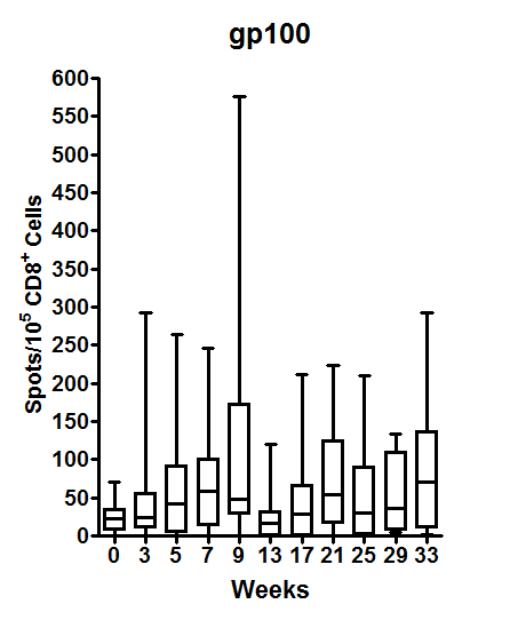


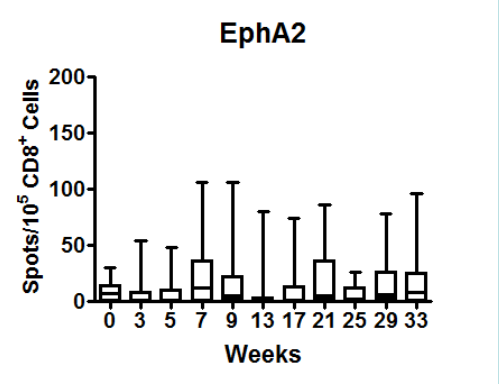


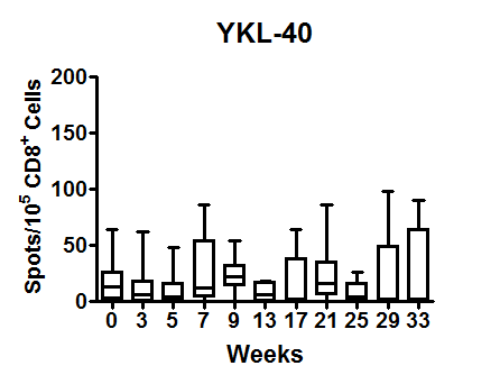
Time course for IFN-γ ESLIPOT assays for all evaluated patients with box plots





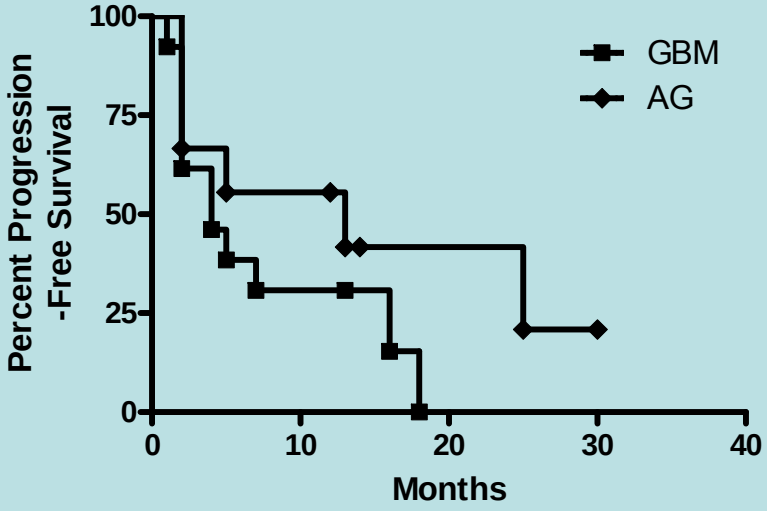




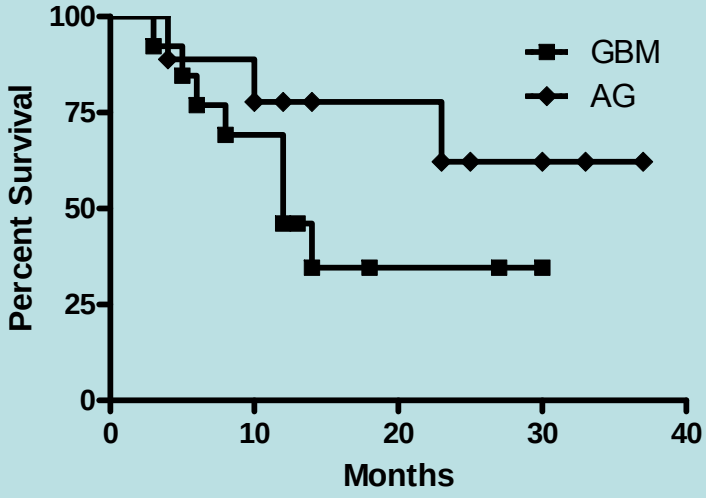


TTP and OS for Each of the Two Tumor Grades (WHO Grade 3 vs. 4)

Time to Progression (TTP)



Overall Survival (OS)



Median TTP
4 months for GBM
13 months for AG

Median OS
12 months for GBM
Undefined for AG (as 5 of 9 patients are still alive with the median follow-up period for 23 months)

	Pt ID	Age/ Gender	Tumor Histol.	Location of Tumor	Prior Therapy	No. Prev Rec.	DC IL-12 (pg)	ELISPOT					Tetramer			RR at Week 9	TTP (Mo)	OS (Mo)
								I	E	Y	G	Pa	I	E	G			
Dose Level 1 (1 x 10^7 DC/dose)	1	57/M	GBM	Rt. Temp/Pa	Res/RT/TMZ/ Mol	1	10					@	P	P	P	PR	7	14
	2	52/M	GBM	Rt. Temporal	Res/RT/TMZ	1	25					@	P	P	P	PD	<2	12
	3	7/M	AA	Rt. Parietal	Resx2/RT/ TMZ/Mol	2	25						N	N	N	SD	5	10
	4	8/	A	Rt. Frontal	SB/RT/TMZ	0	<10	Not Tested					Not Tested			ND*	<2	>35
	5	63/M	GBM	Rt. Parietal	SB/RT/TMZ	0	26						N	N	N	PD	<2	5
	6	5 /M	AC	Lt. Temporal	SB/RT/TMZ	0	27	§		§			P	P	P	SD	>28	>28
	7	37/F	AA	Rt. Temporal	SB/RT/TMZ	0	919	§			§		P	P	P	SD	25	>31
	8	45/F	GBM	Rt. Frontal	SB/RT/TMZ/ Mol	0	480	§		§	§		P§	P§	N	SD	16	>29
	9	43/F	AO	Rt. Frontal	Res/RT/TMZ/ SR	0	24			@			N	N	N	PD	<2	>25
	10	71/F	GBM	Lt. Parietal	Resx2/RT/ TMZ/CE	2	<10		@	@			N	N	N	SD	5	>25
	14	4 /M	GBM	Lt. Front/Temp	Res/RT/TMZ	0	551		@	@	@		N	N	N	SD	>16	>16
Dose Level 2 (3 x 10^7 DC/dose)	11	54/M	GBM	Rt. Temporal	Res/RT/TMZ/ Mol	2	38	Not Tested					Not Tested			ND*	<2	3
	12	3 /F	AO	Lt. Frontal	SB/RT/TMZ	0	111	§	§	§			P§	N	N	SD	13	>23
	13	46/M	AA	Rt. Parietal	Res/RT/TMZ	1	151	Not Tested					Not Tested			ND*	<2	4
	15	1/M	GBM	Multiple	Res/RT/TMZ	0	35				@		N	N	N	SD	4	>15
	16	33/M	AO	Rt. Frontal	Res/RT/ TMZ/Mol	2	985						N	P§	P§	SD	>12	>12
	17	30/F	GBM	Lt. Parietal	Resx2/RT/ TMZ/CW	1	123	@		@			Not Tested			PD	<2	6
	18	61/F	GBM	Bil. Occipital	Res/RT/TMZ/ BI	1	125						N	N	N	PD	<2	5
	19	63/M	GBM	Lt. Temporal	Res/RT/TMZ/ SR	1	199						P§	P§	P	SD	>11	>11
	20	62/M	GBM	Rt. Temporal	Res/RT/TMZ	0	287						P§	P§	P§	PR	>11	>11
	21	38/F	GBM	Rt. Hemi	Res/RT/TMZ/ CPT-Bev	1	27			@			Not Tested			PD	<2	>11
	22	28/M	AA	Brain Stem	SB/RT/TMZ/ Res	0	779	P					Not Tested			SD	>9	>9

Please, do not read — I will summarize these findings for you —

Poly-ICLC as the Key Adjuvant

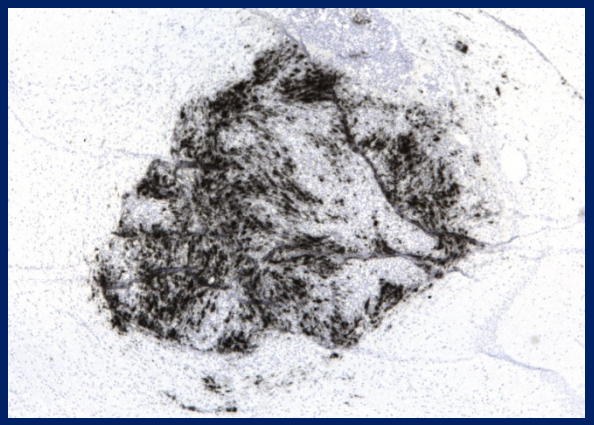
- Polyinosinic-polycytidylic acid (poly-IC) stabilized with poly-lysine and carboxymethylcellulose (Poly-ICLC), has been used as a single agent or combo with TMZ for treatment of malignant glioma (provided by Dr. Andres Salazar [Oncovir]).
- Poly-ICLC stimulates TLR3 RIG-I and MDA-5
- Among the TLRs, TLR3 is most abundantly expressed in the CNS.

Demographics and Clinical Characteristics of Participating Patients

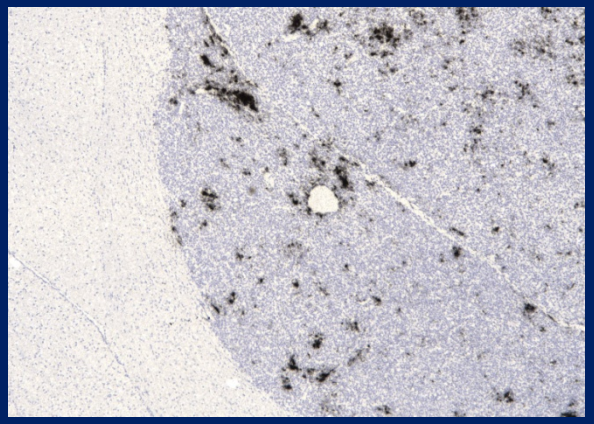
Characteristics	DC Dose Level (No. of DC/dose) 1 (1 x 10^7)	2 (3 x 10^7)	Total (n=22) No. of Patients	%
Received at least one vaccine	11	11	22	
Completed at least 4 vaccines	10	9	19	86
Female (received at least 4 vaccines)	5	4	9	47
Median age, years	52	46	48	
Range	37-71	28-63		28-71
Tumor Histology				
AA	3	2	5	23
AO	1	2	3	14
AOA	1	0	1	4
GBM	6	7	13	59
No. of Previous Recurrences				
0	7	4	11	50
1	2	5	7	32
2	2	2	4	18

Upregulated expression of CXCL10 mRNA in murine GL261 glioma treated with GAA-vaccines and i.m. poly-ICLC (*In situ* hybridization)

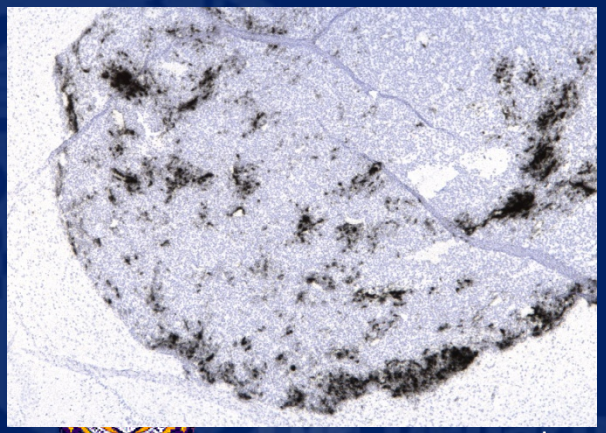
A Vaccine Plus Poly-ICLC



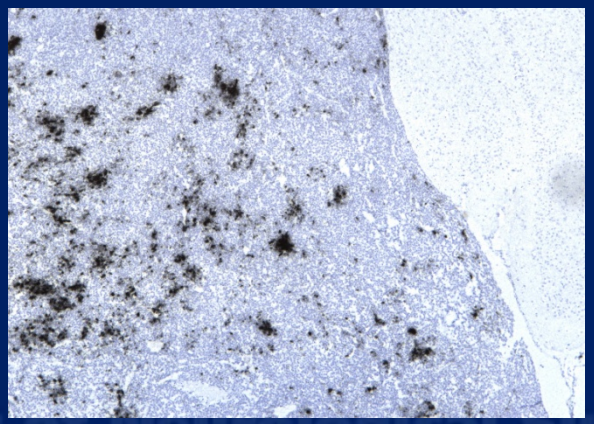
B Vaccine Alone



C Poly-ICLC Alone

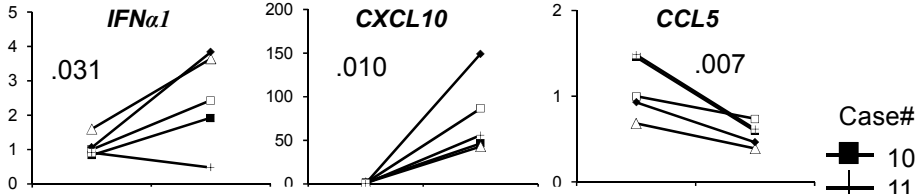


D Mock-Treatment

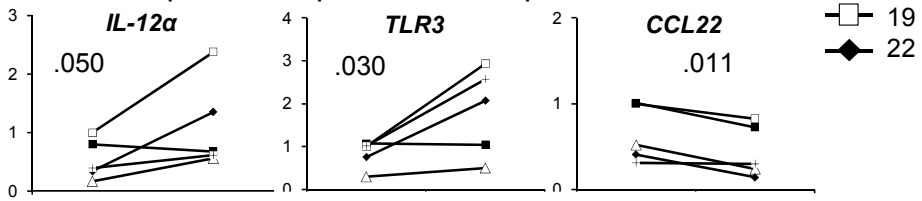


Zhu X. *Et al* Cancer Immunol. Immunother. 2010

RT-PCR (Pre-vac vs. 24-hr Post 1st Vac

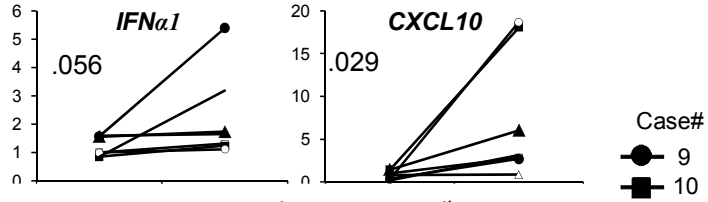


Comparison of pre- vs. 24-hr post 1st vaccine

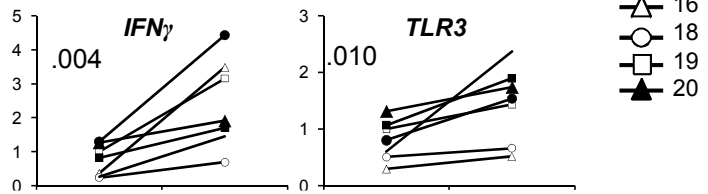


Comparison of pre- vs. 24-hr post 1st vaccine

RT-PCR (Pre-vac vs Post-4th Vac)

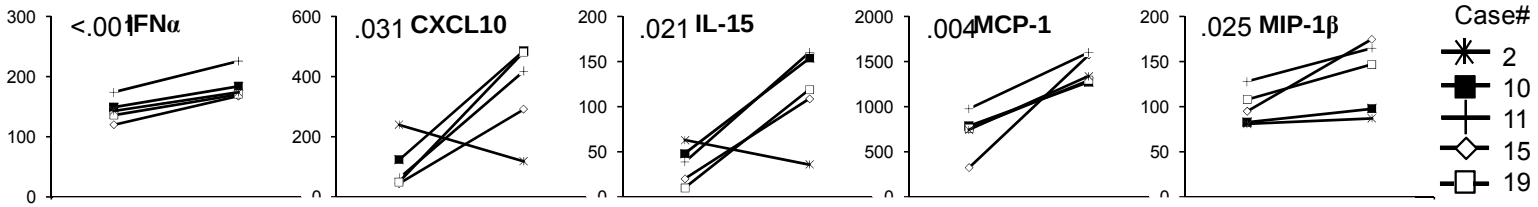


Comparison of pre-1st vs. post-4th vaccine



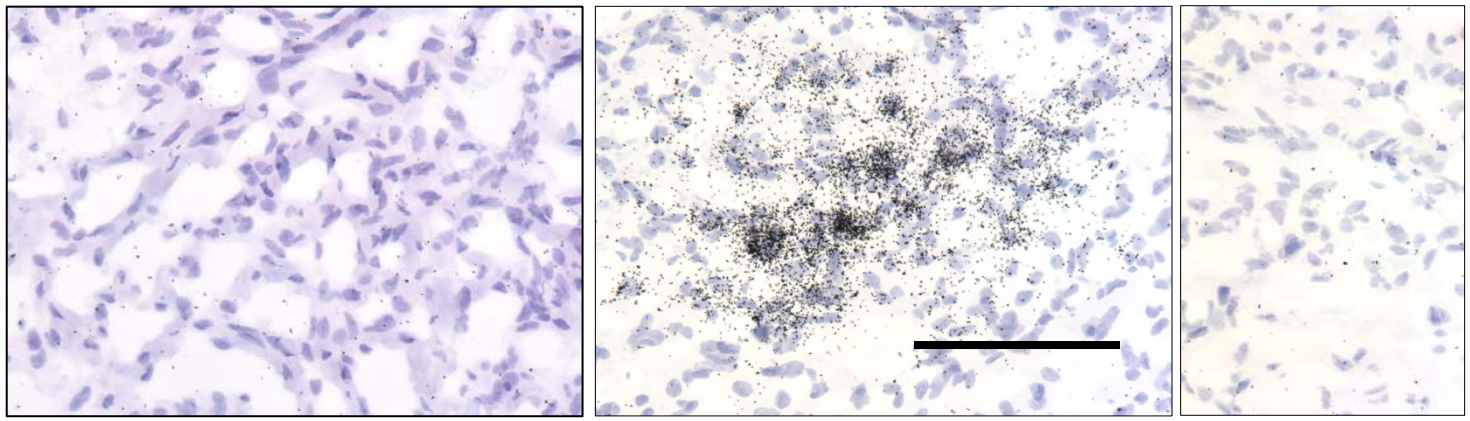
Comparison of pre-1st vs. post-4th vaccine

C Luminex



Comparison of pre-vaccine vs. post 1st vaccine (pg/ml)

D *In Situ Hybridization*



CXCL10 (Pre-Vaccine) CXCL10 (Post-Vaccine) Sense control