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

Okada et al

Data Supplement

Study Design Parameters

The design parameters to evaluate the primary endpoints are as follows.

For safety, as detailed in "Patients and Methods" section, stopping rules were implemented such that a dose level was considered to be excessively toxic, warranting that accrual be halted, if at any time the observed rate of DLT was ≥ 33% and at least 2 DLTs had been observed. With 9 or 10 patients/dose, the study design has the following properties: if the true rate of DLT in this patient population is ≥ 52% at a dose level, there is at least 95% probability that accrual will stop (and that the level will be considered excessively toxic); if the true DLT rate is ≤ 9%, there is 90% probability that the accrual will not stop (and that the level will be considered safe).

For immune response, we considered a dose level would be worthy of further investigation if the response rate is at least 40%. With 10 patients/dose, the criterion has the properties that if the true response rate is < 15%, there is < 5% probability to observe 4 or more responses, and that if the true response rate is >60%, there is <5% probability to observe 3 or fewer responses.

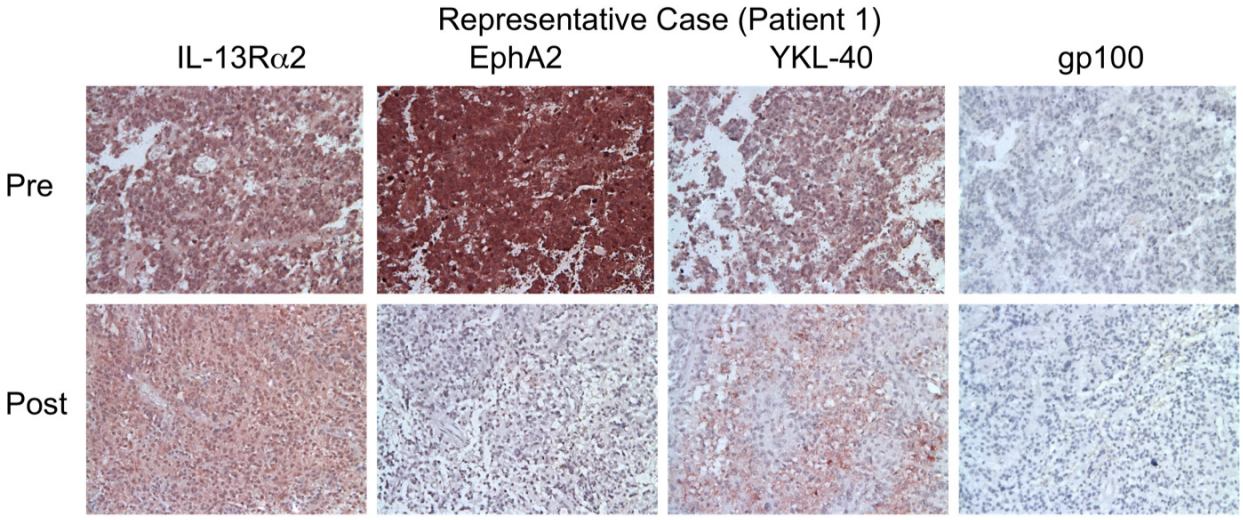
Data Supplement Table DS1. Adverse Events (N=22)

	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		

All AE listed were possibly, probably, or definitely related to the vaccine and/or poly ICLC administration. The numbers represent the number of patients (of 22) experiencing a particular event at any point during the treatment period, with the highest grade reported for any single individual. No grade 3 or grade 4 events observed related to treatment through the 1st booster phase. One patient (Patient 6) demonstrated grade 2 systemic urticaria following the 154th injection of poly-ICLC during the 2nd booster phase; this was considered to be DLT. However, because the relationship was unclear and the patient was progression-free for 22 months by that time, per IRB approval, the patient was re-treated with booster vaccines and poly-ICLC following pre-medication with oral diphenhydramine hydrochloride, and has never demonstrated similar reactions again.

Data Supplement Table DS2. Expression of GAAs targeted by the vaccines

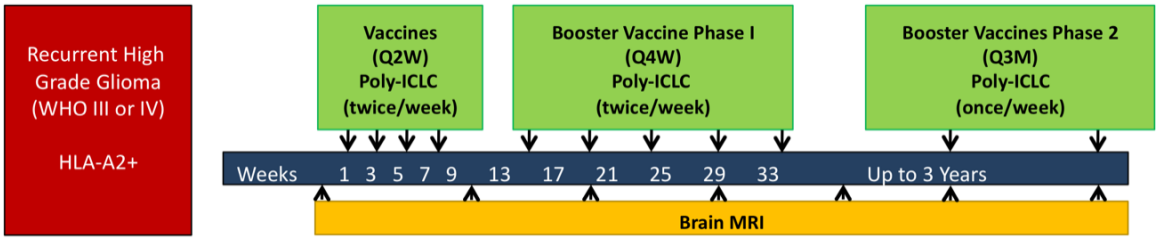
Case #	Pre vs. Post vaccine	IL-13Rα2	EphA2	YKL-40	gp100
1 (GBM)	Pre	2	3	2	0
	Post	2	1	2	0
2 (GBM)	Pre	1	2	1	0
5 (GBM)	Post	1	2	2	1
9 (AO)	Pre	1	2	1	0
	Post	1	1	1	0
10 (GBM)	Post	2	1	2	0
12 (AO)	Pre	2	2	1	1
14 (GBM)	Pre	3	2	2	0



For immunohistocemistry, the following polyclonal antibodies (Ab) and corresponding secondary Ab were used: anti-human(h)IL-13Rα2 (goat IgG; R&D Systems); anti-human EphA2 (H-77) (rabbit IgG; Santa Cruz Biotechnology); anti-human YKL-40 (rabbit IgG; Quidel) and anti-human gp100 (goat IgG; Santa Cruz Biotechnology).

Expression of each GAA was graded as follows: grade 0, negative; 1, weakly positive; 2, moderately positive; 3, strongly positive. Numbers in red font indicate that the patient demonstrated positive ELISPOT or tetramer response against the antigen. Pictures show a representative case (Patient 1). The upper and lower panels show pre- and post-vaccine tissues, respectively. Original magnification was ×20. Please note that pre-vaccine does not mean that tumor tissues were obtained immediately before the vaccination, but obtained at variable time points before the vaccine, including the initial diagnostic biopsy or resection. Likewise, post-vaccine tissues were obtained at variable time points following the last vaccine because re-resection was not always indicated.

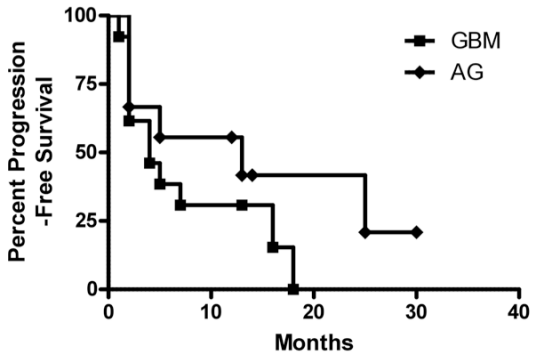
Data Supplement Figure DS1. Flow diagram for the trial



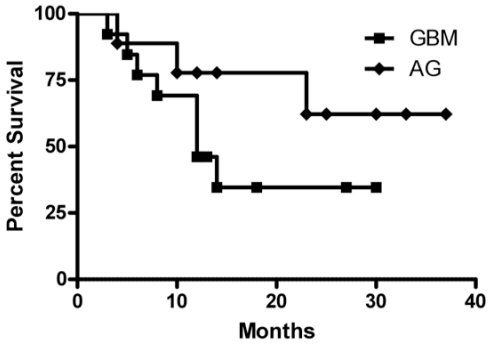
See Patients and Methods section for details of treatment. The second phase of booster vaccines could start any time after Week 37 and administered every 3 months up to 3 years from the first vaccine, unless patients demonstrated major AE or disease progression. The αDC1 vaccines were administered using ultrasound to inguinal lymph nodes (right and left for the first and second vaccines, respectively) and axillary lymph nodes (right and left for the third and forth vaccines, respectively). The site was rotated in the same order for booster vaccines to minimize the potential effects of injection-induced trauma in the microenvironment of the lymph nodes by repeating injections in short periods of time.

Data Supplement Figure DS2.

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)