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Research Article

Enhanced Progression-Free Survival in a Phase 2 Trial of Personal Dendritic Cell Vaccines in Patients with Newly Diagnosed Glioblastoma

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Abstract

Standard therapy of aggressive glioblastoma (GBM), which includes maximum safe surgical resection and concurrent radiation/temozolomide (RT/TMZ) followed by maintenance TMZ, is associated with poor progression-free survival (PFS) and overall survival (OS). Adding an immunotherapy such as AV-GBM-1, a personal vaccine consisting of autologous dendritic cells (DC) pulsed with autologous tumor antigens (ATA) from self-renewing tumor cells, may improve PFS and OS. A multi-center 60-patient phase 2 trial demonstrated feasibility and safety of AV-GBM-1 and an encouraging 10.4 months median PFS, which is about 50% longer than observed in numerous previous trials conducted in similar patients. Age, performance status, *MGMT* promoter methylation and *IDH* mutation were still important prognostic stratifications for AV-GBM-1 therapy. The average OS/PFS ratio was 2.25 (range 2.1 to 3.0) for 10 randomized-trial treatment arms not containing bevacizumab, and defining PFS and OS from enrollment prior to starting RT/TMZ, in patients with newly diagnosed GBM; the ratio for AV-GBM-1 was only 1.6. Concurrent bevacizumab and/or tumor treating fields did not account for the increased PFS observed with AV-GBM-1. Median PFS and OS were reached two and six months respectively after the last vaccine injections, suggesting there might be an advantage for treating longer than eight months from enrollment.

Keywords: Glioblastoma; Dendritic cell vaccine; Personal vaccine; Neoantigens; Autologous tumor antigens; Phase 2; Progression-free survival.

Introduction

In a landmark randomized trial that established the standard aggressive therapeutic approach for newly diagnosed glioblastoma (GBM), patients who underwent a maximum safe surgical section, then a treatment plan of concurrent radiation therapy (RT) and temozolomide (TMZ) chemotherapy, followed by six four-weekly cycles of additional temozolomide, had a median progression-free survival (PFS) of 6.9 months and overall survival (OS) of 14.6 months from enrollment, which took place just prior to starting RT/TM [1]. This study also established that methylation which silences the O6-methyl-DNA methylguanine transferase (*MGMT*) gene promoter is associated with better OS and a better response to TMZ chemotherapy [2]. Since then, several large randomized trials in patients with newly diagnosed GBM have failed to identify an approach that enhances survival beyond that associated with this standard approach. This has included the addition of the anti-vascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab [3,4], the small molecule angiogenesis-inhibitor cilengitide [5], the anti-EGFRvIII monoclonal antibody chemoimmunojugate depatuxizumab mafodotin [6], the anti-programmed death-1 monoclonal antibody nivolumab [7], and the small molecule proteozome inhibitor marizomib [8]. Large randomized trials that failed to identify improved survival after recovery following RT/TMZ, included the addition of dose-dense TMZ administration [9], rindopepimut, a mutated epidermal growth factor receptor (EGFR)vIII mutated peptide linked to keyhole lymphocyte hemocyanin [10], and the dendritic cell-autologous tumor antigen vaccine DCVax-L [11]. The only positive randomized trial in such patients tested the addition of electrodes to the shaved scalps of patients as part of tumor treating fields (TTF), which utilizes alternating low-frequency electrical fields that affect cells [12]. Of note, the two studies with the longest OS in the post RT/TMZ setting excluded patients who had evidence of progressive disease (PD) or pseudoprogression (PsPD) after recovery from RT/TMZ [11,12], which represents about 20% of patients. Thus, there continues to be an unmet need for a therapy that can be added to standard treatment for all patients, that does not add to toxicity, and that is associated with improved survival [13].

The dendritic cell vaccine AV-GBM-1 is a personal dendritc cell-autologous tumor cell (DC-ATA) vaccine that may improve survival in GBM patients by inducing or enhancing anti-tumor immune responses. This product consists of autologous dendritic cells (DC) that are loaded with autologous tumor antigens (ATA) from a lysate of irradiated autologous tumor cells that have been self-renewing in a short-term tissue culture. Similar personal DC products have been associated with minimal toxicity and suggestion of efficacy in patients with metastatic melanoma [14-22], metastatic renal cell cancer [23], and locally advanced hepatocellular cancer [24]. In all of these trials the source of antigen was irradiated autologous tumor cells that were self-renewing in cell culture [25,26]. AV-GBM-1 differs from two other autologous antigen-loaded autologous DC vaccines tested in GBM, namely DCVax-L [11,27], and audencel [28], in that AV-GBM-1 is loaded with a lysate of irradiated self-renewing autologous tumor cells, rather than a whole-tumor lysate. This approach enriches for neoantigens that are present in tumor initiating cells, including tumor stem cells and early progenitor cells that have a proliferative advantage in cell culture. Furthermore, this approach minimizes exposure to antigens from non-tumor cells (hematopoietic, normal glial, stromal, and immune cells) that are contained in a lysate of whole tumor. In addition, AV-GBM-1 is admixed with granulocyte-macrophage colony stimulating factor (GM-CSF) at the time of injection, which may enhance survival and migration of DC-ATA after subcutaneous injection [29].

As reported elsewhere, in a single arm phase 2 trial conducted in patients with a new diagnosis of GBM and no prior diagnosis of lower grade glioma, short-term cell lines were successfully estabished for 97% of patients, a satisfactory peripheral blood mononuclear cell (PBMC) product was obtained for 97% of patients, no SAE or grade 3 or 4 AE were attributed to the vaccine during treatment with up to 8 subcutaneous injections over six months; median OS was 16.0 months and median PFS 10.4 months from the date of enrollment just prior to initiating standard concurrent RT/TMZ [30-32]. The current article focuses on the encouraging PFS observed in this trial and in subsets of patients defined by age, Karnofsky performance status (KPS), O6-methyl-DNA methylguanine transferase (*MGMT*) gene promoter methylation status, and *IDH* mutations in the isocitrate dehydrogenase (*IDH*) gene, on controversies surrounding the definition of PFS, on correlations between PFS with OS in various clinical trials, and on the association between PFS and OS and the duration of vaccine therapy in the current trial.

Methods

The single-arm phase 2 trial was performed as detailed elsewhere [30-32]. This study was registered at clinicaltrials.gov (NCT03400917) and was conducted according to the Declaration of Helsinki and per Good Clinical Practice. The clinical protocol was approved by an investigational review board at each clinical site. All patients provided written informed consent prior to participation. Key eligibility criteria were (1) new diagnosis of GBM without a prior diagnosis of lower grade glioma, (2) age 70 years or less at the time of surgery, (3) KPS of 70 or higher at the time of intent-to-treat enrollment prior to RT/TMZ, (4) availability of a successful short-term cell line for production of an

autologous tumor cell lysate, (5) collection of a sufficient number of monocytes to differentiate into autologous DC to create the DC-ATA vaccine, and plans to proceed with concurrent RT/TMZ. The vaccine was manufactured while the patient was undergoing RT/TMZ. After recovery from RT/TMZ, patients received three weekly subcutaneous vaccine injections and then were dosed every four weeks concurrently with TMZ for up to a total of eight doses. Thus, the vaccine was administered over six months from the start of therapy, which was about eight months from the time of enrollment.

The manufacturing of DC-ATA has been described previously [15,16,18,19]. Briefly, a short-term cell culture is established from resected tumor and a leukapheresis procedure is performed to collect peripheral blood mononuclear cells (PBMC) that are differentiated into DC by culturing monocytes in the presence of GM-CSF and interleukin-4 (IL-4) for six days. The DC are then incubated with ATA for 18 to 24 hours to load antigen. In previous trials DC-ATA were manufactured by incubating DC with intact irradiated tumor cells. For the current trial AV-GBM-1 was manufactured by incubating DC with the cryopreserved lysate of irradiated tumor cells.

Survival was measured from the date of intent-to-treat enrollment just prior to initiation of RT/TMZ, and also from the date of first vaccine injection after recovery from RT/TMZ. PFS was determined by the earlier of the date of death or date of PD as defined by the managing physician. For reasons that are discussed in detail later in this paper, specific intervals for magnetic resonance imaging (MRI) scans, and a specific definition of PD were not prescribed in this protocol, and there was no central review of MRI scans. Rather PD was defined by the neuro-oncology principal investigators and/or the patient's managing physician on the basis of clinical assessment and MRI scans per standards of care [33]. For purposes of the current study, PFS was analyzed in subsets of patients defined by age 60-69 years *vs* less than 60, KPS 90 or 100 *vs* 70 or 80, methylation of the *MGMT* gene promoter *vs* unmethylated, and *IDH* gene mutation *vs* wildtype.

For historical comparisons, randomized phase 3 trials conducted in patients with newly diagnosed GBM were identified from PubMed.gov, ClinicalTrials.gov, and Google Search. One randomized phase 2 trial also was included for comparisons because it also tested an autologous DC-ATA vaccine [28]. In addition to the median PFS for all patients, several trials also provided median PFS for cohorts defined by *MGMT* promoter methylation. Because of data showing that PFS is prolonged in association with the addition of bevacizumab [3,4], or TTF [12], comparisons were made by cohorts defined by treatments that were given concurrently with AV-GBM-1, including no other treatment, TMZ alone, TMZ with bevacizumab and TMZ with TTF. PFS for AV-GBM-1 was compared to PFS reported in publication of previous trials for newly diagnosed GBM patients with careful attention to the definition of the starting timepoint used for calculating PFS and OS. The ratios of the medians of PFS and OS were determined by study arms of various trials.

The relationship between PFS and OS was expressed by the ratio of OS/PFS. Kaplan-Meier survival curves were generated using GraphPad Prism 9. Survival curves were compared by an unadjusted Mantle-Cox log rank test.

Results

Details regarding manufacturing feasibility, patient characteristics, adverse events, and OS are published elsewhere [30-32]. 60 patients were enrolled prior to starting RT/TMZ with the intent-to-treat after recovery from RT/TMZ; 57 patients received at least one dose of vaccine after recovery from RT/TMZ. Vaccine injections were well-tolerated and no patients discontinued AV-GBM-1 because of toxicity [30-32]. Patients received a median of 6.9 doses over 6 months and 68% received all eight planned doses. [30-32]. Individual patients were injected with about the same number of cells on each occasion, but as in previous trials in other malignancies, interpatient variation ranged from one to twenty-six million cells per dose. The first dose was given about two months after enrollment and the eigth dose was given about eight months after enrollment.

PFS was 10.4 months from date of enrollment prior to concurrent RT/TMZ (95% CI 8.7-11.8), and 8.5 months (95% CI 6.5-9.1) from the date of first injection after recovery from RT/TMZ [30-32]. Figure 1 is a Kaplan-Meier plot of PFS for all 60 patients with follow up to March 2022, including three who withdrew prior to receiving any vaccine injections. Those three were censored at the time they were last known to be alive; one had already experienced PD and is included in the ITT analysis from the date of enrollment prior to starting RT/TMZ. Of the remaining 57 patients, as of the end of March 2022 two were still progression-free after completing 36 months of follow up, and 6 were still progression-free when censored between 25 and 28 months from enrollment. For purposes of defining PFS, only four patients had PD declared based on death dates at 1.7, 4.2, 5.1 and 5.6 months; the other 45 had a PD designation prior to death. The longest survival in a patient who had PD, was a patient declared to have PD at 3.7 months, but still alive and in long-term follow at 22.8 months, 19.1 months later.

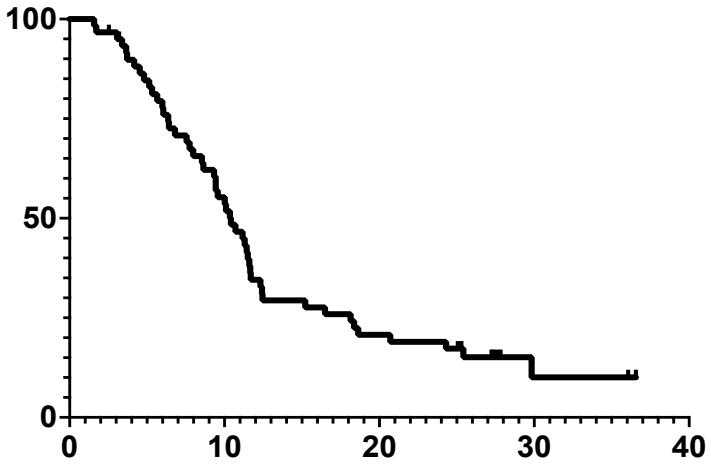


Figure 1: Progression-Free Survival from ITT Enrollment. Median =10.4 (95% CI 8.7-11.8); PD=progessive disease or death.

Months	0	6	12	18	24	30	36
# at risk	60	45	20	12	9	2	2
# PD	0	13	38	46	49	50	50
# censored	0	2	2	2	2	8	8

Table 1 shows PFS for this trial compared to PFS of randomized trials that also measured PFS from enrollment just prior to starting RT/TMZ. Median PFS were quite similar and ranged from 6.1 to 7.5 months, except for the AV-GBM-1 trial and the two trials that included bevacizumab [3,4]. Bevacizumab is known to effect tumor vascularity in a manner that decreases the appearance of edema that is typically used in defining disease progression radiographically [34,35]. It is also interesting to note the relationship between PFS and OS. In every arm of every trial the ratio is between 2 and 3, except for AV-GBM-1 and the two treatment arms that contained bevacizumab [3,4]. The average OS/PFS ratio for study arms that did not include bevacizumab and were not limited to a subset of GBM patients was 2.25. The discrepancy for the bevacizumab arms is presumably due to the masking of disease progression on MRI scans, but the explanation for the low ratio for AV-GBM-1 is not clear, but possibly could be the result of a favorable effect while vaccine was being delivered, that rapidly ebbed after the vaccine was discontinued after about eight months from enrollment in 68% of patients. The other 32% discontinued AV-GBM1 because of PD.

Study	**Standard RT/TMZ + TMZ**	**# Pts**	**Median PFS**	**Median OS**	**Ratio OS/PFS**	**Comment**
AIVITA	+ AV-GBM-1	60	10.4	16.0	1.5	
Stupp 2005	Standard	287	6.9	14.6	2.1	
Gilbert 2013	± dose dense TMZ	833	7.5	16.0	2.1	
Gilbert 2014	+ placebo	309	7.3	16.1	2.2	
Gilbert 2014	+ bevacizumab	312	10.7	15.7	1.5	
Chinot 2014	+ placebo	463	6.2	16.7	2.7	
Chinot 2014	+ bevacizumab	458	10.6	16.8	1.6	
Bruchroithner 2018	Standard	42	6.9	18.7	2.7	
Bruchroithner 2018	+ Audencel	34	6.7	18.5	2.8	
Roth 2021	+ Placebo	@375	6.1	15.9	2.6	

Roth 2021	Marizomib	@375	6.2	15.7	2.5	
Lassman 2022	Placebo	316	6.3	18.7	3.0	Only EGFR amplified
Lassman 2022	+depatuxizumab mafodotin	323	8.0	18.9	2.4	Only EGFR amplified

Table 1: Progression-free survival (PFS) and overall survival (OS) from enrollment prior to RT/TMZ for AV-GBM-1 and treatment arms from GBM randomized trials; RT=radiation therapy, TMZ=temozolomide; EGFR=epidermal growth factor receptor. It is estimated that 50% of GBM patients have EGFR amplification, and about 30% have EGFRvIII mutations; Depatuxizumab mafodotin (depatux-m) is an antibody drug conjugate: a monoclonal antibody that binds activated EGFR (wild-type and EGFRvIII mutant) linked to a microtubule-inhibitor toxin.

Methylation of the *MGMT* promoter is known to be associated with better OS and a better response to TMZ and other alkylator chemotherapies. Table 2 shows the PFS and OS based on populations of patients with a methylated *MGMT* promoter, compared to populations with an unmethylated *MGMT* promoter. Two of these trials only enrolled patients with a methylated *MGMT* gene promoter (or indeterminate) and therefore were not included in Table 1 [5,7]. Among patients with *MGMT* promoter methylation, in every trial arm the median PFS was over 10 months, and median OS over 20 months. OS/PFS ratios were 2.0 or above for all, including AV-GBM-1, except study arms containing bevacizumab (1.6). In the three studies with PFS defined for patients with an unmethylated *MGMT* promoter, AV-GBM-1 was the only treatment associated with a PFS of greater than nine months. OS/PFS ratios were less than 2.0 for AV-GBM-1 and bevacizumab. This raises the possibility that AV-GBM-1 had a more pronounced effect on PFS in patients with non-methylated tumors.

Methylated *MGMT* promoter in patients enrolled prior to RT/TMZ						
Study	**Standard RT/TMZ + TMZ**	**# Pts**	**Median PFS**	**Median OS**	**OS/PFS Ratio**	**Comment**
AIVITA	+ AV-GBM-1	22	11.4	22.8	2.0	
Hegi 2005	Standard	46	10.3	21.7	2.1	
Stupp 2014	+ Placebo	273	10.7	26.3	2.5	Only methylated
Stupp 2014	+ Cilengitide	272	13.5	26.3	2.0	Only methylated
Gilbert 2014	± bevacizumab	621	14.1	23.2	1.6	
Weller 2021	+ Placebo	@358	10.3	28.9	2.8	Only methylated
Weller 2021	+ Nivolumab	@358	10.6	32.1	3.0	Only methylated
MGMT non-methylated promoter for patients enrolled prior to RT/TMZ						
AIVITA	+ AV-GBM-1	38	10.0	13.3	1.3	
Hegi 2005	Standard	60	5.3	12.7	2.4	
Gilbert 2014	± bevacizumab	621	8.2	14.3	1.7	

Table 2: Median survivals (months) based on *MGMT* promoter methylation; *MGMT*= O^6-methyl-DNA methylguanine transferase; PFS=progression-free survival; OS=overall survival.

Table 3 shows PFS from first injection after recovery from RT/TMZ in this trial, compared to PFS in other randomized trials that defined PFS from enrollment after recovery from RT/TMZ. However, two of these trials, which had much longer median OS compared to the others, excluded patients who were felt to have PD or Ps-PD following RT/TMZ, which results in a patient population with more favorable survival prognosis [11,12], while the AV-GBM-1 trial included all patients based on intent-to-treat enrollment prior to RT/TMZ. Despite this, the median OS for AV-GBM-1 was longer than in any other trial, but this did not translate into longer survival. Every OS/PFS ratio was in the range of 2 to 4 except for the 1.6 associated with AV-GBM-1. Because patients were allowed to cross over to DCVax-L at the time of PD, OS data in that trial was compared to external controls [11], and there was no OS analysis for correlation in the placebo arm, and no data was available based on *MGMT* methylation. The small Austrian randomized phase 2 trial did include data based on *MGMT* methylation, but the numbers of patients were under 15 in all subsets, and therefore not included in the table [28].

Study	Standard RT/TMZ + TMZ	# pts	Median PFS	Median OS	OS/PFS Ratio	Comment
AIVITA	+ AV-GBM-1	57	8.5	14.0	1.6	
Gilbert 2013	Standard	411	5.5	16.6	3.0	
Gilbert 2013	+ dose dense TMZ	422	6.7	14.9	2.2	
Weller 2017	+ Placebo	374	5.6	14.1	2.5	EGFRvIII+ only[1]
Weller 2017	+ Rindopepimut	371	7.1	14.8	2.1	EGFRvIII+ only[1]
Stupp 2017	Standard	229	4.0	16.0	4.0	Excluded PD-PsPD[2]
Stupp 2017	+ TTF	458	6.7	20.9	3.1	Excluded PD-PsPD[2]
Mulholland 2022	+ Placebo	99	7.6	NA	NA	Excluded PD-PsPD[2]
Mulholland 2022	+ DCVax-L.	232	6.2	19.3	3.1	Excluded PD-PsPD2[2]

Table 3: Median survivals (months) from time-point after recovery following concurrent radiation therapy and temozolomide; PFS=progression-free survival; OS=overall survival; TTF=tumor treating fields; PD=progressive disease, PsPD=pseudo progressive disease[1]About 30% of GBM tumors are EGFRvIII+[2]About 20% of patients have PD or Ps-PD based on clinical impression and MRI scans following RT/TMZ.

Table 4 shows the relationship between PFS and OS in subsets of patients defined by *MGMT* promoter methylation from a time point shortly after recovery from RT/TMZ. Once again the only OS/PFS ratio less than 2.0 was for AV-GBM-1 and only in the unmethylated patients. PFS data was not readily available for two of the trials [11,12]. Once again OS was longer in the two studies that excluded patients with PD or PsPD after completion of RT/TMZ, but the differences were much greater in the patients with *MGMT* promoter methylation than in those who were unmethylated.

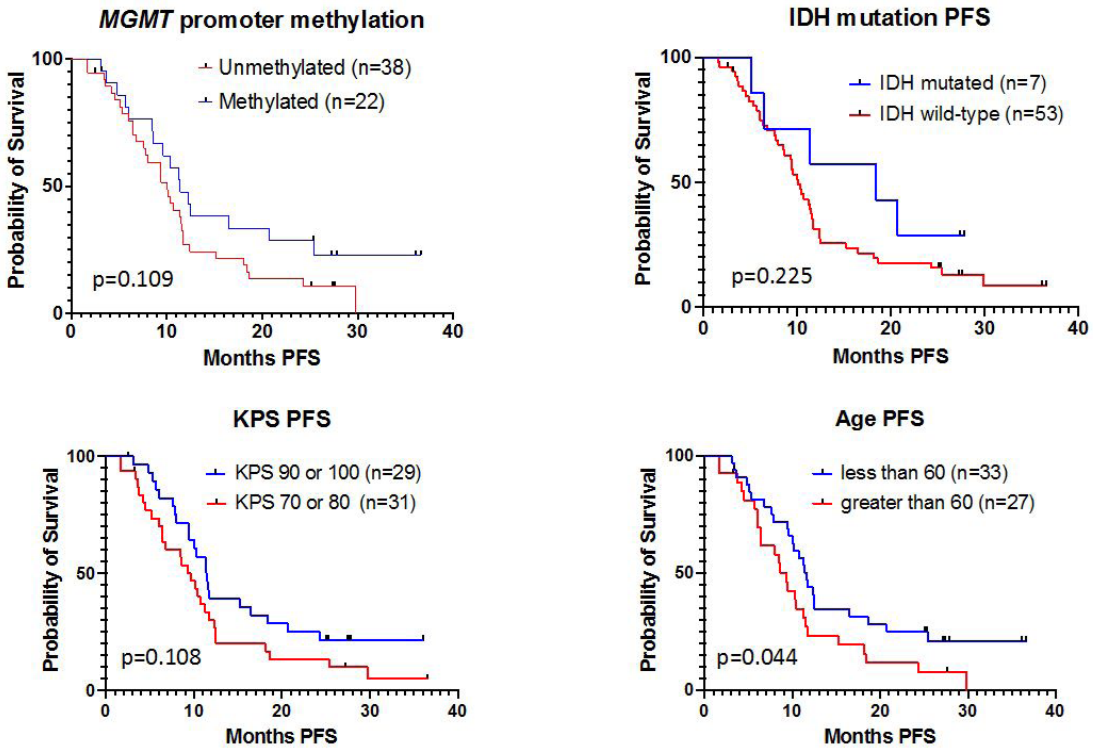
Methylated *MGMT* Promoter						
Study	**Standard RT/TMZ + TMZ**	**# pts**	**Median PFS**	**Median OS**	**OS/PFS Ratio**	**Comment**
AIVITA	+ AV-GBM-1	22	11.4	22.8	2.0	
Gilbert 2013	Standard	122	6.5	21.4	3.3	
Gilbert 2013	+ dose dense TMZ	122	10.1	20.2	2.0	
Stupp 2017	Standard	77	NA	21.2	NA	Excluded PD-PsPD[1]
Stupp 2017	+ TTF	137	NA	31.6	NA	Excluded PD-PsPD[1]
Mulholland 2022	+ DCVax-L	199	NA	30.2	NA	Excluded PD-PsPD[1]
Unmethylated *MGMT* Promoter						
AIVITA	+ AV-GBM-1	38	10.0	13.3	1.33	
Gilbert 2013	Standard	254	5.1	14.6	2.86	
Gilbert 2013	+ dose dense TMZ	262	6.0	13.3	2.22	
Stupp 2017	Standard	95	NA	14.7	NA	Excluded PD-PsPD[1]

Stupp 2017	+ TTF	209	NA	16.9	NA	Excluded PD-PsPD[1]
Mulholland 2022	+ DCVax-L	349	NA	14.9	NA	Excluded PD-PsPD[1]

Table 4: Median survivals (months) based on *MGMT* promoter methylation with survival measured from a time-point after recovery following concurrent radiation therapy and temozolomide; *MGMT*= O^6-methyl-DNA methylguanine transferase RT/TMZ=radiation and temozolomide; pts=patients, PFS=progression-free survival; OS=overall survival; PD=progressive disease, PsPD=pseudo progressive disease; TTF=tumor treating fields

[1]About 20% of patients have PD or Ps-PD based on clinical impression and MRI scans following RT/TMZ.

Figure 2 shows PFS for cohorts defined by variables that are prognostic for overall survival, including *MGMT* promoter methylation, *IDH* mutation, KPS, and age. The relative differences in curves are as would be predicted, but because of the small numbers, only the difference associated with age was statistically significant. Collectively these curves suggest that treatment with AV-GBM-1 does not dramatically impact the PFS outcomes expected in these prognostically stratified subgroups. Had the curves been identical, depending on the PFS observed, one may have inferred that AV-GBM-1 was associated with an improved PFS in the prognostically inferior cohort, or associated with a decreased PFS in the prognostically superior subgroup. However, in each cohort it appears that PFS was similar between the prognostic subgroup during the months while AV-GBM-1 was being given, but the curves separated shortly after the eight-month time point when the last vaccines were administered.



Variable	Standard RT/TMZ + TMZ	# Pts	Median PFS	Median OS	OS/PFS Ratio	P value for PFS	P value for OS
All patients	All Patients	60	10.4	16.0	1.6	NA	NA
MGMT promoter	Methylated	22	11.4	22.8	2.0	0.109	0.142
MGMT promoter	Unmethylated	38	10.0	13.4	1.3		
IDH	Mutated	7	18.4	NR-27	1.5 -2.0	0.225	0.653
IDH	Wild-type	53	10.1	14.7	1.5		
KPS	90 or 100	29	11.4	20.5	1.8	0.108	0.033
KPS	70 or 80	31	9.6	13.0	1.4		
Age	Less than 60	33	11.6	19.5	1.7	0.044	0.136
Age	60 or greater	27	9.3	13.0	1.4		

Figure 2: PFS for cohorts defined by variables that are prognostic for overall survival, including *MGMT* promoter methylation, *IDH* mutation, KPS, and age; RT=radiation therapy; TMZ=temozolomide chemotherapy; PFS=progression-free survival; OS =overall survival , NA=not applicable, *MGMT*= O6-methyl-DNA methylguanine transferase; *IDH*=isocitrate dehydrogenase; KPS=Karnosky Performance Status; NR-27=not reached at 27 months

Figure 3 shows the relationship between PFS and TMZ-based regimens that were given concurrently with the AV-GBM-1 vaccine. Not surprisingly, the worse PFS was in the cohort of patients who were felt to be too ill to receive TMZ concurrently with vaccine ($p<0.0001$). The median PFS was quite similar for TMZ alone, TMZ plus bevacizumab, and TMZ + TTF, although there appears to have been some separation of these curves after about 10 months of follow up. This shows that neither concurrent bevacizumab nor concurrent TTF is the explanation for the prolonged PFS. Managing physicians often add bevacizumab to TMZ to decrease edema in patients who appear to have PD or PsPD following RT/TMZ. This may allow them to decrease the use of immunosuppressive corticosteroids; so, the TMZ + bevacizumab cohort may have had a worse prognosis. However, the PFS comparisons of TMZ *vs* TMZ + bevacizumab, and TMZ + TTF *vs* TMZ + bevacizumab did not differ (p values of 0.103 and 0.358 respectively). There is also no difference between TMZ vs the combined subsets of TMZ with bevacizumab and/or TTF.

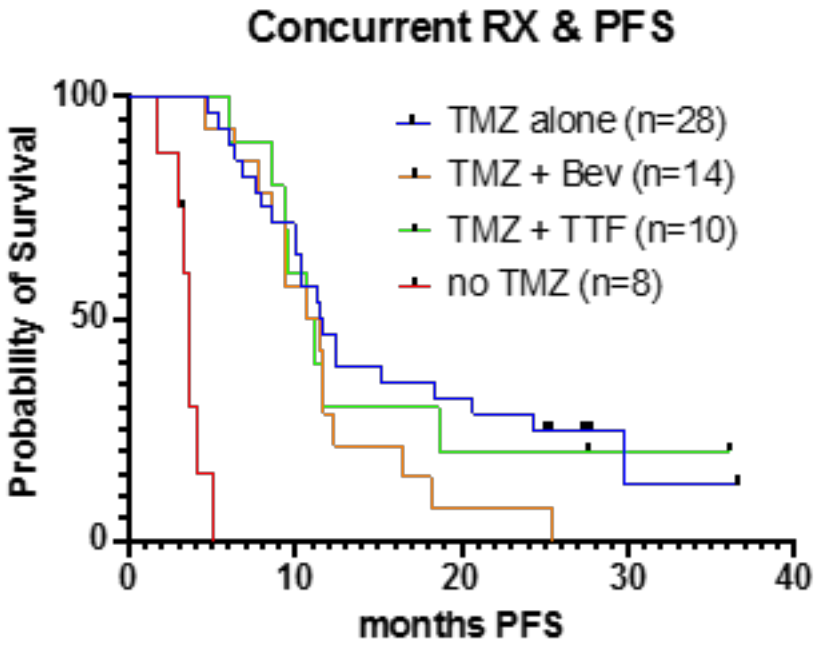


Figure 3: Progression-free survival and its relationship to temozolomide-based regimens given concurrently with vaccine;

Medians:	TMZ alone	11.6 mos
	TMZ + Bev	11.4 mos
	TMZ + TTF	10.9 mos
	No TMZ	3.6 mos

RX=therapy, PFS=progression-free survival, TMZ=temozolomide, Bev=bevacizumab, TTF=tumor treating fields.

Discussion

In patients with recurrent malignant glial tumors, 6-month PFS was shown to be a good predictor of OS [36], but the relationship between PFS and OS in primary GBM is less clear. This detailed analysis was undertaken because the median PFS associated with AV-GBM-1 was about 50% longer than observed in numerous previous trials that were conducted in similar patients, but this apparent improvement in PFS did not translate into increased OS. As can be seen in Table 1, In trials that measured survival from enrollment prior to initiation of RT/TMZ, the 10 study arms that did not include bevacizumab had OS/PFS ratios in the range of 2 to 3, with an average of 2.25. Based on the consistency of the relationship between PFS and OS, in the AV-GBM-1 trial one might have expected an OS of 23.4 months (2.25 X 10.4) rather than the 16.0 months that was observed. There are several possible explanations for this, including: [1] increase in PFS because of concurrent use of bevacizumab, [2] increase in PFS because of concurrent use of TTF, [3] use of a more restrictive definition of PD in the AV-GBM-1 trial compared to PD definitions used in previous trials, [4] initial induction or enhancement of an effective anti-tumor immune response by AV-GBM-1 that subsequently waned or was overcome by immune resistance, and [5] a therapeutic benefit of AV-GBM-1 that only persisted while the vaccine injections were being administered, and then waned within a few weeks to months. These possible explanations are addressed in the following paragraphs.

The concern that concurrent use of bevacizumab confounded the interpretation of PD was reasonable based on previous trials that reported a longer PFS for patients treated with the anti-VEGF antibody [3,4]. This was also supported by the OS/PFS ratios that were less than 2.0 for only the two study arms that tested bevacizumab with TMZ [3,4], and for AV-GBM-1. However, the data show that this is not the explanation. First, only 14 of the 60 patients received bevacizumab concurrently with the vaccine and maintenance TMZ. Second, as shown in Figure 3, the median PFS

and PFS curves were similar for TMZ alone, TMZ + TTF, and TMZ + bevacizumab. In fact, the curve with the poorest long-term PFS among these three was the TMZ-bevacizumab arm, perhaps because investigators chose to add bevacizumab only in those patients who had PD or PsPD after recovery from RT/TMZ.

The suggestion that addition of TTF may have improved PFS was plausible given that a randomized trial showed that the addition of TTF to maintenance TMZ was associated with prolonged PFS [12]. However, the data show that this is not the explanation. First, only 10 of the 60 patients underwent TTF treatment while receiving the vaccine and maintenance TMZ. Second, as shown in Figure 3, the median PFS and PFS curve for concurrent TMZ + TTF was similar to concurrent TMZ alone. The patient numbers are quite small, but the curves do not suggest that adding TTF resulted in better PFS than giving TMZ alone with the vaccine. The addition of AV-GBM-1 to maintenance TMZ may have improved the associated PFS, while there was no additional advantage in the patients treated with TMZ + TTF + AV-GBM-1 because, based on mechanism of action, TTF may have interfered with positive immune cell responses induced by AV-GBM-1. Of course, an alternative explanation would be that AV-GBM-1 decreased the beneficial effects of TTF, but a biological explanation for this is not obvious.

The definition of PD to define PFS in GBM has been evolving for more than three decades, and remains imprecise and controversial [37,38]. The "MacDonald criteria" were designed to define a response in phase II trials of systemic therapies for patients who had recurred following surgery and/or radiation for glial tumors [39]. In trying to define what is an objective response resulting from treatment of GBM, investigators also had to define what constitutes objective disease progression. PD was defined by a 25% or greater increase in the product of the cross-sectional diameters of tumor as measured by computerized axial tomography (CT) or MRI, radiographic identification of new sites of tumor, or neurologic deterioration on a stable or increasing dose of corticosteroids that was considered due to progression rather some other cause. This has remained the cornerstone of subsequent definitions of PD. Twenty years later these criteria were modified and referred to by the name of the Response Assessment in Neuro-Oncology (RANO) working group that developed them [40]. A major impetus to the revisions was recognition that about 20% of patients who have undergone concurrent chemoradiation have PsPD, a transient tumor enhancement by CT or MRI that cannot be reliably differentiated from true tumor progression. During the first 12 weeks after completion of chemoradiotherapy, PD was to only be declared radiographically if there was new enhancement beyond the field of radiation or histologic confirmation of viable tumor. After the first 12 weeks following completion of chemoradiotherapy, PD continued to be defined by a 25% or greater increase in the product of the cross-sectional diameters of tumor, new sites of tumor, or neurological deterioration attributed to tumor. For patients receiving antiangiogenic agents and receiving corticosteroids, PD could be declared based on an increase in the T2/FLAIR on MRI scan. Five years later an additional modified version referred to as immuneRANO (iRANO) was proposed for use in patients receiving immunotherapies that might induce tumor inflammation as part of their mechanism of action at any time during, and possibly following, treatment [41]. A key change was that if PD was to be declared based only on radiographic assessment, then a confirmatory scan was needed three months later before declaring PD, and that patients could continue to receive immunotherapy in the meantime. In clinical practice, in the absence of unacceptable toxicity, declaration of PD by the managing physician is typically associated with a change in clinical management that may include a change in therapeutic agents, or discontinuation of therapy. Two years later there were recommendations for additional changes, so-called modified RANO (mRANO) criteria that included recommendations regarding interpretation of MRI scans, and a recommendation to use post RT/TMZ imaging to establish a new baseline for determining PD [42]. Of the studies summarized in Tables 1 to 4, MacDonald criteria were used in seven [1, 3-6, 11,12], RANO in four [6-8, 10], and a modified MacDonald in one [28]. None of these studies reported using iRANO or mRANO. In the AV-GBM-1 trial PD was defined by managing physicians and investigators rather than by a central radiologic review and the clinical protocol did not prescribe the specific criteria to be used. What was most important was that on the basis of clinical findings, imaging, and changes over time, the treating physician had become convinced that the patient was experiencing PD which justified a change in clinical management. By the time AV-GBM-1 trial was conducted, neurooncology investigators were aware of the problems encountered in trying to determine PD in the DCVax-L DC-vaccine trial because of vaccine-associated inflammation [11,27], which may have resulted in more conservatism in declaring PD during vaccine therapy in the AV-GBM-1 trial. As discussed earlier, in trials to date the OS/PFS ratios were similar regardless of criteria, except for the study arms that included bevacizumab, or AV-GBM-1. From Tables 1 and 2, the average OS/PFS ratio for the earlier six non-bevacizumab study arms that specified MacDonald criteria was 2.4 [1,3,5,9]; the average for the more recent six arms in studies that specified RANO criteria was 2.7 [7,8,11]. In the post RT/TMZ setting (Table 3), the three study arms that excluded patients with PD or PsPD and used MacDonald criteria had a OS/PFS average of 3.4, while the two study arms that did not exclude such patients had an average of 2.6. With regards to AV-GBM-1, In the absence of centralized review committees reviewing serial scans and separately applying MacDonald, RANO, iRANO, and mRANO criteria, it is not possible to determine whether the investigator determinations of PD were delayed compared to these other trials, and if so, by how much.

The possibility of initial induction or enhancement of an effective anti-tumor immune response by AV-GBM-1 that subsequently waned or was overcome by immune resistance remains a possibility, but we currently do not have definitive data to address this. It is possible that a positive immune response may wane within a few months of discontinuing treatment, in which case a longer duration of treatment may be beneficial. It is also possible that after induction or enhancement of the immune response that an inhibitory immune response will negate the positive effects, in which case the addition of immune checkpoint inhibitor therapy may be needed to maintain the clinical benefit.

Patients who did not receive all eight planned AV-GBM-1 doses discontinued therapy because of PD; so it is not surprising that their PFS was much less than the more than 24 months for the 39 who received all eight doses. For all patients, the median PFS of 10.4 months was reached about two months after the planned last injection. It may be that the apparent PFS benefit associated with AV-GBM-1 only persisted while the vaccine injections were being administered, and then waned about two months after the last injection, which for 68% of patients was about 10 months from enrollment. It is possible that PFS, and especially OS would have been longer had the every 4-weeks injections been continued for a longer time. In contrast to the completed phase 2 trial, in which eight doses were injected over six months, in an FDA-approved phase 3 randomized trial, AV-GBM-1 is to be given for up to 21 doses over 18 months, but once an investigator has concluded that the patient is experiencing PD, the vaccine must be discontinued.

There are other theoretical reasons why continuing AV-GBM-1 for a longer period may benefit GBM patients. GBM is known to harbor quiescent cancer stem cells, or tumor-initiating cells, that evade anti-proliferative therapies [43], so continued targeting may be required to eventually deplete this latent cancer stem cell population. Immune cells have only limited access to the brain due to the presence of the blood brain barrier (BBB) and the lack of resident dendritic cells [44], although activated T cells can pass the BBB and diffusely penetrate the brain parenchyma and produce anti-tumor effects [45-47]. Continued treatment may be needed to produce sufficient numbers of T cells activated against various ATA. The hostile and inhibitory microenvironment of GBM is another major challenge to immunotherapy. The activity of immune cells may be suppressed by immunosuppressive cytokines such as prostaglandin IL-10, TGFβ and E2 [48], and the presence of inhibitory cell-surface antigens such as programmed death 1/programmed death-ligand 1 [49]. Furthermore, trafficking of immunosuppressive cells such as myeloid derived suppressor cells and regulatory T cells into the brain may create other physical and metabolic blockades [50,51]. Continued treatment may be needed to overcome these inhibitory obstacles.

Conclusions

AV-GBM-1 was associated with an apparent prolonged PFS that did not translate into the OS benefit one would have expected based on the OS/PFS ratios observed in previous randomized therapeutic trials in patients with newly diagnosed GBM. This analysis showed that concurrent administration of bevacizumab and/or TTF with TMZ in a minority of patients is not the explanation for this lack of correlation. It is possible that use of more conservative criteria by managing physicians to define PD may account for the apparent increase in PFS without an effect on OS, since all trials published to date have used MacDonald or RANO criteria in guiding PD determination. On the other hand, it is also possible that AV-GBM-1 treatment was discontinued too early in patients who were benefitting, and that a longer duration of treatment could provide more survival benefit.

Author Contributions

Conceptualization, Robert Dillman and Daniela Bota; Data curation, Robert Dillman; Formal analysis, Thomas Taylor and Robert Dillman; Funding acquisition, Hans Keirstead; Investigation, David Piccione, Christopher Duma, Santosh Kesari, Ranato LaRocca, Robert Aiken, Jose Carrillo and Daniela Bota; Methodology, Gabriel Nistor and Robert Dillman; Project administration, Robert Dillman; Supervision, David Piccione, Christopher Duma, Santosh Kesari, Ranato LaRocca, Mehrdad Abedi, Gabriel Nistor, Robert Dillman and Daniela Bota; Writing – original draft, Robert Dillman; Writing – review & editing, David Piccione, Christopher Duma, Santosh Kesari, Ranato LaRocca, Robert Aiken, Thomas Taylor, Jose Carrillo, Mehrdad Abedi, Gabriel Nistor, Hans Keirstead, Robert Dillman and Daniela Bota.

Institutional Review Board Statement

The clinical protocol for this study was approved by the Western Institutional Review Board (WIRB) 20182582. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of each of the participating institutions.

Informed Consent Statement

Written Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Study can be made available from AIVITA Biomedical, Inc. based on reasonable request.

Conflicts of Interest

Employees of AIVITA Biomedical, Inc., G.N., H.K., R.D. The funding sponsor (AIVITA Biomedical, Inc.) was involved

in the choice of research project, design of the study, and in the collection, analyses and interpretation of data, and in the writing of the manuscript and the decision to publish the results.

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