Phase I/II Study of Dianhydrogalactitol in Patients with Recurrent Malignant Glioma

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ABSTRACT # ANT-26: Glioblastoma multiforme (GBM) is the most common brain cancer. Front-line systemic therapy with temozolomide is often ineffective due to O^6-methylguanine-DNA-methyltransferase (MGMT)-mediated resistance. Dianhydrogalactitol (VAL-083) is a bi-functional DNA N^7 cross-linking agent that crosses the blood-brain barrier that has demonstrated cytotoxic activity independent of MGMT *in vitro*. The main goal of this clinical trial was to determine an appropriate dose for Phase II/III trials in refractory GBM. **METHODS:** Open-label, single-arm Phase I/II dose-escalation study in patients with histologically-confirmed GBM, previously treated with radiation and must have failed both temozolomide and bevacizumab, unless contraindicated. The study utilized 3+3 dose-escalation design. Patients received VAL-083 on days 1, 2, 3 of a 21-day cycle. **RESULTS:** 29 GBM patients were enrolled across 8 dose cohorts ranging from 1.5 to 50 mg/m^2/d. No drug-related serious adverse events were reported, and myelosuppression was mild at doses ≤40 mg/m^2/d. Dose limiting toxicities (DLT) consisting of thrombocytopenia were observed at 50 mg/m^2/d. Platelet nadir occurred around day 20, and DLT-related symptoms resolved rapidly and spontaneously without concomitant treatment. Pharmacokinetic analyses show dose-dependent linear systemic exposure with a short 1-2h plasma terminal half-life; average C_{max} was 781 ng/mL (5.3µM) at 40 mg/m^2/d resulting in estimated CNS concentrations within the IC_{50} range observed for multiple GBM cell-lines *in vitro*. Preliminary analysis shows increasing dose-dependent median survival with median OS=9.2 months at doses ≥30 mg/m^2/d vs. 5.1 months at doses <10 mg/m^2/d. A small cohort (n=3) at an interim 45 mg/m^2/d dose was studied and DLTs were observed. Thus, 40 mg/m^2/d was confirmed as the maximum tolerated dose. An expansion cohort of 14 patients has been enrolled at 40 mg/m^2/d. **CONCLUSIONS:** VAL-083 dosing appears limited by myelosuppression; however, 40 mg/m^2/d dose exhibited favorable safety profile, with a trend toward improved survival vs. lower doses. Updated safety and efficacy data from the expansion cohort will be presented. **ClinicalTrials.gov Identifier** NCT01478178.

TABLE 1: PHASE I PATIENT DEMOGRAPHICS

Characteristics of Patients Receiving VAL-083 in the Phase I dose escalation part of the study.

	DOSE OF VAL-083	
	≤5 mg/m² *(n=10)*	**30&40 mg/m² *(n=6)***
Age: Mean (range)	49 (30-38)	61 (23-71)
Sex: M / F	4 / 6	5 / 1
KPS: Mean (range)	80 (60-100)	85 (60-100)
Initial lesion		
R temporal	2	2
L temporal	2	0
R parietal	1	2
L parietal	3	0
R frontal	1	1
L frontal	0	2
L ventricular	0	0
R occipital	0	0
Not reported	1	0
Recurrence		
R temporal	2	2
L temporal	1	0
R parietal	1	1
L parietal	2	0
R frontal	1	2
L frontal	1	0
L ventricular	0	1
R occipital	0	1
L cerebellum	1	0
not reported	1	1
Prior Therapy		
temozolomide	10	6
bevacizumab	9	6
Salvage Therapy Prior to VAL-083		
temozolomide re-challenge	3	1
irinotecan	3	0
PDGFR Antibody	2	0
vorinostat	1	0
bevacizumab re-challenge	2	2
radiation re-challenge	1	0
carboplatin	1	1
onartuzumab	1	0
Gleevec	1	0
BKM-120 (combination with bev)	0	1
Gliadel®	0	1
CCNU	0	2

REFERENCES

1. ***Eagan R.T. et al.*** *Cancer Treat Rep. 1982; 66(8):1647-49*
2. ***Eckhardt et al.*** *Cancer Treat Rep. 1977; 61(5):841-7*
3. ***Iwamoto et al.*** *Neurology 2009; 73(15):1200-1206*
4. ***Zhai et al.*** *Canadian Cancer Research Conference 2015*
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6. ***Steino et al.*** *AACR 2015 Abstract #751*
7. ***Wang et al.*** *Ann Surg Oncol 2014; 21: 1337-1344*

SAFETY & TOLERABILITY, Phase I:

Dose escalation was undertaken at a 3+3 design beginning at 1.5 mg/m^2/d on day 1,2,3 of a 21 day cycle. No drug-related adverse events were observed at doses ≤20 mg/m^2/d. Myelosuppression was mild at doses up to 40 mg/m^2/d.

DLT was observed at 50 mg/m^2/d in 2 of 6 patients, defined as 1 patient with Grade 4 thrombocytopenia and 1 patient with Grade 3 thrombocytopenia with hemorrhage (Table 2). DLT generally resolved rapidly and spontaneously. Observations of DLT (G4 thrombocytopenia) were also made in an interim cohort (n=3) at 45 mg/m^2/d, which was initiated in parallel with a Phase II expansion cohort to further explore the therapeutic window of this dosing regimen, thereby confirming 40 mg/m^2/d as MDT with this regimen.

Table 2. Hematologic toxicities observed in patients in the Phase I dose escalation part and the interim 45 mg/m^2/d dose cohort of this clinical trial (CTCAE Grading).

	Cohort	1 – 5	6	7	8	9 (interim)
Dose		***≤20 mg/m²/d***	***30 mg/m²/d (n=3)***	***40 mg/m²/d (n=3)***	***50 mg/m²/d (n=6)***	***45 mg/m²/d (n=3)***
Anemia *(Hct; Hg)*	G1	-	-	1/3 *(33.3%)*	5/6 *(83.3%)*	
	G2	-	-	-	-	
	G3	-	-	-	-	
	G4	-	-	-	-	
Leukopenia *(WBC)*	G1	-	1/3 *(33.3%)*	1/3 *(33.3%)*	3/6 *(50.0%)*	
	G2	-	-	-	2/6 *(33.3%)*	
	G3	-	-	-	2/6 *(33.3%)*	
	G4	-	-	-	-	
Neutropenia *(neutrophils)*	G1	-	-	1/3 *(33.3%)*	2/6 *(33.3%)*	
	G2	-	-	-	-	
	G3	-	-	-	1/6 *(16.6%)*	
	G4	-	-	-	1/6 *(16.6%)*	2/3 *(66.6%)*
Thrombo-cytopenia *(platelets)*	G1	-	2/3 *(66.6%)*	2/3 *(66.6%)*	1/6 *(16.6%)*	
	G2	-	-	-	-	
	G3	-	-	-	3/6 *(50.0%)**	1/3 *(33.3%)*
	G4	-	-	-	1/6 *(16.6%)**	2/3 *(66.6%)*

**DLT was observed at 50 mg/m^2/d in 2 of 6 patients as defined by Grade 4 thrombocytopenia in 1 of 6 patients (16.6%) and Grade 3 thrombocytopenia with hemorrhage in 1 of 6 patients (16.6%).*

PHARMACOKINETICS, Phase I

Pharmacokinetic (PK) analyses show dose-dependent linear systemic exposure with a short plasma 1-2h terminal half-life; average C_{max} at 40 mg/m^2/d was 781 ng/mL (5.3 µM). The observed PK profile is comparable to published literature[1] (Figures 1 & 2).

Fig 1. PK by dose cohort & published literature

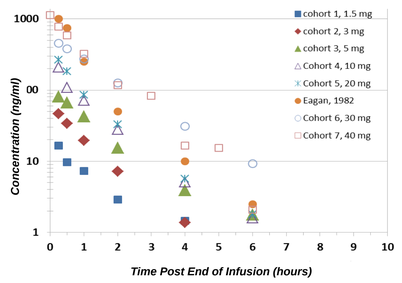
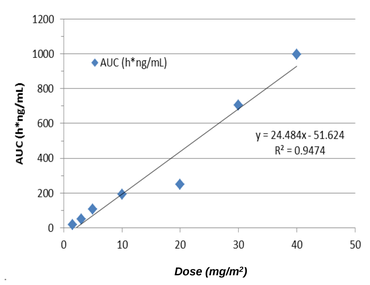


Fig 2. Dose-AUC relationship



We estimated the VAL-083 concentration in CNS tumor by extrapolating CNS exposure based on information in the published literature[2]. This analysis predicts that observed plasma concentrations in current trial result in CNS tissue concentrations exceeding the IC_{50} against glioma cell lines *in vitro* (Table 3).

Table 3. Estimated Tumor Concentration in Human Brain Exceeds in vitro IC_{50}

Dose (day 1,2,3 in 21 day cycle)	Plasma Cmax (µg/mL)	Estimated Maximum Tumor Concentration in Brain (day 3)		IC_{50} in GBM Cell Lines
		(µg/g tissue)	µM	µM
40 mg/m²/d	0.781	0.563	3.86	2 – 4

1.PK was conducted only on Day 1, given the short t-1/2 of ~1h Cmax is assumed to be same for Day 2 & 3.
2.Percent of plasma drug concentration in brain tumor = 44%, Eckhardt, 1977[2]
3.Half-life of drug in human brain tumor tissue = 20h, Eckhardt, 1977[2]
*Volume of 1 g tissue assumed to be 1 mL

ANTI-TUMOR ACTIVITY, Phase I

Subset analysis of low-dose and high-dose cohorts in the Phase I dose escalation part of this clinical trial suggests a dose-response trend that is consistent with the anti-tumor activity predicted by estimated CNS concentrations. In comparison to published literature investigating OS in GBM using currently available salvage therapy[3], these results suggest a meaningful and dose-dependent survival benefit of VAL-083 treatment of patients failing both TMZ and bevacizumab (Table 4).

Table 4. Observed OS in VAL-083 low-dose vs. high-dose cohorts in Phase I dose escalation part of study compared to published literature.[3]

Observed OS following VAL-083 treatment in GBM patients failing TMZ & bevacizumab	**6 mos**	**9 mos**	**12 mos**	**Med OS**
• High dose (30 & 40 mg/m²/d, n=6)	67%	67%	33%	9.2 mos
• Low dose (≤5 mg/m²/d, n=10)	44%	33%	22%	5.1 mos
Predicted Survival following bevacizumab failure[3]				
• Salvage therapy				5.2 mos
• Hospice only				2.0 mos

Update on Status of Phase II Expansion Cohort

Following determination of the MTD at 40 mg/m²/d, a 14-patient Phase II expansion cohort was rapidly enrolled at a dose of 40 mg/m²/d on day 1,2,3 of a 21 day cycle.

The 14th and final patient to be enrolled in the Phase II expansion cohort initiated treatment on October 14, 2015, and all patients enrolled in the Phase II expansion cohort have received at least one cycle of treatment to date.

Safety observations in the Phase II expansion cohort to date are consistent with the Phase I dose-escalation cohort. Generally, observed myelosuppression is mild (G1), with the exception of one patient.

- **One subject previously treated with CCNU developed G4 thrombocytopenia suggesting patients with prior nitrosourea treatment who may exhibit higher susceptibility to thrombocytopenia. The inclusion criteria were modified to account for this observation.**

To date, 20 GBM patients failing standard front-line therapy and bevacizumab have been treated with an assumed well-tolerated therapeutic dose of VAL-083 (30mg/m²/d & 40mg/m²/d). A Kaplan Meyer survival estimate based on interim analysis of patients enrolled in the Phase II expansion cohort is consistent with observations made in the Phase I dose-escalation portion of the study. Preliminary analysis suggests a potentially meaningful survival benefit in this population following treatment with VAL-083 at doses ≥30 mg/m²/d in comparison to published reports for the same population[3] (Figure 3).

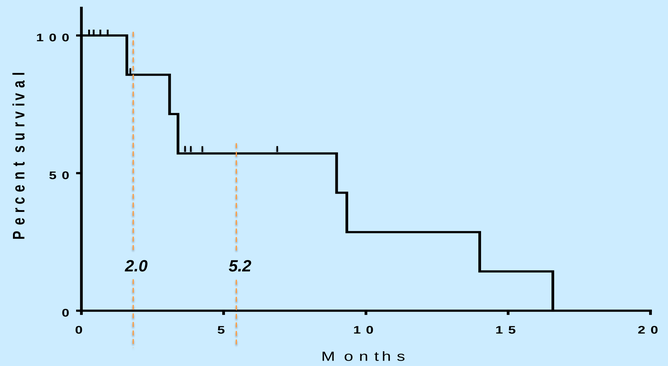
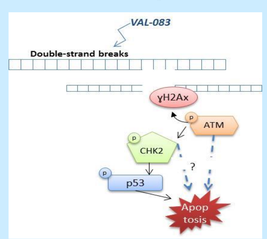


Figure 3. Kaplan-Meier plot is based on 6 patients in the Phase I dose escalation part of this study combined with available data from the ongoing Phase II part of this study. In 2009, Iwamoto reported a median overall survival of 2.0 months after failing bevacizumab for patients receiving only hospice care, and 5.2 months after failing bevacizumab for patients receiving further salvage therapy.[3] The observed median survival to date following treatment with VAL-083 is 9.2 months.

VAL-083 induces phosphorylation of H2A.X, a hallmark of double-strand DNA breaks, leading to cell cycle arrest in the late G2/S phase.[4] H2AX is a histone involved in the CHK2 checkpoint activation pathway, a key component of the body's immune response to DNA damage resulting in down-stream signaling ultimately resulting in apoptosis.

We have demonstrated that VAL-083 antineoplastic activity is independent of MGMT expression against multiple GBM cell lines and active against GBM CSCs.[5] We also recently reported that the cytotoxic activity of VAL-083 appears to be less dependent on wild type p53 in comparison to other chemotherapeutic agents.[6] Alteration in p53 has been correlated with poor patient outcomes in GBM. In particular, gain-of-function mutant p53 is strongly associated with a poor prognosis for overall survival in patients with glioblastoma, potentially by increasing MGMT expression thereby decreasing chemosensitivity to TMZ.[7]

Taken together with historical and recently demonstrated clinical activity, these results suggest a distinct anti-cancer mechanism for VAL-083 which has the potential to overcome chemo-resistance and surpass the standard of care in the treatment of GBM



CONCLUSIONS & NEXT STEPS

- ***VAL-083 at 40 mg/m²/d is a well-tolerated dosing regimen that has demonstrated potential to improve survival outcomes in post-bevacizumab refractory GBM.***
- ***Patient outcomes from the ongoing Phase II expansion cohort will provide insights to support design of registration directed Phase II/III clinical trials.***