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

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ABSTRACT #CT074 : Glioblastoma (GBM) is the most common brain cancer. Front-line systemic therapy with temozolomide (TMZ) is often ineffective due to O^6-methylguanine-DNA-methyltransferase (MGMT)-mediated resistance and patients with recurrent glioma have limited treatment options and very poor prognosis. Dianhydrogalactitol (VAL-083) is a bi-functional alkylating agent that readily crosses the blood-brain barrier and has demonstrated activity against GBM in prior NCI-sponsored clinical trials. VAL-083 induces cross-links at N^7 of guanine causing double-strand DNA breaks and apoptosis independent of MGMT expression against multiple GBM cell lines and cancer stem cells *in vitro*. VAL-083 cytotoxic activity also appears to be less dependent on wild type p53 compared to other alkylating agents. The main goal of this clinical trial was to determine an appropriate dose for VAL-083 for advancement to Phase II/III trials as a potential new treatment for refractory GBM. **METHOD:** Open-label, single-arm Phase I dose-escalation study (3+3 design). Patients receive VAL-083 IV on days 1, 2, and 3 of a 21-day cycle, until MTD is reached. In Phase II, additional patients with GBM are treated at the MTD to gather further safety and outcomes data. Patients must have histologically confirmed GBM, previously treated with surgery and radiation and must have failed both TMZ and bevacizumab, unless contraindicated. **RESULTS:** Phase I has been completed and 40 mg/m^2/d confirmed as the MTD. 29 GBM patients were enrolled in Phase I across 9 dose cohorts (1.5 - 50 mg/m^2/d). Myelosuppression was mild; no drug-related serious adverse events were reported at doses ≤40 mg/m^2/d. Dose limiting toxicities (DLT), consisting of thrombocytopenia, were observed at 50 mg/m^2/d and at an interim 45 mg/m^2/d cohort. Platelet nadir occurred around day 20 and resolved rapidly and spontaneously. Pharmacokinetic analyses show dose-dependent linear systemic exposure with a short 1-2h plasma terminal half-life; average C_{max} 781 ng/mL (5.3μM) at 40 mg/m^2/d resulting in estimated CNS concentrations within the IC_{50} range observed for GBM cell-lines *in vitro*. A 14 patient Phase II expansion cohort was enrolled at 40 mg/m^2/d. Safety observations in the Phase II expansion cohort to date are consistent with Phase I. Observed myelosuppression is mild, with the exception of 1 patient previously treated with CCNU who developed grade 4 thrombocytopenia. To date, 20 GBM patients (6 patients in Phase I and 14 patients in Phase II) have been treated with VAL-083 at therapeutic doses of 30 or 40 mg/m^2/d. **CONCLUSIONS:** VAL-083 at 40 mg/m^2/d on days 1, 2, 3 of a 21-day cycle exhibits a favorable safety profile and the Phase I part of the study showed a trend toward clinically meaningful improved survival in refractory GBM patients. Updated safety and outcomes data from the Phase II expansion cohort will be presented.
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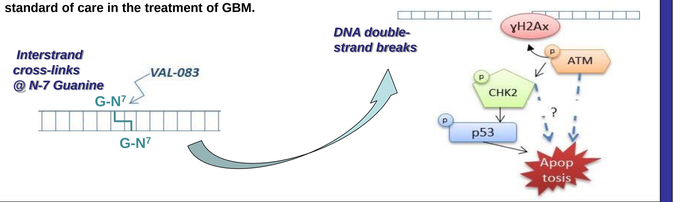
VAL-083 MECHANISM OF ACTION (see abstract #2985):

VAL-083 is a structurally unique bifunctional alkylating agents that alkylates N7-guanine and induces rapid formation of DNA interstrand crosllinks.[1,2] VAL-083 treatment leads to persistent and irreversible phosphorylation of H2A.X, a hallmark of DNA double-strand breaks (DSBs), leading to cell cycle arrest in the late G2/S phase and ultimately apoptosis. VAL-083 induces activation and phosphorylation of Ataxia Telangiectasia Mutated (ATM), a protein kinase recruited and activated by DSBs, leading to activation of the ATM-CHK2 checkpoint pathway and the downstream Homologous Recombination (HR) DNA repair pathway.[3] Deficient DNA repair pathways are a hallmark of cancer cell development, and VAL-083-induced DSBs are not repaired in cancer cells with deficient HR, leading to cancer cell death.

We have further demonstrated that VAL-083 cytotoxic activity is independent of MGMT expression against all tested GBM cell lines, including GBM cancer stem cells, and the mechanism of VAL-083 thus differs significantly from both TMZ and BCNU, both sensitive to MGMT expression.[4,5] 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.



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PHARMACOKINETICS

Pharmacokinetic (PK) analyses show dose-dependent linear systemic exposure with a short plasma 1-2h terminal half-life; average Cmax at 40 mg/m^2/d was 781 ng/mL (5.3 μM). The observed PK profile is comparable to published literature[8] (Figures 1 & 2).

Fig 1. PK by dose cohort & published literature

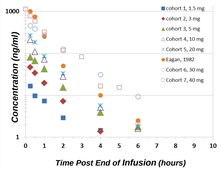
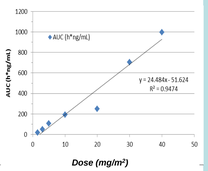


Fig 2. Dose-AUC relationship



VAL-083 concentration in CNS tumor by extrapolating CNS exposure was estimated based on published literature[9]. 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 1).

Table 1. 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/m^2/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[9]
3. Half-life of drug in human brain tumor tissue = 20h, Eckhardt, 1977[9]

*Volume of 1 g tissue assumed to be 1 mL

SAFETY & TOLERABILITY:

Protocol DLM-10-001 was designed to establish a safe dosing regimen for VAL-083 in refractory glioblastoma patients for advancement to registration-directed clinical trials. GBM patients enrolled must have recurrent GBM and have failed both temozolomide and bevacizumab unless one or both are contraindicated. Table 5 describes prior treatments.

Table 5: Prior Treatment of 48 GBM Patients in DLM-10-001

Temozolomide (TMZ)	48 *(100%)*	Bevacizumab (BEV)	46 *(95.8%)*
Radiotherapy (XRT)	47 *(97.9%)*	Failed TMZ + BEV	46 *(95.8%)*
TMZ + XRT	47 *(97.9%)*	OTHER TREATMENTS	38 *(79.2%)*

In studies of VAL-083 conducted by the National Cancer Institute (NCI) in the 1970s and 1980s, a variety of VAL-083 dosing regimens were used to treat cancer patients. The most common regimen was 25-30 mg/m^2/day every 5 days, with re-treatment every 5 weeks.

DelMar's dosing regimen uses a cycle of treatment consisting of intravenous VAL-083 administered on days 1, 2 and 3 of a 3 week cycle. The three day dose regimen was developed to be more patient-friendly than a five day sequence and to take advantage of a shorter platelet nadir and recovery period observed in the literature.

In Phase I dose escalation, no serious adverse events (SAEs) related to VAL-083 were encountered at doses up to 40mg/m^2/day. This dose has the potential advantage of higher acute dose and dose density in comparison to the NCI dosing regimen (Table 6).

Table 6: Comparative exposure NCI vs. DLM-10-001 dosing regimen

DOSING REGIMEN	Single Dose	Cumulative Dose *(@ 35 days)*	Dose Density *(dose per week)*
25 mg/m^2/daily x 5 every 5 weeks	25 mg/m^2	125 mg/m^2	25mg/m^2/week
40 mg/m^2/daily x 3 every 3 weeks	40 mg/m^2	240 mg/m^2	40mg/m^2/week

MGMT & IDH1

High expression of MGMT protein (unmethylated MGMT promoter) and wild-type IDH1 have been correlated with poor outcomes in GBM. The methylation status of the MGMT promoter was characterized by PCR and/or ELISA for nineteen GBM patients enrolled in DLM-10-001; IDH1 status was reported in eleven patients; both were reported in four patients. (Table 2)

Table 2. MGMT and IDH mutation status in DLM-10-001

MGMT Status		IDH1		Both Reported
Unmethylated / High (>67 ng MGMT/mg total protein)	84.2%	Wild Type:	90.9%	Unmethylated & IDH^{wt}: 100%
Methylated / Low (<45ng MGMT/mg total protein)	15.8%	Mutant:	9.1%	

Tumor Response and Outcomes Analysis

Patients were not re-resected prior to treatment with VAL-083 and therefore had a growing recurrent GBM at the time of enrollment. Patients were monitored for tumor response by MRI (RANO). Consistent with un-resected refractory GBM, median progression free survival (PFS) was short at 1.2 months (range 0.2 – 20.1 months). Five of 48 (10.5%) of GBM patients enrolled in study DLM-10-001 were reported to have stable disease as their best response following treatment with VAL-083; 43 (89.5%) reported progressive disease.

Study DLM-10-001 is ongoing and outcomes analyses are continuing.

Ad-hoc subgroup analysis of Phase 1 (dose-escalation) supports an observed dose response trend. (Table 3).

Based on observed pharmacokinetics, DelMar believes that doses equal to or above 20 mg/m^2 daily x 3 days every 21 days may deliver sufficient level of VAL-083 to brain tumors to achieve therapeutic benefit. GBM patients failing bevacizumab have a poor prognosis with expected survival under five months.

To date, more than half of patients receiving an assumed therapeutic dose of VAL-083 have survived more than six months following bevacizumab failure; more than 40% have survived for 9 months or are currently alive and more than 20% have survived for 12 months or are currently alive following bevacizumab failure (Table 4).

Table 3. Phase 1 survival at 6, 9 and 12 months for refractory GBM patients receiving high dose (30 or 40 mg/m^2/d) or low dose (up to 5 mg/m^2/d) VAL-083

Dose Cohort Subgroups	6 months	9 months	12 months
High (30 & 40 mg/m2n=6)	67%	67%	33%
Low (up to 5mg/m2n=10)	44%	33%	22%

Table 4: DLM-10-001 Survival at Therapeutic Doses (>20mg/m^2) to date (study ongoing)

6 months	9 months	12 months
52%	43%	22%

Higher grade and increasing frequency of hematologic toxicities were observed at VAL-083 doses above 40mg/m^2/day. Consistent with the published literature, the observed dose limiting toxicity for VAL-083 is myelosuppression, mainly thrombocytopenia. The platelet nadir occurs at approximately day 18, and recovery is rapid and spontaneous following treatment arrest.

Table 7. Worst observed NCI CTCAE grade in Phase 1: All post-baseline visits through end of study

Hematologic Parameter		VAL-083 Dose (mg/m^2 IV daily x 3 every 21 days)				
		≤20	30	40	45	50
CTCAE Grade		Hematologic adverse events observed by number of subjects treated (%)				
Anemia (Hg)	G1	8/17 (47.0%)	2/3 (67%)	1/3 (33%)	2/4 (50%)	5/7 (71%)
	G2	1/17 (5.9%)	-	1/3		1/7 (14%)
	G3	2/17 (11.8%)	-	-		-
	G4	-	-	-		
Leukopenia (WBC)	G1	4/17 (23.5%)	1/3 (33%)	1/3 (33%)	-	3/7 (43%)
	G2	-	-	1/3 (33%)	-	2/7 (29%)
	G3	-	1/3 (33%)	-	-	3/7 (43%)
	G4	-	-	-	2/4 (50%)	-
Neutropenia (ANC)	G1	2/17 (11.85)	1/3 (33%)	-	-	-
	G2	-	1/3 (33%)	-	-	-
	G3	-	-	-	-	3/7 (43%)
	G4	-	-	-	2/4 (50%)	1/7 (14%)
Thrombocytopenia (Platelets)	G1	5/17 (29.4%)	3/3 (100%)	3/3 (33%)	-	2/7 (29%)
	G2	1/17 (5.9%)	-	-	-	1/7 (14%)
	G3	-	-	-	1/4 (25%)	3/7 (43%)
	G4	-	-	-	2/4 (50%)	1/7 (14%)
Number of subjects with dose-limiting toxicities (DLTs)		**0**	**0**	**0**	**2**	**2**

14 additional patient were enrolled in a Phase 2 expansion cohort. Consistent with Phase 1, the VAL-083 dose of 40 mg/m^2/daily x 3 every 21 was generally well-tolerated in Phase 2. **With only one exception, no dose limiting toxicities were observed in Phase 2.**

One subject previously treated with CCNU reported Grade 4 thrombocytopenia (platelet count 10,000/μL on Day 17). As a result of this observation, the protocol inclusion criterion for platelet count was increased to150,000/μL for patients receiving prior nitrosourea within 12 weeks preceding enrollment.

Conclusions and Next Steps

- ***A well-tolerated VAL-083 dosing regimen of 40 mg/m^2/daily x 3 every 21 days has been selected for advancement into a Phase 3 study of VAL-083 for refractory GBM***
- ***Phase 3 study design and initiation shall be determined in consultation with the USFDA during a meeting planned for the first half of 2016***
- ***Outcomes analysis of DLM-10-001 are ongoing. Results to date support the potential of VAL-083 to offer a new treatment option to GBM patients who have failed or are unlikely to respond to currently available treatment regimens***