Dianhydrogalactitol (VAL-083) offers potential therapeutic alternatives in the treatment of pediatric brain tumors

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ABSTRACT # A51:

Background: Medulloblastoma (MB) is the most common malignant pediatric brain tumor, accounting for 15-30% of all childhood intracranial neoplasms. High grade gliomas (HGG) are much rarer in children than in adults, comprising only 5%–10% of childhood brain tumors. Although multidisciplinary treatment has improved the 5-year survival rates in children significantly, the prognosis for recurrent MB and HGG remains poor with median overall survival <1 year. Temozolomide (TMZ) is frequently employed in the treatment of MB and pediatric HGG; however, clinical evidence is lacking and poor outcomes due to high-expression of the repair protein O^6-methylguanine-DNA methyltransferase (MGMT), which is correlated with TMZ resistance, have been reported. Dianhydrogalactitol (VAL-083) is a structurally unique bi-functional alkylating agent causing DNA crosslinks at N^7 position of guanine. VAL-083 readily crosses the blood brain barrier and has been shown to accumulate in brain tumor tissue. Furthermore, VAL-083 demonstrated clinical activity against MB and HGG in historical NCI-sponsored clinical studies. We have recently shown that VAL-083 demonstrates cytotoxic activity in GBM independent of MGMT expression *in vitro* and *in vivo*. We have further shown that VAL-083 is highly effective against GBM cancer stem cells (CSC) and non-CSC and that it acts as a radiosensitizer in GBM cell lines, *in vitro*. VAL-083 is currently in phase II clinical trials for recurrent GBM in adults. In the current adult GBM clinical trial VAL-083 displayed a favorable safety-profile and preliminary analysis supports a survival benefit at doses chosen for further investigation. Based on these recent results and data supporting VAL-083's clinical activity in historical MB and HGG studies, we sought to investigate the cytotoxic activity of VAL-083 as a potential therapeutic alternative for pediatric brain tumors by studying the drug against MB and pediatric HGG cell lines *in vitro*.

BACKGROUND

VAL-083 is currently undergoing Phase II clinical studies as a potential treatment for refractory glioblastoma multiforme (GBM) in adults and has received orphan drug designation in EU and the U.S. for the treatment of gliomas. VAL-083 is a bifunctional alkylating agent causing interstrand DNA crosslinks at N7 of guanine leading to cell cycle arrest in the S/G2 phase and cell death due to DNA double strand breaks.[1,2,3] The mechanism is believed to be distinct from the mechanisms of other alkylating agents (e.g. temozolomide, cisplatin or BCNU) and we have recently shown that VAL-083 overcomes TMZ-resistance and cisplatin-resistance, *in vitro*. Recent research suggests that the cytotoxic mechanism is much less dependent on p53 for activity in comparison to commonly used agents, such as platinum-based chemotherapies. VAL-083 has demonstrated activity against medulloblastoma and glioma cell lines *in vitro*, including TMZ-resistant GBM cancer stem cells (CSC).[4,5] During the 1970s and 1980s, VAL-083 was investigated in a number of NCI-sponsored clinical trials, both as a stand-alone therapy and in combination with other chemotherapeutic regimens. Trials showed clinical activity in a range of brain tumor types, including medulloblastoma[6,7] and high-grade gliomas[8]. In a Phase II clinical trial in refractory medulloblastoma, treatment with dibromodulcitol (DBD) - which is spontaneously hydrolyzed *in situ* to VAL-083 - resulted in stable disease for 40% of patients (8/20) whose tumors had recurred following extensive prior chemo-therapy, including vincristine, CCNU and procarbazine.[1] This suggests a lack of cross-resistance between VAL-083 chemotherapeutic drugs commonly used in the treatment of medulloblastoma today, and supports the potential utility of VAL-083 in the treatment of chemo-resistant medulloblastoma, including SHH subgroup patients with p53 mutations and group 3 tumors as a single agent or as part of combination therapeutic regimens.

Table 1. Historical clinical trials showing activity of VAL-083 for the treatment of medulloblastoma

Reference	Enrollment/ Evaluation	Design	Drug dose and regimen	Diagnosis Inclusion	Results
Levin V, *et al*. 1984[6]	20/20	Open-label, single arm dibromodulcitol (DBD)→VAL-083	DBD: oral 100 mg/m^2 daily x 45 days or until WBC or platelet count <50% of pretreatment	Chemo-resistant recurrent medulloblastoma (adult and pediatric)	40% Stable disease (8/20) 10% Tumor regression (2/20) No progression after 4 years (4/20)
Finklestein *et al.* 1985[7]	9/8	Open-label, single arm VAL-083	VAL-083: IV 50 mg/m^2/day twice a week for 4 weeks	Pediatric cyclophosphamide-resistant refractory medulloblastoma	13% partial response (1/8)

Table 2. Historical clinical data of TMZ or VAL-083 in GBM support the potential for comparable or enhanced survival to standard-of-care chemotherapy

Chemotherapeutic Agent	MGMT Promoter Status	XRT Alone	XRT + Chemotherapy	p-value
		median survival (mo.)		
LOMUSTINE[9]	n/a	n/a	**11.9**	n/a
CARMUSTINE[9]	n/a	n/a	**9.2 - 11.5**	n/a
SEMUSTINE[9]	n/a	n/a	**8.0**	n/a
TMZ[10]	Methylated *(low MGMT expression)*	15.3	**21.7**	0.007*
	Unmethylated *(high MGMT expression)*	11.8	**12.7**	0.06
VAL-083[8]	n/a	8.8	**16.8**	0.02*

**statistically significant improvement p-value by log-rank test*

References

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Proposed clinical trial design

Results of further *in vitro* research will be used to guide clinical strategy for development of VAL-083 as a potential new therapy for treatment of pediatric MB and pediatric HGG. The goal is to stratify patients based on their specific molecular subtype and target VAL-083-responsive difficult-to-treat tumors. However initially, **VAL-083 will be tested in recurrent patients with MB or HGG**. By focusing on "high-risk molecular" patients stratified based on their molecular subtype, we hope to provide a new treatment option for difficult-to treat pediatric tumors of the central nervous system such as HGG, SHH subgroup patients with p53 mutations and group 3 tumors

Primary goal

To establish the maximum tolerated dose in children

Secondary goals

To estimate the efficacy of VAL-083, as measured by objective radiographic response
To evaluate progression-free survival (PFS) at 6 months
To evaluate median OS
To evaluate the safety profile of VAL-083 treatment

Study duration

The study will be considered complete when the last patient either experiences disease progression or an intolerable toxicity, or withdraws from the study.

Figure 2. Cell viability analysis at day 6 post treatment for paired CSC and non-CSC MGMT-expressing 7996 GBM cells. MGMT-expressing GBM cancer cells and MGMT-expressing GBM cancer stem cells were treated with either TMZ at high doses (50μM) or VAL-083 at a clinically relevant dose (5μM) either with or without 2Gy of radiation and compared to untreated control (nul). TMZ exhibited no activity against MGMT-expressing GBM tumor cells in comparison to control with or without radiation. However, VAL-083 was active against both MGMT-expressing GBM tumor cells and MGMT-expressing GBM cancer stem cells.

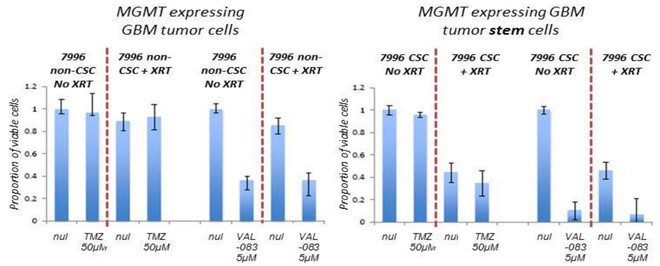


Figure 1. VAL-083 is active against medulloblastoma cell lines with SHH characteristics, including p53 mutated Daoy and UW228

VAL-083 inhibited growth of four pediatric medulloblastoma cell lines, *in vitro*. VAL-083 was most active in Daoy and UW228 cells, which are known to have the particularly difficult-to-treat combination of SHH characteristics and p53 mutations. This suggests that VAL-083 may be a valuable treatment option for chemo-resistant medulloblastoma of the SHH subtype with p53 mutations.

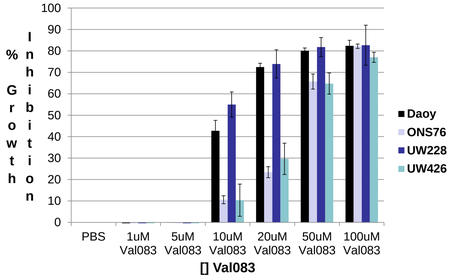
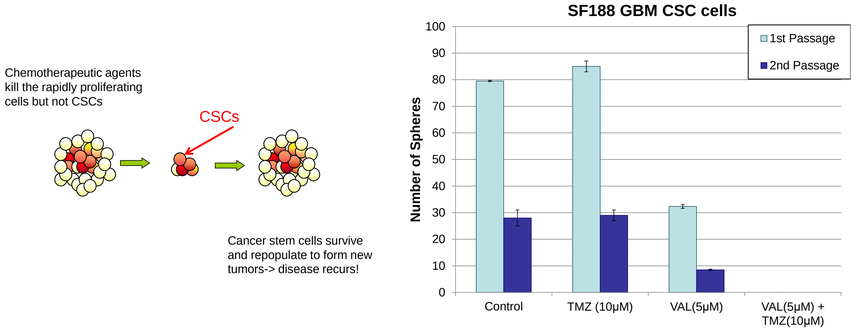


Figure 3. VAL-083 inhibited self-renewal of pediatric GBM cancer stem cells (CSC) SF188. Sequential combination with TMZ completely inhibited self-renewal of SF188.

Brain cancer stem cells are thought to give rise to relapse of the brain tumor as they are resistant to conventional therapies. Using a 10-day colony formation assay, VAL-083 (5 μM) alone suppressed SF188 growth by ~90%. VAL-083 in combination with TMZ completely inhibited the formation of spheres. For the combination treatment, cells were treated with 10 μM TMZ first (3d) followed by 5 μM VAL-083 (3d).



CONCLUSIONS

- **Historical clinical activity combined with modern research suggests VAL-083 may be valuable as a therapy for difficult-to-treat or resistant pediatric brain tumors**
  - ✓ **VAL-083 retains cytotoxic activity in the presence of p53 mutations in multiple models including p53 mutated medulloblastoma cells with SHH characteristics**
  - ✓ **VAL-083 overcomes MGMT-related TMZ-resistance in GBM cancer stem cells (CSCs) and non-CSCs**
  - ✓ **VAL-083 is active against resistant CSCs and the combination with TMZ completely inhibits self-renewal of pediatric GBM CSCs**