Dianhydrogalactitol (VAL-083) has the potential to overcome major challenges in the treatment of DIPG

Anne Steino[1], Xiaodong Yang[2,3], Beibei Zhai[4,5], Jeffrey A. Bacha[1], Dennis M. Brown[1], Mads Daugaard[4,5], Sabine Mueller[2,3]

[1]DelMar Pharmaceuticals, Inc., Vancouver, BC, Canada and Menlo Park, CA, USA; [2]Department of Neurosurgery and [3]Department of Neurology and Pediatrics, University of California, San Francisco, CA, USA; [4]Vancouver Prostate Centre, Vancouver, BC, Canada; [5]Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

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Despite decades of clinical trials, children with diffuse intrinsic pontine gliomas (DIPG) continue to have a very poor prognosis and dismal survival. DIPG is inoperable and standard treatment is radiation alone. Major obstacles to the successful treatment of DIPG include:

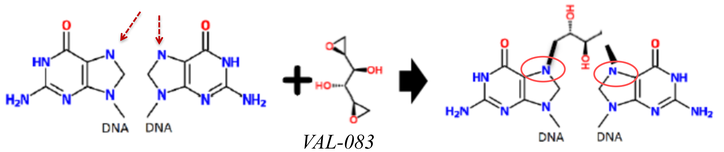
1) *Intact blood brain barrier impeding drug penetration*
2) *Inherent tumor cell resistance mechanisms to conventional chemotherapeutics*
3) *Lack of drug-induced potentiation of radiotherapy*

VAL-083 is a novel bi-functional DNA targeting agent that readily ***crosses the blood-brain barrier and accumulates in brain tumor tissue***. In prior NCI-sponsored clinical trials, VAL-083 was well-tolerated and demonstrated activity against pediatric brain tumors, including pediatric high-grade glioma and medulloblastoma[1,2]. VAL-083 ***overcomes MGMT-related resistance mechanisms*** and is equally active against GBM cancer stem cells and non-stem cells and ***potentiates the effect of radiation in adult GBM cells***, *in vitro*.

VAL-083 overcomes MGMT-mediated chemoresistance

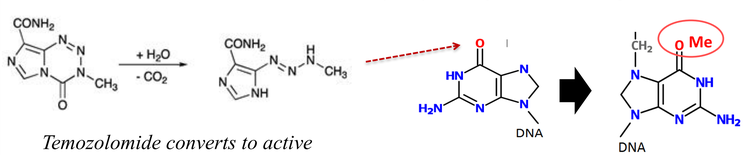
VAL-083 is a novel bi-functional DNA targeting agent that rapidly induces interstrand cross-links at N7-guanine, leading to DNA double-strand breaks (DSBs) and ultimately cell death. VAL-083's N7-targeting mechanism differs from TMZ and nitrosoureas, enabling VAL-083 to overcome MGMT-mediated chemoresistance.

Mechanism of VAL-083 via crosslinks at N7 of guanine



2 guanine bases on DNA — *VAL-083* — *VAL-083 – interstrand crosslink (ICL)*

Mechanism of temozolomide (TMZ) via alkylation at O6 of guanine



Temozolomide converts to active state (MTIC) at physiological pH — *Alkylation of O6 guanine*

FIGURE 1. *The N7-targeting mechanism of action of VAL-083 differs from those of O6-alkylating agents like temozolomide and nitrosoureas.*

References

1. Levin V, et al. J Neurosurg 1984(61): 1063-68
2. Finklestein JZ, et al. Cancer Treat Rep 1985 (69): 1331-33
3. Fouse S, et al. *Neuro-Oncology* 2014(16)
4. Hu, S.E et al. Cancer Res. 2012(72)

CONCLUSIONS

- **Historical clinical activity combined with modern research suggests VAL-083 may be valuable, either alone or as part of a combination therapy, for difficult-to-treat or resistant pediatric high-grade gliomas including DIPG**
  - ✓ **VAL-083 is active against DIPG cell lines with varying genetic profiles including p53 and H3.3/H3.1 K27M mutations.**
  - ✓ **VAL-083 is synergistic with Wee1 inhibitor AZD1775 against DIPG cell lines with varying genetic profiles and pediatric GBM cells.**
  - ✓ **Low-dose VAL-083 acted as a radiation potentiator against adult GBM cancer stem cells (CSCs) and non-CSCs.**
  - ✓ **VAL-083 overcomes MGMT-related TMZ-resistance in GBM CSCs and non-CSCs.**

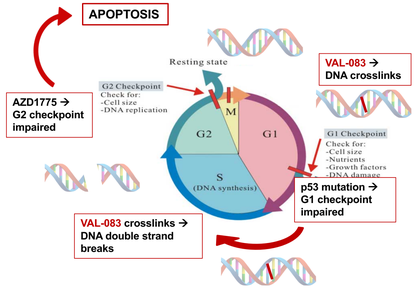


FIGURE 2. VAL-083 induces interstrand crosslinks leading to replication-induced double-strand breaks and S/G2 cell cycle arrest. AZD1775 eliminates the G2 checkpoint allowing cells with DNA damage to continue their cell cycle leading to cell death.

VAL-083 displayed synergy with Wee1 inhibitor AZD1775 in DIPG and GBM cells.

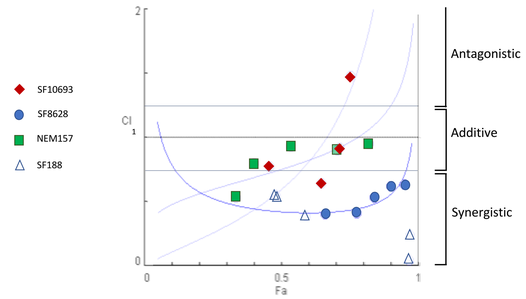


FIGURE 4. *VAL-083 exhibited synergy with Wee1 inhibitor AZD1775 in DIPG cell lines SF8628, pediatric GBM cell line SF188 and additive effect in DIPG cell line NEM 157. Proliferation/viability was quantified using CellTiter-Glo® after 3 days of concomitant treatment.*

VAL-083 was active against pediatric DIPG and GBM cells, *in vitro.*

VAL-083 inhibited proliferation/growth of three biopsy-derived pediatric DIPG and one GBM cell line with varying genomic profiles, *in vitro*.

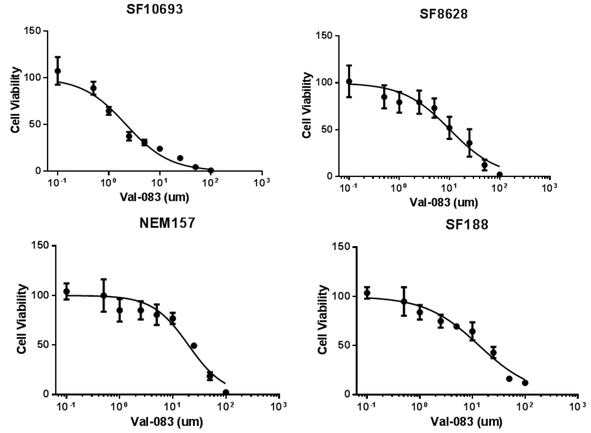


TABLE 1: IC_{50} values of VAL-083 in SF10693, SF8628, NEM157 and SF188 tumor cells. N=3

Cell line	SF10693	SF8628	NEM157	SF188
Tumor type	DIPG	DIPG	DIPG	GBM
Histone mutations	H3.1 K27M	H3.3 K27M	H3.3 K27M	WT
p53	WT	WT	Mutant	Mutant
VAL-083 IC_{50}	1 μM	5 μM	10 μM	10 μM

FIGURE 3. *VAL-083 inhibited proliferation/growth of three biopsy-derived pediatric DIPG and one GBM cell lines with varying genomic profiles, in vitro. Proliferation/viability was quantified using CellTiter-Glo® Luminescent Cell Viability Assay Kit after 3 days of treatment.*

VAL-083 overcame TMZ-resistance in both GBM tumor cells and GBM tumor stem cells (CSCs) independent of MGMT.

In addition, when CSC cultures were treated with low dose VAL-083 (1μM) with or without 2Gy radiation, VAL-083 acted as a radio-potentiater against CSC's in all cultures tested[3].

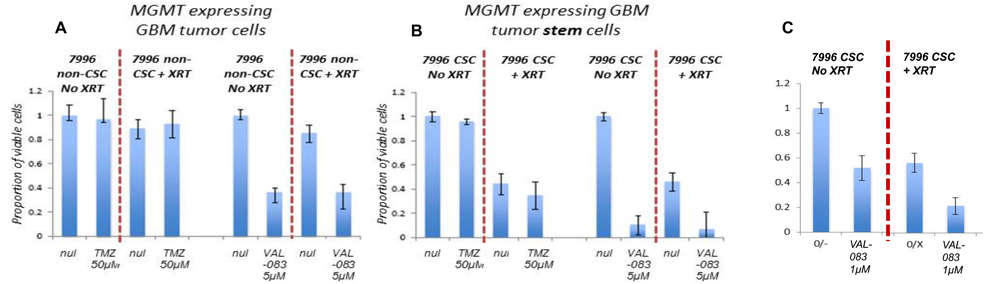


FIGURE 5. *Cell viability analysis at day 6 post treatment for the paired non-CSC (A) and CSC (B,C) MGMT-expressing 7996 cultures. MGMT expressing GBM tumor cells and GBM tumor stem cells were treated with TMZ (50 μM) or VAL-083 5 μM (A, B) or 1 μM (C) either with or without radiation (2Gy).[3]*

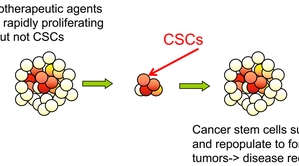


TABLE 2: IC_{50} values of VAL-083, TMZ and lomustine in SF188, Med8a and adult T98G tumor cells. N=3

Cell line		SF188	Med8a	T98G[4]
MGMT expression		High	Low	High
p53 status		Mutant	Wild type	Mutant
IC_{50}	**VAL-083**	**0.4 μM**	**1.6 μM**	**1.8 μM**
	TMZ	>>100 μM	15.2 μM	>>100 μM
	lomustine	5.5 μM	6.8 μM	n/a