Dianhydrogalactitol (VAL-083) overcomes chemoresistance in pediatric malignant brain tumors and displays synergy with topoisomerase inhibitors

Beibei Zhai[1,2], Anne Steino[3], Jeffrey Bacha[3], Dennis M. Brown[3], Jie Zhang[5], Theodore Nicolaides[5], Mads Daugaard[1,2]

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

ABSTRACT #5248

More children die from brain cancer than from any other disease. Medulloblastoma (MB) and pediatric high-grade gliomas (pHGG) are the most common malignant brain cancers in children. Children with pHGG have few therapeutic options and 5-year survival is less than 20%. Treatment includes surgery, radiotherapy and various chemotherapeutic combinations often including topoisomerase inhibitors and/or temozolomide (TMZ). The expression of the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) is strongly correlated with TMZ-resistance and is highly expressed in many pHGG, and deficient DNA mismatch repair (MMR) (25% of pHGG) confers a secondary mechanism of TMZ-resistance.

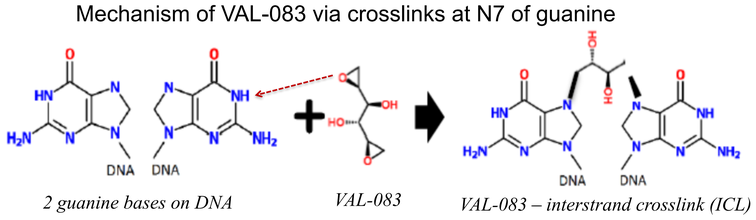
VAL-083 is a novel bi-functional DNA targeting agent that readily ***crosses the blood-brain barrier and accumulates in brain tumor tissue***. In prior NCI-sponsored clinical trials, ***VAL-083 was well-tolerated*** and demonstrated activity against pediatric brain tumors, including pHGG and MB. VAL-083 overcomes MGMT-related resistance mechanisms and is equally active against HGG cancer stem cells and non-stem cells, *in vitro*. Here, we show that VAL-083 overcomes resistance to TMZ and is active against HGG and MB cell lines, independent of their MGMT, MMR and p53 status *in vitro*. We further show that VAL-083 displays synergy with topoisomerase I and II inhibitors *in vitro*.

BACKGROUND

VAL-083 overcomes MGMT-mediated chemoresistance

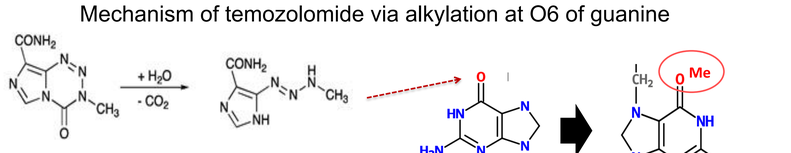
VAL-083 is a novel bi-functional DNA targeting agent that rapidly induces interstrand cross-links at N7-guanine, leading to DNA double-strand breaks (DSBs) and ultimately cell death. The N7-targeting mechanism differs from TMZ and nitrosoureas, enabling VAL-083 to overcome MGMT-mediated chemoresistance.

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 via alkylation at O6 of guanine



Temozolomide converts to active state (MTIC) at physiological pH — *guanine base on DNA* — *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.*

VAL-083 is a DNA-targeting agent with a unique mechanism of action

VAL-083 is a bifunctional DNA-targeting agent, with a mechanism of action that differs from other DNA-targeting agents.[1] VAL-083 rapidly introduces DNA interstrand crosslinks (ICLs) at the N^7-position of guanine leading to persistent DNA DSBs, S/G2 phase cell cycle arrest and activation of the homologous recombination (HR) repair pathway**.** The DNA DSBs and HR activation persists for 24-72h after VAL-083 pulse treatment, ultimately ***leading to cell death through two parallel pathways: p53-dependent*** *and* ***p53-independent*** (Figure 2).[2]

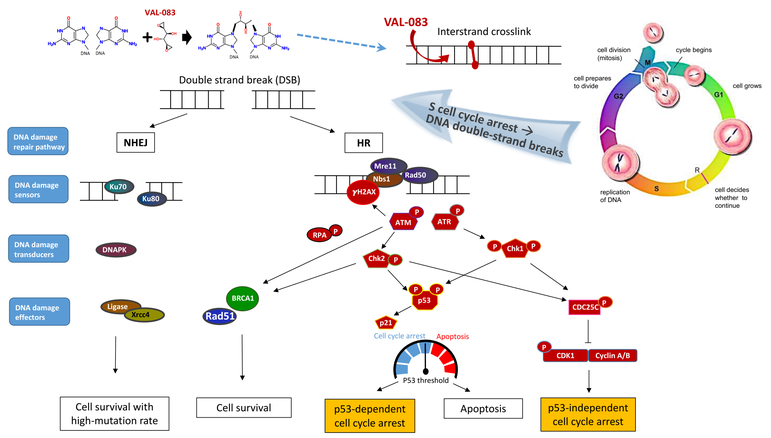


FIGURE 2. *Mechanisms of action of VAL-083 induced chemotherapeutic cytotoxicity. Apoptosis can be induced through either p53 dependent or p53 independent pathways. Red color signifies VAL-083-induced activation.[1,2]*

This distinct mechanism of action of VAL-083 suggests that VAL-083 may offer a treatment alternative against tumors with p53-, MMR-, or MGMT-mediated resistance to chemotherapeutic agents, including temozolomide and nitrosoureas.

VAL-083 ACTIVITY IS INDEPENDENT OF MGMT AND MMR DNA DAMAGE RESPONSE

The mechanism of action of VAL-083 differs from other alkylating agents and ***overcomes both MGMT- and MMR-related resistance to temozolomide, in vitro.***

VAL-083 cytotoxic activity overrides MGMT-mediated chemoresistance to TMZ and lomustine in pediatric (SF188) and adult (T98G) HGG and medulloblastoma (Med8a) cell lines, independent of p53-status (Figure 3).

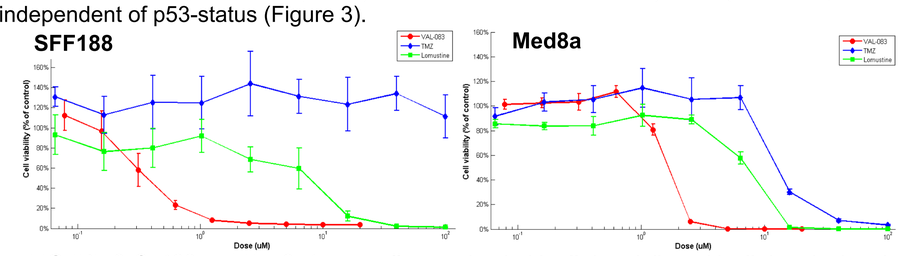


FIGURE 3. SF188 and Med8a tumor cells were treated for 3 days followed by 3 days in drug-free media with VAL-083, temozolomide or lomustine. Viable cells were quantified using CellTiterGlo. N=6.

TABLE 1: *IC_{50} values of VAL-083, TMZ and lomustine in pHGG SF188, MB Med8a and adult HGG T98G tumor cells. N=3*

Cell line		SF-188	Med8a	T98G[3]
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

VAL-083 cytotoxic activity is independent of the cancer cell mismatch repair (MMR) status, suggesting that VAL-083 can overcome this secondary TMZ-resistance mechanism (Figure 4).

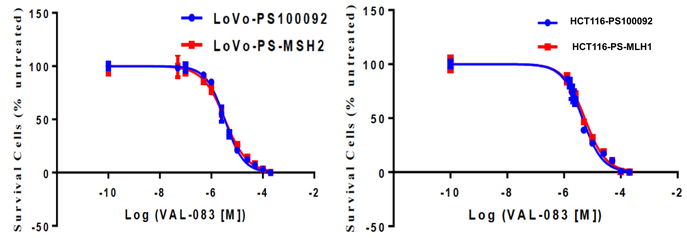


FIGURE 4. *Cytotoxicity of VAL-083 in isogenic human colorectal cancer cell lines using the crystal violet assay. MMR-proficient cell lines, HCT116-PS-MLH1 and LoVo-PS-MSH2, were established by lentiviral infection. HCT116-PS100092 is the MLH1-deficient cell line, HCT116-PS-MLH1 is the MLH1-proficient cell line; LoVo-PS100092 is the MSH2-deficient cell line, and LoVo-PS-MSH2 is the MSH2-proficient cell line. N=3.*

VAL-083 POTENTIATES RADIATION AND IS ACTIVE AGAINST GBM CANCER STEM CELLS

VAL-083 (5 µM) overcame TMZ-resistance in both HGG tumor cells and HGG tumor stem cells (CSCs) independent of MGMT (Figure 5). 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 (Figure 5C)[9].

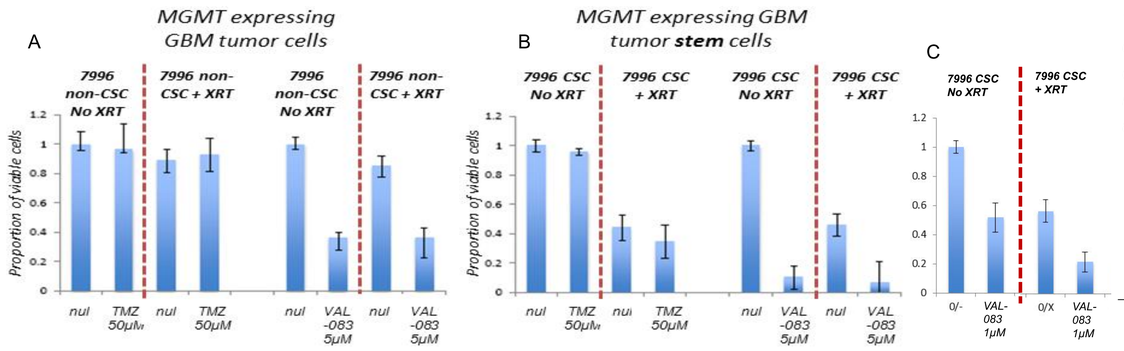


FIGURE 5. *Cell viability analysis at day 6 post treatment for the paired (A) non-CSC and (B,C) CSC MGMT-expressing 7996 cultures. MGMT expressing GBM tumor cells and GBM tumor stem cells were treated with TMZ (50 µM) or VAL-083 (1 or 5 µM) either with or without radiation (2Gy).[9]*

TABLE 2: *Historical data supporting radio-potentiating abilities of VAL-083. cReported median survival of VAL-083 in combination with radiotherapy, and the benefit versus radiotherapy alone is similar or superior to other alkylating agents.*

			Nitrosourea therapy		
XRT +	VAL-083 *(Eagan 1979)[4]*	TMZ *(Stupp 2005)[5]*	BCNU *(Walker 1976)[6]*	CCNU *(Reagan 1976)[7]*	ACNU *(Takakura 1986)[8]*
Median survival (months)	16.8	14.6	12.5	13.0	8.8
Benefit vs. XRT alone	***8.4***	*2.5*	*2.5*	*1.2*	*n/a*

VAL-083 DISPLAYS SYNERGY WITH TOPOISOMERASE INHIBITORS

The distinct mechanism of action of VAL-083 makes it a valuable partner for combination therapies with agents already used in the treatment of GBM and other CNS tumors.

- As VAL-083 induces cell cycle arrest initially in S- followed by G2/M-phase, we predicted synergy with agents that require cancer cells to be in S/G2-phase for maximum effect, including topoisomerase inhibitors. As expected, **VAL-083 demonstrated synergy with etoposide** *(TOP-2 inhibitor)* **and camptothecin** *(TOP-1 inhibitor)* (Table 3).

TABLE 3. *VAL-083 demonstrates synergy with etoposide (TOP2 inhibitor) and camptothecin (TOP1 inhibitor) in PC3 prostate and A549 NSCLC cancer cells. CI values for the cytotoxic effect (Fa). CI<1 shows synergy. N=4-5.[8]*

Cell line	Etoposide *(topoisomerase II inhibitor)*		Camptothecin *(topoisomerase I inhibitor)*	
	Cytotoxic effect (**Fa**)	Combination index (**CI**)	Cytotoxic effect (**Fa**)	Combination index (**CI**)
PC3	**ED50**	0.58	**ED75**	0.68
	ED75	0.48	**ED90**	0.59
	ED90	0.42	**ED95**	0.54
A549	**ED50**	0.72	**ED85**	0.94
	ED75	0.88	**ED90**	0.87
	ED80	0.94	**ED95**	0.77

Molar ratios: VAL-083:etoposide 5:1 in PC3 and 5:1 in A549; VAL-083:camptothecin 250:1 in PC3 and 212:1 in A549

Chemotherapy	Temozolomide[12]	Nitrosoureas[11,12]	VAL-083[9,10,11]
Cytotoxic target	O6-Guanine	O6-Guanine	N7-Guanine
DNA damage	Base mismatch Single-strand break	Interstrand crosslinks (G-C) Double-strand break	Interstrand crosslinks (G-G) Double-strand break
Cell cycle arrest	G2/M	G2/M	Late S/G2
ATM-Chk2	activated	activated	activated
MGMT	dependent	dependent	independent
MMR	dependent	independent	independent
p53	dependent	dependent	independent

CONCLUSIONS

- **VAL-083 displays a distinct anti-cancer mechanism enabling it to overcome MGMT-mediated chemo-resistance to temozolomide and nitrosoureas**
- **VAL-083 is able to overcome MMR-mediated chemo-resistance, *in vitro***
- **Low-dose VAL-083 potentiates radiation therapy**
- **VAL-083 displays synergy with topoisomerase inhibitors, *in vitro***
- **VAL-083 is equally active against GBM cancer stem cells and non-cancer stem cells, *in vitro***

References:

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