Dianhydrogalactitol (VAL-083) overcomes p53-mediated chemo-resistance and displays synergy with topoisomerase inhibitors

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Abstract #HGG-02

Children with pediatric high-grade gliomas (pHGG) have few therapeutic options and 5-year survival is less than 20%. Relapsed pHGG have a uniformly fatal outcome despite all available treatments. The standard treatment for HGG is surgery followed by radiotherapy and temozolomide (TMZ) although the efficacy of TMZ in children is negligible. 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 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 medulloblastoma (MB). VAL-083 rapidly induces interstrand cross-links at N7-guanine causing DNA double-strand breaks and persistent activation of the homologous recombination DNA repair pathway. VAL-083 overcomes TMZ-resistance and is equally active against GBM cancer stem cells (CSCs) and non-CSCs independent of MGMT status, *in vitro*. We recently showed that VAL-083 induces irreversible S/G2-phase cell-cycle arrest, thus proposing synergy with S-phase specific chemotherapeutics, including topoisomerase inhibitors (TOPi).

RESULTS: We report strong VAL-083 activity in Med8a (MB, p53-wild type) and SF188 (pHGG, p53-mutated) tumor cells, independent of MGMT. Further, we report synergy between VAL-083 and inhibitors of both topoisomerase I and II (camptothecin and etoposide) in A549 and PC3 cancer cell lines. Our results support a distinct anti-cancer mechanism for VAL-083 compared to standard HGG chemotherapy, and support the potential of VAL-083 to overcome resistance to TMZ and nitrosoureas and for synergy as part of a TOPi-combination regimen.

BACKGROUND

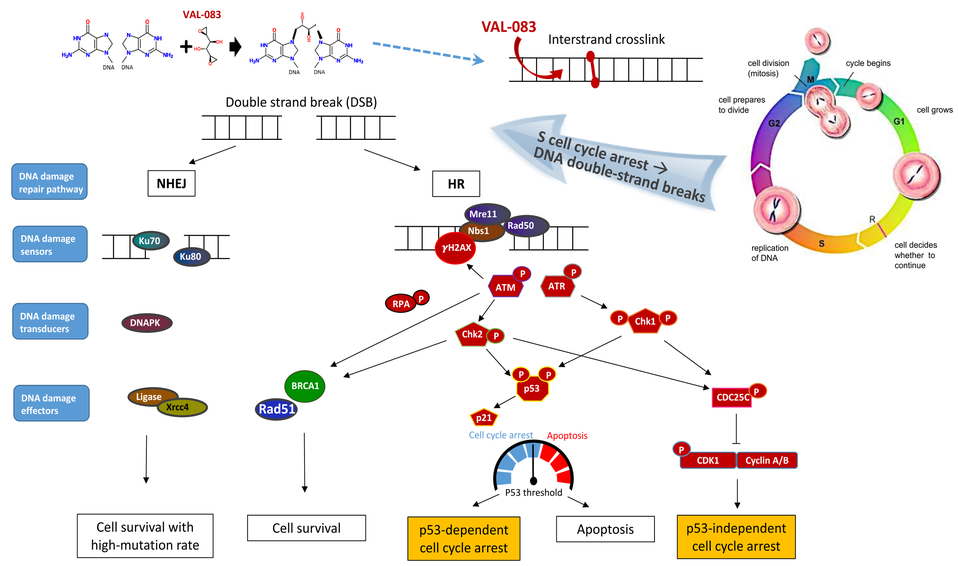

FIGURE 1. VAL-083 induces interstrand crosslinks at N7-guanines leading to DNA double-strand breaks and activation of the homologous recombination (HR) DNA repair pathway, mediating cell cycle arrest through p53-dependent and p53-independent pathways[1,2,3]. Red color signifies demonstrated activation/expression after VAL-083 treatment.

CONCLUSIONS

- **VAL-083 displays a distinct anti-cancer mechanism enabling it to overcome MGMT-mediated chemo-resistance to temozolomide and nitrosoureas**
- **VAL-083 displays synergy with topoisomerase inhibitors**
- **VAL-083 displays synergy with temozolomide**
- **VAL-083 induces persistent cell cycle arrest through a p53-dependent and a p53-independet pathway**
- **VAL-083 displays activity against GBM cancer stem cells**
- **VAL-083 is able to overcome MMR-mediated chemo-resistance**

Chemotherapy	Temozolomide[7]	Nitrosoureas[6,7]	VAL-083[2,4,5,6]
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

CURRENT VAL-083 GBM CLINICAL TRIALS.

Scan your smart phone and link to VAL-083 trials on clinicaltrials.gov

1. ***Enrolling:*** *Open label single-arm, biomarker-driven, **Phase 2** study of VAL-083 in patients with MGMT-unmethylated, bevacizumab-naive recurrent glioblastoma*
2. ***Planned (2017):*** *Pivotal, randomized multi-center **Phase 3** study in bevacizumab-failed GBM.*
3. ***Planned (2017):*** *Open label, single-arm, biomarker-driven, **Phase 2** study of VAL-083 and radiation therapy patients with in newly diagnosed MGMT-unmethylated GBM*

MGMT-INDEPENDENCE

VAL-083 cytotoxic activity overrides MGMT-mediated chemoresistance to temozolomide (TMZ) and lomustine.

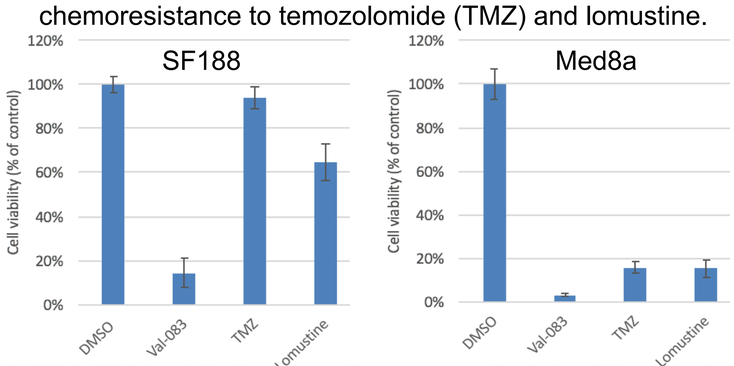

FIGURE 2. SF188 and Med8a tumor cells were treated for 3 days followed by 3 days in drug-free media with VAL-083, temozolomide or lomustine at their respective IC50 concentrations (see table 2). Viable cells were quantified using cell titer glo. N=3.

		SF-188	Med8a
MGMT expression		High	Low
IC50	**VAL-083**	0.4 µM	1.6 µM
	TMZ	>>100 µM	15.2 µM
	Lomustine	5.5 µM	6.8 µM

TABLE 2: IC50 values of VAL-083, TMZ and lomustine in SF188 pHGG and Med8a MB tumor cells. N=3

VAL-083 IS ACTIVE AGAINST GBM CSCs

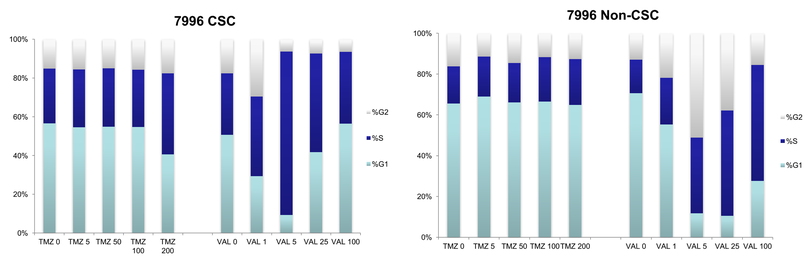

FIGURE 3. VAL-083 is active against TMZ-resistant GBM stem and non-stem cells at low µM doses, suggesting the ability to overcome TMZ-resistance in chemo-refractory GBM cancer stem cells.[4]

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 induce cell cycle arrest 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).
- VAL-083 demonstrated **synergy with TMZ in GBM cancer stem cells** completely eliminating cancer stem cell spheres after 2 passages (Figure 4).[5]

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

VAL-083 DISPLAYS SYNERGY WITH TEMOZOLOMIDE

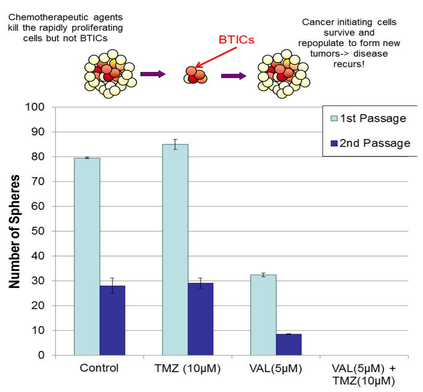

FIGURE 4. VAL-083 demonstrates potential synergy with TMZ in GBM cancer stem cell line BT74. N=3.

MMR-INDEPENDENCE

VAL-083 cytotoxic activity is independent of cancer cell's Mismatch Repair (MRR)-status.

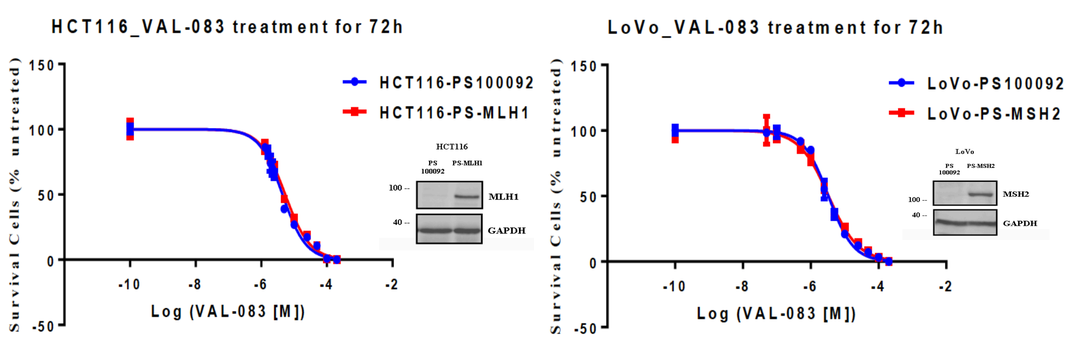

FIGURE 5. Cytotoxicity of VAL-083 in isogenic human colorectal cancer cell lines using the crystal violet assay. MMR-proficient cell lines, HCT116-PS-MLH1 and LoVo-PS-MSH2, were established by lentiviral infection. HCT116-PS100092 is the MLH1-deficient cell line, HCT116-PS-MLH1 is the MLH1-proficient cell line; LoVo-PS100092 is the MSH2-deficient cell line, and LoVo-PS-MSH2 is the MSH2-proficient cell line. N=3.

References:

1. ***Zhai et al.*** *AACR meeting 2017, Abstr. #6431*
2. ***Steino et al.*** *AACR meeting 2017, Abstr. #1429*
3. ***Peng et al.*** *Acta Pharmacologica Sinica 2017: 1–10*
4. ***Fouse S, et al.*** *SNO annual meeting 2014, Abstract #ET-18*
5. ***Hu K, et al.*** *Cancer Research, Apr15, 2012; Volume 72 (8) Suppl. 1*
6. ***Institoris E, et al.*** *Cancer Chemother Pharmacol 1989;24(5):311-3*
7. ***Ramirez, YP, et al.*** *Pharmaceuticals 2013, 6(12), 1475-1506 (review)*
8. ***Zhai B, et al.*** *ENA meeting 2016, Abstr. #363*