Distinct mechanism-of-action of dianhydrogalactitol (VAL-083) overcomes chemoresistance in glioblastoma

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Abstract #08.57

BACKGROUND

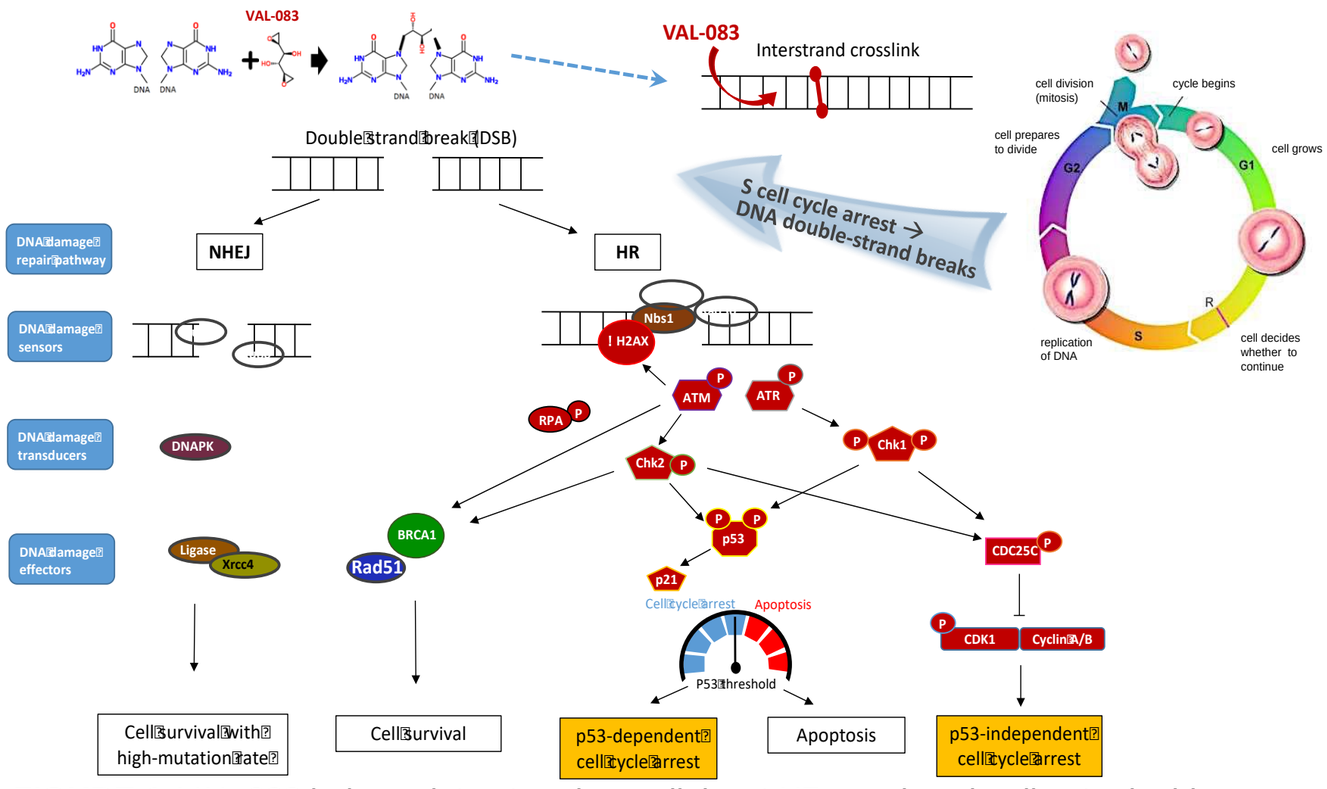


FIGURE 1. VAL-083 induces interstrand crosslinks at N7 guanines leading to double-strand breaks and HR activation independent of MGMT, and mediating cell cycle arrest through p53-dependent and p53-independent pathways[1,2,3]. Red color signifies demonstrated activation/expression after VAL-083 treatment.

VAL-083 is a bi-functional DNA targeting agent that readily **crosses the blood-brain barrier** and has shown activity against GBM in prior NCI-sponsored clinical trials. A Phase I/II clinical trial studying VAL-083 in recurrent GBM, after TMZ and bevacizumab failure, suggested the potential of VAL-083 to offer a clinically meaningful survival benefit. The mechanism-of-action of VAL-083 differs from other chemotherapeutic agents, including TMZ and nitrosoureas, inducing interstrand cross-links at guanine-N^7 causing DNA double-strand breaks and cancer cell death.

CURRENT VAL-083 GBM CLINICAL TRIALS (SEE ABSTR. #09.57)

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*

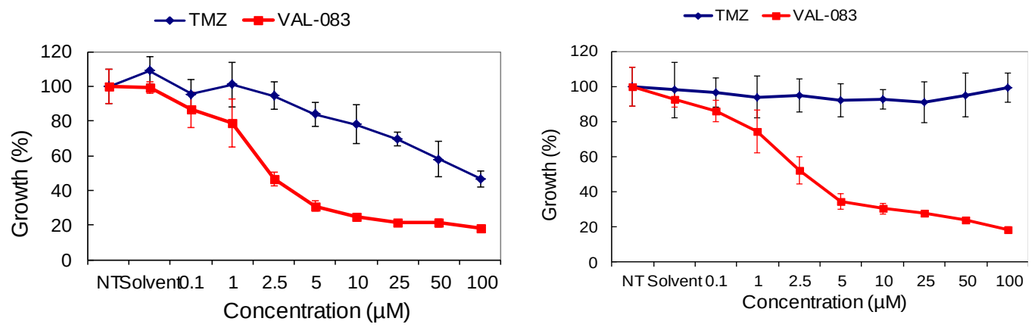
CONCLUSIONS

- **VAL-083 displays a distinct anti-cancer mechanism enabling it to overcome MGMT-mediated temozolomide-resistance**
- **VAL-083 is equally active against GBM cancer stem cells and non-stem cells, independent of MGMT**
- **VAL-083 mediates persistent DNA double-strand breaks and activation of the HR DNA repair pathway**
- **VAL-083 displays increased potency in cancers with impaired HR**
- **VAL-083 displays synergy with topoisomerase and PARP inhibitors**

Alkylating agent	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

MGMT-INDEPENDENCE

VAL-083 cytotoxic activity is independent of MGMT-mediated temozolomide-resistance.



GBM Cell Line	*U251*	*T98G*
MGMT promoter methylation	Methylated (low expression)	Unmethylated (high expression)
IC_{50} VAL-083	2.5μM	2.5μM
IC_{50} TMZ	10.0μM	>>100μM

FIGURE 2. TMZ vs. VAL-083 in Adult GBM Cell Lines (3000 cells/well, 72-h exposure)[5]

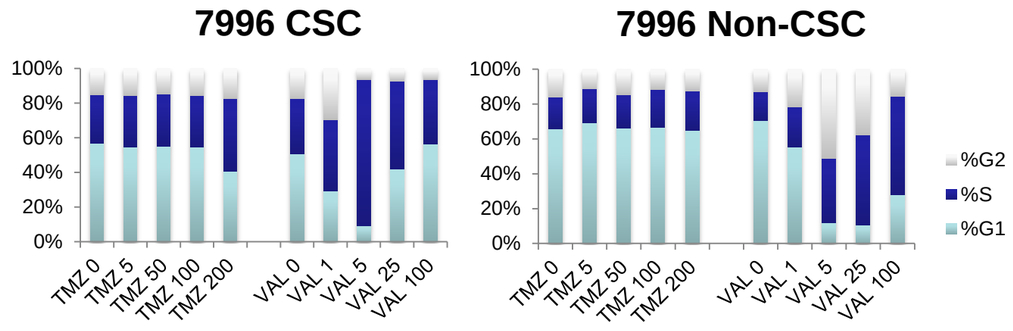


FIGURE 3. VAL-083 is active against TMZ-resistant GBM stem and non-stem cell GBM cultures at low μM doses, suggesting the ability to overcome TMZ-resistance in chemo-refractory GBM cancer stem cells.[4]

VAL-083 DISPLAYS SYNERGY WITH TEMOZOLOMIDE AND 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.

- VAL-083 demonstrated **synergy with temozolomide in GBM cancer stem cells** completely eliminating cancer stem cell spheres after 2 passages (Figure 5).[5]
- 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** *(topoisomerase II inhibitor)* **and camptothecin** *(topoisomerase I inhibitor)* (Table 2).

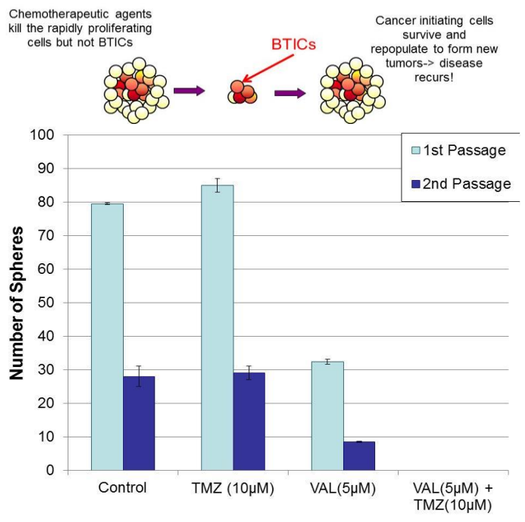


FIGURE 5. VAL-083 demonstrates potential synergy with temozolomide in GBM cancer stem cell line BT74. N=3.

TABLE 2. VAL-083 demonstrates synergy with etoposide (topoisomerase II inhibitor) and camptothecin (topoisomerase I inhibitor) in PC3 prostate and A549 NSCLC cancer cells. CI values for the cytotoxic effect (Fa). CI<1 shows synergy. N=4-5.

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 ACTIVATES HR PATHWAY

FIGURE 4. Pulse treatment (1 hr) with VAL-083 leads to persistent (>72 hr) cell cycle arrest, DNA double strand breaks (H2AXP) and activation of the HR DNA damage repair pathway (ATMP) in U251 GBM cancer cells.

MMR-INDEPENDENCE

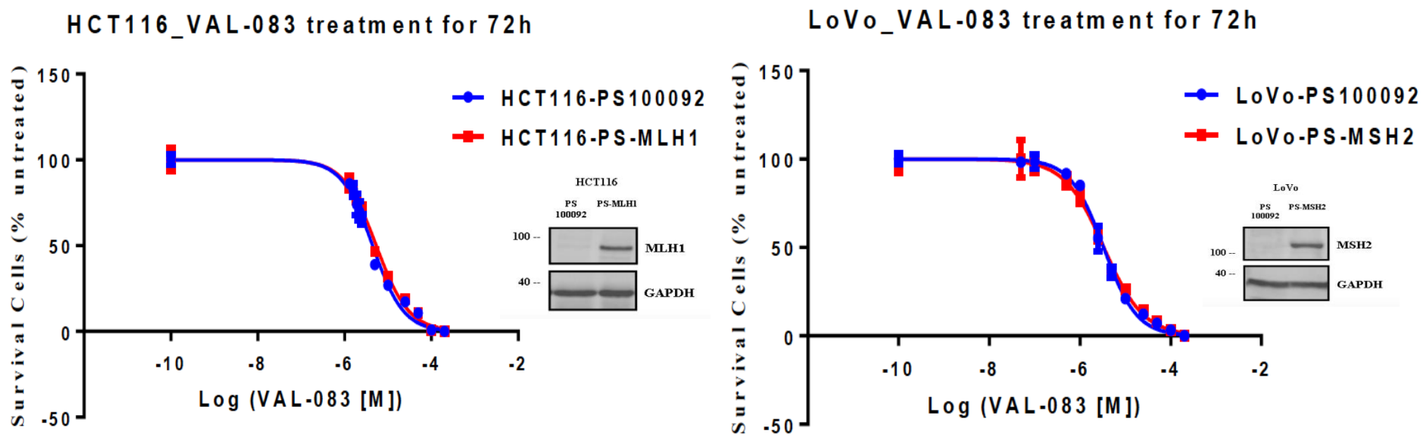


FIGURE 6. MMR-independency of VAL-083. Cytotoxicity of VAL-083 in two pairs of isogenic human colorectal cancer cell lines were performed by the crystal violet assay. MMR-proficient cell lines, HCT116-PS-MLH1 and LoVo-PS-MSH2, were established by lentiviral infection. HCT116-PS100092 is the MLH1-deficient 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:

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