Molecular mechanisms of dianhydrogalactitol (VAL-083) in overcoming GBM chemo-resistance

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ABSTRACT # 363

MECHANISM-OF-ACTION

VAL-083 targets N7 of guanine leading to DNA interstrand crosslinks, irreparable DNA double strand breaks, persistent S/G2-phase cell cycle arrest, and activation of the HR DNA repair pathway.[1,2]

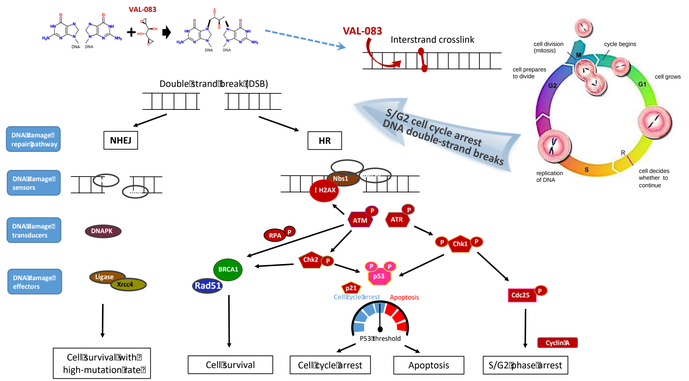


FIGURE 1. VAL-083 induces interstrand crosslink leading to double-strand breaks, S/G2 phase arrest and HR activation. Red color signifies demonstrated activation/expression after VAL-083 treatment.

VAL-083 ACTIVATES HR PATHWAY

VAL-083 treatment induces activation of the HR pathway, reflecting the cancer cell's attempt to repair the VAL-083-induced DNA double-strand breaks. This suggests increased VAL-083 activity in cancers known to frequently be HR-impaired (e.g. BRCA- or PTEN-deficient), including GBM and ovarian cancer. As expected, **the potency of VAL-083 activity was increased (IC_{50} was reduced) when HR was impaired**, demonstrating that VAL-083 induced DNA-lesions are repaired via HR (Figure 2). Furthermore, hypoxic cancer cells are known to downregulate their HR pathway, suggesting increased activity of VAL-083 in hypoxic tumors like GBM and in cancer stem cells.[3] Bevacizumab treatment increases hypoxia in the tumor, presumably further impairing HR.[4] This suggests VAL-083 as a treatment option in HR-deficient or hypoxic cancers either alone or as part of a combination treatment with bevacizumab. Research is underway to test this hypothesis.

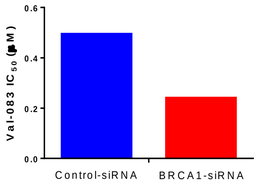


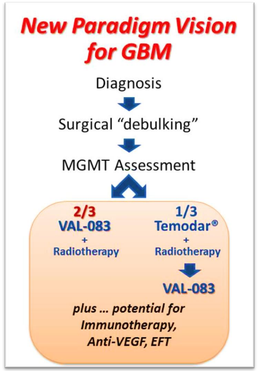
FIGURE 2.
VAL-083 activity is increased in HR-impaired A2780 ovarian cancer cells. HR was inhibited in A2780 ovarian tumor cells by down-regulation of BRCA1 with siRNA oligos for 24h and then exposing to VAL-083 for 5 days to assess IC_{50}.

CONCLUSION & NEXT STEPS

- **VAL-083 induces irreparable DNA double strand breaks, irreversible S/G2-phase arrest and activation of the homologous recombination DNA repair pathway.**
- **VAL-083's unique cytotoxic mechanism is MGMT-independent and able to overcome TMZ-resistance in GBM cancer stem cells and non-stem cells, *in vitro***
- **VAL-083 potentiates radiation in GBM cancer stem cells, *in vitro***
- **VAL-083 activity appears independent of p53**
- **VAL-083 displays synergy with a number of agents used in the treatment of GBM and other cancers, including temozolomide, etoposide, camptothecin, olaparib and platinum-based chemotherapy.**

THREE ADDITIONAL GBM CLINICAL TRIALS ARE PLANNED

1. ***A pivotal, randomized multi-center Phase 3 study measuring survival outcomes compared to a "physicians' choice" control for the treatment of bevacizumab-failed GBM.***
2. ***An open label single-arm, biomarker-driven, Phase 2 study of VAL-083 in patients with MGMT-unmethylated, bevacizumab-naive recurrent glioblastoma****clinicaltrials.gov identifier: NCT02717962*
3. ***An open label, single-arm, biomarker-driven, Phase 2 study of VAL-083 and radiation therapy patients with in newly diagnosed MGMT-Unmethylated GBM***



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2. ***Zhai et al.*** *AACR annual meeting 2016*
3. ***Bindra et al.*** *Mol & Cell Biol. 2004:24(19):8504-18*
4. ***Fack et al.*** *Acta Neuropathol. 2015;129:115-131*
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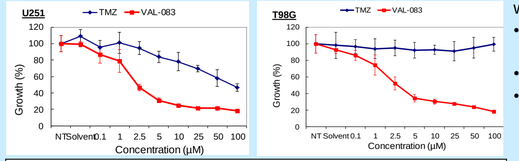
MGMT-INDEPENDENCE

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

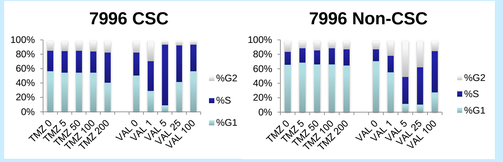


FIGURE 4. VAL-083 is active against TMZ-resistant GBM stem and non-stem cell GBM cultures at low μM doses, suggesting the ability to overcome TMZ-resistance in chemorefractory GBM cancer stem cells.[6]

VAL-083 POTENTIATES RADIATION

Dianhydrogalactitol potentiates the cytotoxic effect of radiation in GBM CSC cultures, *in vitro*.

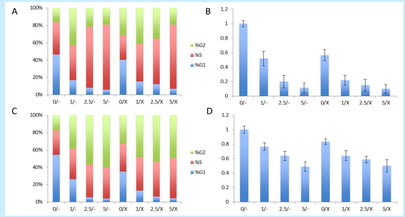


FIGURE 5. **VAL-083 acts as radiosensitizer in GBM CSC cells.** VAL-083 was added to primary CSC cultures at various doses (1, 2.5 and 5 μM) with or without irradiation (2 Gy). Shown are cell cycle profile analysis at day 4 post treatment (A,C) and cell viability analysis at day 6 post treatment (B,D) for two different patient-derived CSC cultures, 7996 (A,B) and 8565 (C,D). [6]

VAL-083 DISPLAYS SYNERGY WITH TEMOZOLOMIDE, ETOPOSIDE, CAMPTOTHECIN, OLAPARIB AND CISPLATIN

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.

- We have demonstrated **synergy with temozolomide in GBM cancer stem cells** completely eliminating cancer stem cell spheres after 2 passages (Figure 6).[5]
- VAL-083 demonstrated **synergy with PARP inhibitor olaparib** (Figure 7).
- As VAL-083 induce cell cycle arrest in S/G2-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 1).
- **VAL-083 also demonstrated synergy with cisplatin and oxaliplatin** in NSCLC cell lines, suggesting distinct mechanism-of-action from the platinum-based agents (Figure 8).[7]

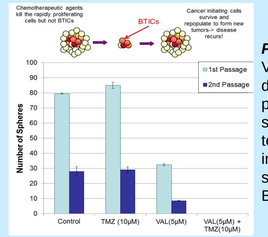


FIGURE 6. VAL-083 demonstrates potential synergy with temozolomide in GBM cancer stem cell line BT74. N=3.

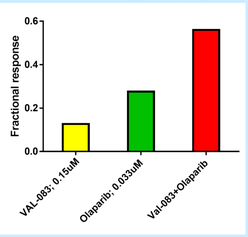
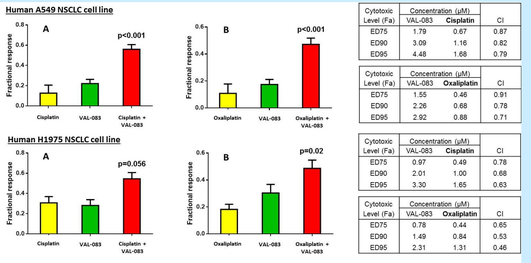


FIGURE 7. VAL-083 demonstrates synergy with PARP inhibitor olaparib in ovarian cancer cell line A2780. Mean, N=3.

TABLE 1. VAL-083 demonstrates synergy with etoposide (topoisomerase II inhibitor) and camptothecin (topoisomerase I inhibitor) in PC3 prostate and A549 NSCLC cancer cells. The tables show CI values for the cytotoxic effect (Fa), achieved at indicated drug combinations. 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 ratio VAL-083:etoposide was 5:1 in PC3 and 5:1 in A549; molar ratio VAL-083:camptothecin was 250:1 in PC3 and 212:1 in A549



Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Cisplatin	CI
ED75	1.79	0.67	0.87
ED90	3.09	1.16	0.82
ED95	4.48	1.68	0.79

Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Oxaliplatin	CI
ED75	1.55	0.46	0.91
ED90	2.26	0.68	0.78
ED95	2.92	0.88	0.71

Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Cisplatin	CI
ED75	0.97	0.49	0.78
ED90	2.01	1.00	0.68
ED95	3.30	1.65	0.63

Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Oxaliplatin	CI
ED75	0.78	0.44	0.65
ED90	1.49	0.84	0.53
ED95	2.31	1.31	0.46

FIGURE 8. VAL-083 demonstrates synergy with cisplatin (A) or oxaliplatin (B) on A549 and H1975 NSCLC cells. The tables shows CI values for the cytotoxic level (Fa) shown, achieved at indicated drug concentrations. CI<1 shows synergy. Mean +/- SE, N=4-7.