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

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ABSTRACT # DDIS-11

Glioblastoma (GBM) is the most aggressive malignant brain tumor. The heterogeneous nature of GBM tumors and their highly chemo-resistant cancer stem-cells (CSCs) comprise significant clinical challenges. Most GBM tumors express O^6-methylguanine-DNA-methyltransferase (MGMT) causing intrinsic chemo-resistance to temozolomide and CCNU. Even tumors initially responsive to temozolomide, often recur with deficient DNA mismatch repair system (MMR) leading to acquired chemo-resistance to temozolomide. Alteration in p53, particularly gain-of-function mutations, are correlated with poor prognoses in GBM, potentially by increasing MGMT-expression and temozolomide resistance. Second-line treatment with bevacizumab to inhibit angiogenesis, also induces intra-tumor hypoxia, has not improved overall survival. This is possibly due to GBM CSCs rapidly adapting to hypoxia by upregulating glucose-uptake and increasing invasiveness. Dianhydrogalactitol (VAL-083) is a bi-functional alkylating agent that readily crosses the blood-brain barrier, accumulates in brain tumor tissue and has demonstrated activity against GBM in prior NCI-sponsored clinical trials. VAL-083 induces interstrand cross-links at guanine-N^7 causing DNA double-strand breaks and cell-death. VAL-083 is equally active against GBM CSCs and non-CSCs, and the activity is MGMT-independent and appears minimally dependent on wild-type p53, *in vitro*. A Phase I/II clinical trial studying VAL-083 in recurrent GBM, after temozolomide and bevacizumab failure, suggested the potential of VAL-083 to offer clinically meaningful survival benefits. Here we report a distinct mechanism-of-action of VAL-083, showing that VAL-083 leads to irreversible S-phase cell-cycle arrest, activation of the homologous recombination (HR) pathway and ensuing cell-death, through mechanisms independent of MGMT and MMR. Based on the results we examined the cytotoxic activity and synergistic properties of VAL-083 in combination with relevant chemotherapeutic agents used in the treatment of GBM and other CNS tumors. Our results demonstrate a distinct anti-cancer mechanism for VAL-083, enabling it to overcome resistance to TMZ and cisplatin and to display synergy with topoisomerase inhibitors, etoposide or camptothecin.

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]

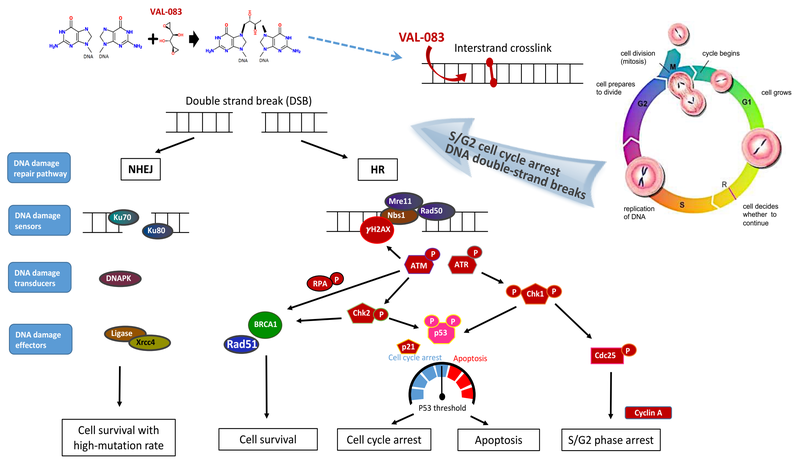
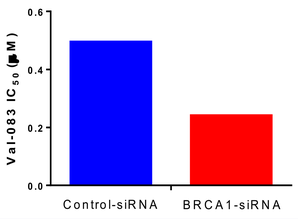


FIGURE 1. VAL-083 induces interstrand crosslink leading to double-strand breaks, S/G2 phase arrest and HR activation. Red color signifies demonstrated activation/expression after VAL-083 treatment.

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}.



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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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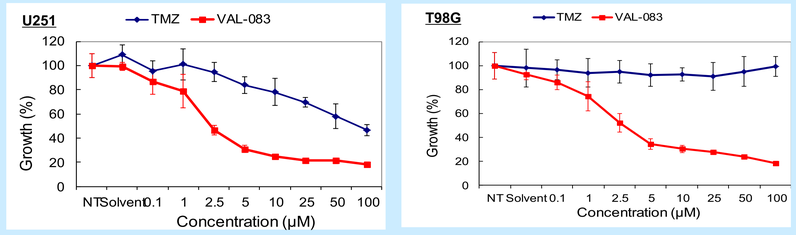
7. ***Steino et al.*** *AACR annual meeting 2015*

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
	TMZ	10.0μM	>>100μM

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

VAL-083 ACTIVITY AGAINST TMZ-RESISTANT GBM CANCER STEM CELLS

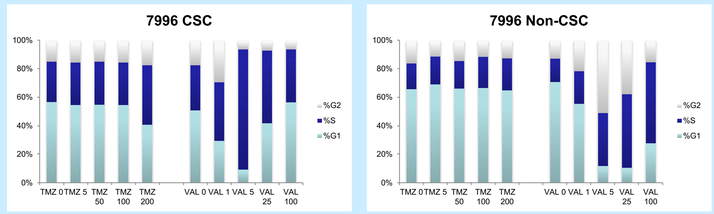


FIGURE 4. VAL-083 is active against TMZ-resistant GBM stem and non-stem cell GBM cultures at low μM doses, suggesting the ability to overcome TMZ-resistance in chemorefractory GBM cancer stem cells.[6]

VAL-083 POTENTIATES RADIATION

Dianhydrogalactitol potentiates the cytotoxic effect of radiation in GBM CSC cultures, *in vitro*.[6]

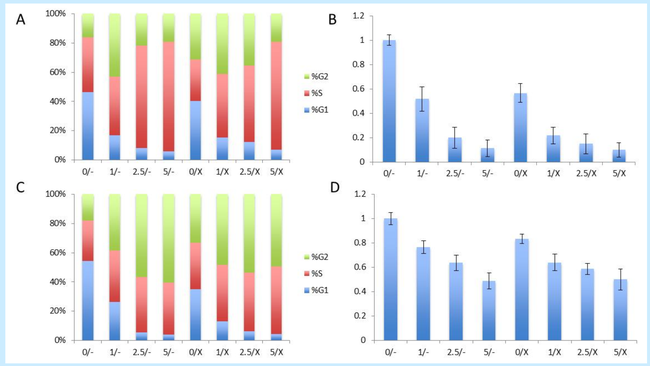


FIGURE 5. **VAL-083 acts as a radiosensitizer in primary GBM CSC cultures.** VAL-083 was added to primary CSC cultures at various doses (1, 2.5 and 5 μM) with or without irradiation (2 Gy). Shown are cell cycle profile analysis at day 4 post treatment (A,C) and cell viability analysis at day 6 post treatment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

CONCLUSION & NEXT STEPS

The mechanism-of-action of VAL-083 is distinct from other alkylating agents used in the treatment of CNS tumors (Table 2).

Alkylating agent	Temozolomide[5]	BCNU/CCNU[1,5]	Cisplatin/carboplatin[5,6]	VAL-083[1,2,3,4]
Cytotoxic target	O6-Guanine	O6-Guanine	N7-Guanine	N7-Guanine
DNA damage	Base mismatch Single-strand break	Interstrand crosslinks (G-C) Double-strand break	Intrastrand crosslinks (G-G) Double-strand break	Interstrand crosslinks (G-G) Double-strand break
Cell cycle arrest	G2/M	G2/M	G2	Late S/G2
ATR-Chk1	activated	activated	activated	activated
ATM-Chk2	activated	activated	activated	activated
MGMT	dependent	dependent	independent	independent
MMR	dependent	independent	dependent	independent
p53	dependent	dependent	dependent	Less dependent

Table 2. MGMT: O6-alkylguanine DNA alkyltransferase; MMR: mismatch repair

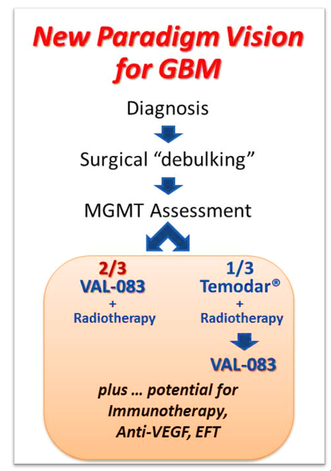
Conclusions

- **VAL-083 induces irreparable DNA double strand breaks, irreversible S/G2-phase arrest and activation of the homologous recombination DNA repair pathway.**
- **VAL-083 cytotoxic activity 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 CNS tumors, including temozolomide, etoposide, camptothecin and platinum-based chemotherapy.**

THREE ADDITIONAL GBM CLINICAL TRIALS ARE PLANNED

SNO2016 Abstract #ACTR-42

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***



VAL-083 DISPLAYS SYNERGY WITH TEMOZOLOMIDE, ETOPOSIDE, CAMPTOTHECIN 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]
- 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 7).[7]

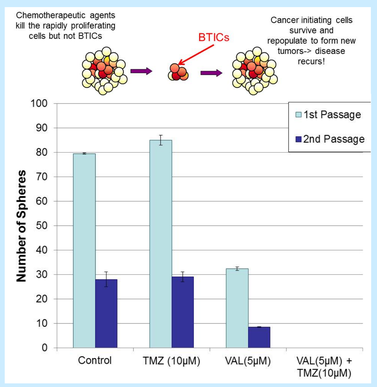
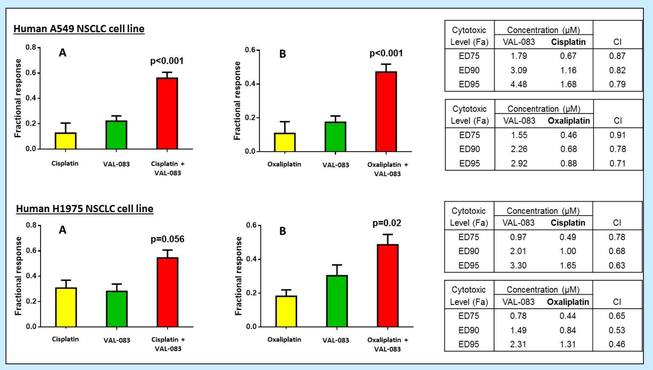


FIGURE 6. VAL-083 demonstrates potential synergy with temozolomide in GBM cancer stem cell line BT74. N=3.

A	**VAL-083 in combination with etoposide (topoisomerase II inhibitor), 72 hr treatment.**		
Cell line (molar ratio VAL-083:etoposide)		Cytotoxic effect (**Fa**)	Combination index (**CI**)
PC3 (5:1)		**ED50**	0.65
		ED75	0.59
		ED90	0.55
A549 (5:1)		**ED50**	0.72
		ED75	0.88
		ED80	0.94
B	**VAL-083 in combination with camptothecin (topoisomerase I inhibitor), 72 hr treatment.**		
Cell line (molar ratio VAL-083:camptothecin)		Cytotoxic effect (**Fa**)	Combination index (**CI**)
PC3 (250:1)		**ED75**	0.83
		ED90	0.66
		ED95	0.56
A549 (212:1)		**ED85**	0.94
		ED90	0.87
		ED95	0.77

CI: 0.8-0.9 Slight synergism
CI: 0.6-0.8 Moderate synergism
CI: < 0.6 Strong synergism

TABLE 1. VAL-083 demonstrates synergy with A) etoposide (topoisomerase II inhibitor) and B) 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 concentrations. N=3.



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 7. 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.