The distinct cytotoxic mechanism of dianhydrogalactitol (VAL-083) overcomes chemoresistance and provides new opportunities for combination therapy in the treatment of glioblastoma

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ABSTRACT #5264

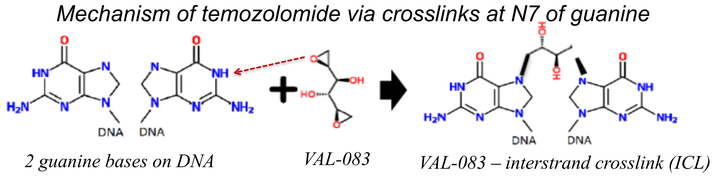
Treatment of glioblastoma (GBM) includes surgery and chemoradiation with temozolomide. Due to chemoresistance, nearly all tumors recur and 5-year survival is less than 3%. Various treatments, including anti-angiogenic treatment with bevacizumab, nitrosoureas and topoisomerase inhibitors, have failed to improve overall survival in recurrent GBM (rGBM). GBM tumors expressing O^6-methylguanine-DNA-methyltransferase (MGMT) are resistant to temozolomide and nitrosourea, and deficient DNA mismatch repair (MMR) confers secondary resistance to temozolomide. Our recent phase I/II trial in rGBM patients previously treated with temozolomide and bevacizumab, suggested that VAL-083 may offer a clinically meaningful survival benefit for rGBM patients. VAL-083 is a first-in-class bi-functional DNA-targeting agent that ***readily crosses the blood-brain barrier*** and accumulates in brain tumor tissue. 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 rapidly induces interstrand cross-links at N7-guanine, causing DNA double-strand breaks and persistent activation of the homologous recombination (HR) DNA repair pathway. Furthermore, VAL-083 potency is increased in HR-deficient cancer cells, suggesting increased cytotoxicity in HR-impaired tumors. Hypoxic GBM cells downregulate HR activity, thus proposing increased VAL-083 potency in hypoxic tumors. We demonstrated that VAL-083 induces irreversible S/G2-phase cell cycle arrest, thus proposing synergy with S-phase specific chemotherapeutics, including topoisomerase and PARP inhibitors. Our results support the potential of VAL-083 to i) overcome resistance to temozolomide, and ii) display synergy as part of combinatory therapies with topoisomerase or PARP inhibitors.

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 temozolomide via crosslinks at N7 of guanine



Mechanism of temozolomide via alkylation at O6 of guanine

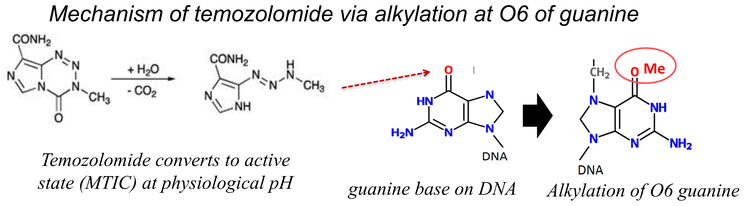


FIGURE 1. *The N7-targeting mechanism of action of VAL-083 differs from those of O6-alkylating agents like temozolomide and nitrosoureas.*

VAL-083 IS A DNA-TARGETING AGENT WITH A UNIQUE MECHANISM

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 ad 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]

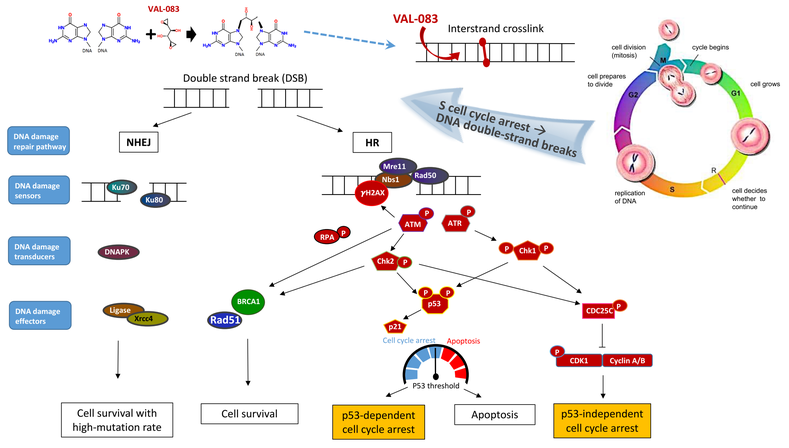


FIGURE 2. *Mechanisms of action of VAL-083 induced chemotherapeutic cytotoxicity. Apoptosis can be induced through either p53 dependent or p53 independent pathways. Red color signifies VAL-083-induced activation.*[1,2]

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

VAL-083 MEDIATES DNA DOUBLE STRAND BREAKS, S/G2 PHASE CELL CYCLE ARREST AND ACTIVATES THE HOMOLOGOUS RECOMBINATION (HR) DNA REPAIR SYSTEM

VAL-083 pulse treatment led to increased Rad51, BRCA1, RPA32 and γH2A.X foci formation in A549 lung cancer cells (Figure 3) and increased γH2A.X and ATM activation and S/G2 cell cycle arrest U251 GBM cancer cells for up to 72 hours (Figure 4), suggesting ***VAL-083-mediated DNA double strand breaks and persistent activation of the HR DNA damage repair system***

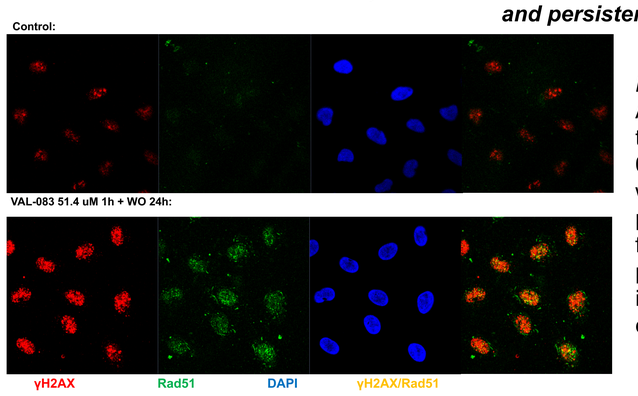


FIGURE 3. Serum-starved A549 lung cancer cells were treated with 51.4 µM VAL-083 for 1h, followed by washout for 24h. Cells were pre-extracted in CSK buffer for 5 min at 4°C, fixed in 4 % paraformaldehyde, washed in PBS and stained with corresponding antibodies.

A549 cells	γH2A.X +	BRCA1 +	Double positive
Control	21.59 %	10.23 %	10.23 %
VAL 1h + WO 24h	81.72 %	72.04 %	67.74 %

A549 cells	γH2A.X +	Rad51 +	Double positive
Control	23.73 %	1.69 %	0
VAL 1h + WO 24h	87.18 %	74.36 %	67.95 %

A549 cells	γH2A.X +	RPA32 +	Double positive
Control	16.26 %	1.63 %	0.81 %
VAL 1h + WO 24h	87.06 %	83.53 %	80 %

VAL-083 TREATMENT MEDIATES PERSISTENT DSBs, HR ACTIVATION AND S/G2 PHASE CELL CYCLE ARREST

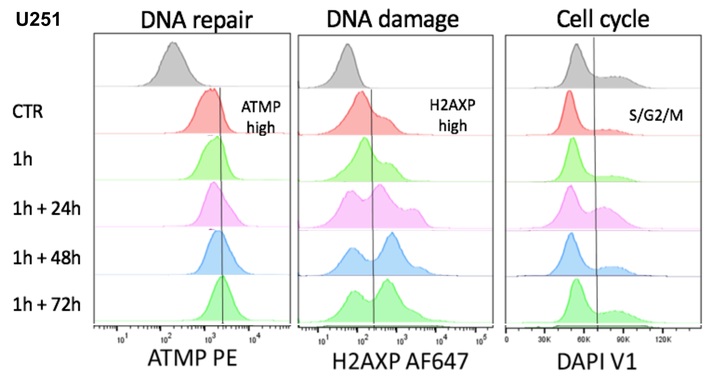


FIGURE 4. VAL-083 pulse treatment led to phosphorylation of DNA DSB marker H2AX, HR component ATM and mediates cell cycle arrest at S/G2 phase in U251 GBM cells (50 µM VAL-083 pulse treatment for 1 hr)

VAL-083 DISPLAYS SYNERGY WITH TOPOISOMERASE INHIBITORS

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** *(TOP-2 inhibitor)* **and camptothecin** *(TOP-1 inhibitor)* (Table 1).

TABLE 1. *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.*

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 CYTOTOXICITY IS INCREASED IN HR-IMPAIRED CANCER CELLS AND DISPLAYS SUPERADDITIVITY WITH PARP INHIBITORS (PARPi)

VAL-083 cytotoxicity against ovarian cancer A2780 cells was increased (IC50 decreased) when BRCA1 was impaired (HR impaired) (Figure 5A). This suggests ***increased activity in ovarian cancer with dysfunctional BRCA1*** and further suggests the potential for synergy with PARPi. ***VAL-083 in combination with PARPi olaparib, talazoparib or veliparib was superadditive*** in BRCA1-proficient ovarian cancer cells (Figure 5).
Studies of VAL-083 in combination with PARPis in BRCA1-deficient ovarian cancer cells are ongoing.

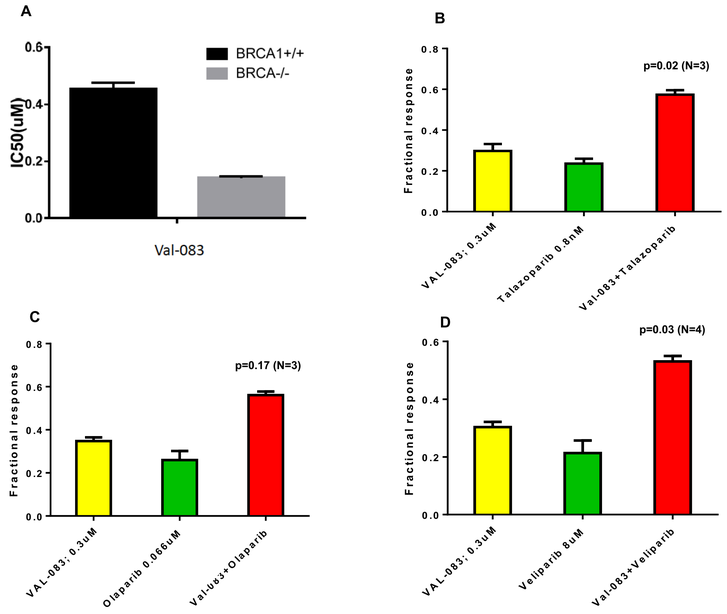
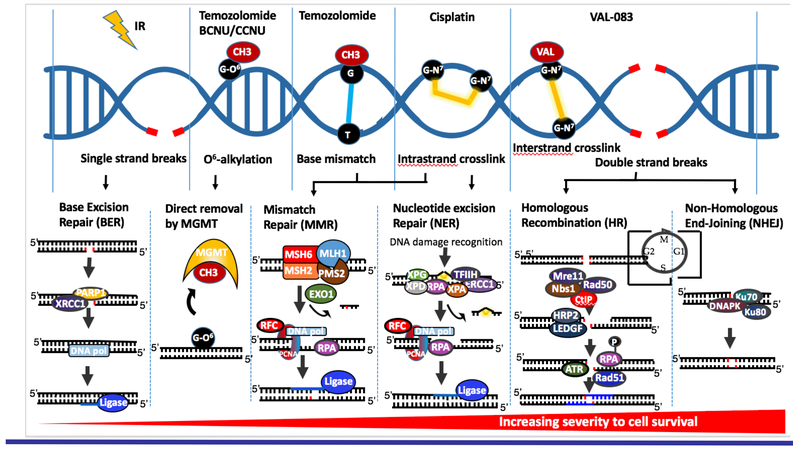


FIGURE 5. VAL-083 cytotoxicity against BRCA1-proficient (BRCA1+/+) and -deficient (BRCA1-/-) ovarian cancer cells A2780 (A). VAL-083 cytotoxicity in combination with PARPi talazoparib (B), olaparib (C) or veliparib (D) in BRCA1-proficient ovarian cancer cells A2780 .



CONCLUSIONS

- **VAL-083 mediates persistent DNA double strand breaks, activates the HR repair system and mediates S/G2 cell cycle arrest**
- **VAL-083 displays synergy with topoisomerase I and II inhibitors**
- **Val-083 activity is increased in HR (BRCA1) impaired ovarian cancer cells**
- **VAL-083 displays superadditivity with PARPis talazoparib, olaparib and veliparib**

References:

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