Abstract #: 6431

Molecular Mechanisms of Dianhydrogalactitol (VAL-083) in Overcoming Chemoresistance in Glioblastoma

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Summary: Glioblastoma (GBM) is the most common CNS tumor. Standard treatments for glioblastoma (GBM) include surgery, radiation and chemotherapy with temozolomide (TMZ). Nearly all tumors recur and 5-year survival is less than 3%, largely due to chemoresistance. Evidence shows that cancer cells utilize DNA damage repair pathways to overcome cytotoxic effects of chemotherapy. GBM tumors expressing O^6-methylguanine-DNA-methyltransferase (MGMT) display intrinsic chemoresistance to TMZ and nitrosoureas, while a deficient DNA mismatch repair (MMR) system confers chemoresistance to TMZ and platinum agents. Alterations in p53, particularly gain-of-function mutations, are correlated with increased MGMT-expression and poor prognoses in GBM. 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 cancer cell death. VAL-083 is equiactive against GBM cancer stem cells (CSCs) and non-CSCs independent of MGMT and p53 status, *in vitro*. We recently showed that VAL-083 leads to irreversible S/G2-phase cell cycle arrest, proposing synergy with S-phase specific chemotherapeutics, including topoisomerase and PARP inhibitors. VAL-083 further showed persistent activation of the homologous recombination (HR) DNA repair pathway and its potency was increased when HR was impaired, demonstrating that VAL-083-induced lesions are repaired via HR suggesting increased VAL-083 potency in HR-impaired tumors. Here, VAL-083 cytotoxicity and DNA damage response was evaluated by crystal violet assays, Western blot and flow cytometry. We report synergy between VAL-083 and etoposide or camptothecin in A549 and PC3 cancer cell lines. Our results demonstrate a distinct anti-cancer mechanism for VAL-083, resulting in the ability to overcome resistance to TMZ and nitrosoureas, increased activity in cancers with impaired HR and synergy with etoposide or camptothecin.

MECHANISM-OF-ACTION

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

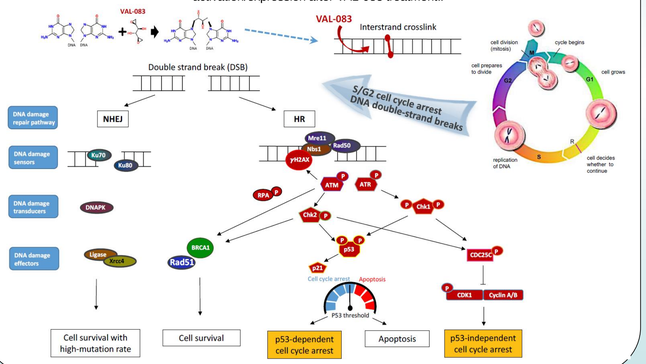


Figure 2: Cytotoxic effects of VAL-083 in different cancer cell lines.

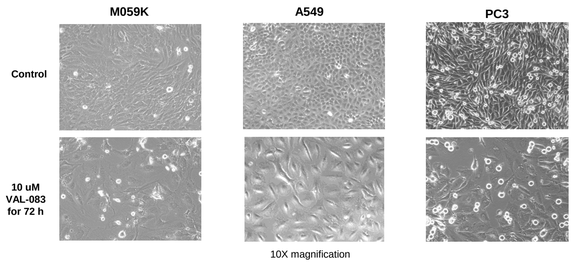
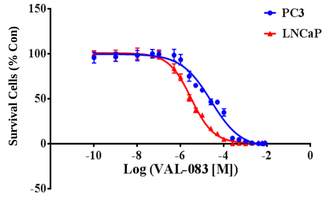


Figure 3: IC_{50} of VAL-083 treatment in different cancer cells for 72h.



	PC3	LNCaP	H2122	H1792
IC_{50} (uM)	25.7	3.06	12.23	4.57
IC_{50} range (uM)	19.94 – 33.1	2.74 – 3.41	8.68 – 17.22	4.06 – 5.13

Figure 4: VAL-083 treatment led to cell cycle arrest at S, followed by G_2/M phase [3] (serum starvation for 24 h before 5 uM VAL-083 treatment).

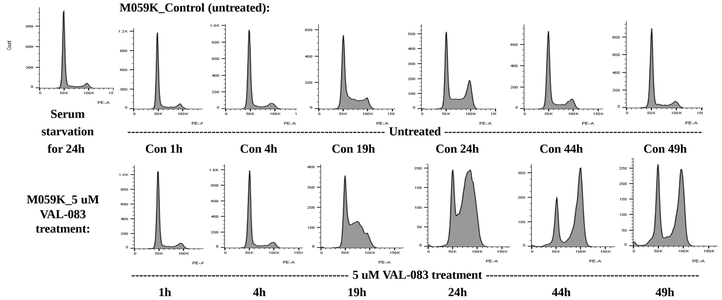
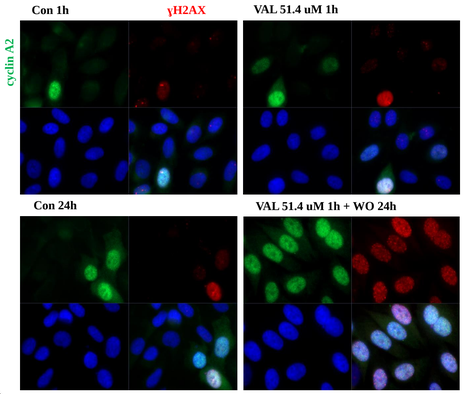
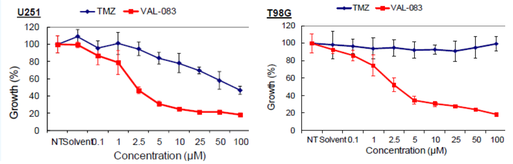


Figure 5: VAL-083 pulse treatment induced replication-dependent DNA damage. Serum starvation for 24 h before VAL-083.



PC3 cells:	γH2A.X + cyclin A2 +
Con 1h	2.8 %
VAL 1h	2.6 %
Con 24h	16.5 %
VAL 1h + WO 24h	90.3 %

Figure 6: VAL-083 cytotoxic activity is independent of MGMT-mediated temozolomide-resistance.



GBM Cell Lines		U251	T98G
MGMT promoter methylation status		Methylated (low MGMT expression)	Unmethylated (high MGMT expression)
IC_{50} (uM)	VAL-083	2.5	2.5
	TMZ	10	>> 100

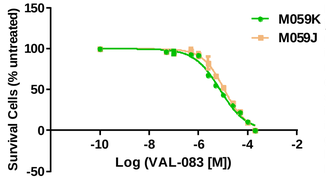


Figure 7: Non-homologous end joining (NHEJ) did not play critical role in VAL-083-mediated DNA damage signaling, supporting HR involvement. M059K is NHEJ-proficient glioblastoma cell line and M059J is NHEJ-deficient cell line.

Figure 8: VAL-083 pulse treatment activated DNA damage signaling pathway (HR) as demonstrated by expression of phospho-ATM (S1981), phospho-Chk2 (T68), phospho-RPA32 (S33) and γH2A.X which persisted for 24 - 48 h after removal of VAL-083 from the medium.

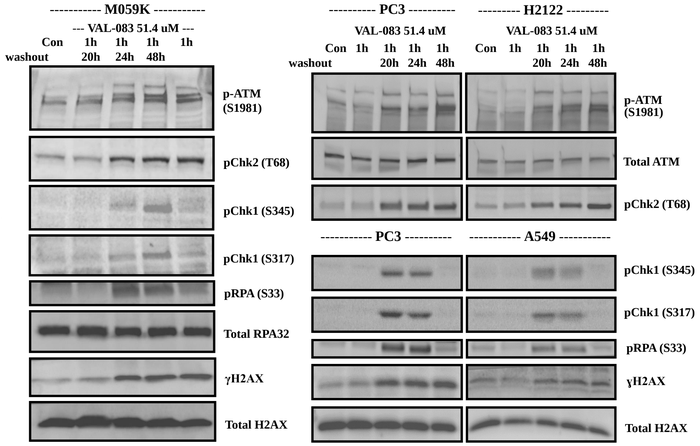


Table 1. VAL-083 demonstrates synergy with etoposide (topoisomerase II inhibitor) and camptothecin (topoisomerase I inhibitor) in PC3 and A549 cancer cells. The table shows CI values for the cytotoxic effect (Fa), achieved at indicated drug combinations. CI<1 shows synergy. N=4-5.

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 4.6:1 in PC3 and 5.1:1 in A549; molar ratio VAL-083:camptothecin was 250:1 in PC3 and 212:1 in A549.

Ongoing Research and Conclusions:

1. VAL-083 displayed broad anti-tumor activity in various cancer cells.
2. VAL-083 treatment causes durable DNA crosslinks leading to irreparable DNA double-strand breaks, S/G_2 phase cell-cycle arrest and cell death in cancer cells.
3. Elucidation of the molecular mechanisms underlying VAL-083 cytotoxicity in cancer cells will offer help for effective combination therapies.
4. Lentiviral constructs will be employed to investigate the role of MMR system in VAL-083-induced DNA damage signaling.
5. Combination treatment with VAL-083 and topoisomerase I/II inhibitors will be tested in GBM cell lines.

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References: 1. Institoris E, et al. Cancer Chemother Pharmacol (1989)24:311-3. 2. Zhai et al. AACR annual meeting 2016. 3. Peng C, et al. Acta Pharmacol Sin (2017). doi: 10.1038/aps.2016.154. [Epub ahead of print]