Dianhydrogalactitol (VAL-083) causes irreparable DNA double-strand breaks, S/G2 phase cell-cycle arrest and tumor cell death in an MGMT independent manner offering a unique treatment paradigm for GBM

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VAL-083

VAL-083 is a bifunctional alkylating agent causing alkylation of N^7-guanine leading to interstrand DNA crosslinks[1] and DNA double strand breaks (DSB). We have previously shown that VAL-083´s cytotoxic activity is independent of MGMT in contrast to temozolomide (TMZ) and nitrosoureas (Fig 2).[2] Likely due to its different mechanism, VAL-083 has also been shown to overcome both BCNU-resistance[1] and TMZ-resistance[2,3] *in vitro*. We have previously demonstrated VAL-083 is active against GBM cancer stem cells (CSCs) and acts as a radiosensitizer in GBM CSCs, *in vitro*.[2] We have also previously shown that VAL-083 circumvents cisplatin-resistance and is less dependent on p53 activity than cisplatin suggesting a distinct mechanism of action for VAL-083 from other alkylating agents used in the treatment of brain cancer (Table 1).[4] VAL-083 readily crosses the blood-brain barrier, accumulates in brain tumor tissue and has shown activity in prior NCI-sponsored clinical trials against CNS tumors, including GBM and medulloblastoma. VAL-083 has received orphan drug designation in the U.S. for the treatment of gliomas, medulloblastoma and ovarian cancer; and in Europe for gliomas and is approved in China for the treatment of chronic myelogenous leukemia and lung cancer.

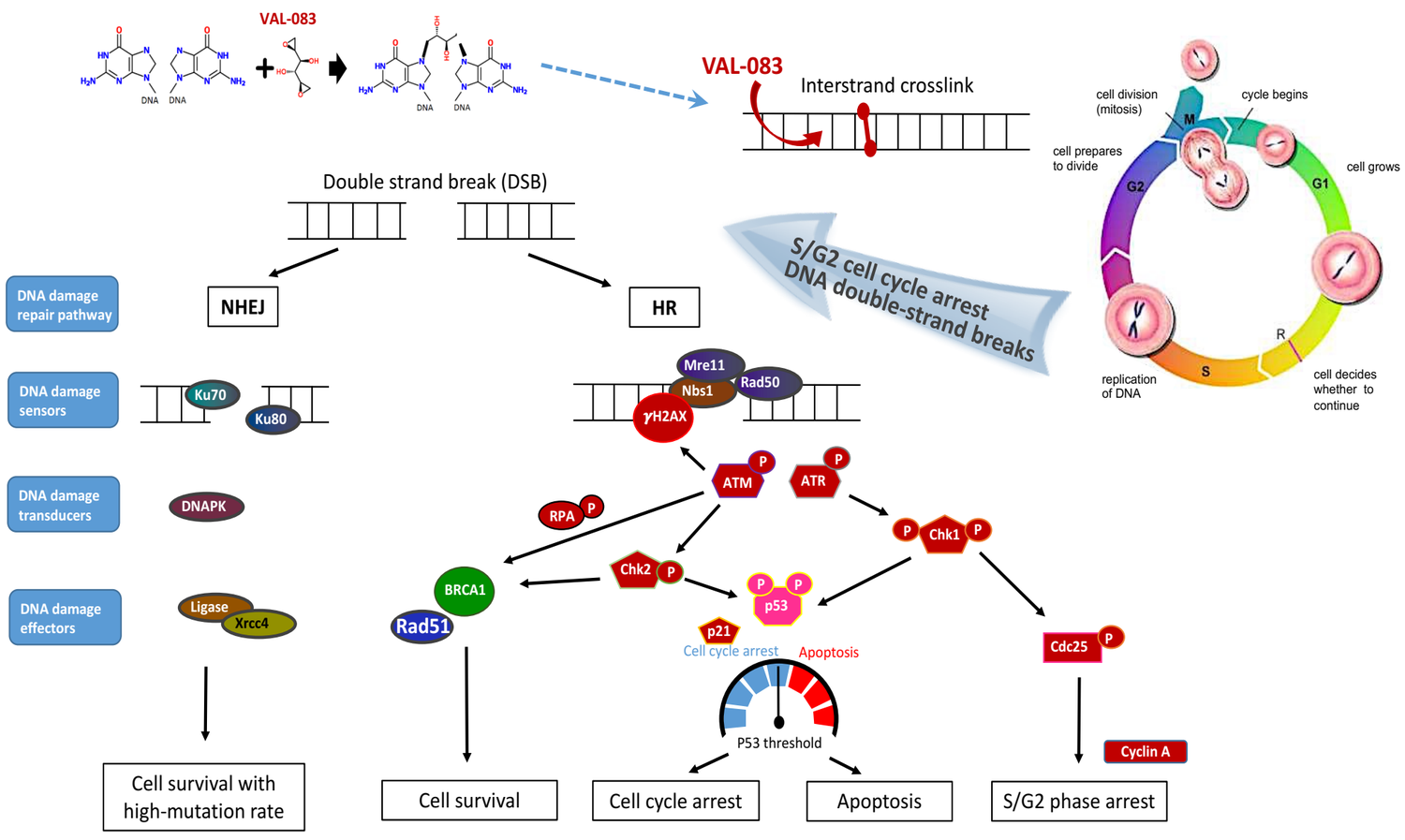


Fig 1. VAL-083 induces interstrand crosslink leading to double-strand breaks, S/G2 phase arrest and HR activation. Red color signifies demonstrated activation/expression after VAL-083 treatment.

Here we report new insights into VAL-083 mechanism of action by showing that VAL-083 rapidly induces interstrand DNA cross-links leading to irreparable DNA double-strand breaks, irreversible S/G_2 cell-cycle arrest, activation of the HR DNA repair pathway and cancer cell death caused by replication-dependent DNA damage. In addition to Chk1 and ATR phosphorylation[4], VAL-083 pulse-treatment leads to persistent phosphorylation of histone variant H2A.X (γH2A.X), ATM, Replication Protein A (RPA32) and Chk2 (Fig 3). VAL-083 induced persistent S/G2 phase cell cycle arrest in cells with DNA double-strand breaks (Table 2).

GBM cell lines

SF188	T98G
Methylated MGMT promoter status	Unmethylated MGMT promoter status

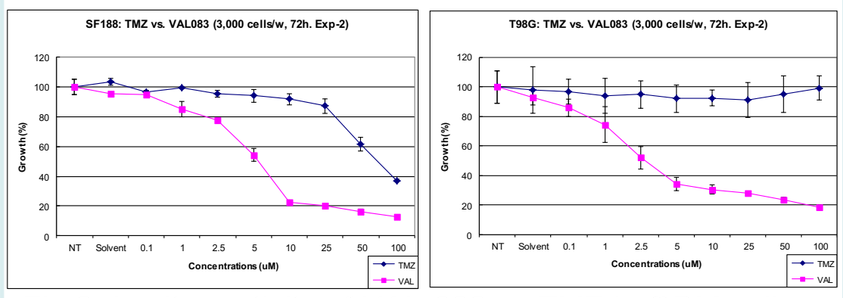


Fig 2. In vitro activity of VAL-083 vs.TMZ in GBM cell lines SF188 (MGMT-), and T98G (MGMT+) as impacted by expression of MGMT repair enzyme.[3]

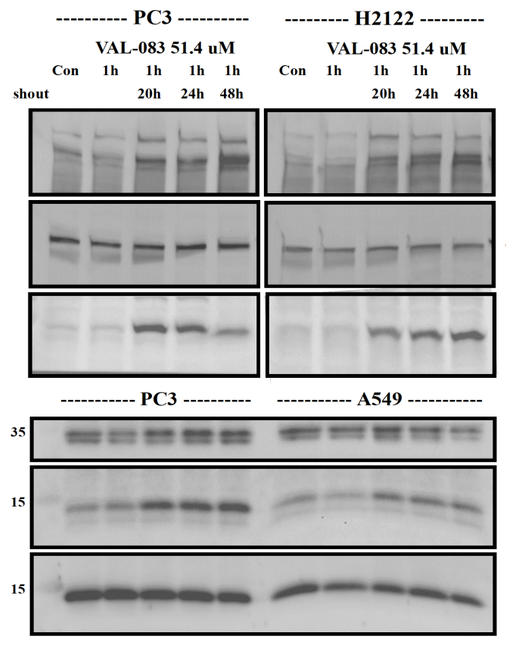


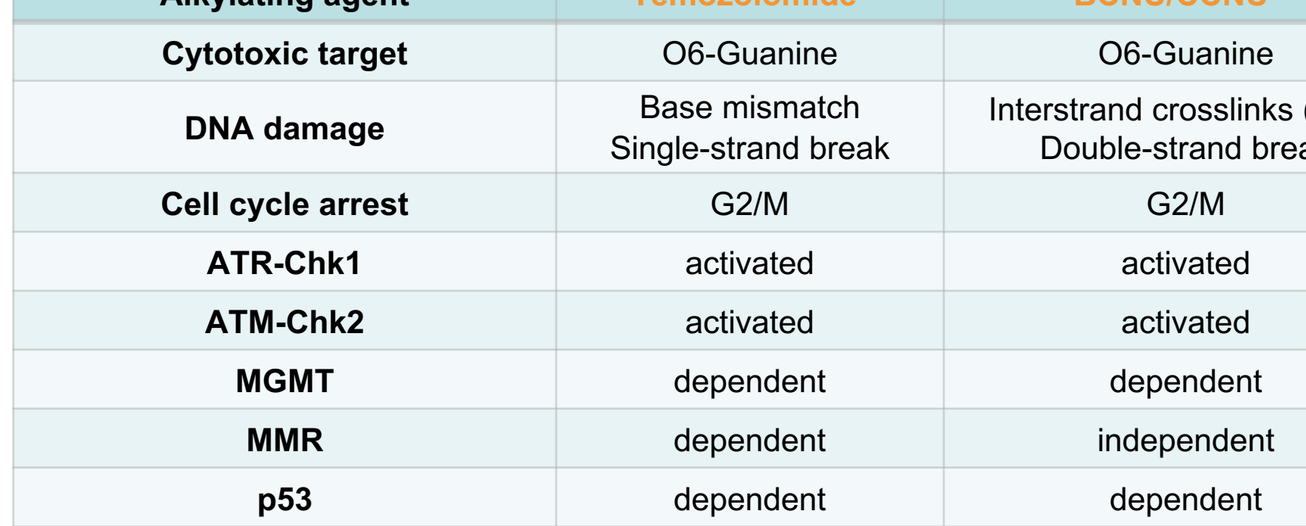
Fig 3. VAL-083 pulse treatment activated HR DNA damage signaling pathway as demonstrated by expression of phospho-ATM (S1981), phospho-Chk2 (T68), phospho-RPA32 (S33) and γH2A.X which persisted for 24 - 48 h after removal of VAL-083 from the medium.

Table 2. VAL-083 pulse treatment induced co-localized DNA double-strand breaks (γH2A.X) and S/G2 phase cell cycle arrest (cyclin A2).

PC3 cells	γH2A.X + cyclin A2+
Con 1h	2.8 %
VAL-083 1h	2.6 %
Con 24h	16.5 %
VAL-083 1h +WO 24h	90.3 %

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
Cross blood-brain barrier?	yes	yes	no	yes

Table 1. MGMT: O6-alkylguanine DNA alkyltransferase; MMR: mismatch repair



CONCLUSIONS & FUTURE DIRECTIONS

- VAL-083 induces irreparable DNA double-strand breaks, irreversible S/G2 cell-cycle arrest and activation of the homologous recombination DNA repair pathway
- VAL-083 has a unique molecular mechanism that differs from both temozolomide, nitrosoureas or cisplatin/carboplatin and is less dependent on p53
- VAL-083's mechanism is resistant to important DNA-repair strategies employed by cancer cells to escape effects of alkylating agents commonly used in the treatment of GBM
- VAL-083 is being advanced in clinical trials for GBM and other indications

References

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