Dianhydrogalactitol (VAL-083) synergizes with topoisomerase inhibitors to overcome homologous recombination repair activity in glioblastoma and prostate cancer cells

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ABSTRACT #1369

Dianhydrogalactitol (VAL-083) is a bi-functional DNA-damaging agent that targets N^7-guanines and causes DNA inter-strand crosslinks. **VAL-083 is a small water-soluble molecule that readily crosses blood-brain-barrier and accumulates in brain tumor tissue**, making it a good candidate for targeting brain malignancies, such as glioblastoma multiforme (GBM). VAL-083 has demonstrated anti-tumor activity in prior NCI-sponsored clinical trials in brain tumors and other cancer types[1,2]. Previous research in our group demonstrated VAL-083-induced DNA inter-strand crosslinks that lead to replication-dependent DNA double strand break (DSB) lesions in non-small cell lung cancer (NSCLC) cell lines. The NSCLC cancer cells attempted to repair the DSBs by homologous recombination (HR)[3,4] and were arrested in S phase prior to cell death. Here, we show that the the observed mechanism-of-action of VAL-083 in NSCLC cells translate to GBM and prostate cancer cell lines. As expected from the mechanism-of-action, we demonstrate synergy between VAL-083 and topoisomerase inhibitors in GBM and prostate cancer cells lines.

VAL-083 MECHANISM-OF-ACTION

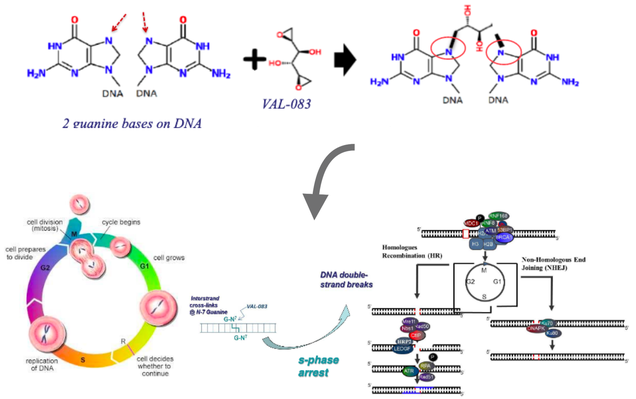

FIGURE 1. *VAL-083 crosslinks DNA at N^7-guanines leading to S/G2 cell cycle arrest, DNA double-strand breaks and cancer cell death[3-6].*

REFERENCES

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3. Zhai B, et al. Cancer Res. July 2017: 77(13), #2483
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VAL-083 SHOWED BROAD CYTOTOXIC EFFECT AGAINST A PANEL OF GBM AND PROSTATE CANCER CELL LINES

Cell line	VAL-083_IC_{50} (μM)	IC_{50} range (μM)
GBM		
M059K	11.0	9.7 – 12.4
M059J	14.5	13.0 – 16.1
Prostate		
PC-3	24.2	15.5 – 37.7
LNCaP	3.1	2.9 – 3.4
DU-145	2.2	1.9 – 2.4
PC-3-DR	22.3	17.8 - 27.8

TABLE 1. (A) *Two human GBM and four prostate cancer cell lines were treated with different concentrations of VAL-083 (0, 100 nM, 500 nM, 1 μM, 2.5 μM, 5 μM, 10 μM, 25 μM, 50 μM, and 100 μM) for 72h. Cell death was determined by crystal violet assay and IC_{50} values were calculated by sigmoidal dose-response curve fitting using GraphPad Prism 6 (n=3).*

RESULTS: *VAL-083 showed cytotoxicity against all six GBM and prostate cancer cell lines tested.*

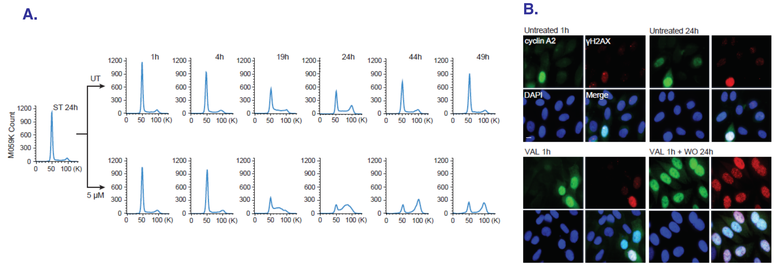

FIGURE 2. (A) *Serum-deprived (ST for 24h) M059K GBM cells were treated with or without 5μM VAL-083 for the indicated time periods. Following treatment, cell cycle analyses were performed using PI staining by flow cytometry. Similar results were seen for PC-3 prostate cancer cells.* ***(B)*** *PC-3 prostate cancer cells were synchronized at G_0/G_1 cell cycle phase by 24h serum starvation before treatment with 50μM VAL-083 for 1h in complete medium. After treatment, VAL-083 was removed from the culture and cells were incubated in complete medium for 24h (VAL 1h + WO 24h). Cells were subsequently fixed, permeabilized, and immunostained with anti-cyclin A2 (green) and anti-γH2AX (red) antibodies. Representative IF images shown from two independent experiments. Scale bar = 10 μm.*

RESULTS: *In GBM cells, VAL-083 treatment led to increased PI stain, indicating cell cycle arrest in S-phase. In prostate cancer cells, VAL-083 treatment led to increase in γH2AX (DNA double strand breaks) and cyclin A2 (S-phase arrest), indicating replication-dependent DNA damage and cell-cycle arrest in S-phase.*

VAL-083 TREATMENT ACTIVATED THE HOMOLOGOUS RECOMBINATION PATHWAY IN GBM AND PROSTATE CANCER CELL LINES

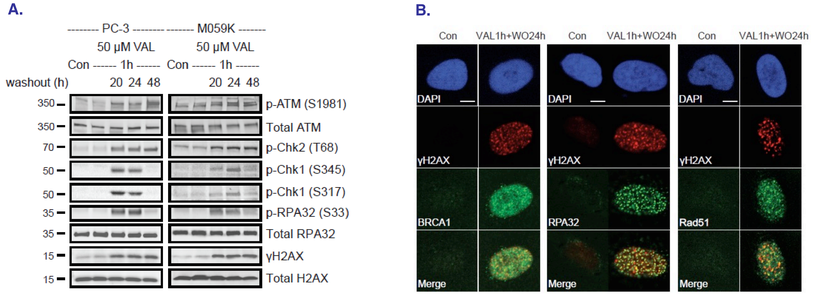

FIGURE 3. (A) *M059K GBM and PC-3 prostate cancer cells were synchronized at G0/G1 cell cycle phase by serum starvation for 24h. The quiescent cells were then treated with 50μM VAL-083 in complete medium for 1h followed by washout of the drug and additional incubation for another 20, 24, or 48h in complete medium. Cell lysates were subsequently extracted and analyzed by western blot for HR mediators using indicated antibodies. Representative images shown from one of three independent experiments.* ***(B)*** *Quiescent PC-3 prostate cancer cells were treated with 50μM VAL-083 for 1h in complete medium. After the treatment, cells were washed and incubated for another 24h. Cells were pre-extracted by cytoskeletal buffer, fixed, permeabilized, and probed with corresponding antibodies detecting indicated proteins (BRCA1, RPA32, Rad51, and γH2AX). Representative confocal images shown from one of three independent experiments. Scale bar = 5μm.* ***(C)*** *M059K cells were knocked down of BRCA1 by transient transfection using three non-overlapping BRCA1-targeting siRNAs (B1, B4, or B5) or negative control siRNA (C). After 24h transfection, cells were treated with different concentrations of VAL-083 for 5 days before crystal violet assay. IC50 values for VAL-083 were calculated using GraphPad Prism 6. The data are presented as mean ± SD. BRCA1-knockdown was confirmed by western blot. GAPDH = loading control. *p-value<0.05, **p-value<0.01, n=3.*

RESULTS: *VAL-083-induced DNA damage lead to activation of the homologous recombination repair pathway in GBM and prostate cancer cells and the potency of VAL-083 cytotoxicity was increased in cells with impaired BRCA1.*

VAL-083 DISPLAYED SYNERGY WITH TOPOISOMERASE INHIBITORS

TABLE 2. VAL-083 combination treatment with topoisomerase inhibitors in GBM cell line M059K

VAL-083 + etoposide		VAL-083 + irinotecan	
Cytotoxic effect (Fa)	Combination Index (CI)	Cytotoxic effect (Fa)	Combination Index (CI)
ED60	0.81 ±0.12	ED50	0.78 ± 0.09
ED75	0.62 ± 0.11	ED75	0.63 ± 0.09
ED90	0.42 ± 0.09	ED90	0.53 ± 0.10

TABLE 2. *M059K GBM cells were treated with VAL-083 in combination with topoisomerase inhibitors etoposide or irinotecan with fixed dose ratios for 72h in complete medium followed by crystal violet assay. Molar ratios of VAL-083:etoposide and VAL-083:irinotecan were 2.5:1 and 3.4:1, respectively. CI values were determined using Chou-Talalay method and Calculsyn software (CI < 1, synergism; CI = 1, additivity; CI > 1, antagonism). The data represent mean ± SD, n=3-4.*

RESULTS: *VAL-083 showed synergistic effects with topoisomerase inhibitors in combination treatment in M059K GBM cells.*

TABLE 3. VAL-083 combination treatment with topoisomerase inhibitors in prostate cancer cell line PC-3

VAL-083 + etoposide		VAL-083 + irinotecan		VAL-083 + Camptothecin	
Cytotoxic effect (Fa)	Combination Index (CI)	Cytotoxic effect (Fa)	Combination Index (CI)	Cytotoxic Effect (Fa)	Combination Index (CI)
ED50	0.58 ±0.10	ED75	0.78 ± 0.22	ED75	0.68 ± 0.07
ED75	0.48 ± 0.03	ED85	0.59 ± 0.12	ED90	0.59 ± 0.03
ED90	0.42 ± 0.05	ED90	0.49 ± 0.07	ED95	0.54 ± 0.03

TABLE 3. *PC-3 prostate cancer cells were treated with VAL-083 in combination with topoisomerase inhibitors etoposide, camptothecin, or irinotecan with fixed dose ratios for 72h in complete medium followed by crystal violet assay. Molar ratios of VAL-083:etoposide, VAL-083:camptothecin and VAL-083:irinotecan were 4.6:1, 250:1 and 2.1:1, respectively. CI values were determined using Chou-Talalay method and Calculsyn software (CI < 1, synergism; CI = 1, additivity; CI > 1, antagonism). The data represent mean ± SD, n=3-4.*

RESULTS: *VAL-083 showed synergistic effects withal three topoisomerase inhibitors in combination treatment in PC-3 prostate cancer cells.*

CONCLUSIONS

- VAL-083 mediates irreparable DNA double strand breaks leading to cell cycle arrest and cancer cell death in GBM and prostate cancer cells.
- VAL-083 activates the homologous recombination pathway in GBM and prostate cancer cells.
- VAL-083 is synergistic with inhibitors of both topoisomerase I (camptothecin and irinotecan) and II (etoposide) in GBM and prostate cancer cells.
- These results provide rationale for clinical investigation of VAL-083 either as single agent or as part of combination regimens in the treatment of cancer patients.