Dianhydrogalactitol (VAL-083) has a distinct mechanism of action that suggests combination with PARP inhibitors as an effective therapeutic strategy

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Ovarian cancer is normally treated with platinum (Pt)-based chemotherapy, but patients frequently develop Pt-resistance. Dysfunctional p53 is implicated in Pt- resistance, comprising a therapeutic challenge in high grade serous ovarian cancer (HGSOC), where p53 is universally mutated (~96%)[1]. Attempts to overcome Pt-resistance in HGSOC include agents blocking the DNA repair pathways, most notably the PARP inhibitors (PARPi), leading to the accumulation of DNA double strand breaks (DSBs) and cancer cell death. However, PARPi resistance frequently arises, leading to a 5-year survival rate of only 40% in this cancer. VAL-083 is a first-in-class DNA damaging agent with demonstrated clinical activity against a range of cancers, including ovarian. VAL-083 rapidly induces interstrand cross-links at guanine N7 leading to DSBs, activation of the homologous recombination (HR) DNA repair pathway, S/G2 cell cycle arrest and cancer cell death. Notably, VAL-083 induces cell death through two parallel pathways - one p53-independent and one p53-dependent and we have shown that VAL-083 is able to overcome cisplatin resistance in a panel of ovarian cancer cells, independent of p53 status. We have also shown that VAL-083 maintains activity independent of prominent DNA repair mechanisms such as non-homologous end-joining (NHEJ) and mismatch repair (MMR) implicated in resistance to chemotherapeutics, including cisplatin and PARPi. Cancer cells thus rely heavily on a functional HR pathway for repair of VAL-083-induced DSBs, proposing combination therapy with agents further inducing DSBs or blocking their repair, including PARPi. Taken together, these data propose VAL-083's potential for targeting Pt-resistant HGSOC and for combination treatment with PARPi.

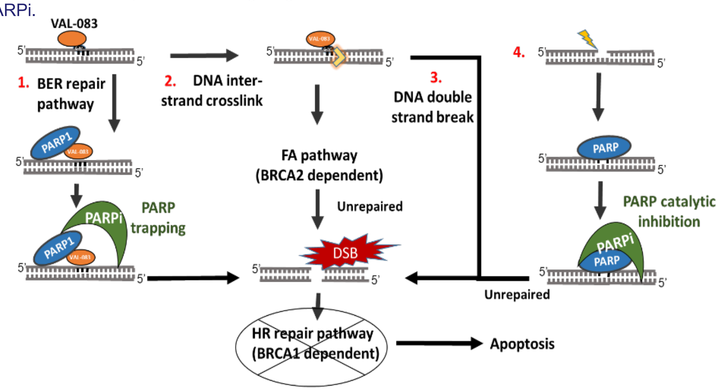

FIGURE 1. VAL-083 synergy with PARPi is anticipated to work through multiple mechanisms leading to DSBs overwhelming HR repair: (1) VAL-083 targets guanine N7, activating the PARP-dependent BER pathway. PARPi trap the PARP1-DNA complex[2], causing the lesion to turn into DSBs; (2) VAL-083 rapidly forms DNA ICLs, activating the Fanconi Anemia (FA) repair pathway, frequently deficient in ovarian cancer. Unrepaired ICLs turn into DSBs; (3) VAL-083-induced ICLs become DSBs during DNA replication in the cell cycle S-phase[3]; and (4) spontaneously occurring single strand breaks require PARP for repair. PARPi inhibit PARP, causing the SSBs to turn into DSBs.[4]

References

1. The Cancer Genome Atlas Research Network. *Nature*. 474(7353): 609–615, 2011
2. Murai J, et al. *J Pharmacol Exp Ther.* 349(3):408-16, 2014
3. Zhai B, et al. *Neuro-Oncology*, Volume 18, Issue suppl_6, November 2016
4. Underhill C, et al. *Ann Oncol.* 2011. 22(2):268-79

CONCLUSIONS & FUTURE DIRECTIONS

- **Historical clinical activity combined with recent preclinical data, suggests VAL-083 may overcome Pt-resistance either alone or as part of a combination treatment with PARP inhibitors.**
  - VAL-083 is able to overcome cisplatin-resistance in ovarian cancer cells independent of p53 status.
  - VAL-083 can synergize with PARP inhibitors in both a BRCA-proficient and -deficient setting, particularly olaparib, talazoparib and niraparib.
  - VAL-083 displays increased activity in HR-deficient ovarian cancer cells, suggesting VAL-083 as a treatment alternative for HR-impaired ovarian cancer.

VAL-083 REPROVe Trial *(NCT03281681)*

Phase 1-2 Trial in Recurrent Platinum-Resistant Ovarian Cancer

- IND has been allowed by US FDA.
- *Primary endpoint:* Demonstration of overall response rate (ORR) benefit compared to historical control, as determined using RECIST v1.1.
- *Secondary endpoints:* Safety & tolerability, progression free survival, duration of response, overall survival, pharmacokinetics and evaluation of symptoms using the FOSI index.

VAL-083 HAS THE ABILITY TO OVERCOME CISPLATIN RESISTANCE IN OVARIAN CANCER CELLS AND DISPLAYS INCREASED ACTIVITY WHEN BRCA IS IMPAIRED.

VAL-083 overcomes cisplatin-resistance in a panel of ovarian cancer cell lines, independent of their p53 status (Table 1). In addition, VAL-083 cytotoxic activity was increased (IC_{50} decreased) when BRCA was knocked down (Figure 2). This ***suggests VAL-083 as a treatment alternative for Pt-resistant ovarian cancer***, including HR-impaired ovarian cancers.

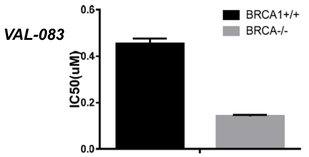

FIGURE 2. VAL-083 cytotoxic activity was increased (IC_{50} decreased) when BRCA was knocked down (BRCA-/-) compared to control siRNA (BRCA1+/+).

TABLE 1. Characteristics and cytotoxicity of VAL-083 against a panel of ovarian cancer cell lines with wild type p53 (A2780), knocked out (A2780 p53-/-) or mutant p53 (2780CP, OVCAR10, HEY and OVCA-433) harboring P72R, V172F, and/or G266R mutations. All five cell lines are believed to be HR proficient. IC_{50} values are the mean of 3 independent studies.

Cell line	A2780 p53 +/+	A2780 p53 -/-	2780CP	OVCAR-10	HEY	OVCA-433
Histology	Unknown	Unknown	Unknown	Adenocarcinoma	HGSOC	HGSOC
P53 status	WT	Knocked out	V172F	V172F, G266R	P72R	P72R
Cisplatin sensitivity/resistance	Sensitive	Less sensitive	Resistant	Resistant	Resistant	Resistant
VAL-083 IC_{50}	0.6 µM	1.3 µM	2.2 µM	3.6 µM	2.1 µM	2.3 µM

THE CYTOTOXIC ACTIVITY OF VAL/083/PARPI COMBINATION IS SUPERADDITIVE AGAINST OVARIAN CANCER CELLS, BOTH BRCA-PROFICIENT AND BRCA-DEFICIENT.

VAL-083 produces superadditivity in combination with PARP inhibitors olaparib, talazoparib and niraparib in both HR-proficient (control siRNA) and HR-deficient (BRCA1 siRNA) settings. VAL-083 combination with rucaparib produced superadditive cytotoxicity in the BRCA-deficient setting, while VAL-083 combination with veliparib was no more than additive. These results demonstrate that ***VAL-083 can synergize with some PARP inhibitors*** in both a BRCA-proficient and -deficient setting.

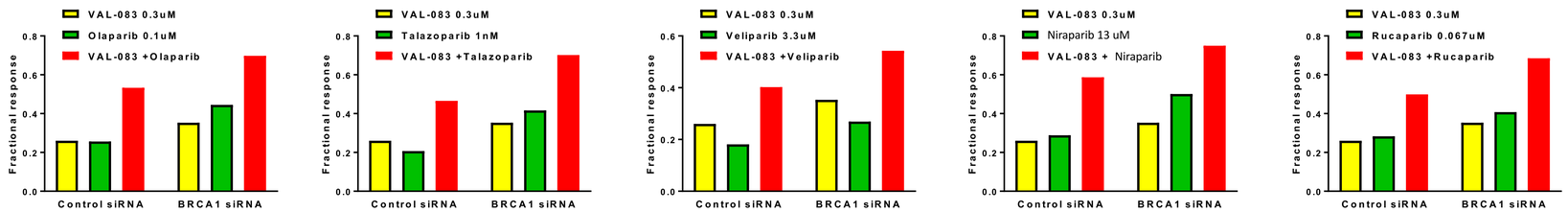

FIGURE 3. Ovarian A2780 tumor cells were treated with control (left panels) or BRCA1 siRNA (right panels) for 24 hr and washed. After a further 24 hr to allow cell attachment, cells were exposed to VAL-083 and/or one of the PARP inhibitors indicated at a concentrations that individually give in control cells about a 20-25% fractional affect (Fa or growth inhibition). After 5 days, the cells were processed for the MTT assay.

TABLE 2. VAL-083 combination with PARP inhibitors in A2780 ovarian cancer cells. The percentages shown in the table refers to the increase in cytotoxic activity above that predicted for simple additivity in each case. HR-proficient A2780 ovarian cancer cells: no BRCA siRNA treatment; HR-deficient cells: BRCA1-siRNA treated A2780 ovarian cancer cells (~90% BRCA1 knockdown).

VAL-083 + PARP inhibitor	VAL-083 + olaparib	VAL-083 + talazoparib	VAL-083 + veliparib	VAL-083 + niraparib	VAL-083 + rucaparib
HR-proficient (BRCA1)	18.4%	12.5%	2.0%	24.0%	6.2%
HR-deficient (BRCA1 knockdown)	8.9%	12.6%	2.9%	10.7%	11.0%