Activity of dianhydrogalactitol (VAL-083) in ovarian tumor models, sensitive and resistant to cisplatin

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ABSTRACT

Purpose: Platinum drug resistance is a major clinical impediment in ovarian cancer. It is ascribed to several mechanisms, with dysfunctional p53 playing a critical role, particularly in the highly lethal high-grade ovarian serous carcinoma, where p53 mutations are 96%[1]. The goal of this study was to examine dianhydrogalactitol (VAL-083) for its potential in an ovarian tumor panel representing different p53 statuses and cisplatin-resistance. VAL-083 has demonstrated clinical activity against a range of tumor types, including ovarian cancer, in historical NCI-sponsored clinical studies, but its activity in a cisplatin-resistance setting is not elucidated. VAL-083 is a bi-functional alkylating agent with a distinct mechanism of action, forming DNA interstrand cross links at the N7 position of guanine leading to DNA double strand breaks, whereas cisplatin predominantly forms intrastrand DNA cross-links.
Methods: The tumor panel was composed of cisplatin-sensitive wild type (wt) p53 ovarian cancer cell line A2780, cisplatin-resistant A2780-derived heterozygous p53-V172F mutant 2780CP/Cl-16 cells, and three other cisplatin-resistant models harboring V172F/G266R (OVCAR-10) or P72R (Hey and OVCA-433) p53 mutations. Cells were exposed to cisplatin, VAL-083 or olaparib (A PARP inhibitor) or combinations, and the IC_{50} cytotoxic parameter was determined by fitting the 5-day MTT cell survival data to a 4-parameter sigmoidal curve. MTT assay was used in conjunction with Bliss Independence Model and combination index approaches to assess VAL-083/cisplatin and VAL-083/olaparib combinations. Homologous recombination (HR) pathway was inhibited in A2780 ovarian tumor cells by down-regulation of BRCA1 with siRNA oligos for 24h and then exposing to VAL-083 for 5 days to assess IC_{50}.
Results: The IC_{50} of cisplatin in the cisplatin-sensitive A2780 model was 0.2-0.3 μM. In contrast, the IC_{50} in the four cisplatin-resistant models (2780CP/Cl-16, OVCAR-10, Hey and OVCA-433) was 3-8 μM; a 10- to 27-fold increase. The corresponding IC_{50} for VAL-083 was about 0.5 μM in A2780 cells and 2-4 μM in the four cisplatin-resistant models; a 4- to 7-fold increase, suggesting a distinct mode of action of VAL-083 and the ability to circumvent 70-85% of cisplatin-resistance. In addition, these results further indicate that VAL-083 activity is less dependent on p53 status. The apparent differences in the mechanism between cisplatin and VAL-083 prompted a combination study with the two agents, and consistent synergy was demonstrated.
Conclusions: VAL-083 is able to circumvent 70-85% cisplatin-resistance in ovarian tumor models, and its activity is less dependent on p53 status. Together with overt synergy between VAL-083 and cisplatin, our results demonstrate the effectiveness of VAL-083 against cisplatin-resistant ovarian cancer as a single agent or in combination with cisplatin. We further showed VAL-083 potentiation by HR inhibition, suggesting HR-dependent repair of VAL-083-induced DNA lesions. Together with VAL-083 super-additivity with PARP inhibitor olaparib, our results suggest VAL-083 as a possible treatment alternative in cisplatin-resistant and HR impaired ovarian cancer, as a single agent or in combination with a PARP inhibitor.
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BACKGRUND

Ovarian cancer is the leading cause of death from gynecologic cancers in North America. Although initially responsive to standard-of-care chemotherapy based on platinum-taxane combinations, most tumors recur. Recurrent ovarian cancer has a poor prognosis with median survival of 12-24 months and 5-year survival of ~20%. Therefore, there is a major clinical need for treatment with alternatives that can circumvent resistance to standard-of-care chemotherapy.[1]
VAL-083 is a bifunctional alkylating agent causing interstrand DNA crosslinks at N7 of guanine, independent of MGMT and MMR repair mechanisms, which is believed to be distinct from the mechanisms of other alkylating agents targeting DNA at N7 of guanine (e.g. cisplatin/carboplatin or BCNU).[2,3] In prior clinical studies sponsored by the US National Cancer Institutes, VAL-083 exhibited clinical activity against a number of cancers including ovarian,[4] cervical, lung, brain and hematologic malignancies. VAL-083 is approved in China for the treatment of chronic myelogenous leukemia and lung cancer. DelMar Pharmaceuticals recently completed a Phase I/II clinical study of VAL-083 in refractory glioblastoma (GBM) in the United States (**ClinicalTrials.gov Identifier** NCT01478178) and VAL-083 has received orphan drug designation in the U.S. for the treatment of ovarian cancer and malignant glioma. We have previously shown that the unique cytotoxic mechanism of VAL-083 overcomes mechanisms of resistance in chemo-resistant subgroups of NSCLC and GBM. In particular, activity of VAL-083 appears to be independent of p53-related resistance to platinum-based chemotherapies in NSCLC cell lines. These observations are guiding the development of planned clinical trials in GBM and NSCLC. Since platinum-based chemotherapy forms the basis of therapeutic regimens in ovarian cancer treatment, and p53-mediated resistance remains an unmet medical need and harbinger of poor outcomes, we hypothesized that a similar opportunity may be represented in ovarian cancer. We furthermore hypothesize that VAL-083 will synergize with HR dysfunction and PARP inhibitors in cisplatin-resistant ovarian cancer. This synergy is expected to be particularly potent if the HR pathway is impaired by BRCA mutations or other mutations in the HR pathway leading to BRCAness phenotypes, as in refractory high grade serous ovarian cancer. Such synergistic efficacy could be especially advantageous, considering that only ~30-50% of patients treated with platinum/olaparib combination for HR-defective cancers experience response, mostly partial.[1] This preclinical study thus seeks to investigate the potential for VAL-083 as a treatment alternative in chemo-resistant ovarian cancer, which, juxtaposed against VAL-083 activity in clinical historic trials, may form the basis for a new strategy in the treatment of chemo-resistant ovarian cancer.

RESULTS

VAL-083 activity in five wild-type p53 ovarian cancer cell lines

The activity of VAL-083 was examined in wild-type p53 ovarian cancer cell lines: cisplatin-sensitive A2780, and cisplatin-resistant 2780CP-16, OVCAR-10, Hey and OVCA-433 tumor cells. The IC_{50} for VAL-083 in the cisplatin-resistant cell-lines 2780CP-16, OVCAR-10, Hey and OVCA-433 were 4- to 7-fold greater than for A2780; while the corresponding IC_{50} values for cisplatin in these models were 10- to over 25-fold greater. **These results demonstrate that VAL-083 is able to circumvent 70-85% of cisplatin-resistance and further suggest distinct modes of action for the two drugs.**

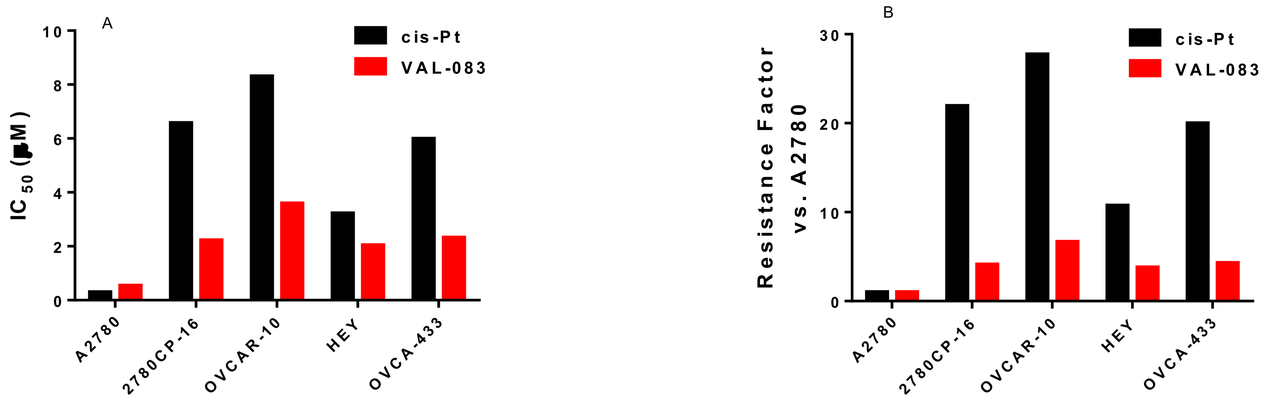


Figure 1. Cytotoxicity (A) and resistance factors (B) of cisplatin and VAL-083 in wild-type p53 ovarian cancer cell lines. Resistance Factor is calculated the ratio of IC_{50} in the resistant cell line to the IC_{50} in A2780.

Limited dependence of VAL-083 on p53 status

The dependence on p53 status was investigated in isogenic models with (HCT-116$^{p53-/-}$) or without (HCT-116$^{p53+/+}$) p53 knockout. Loss of p53 increased resistance to cisplatin and oxaliplatin by 3- and 6-fold, respectively, whereas the increase in resistance to VAL-083 was <2-fold. **This suggest a mechanism of VAL-083 that is less dependent on wild-type p53 than cisplatin**.

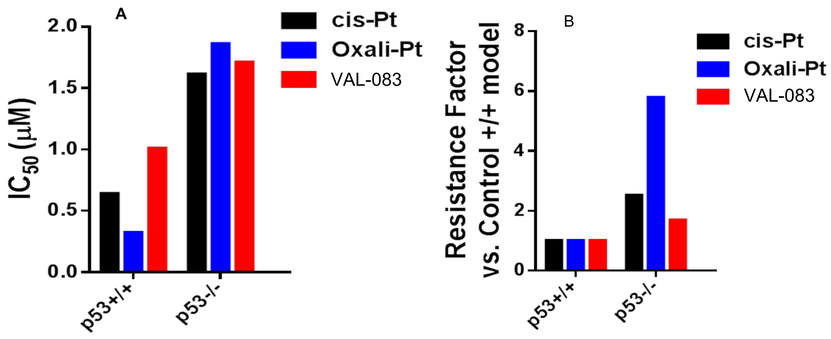


Figure 2. IC_{50} values (A) and resistance factors (B) for cisplatin, oxaliplatin and VAL-083 in molecularly engineered isogenic models of HCT-116 with (p53+/+) or without (p53-/-) p53.

The potency of VAL-083 is increased when HR is impaired

The activity of VAL-083 was examined in A2780 cells with impaired HR DNA repair system by knocking down BRCA1, which is required for HR proficiency. The potency of VAL-083 activity was increased (IC_{50} was reduced) when HR was impaired, demonstrating that VAL-083 induced DNA-lesions are repaired via HR. **This suggests VAL-083 as a treatment option in HR-deficient ovarian cancer** either alone or as part of a combination treatment.

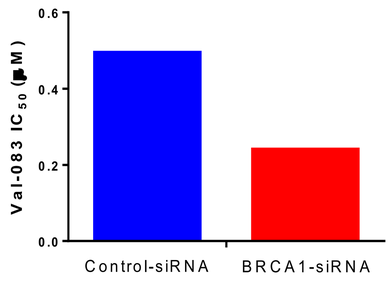
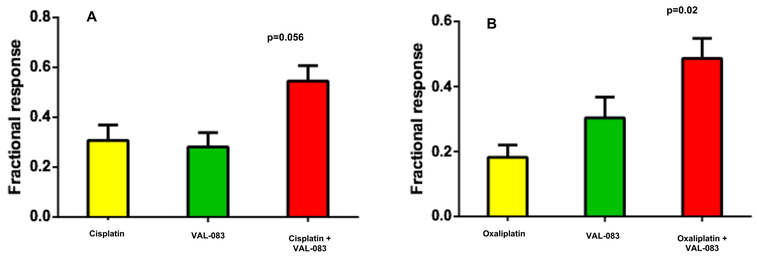


Figure 3. HR was inhibited in A2780 ovarian tumor cells by down-regulation of BRCA1 with siRNA oligos for 24h and then exposing to VAL-083 for 5 days to assess IC_{50}.

Combination of VAL-083 with cisplatin or oxaliplatin in p53 mutant H1975 NSCLC

The combination of VAL-083 with either cisplatin (A and C) or oxaliplatin (B and D) in the human H1975 model demonstrated significant superadditivity ($p \leq 0.06$; A and B) and synergy (CI<1; C and D). These results suggest non-overlapping mechanism of action between the platinum drugs and VAL-083, and **support the potential for synergistic benefit for a combination of VAL-083 and platinum-based therapies in the treatment of cisplatin-resistant cancers**.



Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Cisplatin	CI
ED75	0.97	0.49	0.78
ED90	2.01	1.00	0.68
ED95	3.30	1.65	0.63

Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Oxaliplatin	CI
ED75	0.78	0.44	0.65
ED90	1.49	0.84	0.53
ED95	2.31	1.31	0.46

Figure 4. Cytotoxicity of VAL-083 in combination with cisplatin or oxaliplatin in the H1975 cell line. Fa: Fraction of cells affected. ED75: effective dose that kills 75% of cells.

VAL-083 combination with PARP inhibitor olaparib is super-additive

The activity of VAL-083, PARP inhibitor olaparib and a combination of the two was examined in A2780 ovarian cancer cells. The VAL-083/olaparib combination was super-additive, suggesting potential for synergistic interaction. **This suggests the potential for a synergistic benefit for a combination of VAL-083 and PARP inhibitors in ovarian cancers.**

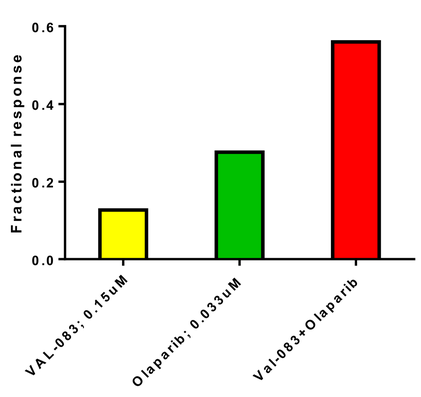


Figure 5. A2780 cells were exposed to 0.15mM VAL-083 ±0.033mM of PARP inhibitor olaparib individually or simultaneously and assessed for cytotoxicity 5 days later.

CONCLUSIONS & FUTURE DIRECTIONS

- VAL-083 demonstrated cytotoxic activity against all ovarian cancer cell lines tested
- VAL-083 was able to circumvent 70-85% of cisplatin-resistance in an ovarian cancer cell line panel with several known p53 mutations
- VAL-083 is substantially less dependent on wild-type p53 for cytotoxic activity
- VAL-083 has a distinct mode of action from platinum-based chemotherapy currently used in the treatment of ovarian cancer
- VAL-083 displays significant synergy with cisplatin in p53 mutant cell line H1975
- VAL-083 potency is increased when HR is impaired, suggesting that VAL-083-induced DNA lesions are repaired via the HR pathway
- VAL-083 displays super-additivity with PARP inhibitor olaparib in ovarian cancer cell line A2780
- Taken together, these results support VAL-083 as a viable treatment option for ovarian cancer patients failing platinum-based therapy, particularly in a HR-impaired setting. They further suggest a potential benefit of therapeutic combination regimens containing VAL-083/platinum or VAL-083/olaparib

References

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