A comparison of mechanisms and cytotoxic activity of dianhydrogalactitol (VAL-083) to cisplatin in ovarian tumor models harbouring wild-type and mutant p53

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ABSTRACT # A1:

Ovarian cancers are usually treated with platinum-based therapies, which produce a 70% response rate. However, many patients relapse as tumors become resistant to cisplatin and carboplatin, resulting in a 5-year survival rate of about 20%. Onset of drug resistance is a major factor limiting the clinical utility of platinum-based therapeutic regimens drugs and, therefore, new agents are needed to circumvent resistance. Dianhydrogalactitol (VAL-083) is a bi-functional alkylating agent, whose cytotoxic activity is due to the formation of DNA cross links at the N7 position of guanine. Unlike cisplatin and carboplatin, which predominantly form intrastrand DNA cross-links, VAL-083 derives its anti-cancer activity interstrand DNA cross-links. More importantly, VAL-083 has demonstrated clinical activity against a range of tumor types, including ovarian cancer in historical NCI-sponsored clinical studies. Platinum drug resistance is normally ascribed to several mechanisms, with mutation in wild-type p53 playing a critical role, particularly in high-grade ovarian serous carcinoma (HGOSC), where the incidence of this mutation can be substantial. However, recent analysis of the TCGA database indicates that the survival rate in wild-type p53 HGOSC is no better or perhaps even worse. Using a 5-day MTT assay and fitting a sigmoidal curve to the dose-response data to determine IC_{50} values, we have similarly found that resistance to cisplatin in a panel of ovarian tumor models is greater when p53 is wild-type (median IC_{50}: 4-7 µM vs. 1-2 µM for mutant/null p53 models; in comparison, sensitive wild-type p53 A2780 cells have an IC_{50} of 0.2-0.3 µM). Previous studies in our lab suggest that factors downstream from p53, including MDM4 and p21, may contribute to cisplatin-resistance in ovarian cancer models with high cisplatin-resistance and wild-type 53, like 2780CP-16. We thus sought to investigate the potential of VAL-083 to circumvent cisplatin-resistance in five p53 wild-type ovarian cancer models: one cisplatin-sensitive A2780, and four cisplatin-resistant 2780CP-16, OVCAR-10, Hey and OVCA-433. IC_{50} values of VAL-083 were generated using the MTT assay and sigmoidal curve fitting of data, as described above. The baseline IC_{50} for VAL-083 against A2780 cell was about 0.5 µM. 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; however, VAL-083 was substantially more potent in comparison to cisplatin in these models where corresponding IC_{50} values were 10- to over 25-fold greater. These results demonstrate that there is only partial cross-resistance between cisplatin and VAL-083 further suggesting distinct modes of action for the two drugs. In order to examine whether the partial circumvention of cisplatin-resistance was p53-dependent, cytotoxicity was determined in isogenic HCT-116$^{p53-/-}$ and HCT-116$^{p53+/+}$ models. These studies demonstrated that loss of p53 increased resistance to cisplatin by 2-5-fold whereas loss of p53 only increased resistance to VAL-083 about 1.7-fold. These results suggest that VAL-083 is less dependent on p53 for its cytotoxic activity. Immunoblots confirmed this in 2780CP-16 cells, where VAL-083 was more effective than cisplatin at increasing p53 and p21 levels, and induced relatively greater Ser-15 and Ser-20 phosphorylations, further supporting different modes of action for the two drugs. In contrast, both drugs were equally effective in inducing these markers of DNA damage in A2780 cells. The non-overlapping mechanisms of action suggested a potential therapeutic benefit for combinations of VAL-083 with cisplatin. We have previously reported that the combination of VAL-083 with cisplatin in wild-type p53 NSCLC models H460 and A549, and mutant p53 NSCLC H1975 demonstrated significant super-additivity ($p<0.05$) and synergy (CI < 1) in all three cell-lines. Taken together these results demonstrate the effectiveness of VAL-083 against refractory cisplatin-resistant ovarian cancers and raise the potential for treatment of platinum-resistant ovarian cancers or a combination regimen with cisplatin. (Supported in part by NCI RO1 CA160687 to ZHS).

BACKGROUND

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 (Fig. 1) is a bifunctional alkylating agent causing interstrand DNA crosslinks at N7 of guanine (Fig. 2), which is believed to be distinct from the mechanisms of other alkylating agents targeting DNA at N7 of guanine (e.g. cisplatin or carboplatin).[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. VAL-083 is currently undergoing clinical studies for refractory glioblastoma multiforme (GBM) in the United States (**ClinicalTrials.gov Identifier** NCT01478178) and has received orphan drug designation in Europe and the U.S. for the treatment of malignant gliomas. 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 NSCLC cell lines. These observations have guided the development of planned clinical trials in 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. 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.

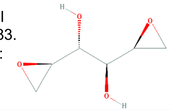

Figure 1. Chemical structure of VAL-083.
Molecular Formula: $C_8H_{10}O_4$.
MW: 146.1 g/mol

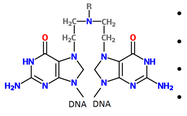

Figure 2. N7-guanine interstrand DNA crosslink

- **Cross-link at N7 position of guanine**
- **Interstrand cross-links**
- **Double-strand DNA breaks**
- **Apoptosis & Cell Death**

References

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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 there is only partial cross-resistance between cisplatin and VAL-083, further suggesting distinct modes of action for the two drugs.

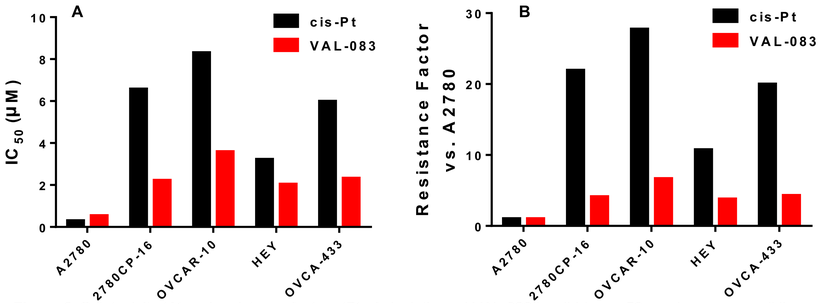


Figure 3. Cytotoxicity (A) and resistance factors (B) of cisplatin and VAL-083 in wild-type p53 ovarian cancer cell lines. Resistance Factor is calculated the ratio of IC_{50} in the resistant cell line to the IC_{50} in A2780.

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.

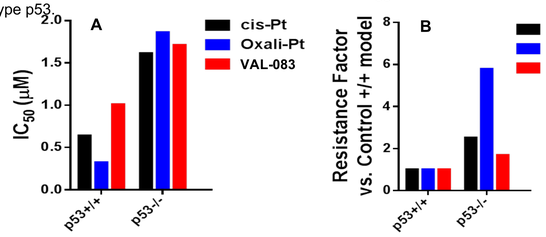


Figure 4. IC_{50} values (A) and resistance factors (B) for cisplatin, oxaliplatin and VAL-083 in molecularly engineered isogenic models of HCT-116 with (p53+/+) or without (p53-/-) p53.

VAL-083 effect on p21 and p53 Ser-15/Ser-20 phosphorylation

Mutations in p53 plays a critical role in the emergence of cisplatin resistance. However, cisplatin resistance frequently appears in ovarian cancers in spite of wild-type p53 due to factors deregulating p53 induction, activation via phosphorylation and transactivation function. In A2780 cells, VAL-083 and cisplatin at equitoxic concentrations had similar effects on p53 induction/activation via phosphorylation and p21 induction. However, in cisplatin-resistant 2780CP-16 cells, VAL-083 was more effective than cisplatin at increasing p53 and p21 levels, and at inducing relatively greater Ser-15 and Ser-20 phosphorylations in p53. This further supports the conclusion of different modes of action for VAL-083 and cisplatin.

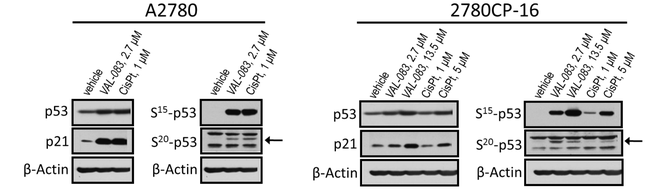
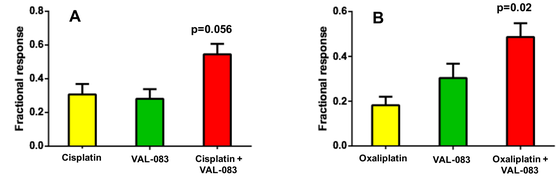


Figure 5. Immunoblots of p53 and p21 inductions and post-translational phosphorylation of p53

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 ($p\leq0.06$; 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.



C

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

D

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 6. 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.

CONCLUSIONS

- **VAL-083 demonstrates cytotoxic activity against all tested ovarian cancer cell lines, including cisplatin-resistant cell lines with known p53 mutations, *in vitro***
- **VAL-083 is substantially less dependent on wild-type p53 for cytotoxic activity and appears to have a distinct mode of action versus platinum-based chemotherapy currently used in the treatment of ovarian cancer**
- **VAL-083 increases p53 and p21 levels more effectively than cisplatin in cisplatin-resistant ovarian cancer cells, *in vitro***
- **VAL-083 displays significant synergy with cisplatin in p53 mutant cell line H1975, *in vitro***
- **Taken together, these results support VAL-083 as a viable treatment option for ovarian cancer patients failing platinum-based therapy, and suggests a potential benefit of combination therapeutic regimens containing platinum and VAL-083**