Enhanced in vitro activity of dianhydrogalactitol (VAL-083) in combination with platinum drugs: impact of p53 and platinum-resistance

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BACKGROUND

VAL-083 is a bifunctional alkylating agent causing alkylation of N^7-guanine leading to interstrand DNA crosslinks and DNA double strand breaks (DSB). This mechanism is distinct from other alkylating agents used in the treatment of cancer (Table 1). Likely due to its different mechanism, VAL-083 has also been shown to overcome both BCNU-resistance[3] and temozolomide-resistance[4] *in vitro*. In prior clinical studies sponsored by the US National Cancer Institutes, VAL-083 exhibited clinical activity against a number of tumor-types in a number of cancers including lung, brain, cervical, ovarian and hematologic malignancies. VAL-083 is approved in China for the treatment of chronic myelogenous leukemia and lung cancer and has received orphan drug designation in the U.S. for the treatment of gliomas and medulloblastoma and in Europe for gliomas.

***Table 1**. VAL-083 has a distinct mechanism of action from other DNA modifying agents.*

	VAL-083[3,4]	Temozolomide[5]	BCNU[3,5]	Cisplatin[5,6]
Pharmacophore	Epoxide	Diazomethane	Nitrosourea	Dichloroplatinum
DNA target	N7-Guanine	O6-Guanine	O6-Guanine	N7-Guanine
DNA damage	Bifunctional alkylation, interstrand crosslinks	Monofunctional alkylation, G-T mismatch, SSB	Bifunctional alkylation, interstrand crosslinks	Intrastrand crosslinks (G-G)
ICL formation	+ (rapid, G-G)	-	+ (slow, G-C)	-
DNA DSB	+	-	+	(+)
Cell cycle arrest	Late S/G2	G2/M	G2/M	G2/M
DNA repair pathways	HR	MGMT, MMR	MGMT, BER, HR	NER, MMR
MGMT sensitive	-	+	+	-

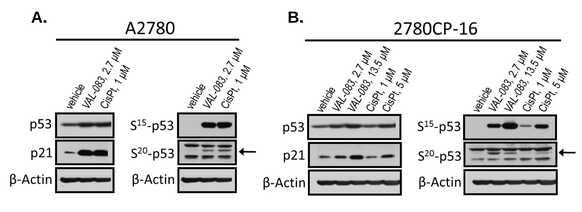
MGMT: O6-alkylguanine DNA alkyltransferase; ICL: interstrand crosslinks; DSB: doubles strand breaks, MMR: mismatch repair; BER: base excision repair; HR: homologous repair; NER: nucleotide excision repair

ABSTRACT # 5279:

Cisplatin is an important frontline drug for ovarian carcinoma and non-small cell lung cancer (NSCLC). However, the initial response rates of up to 70% are usually followed by relapse due to the onset of drug resistance. Mechanistically, platinum resistance is multifactorial, with loss of p53 function (by mutation or inhibitory protein binding to wild-type (wt) p53) playing a central role. The appearance of drug resistance is a major clinical barrier and, therefore, new agents are needed to overcome this limitation. Dianhydrogalactitol (VAL-083) is a bi-functional alkylating agent that induces DNA interstrand cross-links at N7-guanine, a mechanism that is distinct from the intrastrand cross-links by platinum-based drugs. VAL-083 is clinically approved in China for lung cancer, and is undergoing clinical trial in the US for glioma. In the present study, we have examined the *in vitro* cytotoxicity of VAL-083 as a single agent and in combination with cisplatin or oxaliplatin using the 5-day MTT assay. In the isogenic HCT-116$^{p53+/+}$ and HCT-116$^{p53-/-}$ colorectal models, loss of p53 increased IC_{50} of (or resistance to) cisplatin and oxaliplatin by 3 to 6-fold, whereas resistance to VAL-083 was increased only 1.7-fold by loss of p53. These results indicate that the cytotoxicity of VAL-083 is less impacted than the platinum drugs by loss of p53. When tested in cisplatin-sensitive (A2780) vs. cisplatin-resistant wt p53 ovarian tumor models (2780CP-16, OVCAR-10, Hey and OVCA-433) there was a 10 to 40-fold increase in IC_{50} for cisplatin, while the corresponding increase in IC_{50} for VAL-083 was only 4 to 7-fold. This indicates only partial cross-resistance between VAL-083 and cisplatin and thus suggests a distinct mode of action for VAL-083 as compared to cisplatin. To further investigate, immunoblots were developed after a 24-h exposure of isogenic A2780 or 2780CP-16 models to cisplatin or VAL-083. The two drugs were equally effective at stabilizing and activating p53 in A2780 cells. However, in cisplatin-resistant 2780CP-16 cells, VAL-083 was more effective than cisplatin at increasing p53 and p21 levels, and at inducing Ser-15 and Ser-20 phosphorylation of p53. This is consistent with the ability of VAL-083 to circumvent cisplatin resistance and demonstrated that this alkylating agent also has the capacity to partially restore wt p53 function in ovarian tumor cells. The independent mode of actions of these drugs suggested the potential for combining VAL-083 with cisplatin or oxaliplatin. These combinations in wt p53 (H460 and A549) and mutant p53 (H1975 and H157) NSCLC models demonstrated significant super-additivity ($p<0.05$) and/or synergy (CI < 1). Taken together, these results demonstrate the antitumor activity of VAL-083 against both wt and mutant p53 cancers and raise the clinical potential for treatment in a combination setting with platinum drugs.

VAL-083 effect on p53 activation via phosphorylation and DNA damage signaling

Although mutated, $p53^{V172F}$ in 2780CP-16 cells has the potential to be activated. In A2780 cells (A), VAL-083 and cisplatin at equitoxic concentrations had similar effects on p53 phosphorylation and p21 induction. In cisplatin-resistant 2780CP-16 cells (B) however, VAL-083 was more effective than cisplatin at activating p53 and p21, suggesting that VAL-083 can circumvent cisplatin-resistance. Cisplatin-induced DNA damage is mediated via ATM, ATR, Chk1 and Chk2 kinases. In A549 cells (C), DNA damage signaling by VAL-083 also involved these kinases, as indicated by activation (phosphorylation) of kinases in a time and dose-dependent manner. These results suggest that factors downstream from ATM and ATR or other signaling pathways are responsible for VAL-083 circumvention of cisplatin-resistance.



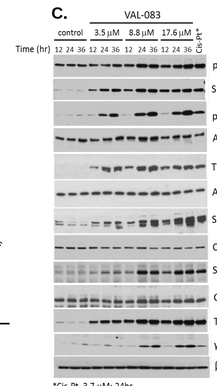


Figure 3. Immunoblots for p53 activation (A), p21 induction (B) and DNA damage signaling (C).

Superadditivity/synergy of VAL-083 with platinum drugs

The combination of VAL-083 with either cisplatin or oxaliplatin in four human NSCLC models is superadditive ($p<0.05$; Figs. 4 & 5) and/or synergistic (CI < 1; Table 2).

Figure 4. VAL-083 with cisplatin

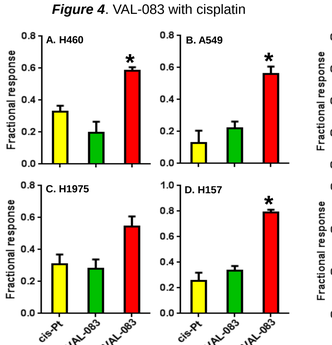


Figure 5. *VAL-083 with oxaliplatin*

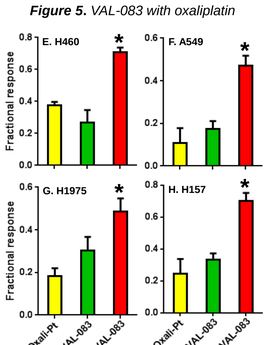


Table 2. CI of VAL-083 + Pt

A. H460 (VAL-083 + cis-Pt)

Cytotoxic Level (Fa)	Concentration (µM) VAL-083	Cisplatin	CI
ED75	0.42	0.38	0.92
ED90	0.92	0.85	0.91
ED95	1.58	1.45	0.90

B. A549 (VAL-083 + cis-Pt)

Cytotoxic Level (Fa)	Concentration (µM) VAL-083	Cisplatin	CI
ED75	0.42	0.38	0.92
ED90	0.92	0.85	0.91
ED95	1.58	1.45	0.90

C. H1975 (VAL-083 + cis-Pt)

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. H157 (VAL-083 + cis-Pt)

Cytotoxic Level (Fa)	Concentration (µM) VAL-083	Cisplatin	CI
ED75	6.84	4.59	0.86
ED90	10.26	7.57	0.82
ED95	13.51	10.63	0.79

E. H460 (VAL-083 + oxali-Pt)

Cytotoxic Level (Fa)	Concentration (µM) VAL-083	Oxaliplatin	CI
ED75	0.29	0.21	0.86
ED90	0.51	0.37	0.82
ED95	0.73	0.54	0.81

F. A549 (VAL-083 + oxali-Pt)

Cytotoxic Level (Fa)	Concentration (µM) VAL-083	Oxaliplatin	CI
ED75	0.29	0.21	0.86
ED90	0.51	0.37	0.82
ED95	0.73	0.54	0.81

G. H1975 (VAL-083 + oxali-Pt)

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

H. H157 (VAL-083 + oxali-Pt)

Cytotoxic Level (Fa)	Concentration (µM) VAL-083	Oxaliplatin	CI
ED75	6.84	5.00	0.97
ED90	10.26	8.76	1.00
ED95	13.51	12.84	1.03

VAL-083 dependence on p53 status and mode of cell death

Dependence on p53 status was examined in isogenic HCT-116$^{p53+/+}$ and HCT-116^{p53-} cell lines. 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 (Fig. 1A & 1B). This suggest a mechanism of VAL-083 that may be independent of p53 status. This is supported in part by facile apoptotic cell death in both wild-type p53 (A549) and mutant p53 (H1975) cell lines (Fig. 1C).

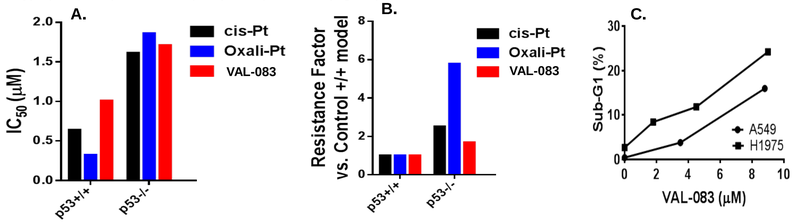


Figure 1. IC_{50} values (A) and resistance factors (B) for cisplatin, oxaliplatin and VAL-083 in molecularly engineered isogenic models of HCT-116 with (p53+/+) or without (p53-/-) p53. Apoptosis (sub-G1) in A549 and H1975 cells (C).

VAL-083 circumvents cisplatin-resistance in a small ovarian panel

The activity of VAL-083 was examined in ovarian cancer cell lines with varying p53 status (indicated below): cisplatin-sensitive A2780 (wild-type), and cisplatin-resistant 2780CP-16 (heterozygous V172F mutant), OVCAR-10 (V172F/G266R mutant), Hey (wild-type) and OVCA-433 (wild-type) tumor cells. The IC_{50} values 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.

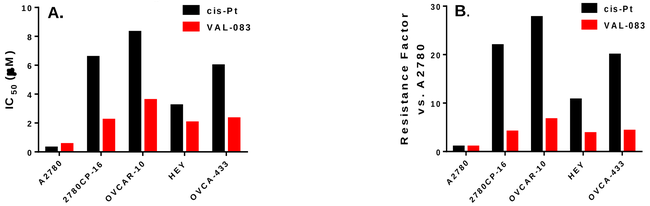


Figure 2. Cytotoxicity (A) and resistance factors (B) of cisplatin and VAL-083 in wild-type p53 ovarian cancer cell lines

METHODS:

The analysis of toxicity, depicted as IC_{50} (concentration inhibiting cell growth by 50%) or as Fraction of cells affected (Fa) at a specified concentration of drug A alone, drug B alone, or a combination of drug A + drug B, is based on the data generated from the MTT growth inhibition assay following a 5-day drug exposure period. The IC_{50} values were generated by fitting Fa values from a range of drug concentrations to a four-parameter logistic dose-response sigmoidal equation. Resistance factors were calculated as the ratio of IC_{50} in the specific tumor model to the IC_{50} in HCT-116$^{p53+/+}$ or A2780 cells. Synergy was assessed from the combination index (CI) using the Chou and Talalay[1] approach for synergism and superadditivity based on the Bliss Model using the comparison between experimental and predicted values for the cytotoxicity of the combination. Significant difference between experimental vs. predicted by Student's t-test. The determination of predictive additive effect of [drug A + drug B] combination is based on the equation described by Tallarida[2] as follows:

Additive effect of [drug A + drug B] = FaA + (1-FaA)FaB

Where FaA or FaB = Fraction of cells affected (Fa) by drug A alone or drug B alone. Data, where appropriate, are provided as Mean ± SE of N=3-7.

References

1. Chou TC, Talalay P. Adv Enzyme Regul 1984;22:27-55
2. Tallarida RJ. J Pharmacol Exp Ther 2001;298:265-72.
3. Institoris E, et al. Cancer Chemother Pharmacol 1989;24(5):311-3
4. Fouse S, et al. SNO annual meeting 2014, Abstract #ET-18
5. Ramirez, YP, et al. Pharmaceuticals 2013, 6(12), 1475-1506 (review)
6. Martin, LP, et al. Clin Cancer Res 2008;14(5): 1291-95 (review)

CONCLUSIONS:

- **VAL-083 induced apoptosis independent of p53 status, and appears to have a distinct mode of action from cisplatin and oxaliplatin;**
- **VAL-083 demonstrated ability to circumvent cisplatin-resistance in all ovarian cell lines tested, including mutant p53 models 2780CP-16 and OVCAR-10;**
- **VAL-083 was active against NSCLC cancer cell lines H1975, H157 and H460 harboring T790M, p53 and/or KRAS mutations, known to confer resistance to currently available therapies;**
- **VAL-083 demonstrated superadditivity/synergy against NSCLC cell lines, including TKI-resistant/mutant p53 H1975 and TKI-sensitive/wild-type p53 A549 models, when combined with either cisplatin or oxaliplatin, *in vitro;***
- **These results support VAL-083 as a viable treatment option for refractory NSCLC and ovarian cancer patients failing platinum-based therapy;**
- **These results also support the potential benefit of VAL-083/platinum combination therapy in newly diagnosed NSCLC patients.**