In vitro activity of dianhydrogalactitol alone or with platinum drugs in the treatment of non-small cell lung cancer

Anne Steino[1], Guangan He [2], Jeffrey A. Bacha[1], Sarath Kanekal[1], Dennis M. Brown[1], Zahid H. Siddik[2]

[1]DelMar Pharmaceuticals, Inc., Vancouver, Canada and California, USA; [2]The University of Texas MD Anderson Cancer Center, Houston, TX

ABSTRACT # 751:

The median overall survival time for patients with stage IV non-small cell lung cancer (NSCLC) is 4 months, and 1- and 5-year survival is less than 16% and 2%, respectively. NSCLC is usually treated with surgery followed by treatment with either Tyrosine Kinase Inhibitors (TKIs) or platinum-based regimens. Unfortunately TKI resistance has emerged as a significant unmet medical need, and long-term prognosis with platinum-based therapies is poor. Dianhydrogalactitol (VAL-083) is a structurally unique bi-functional alkylating agent mediating interstrand DNA crosslinks at N^7 of guanine. It has previously demonstrated activity against NSCLC in preclinical and clinical trials, and is approved for treatment of lung cancer in China, suggesting that it may be a therapeutic option for drug-resistant NSCLC. However, the underlying basis for its activity remains unclear. Therefore, we aimed to investigate *in vitro* i) the role of p53 status in the activity of VAL-083 , ii) VAL-083 activity in comparison to cisplatin and oxaliplatin, and iii) the combination of VAL-083 with cisplatin or oxaliplatin.

The dependence on p53 status was investigated in isogenic models with (HCT-116$^{p53-/-}$) or without (HCT-116$^{p53+/+}$) p53 knockout. The cytotoxic activity of VAL-083 was tested in a panel of 9 human NSCLC cell lines, of which 4 were wild-type (wt) p53, 4 were mutant p53 and 1 was null for p53. The potential for combination was investigated by determining superadditivity and assessing synergy using the criteria of combination index (CI) of <1, obtained by following the Compusyn constant-dose ratio protocol. Cytotoxicity in all cases was monitored on day 5 with the MTT assay. Studies in HCT-116 models demonstrated that loss of p53 increased resistance to cisplatin and oxaliplatin by 3- and 6-fold, respectively, whereas resistance to VAL-083 was <2-fold. As single agents, VAL-083, cisplatin and oxaliplatin displayed cytotoxic activity in all 9 NSCLC cell lines to varying degrees, with H460 being the most sensitive to the three agents (IC_{50} < 0.5 uM). The IC_{50} in the other cell lines ranged from 0.9 to 6.1 μM, 0.5 to 2.2 μM and 0.6 to 2.6 μM for VAL-083, cisplatin and oxaliplatin, respectively, and there was no overt difference in drug sensitivity between the wt and mutant/null p53 group. This suggests that either wt p53 is not activated and/or other genetic alterations attenuate cytotoxic activities. If the agents have similar mode of action, then combinations may only demonstrate cytotoxic additivity. However, the combination of VAL-083 with cisplatin or oxaliplatin in the A549 NSCLC model, demonstrated significant superadditivity (p<0.05) and synergism (CI < 1) for both combinations. This strongly favors non-overlapping mechanism of action between the platinum drugs and VAL-083.

In conclusion, VAL-083 is less dependent on p53 for its activity, and demonstrates superadditivity/synergy in NSCLC cells when combined with either cisplatin or oxaliplatin.

BACKGROUND

VAL-083 is a bifunctional alkylating agent causing interstrand DNA crosslinks at N7 of guanine, which is believed to be distinct from the mechanisms of other alkylating agents (e.g. cisplatin or BCNU). VAL-083 has demonstrated activity against a range of NSCLC cell lines *in vitro* (see table 1). Furthermore, VAL-083 demonstrated activity against tyrosine kinase inhibitor resistant NSCLC both *in vitro* and *in vivo*[3]. 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. VAL-083 is currently undergoing clinical studies for refractory glioblastoma multiforme in the United States and has received orphan drug designation in Europe and the U.S. for the treatment of gliomas.

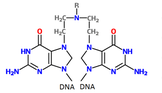


Figure 1. N7 guanine interstrand crosslinked DNA

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

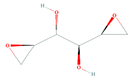


Figure 2. Chemical structure of VAL-083.
Molecular Formula: $C_6H_{10}O_4$. Molecular Weight: 146.1 g/mol

Table 1. **LC_{50} Mean values for VAL-083. NCI 6 day Cancer Screen 08/2002. August 2002.**

Cell panel	Cell Line	Log LC_{50}	LC_{50}
Non-Small Cell Lung Cancer	A549/ATCC	-3.9	
	EKVX	-3.9	
	HOP-62	-4.4	
	HOP-92	-4.0	
	NCI-H226	-3.9	
	NCI-H23	-4.0	
	NCI-H322M	-4.0	
	NCI-H460	-4.1	
	NCI-H522	-4.1	

Table 2. When tested side-by-side in a standard syngeneic mouse fibrosarcoma model (RIF-1 cell-line in C3H mice), VAL-083 was superior to cisplatin in tumor growth delay *in vivo*. Mice were treated with a single IP injection of either cisplatin, VAL-083 or VAL-083 followed immediately by cisplatin.

Combination treatment of with cisplatin produced a more than additive effect by delaying growth 8.65 days.

Treatment	Dose (mg/kg)	Days to 4 x Median tumor size	Tumor delay (days)
Untreated	-	6.29	0.00
Cisplatin	4	7.75	1.45
VAL-083	10	11.45	5.16
VAL-083 + cisplatin	10 + 4	14.94	8.65

METHODS:

The analysis of cytotoxicity, 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 MTT growth inhibition data using a 5-day drug exposure protocol. 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 Factor of HCT-116$^{p53-/-}$ was calculated as the ratio of IC_{50} in this tumor model to the IC_{50} in HCT-116$^{p53+/+}$. The determination of predictive additive effect of [drug A + drug B] combination is based on the equation described by Tallarida[1] 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.

The combination of VAL-083 with either cisplatin or oxaliplatin was examined using the Chou and Talalay[2] approach for synergism, which was assessed from the combination index (CI), and using the comparison between predicted and experimental values for the cytotoxicity of the combination for a superadditive effect.

References:

1. Tallarida RJ. Drug synergism: its detection and application. J Pharmacol Exp Ther 2001;298:265-72.
2. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 1984;22:27-55.
3. Steino A, *et al.* The unique mechanism of action of VAL-083 may provide a new treatment option for chemo-resistant non-small cell lung cancer. AACR-NHICR 2014, #A65.

RESULTS

VAL-083 dependence 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.

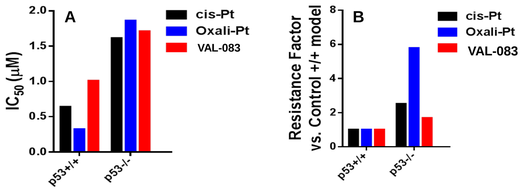


Figure 3. IC_{50} values (A) and resistance factors (B) for cisplatin, oxaliplatin and VAL-083 in molecularly engineered isogenic models of HCT-116 with (p53+/+) or without (p53-/-) p53.

VAL-083 showed cytotoxic activity against all NSCLC cell lines in a human NSCLC panel

VAL-083, cisplatin and oxaliplatin were tested in a panel of 9 human NSCLC cell lines, of which 4 were wild-type (wt) p53, 4 were mutant (mu) p53 and 1 was null for p53. There was no overt difference in drug sensitivity between the wt and mu/null p53 group. VAL-083 was active against all tested NSCLC cell lines, including TKI-resistant cell lines H1975, H460, and H1299, and VAL-083 activity was independent of p53 status.

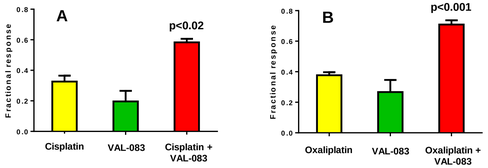
NSCLC Tumor Models							
		IC_{50} (μM)					
		Cisplatin		Oxaliplatin		VAL-083	
Cell Line	p53 Status	Mean	SE	Mean	SE	Mean	SE
H460	wt	0.45	0.052	0.36	0.014	0.49	0.050
A549	wt	0.74	0.106	0.57	0.059	1.76	0.314
H838	wt	1.18	0.092	2.63	0.041	4.62	0.421
H226	wt	1.82	0.156	0.82	0.023	6.11	0.984
H1975	mu	0.45	0.049	0.51	0.031	0.90	0.152
SkLU1	mu	0.89	0.019	2.02	0.473	2.72	0.022
H2122	mu	1.07	0.123	1.42	0.066	2.84	0.304
H157	mu	2.16	0.136	2.04	0.128	4.48	0.415
H1299	null	1.20	0.073	0.64	0.037	2.37	0.120
N = 3							

Table 3. Cytotoxicity of VAL-83, cisplatin and oxaliplatin in a human NSCLC tumor panel.

Combination of VAL-083 with cisplatin or oxaliplatin in H460, A549 and H1975 NSCLC

The combination of VAL-083 with either cisplatin or oxaliplatin in the human NSCLC models demonstrated significant superadditivity (p≤0.05) and/or synergism (CI<1) for both combinations. Significantly, this cytotoxic effect of VAL-083 in combination with either platinum drug was observed in both TKI-resistant (H1975 and H460) and TKI-sensitive (A549) NSCLC cells. 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 lung cancer.

Human H460 NSCLC cell line

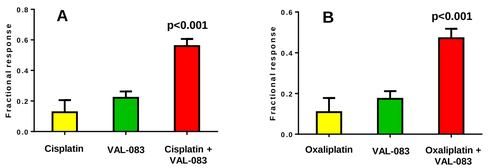


Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Cisplatin	CI
ED75	0.42	0.38	0.92
ED90	0.92	0.85	0.91
ED95	1.58	1.45	0.90

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

Figure 4. The cytotoxic effect of VAL-083 in combination with cisplatin (**A**) or oxaliplatin (**B**) on H460 cells. The table provides CI values for the Fa shown and achieved at indicated drug concentrations. Data, where applicable, are shown as Mean +/- SE, N=4.

Human A549 NSCLC cell line

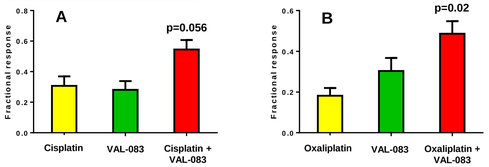


Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Cisplatin	CI
ED75	1.79	0.67	0.87
ED90	3.09	1.16	0.82
ED95	4.48	1.68	0.79

Cytotoxic Level (Fa)	Concentration (μM) VAL-083	Oxaliplatin	CI
ED75	1.55	0.46	0.91
ED90	2.26	0.68	0.78
ED95	2.92	0.88	0.71

Figure 5. The cytotoxic effect of VAL-083 in combination with cisplatin (**A**) or oxaliplatin (**B**) on A549 cells. The table provides CI values for the Fa shown and achieved at indicated drug concentrations. Data, where applicable, are shown as Mean +/- SE, N=7.

Human H1975 NSCLC cell line



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 6. The cytotoxic effect of VAL-083 in combination with cisplatin (**A**) or oxaliplatin (**B**) on H1975 cells. The table provides CI values for the Fa shown and achieved at indicated drug concentrations. Data, where applicable, are shown as Mean +/- SE, N=4.

CONCLUSIONS & NEXT STEPS:

- **VAL-083 demonstrated cytotoxic activity in all tested NSCLC cell lines, including TKI-resistant cell lines, *in vitro***
- **VAL-083 demonstrated superadditivity/synergy against NSCLC cell lines H460, A549 and H1975 when combined with either cisplatin or oxaliplatin, *in vitro***
- **VAL-083 is less dependent on wild-type p53 for its activity than both cisplatin and oxaliplatin, and appears to have a distinct mode of action**
- **Taken together, these results support VAL-083 as a viable treatment option for NSCLC patients failing platinum-based therapy, and support the potential benefit of a VAL-083/ platinum combination therapy in newly diagnosed patients**
- **Clinical investigations of VAL-083 in NSCLC to explore activity in patients who are relapsed or refractory to currently available therapies**