Dianhydrogalactitol (VAL-083) enhances activity of platinum drugs in non-small cell lung cancer

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ABSTRACT # A159:

Non-small cell lung cancer (NSCLC) is treated with surgery followed by chemotherapy with either tyrosine kinase inhibitors (TKIs) or platinum-based regimens, but long term prognosis is poor. Dianhydrogalactitol (VAL-083) is a bi-functional alkylating agent with proven activity against NSCLC in preclinical and clinical studies. VAL-083 is approved for the treatment of lung cancer in the Peoples Republic of China (PRC, Approval No. Guayo Zhunzi H45021133); however, clinical use has been limited by lack of mechanistic data. Here we aimed to investigate *in vitro* i) the role of p53 status in VAL-083 activity, ii) VAL-083 cytotoxicity in a panel of NSCLC cell lines, iii) the combination of VAL-083 with cisplatin or oxaliplatin in NSCLC cells. We further studied the combination of VAL-083 with cisplatin in NSCLC *in vivo*. Dependence on p53 status was investigated in isogenic HCT-116$^{p53-/-}$ and HCT-116$^{p53+/+}$ models. VAL-083, cisplatin and oxaliplatin cytotoxicity was tested in a panel of 9 human NSCLC cell lines: 4 wt, 4 mutant and 1 null for p53. The combination potential for VAL-083 with cisplatin or oxaliplatin was investigated in 3 human NSCLC cell lines; H460 (p53wt), A549 (p53wt) and H1975 (p53mut) by determining superadditivity and synergy using the criteria of combination index (CI)<1. Cytotoxicity was monitored on day 5 with the MTT assay. The *in vivo* activity of VAL-083 (2, 2.5, or 3 mg/kg i.p.) in combination with cisplatin (2 mg/kg i.v.) was tested in Rag2 mice bearing A549 xenograft tumors. Studies in HCT-116 models showed that loss of p53 increased resistance to cisplatin and oxaliplatin by 3- and 6-fold, respectively, while resistance to VAL-083 was <2-fold, suggesting a more p53-independent mechanism for VAL-083. As single agents, VAL-083, cisplatin and oxaliplatin showed good cytotoxicity in all NSCLC cell lines, with TKI-resistant cell line H460 as the most sensitive (IC_{50} < 0.5 uM). The combination of VAL-083 with cisplatin or oxaliplatin in H460, A549 and H1975 cells demonstrated significant super-additivity ($p<0.05$) and synergism (CI < 1) for both combinations in all 3 cell lines. This strongly favors non-overlapping mode of action between the platinum drugs and VAL-083 and demonstrates synergism in TKI-resistant cell lines. In the *in vivo* model, tumor growth delays of 11, 18 and 25 days were observed for cisplatin combined with 2, 2.5 or 3 mg/kg VAL-083, respectively, while no tumour growth delay was seen between untreated and cisplatin. The median survival time was increased by 2 days for cisplatin alone, while VAL-083 (2, 2.5 and 3 mg/kg) combined with cisplatin increased survival by 17, 17, and 14 days, respectively. In conclusion, when combined with cisplatin or oxaliplatin, VAL-083 demonstrates superadditivity/synergy against NSCLC cells, independent of their p53 status. Further, VAL-083 in combination with cisplatin significantly increased median survival *in vivo*. These results strongly suggest a potential for VAL-083 as part of combination treatment with platinum drugs for NSCLC, including drug-resistant phenotypes. A clinical trial is planned under the context of the existing PRC approval to investigate these observations in a clinical setting. Results, if favorable, will support expanded clinical use of VAL-083 in PRC and will serve as the basis for global development of VAL-083 as a potentially important chemotherapeutic agent in the treatment of NSCLC.

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). Historically, VAL-083 has demonstrated activity against a range of NSCLC cell lines, *in vitro*. In prior clinical studies sponsored by the US National Cancer Institutes (NCI), VAL-083 exhibited clinical activity against a number of tumor-types, including lung, brain, cervical, ovarian and hematologic malignancies, and is approved in China for the treatment of chronic myelogenous leukemia and lung cancer.. More recently, we have shown that VAL-083 demonstrated activity against platinum and TKI-resistant NSCLC both *in vitro* and *in vivo*[3]. 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. Our research has been aimed at determining whether VAL-083's historical clinical activity combined with a modern understanding of its cytotoxic mechanism and tumor biology provides an opportunity to effectively address current unmet medical needs in the treatment of cancer.

Figure 1. Chemical structure of VAL-083. Molecular Formula: $C_8H_{10}O_4$. Molecular Weight: 146.1

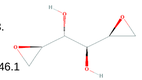
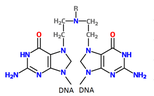


Figure 2. N7 guanine interstrand crosslinked DNA



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

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 **VAL-083** 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 Bliss Independence Model and is defined by the equation described by Tallarida[1] as follows:

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

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:

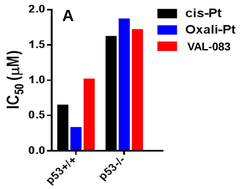
1. Tallarida RJ. Drug synergism: its detection and application. J Pharmacol Exp Ther 2001;298:265-72.
2. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 1984;22:27-55.
3. Steino A, *et al.* The unique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

VAL-083 showed cytotoxic activity against all NSCLC cell lines in a human NSCLC panel, including TKI-resistant cell lines

VAL-083, cisplatin and oxaliplatin were tested in a panel of 9 human NSCLC cell lines, of which 4 were wild-type p53 (H460, A549, H838, H226), 4 were mutant p53 (H1975, SkLU1, H2122, H157) and 1 was null for p53 (H1299). 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.

VAL-083 mechanism is less dependent on p53

The dependence on p53 status was investigated in isogenic models with (HCT-116$^{p53-/-}$) or without (HCT-116$^{p53+/+}$) p53 knockout (see Fig 3). 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.



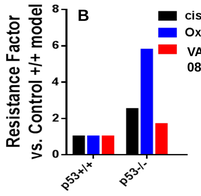


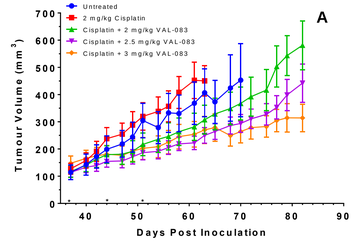
Figure 3. IC_{50} values (A) and resistance factors (B) for cisplatin, oxaliplatin and VAL-083 in molecularly engineered isogenic models of HCT-116 with (p53+/+) or without (p53-/-) p53. Resistance factors are the ratio of IC_{50} in the p53-/- model vs. the p53+/+ model

CONCLUSIONS

- **Our results support VAL-083 as a viable treatment option for NSCLC patients who fail to respond to standard-of-care platinum-based therapy or TKI therapy, and also support potential therapeutic benefit of a VAL-083 + platinum combination regimens in newly diagnosed patients**
  - ✓ ***VAL-083 demonstrated cytotoxic activity in all tested NSCLC cell lines, including TKI-resistant cell lines***
  - ✓ ***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 p53 for its activity than both cisplatin and oxaliplatin, and appears to have a distinct mode of action in comparison to platinum-based chemotherapy***
  - ✓ ***The combination of VAL-083 with cisplatin significantly decreased tumor growth and increased median survival time in a xenograft A549 in vivo model of NSCLC***
- **Clinical investigations of VAL-083 in NSCLC to explore activity in patients with relapsed or refractory NSCLC are planned**

VAL-083/cisplatin combination increase median survival time in A549 NSCLC model, *in vivo*

Rag2 mice bearing A549 xenograft tumors were treated with vehicle (gr. 1), 2 mg/kg cisplatin alone (gr. 2), or cisplatin in combination with VAL-083 (2, 2.5, or 3 mg/kg) (gr. 3-5). As seen in table 2, VAL-083 in combination with cisplatin significantly and dose-dependently decreased tumor growth in the animals and increased median survival time compared to both cisplatin treatment alone and to untreated control.



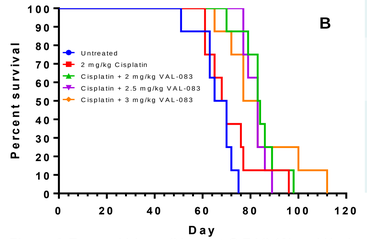


Table 2. Analysis parameters for the 5 groups in A549 model

Treatment	TD*	TGD* (days)	TGI*	Median survival time (days)	P value**
Untreated control	11	0	0	67.5	n/a
Cisplatin 2 mg/kg	10	0	0%	69	0.70
VAL-083 2 mg/kg cisplatin 2 mg/kg	22	11	40%	83.5	0.08
VAL-083 2.5 mg/kg cisplatin 2 mg/kg	29	18	47%	83	0.03
VAL-083 3 mg/kg cisplatin 2 mg/kg	36	25	56%	80	0.02

*TD: Tumor Doubling Time (150-300mm^3); TGD: Tumor Growth Delay; TGI: Tumor Growth Inhibition= [(TVcontrolDay63 – TVcontrolDay37)-(TVtxDay63-TvtxDay37) x 100%]/(TVcontrolDay63 – TVcontrolDay37)

**Unpaired t-test of tumor growth compared to untreated control, from day 37 to 63

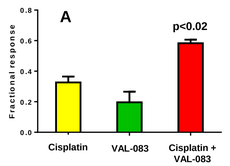
Figure 4. Tumour Volume (Means ± S.E.M) (**A**) and Kaplan Meier Survival (**B**) of Rag2 Mice Bearing A549 xenograft tumours following treatment with cisplatin and VAL-083. *Excluded data if ≤ 2 mice per group, and early ulceration/low tumour volume <50mm3 at the start of treatment, 8 mice per group. Table 2 displays the numbers depicted in A and B.

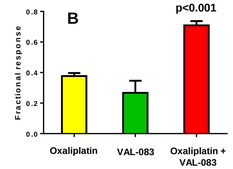
VAL-083 displays synergy in combination with cisplatin or oxaliplatin in NSCLC cells H460, A549 and H1975, *in vitro*

The combination of VAL-083 with either cisplatin or oxaliplatin in three human NSCLC cell lines demonstrated significant superadditivity ($p\leq0.06$) and/or synergism (CI<1) for both combinations, *in vitro* (Fig. 5-7). 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, irrespective of their p53 status.

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, including TKI-resistant NSCLC.

Human H460 NSCLC cell line (p53 wt, EGFR wt)



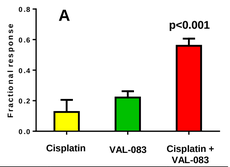


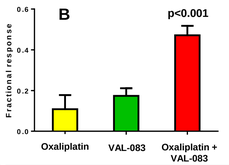
Cytotoxic Level (Fa)	Concentration (μM) VAL-083	**Cisplatin**	CI
ED75	0.42	0.38	0.92
ED90	0.92	0.85	0.91
ED95	1.58	1.45	0.90

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 5. The cytotoxic effect of VAL-083 in combination with cisplatin (**A**) or oxaliplatin (**B**) against H460 cells. Data, where applicable, are shown as Mean +/- SE, N=4. Fa: Fraction of cells affected. The tables provide CI values for the Fa shown and achieved at indicated drug concentrations, i.e. ED75: effective dose that kills 75% of cells.

Human A549 NSCLC cell line (p53 wt, EGFR wt)



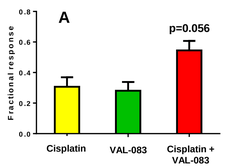


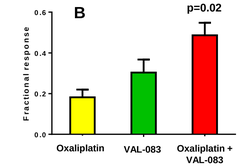
Cytotoxic Level (Fa)	Concentration (μM) VAL-083	**Cisplatin**	CI
ED75	1.79	0.67	0.87
ED90	3.09	1.16	0.82
ED95	4.48	1.68	0.79

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 6. The cytotoxic effect of VAL-083 in combination with cisplatin (**A**) or oxaliplatin (**B**) against A549 cells. Data, where applicable, are shown as Mean +/- SE, N=7. Fa: Fraction of cells affected. The tables provide CI values for the Fa shown and achieved at indicated drug concentrations, i.e. ED75: effective dose that kills 75% of cells.

Human H1975 NSCLC cell line (p53 mut, EGFR mut T790M)





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 7. The cytotoxic effect of VAL-083 in combination with cisplatin (**A**) or oxaliplatin (**B**) against H1975 cells. Data, where applicable, are shown as Mean +/- SE, N=4. Fa: Fraction of cells affected. The tables provide CI values for the Fa shown and achieved at indicated drug concentrations, i.e. ED75: effective dose that kills 75% of cells.