The unique mechanism of action of VAL-083 may provide a new treatment option for chemo-resistant non-small cell lung cancer

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ABSTRACT # A65

WHO predicts that the incidence of lung cancer may exceed 1 million cases per year by 2025 with non-small cell lung cancer (NSCLC) representing up to 90% of newly diagnosed cases. The median overall survival time for patients with stage IV NSCLC is 4 months, while 1- and 5-year survival is less than 16% and 2%, respectively. Metastatic NSCLC is usually treated with either Tyrosine Kinase Inhibitors (TKIs) (e.g. gefitinib) or platinum-based regimens (e.g. cisplatin). TKIs have resulted in vastly improved outcomes for patients with EGFR mutations; however, TKI resistance has emerged as a significant unmet medical need, and long-term prognosis with platinum-based therapies is poor. Additionally, the incidence of brain metastases is high in patients with NSCLC with a poor prognosis.

Dianhydrogalactitol (VAL-083) is a structurally unique bi-functional alkylating agent mediating interstrand DNA crosslinks targeting N7 of guanine, thus differing in mechanism from TKIs and cisplatin. VAL-083 is approved for treatment of lung cancer in China and has documented activity against NSCLC in historical NCI-sponsored clinical trials in the United States; however, specific questions regarding the efficacy of VAL-083 in comparison to cisplatin and in TKI-resistant NSCLC have to our knowledge not been addressed before. Further, VAL-083 crosses the blood-brain barrier and accumulates in tumor tissue. VAL-083 has demonstrated activity against NSCLC in preclinical and clinical trials, suggesting VAL-083 may be a therapeutic option for drug-resistant NSCLC and NSCLC patients with brain metastasis. When tested side-by-side in a standard syngeneic mouse fibrosarcoma model (RIF-1 cell-line in C3H mice), VAL-083 demonstrated superiority to cisplatin in tumor growth delay. For mice treated with a single IP injection of VAL-083 (10 mg/kg) tumor growth was delayed by 5.6 days compared to control, versus 1.5 days for mice treated with single dose cisplatin (4 mg/kg). Combination treatment of VAL-083 and cisplatin produced a more than additive effect by delaying growth 8.7 days.

Previous clinical studies showing VAL-083 activity in NSCLC combined with the new data on synergy with cisplatin, makes VAL-083 a promising alternative for NSCLC with brain metastases as well as chemo-resistant NSCLC.

In vitro: The cytotoxic effect of VAL-083 in combination with cisplatin or oxaliplatin was tested in NSCLC cell-lines A549 and H1975. The results show additive and more than additive effects of combining VAL-083 with cisplatin or oxaliplatin in both cell-lines.

In vivo: In two separate studies we evaluated the activity of VAL-083 in in vivo models of EGFR-TKI-resistant NSCLC in comparison to cisplatin. Rag2 mice bearing subcutaneous human lung adenocarcinoma xenograft tumors of either TKI-sensitive (A549) or TKI-resistant (H1975) origin were treated. VAL-083 was given i.p. 3 times/week for 3 weeks, and the in vivo efficacy of VAL-083 in controlling tumor growth compared to cisplatin (5 mg/kg). Saline was used as control treatment. Disease progression was evaluated by tumor volume, clinical observations and body weight measurements. Blood samples were analyzed for CBC/differential analyses to assess myelosuppression or other changes in blood chemistry.

A549: Tumour growth delay of 26 days was observed in animals treated with 3 mg/kg VAL-083 compared to controls, versus a 4-day delay for mice treated with cisplatin. Mean tumor volume on day 68 was significantly reduced in animals treated with 3 mg/kg VAL-083 compared to controls (p=0.001).

H1975: Treatment was stopped after 6 doses of VAL-083 in the 4 mg/kg group due to significant body weight loss and the mice quickly recovered. Median survival time in mice treated with 4 mg/kg VAL-083 was 41 days compared to 31 days for all other treatment and control groups. Mean tumor volume on day 31 was significantly reduced in animals treated with 4 mg/kg VAL-083 compared to control (p=0.004).

Taken together, the results suggest that VAL-083 is superior to cisplatin in both TKI-sensitive and resistant tumor models, has synergistic effect in combination with cisplatin, and suggest clinical potential in TKI-resistant NSCLC. This data provides direction to clinical research aimed at influencing practice patterns under VAL-083's current label and support expanded global development.

METHODS

In vivo models:

Cell number for inoculation was:

- **A549:** $5x10^6$ cells in an injection volume of 50 µL per animal
- **H1975:** $2x10^6$ cells in an injection volume of 50 µL per animal

Treatment was initiated at average tumor volume 100-150 mm^3.

Table 1. Treatment protocol for testing the efficacy of VAL-083 in comparison to cisplatin in the treatment of female Rag2 mice bearing subcutaneous A549 or H1975 human lung adenocarcinoma xenograft tumors.

Group Name	Dose (mg/kg)		No. mice	Admin. Route	Volume (uL/20g)	Timepoint/ Schedule
	A549	H1975				
1. Untreated	-	-	10	n/a	n/a	n/a
2. Cisplatin	5.0	5.0	10	i.v.	200	Q7D X 3
3. VAL-083	1.5	2.0	10	i.p.	200	M, W, F X 3
4. VAL-083	3.0	3.0	10	i.p.	200	M, W, F X 3
5. VAL-083	6.0	4.0	10	i.p.	200	M, W, F X 3

Body weight loss was observed in mice treated with 5 mg/kg cisplatin (group 2) and 4 mg/kg VAL-083 (group 5). Treatment was stopped after 6 doses of VAL-083 in the 4 mg/kg group due to significant body weight loss, and the then mice quickly recovered.

In vitro models:

The *in vitro* activity of VAL-083 in combination with cisplatin was tested in NSCLC cell-lines **A549** and **H1975.** Cells were treated with VAL-083 and cisplatin or oxaliplatin, simultaneously, using IC10-30 concentrations of the individual agents, and cytotoxicity was monitored on day 5 with the colorimetric MTT assay. P-values were calculated by Student's t-test analysis of experimental values vs. predicted additive values for the treatment combinations.

TGI = Tumor Growth Inhibition = $\frac{(TVcontrolDay68 - TVcontrol,int)-(TVtxDay68-TVtx,int) \times 100\%}{(TVcontrolDay68 - TVcontrol,int)}$

TGD = Tumor Growth Delay = DTtx – Dtcontrol

TV = Tumor volume; tx = treatment; int = initial; DT= Doubling time for mean tumor volume from 200mm^3 to 400mm^3 (for A549) and 300mm^3 to 600mm^3 (for H1975)
MTV: Mean Tumor Volume (mm^3); TCR: Tumor Control Ratio

Conclusions & Next Steps

VAL-083 is a potent chemotherapy with activity against a range of tumor types. Taken together these results suggest:

- **VAL-083 demonstrated superior activity to cisplatin in both TKI-sensitive (A549) and TKI-resistant (H1975) NSCLC tumor models *in vivo.***
- **As evidenced by a statistically significant survival benefit *in vivo,* VAL-083 maintains activity where platinum-based regimens have little or no effect**
- **VAL-083 has a super-additive effect when combined with cisplatin or oxaliplatin in both TKI-sensitive (A549) and TKI-resistant (H1975) NSCLC cell-lines *in vitro,***
- **VAL-083 with cisplatin is better than additive *in vivo*.**

Taken together, these results support VAL-083 as a viable treatment option for NSCLC patients failing platinum based and TKI-therapy, and support the potential benefit as part of a platinum-based combination therapy in newly diagnosed patients.

Such observations have important immediate implications in the treatment of NSCLC in China, where VAL-083 is approved for as a chemotherapy for the treatment of lung cancer, and for future clinical development in the rest of the world.

Next steps include clinical validation of our results. Such work could be undertaken in China in a post-approval setting.

BACKGROUND

VAL-083 *(dianhydrogalactitol)* is a first-in-class alkylating agent with a novel cytotoxic mechanism distinct from other alkylating agents used in the treatment of cancer.

In historical studies sponsored by the National Cancer Institute in the United States, VAL-083 exhibited clinical activity against a range of tumor types including CNS tumors, solid tumors and hematologic malignancies. VAL-083 is approved in China for the treatment of chronic myelogenous leukemia (CML) and lung cancer *(Approval No. Guoyao Zhunzi H45021133; manufactured by Guangxi Wuzhou Pharmaceutical (Group) Co. Ltd.)*

Figure 1. Commercial package and chemical structure of VAL-083. Molecular Formula: $C_6H_{10}O_4$. Molecular Weight: 146.1 g/mol

DelMar Pharmaceuticals and Guangxi Wuzhou Pharmaceuticals are collaborating in the development of VAL-083. Currently DelMar is using this product in Phase I/II clinical trials in the United Stated as a potential treatment for refractory glioblastoma multiforme (clinicaltrials.gov identifier: NCT01478178).

Non-small cell lung cancer (NSCLC) represents approximately 90% of lung cancer cases diagnosed in China.

Standard NSCLC treatment includes surgical resection, radiotherapy and primarily platinum-based chemotherapy regimens. Some patients exhibiting TKI-mutation may receive tyrosine kinase (TKI) therapy, often following failure of platinum based chemotherapy. Patients with refractory or resistant NSCLC have limited therapeutic options.

In spite of promising historical data, use of VAL-083 in China in the treatment of lung cancer has been limited to date. We have investigated the potential utility of VAL-083 in the context of modern NSCLC treatment namely:

- ***How does the activity of VAL-083 compare to standard platinum therapy?***
- ***Can VAL-083 be combined with platinum based therapy?***
- ***Can VAL-083 treat NSCLC that is resistant to platinum-based chemotherapy or TKIs?***

RESULTS

In vivo

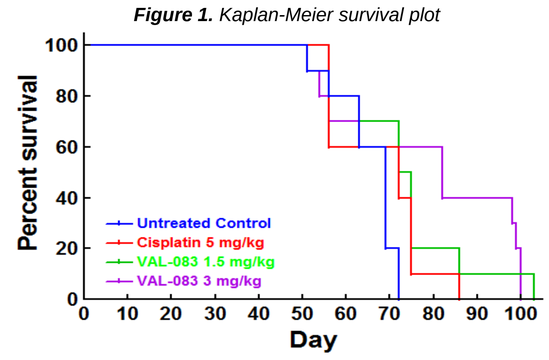
A549 (TKI-sensitive): A significant survival benefit was observed with VAL-083 at 3mg/kg as shown in Figure 1 and Table 2. A log-rank statistical test (Mantel-Cox) was performed indicating p value of 0.0446 indicating a significant difference between the survival curves. A tumour growth delay of 26 days was observed in animals treated with 3 mg/kg VAL-083 compared to untreated controls, versus positive control, 5 mg/kg Cisplatin, which resulted in a tumour growth delay of 4 days compared to untreated controls. Mean tumor volume on day 68 was significantly reduced in animals treated with 3 mg/kg VAL-083 (p=0.001) compared to untreated control. These observations suggest that VAL-083 maintains activity where cisplatin fails to gain a statistically significant benefit.

Table 2. Analysis parameters for groups 1-4 in A549 model

Treatment	MTV* at day 68	TCR* at day 68	TGD* (Days)	TGI* (%)	P value**	Median survival (days)
Control	638	1	0	0	n/a	69
Cisplatin 5 mg/kg	460	0.72	4	29 %	0.059	72
VAL-083 1.5 mg/kg	440	0.69	9	32 %	0.069	73.5
VAL-083 3 mg/kg	303	0.47	26	55 %	0.001	82

**Unpaired t-test of tumor volume on day 68, treatment compared to untreated control

Figure 1. *Kaplan-Meier survival plot*



H1975 (TKI-resistant): A significant survival benefit was observed with VAL-083 at 4mg/kg as shown in Figure 2 and Table 3. The median survival time for mice treated with 4 mg/kg VAL-083 was 41 days compared to 31 days for all other treatment and control groups. A log-rank statistical test (Mantel-Cox) was performed indicating p value of 0.0009 indicating a significant difference between the survival curves. Mean tumor volume on day 31 was significantly reduced in animals treated with 4 mg/kg VAL-083 compared to control (p=0.004). These observations suggest that VAL-083 maintains activity where cisplatin fails to gain a statistically significant benefit., even in a TKI-resistant setting

Table 3. Analysis parameters for groups 1-5 in H1975 model

Treatment	MTV* at day 31	TCR* at day 31	P value**	Median survival (days)
Control	459	1	n/a	31
Cisplatin 5 mg/kg	381	0.83	0.102	31
VAL-083 2 mg/kg	396	0.87	0.490	31
VAL-083 3 mg/kg	383	0.84	0.769	31
VAL-083 4 mg/kg	262	0.57	0.004	41

**Unpaired t-test of tumor volume on day 68, treatment compared to untreated control

Figure 2. *Kaplan-Meier* survival plot

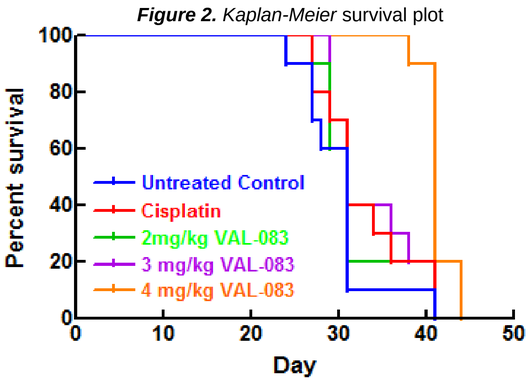


Table 4.

In a separate, standard model of anti-cancer activity, VAL-083 was superior to cisplatin in tumor growth delay. Mice were treated with a single IP injection of either cisplatin, VAL-083 or VAL-083 followed immediately by cisplatin.

Interestingly, Combination treatment of with cisplatin produced a more than additive effect by delaying growth 8.65 days. When tested side-by-side in a standard syngeneic mouse fibrosarcoma model (RIF-1 cell-line in C3H mice).

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

in vitro

We investigated the cytotoxic effect of VAL-083 alone or in combination with cisplatin (A) or oxaliplatin (B).

Figure 3 shows the cytotoxic effect of VAL-083 alone or in combination with cisplatin (Figure 3A) or oxaliplatin (Figure 3B) on A549 NSCLC cells.

Figure 4 shows the cytotoxic effect of VAL-083 alone or in combination with cisplatin (Figure 4A) or oxaliplatin (Figure 4B) on H1975 NSCLC cells.

VAL-083 in combination with either cisplatin or oxaliplatin has a more than additive cytotoxic effect on both TKI-resistant (H1975) and TKI-sensitive (A549) NSCLC cells.

These results support the potential for synergistic benefit for a combination of VAL-083 and platinum-based therapies, similar to those results observed *in vivo.*

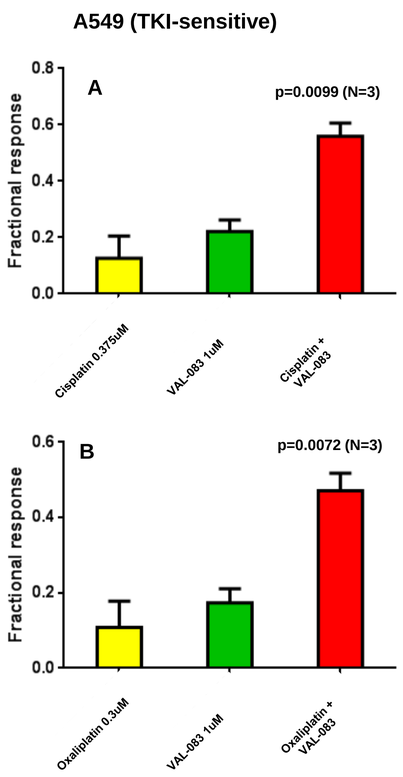


Figure 3. The cytotoxic effect of VAL-083 in combination with cisplatin (A) or oxaliplatin (B) on A549 cells. Data are shown as Mean +/- SE.

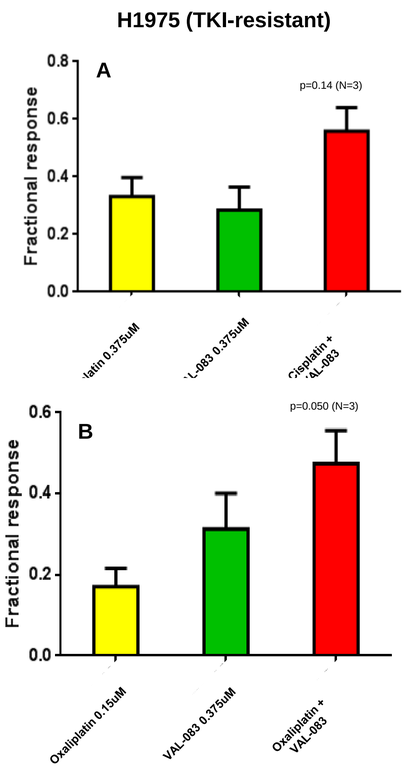


Figure 4. The cytotoxic effect of VAL-083 in combination with cisplatin (A) or oxaliplatin (B) on H1975 cells. Data are shown as Mean +/- SE.