In vivo efficacy of VAL-083 in the treatment of non-small cell lung cancer

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Abstract #: 824

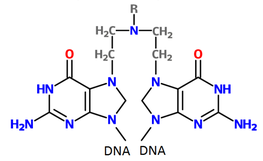
The median overall survival time for patients with stage IV non-small cell lung cancer (NSCLC) is 4 months, and 1- and 5-year survival is less than 16% and 2%, respectively. NSCLC is usually treated with surgery followed by treatment with either Tyrosine Kinase Inhibitors (TKIs) (e.g. erlotonib, gefitinib) 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. VAL-083 is a structurally unique bi-functional alkylating agent mediating interstrand DNA crosslinks at targeting N^7 of guanine, thus differing in mechanism of action from TKIs and cisplatin. VAL-083 crosses the blood-brain barrier and accumulates in tumor tissue. VAL-083 has demonstrated activity against NSCLC in preclinical and clinical trials, both as a single agent and in combination with other treatment regimens, suggesting VAL-083 may be a therapeutic option for drug-resistant NSCLC and NSCLC patients with brain metastasis. VAL-083 is approved for treatment of lung cancer in China and has documented activity against NSCLC in historical NCI-sponsored clinical trials; 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.

The purpose of this study is to evaluate the activity of VAL-083 in *in vivo* models of drug-resistant NSCLC in comparison to other drugs, including cisplatin. Rag2 mice bearing subcutaneous human lung adenocarcinoma xenograft tumors of either TKI-resistant (H1975) or TKI-sensitive (A549) origin were treated. The results will provide direction to clinical research aimed at influencing practice patterns under VAL-083's current label and support expanded global development.

Two human NSCLC cell lines, A549 (TKI-sensitive) and H1975 (TKI-resistant), were used for xenograft tumor models in female Rag2 mice. 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. Disease progression is evaluated by tumor volume, clinical observations and body weight measurements. Blood samples are analyzed for CBC/differential analyses to assess myelosuppression or other changes in blood chemistry.

Background

VAL-083 is a bifunctional alkylating agent causing methylation of N^7-guanine and interstrand DNA crosslinks, 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 3)



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

Figure 1. N7 guanine interstrand crosslinked DNA

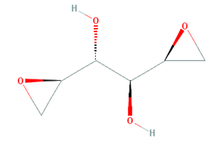


Figure 2. Chemical structure of VAL-083. Molecular Formula: $C_6H_{10}O_4$. Molecular Weight: 146.1 g/mol

Table 3. **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	

Conclusions

In an established model of non-small cell lung cancer, VAL-083 provided superior efficacy (Tumor Growth Delay) and safety (Body Weight loss) in the treatment of TKI-susceptible (A549) tumors in comparison to standard cisplatin therapy. Preliminary results further suggest that VAL-083 is superior to cisplatin treatment in TKI-resistant (H1975) tumors.

These results suggest that VAL-083 may be a viable treatment option for NSCLC patients failing TKI-therapy, especially where platinum-based therapy has already failed or is predicted to give sub-optimal results. These data 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.

Methods

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 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 human lung adenocarcinoma xenograft tumors.

Group Name	No. mice	Admin. Route	Volume (uL/20g)	Timepoint/ Schedule
Untreated control	10	n/a	n/a	n/a
Cisplatin, 5 mg/kg	10	i.v.	200	Q7D X 3
VAL-083, 1.5 mg/kg	10	i.p.	200	M, W, F X 3
VAL-083, 3 mg/kg	10	i.p.	200	M, W, F X 3
VAL-083, 6 mg/kg	10	i.p.	200	M, W, F X 3

Table 2. Treatment protocol for testing the efficacy of VAL-083 in comparison to cisplatin in the treatment of female Rag2 mice bearing subcutaneous H1975 human lung adenocarcinoma xenograft tumors.

Group Name	No. mice	Admin. Route	Volume (uL/20g)	Timepoint/ Schedule
Untreated control	10	n/a	n/a	n/a
Cisplatin, 5 mg/kg	10	i.v.	200	Q7D X 3
VAL-083, 2 mg/kg	10	i.p.	200	M, W, F X 3
VAL-083, 3 mg/kg	10	i.p.	200	M, W, F X 3
VAL-083, 4 mg/kg	10	i.p.	200	M, W, F X 3

$$\text{Tumor Growth Inhibition} = \frac{(TVcontrolDay68 - TVcontrolinitial)-(TVtxDay68-TVtxinitial) \times 100\%}{(TVcontrolDay68 - TVcontrolinitial)}$$

Tumor Growth Delay = DTtx - DTcontrol

DT= Doubling time for mean tumor volume from 200mm^3 to 400mm^3

Results A549

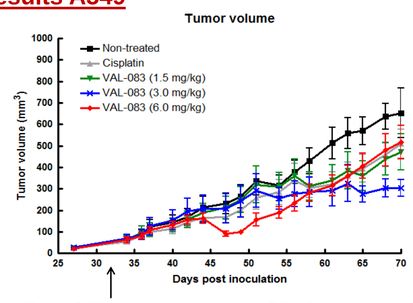


Figure 3. Tumor volume (Means ± S.D.) for all mice until day 70 (last day for untreated control group). Arrow indicates first treatment dose on day 37.

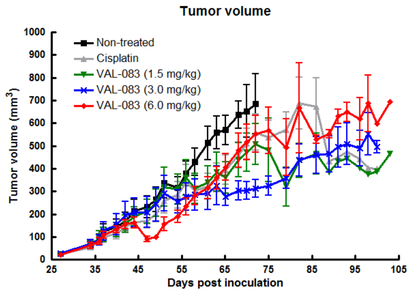


Figure 4. Tumor volume (Means ± S.D.) for the complete duration of the study.

Body weight loss was observed in mice treated with 5 mg/kg Cisplatin (group 2) and 6 mg/kg VAL-083 (group 5). Treatment was stopped after 3 doses of VAL-083 in the 6 mg/kg group due to significant body weight loss.

The doubling times for groups 1-4 were 13, 17, 22 and 39, respectively.

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.

Table 4. Analysis parameters for the 5 groups in A549 model

Treatment	MTV* at day 68	TCR* at day 68	TGD* (Days)	TGI* (%)	P value**
Control	637.8883	1	0	0	n/a
Cisplatin 5 mg/kg	460.305	0.721	4	29 %	0.059
VAL-083 1.5 mg/kg	440.1114	0.69	9	32 %	0.069
VAL-083 3 mg/kg	302.6333	0.474	26	55 %	0.001
VAL-083 6 mg/kg	n/a	n/a	n/a	n/a	n/a

*MTV: Mean Tumor Volume; TCR: Tumor to Control Ratio; TGD: Tumor Growth Delay; TGI: Tumor Growth Inhibition
**Unpaired t-test of tumor volume on day 68, treatment compared to control

Results H1975 (study ongoing)

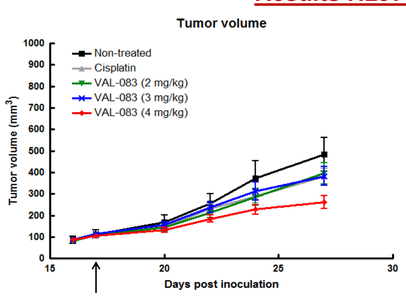


Figure 5. Tumor volume (Means ± S.D.) for all mice until day 27 (study ongoing). Arrow indicates first treatment dose on day 17.

On day 27, body weight loss was observed in mice treated with 5 mg/kg Cisplatin (group 2) and 4 mg/kg VAL-083 (group 5).

Mean tumor volume on day 27 was significantly reduced in animals treated with 4 mg/kg VAL-083 (p=0.012) compared to untreated control.

Table 5. Analysis parameters for the 5 groups in H1975 model

Treatment	MTV* at day 27	TCR* at day 27	P value**
Control	458.54	1	n/a
Cisplatin 5 mg/kg	381.18	0.831	0.233
VAL-083 2 mg/kg	395.82	0.87	0.342
VAL-083 3 mg/kg	382.68	0.835	0.257
VAL-083 4 mg/kg	261.78	0.571	0.012

*MTV: Mean Tumor Volume; TCR: Tumor to Control Ratio.
**Unpaired t-test of tumor volume on day 27, treatment compared to control