Assessment of dianhydrogalactitol (VAL-083) in the treatment of relapsed or refractory non-small cell lung cancer (NSCLC)

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BACKGROUND

VAL-083 is a bi-functional alkylating agent with proven activity against NSCLC in historical NCI-sponsored clinical studies[1-5]. VAL-083 is approved for the treatment of lung cancer in China; however, clinical adoption is limited by lack of modern data related to mechanism-of-action (MoA) and utility in the context of standard-of-care in NSCLC. We have previously demonstrated that VAL-083 circumvents cisplatin-resistance in ovarian cancer cells, *in vitro* and, further, that VAL-083 exhibits superior activity to cisplatin in both *in vitro* and *in vivo* NSCLC models, including TKI-resistant NSCLC.[6] Here we aim to further differentiate VAL-083 from current standard-of-care in NSCLC by investigating *in vitro* i) the distinct MoA of VAL-083, ii) VAL-083 cytotoxicity in a panel of NSCLC cell lines with varying status of p53, T790M and KRAS, and iii) the combination of VAL-083 with cisplatin or oxaliplatin.

NSCLC: Lung cancer, including NSCLC, is treated with surgery and chemotherapy with tyrosine kinase inhibitors (TKIs) or platinum-based regimens. EGFR mutated tumors account for 10-15% and 40% of NSCLC in Western and Asian populations, respectively. In EGFR mutated NSCLC, TKI-treatment produces dramatic initial improvements, but tumors ultimately recur with new mutations, including T790M. Third generation TKI AZD9291 is effective against recurrent NSCLC with T790M, but acquired resistance appears to emerge through RAS signaling, including KRAS mutations. Cisplatin-resistance also represents an unmet clinical need, and long-term prognosis in NSCLC remains poor.

ABSTRACT # B42

The preclinical data presented here strongly support VAL-083 as a potential treatment for platinum- and TKI-resistant/refractory NSCLC as a single agent or as part of a combination therapy.

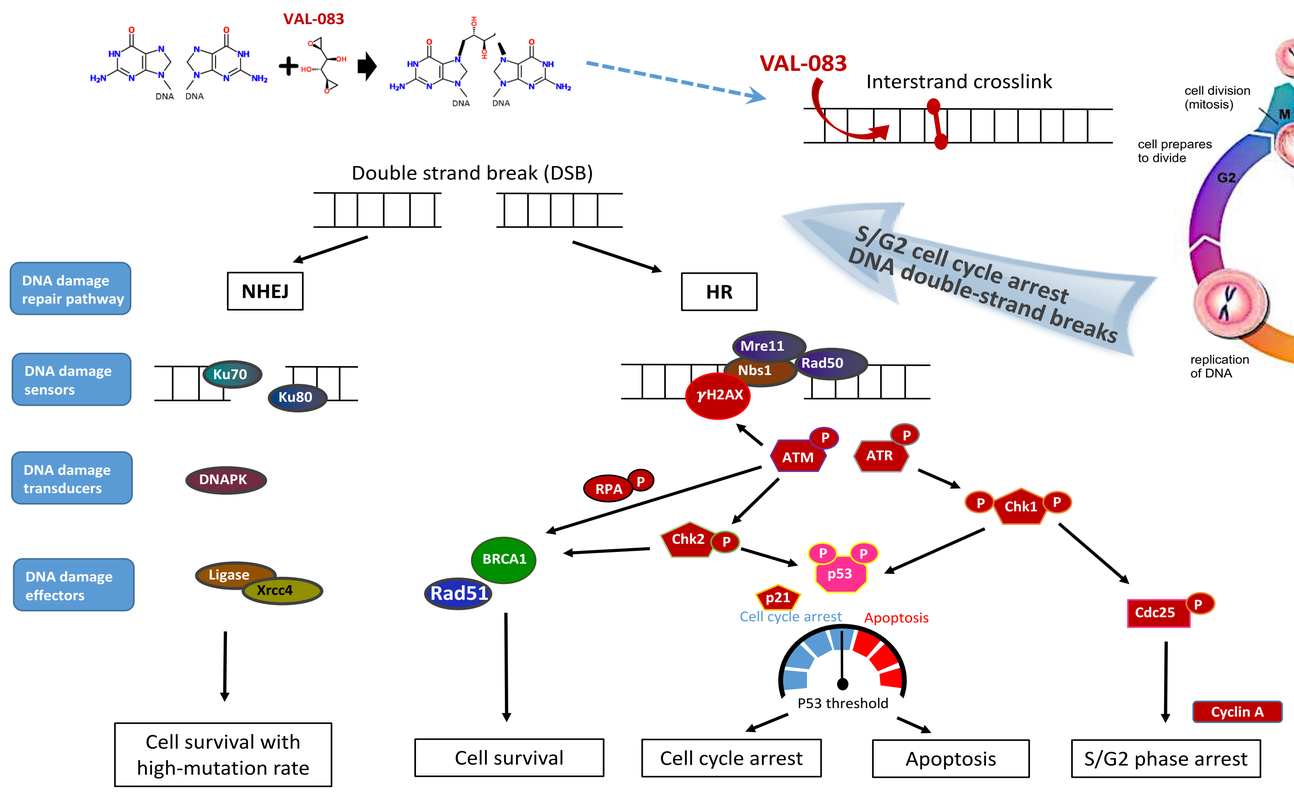


Figure 1. *VAL-083 induces interstrand crosslinks leading to double-strand breaks, S/G2 phase arrest and homologous recombination (HR) activation. Red color signifies demonstrated activation/expression after VAL-083. treatment.*

VAL-083 demonstrated activity in prior clinical trials sponsored by the US National Cancer Institutes. VAL-083 is approved for the treatment of lung cancer in China *(Approval No. Guoyao Zhunzi H45021133)*

Table 2. Historical clinical results with VAL-083 in lung cancer

Reference	Patient Population	Reported Result
Haas et al. (1976[4]) VAL-083 single agent	Advanced lung cancer	**ORR** • 42% sqclc • 25% adeno
Eagan et al. (1977[2]) VAL-083 single agent	Advanced SQCLC	**Regression:** • 15%: sqclc
Eagan et al (1980[5]) VAL-083 combination therapy	Advanced SQCLC	**Regression:** • 27% VAL-083 + adriamycin • 53% VAL-083 + adriamycin + cisplatin
Haas et al. (1981[5]) VAL-083 single agent	Advanced lung cancer	**ORR** • 19%: recurrent disease • 26%: newly d8agnosed
Eagan et al. (1981[6]) VAL-083 combination therapy	Advanced SQCLC	**Regression**: • 54%: VAL-083 + adriamycin + cisplatin

ORR = SD/PR/CR

Consistent with prior published research, VAL-083 was active against all NSCLC cell-lines tested, irrespective of their p53, T790M and KRAS status, **suggesting a MoA that differs from other chemotherapeutic agents** for the treatment of NSCLC, including cisplatin.

Table 3. IC50 values for Val-083 in nine human NSCLC cell lines and their p53 status: 3 wild-type (H460, A549, H226), 4 mutant (H1975, SkLU1, H2122, H157) and 2 null (H838, H1299) for p53.

Cell line (mutation)	p53 status	IC_{50} (VAL-083)	
		Mean (μM)	SE (μM)
H460 (KRAS)	wild type	0.49	0.050
A549 (KRAS)	wild type	1.76	0.314
H226	wild type	6.11	0.984
H1975 (T790M)	mutant	0.90	0.152
SkLU1 (KRAS)	mutant	2.72	0.022
H2122 (KRAS)	mutant	2.84	0.304
H157 (KRAS)	mutant	4.48	0.415
H1299	null	2.37	0.120
H838	null	4.62	0.421

N=3.

VAL-083 treatment caused persistent S/G2 cell-cycle arrest (Fig 2). VAL-083 pulse treatment (1 hr) led to persistent phosphorylation of DNA double-strand break sensors ATM, single-strand DNA-binding Replication Protein A (RPA32), and histone variant H2AX (γH2AX), suggesting activation of the HR DNA repair pathway (Fig 3). VAL-083 pulse treatment (1 hr) induced co-localization of S/G2 phase marker cyclin A2 and HR marker γH2AX in cancer cells (Fig 4 and Table 1). **These results show that VAL-083 induces irreparable double-strand breaks, persistent S/G2 phase arrest and HR activation.**

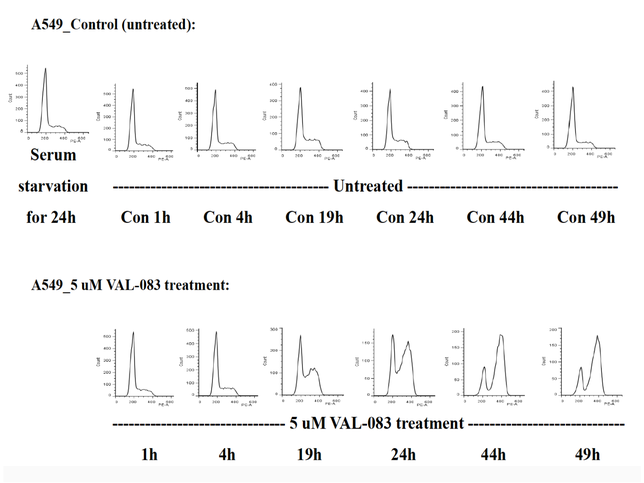


Figure 2. *VAL-083 treatment leads to persistent cell cycle arrest at S/G2 phase.*

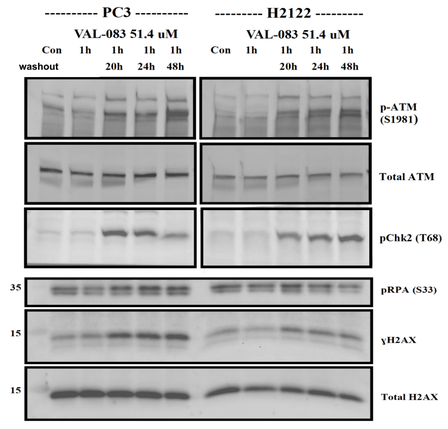


Figure 3. *VAL-083 pulse treatment activated HR DNA damage signaling pathway as demonstrated by expression of phospho-ATM (S1981), phospho-Chk2 (T68), phospho-RPA32 (S33) and γH2AX which persists for 24 - 48 h after removal of VAL-083 from the medium.*

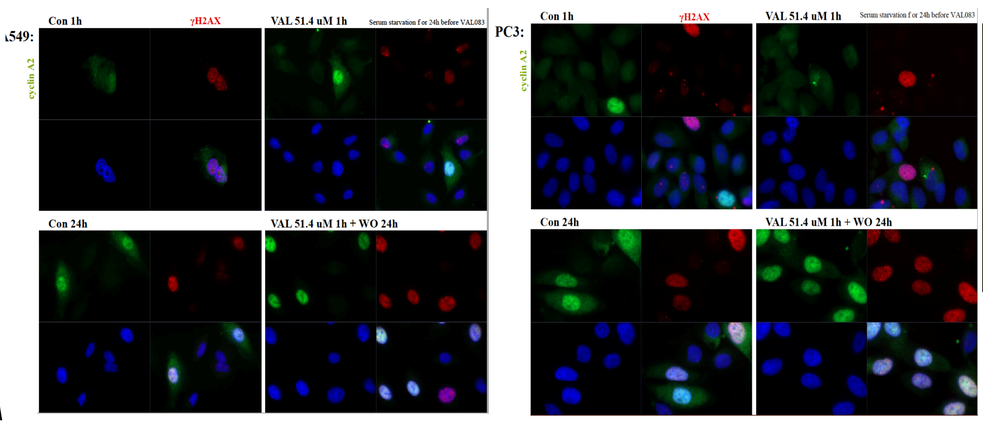
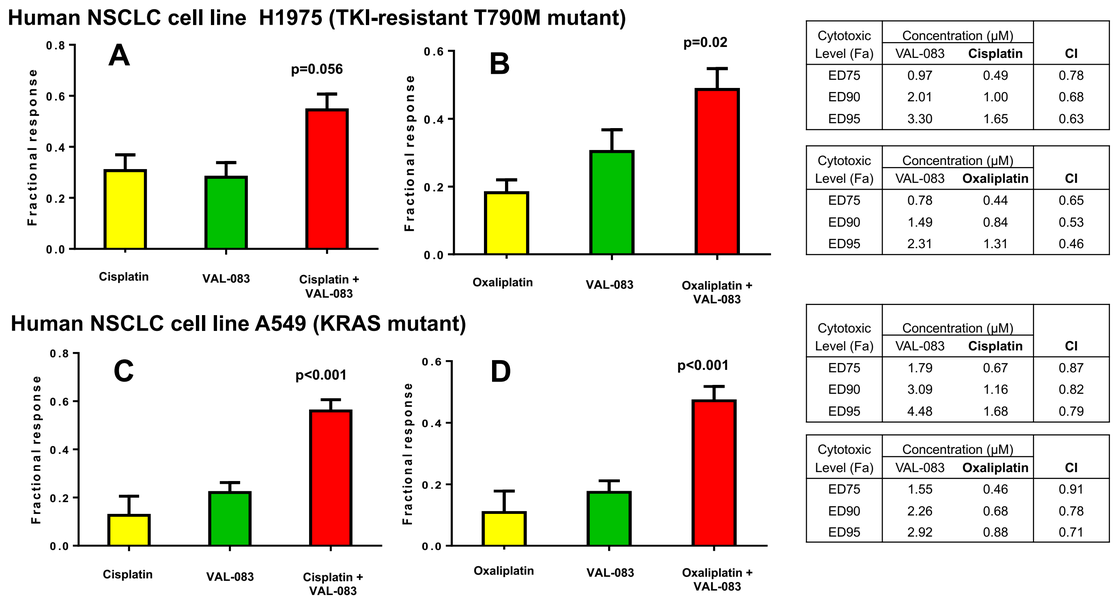


Figure 4. *VAL-083 pulse treatment (1 hr) induced co-localization of γH2AX (DNA double-strand breaks) and cyclin A2 (S/G2 phase cell cycle arrest) in cell lines A549 (left) and PC3 (right).*

PC3 cells	γH2AX + cyclin A2+
Con 1h	2.8 %
VAL-083 1h	2.6 %
Con 24h	16.5 %
VAL-083 1h +WO 24h	90.3 %

Table 1. *VAL-083 pulse treatment (1 hr) induced co-localized DNA double-strand breaks (γH2AX) and S/G2 phase cell cycle arrest (cyclin A2) in 90% of PC3 cells.*

VAL-083 in combination with cisplatin or oxaliplatin demonstrated significant superadditivity ($p<0.05$) and synergism (CI<1) in all NSCLC cell-lines, including TKI-resistant cell lines, independent of T790M (H1975) and KRAS mutations (A549). ***This suggests non-overlapping modes-of-action between the platinum drugs and VAL-083 and demonstrates synergism in TKI-resistant cell-lines irrespective of T790M or KRAS mutations.***



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

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,C**) or oxaliplatin (**B,D**) on H1975 cells (**A,B**), A549 cells (**C,D**) in vitro. Data, where applicable, are shown as Mean +/- SE, N=4-7. 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.*

CONCLUSIONS & FUTURE DIRECTIONS

- ***Historical clinical activity combined with a new understanding of the MoA supports the potential of VAL-083 as a possible solution for the treatment of chemo-refractory NSCLC***
  - ✓ **VAL-083 has a distinct MoA from other chemotherapeutics used in the treatment of NSCLC**
  - ✓ **VAL-083 overcomes TKI-resistance in NSCLC cell lines, including cells with the EGFR mutation T790M and KRAS mutations**
  - ✓ **VAL-083 displays super-additivity and synergy with both cisplatin and oxaliplatin in NSCLC cell lines, including TKI-resistant cells with T790M or KRAS mutations**
  - ✓ **VAL-083 activity is independent of p53 status in a panel of NSCLC cell lines**
- **An open-label post-market clinical trial in China will investigate the activity of VAL-083 in relapsed/refractory NSCLC. Results will provide guidance to physicians under the context of VAL-083's current approval in China, and serve as proof-of-concept for expanded clinical development worldwide**

References:

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4. *Haas CD, et al. Cancer Treat Rep.1981;65(1-2):115-7*
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6. *Steino A, et al. AACR annual meeting 2014, # 824*