DNA damage response to dianhydrogalactitol (VAL-083) in p53-deficient non-small cell lung cancer cells

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ABSTRACT # 1429

Non-small cell lung cancer (NSCLC) treatment usually involves surgery and chemotherapy with tyrosine kinase inhibitors (TKI) in patients with EGFR mutations (10-15% in Western population, 40% in Asian populations) or with platinum-based regimens. Response to TKI treatment is short lived, and tumors recur with new mutations, primarily T790M. Recurrent NSCLC with T790M is sensitive to third generation TKIs, but resistance usually emerges through new mutations, including KRAS. Resistance to cisplatin and carboplatin, partly due to p53 mutation, is also a major clinical limitation and long-term prognosis in NSCLC is poor. Dianhydrogalactitol (VAL-083) is a bi-functional DNA targeting agent with demonstrated clinical activity against NSCLC in historical NCI-sponsored trials and VAL-083 is approved for lung cancer treatment in China. However, the mechanism-of-action of how DNA damage signals are propagated and their effects on NSCLC cells are not fully understood. Therefore, we examined VAL-083 in a panel of 11 human NSCLC cell lines harboring wild-type p53 (H460, A549, H226), mutant p53 (H1975, SkLU1, H2122, H157, H1792, H23) or null p53 (H838, H1299). Importantly, as determined by the 5-day MTT assay, VAL-083 was cytotoxic against all 11 cell lines at low μM concentrations and cytotoxicity was independent of p53 status. We chose 3 TKI-resistant cell lines with different mutation profiles for cell cycle kinetics studies: i) H1975 (p53 mut, EGFR-T790M mut, KRAS wt) with IC_{50} 0.9μM, ii) A549 (p53 wt, EGFR wt, KRAS mut) with IC_{50} 1.8μM, and iii) H157 (p53 mut, EGFR wt, KRAS mut) with IC_{50} 4.5μM. In all 3 cell lines, early response at 18 hr showed dose-dependent increase in cells in S-phase, with continued slow cell cycle progression resulting in accumulation of cells in G2/M phase by 36 hr, suggesting persistent cell cycle arrest. DNA damage signaling was examined by immunoblot analysis in A549 and H1975 cells. In p53-wt A549, VAL-083 induced the phospho-Ser15 form of p53, total p53 and total p21, thus indicating that VAL-083 treatment activated p53 function. On the other hand, in p53-mutant H1975, VAL-083 treatment did not result in consistent p53 or p21 increases, but did readily induce phospho-Ser15 p53. This is consistent with a lack of p53 function, as anticipated for mutant p53 cells. Interestingly, H1975 was 2-fold more sensitive to VAL-083 than A549, suggesting, that in p53-mutant cells, VAL-083 acts through a p53-independent mechanism. Examination of ATR, ATM, Chk1 and Chk2 indicated that DNA damage by VAL-083 prompted phosphorylation of these kinases. Notably, the total anti-apoptotic Chk1 was more prominently reduced in H1975 than A549, which may partly explain the stronger cytotoxicity of VAL-083 in p53-mutant H1975.
These preclinical data strongly support VAL-083 as a potential treatment of mutant p53 and TKI-resistant NSCLC, and indicate DNA damage signaling is mediated via ATM, ATR, Chk1 and Chk2 leading to cell cycle arrest in S- followed by G2/M-phase by two signaling pathways, one p53-dependent and one p53-independent.

MECHANISM-OF-ACTION

VAL-083 targets N^7 of guanine leading to DNA interstrand crosslinks, irreparable DNA double strand breaks, activation of the HR DNA repair pathway and S- followed by G2/M-phase cell cycle arrest by two signaling pathways, one p53-dependent and one p53-independent.[6,7,8]

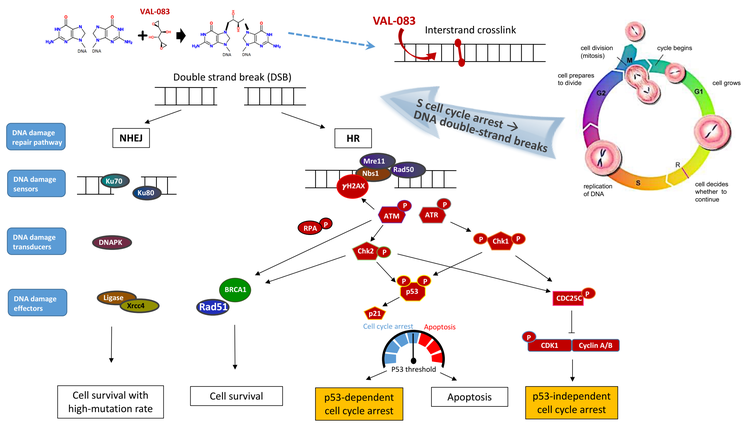

FIGURE 1. VAL-083 induces interstrand crosslinks leading to double-strand breaks and HR activation, mediating cell cycle arrest through a p53-dependent and a p53-independent pathway. Red color signifies demonstrated activation/expression after VAL-083 treatment.

CLINICAL BACKGROUND

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.

TABLE 1. **Historical clinical results with VAL-083 in lung cancer**

Reference	Patient Population	Reported Result
Haas et al. (1976[1]) 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[3]) VAL-083 combination therapy	Advanced SQCLC	Regression: • 27% VAL-083 + adriamycin • 53% VAL-083 + adriamycin + cisplatin
Haas et al. (1981[4]) VAL-083 single agent	Advanced lung cancer	ORR • 19%: recurrent disease • 26%: newly diagnosed
Eagan et al. (1981[5]) VAL-083 combination therapy	Advanced SQCLC	Regression: • 54%: VAL-083 + adriamycin + cisplatin

ORR = SD/PR/CR

References:

1. *Haas CD, et al. Cancer Treat. Rep. 1976;60(5):611-4*
2. *Eagan et al. Cancer Treat Rep. 1977;61(7):1339-45*
3. *Eagan et al. Cancer Treat Rep. 1980;64(1):87-91*
4. *Haas CD, et al. Cancer Treat Rep.1981;65(1-2):115-7*
5. *Eagan et al. Cancer Treat Rep. 1981;65(5-6):517-9*
6. *Institoris & Tamas. Biochem J. 1980:185,659-66.*
7. *Zhai et al. AACR annual meeting 2016*
8. *Peng et al. Acta Pharmacologica Sinica. 2017:1-10*

CONCLUSIONS & FUTURE DIRECTIONS

- ***Historical clinical activity combined with a new understanding of the MoA supports the potential of VAL-083 as a possible 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 activity is independent of p53, EGFR and KRAS status in a panel of NSCLC cell lines**
  - ✓ **VAL-083 activates two signaling pathways leading to S- followed by G2/M-phase cell cycle arrest, one p53-dependent and one p53-independent**
  - ✓ **VAL-083 leads to persistent G2/M phase arrest suggesting irreversible DNA damage, independent of p53 status.**
- **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**

VAL-083 IS ACTIVE AGAINST NSCLC CELLS INDEPENDENT OF P53, EGFR AND KRAS STATUS

Consistent with prior published research, VAL-083 was active against all 11 NSCLC cell-lines tested, irrespective of their p53, EGFR (T790M) and KRAS status, **suggesting a MoA that differs from other chemotherapeutic agents** in the treatment of NSCLC, including platinum-based chemotherapy.
These results further suggest VAL-083 as a **treatment option for chemo-resistant NSCLC, irrespective of the presence of p53, EGFR or KRAS mutations** known to induce resistance to other chemotherapeutics used in the treatment of NSCLC.

TABLE 2. **VAL-083 is active against all 11 NSCLC cell lines independent of p53, EGFR and KRAS status.**

NSCLC cell line	IC_{50} ± SE (μM)	p53	EGFR	KRAS
H460	0.5 ± 0.1	wild type	wild type	*mutant*
A549	1.8 ± 0.3	wild type	wild type	*mutant*
H226	6.1 ± 1.0	wild type	wild type	wild type
H1792	4.6	*mutant*	wild type	*mutant*
H1975	0.9 ± 0.2	*mutant*	*mutant (T790M)*	wild type
SkLU1	2.7 ± 0.0	*mutant*	wild type	*mutant*
H2122	2.8 ± 0.3	*mutant*	wild type	*mutant*
H157	4.5 ± 0.4	*mutant*	wild type	*mutant*
H23	2.6	*mutant*	wild type	*mutant*
H1299	2.4 ± 0.1	null	*amplified*	wild type
H838	4.6 ± 0.4	null	wild type	*mutant*

VAL-083 ACTIVATES TWO SIGNALING PATHWAYS IN NSCLC CELLS, ONE p53-DEPENDENT AND ONE p53-INDEPENDENT

- The distinct mechanism-of-action of VAL-083 leads to cell cycle arrest in S-phase followed by G2/M-phase irrespective of p53, EGFR or KRAS status (Figure 2)
- VAL-083 prompted activation of ATR, ATM, Chk1 and Chk2 in NSCLC cell lines independent of p53, EGFR or KRAS status (Figure 3)
- In p53-wild type A549, VAL-083 induced the phospho-Ser15 form of p53, total p53 and total p21, thus indicating that VAL-083 treatment activated p53 function. On the other hand, in p53-mutant H1975, VAL-083 treatment did not result in consistent p53 or p21 increases, but did readily induce phospho-Ser15 p53. This is consistent with a lack of p53 function, as anticipated for mutant p53 cells. Interestingly, H1975 was 2-fold more sensitive to VAL-083 than A549, suggesting, that in p53-mutant cells, VAL-083 acts through a p53-independent mechanism (Figure 3).

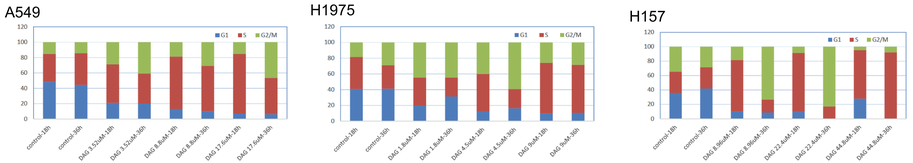

FIGURE 2. VAL-083 (DAG in the figure) treatment leads to cell cycle S-phase arrest at 18 hours followed by G2/M-phase arrest at 36 hours in A549 (p53 wt, EGFR wt, KRAS mut), H1975 (p53 mut, EGFR-T790M mut, KRAS wt) and H157 (p53 mut, EGFR wt, KRAS mut) NSCLC cell lines.

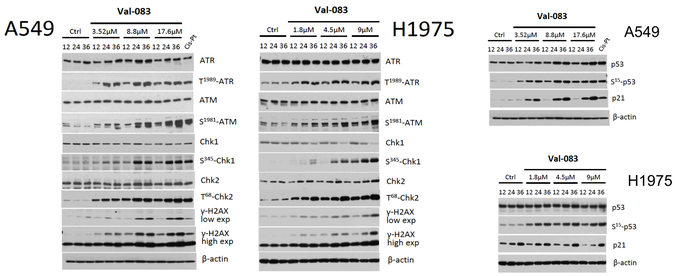

FIGURE 3. DNA damage signaling was examined by immunoblot analysis in A549 and H1975 cells. VAL-083 prompted phosphorylation of ATM, ATR, Chk1 and Chk2 at 12, 24 and 36 hours that, in conjunction with γ-H2AX levels and other data (see abstract #2483), is not only consistent with S and G2/M checkpoint activation, but also suggests persistent DNA double strand breaks and HR activation independent of p53 status.

In p53-wt A549 cells, VAL-083 induced the phospho-Ser15 form of p53, as well as total p53 and total p21, thus indicating that VAL-083 treatment activated p53 function. In p53-mutant H1975, VAL-083 treatment did not result in consistent p53 or p21 increases, but did readily induce phospho-Ser15 p53.