The PLK1 Inhibitor, Onvansertib, Synergizes with Paclitaxel in Small Cell Lung Cancer

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Background

Small Cell Lung Cancer (SCLC) & Current Therapy:

- Key transcription factors: ASCL1, NEUROD1, YAP1, POU2F3 drive SCLC molecular subtypes (A, N, Y, P, respectively).
- SCLC-A (40%-50%) and SCLC-N (25%-30%) constitute the main patient populations, both exhibiting significant neuroendocrine features.
- Prognosis: <7% five-year survival rate [1].
- Treatments: platinum-based chemotherapy in frontline; paclitaxel is used in relapsed/refractory setting (<30% response rate) [2].
- Therapeutic advancements to improve clinical outcomes are necessary.

Polo-like kinase 1 (PLK1):

- Serine/threonine protein kinase.
- Crucial for mitosis, centrosome maturation, chromosome segregation, cytokinesis, DNA repair.
- SCLC dependency on PLK1, especially in p53 and RB1 loss contexts [3]
- PLK1 inhibitors showed promise in preclinical models [4].

Onvansertib:

- Oral, selective PLK1 inhibitor in clinical development.
- Phase 1 identified maximum tolerated dose and evaluated toxicities [5].
- An ongoing Phase 2 study is assessing its safety and efficacy in relapsed extensive-stage SCLC (NCT05450965).

This investigation sought to evaluate the effectiveness of onvansertib in combination with paclitaxel in SCLC preclinical models.

Results

IC_{50} and Synergistic Effects of Onvansertib and Paclitaxel in SCLC Cell Lines

Combination treatment of onvansertib (Onv) and paclitaxel (Ptxl) synergistically inhibited cell growth in SCLC cell lines across different molecular subtypes.

Cell line	SCLC subtype	Mutation Status			IC_{50} (nM)		Synergistic Effects
		RB	P53	PTEN	Onv	Ptxl	Onv + Ptxl
DMS53	A	WT	MUT	WT	50	181	Yes
H146	A	LOF	LOF	WT	76	15	Yes
DMS273	N	LOF	MUT	MUT	80	3	Yes
H526	P	LOF	LOF	WT	15	2	No
H211	P	WT	MUT	WT	21	3	No
DMS114	Y	WT	LOF	WT	39	3	Yes
SW1271	Y	WT	MUT	WT	325	5	Yes
KP11 (murine)	Possibly A	LOF	LOF	NA	97	5	Yes

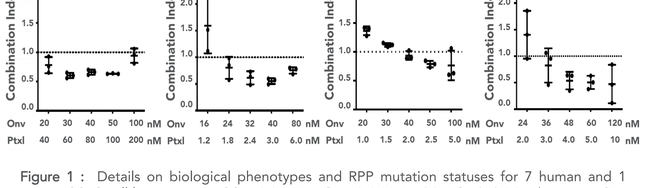


Figure 1 : Details on biological phenotypes and RPP mutation statuses for 7 human and 1 murine SCLC cell lines, across ASCL1 (A), NEUROD1 (N), YAP1 (Y), POU2F3 (P) subtypes. WST1 assay was used to assess IC_{50} and synergy in cell lines treated for 72h with onvansertib (Onv) and paclitaxel (Ptxl). Synergy was determined using the combination index (CI) calculations with CompuSyn. CI <1.0 indicates synergy, categorized into strong (CI < 0.3), moderate (CI 0.3–0.7), or slight (CI 0.7–0.9). CI graphs for 4 cell lines are shown. Wild-type (WT); Mutation (MUT); Loss-of-function (LOF); Not specified in the available information (NA).

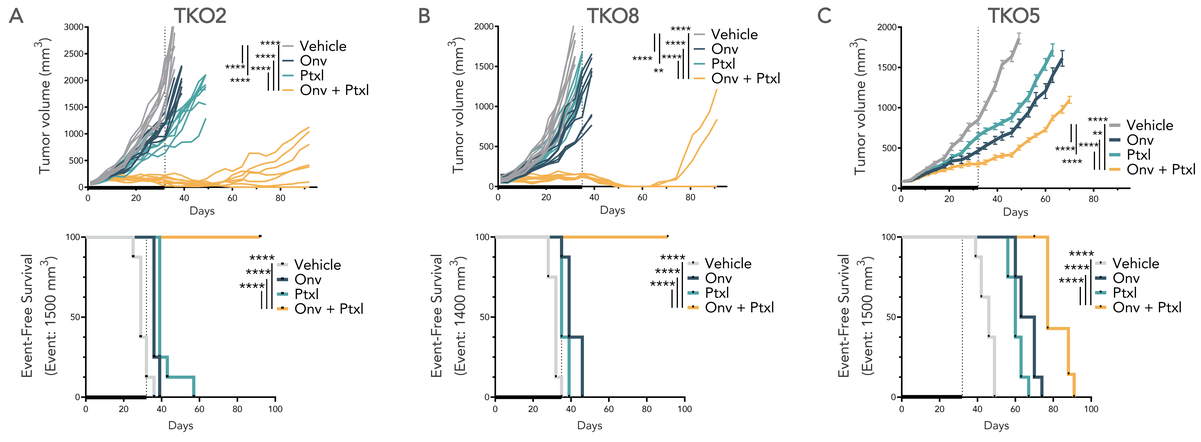
References

1. Ganti et al., Journal of the National Comprehensive Cancer Network 2021
2. Yamamoto et al., Anticancer Research 2006
3. Oser et al., Cancer Discovery 2019
4. Wang et al., Cancer Letters 2018
5. Weiss et al., Investigational New Drugs 2018

Results

Combined Onvansertib and Paclitaxel Treatment Demonstrates Robust Anti-Tumor Efficacy and Extends Effectiveness in Both Cisplatin-Sensitive and -Resistant SCLC Patient Derived Xenograft (PDX) Models

- **All treatments were well-tolerated by all animals throughout the entire study duration.**
- **The combination therapy demonstrated superior efficacy over monotherapies in cisplatin-sensitive and resistant PDX xenografts.**
- **All mice in the combination groups of the two cisplatin-resistant PDX models exhibited tumor regression or stasis from day 10 onwards.**
- **These mice showed sustained tumor regression beyond treatment interruption on day 32 or 35.**
- **All TKO8 mice in the combination group had complete responses (CR), with 6/8 maintaining this response by day 92 (56 days post-treatment interruption).**
- **60 days post-treatment interruption, all mice in the TKO2 combination group were still event-free (TV<1500mm³); those achieving CR maintained it.**



TKO2	Vehicle	Onv	Ptxl	Onv + Ptxl
TGI (d36)	-	41%	63%	99%
Tumor Regression	0% (0/8)	0% (0/8)	0% (0/8)	100% (8/8)
CR	0% (0/8)	0% (0/8)	0% (0/8)	25% (2/8)
Median Survival	29d	36d	39d	NR

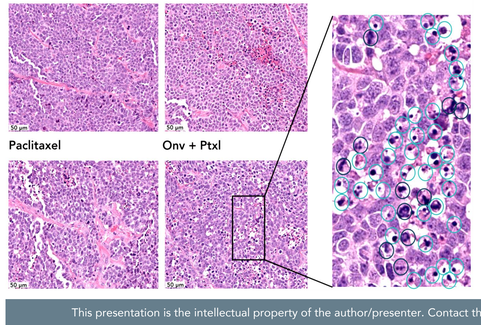
TKO8	Vehicle	Onv	Ptxl	Onv + Ptxl
TGI (d32)	-	53%	35%	93%
Tumor Regression	0% (0/8)	0% (0/8)	0% (0/8)	100% (8/8)
CR	0% (0/8)	0% (0/8)	0% (0/8)	100% (8/8)
Median Survival	32d	39d	35d	NR

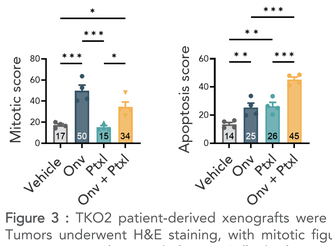
TKO5	Vehicle	Onv	Ptxl	Onv + Ptxl
TGI (d49)	-	56%	46%	72%
Median Survival	46d	67d	60d	77d

Model	Cisplatin Sensitivity	Subtype	Origin	Patient Treatment History
TKO2	Resistant	A	Hepatic metastasis, female, white	Carboplatin/etoposide for 4 cycles → Single agent etoposide → Topotecan
TKO8	Resistant	N	Lung, 76-year-old male, Caucasian	Carboplatin/etoposide for 6 cycles → Paclitaxel for progressive disease
TKO5	Sensitive	A	Lung, 50-year-old male, African American	Radiation + cisplatin/etoposide

Figure 2: TKO2 (**A**), TKO8 (**B**), and TKO5 (**C**) PDX models were treated with vehicle, onvansertib (50mg/kg, orally, 4 times/week), paclitaxel (15mg/kg, intraperitoneally, weekly), or their combination over set periods (▬) (n=8 /group), with tumor growth and body weight monitored. Tumor growth inhibition (TGI) was calculated as 100% x $((V_{control}-V_{treated})/V_{control})$, with regression indicated by TGI > 100% and complete response (CR) by a tumor size of 0 mm³. Statistical tumor volume differences were analyzed using One-Way ANOVA, significant at **p<0.01 and ****p<0.0001. Kaplan-Meier curves for event-free survival (time to reach tumor volumes of 1400 or 1500 mm³) analyzed by Log-rank Mantel Cox test (****p<0.0001). Day (d); Not reached (NR).

Onvansertib-Paclitaxel Combination Induces Mitotic Arrest and Apoptosis in PDX Models





- **Both onvansertib monotherapy and Onv + Ptxl groups exhibited increased mitotic figures.**
- **Monotherapies triggered apoptosis; Onv + Ptxl combination therapy induced significantly more apoptotic cells than either monotherapy alone.**
- **These findings were corroborated through Western-Blot analyses of mitotic and apoptotic markers, as shown in Figure 4.**

Figure 3 : TKO2 patient-derived xenografts were treated for four days with Onv and Ptxl (n=4 per group). Tumors underwent H&E staining, with mitotic figures highlighted in dark blue and apoptotic figures in sea green as exemplar morphologies. Cells displaying mitotic and apoptotic characteristics were manually counted for each tumor (5 fields per tumor) by board-certified pathologists. Data are presented as means ± SEM and were subjected to One-way ANOVA analysis. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Onvansertib-Paclitaxel Combination Inhibits PLK1 Signaling and Disease Targets, and Induces Apoptotic Markers in PDX Models

- **PLK1 phosphorylates TCTP (Translationally-Controlled Tumor Protein) on Serine 46.**
- **Onvansertib alone or with paclitaxel significantly decreased p-TCTP, confirming PLK1 inhibition; both treatments notably induced p-HH3 in TKO2, indicating mitotic arrest.**
- **Onv + Ptxl activated apoptotic markers, cleaved PARP and cleaved caspase-3.**
- **Onv + Ptxl significantly decreased the expression of c-Myc, an oncogene frequently overexpressed in SCLC and linked to aggressive tumor behavior and poor prognosis.**

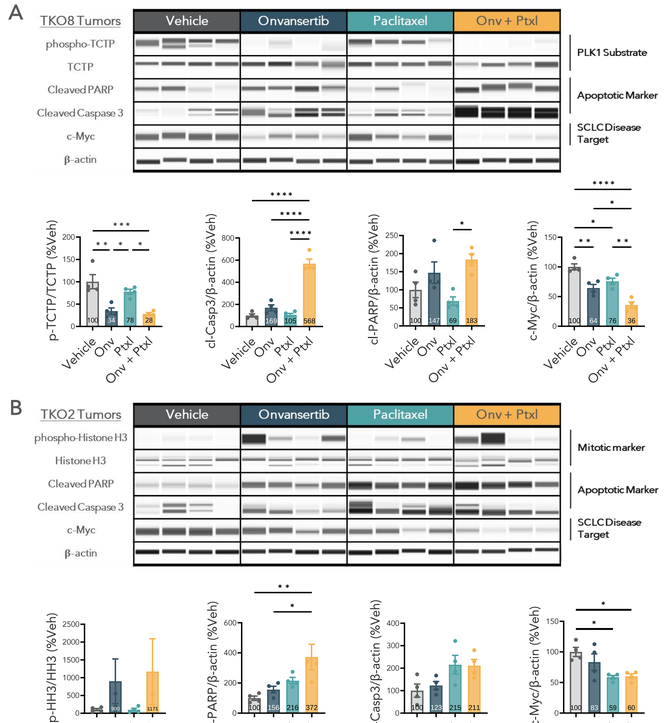


Figure 4: TKO8 (**A**) and TKO2 (**B**) patient-derived xenografts were treated for four days, and tumor were subjected to ProteinSimple immunoblotting. Phospho-TCTP (p-TCTP) and phospho-histone H3 (p-HH3), were normalized against total TCTP and HH3 respectively. Cleaved caspase-3 (cl-Casp3), cleaved PARP (cl-PARP), and c-Myc, were standardized to β-actin. Statistical differences between treatment groups were evaluated using One-Way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

Onvansertib and Paclitaxel: A Promising Combination for SCLC

- Onv + Ptxl synergistically inhibited cell proliferation in SCLC cell lines.
- The combination was well-tolerated, and highly effective in cisplatin-sensitive and -resistant SCLC PDX models. In cisplatin-resistant models, Onv + Ptxl led to tumor regression, with effects lasting 2 months post-treatment.
- The Onv + Ptxl treatment resulted in increased mitotic arrest and apoptosis, along with decreased c-Myc levels in SCLC patient-derived tumors.
- These findings support that combining onvansertib with paclitaxel could emerge as a highly promising treatment strategy for relapsed/refractory SCLC patients.