Abstract #2153

# Cyclin E1 protein overexpression sensitizes ovarian cancer cells to azenosertib (ZN-c3), a novel, selective and orally bioavailable inhibitor of WEE1

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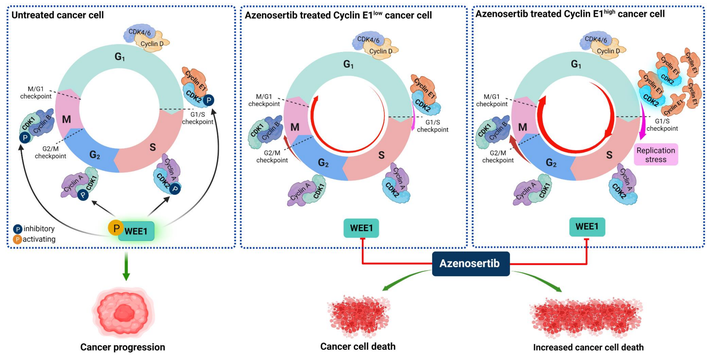
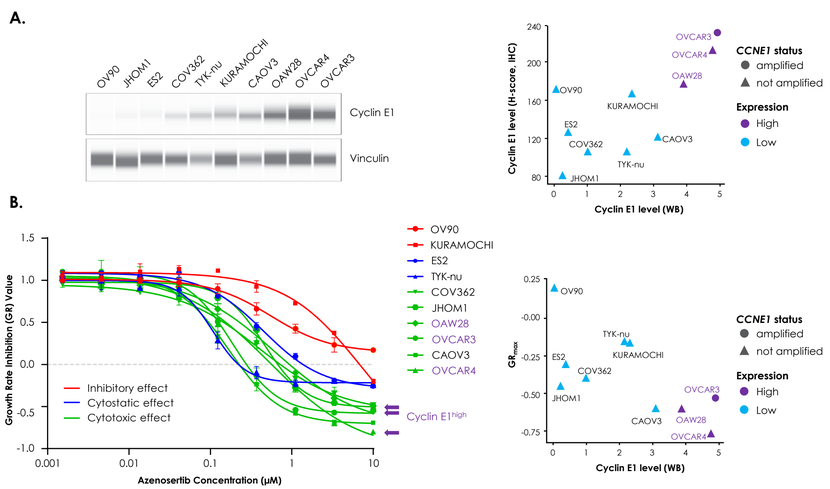
## BACKGROUND

- *CCNE1* gene amplification is a prevalent oncogenic driver in high grade serous ovarian cancer (HGSOC) and is associated with platinum resistance and poor patient outcomes[1,2]. Importantly, Cyclin E1 overexpression can also occur in the absence of gene amplification.
- Overexpressed Cyclin E1 forms a complex with CDK2 to accelerate G1/S transition, resulting in replication stress and increased dependency on the G2/M checkpoint[3].
- WEE1 is a protein kinase that plays a critical role in cell cycle regulation by inactivating both CDK1 and CDK2 through inhibitory phosphorylation on tyrosine 15.
- WEE1 is involved in controlling several stages of cell cycle progression, in particular limiting progression from G1 to S and G2 to M, allowing cells to repair damaged DNA before entering the next phase of cell cycle[3,4].
- Azenosertib (ZN-c3) is a novel, selective, and orally bioavailable WEE1 inhibitor currently in clinical development. WEE1 inhibition by azenosertib abrogates G1/S and G2/M checkpoints, leading to increased cell cycling, premature S and M phase entry, and subsequent cell death.
- We hypothesized that azenosertib treatment would exacerbate replication stress caused by Cyclin E1 overexpression, leading to increased cell death and enhanced anti-tumor activity in Cyclin E1$^{high}$ preclinical models.




## RESULTS

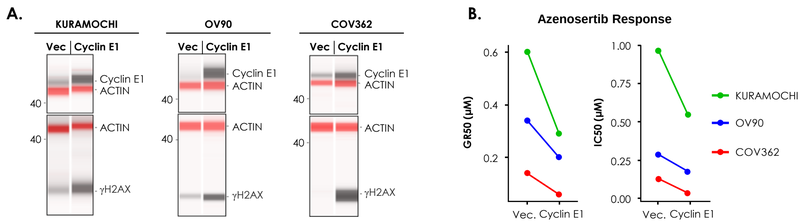
### Figure 1. Cyclin E1$^{high}$ HGSOC cell lines are more sensitive to azenosertib *in vitro*




**A.** OVCAR3 (*CCNE1*amplified, CN=12), OVCAR4 and OAW28 cells express high levels of Cyclin E1 which was determined by Western blot (WB) and immunohistochemistry (IHC). Cyclin E1 expression was normalized to Vinculin.

**B.** All three Cyclin E1$^{high}$ cell lines induce significant cytotoxic effect ($GR_{max}$ < -0.5) while only 43% (3/7) of Cyclin E1$^{low}$ cell lines do. GR and $GR_{max}$ were determined by measurement of cell viability and the GR calculator (http://www.grcalculator.org).

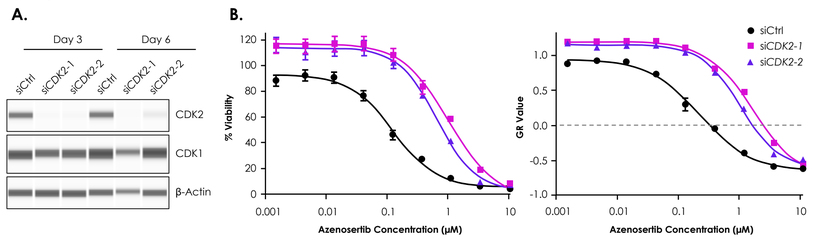
### Figure 2. Cyclin E1 overexpression sensitizes isogenic HGSOC cell lines to azenosertib




**A.** Cyclin E1 overexpression induced by lentiviral transduction was confirmed by WB. Cyclin E1 overexpression significantly increases γH2AX in all cell lines, indicating an increase of replication stress and DNA damage response.

**B.** Cyclin E1 overexpression sensitizes HGSOC cell lines to azenosertib by further decreasing growth rate and cell viability compared to control vector (Vec) cell lines. GR50 and IC50 of azenosertib were determined by CellTiter-Glo assay and the GR calculator.

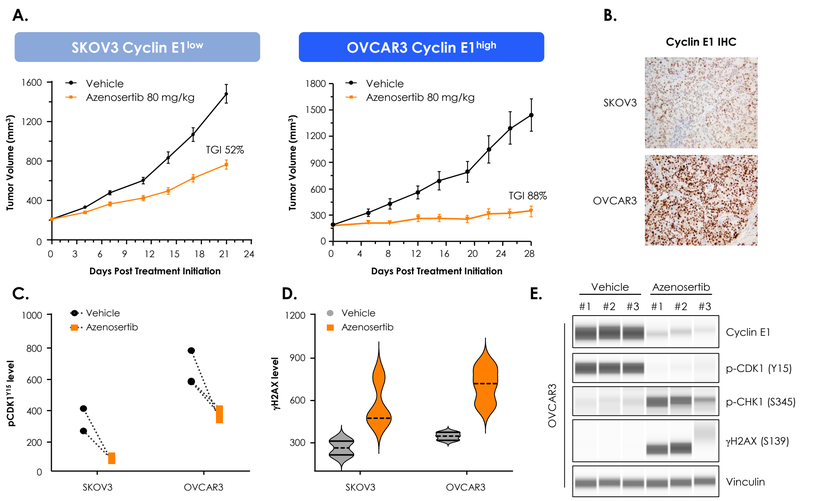
### Figure 3. Sensitivity of Cyclin E1$^{high}$ HGSOC cell line to azenosertib is dependent on CDK2




**A.** Two different *CDK2* siRNAs significantly reduced CDK2 protein expression in Cyclin E1$^{high}$ OVCAR4 cells before and during azenosertib treatment. CDK2 protein level was determined by WB at day 3 and day 6 after transfection

**B.** CDK2 knockdown desensitizes OVCAR4 cells to azenosertib. After 3 days of *CDK2* siRNAs transfection, OVCAR4 cells were treated with azenosertib for 3 days. % viability and GR value were determined by CellTiter-Glo assay and the GR calculator.

### Figure 4. Greater anti-tumor effects of azenosertib in a Cyclin E1$^{high}$ tumor model are associated with increased replication stress



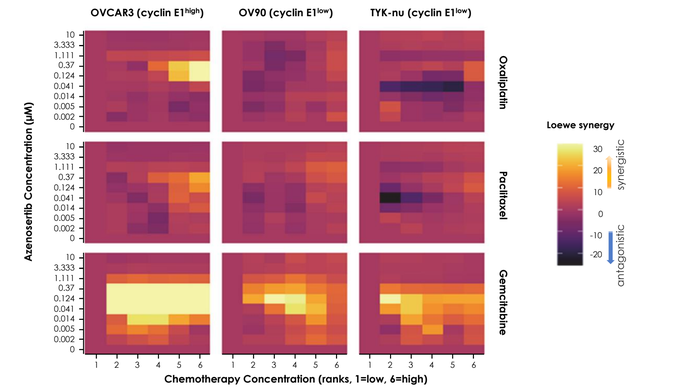

**A.** NOD/SCID mice bearing SKOV3 and OVCAR3 tumors were dosed orally every day for the time as indicated. Treatment was well tolerated. TGI, tumor growth inhibition.

**B.** Baseline Cyclin E1 expression of each model was examined by IHC.

**C and D.** Target engagement of azenosertib (decreased pCDK1) and γH2AX were examined by IHC in tumors after 12 hrs of azenosertib treatment. Y-axis represents the sum of H-score evaluated by 3 independent pathologists.

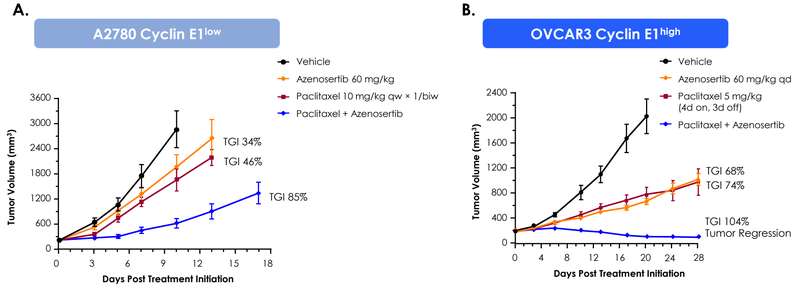
**E.** Replication stress markers pCHK1 and γH2AX were determined by WB in OVCAR3 tumor samples with or without azenosertib treatment for 12 hrs.

### Figure 5. Increased synergy between azenosertib and chemotherapy is observed in Cyclin E1$^{high}$ HGSOC cell line




Cyclin E1$^{high}$ OVCAR3 cells show greater synergistic effects (Loewe synergy score > 10 is synergistic, < -10 is antagonistic) in all chemotherapy and azenosertib combinations than Cyclin E1$^{low}$ OV90 and TYK-nu cells. The drug combination effect was evaluated by measurement of cell viability and 4 calculation methods (ZIP, Bliss, Loewe and HSA) following SynergyFinder guidelines (https://synergyfinder.org). The Loewe score was consistent with other methods of calculating synergy. Scores were capped at 30 for visualization purposes. Each panel represents the summary of 3 to 6 replicates. The concentration of chemotherapy was rank-transformed and its ranges were cell line and chemotherapy specific: oxaliplatin: 0 – 10 μM (OVCAR3, OV90), 0 - 3.3 μM (TYK-nu); paclitaxel: 0 - 0.005 μM (OVCAR3), 0 - 0.02 μM (OV90, TYK-nu); gemcitabine: 0 – 1 μM (OVCAR3), 0 - 0.02 μM (OV90), 0 - 0.01μM (TYK-nu).

### Figure 6. Cyclin E1$^{high}$ tumor model is more sensitive to combination treatment with azenosertib and paclitaxel than Cyclin E1$^{low}$ model




**A and B.** BALB/c nude mice bearing A2780 tumors or NOD/SCID mice bearing OVCAR3 tumors were treated with azenosertib orally every day and paclitaxel intraperitoneally as a single agent or in combination as indicated. All treatments were well tolerated.

## CONCLUSIONS

- Ovarian cancer cell lines with high levels of Cyclin E1 are more sensitive to azenosertib *in vitro* and *in vivo*. Lentiviral-mediated overexpression of Cyclin E1 sensitizes cell lines with low endogenous Cyclin E1 levels to azenosertib treatment.
- Cyclin E1 overexpression results in accumulation of replication stress markers such as γH2AX, which are further enhanced upon azenosertib treatment in tumors.
- Sensitivity of Cyclin E1$^{high}$ ovarian cancer cell line to azenosertib is dependent on CDK2.
- Azenosertib in combination with chemotherapy showed greater synergistic effects in Cyclin E1$^{high}$ ovarian cancer models than Cyclin E1$^{low}$ models *in vitro* and *in vivo*.
- These data support CCNE1 gene amplification and Cyclin E1 overexpression as candidate predictive biomarkers for improved response and outcome to treatment with azenosertib both as monotherapy and in combination with chemotherapy.

## REFERENCES

**1.** Gorski JW, et al. *Diagnostics (Basel)*. 2020;10(5):279.
**2.** Nakayama N, et al. *Cancer.* 2010;116(11):2621-2634
**3.** Kok YP, et al. *Oncogenesis.* 2020;9(10):88.
**4.** Chen X, et al. *Clin Cancer Res.* 2018;24(24):6594-6610.