A study of combinatorial growth inhibition, cell death and DNA damage repair caused by CHK1 inhibitor SRA737 and WEE1 inhibitor Adavosertib in *TP53* mutated cell lines.

#1010

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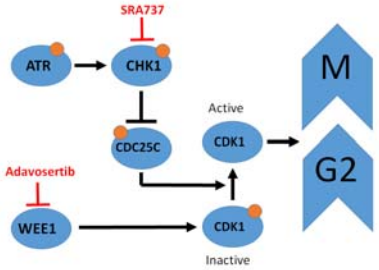
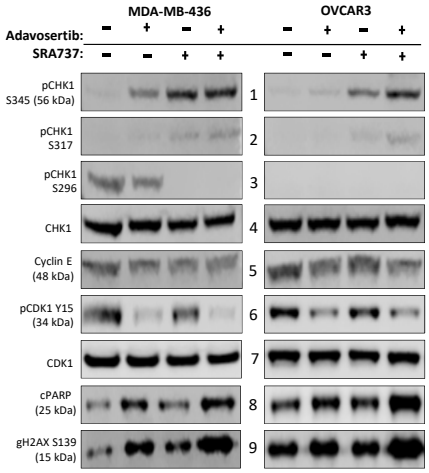
Introduction

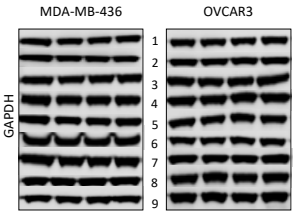


- DNA damage in the S phase could be exogenous e.g. anticancer drugs or endogenous due to high replicative stress cause by oncogene driven cell growth enhanced by a abrogated G1/S checkpoint e.g. *TP53* mutation
- The G2/M checkpoint is crucial to cancer cells as it allows cells to repair DNA in the G2 and S phase
- Abrogation of the G2/M checkpoint will lead to premature entry into mitosis and cell death

Hypothesis: The combination of CHK1 inhibitor (SRA737) and WEE1 inhibitor (Adavosertib) in *TP53*^{M} cell lines will cause additive synergistic cell death due to abrogation of the G2/M checkpoint.

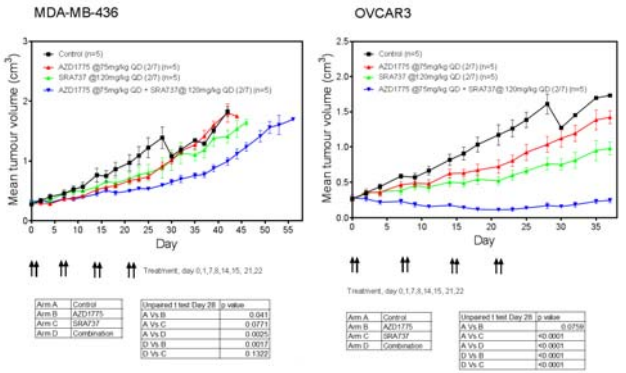
Mechanism of Action





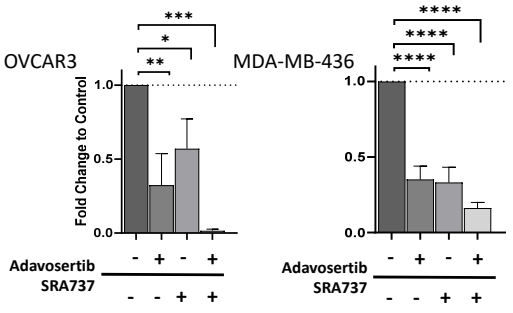
Western blots showing mechanism of action in both single agent and combination at SF_{50} after 24 hours. CHK1 activation at phospho sites S345 and S317 are established markers of DNA damage and levels of S345 have been shown to accumulate upon CHKi. CHK1 S296, however, shows reduction in phosphorylation in MDA-MB-436 cells exposed to SRA737. Downstream inhibition of CDK1 Y15 by the WEE1i Adavosertib was also seen in both cell lines. Markers for extensive DNA damage (cPARP) and repair (gH2AX S139) increased in cells dosed with SF_{50} of both drugs in combination compared to either control or single agents.

In vivo growth inhibition



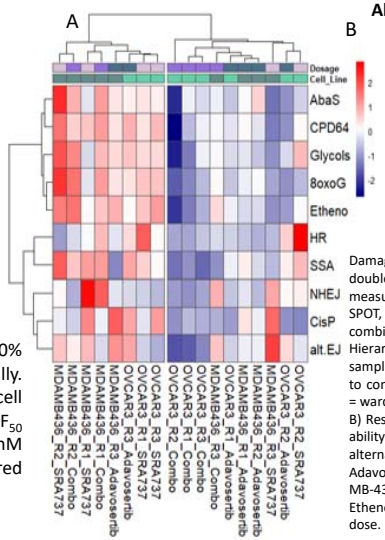
- Doses used in experiment included vehicle control, SRA737 120 mg/kg qd 2/7 x 4 weeks, Adavosertib 75 mg/kg qd 2/7 x 4, or the combination of SRA737 + Adavosertib
- Tumour regressions seen in The OVCAR3 ovarian cancer xenograft models with a *TP53*^{M} with a cyclin E amplification. Tumour growth delay was seen with the combination in the *TP53*^{M} *BRCA*^{M} TNBC breast cancer xenograft model

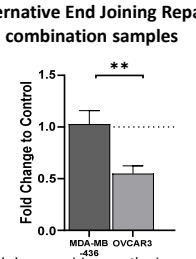
Growth Inhibition

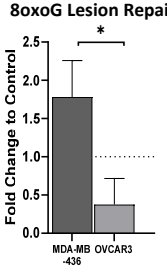


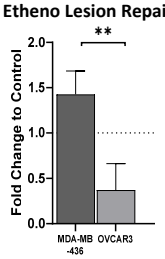
14 day clonogenic assay, studying colony formation. Cells exposed to 50% surviving fraction (SF_{50}), previously determined for each drug individually. The adavosertib SF_{50} concentration for the OVCAR3 and MDA-MB-436 cell lines were 182 nM and 546 nM respectively. Similarly SRA737 SF_{50} concentrations for the OVCAR3 and MDA-MB-436 cell lines were 730 nM and 1819 nM. The combination shows significant growth delay compared to control.

Mechanisms of DNA Damage response









Damaged base excision synthesis repair and double stranded DNA break repair mechanisms, measured with LXRepair NEXT-SPOT and ExSy-SPOT, respectively, across both single agents and combination at SF_{50} after 24 hours. A) Hierarchical clustering heatmap across all samples and repetitions (n=3). Data normalised to control, distance = Euclidean, linkage method = ward.D.

B) Results indicate that OVCAR3 has a decreased ability to repair double stranded DNA breaks via alternative end joining vs MDAMB436 post Adavosertib + SRA737 in combination. C) MDA-MB-436 also displays a greater ability to repair Etheno and 8oxoG lesions post combination dose. P values generated via t-test.

Summary/Conclusions

- CHK1 and WEE1 are critical determinants of the G2/M checkpoint that allow repair of DNA damage caused by exogenous (chemotherapy and radiotherapy) and endogenous (oncogene addicted cell growth or lack of intact G1/S checkpoint due to *TP53* mutation) events .
- The combination of the CHK1 inhibitor SRA737 and the WEE1 inhibitor Adavosertib induce additive growth inhibition in vitro and in vivo, including tumour regressions seen in the OVCAR3 human ovarian cancer xenograft model.
- The mechanisms of DNA damage response is different in the BRCA mutated MDA-MB-436 and Cyclin E amplified OVCAR3 cell lines studied.
- This combination should be clinically explored in hypothesis testing clinical trials and in biomarker stratified cohorts such as cyclin E amplification.

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