Chk1 inhibition and Wee1 inhibition combine synergistically to inhibit cellular proliferation

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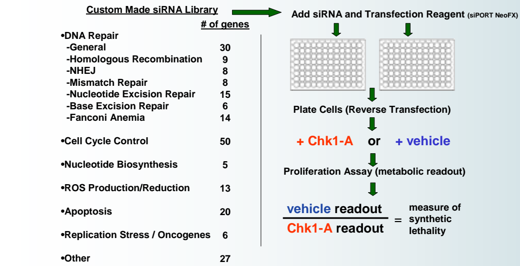
Introduction

Inhibition of the checkpoint kinase Chk1, both as a monotherapy and in combination with DNA damaging cytotoxics, is a promising therapeutic strategy for cancer. However, much remains to be learned in regard to the patient populations that will respond best to a Chk1 inhibitor and the optimal therapeutics to combine with a Chk1 inhibitor. In an effort to discover sensitizing mutations and novel combination strategies for Chk1 inhibition, we performed a 'synthetic lethality' siRNA screen with the selective Chk1 inhibitor Chk1-A. This screen employed a custom made library of siRNAs against 197 genes (3 siRNAs per gene), most of which are involved in cell-cycle control or DNA damage repair. One of the most prominent and consistent hits across runs of the screen performed in PC3, LNCaP, and A549 cell lines was Wee1 kinase. MK-1775 is a small molecule inhibitor of Wee1 that is currently in early stage clinical trials. In confirmation of the results obtained from the siRNA screen, we found that Chk1-A and MK-1775 synergistically inhibited proliferation in multiple cell types. This anti-proliferative synergy correlated with a synergistic induction of apoptosis. We explored the mechanism of the impressive synergy by examining the cellular and biochemical effects of the Chk1-A and MK-1775 combination. We found that co-treatment with the two inhibitors resulted in dramatic decreases in inhibitory phosphorylation of cyclin-dependent kinases 1 and 2, increases in DNA damage, and the collapse of DNA replication. In conclusion, the combination of a Chk1 inhibitor and a Wee1 inhibitor may be an effective treatment strategy for cancer.

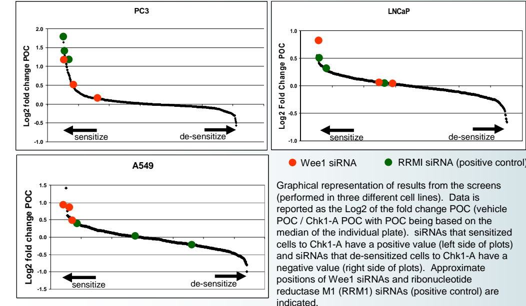
Results

Figure 1: Description of the synthetic lethality siRNA screen



Left – Summary of custom made siRNA library (Ambion). 3 siRNAs per gene were included.
Right – Schematic for synthetic lethality screen.

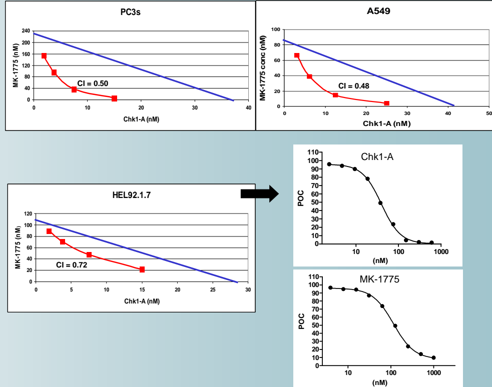
Figure 2: Wee1 a prominent hit in synthetic lethality screens



Graphical representation of results from the screens (performed in three different cell lines). Data is reported as the Log2 of the fold change POC (vehicle POC / Chk1-A POC with POC being based on the median of the individual plate). siRNAs that sensitized cells to Chk1-A have a positive value (left side of plots) and siRNAs that de-sensitized cells to Chk1-A have a negative value (right side of plots). Approximate positions of Wee1 siRNAs and ribonucleotide reductase M1 (RRM1) siRNAs (positive control) are indicated.

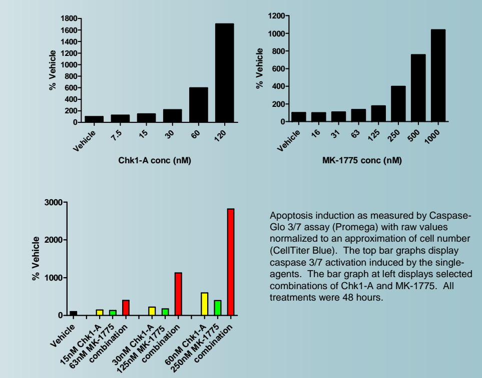
Results

Figure 3: Chk1-A and MK-1775 synergistically inhibit proliferation in multiple cell lines



Top and bottom-left XY plots (isobolograms) are data from proliferation assays (CellTiter Blue readout, Promega) in which Chk1-A and MK-1775 were combined in matrix fashion. The blue line connects the respective single-agent IC_{50}s for both compounds. This line represents the expected combined values that would give 50% POC if the compounds were acting in additive fashion. The red line represents the actual values that combine to give 50% POC. Combination index (CI) was calculated as $(D)_{Chk1\text{-}A} / (D_m)_{Chk1\text{-}A} + (D)_{MK\text{-}1775} / (D_m)_{MK\text{-}1775}$ where (D) represents the actual drug concentrations required to inhibit proliferation 50% in combination and (D_m) represents the concentrations of the single-agents that inhibit proliferation 50%. The lowest CI calculated is displayed on the graph. Bottom-right XY plots are representative dose-response proliferation curves for single-agent Chk1-A and MK-1775 in the HEL92.1.7 line. All treatments were 72 hours.

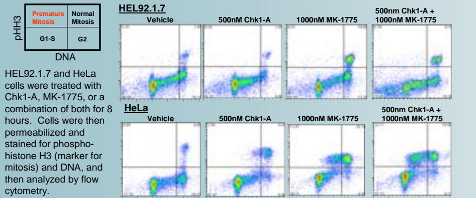
Figure 4: Chk1-A and MK-1775 synergistically induce apoptosis in HEL92.1.7 cells



Apoptosis induction as measured by Caspase-Glo 3/7 assay (Promega) with raw values normalized to an approximation of cell number (CellTiter Blue). The top bar graphs display caspase 3/7 activation induced by the single-agents. The bar graph at left displays selected combinations of Chk1-A and MK-1775. All treatments were 48 hours.

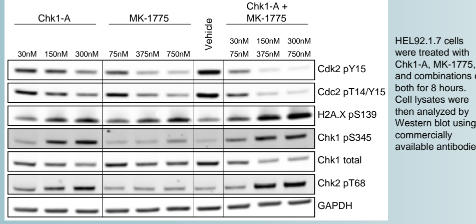
Results

Figure 5: Chk1-A and MK-1775 have differential effects on the cell-cycle, premature mitosis is cell-type dependent



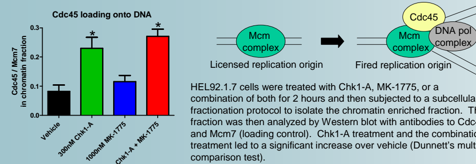
HEL92.1.7 and HeLa cells were treated with Chk1-A, MK-1775, or a combination of both for 8 hours. Cells were then permeabilized and stained for phospho-histone H3 (marker for mitosis) and DNA, and then analyzed by flow cytometry.

Figure 6: Chk1-A and MK-1775 both reduce CDK inhibitory phosphorylation and increase H2A.X phosphorylation, but only Chk1-A leads to strong cell-cycle checkpoint activation



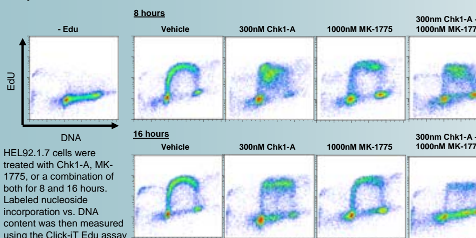
HEL92.1.7 cells were treated with Chk1-A, MK-1775, and combinations of both for 8 hours. Cell lysates were then analyzed by Western blot using commercially available antibodies

Figure 7: Chk1-A but not MK-1775 induces loading of Cdc45 onto DNA



HEL92.1.7 cells were treated with Chk1-A, MK-1775, or a combination of both for 2 hours and then subjected to a subcellular fractionation protocol to isolate the chromatin enriched fraction. This fraction was then analyzed by Western blot with antibodies to Cdc45 and Mcm7 (loading control). Chk1-A treatment and the combination treatment led to a significant increase over vehicle (Dunnett's multiple comparison test).

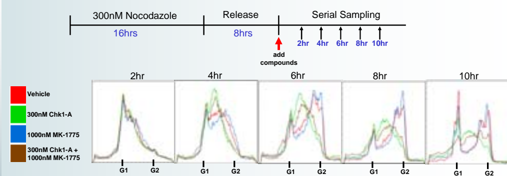
Figure 8: Chk1-A treatment results in collapse of DNA synthesis in S-phase and the addition of MK-1775 enhances this effect

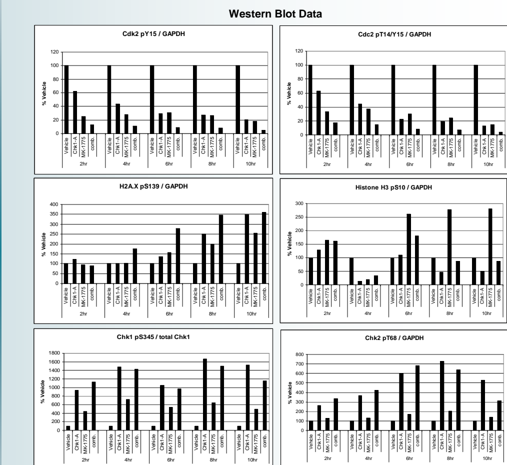


HEL92.1.7 cells were treated with Chk1-A, MK-1775, or a combination of both for 8 and 16 hours. Labeled nucleoside incorporation vs. DNA content was then measured using the Click-iT Edu assay (Invitrogen).

Results

Figure 9: Chk1-A and MK-1775 have differential effects on progression through S-phase in HEL92.1.7 cells





HEL92.1.7 cells were synchronized as shown and then Chk1-A, MK-1775, or a combination of both were added as the bulk of cells entered S-phase. Serial samples of the cells were then taken every 2 hours for analysis of cell-cycle position (propidium iodide staining followed by flow cytometry) and biochemical analysis (Western blotting).

Conclusions

-Chk1 inhibition and Wee1 inhibition synergistically inhibit proliferation and induce apoptosis

-Chk1 inhibition and Wee1 inhibition both lead to reductions in inhibitory phosphorylation of CDKs and an increase in H2A.X phosphorylation, and the combination of the inhibitors enhances these effects

-Chk1 inhibition, but not Wee1 inhibition, leads to excessive loading of Cdc45 onto DNA and collapse of DNA replication early in S-phase

-Wee1 inhibition, but not Chk1 inhibition, leads to early histone H3 phosphorylation and accumulation in mitosis

-Synergistic inhibition of proliferation may be due to the combination of these differential effects on the cell cycle