Investigating Wee1 and Myt1 combined inhibition as a potential cancer therapeutic strategy

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Abstract

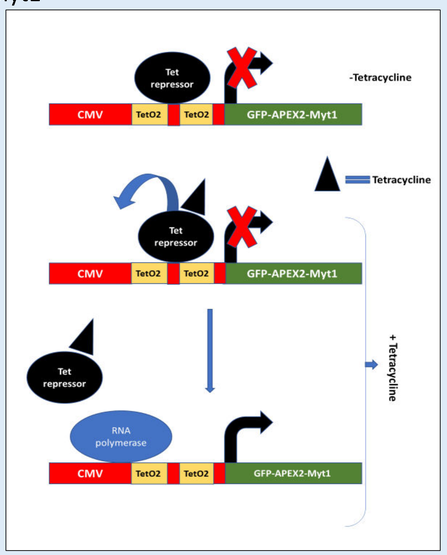
Introduction: Cell cycle is under the surveillance of checkpoints to repair any damage before the cell transits between phases. Wee1 and Myt1 kinases prevent premature entry into mitosis by monitoring the G2/M checkpoint by adding an inhibitory phosphorylation on Cdk1. Wee1 is overexpressed in various tumors and MK-1775, a Wee1 small molecule inhibitor, is currently in phase I/II cancer clinical trials. Although MK-1775 was shown to potentiate the effect of other genotoxic therapies, yet clinical resistance has emerged towards it. In examining the mechanism of MK-1775-mediated cytotoxicity, we identified Myt1 as a resistant factor. Emerging evidence suggests that Myt1 is an important cancer therapeutic target. Hence, we are examining a novel Myt1 kinase small molecule inhibitor, RP-6306 in combination with MK-1775 as a potential synthetic lethal cancer therapy. Aim:. We hypothesize that a combination of MK-1775 and RP-6306, inhibitors against two partially redundant kinases important for adaptation to genotoxic stress, will achieve synthetic lethality while circumventing the issue of resistance development following monotherapies. Model systems used: 1) a cervical cancer cell line that is tetracycline inducible for Myt1 expression; 2) cancer cell lines (breast and cervical) that are resistant to MK-1775 through upregulation of Myt1. Methods: We established the IC50 of RP-6306 in a panel of tumorigenic and non-tumorigenic cell lines using standard crystal violet viability assay. Following that, we tested the combination effect of MK-1775 and RP-6306 in model cell lines listed above using zero potency model of synergy. The effect of the combination treatment on the clonogenicity was evaluated using clonogenic assay. Timelapse microscopy on a High Content Imaging system was used to determine the effect of RP-6306 and MK-1775 on mitotic duration and cell fates. Results: We found that MK-1775 and RP-6306 combination treatment shows synergistic cell killing in a panel of cancer cell lines. Wee1 inhibition shows synergistic cell killing with Myt1 inhibition especially in inducible Myt1 overexpressing cells. Wee1 and Myt1 combined inhibition resists the increase in clonogenic potential of the cancer cells transiently overexpressing Myt1. RP-6306 and MK-1775 combination treatment promotes mitotic arrest leading to cell death in Myt1 overexpressing cells. We also found that the mechanism of cell death in the cells treated with the combination treatment is centromere fragmentation leading to mitotic catastrophe. Conclusions: The combined Wee1 and Myt1 inhibition leads to synthetic lethality. Hence, our findings strongly suggest that the combined MK-1775 and RP-6306 treatment has promising potential to mitigate MK-1775 resistance. Our research contributes to the development of novel potential combination therapy while optimizing and improving the efficacy of MK-1775 treatment for potential clinical use.

Hypothesis

The combined inhibition of (two partially redundant) Myt1 and Wee1 checkpoint kinases will induce synthetic lethality by introducing high replication and mitotic stress leading to cell death.

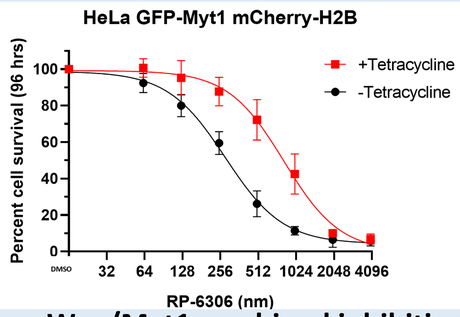
Tetracycline inducible HeLa (Flp-In T-Rex) APEX2-GFP-Myt1 cell line

The promoter is consisted of a cytomegalovirus (CMV) promoter with two copies of the tetracycline operator 2 (tetO2). Addition of tetracycline= Overexpression of Myt1



Results

Transient overexpression of Myt1 mediates resistance to RP-6306 mono-treatments

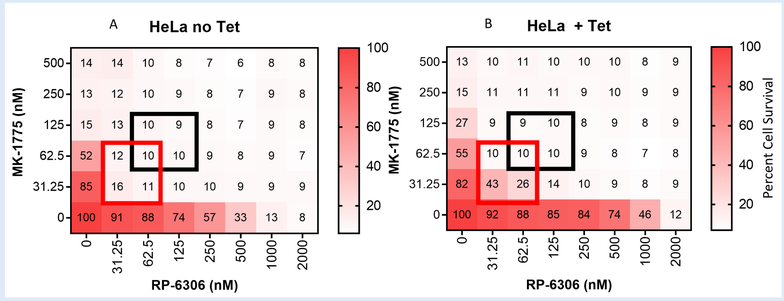


HeLa APEX2-GFP-Myt1 mCherry H2B	RP-6306 IC50 (nM)
- Tetracycline	280.5
+ Tetracycline	819.7

3X

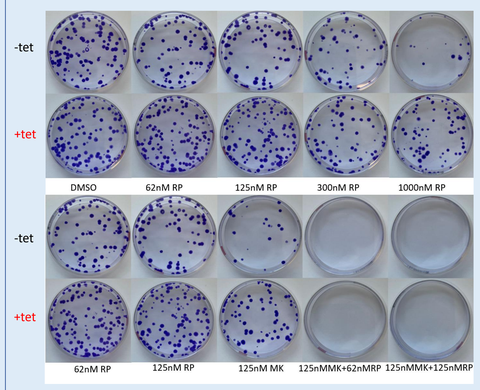
N=3

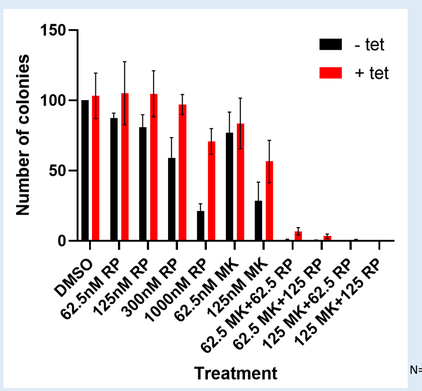
Wee/Myt1 combined inhibition leads to synergistic cell killing



N=3

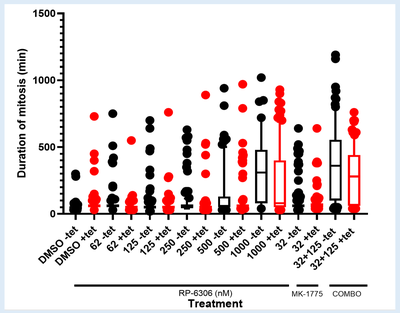
Wee1/Myt1 combined inhibition overcomes the resistance mediated by Myt1 upregulation





N=3

RP-6306 and MK-1775 combination treatment promotes mitotic arrest

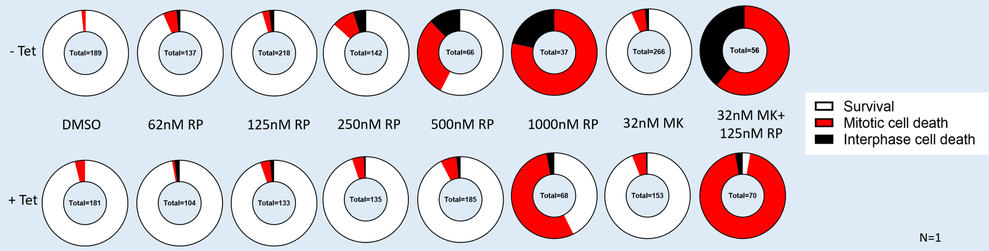


Treatment	-Tet Median	+Tet Median
DMSO	50	60
62	60	50
125	50	50
250	50	50
500	60	60
1000	310	80
32	60	60
32+125	360	280

N=1

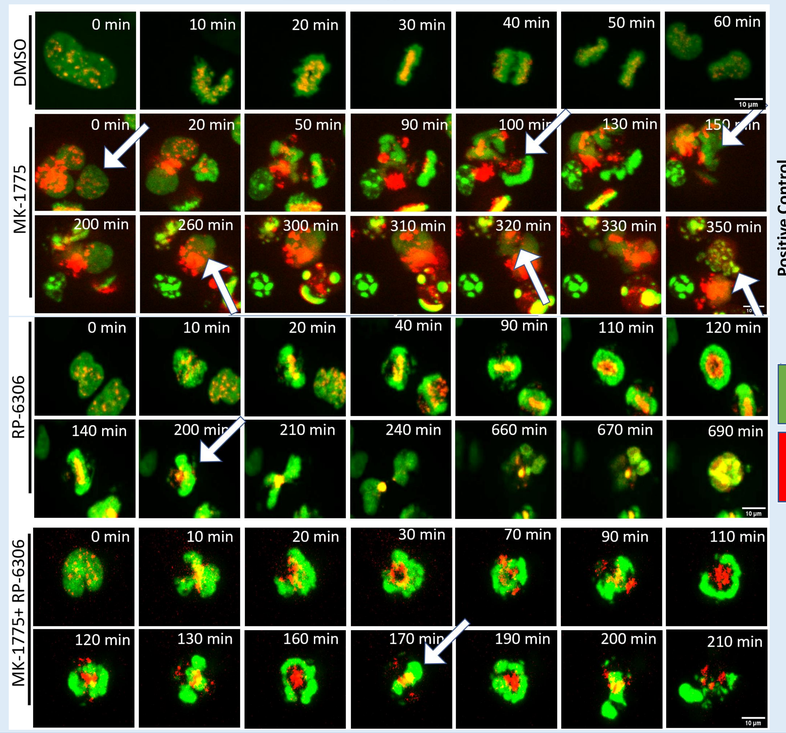
Results

RP-6306 and MK-1775 combination treatment leads to increased cell death



N=1

RP-6306 combined with MK-1775 induces centromere fragmentation resulting in mitotic catastrophe



N=3

Conclusions

1. The combination of MK-1775 (a Wee1 inhibitor) and RP-6306 (selective Myt1 inhibitor) is better than either drug alone and is successful in overcoming resistance mediated by Myt1 upregulation to MK-1775.
2. The combined Wee1 and Myt1 inhibition promotes synthetic lethality.
3. The combination treatment enhances cell death.
4. One of the mechanisms of cell death in the cells treated with the combination treatment is centromere fragmentation leading to mitotic catastrophe.

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REPARE THERAPEUTICS

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