PMV Pharmaceuticals

Systematic Identification of Combination Strategies for p53 Y220C Reactivation

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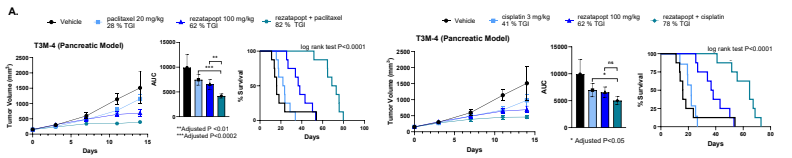
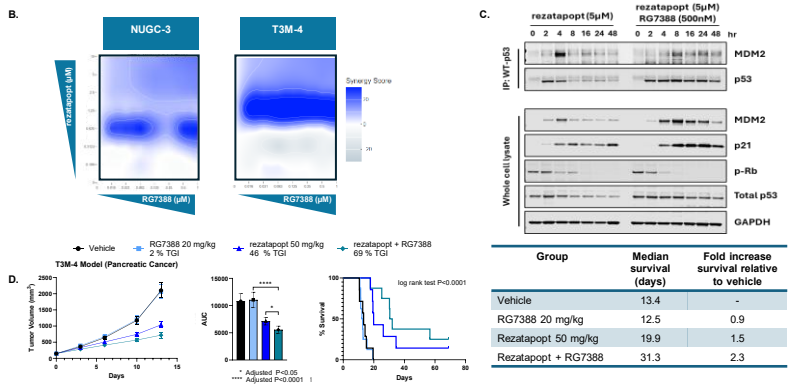
Overview

The investigational p53 reactivator rezatapopt,[1,2] demonstrates single-agent clinical efficacy;[3] however, combinatorial strategies may enhance clinical outcomes. Here, we evaluated rezatapopt in combination with standard-of-care agents (chemotherapy and bevacizumab), MDM2 inhibitors, and a library of U.S. Food and Drug Administration (FDA)-approved compounds. While chemotherapy, bevacizumab, and MDM2 inhibitors showed in vivo efficacy, a high-throughput screen identified the phosphoinositide 3-kinase (PI3K) / AKT / mechanistic target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK) pathways and a natural product as top synergistic candidates. Validation confirmed that PI3Kα inhibition synergized with rezatapopt to deepen apoptosis and tumor growth inhibition in xenograft models. Thus, PI3K/mTOR pathway inhibition may represent a clinically actionable, conserved p53-reactivation vulnerability across diverse histologies harboring a *TP53* Y220C mutation.

Results



Group	Median survival (days)	Fold increase survival relative to vehicle
Vehicle	16.0	-
Paclitaxel 3 mg/kg	22.8	1.4
Cisplatin 3 mg/kg	22.2	1.4
Rezatapopt 100 mg/kg	37.4	2.3
Rezatapopt + paclitaxel	70.9	4.4
Rezatapopt + cisplatin	64.7	4.0



Group	Median survival (days)	Fold increase survival relative to vehicle
Vehicle	13.4	-
RG7388 20 mg/kg	12.5	0.9
Rezatapopt 50 mg/kg	19.9	1.5
Rezatapopt + RG7388	31.3	2.3

Figure 1. Administration of rezatapopt together with chemotherapeutic agents or MDM2 inhibitors had combination effects *in vitro* and *in vivo*. **(A)** Rezatapopt was administered orally as a single agent or in combination with paclitaxel or cisplatin at the indicated doses to p53 Y220C-expressing T3M-4 mouse xenografts. Average tumor volume with % of tumor growth inhibition (TGI) or regression relative to starting volume. Kaplan–Meier curves are shown as % of survival. **(B)** Loewe synergy analysis (bottom panels) of the combination of rezatapopt (0.15–10 μM) and MDM2 inhibitor RG7388 (16–1000 nM) in cell lines NUGC-3 and T3M-4. **(C)** NUGC-3 cells were treated with rezatapopt at 5 μM alone or in combination with RG7388 at 500 nM in a time-course. Cell lysates were subjected to immunoprecipitation with a wild-type p53 (PAb 1620) antibody and immunoblotted for p53 and MDM2. Total cell lysates were also immunoblotted. **(D)** In vivo efficacy of rezatapopt alone or in combination with RG7388 at the indicated doses in T3M-4 xenografts.

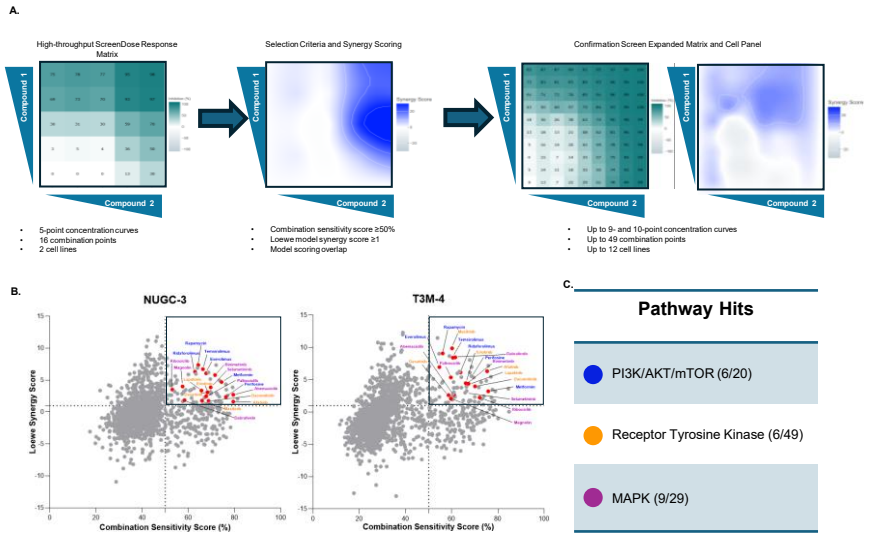


Figure 2. High-throughput screening of FDA-approved library (1,746 compounds) identifies multiple potential combination agents with p53 reactivation. **(A)** Illustration of generalized workflow for the primary high-throughput screening of the FDA-approved anticancer library. **(B)** Synergy-sensitivity plots of results for full library in both cell lines. Compounds meeting predefined ≥1 plate-averaged Loewe synergy score and ≥50% combination sensitivity score thresholds are highlighted in the upper right quadrant boxes. Inhibitors within enriched signatures are highlighted in red and identification is color-coded based on targeted pathway listed in **(C)** with number of agents scoring in the assay over the total agents in the pathway included in the screening library.

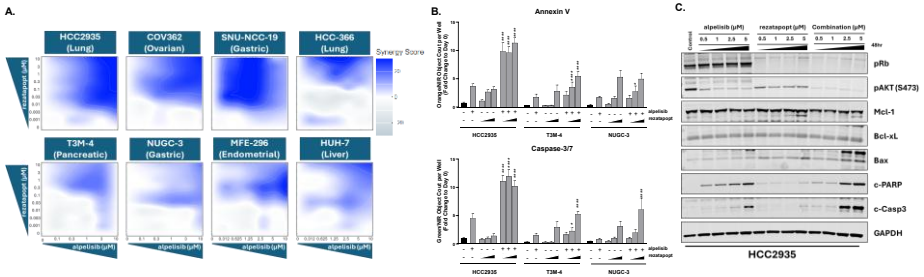


Figure 3: PI3Kα inhibition with alpelisib amplified rezatapopt-mediated apoptotic signaling. **(A)** Loewe synergy contour maps of rezatapopt and alpelisib combinations in 8 p53 Y220C-mutant cell lines. **(B)** Live-cell imaging with Annexin V (IncuCyte) and caspase-3/7 apoptosis assays in indicated cell lines after treatment with alpelisib (5 μM) and rezatapopt (1.25, 2.5, 5 μM) combination for 5 days. **(C)** Western blot analysis of indicated apoptotic and cell-cycle regulators in HCC2935 cells following 48 h treatment with dimethyl sulfoxide (DMSO; control), rezatapopt, alpelisib, or combination at the indicated concentrations. Statistical significance relative to single agent: one-way ANOVA withTukey's multiple comparisons test (* and **** indicate *P* value ≤0.05 and ≤0.0001, respectively).

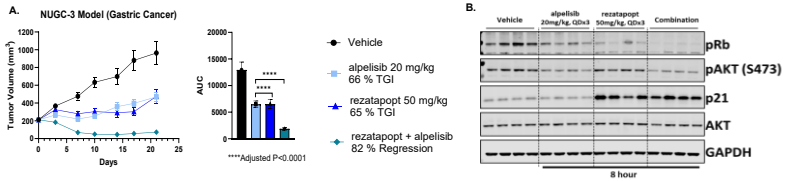


Figure 4: Combined rezatapopt and alpelisib treatment resulted in robust tumor growth inhibition and pharmacodynamic response in vivo. **(A) (B)** Rezatapopt and alpelisib were administered orally at the indicated doses to p53 Y220C-expressing NUGC-3 mouse xenografts. **(C)** Western blot analysis of pharmacodynamic response of indicated pathway markers in tumors at 8 h post 3 doses (Day 3) of rezatapopt and alpelisib alone or in combination.

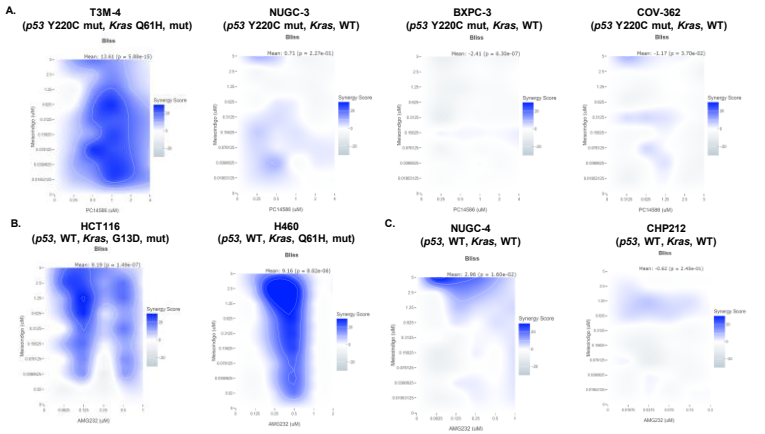


Figure 5: Combination of rezatapopt with meisoindingo effects seen in cells lines with *KRAS* mutations. **(A)** Bliss synergy contour maps of rezatapopt and meisoindingo combination effect was observed only in T3M-4 cells, which harbors both mutant *TP53 Y220C* and mutant *KRAS*. **(B)** Wild-type p53 stabilization through MDM2 inhibition (AMG232) in *KRAS* mutant cell lines demonstrates combination effects similar to p53 reactivation combination. **(C)** WT p53 stabilization through MDM2 inhibition (AMG232) in *KRAS* wild-type lines does not demonstrate combinational effects similar to mutant *KRAS* cell lines **(B).**

Summary

Reactivation of the mutated p53 Y220C with rezatapopt, including combination with standard-of-care agents and MDM2 inhibitors, improved efficacy and survival in mouse models. However, chemotherapy associated toxicity and clinical limitations of MDM2 inhibitors constrain their translational potential, motivating the search for alternative combination strategies. A high-throughput screen of FDA-approved drugs identified synergistic partners targeting the PI3K/AKT/mTOR and MAPK pathways, as well as a natural product with potential activity in KRAS-mutant cell lines. These findings highlight clinically actionable combination strategies with potential translatability into the clinic.

Acknowledgments
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References
1. Puzio-Kuter AM, et al. *Cancer Discov.* 2025;15:1159–1179; 2. Vu BT, et al. *ACS Med Chem Lett.* 2024;16:34–39; 3. Dumbrava EE, et al. *N Engl J Med.* 2026;394:872–883.

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