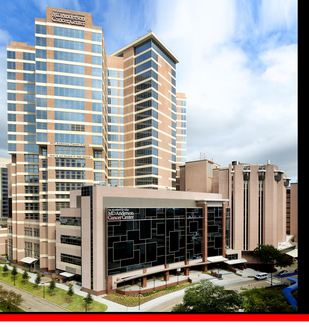

Mechanism of APR-246 and Sensitization of Cells to Targeted Agents

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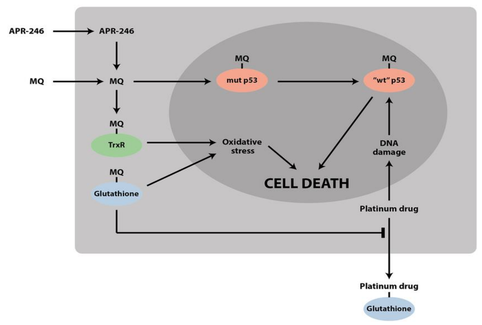
Introduction

APR-246

♦APR-246 (APR) is a novel agent proposed to reactivate mutant p53 and consequential downstream biological effects, including induction of apoptosis.

♦The mechanism by which APR-246 reactivates p53 and disrupts cellular redox along with sub sequential cytotoxic effects remain unknown.

Schematic of known APR-246 Mechanism



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Chronic Lymphocytic Leukemia (CLL)

♦CLL is a B-cell malignancy most commonly treated with venetoclax or ibrutinib.

♦ Deletion of chromosome 17p and mutations of p53 hallmark poor prognosis in patients with CLL.

♦5-8% of CLL patients incur dell17p, while p53 mutations exist in 10-15% of untreated CLL cases (40-50% in refractory disease).

♦CLL patients with mutated p53 treated with frontline targeted therapies, venetoclax and ibrutinib, experience shorter progression-free survival.

Materials and Methods

■ Cell lines: CLL isogenic HG3 cell lines: wild-type (WT), knock-out (KO), two lines with hot-spot induced mutations: R175H and R248Q, and Mec1 cell line.

■Cell viability quantified using FITC-Annexin/Propidium Iodide Flow Cytometry.

■P53 and Glutathione (GSH)-associated protein expression confirmed via Immunoblot.

■Cellular ROS detected by DCFDA fluorescence through flow cytometry.

■Intracellular GSH measured by Promega GSH-Glo Assay via luminometer.

Isogenic HG3 Cell Lines

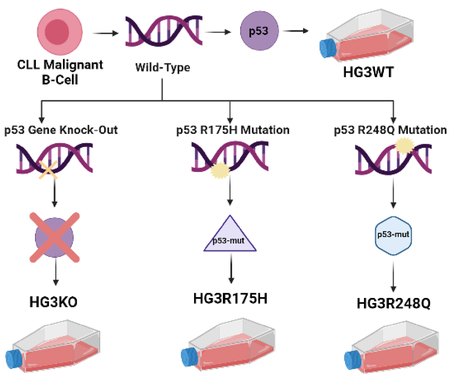

Figure 1. Schematic of Isogenic HG3 cell lines

Objectives

1. Determine role of p53 in the mechanism of APR-246-induced cytotoxicity
2. Evaluate APR-246 alone and in combination with venetoclax and ibrutinib in isogenic cell line with different p53 background

Role of p53 in APR-246 Cytotoxicity

Does p53 status relate to APR-246 induced cytotoxicity?

p53/GSH Protein Immunoblot

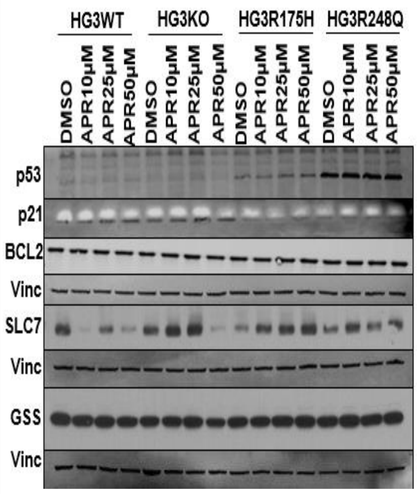

Figure 2. HG3 cell line p53 protein expression was visualized via immunoblot. HG3WT had minimal expression of p53. HG3R175H and HG3R248Q both had increases in p53 expression when compared to WT.

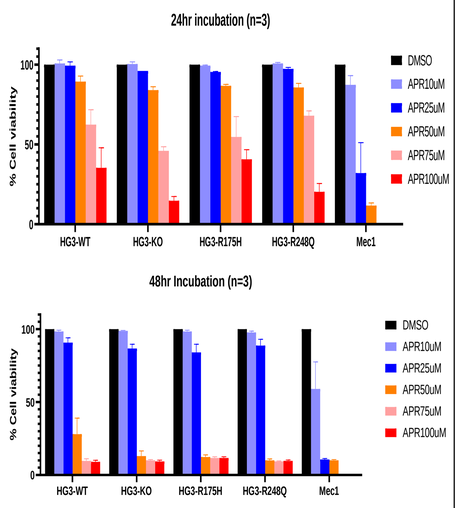

Figure 3. After both 24hrs and 48hrs of treatment with increasing doses of APR-246, there is no significant difference in cytotoxicity between HG3 cell lines and Mec1. Mec1 was not treated with APR75μM or APR100μM due its sensitivity to higher doses.

	Time	HG3-WT	HG3-KO	HG3-R175H	HG3-R248Q	Mec1
IC_{50}	24hr	68.29	66.02	61.55	76.97	24.18
	48hr	39.06	32.36	29.73	30.96	19.91

Table 1. IC50 values for HG3 and Mec1 cell lines after 24- and 48-hour treatment with APR-246

Does APR-246 treatment induce oxidative stress?

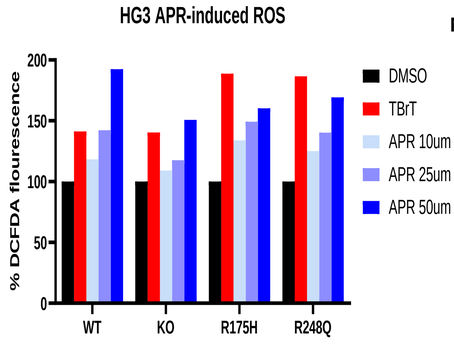

Figure 4. HG3 cell lines were treated with TBrT and increasing doses of APR-246. APR50μM produced the highest DCFDA fluorescence, indicating the highest levels of cellular ROS.

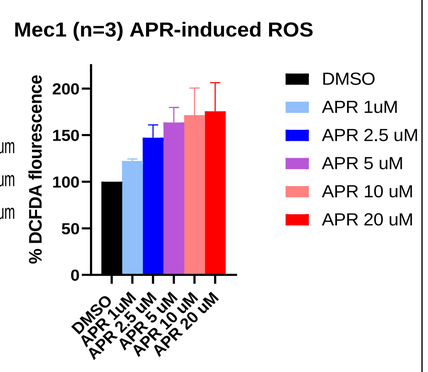

Figure 5. Mec1 cell line was treated with increasing doses of APR-246 and analyzed for DCFDA fluorescence. Treatment with APR20μM produced the highest levels of ROS in comparison to lower doses.

Is there a cytotoxicity-related decline in glutathione after treatment with APR-246?

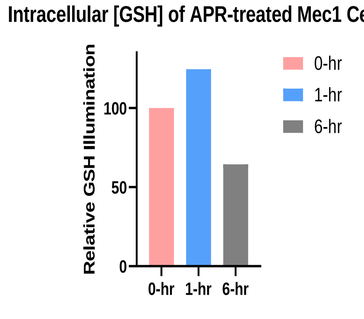

Figure 6. Mec1 cells were treated with 50μM of APR-246 for six hours. Relative illumination at 1 hour was 124, while illumination at 6 hours was 64, indicating that intracellular GSH concentration decreased by half.

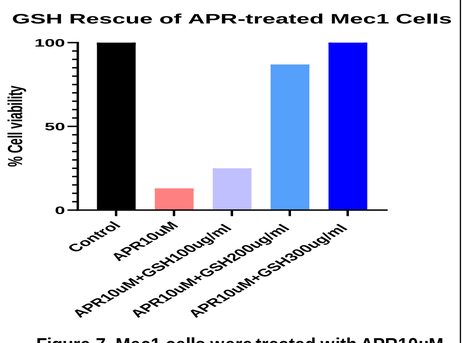

Figure 7. Mec1 cells were treated with APR10μM and increasing amounts of glutathione. After 24-hr incubation, cell viability was analyzed showing that Mec1 cells were rescued from cell death as dosage of GSH increased.

Role of APR-246 in Sensitization of Cells

Does combining APR-246 with each venetoclax (VTX) and ibrutinib (IBR), sensitize all HG3 cell lines to treatment?

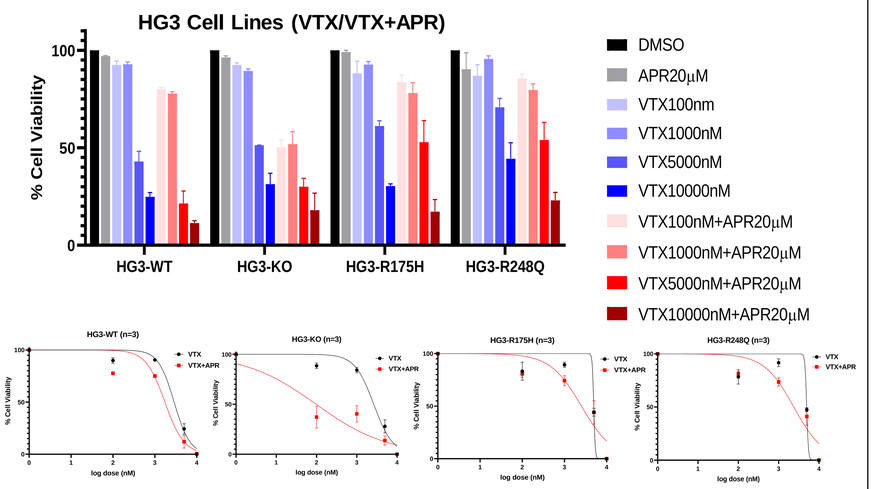

Figure 8. HG3 Cell lines were administered increasing doses of both alone and in combination with 20μM APR-246 for 72hrs and triplicated. In all four cell lines with varying p-53 status, venetoclax combined with APR20μM induced significantly more cell death than venetoclax as a single agent. This effect was more pronounced at higher VTX doses.

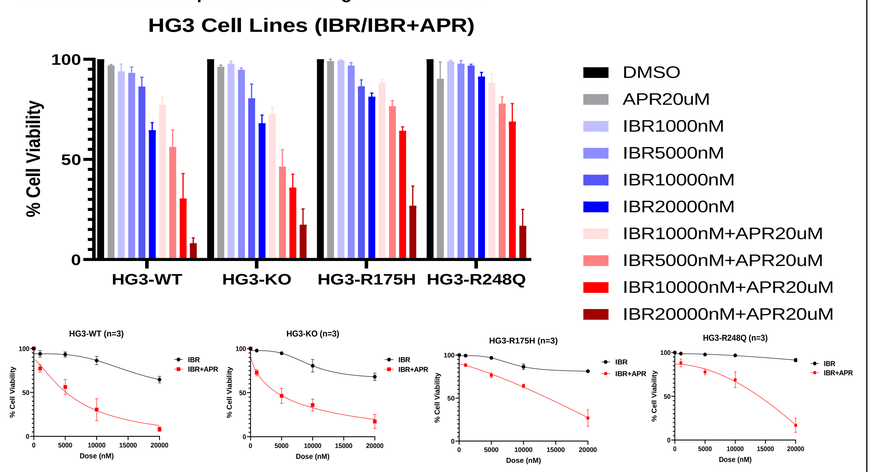

Figure 9. HG3 Cell lines were administered increasing doses of both ibrutinib alone and in combination with APR-246 for 72hrs and triplicated. In all four cell lines with varying p-53 status, ibrutinib combined with APR20μM induced significantly more cell death than ibrutinib as a single agent.

	HG3-WT		HG3-KO		HG3-R175H		HG3-R248Q	
Treatment	**VTX**	**VTX+APR**	**VTX**	**VTX+APR**	**VTX**	**VTX+APR**	**VTX**	**VTX+APR**
IC_{50}	3.46	3.24	3.42	1.989	3.695	3.403	3.697	3.376
R^2	0.9753	0.9272	0.9578	0.8392	0.9169	0.8361	0.8941	0.871
Treatment	**IBR**	**IBR+APR**	**IBR**	**IBR+APR**	**IBR**	**IBR+APR**	**IBR**	**IBR+APR**
IC_{50}	***	6764	***	7400	***	14027	***	14566
R^2	0.8256	0.8431	0.8349	0.9032	0.8945	0.9013	0.7118	0.877

Table 2. IC50 and R^2 Values for VTX, VTX+APR, IBR, and IBR+APR in HG3 cell lines
***** IC_{50}values for IBR alone surpassed 20000nM, so could not be calculated**

Conclusions

♦APR-246 is equally efficacious in cells with proficient, deficient, or mutated p53.

♦Mode of action of APR-246 is independent of p53 status, and glutathione depletion appears to be the primary mechanism.

♦APR-246 sensitizes CLL malignant B-cells to treatment with targeted therapies venetoclax and ibrutinib.

Why do these findings matter?

1. APR-246 may be useful in treating all CLL patients rather than solely p53-mutated.
2. APR-246 shows promise for treatment of other malignancies.
3. A phase III clinical trial has been designed for patients with CLL to test APR-246 with ibrutinib or venetoclax.

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