# COMPARATIVE ANALYSIS OF WRN INHIBITORS AND DHX9 INHIBITOR IN A CANCER CELL PANEL-INSIGHTS INTO MSI STATUS-DEPENDENT DRUG RESPONSES AND RESISTANCE MECHANISM EXPLORATION

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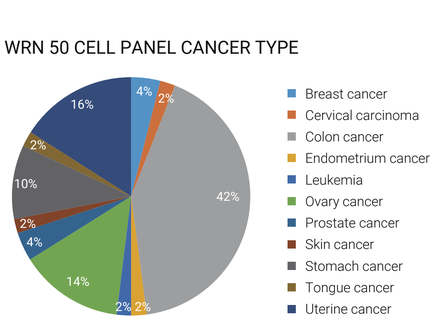
Poster Number: P246

## Introduction

High-throughput screening of a cancer cell panel represents a pivotal instrument in the armamentarium of drug discovery and development. The sensitivity profiles of these cell lines serve as a cornerstone for the refinement of in vivo model systems and for the stratification of patient cohorts in clinical trials. Werner Syndrome RecQ Helicase (WRN), a member of the RecQ helicase family, is instrumental in the preservation of genomic integrity. Its multifaceted role encompasses DNA repair, replication, recombination, and telomere maintenance. WRN has garnered significant attention as a potential therapeutic nexus in oncology, particularly within the context of tumors exhibiting microsatellite instability (MSI). The synthetic lethality between WRN and MSI has been the subject of intensive research, demonstrating that WRN inhibition is selectively toxic to cancer cells harboring a high microsatellite instability (MSI-H) phenotype, which is a characteristic observed across a spectrum of malignancies, including colorectal, endometrial, and gastric cancers.

## ICECP WRN and DHX9 Cancer Cell Panel



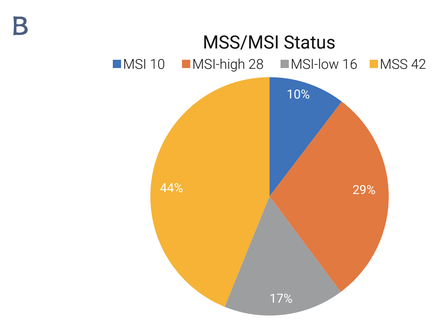
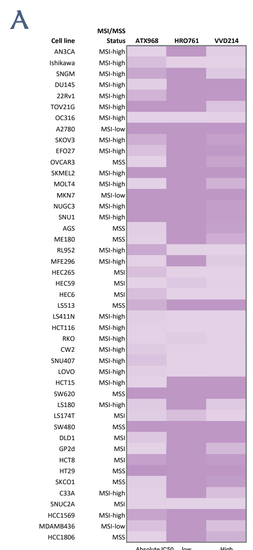
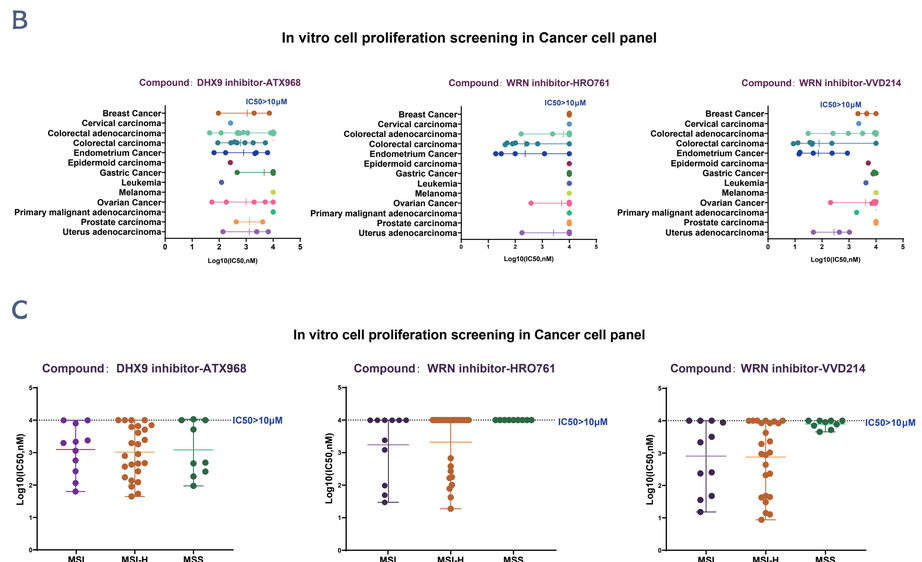




Figure 1. A WRN/DHX9 Cancer cell panel construction including cancer type(A) and MSI/MSS status(B).

## Comparison of Different WRNi and DHX9i in Cancer Cell Panel



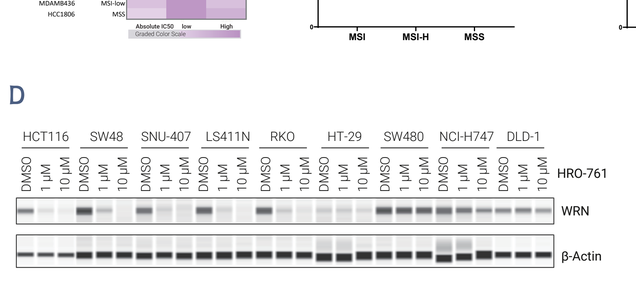



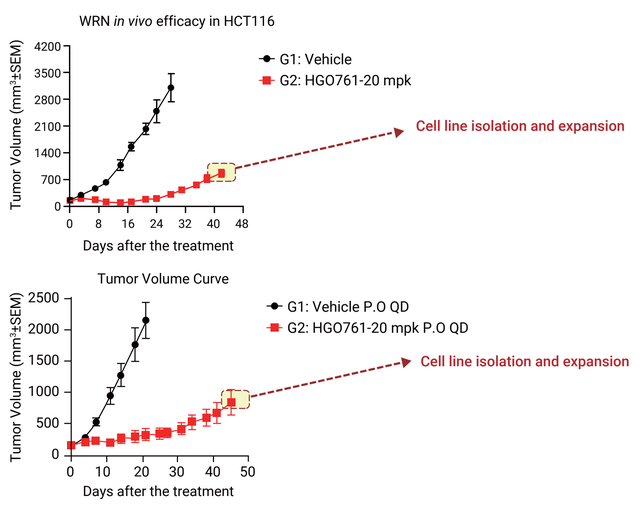

Table 1 Cell panel screening assay condition

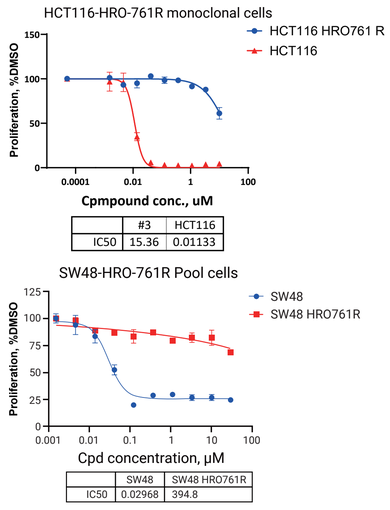
| Drug Name | Target | Characteristic | Highest Phase | Assay format | Duration time | Method |
|---|---|---|---|---|---|---|
| HRO761 | WRN | Small Molecule | Phase I | 384 well plate | 7 days | Cell Titer Glo |
| VVD214 | WRN | Small Molecule | Phase I | | | |
| ATX-968 | DHX9 | Small Molecule | Preclinical | | | |

Figure 2. A, IC50 heatmap showing ATX968 (DHX9i), HRO761 (WRNi), and VVD214 (WRNi) efficacy across a cancer cell panel. ATX968 exhibited broader effective cell line spectrum than the other two compounds. B, ATX968 (DHX9i) showed efficacy across a wider cancer type range. Among WRN inhibitors, VVD214 demonstrated notable activity. C, In MSS, MSI, and MSI-H cell lines, ATX968 inhibited all tested subtypes, while WRN inhibitors HRO761 and VVD214 primarily affected MSI cell lines, with some MSI resistance observed. D, WRN degradation assessed in HRO761-sensitive (HCT116, SW48, SNU407, LS411N, RKO, HT-29) and -resistant (SW480, NCI-H747, DLD1) cell lines correlated with proliferation inhibition effects. Table 1 summarizes cell panel screening conditions: Cells were seeded in 384-well plates and treated with compounds for 7 days.

## WRN Resistant Cell Line Generation

### Resistant cell line generation



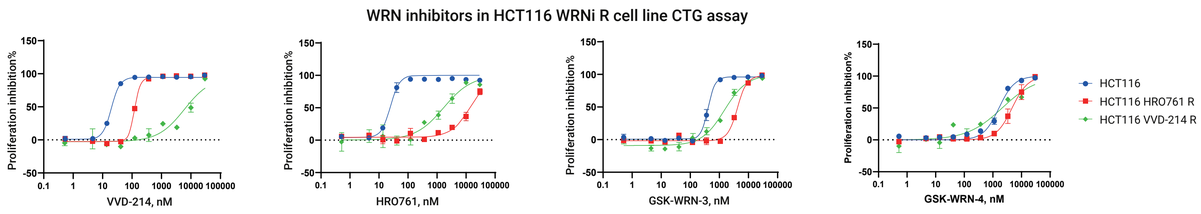




| | #3 | HCT116 |
|---|---|---|
| IC50 | 15.36 | 0.01133 |

| | SW48 | SW48 HRO761R |
|---|---|---|
| IC50 | 0.02968 | 394.8 |

Figure 3. Establishment of WRNi resistant cell lines. During in vivo drug efficacy assessment, tumors initially regressed but resumed growth around day 40 of treatment. Tumor cells were subsequently isolated and subjected to in vitro culture with sustained drug exposure to propagate resistance, ultimately yielding HRO761 resistant cell lines.

### WRNi and DHX9i Efficacy in WRNi Resistant Cell Line



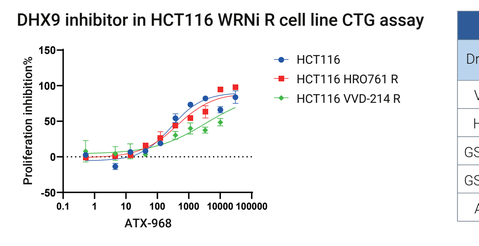




Table 2 IC50 Summary

| Drug Name | Target | HCT116 | HCT116 HRO761 R | | HCT116 VVD214 R | |
|---|---|---|---|---|---|---|
| | | IC50, nM | IC50, nM | Resistant Index(RI) | IC50, nM | Resistant Index(RI) |
| VVD-214 | WRN | 19.69 | 122.20 | 6.21 | 5980.00 | 303.71 |
| HRO-761 | WRN | 25.25 | 148852.00 | 5895.13 | 1692.00 | 67.01 |
| GSK-WRN-3 | WRN | 424.20 | 4271.00 | 10.07 | 1495.00 | 3.52 |
| GSK-WRN-4 | WRN | 1634.00 | 4692.00 | 2.87 | 2104.00 | 1.29 |
| ATX-968 | DHX9 | 278.10 | 423.80 | 1.52 | 3428.00 | 12.33 |

Figure 4. WRN inhibitors (HRO761, VVD214) and DHX9 inhibitor (ATX-968) were tested in HRO761 resistant and VVD214 resistant cell lines. Results showed that in HRO761 resistant cell lines, only HRO761 exhibited strong resistance, while other WRN inhibitors and DHX9 inhibitors showed weak resistance. Similarly, in VVD214 resistant cell lines, only VVD214 had strong resistance, and HRO-761 showed some resistance, and other tested inhibitors displayed weak resistance. This indicates that different inhibitors have distinct resistance mechanisms. IC50 values are summarized in Table 2.

## Resistance Mechanism Exploration

### WRN Degradation Evaluation in Resistance Cell Lines

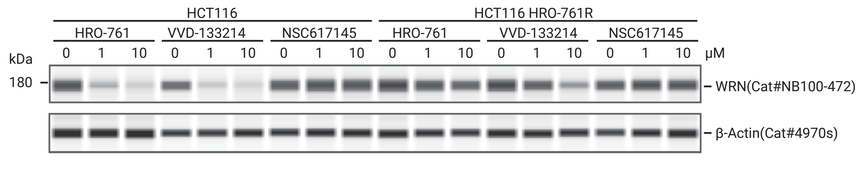



Figure 5. HCT116 and HCT116 HRO 761 resistant cells were exposed to HRO761, VVD - 214, or NSC617145. WRN protein levels were assessed via JESS. Results demonstrated that, unlike in wild type cells, these compounds failed to degrade WRN in resistant cells, indicative of a key resistance mechanism.

### Small Molecule Library Combination Study

- Small molecule library containing 300 compounds
- Compound duration-7 days
- Readout: Proliferation inhibition%

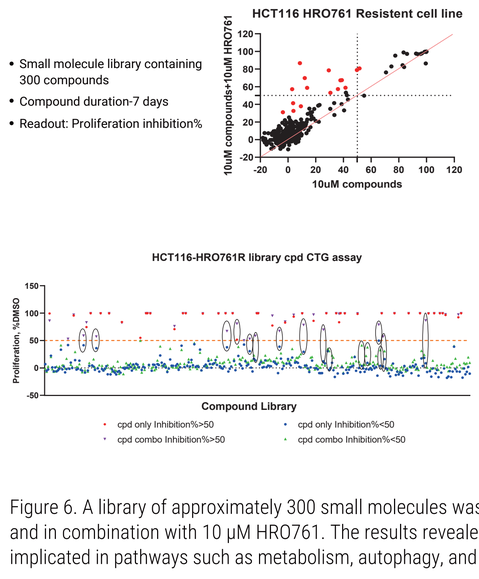



Table 3 compounds summary with suspected synergesitc effect

| Compound Name | 10uM Only | Combined with 10uM HRO761 | Target | Pathway |
|---|---|---|---|---|
| Nintedanib | | | FGFR; PDGFR; VEGFR | Protein Tyrosine Kinase/RTK |
| Dasabuvir | | | DNA/RNA Synthesis; HCV | Anti-infection; Cell Cycle/DNA Damage |
| (Z)-Guggulsterone | | | Akt; Angiotensin-converting Enzyme (ACE); Apoptosis; FXR; SARS-CoV; VEGFR | Anti-infection; Apoptosis; Metabolic Enzyme/Protease; PI3K/Akt/mTOR; Protein Tyrosine Kinase/RTK |
| Rilpivirine (hydrochloride) | | | MMP; SARS-CoV | Anti-infection; Metabolic Enzyme/Protease |
| Everolimus | | | Apoptosis; Autophagy; Bacterial; FKBP; mTOR | Anti-infection; Apoptosis; Autophagy;Cell Cycle/DNA Damage; Immunology/Inflammation; PI3K/Akt/mTOR |
| Danthron | | | AMPK; Autophagy; Bacterial; Virus Protease | Anti-infection; Autophagy; Epigenetics; PI3K/Akt/mTOR |
| Griseofulvin | | | Antibiotic; Apoptosis; Bcl-2 Family; Caspase; Endogenous Metabolite; Fungal; Microtubule/Tubulin; Wee1 | Anti-infection; Apoptosis; Cell Cycle/DNA Damage; Cytoskeleton; Metabolic Enzyme/Protease |
| Temsirolimus | | | Apoptosis; Autophagy; Bacterial; mTOR | Anti-infection; Apoptosis; Autophagy; PI3K/Akt/mTOR |
| Propyl gallate | | | Others | Others |
| Azelnidipine | | | Calcium Channel; MEK | MAPK/ERK Pathway; Membrane Transporter/Ion Channel; Neuronal Signaling |
| Alfuzosin (hydrochloride) | | | Adrenergic Receptor | GPCR/G Protein; Neuronal Signaling |
| Masoprocol | | | Lipoxygenase | Metabolic Enzyme/Protease |
| Prussian blue insoluble | | | Bacterial | Anti-infection |
| Megestrol acetate | | | Autophagy; HIV; Progesterone Receptor | Anti-infection; Autophagy; Vitamin D Related/Nuclear Receptor |
| Amlodipine | | | Calcium Channel | Membrane Transporter/Ion Channel; Neuronal Signaling |
| Tetrahydropalmatine (hydrochloride) | | | Dopamine Receptor | GPCR/G Protein; Neuronal Signaling |

Figure 6. A library of approximately 300 small molecules was assessed in the HRO761 Resistant cell line at a concentration of 10 μM, both as single agents and in combination with 10 μM HRO761. The results revealed several compounds that demonstrated potential synergistic effects. These compounds were implicated in pathways such as metabolism, autophagy, and cell cycle regulation, details showed in table 3.

## Bioinformatics Analysis for Cell Panel Screening

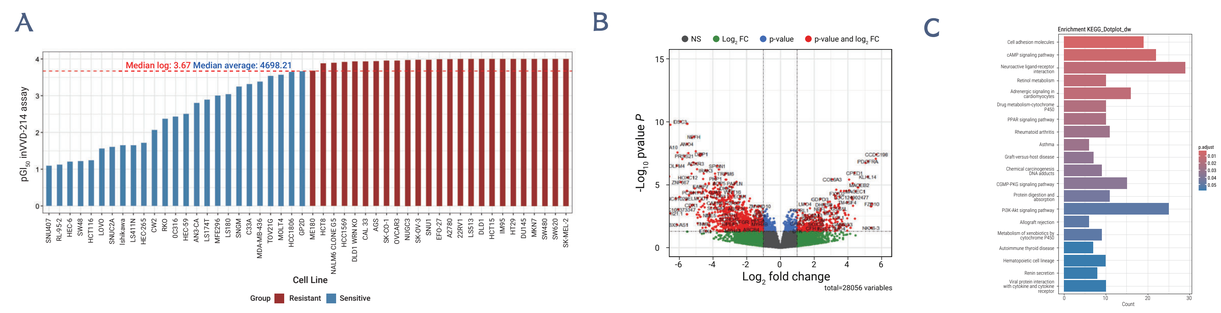



Figure 7. Bioinformatics analyses of Cell panel screening, the IC50 data was clustered into sensitive and resistant group, and bioinformatics was performed using pulished cell lines RNA seq Data, the results reveal significant molecular differences between drug-resistant and sensitive samples, with key differentially expressed genes shown by volcano plot and distinct gene expression profiles demonstrated by PCA. KEGG pathway enrichment analysis highlights critical pathways involved in cellular response to drug treatment, including upregulation of protein digestion and absorption pathways, and downregulation of cell adhesion molecules and cAMP signaling pathways in resistant samples.

## Bioinformatics Analysis for WRNi Resistant Cell Line

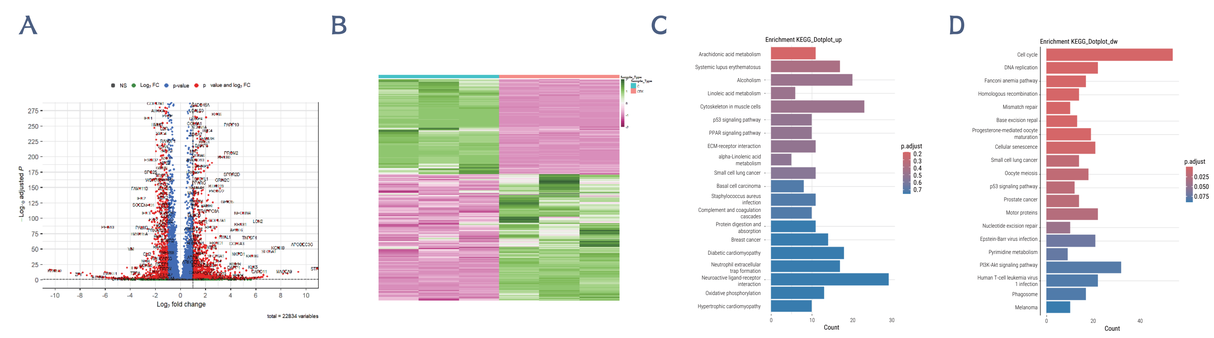



Figure 8. Bioinformatics analyses was performed cased on RNAseq data of HCT116 WT and HCT116 HRO761 R esistant cell lines. A, The RNA-seq heatmap reveals distinct gene expression patterns between WT and resistant cell lines, with notable differences in expression levels across various genes. B, The volcano plot further identifies specific genes exhibiting significant expression changes, highlighting potential key players in the observed biological variations. C,D, KEGG enrichment analysis of upregulated genes points to activation of pathways such as arachidonic acid metabolism and systemic lupus erythematosus, while downregulated genes are enriched in pathways like the cell cycle and DNA replication.

## Summary

This study presents research on WRN and DHX9 inhibitors in cancer treatment, highlighting their potential as therapeutic targets. It covers high throughput screening of cancer cell panels, showing ATX968's broad efficacy and WRN inhibitors' main impact on MSI cell lines, with noted resistance. Resistant cell line generation and mechanism exploration reveal distinct resistance mechanisms among inhibitors and some compounds' synergistic effects with HRO761. Bioinformatics analysis uncovers molecular differences and key pathways in drug resistant vs. sensitive samples. Overall, the study offers vital insights for developing WRN targeted drugs, understanding resistance mechanisms, and creating combination therapy strategies, advancing precise treatment for cancers like MSI phenotyped ones.