Novel WRN drug resistant CDX models

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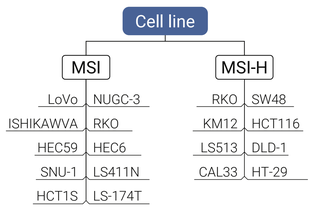
Abstract

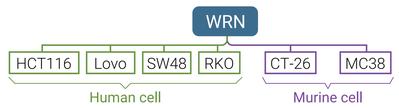
Microsatellite Instability (MSI) is a condition characterized by the presence of mutations in microsatellite regions of DNA, which can lead to the accumulation of mutations in these regions and result in genomic instability. This phenomenon occurs in various cancer types. Werner helicase (WRN) plays a crucial role in highly MSI (MSI-H) cancer cells, causing synthetic lethality when WRN is inhibited or knocked out. Previous studies have revealed that WRN inhibitors, such as HRO761 and VVD-214, can effectively kill MSI-H cancer cells, including human colorectal cell lines HCT116 and SW48.

However, the development of resistance to WRN inhibitors is a potential challenge that needs to be addressed. In this study, we aimed to establish WRN inhibitor-resistant cell lines to investigate the mechanisms of resistance and to identify potential strategies to overcome it. We developed WRN inhibitor-resistant cell lines against HRO761 and VVD-214 through a combination of in vivo and in vitro drug tolerance approaches. Drug-resistant tumor tissues were collected after in vivo drug resistance was observed, characterized by tumor regrowth under treatment with 20 mg/kg HRO761 and 5 mg/kg VVD-214. These tissues were then dissected for primary cell culture, and drug-resistant cell lines were established by escalating drug concentrations in vitro.

The WRN inhibitor-resistant cell lines were genotyped and phenotyped using Short Tandem Repeat (STR) analysis and IC50 assays for cell growth. The drug-resistant index was found to be more than 5 times that of the parental cells, confirming the successful establishment of different WRN inhibitor-resistant HCT116 cell lines. These resistant cell lines will be valuable for the discovery of anti-cancer drugs targeting resistance to WRN inhibitors.

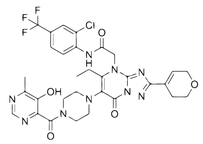
1. ICE WRN related human and murine cell lines.



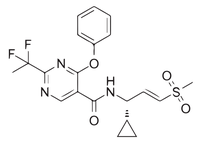


HCT116 and SW48 cells with MSI-H characteristics were used to generated the in vivo drug-resistant models for WRN target therapies

WRNi Molecular Comparison: VVD-214 and HRO761



HRO761, identified through an innovative hit-finding and lead-optimization strategy, is a potent and selective allosteric WRN inhibitor that binds at the interface of the D1 and D2 helicase domains, locking WRN in an inactive conformation.

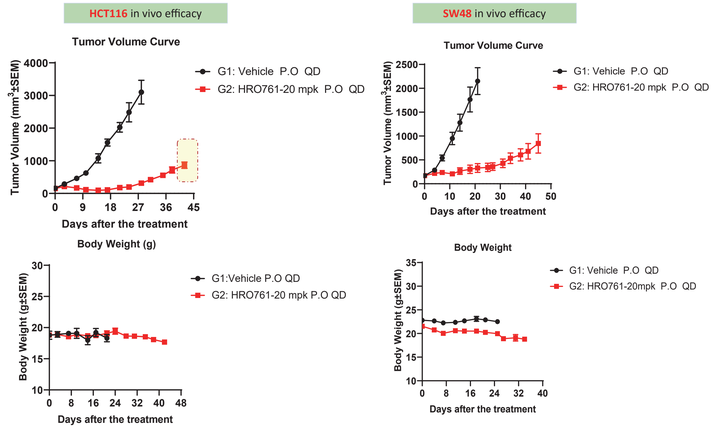


VVD-214 is a synthetic synthetic-lethality allosteric inhibitor of WRN helicase, with an IC50 of 0.1316 μM. It covalently binds to cysteine 727 of WRN, thereby inhibiting ATP hydrolysis and helicase activity. In MSI-H cancers, VVD-214 induces cell death by causing double-strand DNA breaks and nuclear swelling.

Characteristics	VVD-214	HRO761
Mechanism	Covalent binding to WRN, inhibits ATP hydrolysis	Non-covalent binding, induces WRN structural changes
Structure	Molecular formula $C_{20}H_{21}F_2N_3O_4S$	Molecular weight 702 Da
Preclinical Results	Tumor regression in MSI-H models	Selective inhibition of MSI cancer cells
Clinical Trials	Phase I for MSI/dMMR solid tumors	Phase I for high MSI/dMMR tumors

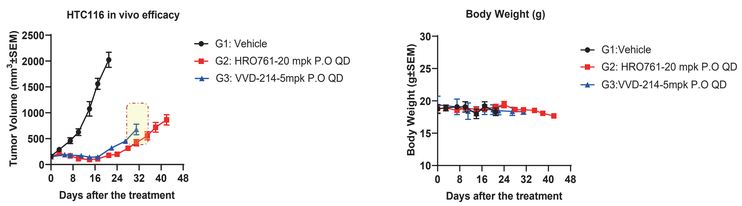
Table 1: VVD-214 and HRO761 are WRN inhibitors with distinct mechanisms. VVD-214 uses covalent binding, while HRO761 employs non-covalent binding. Both showed promising therapeutic effects in preclinical MSI-H cancer models and are in Phase I clinical trials for MSI/dMMR tumors.

2. In vivo WRNi drug resistant CDX models of HCT116 and SW48 cells



	TGI (%)	T/C (%)	*p* value
HCT116 CDX Model	94.43	10.61	＜0.0001
SW48 CDX Model	93.51	12.16	0.0013

Figure 2: Tumor Volume and Body Weight changes in the HCT16 and SW48 CDX model following treatment with HRO761.
The mice are in good condition, and there is no statistical difference in weight changes between groups.



	TGI (%)	T/C (%)	*p* value
HRO761	94.43	10.61	＜0.0001
VVD-241	85.56	21.22	＜0.0001

Figure 3: Tumor Volume and Body Weight changes in the HCT16 CDX model following treatment with both HRO761 and VVD-214.
The mice are in good condition, and there is no statistical difference in weight changes between groups.

3. Isolation and establishment of WRNi-drug resistant cell line

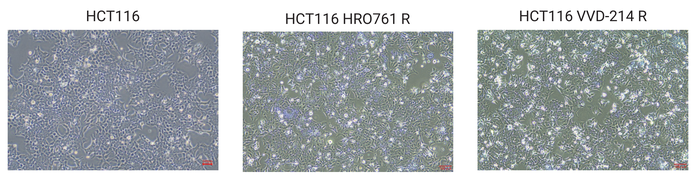
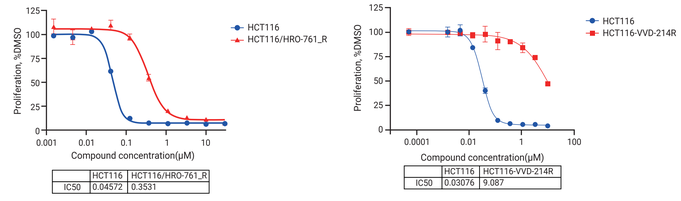


Figure 4: Morphology of HCT116 ,HCT116 HRO761 R and HCT116 VVD214 R
We excised the tumors treated with HRO761 and VVD-214 and further induced them with the same drugs in vitro to develop drug-resistant strains of HRO761 and VVD-214 WRNi drugs.

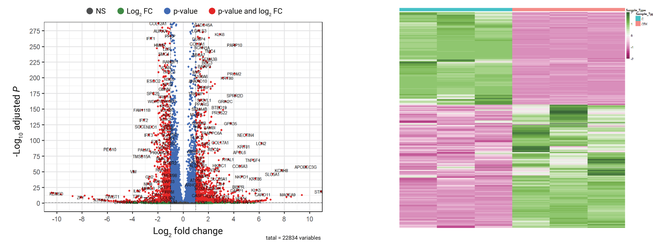


	HCT116	HCT116/HRO-761_R
IC50	0.04572	0.3531

	HCT116	HCT116-VVD-214R
IC50	0.03076	9.087

Figure 5: Drug resistance index of HCT116 was determined using IC50 of WRNi drugs treatments
Drug resistance index was determined by comparison the IC50 between parental and resistant cell lines, the drug resistance index of HCT116 HRO761 R and HCT116 VVD-214 R is 7.72 and 295.42, respectively.

4.Transcriptome sequencing analysis of drug-resistant strains



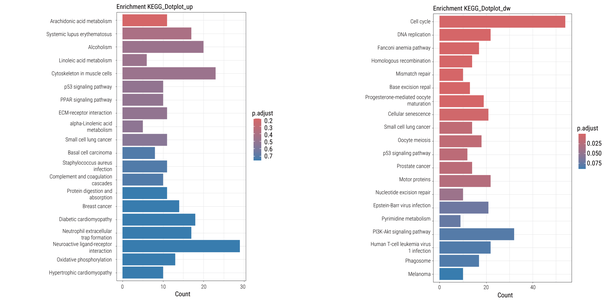


Figure 6: RNA seq data of HCT116 HRO761 R .
RNA sequencing was performed on HCT116 HRO761 R cells, providing a detailed analysis of gene expression changes in HCT116 cells and offering valuable insights into the molecular mechanisms underlying resistance and wild-type phenotypes. The identified genes and pathways may serve as potential targets for further research and therapeutic development.

Summary

Our study systematically investigated the emergence of in vivo resistance to WRN inhibition and successfully established corresponding drug-resistant cell models. Through prolonged exposure of MSI-H cell lines, including HCT116 and SW48, to WRN inhibitors HRO761 and VVD-214, we observed the gradual development of resistance to these compounds. This research represents the first comprehensive characterization of WRNi-resistant cell lines through in vivo efficacy evaluation, transcriptomic sequencing analysis, and detailed morphological assessment. These findings provide valuable insights into the mechanisms of cancer drug resistance and may contribute to the development of more effective therapeutic approaches for MSI-H tumors.

References

1. Ferretti S, Hamon J, de Kanter R , et al. Discovery of WRN inhibitor HRO761 with synthetic lethality in MSI cancers[J]. Nature, 2024, 629(8011): 443-449.

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