WRN under the scalpel: Helicase-domain hotspot & splice-site knock-ins in HCT116 and RKO

Yue Huang, Xiaomeng Gou, Jinying Ning, Feng Hao*

KYinno Biotechnology Co., Ltd. F2 Building, Yizhuang Biomedical Park, Beijing 101111 China Correspondence: Feng.hao@kyinno.com

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Abstract

Background: WRN is a synthetic-lethal target in MSI-H cancers, and allele-specific resistance is likely to emerge as WRN inhibitors advance. Endogenous point-mutation models in the native genomic context can better define target engagement, resistance liabilities, and chemotype-specific vulnerabilities than overexpression systems.

Methods: We generated an endogenous WRN mutant panel in isogenic MSI-H colorectal cancer cell lines (HCT116 and RKO) using CRISPR-Cas9 HDR with ssODN donors. Engineered alleles included C727A, C727S, G729D, F730L, I852F, G729D+I852F, and c.1577-1G>C. Clones were sequence-verified, STR-authenticated, and confirmed mycoplasma-free. Sensitivity to two WRN inhibitor chemotypes, VVD-214 and HRO761, was assessed by 72–96 h viability assays with 10-point dose-response curves.

Results: Wild-type HCT116 was highly sensitive to VVD-214 and HRO761 (48.47 and 86.89 nM), and parental RKO also remained responsive (366–720 nM). C727A conferred strong resistance to VVD-214 (>10 μM) while retaining near-wild-type HRO761 sensitivity (153–179 nM), whereas C727S caused dual resistance. In contrast, I852F preserved VVD-214 potency (62–77 nM) but abolished HRO761 activity (>10 μM). G729D and F730L caused bilateral right-shifts, more pronounced for HRO761, and G729D+I852F fully eliminated HRO761 activity while maintaining submicromolar VVD-214 sensitivity. The splice-site variant c.1577-1G>C remained hypersensitive to VVD-214 (36–37 nM) but showed reduced sensitivity to HRO761 (~1.11 μM).

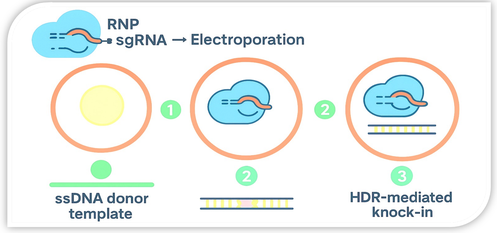
Impact: This endogenous WRN allele panel resolves chemotype-specific resistance mechanisms and supports backup selection, biomarker discovery, and patient-stratification strategies for WRN-inhibitor development in MSI-H cancers.oth the chemical and translational levels.

Cell ID	Cell name
KC-4274	293T-WRN-C727A-KI
KC-4310	HCT116-WRN-C727A-KI
KC-4313	RKO-WRN-C727S-KI
KC-4425	HCT116-WRN-C727S-KI
KC-5413	HCT116-WRN-I852F-KI

Cell ID	Cell name
KC-5599	HCT116-WRN-G729D-KI
KC-5598	HCT116-WRN-G729D-I852F-KI
KC-5672	HCT116-WRN-F730L-KI
KC-5674	HCT116-WRN-1577-1G>C-KI
KC-6116	HCT116-WRN-G729S-KI

……(Not an exhaustive list—contact us for details)

Pattern Diagram and Validation

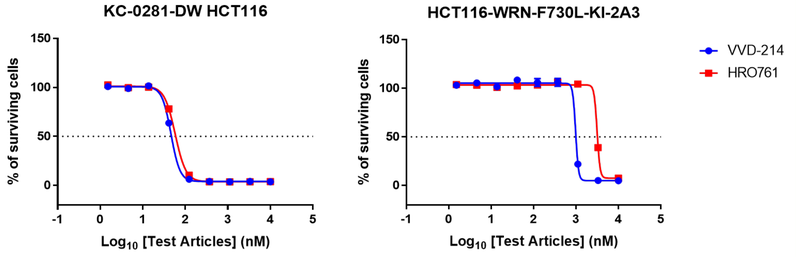


HCT116-WRN-F730L-KI

In Vitro Assay

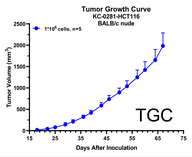
Cell ID	Cell line	Compound IC50(nM)	
		VVD-214	HRO761
KC-0281	HCT116	48.47	86.89
KC-4425	HCT116-WRN-C727S 3A1	10000	3534.19
KC-4310	HCT116-WRN-C727A 1A2	10000	179.04
KC-5413	HCT116-WRN-I852F-KI-2C6	77.17	10000
KC-5598	HCT116-WRN-G729D-I852F-KI-1A3	782.2	10000
KC-5599	HCT116-WRN-G729D-KI-1B1	1340	7994
KC-5674	HCT116-WRN-1577-1G>C-KI-2A4	36.25	1110
KC-5672	HCT116-WRN-F730L-KI-2A3	982.1	3149
KC-6116	HCT116-WRN-G729S-KI 1A2	344.9	10000
KC-0678	RKO	366	720.26
KC-4313	RKO-WRN-C727S 1A1	10000	10000
KC-4314	RKO-WRN-C727S 1A2	10000	10000

1000
10

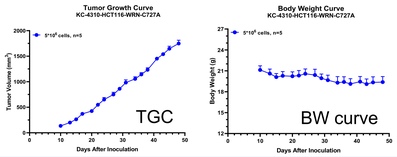


Xenograft-based *in vivo* validation (BALB/c nude)

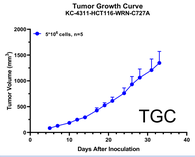
KC-0281-HCT116



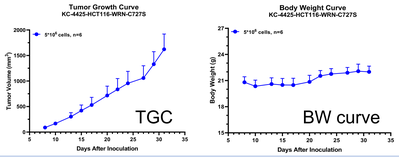
KC-4310-HCT116-WRN-C727A



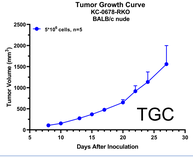
KC-4311-HCT116-WRN-C727A



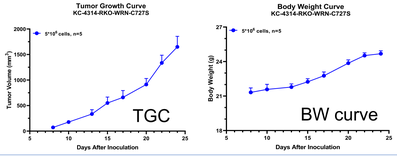
KC-4425-HCT116-WRN-C727S



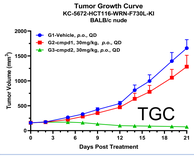
KC-0678-RKO



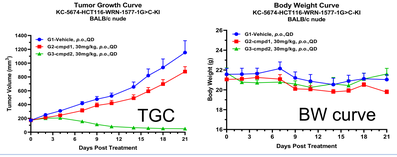
KC-4314-RKO-WRN-C727S



KC-5672-HCT116-WRN-F730L



KC-5674-HCT116-WRN-1577-1G>C



Conclusion

Endogenous WRN point-mutation models generated by CRISPR/Cas9 HDR reveal chemotype-specific resistance mechanisms and allele-dependent vulnerabilities in MSI-H colorectal cancer. Isogenic panels in HCT116 and RKO support pharmacology studies, enabling backup compound selection, biomarker discovery, and patient stratification for WRN inhibitor development.

Contact: Dr. Feng Hao, Email: Feng.hao@kyinno.com Website: http://www.kyinno.com Database: http://innopedia.kyinno.com