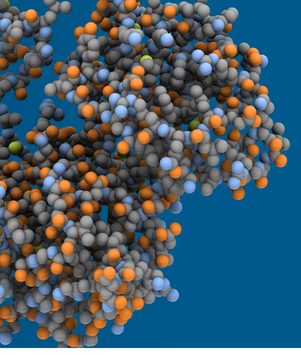

Preclinical Characterization of NTX-452, a Potent, Selective and Highly Efficacious WRN Inhibitor for the Treatment of MSI-H Tumors

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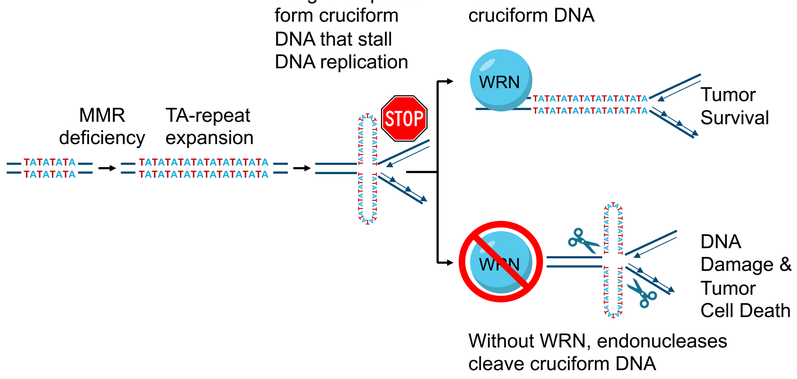
BACKGROUND

Microsatellite instability (MSI-H) is a phenotypic consequence of defective mismatch repair (dMMR) and occurs in various tumor types, including up to 15% of colorectal, 10% of gastric, and 30% of endometrial cancers. While the treatment landscape of MSI-H is improving with the use of immune checkpoint inhibitors, significant unmet medical need remains across several MSI-H tumor types that fail to respond, or eventually relapse, with current standard of care therapies. Werner Syndrome Helicase (WRN) has been validated as a promising synthetic lethal drug target for MSI-H tumors, thus inhibitors of WRN may offer a novel therapeutic option for patients with MSI-H tumors.

Figure 1: MSI-H Tumors Require WRN Helicase Activity for Survival



MATERIALS AND METHODS

We developed a series of novel, allosteric, potent and selective inhibitors of WRN helicase activity with best-in-class potential. Drug properties, safety, selectivity, potency and mechanism of action of one inhibitor, NTX-452, was characterized in preclinical assays. NTX-452 was also tested for its synthetic lethal potency in MSI-H and MSS tumor cells. Additionally, the pharmacodynamic activity and anti-tumor efficacy of NTX-452 was determined in cell line derived xenograft (CDX) and patient derived xenograft (PDX) tumor models.

RESULTS

- NTX-452 is a potent and selective WRN inhibitor (WRNi) with favorable drug-like properties (**Table 1**):
  - Non-covalent, reversible inhibition of WRN helicase activity without affecting exonuclease activity
  - High selectivity for WRN, with no inhibition of BLM (closest RecQ family member) and low/no off-target effects in a panel of enzymes, receptors, and ion channels, including hERG and CYPs
  - Negative in *in vitro* genotoxicity assays (AMES and MNT)
  - Excellent metabolic stability, low *in vivo* clearance, good oral bioavailability and high oral exposures in rodents and non-rodents
- NTX-452 treatment of MSI-H tumor cells triggers a DNA damage response that suppresses cell viability and promotes cell death, an effect that is not observed in MSS cells, confirming synthetic lethality (**Fig 2**, **3**)
- NTX-452 treatment leads to low dose tumor regression and complete responses in multiple human MSI-H CDX and PDX models:
  - Colorectal cancer and endometrial CDX (SW48, LS411N, HCT116, LoVo, less-sensitive RKO, Ishikawa) (**Fig 5**, **6**),
  - Colorectal cancer PDX model refractory to anti-PD1 (**Fig 7**)
  - Gastric PDX and chemo-refractory PDX models (**Fig 7**, **8**)
  - Highly efficacious across diverse CRC and Gastric PDX tumor models (**Fig 8**)
- These results highlight the broad potential of NTX-452 in MSI-H tumors when evaluated alongside irinotecan and other non-covalent WRNi in development such as HRO761 (Novartis)

Table 1: Drug Profile of Nimbus Non-Covalent WRN Inhibitor NTX-452

	Test System	NTX-452
Mechanism		Non-Covalent
Biochemical	WRN ATPase IC_{50} (μM)	0.009
	BLM ATPase IC_{50} (μM)	>100
	WRN DNA unwinding IC_{50} (μM)	0.007
	WRN Exonuclease IC_{50} (μM)	>100
Cell 24h p21 EC_{50} MSI-H vs. MSS	MSI-H: SW48, HCT116 (μM)	0.04, 0.04
	MSS: SW620, SKCO-1 (μM)	>10
Cell 5-day Viability IC_{50} MSI-H vs. MSS	MSI-H: SW48, HCT116 (μM)	0.02, 0.02
	MSS: SW620, SKCO-1 (μM)	>10
PK	Mu CL / T1/2 (h) / Vss (L/Kg) / %F	1.8 / 1.3 / 0.2 / 84
	R CL / T1/2 (h) / Vss (L/Kg) / %F	6.8 / 3.2 / 0.7 / 80
	D CL / T1/2 (h) / Vss (L/Kg) / %F	2.4 / 6.4 / 1.1 / 52
	NHP CL / T1/2 (h) / Vss (L/Kg) / %F	0.33 / 8.0 / 0.2 / 57
In Vivo Efficacy MSI-H: Fully efficacious Regression Dose	SW48, HC116 Xenograft Tumors	5 mg/kg PO QD
Safety	hERG IC_{50} (μM)	>30
	CYP IC_{50} / TDI / PXR activation	> 30 μM/ No/ No
	Ames and MNT	Negative
	Off target and Safety panels	No Liability
Predicted Human Dose		Low

Figure 2: NTX-452 Suppresses Viability of Human MSI-H Cells & MSI-H Patient Derived Organoids *In Vitro*

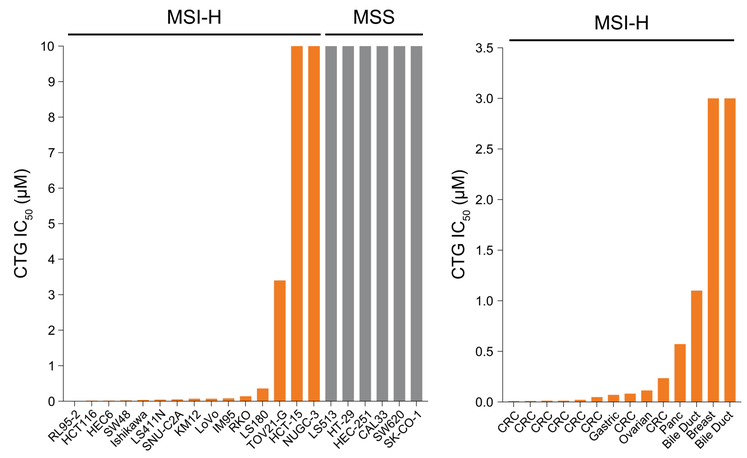


Figure 3: NTX-452 Elicits a DNA Damage Response in MSI-H Tumor Cells *In Vitro*

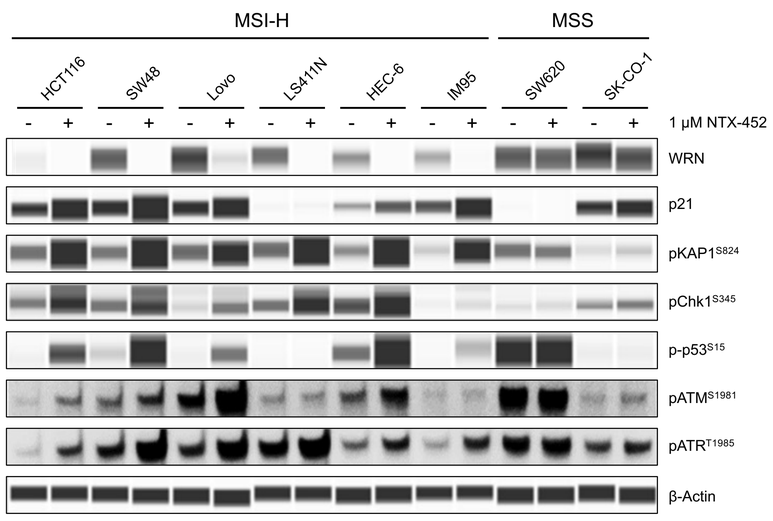


Figure 4: NTX-452 Elicits a p21 and γ-H2AX Pharmacodynamic Response in Human MSI-H SW48 CRC Xenograft Tumors *In Vivo*

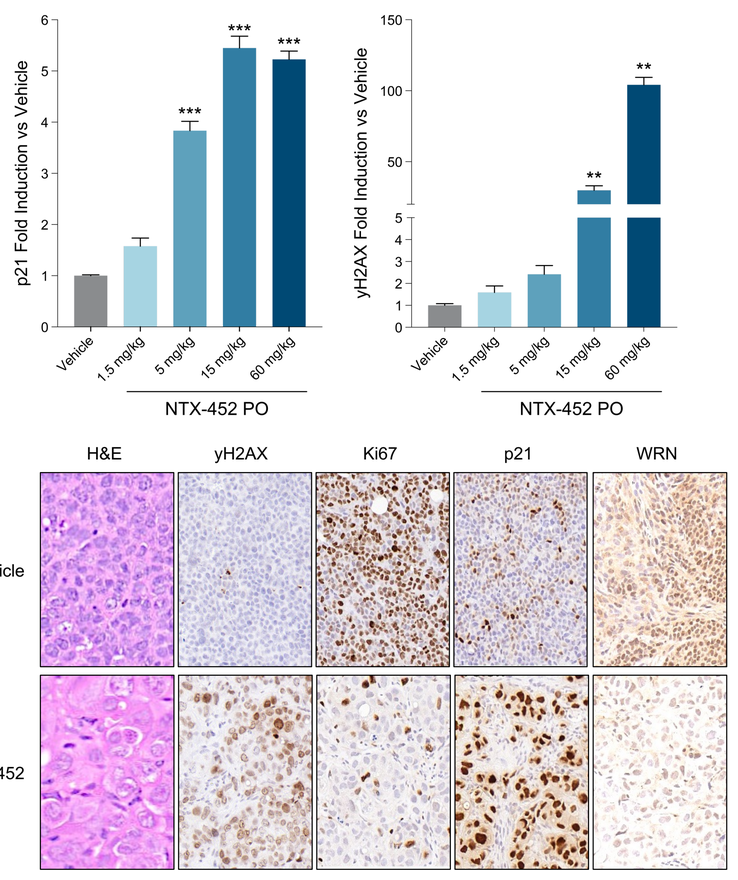


Figure 5: NTX-452 Promotes Tumor Regression and Complete Responses of MSI-H SW48 CRC CDX Tumors *In Vivo*

- QD oral dosing for 20-days
- Regressions achieved at 5mg/kg PO QD
- NTX-452 is well tolerated

Figure 6: Daily Oral NTX-452 Treatment Promotes Durable Tumor Regression and Complete Responses of Multiple MSI-H CDX Tumor Models *In Vivo* at Low Doses with No Effect in the MSS SW620 Model

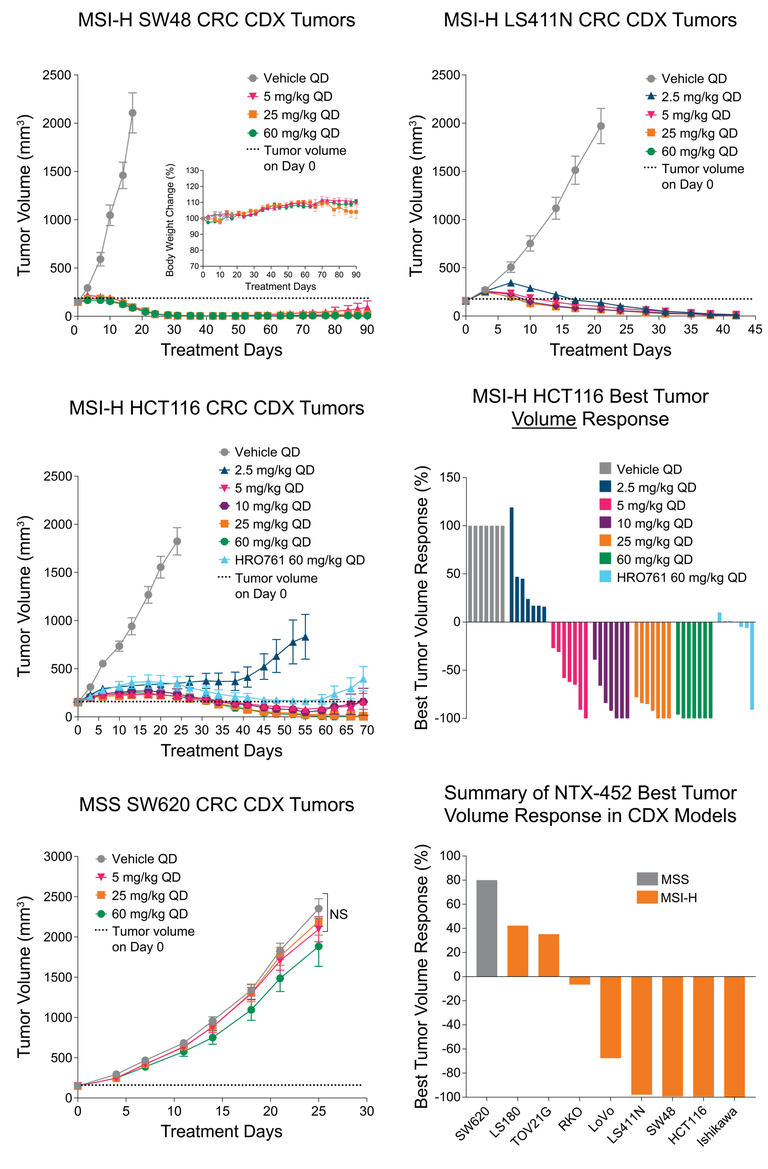


Figure 7: NTX-452 Promotes Durable Tumor Regression and Complete Responses in MSI-H PDX Models Refractory to Immunotherapy (PD1) and Chemotherapy

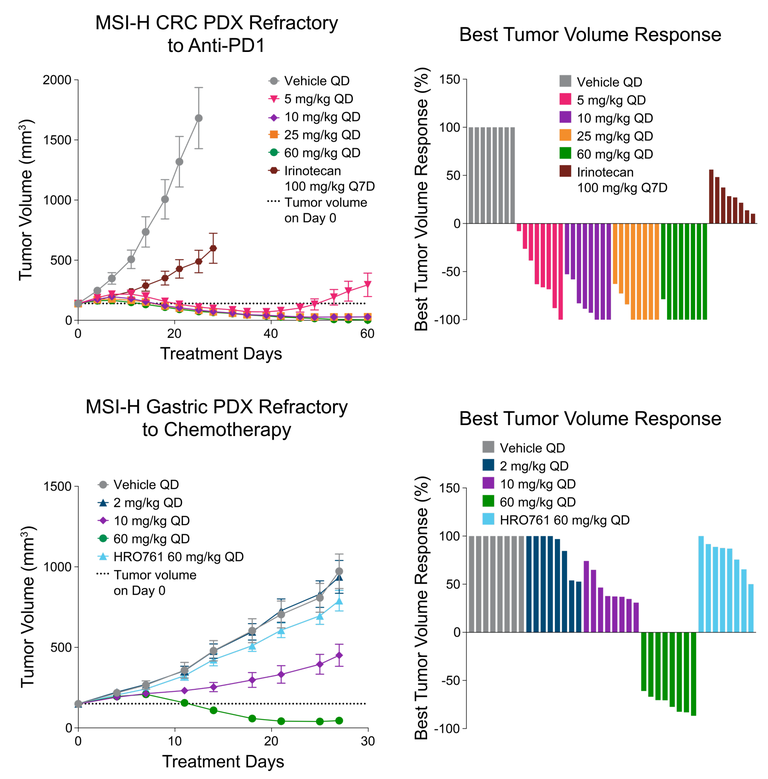
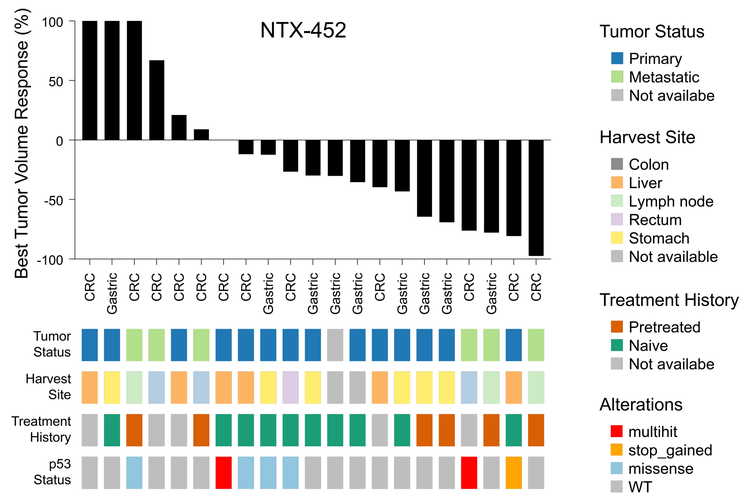


Figure 8: NTX-452 is Highly Efficacious Across MSI-H CRC and Gastric Cancer PDX Tumor Models with Diverse Genomic and Clinical Characteristics



CONCLUSIONS

- Our WRNi candidate, NTX-452, demonstrates excellent drug properties, robust *in vivo* efficacy across multiple preclinical MSI-H CDX and PDX tumor models, and the potential for a low efficacious dose in humans.
- These findings position NTX-452 as a promising therapeutic option for MSI-H cancer patients and support exploration in a clinical study.

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