Discovery of NTS071, an orally bioavailable, highly potent and selective small molecule p53 Y220C reactivator

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Abstract #109

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BACKGROUND

- The *TP53* gene encodes a tumor suppressor protein that binds to DNA and transcriptionally activates a network of target genes responsible for cell-cycle checkpoint, apoptosis, DNA repair, and other cellular processes.
- The normal function of p53 protein is inactivated by different mutations in about half of all cancer cases. Y220C is one of the most frequent *TP53* missense mutations and is associated with approximately 100,000 new cases per year worldwide.
- Reactivation of the loss-of-function p53 mutant Y220C shows promise in treating solid tumors as demonstrated by the initial clinical data of PC14586 (Rezatapopt), while still leaving room for a more efficacious and safer compound.
- NTS071 is a highly potent and selective p53 Y220C reactivator, whose discovery was facilitated by Nutshell's proprietary AlloStar™ AI-powered computational platform. NTS071 exhibits superior *in vitro* potency, more desirable ADMET properties and better *in vivo* antitumor efficacy than PC14586.

TARGETING HYPOTHESIS AND EXPERIMENTAL VALIDATION

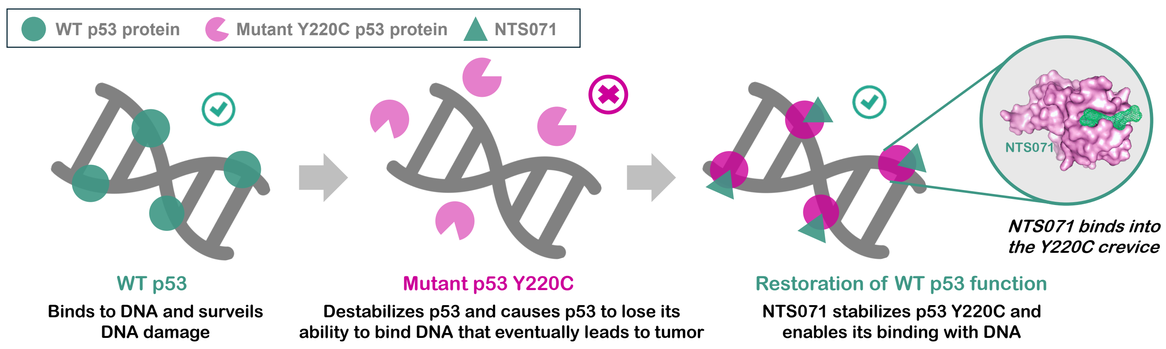

Figure 1. Targeting hypothesis and molecular docking result of NTS071 in p53 Y220C protein.

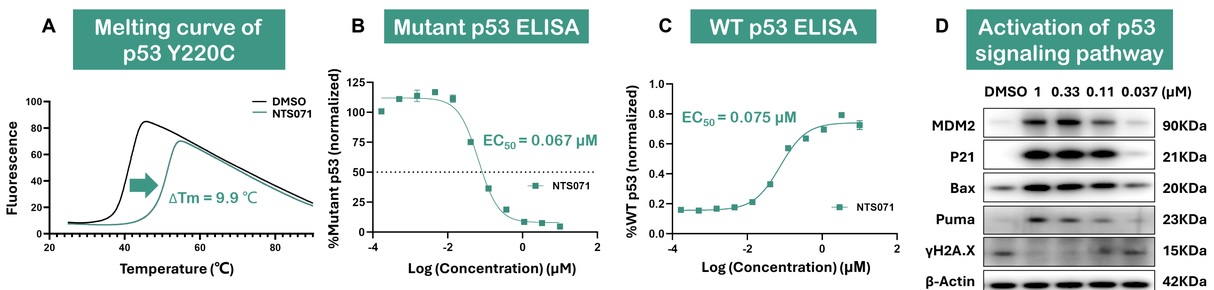

Figure 2. NTS071 restores p53 Y220C conformation and transcriptional activity.

A. NTS071 promotes the thermal stability of p53 Y220C protein at the testing concentration of 11 μM.
B & C. NTS071 reduces the mutant p53 protein and induces the changing of conformation to p53 WT in NUGC-3 cells.
D. NTS071 leads to the activation of p53 signaling pathway in a dose-dependent manner, with increasing protein expression of MDM2, p21, Bax, Puma and decreasing level of γH2A.X observed after 24 h incubation of NTS071 in NUGC-3 cells.

COMPARISON OF COMPOUND PROFILES

- NTS071 exhibits superior biochemical potency and more desirable ADMET properties than PC14586.

	PMV Pharmaceuticals PC14586	Nutshell Therapeutics NTS071
Y220C DNA binding HTRF assay SC_{150} (nM)	14	0.7
Liver microsome stability h/d/r/m $T_{1/2}$ (min)	41 / 12 / 30 / 27	>256 / 167 / 119 / 110
Hepatocyte stability h/d/r/m $T_{1/2}$ (min)	61 / 157 / 73 / 164	>512 / >512 / >512 / >512
Plasma protein binding h/d/r/m Fu (%)	0.3 / 5.0 / 1.8 / 1.3	2.8 / 7.6 / 2.3 / 5.7
CYP 1A2/2C9/2C19/2D6/3A IC_{50} (μM)	>50 / 47 / 20 / >50 / 1.6	>50 / >50 / >50 / >50 / >50
hERG patch clamp IC_{50} (μM)	19	>30
Mouse PK CL (IV 1 mg/kg); AUC_{0-t} / F% (PO 10 mg/kg)	18 mL/min/kg; 10 hr*μM / 63%	15 mL/min/kg; 16 hr*μM / 88%
Rat PK CL (IV 1 mg/kg); AUC_{0-t} / F% (PO 10 mg/kg)	7.1 mL/min/kg; 36 hr*μM / 84%	3.2 mL/min/kg; 58 hr*μM / 70%
Dog PK CL (IV 1 mg/kg); AUC_{0-t} / F% (PO 5 mg/kg)	23 mL/min/kg; 5.5 hr*μM / 91%	8.9 mL/min/kg; 15 hr*μM / 106%

Table 1. Comparison of the overall properties of PC14586 and NTS071.

INHIBITORY ACTIVITY ON CELL PROLIFERATION

- NTS071 potently inhibits the growth of various cancer cell lines harboring Y220C mutation (2~7 fold more potent than PC14586) and displays a good selectivity against p53 WT or other p53 mutant cells.

Cell line	Tumor type	*TP53* status	PC14586 IC_{50} (μM)	NTS071 IC_{50} (μM)
HCC2935$	NSCLC	Y220C	0.16	0.059
NUGC-3‡	GC	Y220C	0.44	0.064
BxPC3#	PC	Y220C	0.27	0.075
Huh7#	HCC	Y220C	0.28	0.097
HCC366$	NSCLC	Y220C	0.40	0.11
NCIH2342$	NSCLC	Y220C	0.27	0.12
KON#	HNSCC	Y220C	0.41	0.19
COV362#	OC	Y220C	0.56	0.23
MFE296#	EC	Y220C	1.22	0.25
NCIH2085#	NSCLC	Y220C	0.81	0.33
HCT116#	CRC	WT	5.09	>10
NUGC-4#	GC	WT	>10	>10
A549#	NSCLC	WT	>10	>10
TOV-112D#	OC	R175H	>10	>10
SU.86.86#	PC	G245S	>10	>10
OVCAR-3#	OC	R248Q	>10	>10

Table 2. Comparison of PC14586 and NTS071 for their anti-proliferation activities in cancer cells with different p53 mutations.

The experiments were evaluated using either CellTiter-Glo™ or CCK8 methods after incubation with compounds for 5‡, 7#, or 10$ days.

IN VIVO ANTITUMOR EFFICACY IN MULTIPLE CDX MODELS

- NTS071 shows excellent dose-dependent *in vivo* antitumor efficacy in multiple CDX models harboring Y220C mutation; The minimum effective doses of NTS071 were 2-4 fold less than those of PC14586.

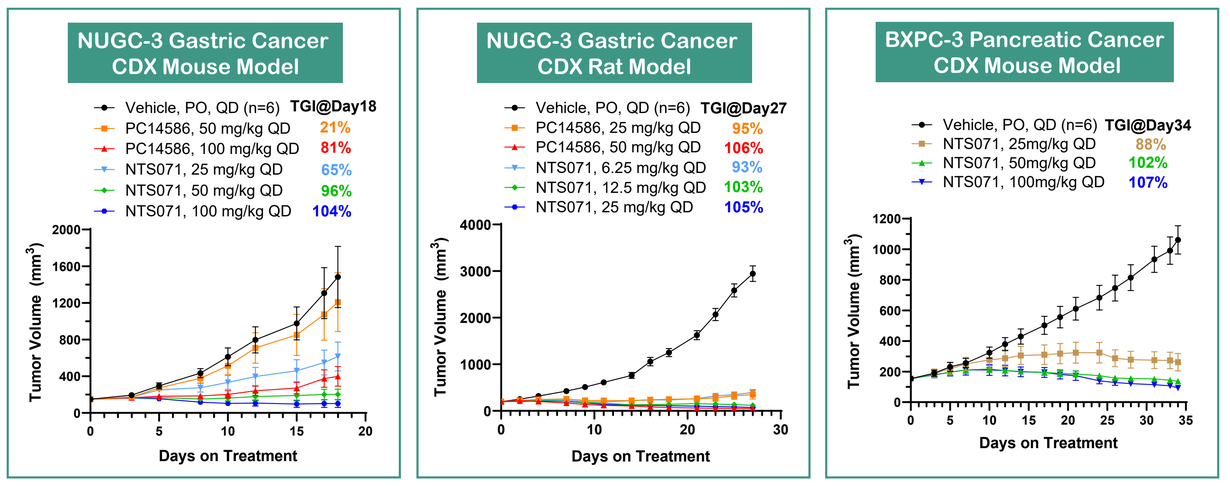

Figure 3. *In vivo* efficacy of NTS071 in NUGC-3 gastric cancer xenograft mouse/rat models, and BXPC-3 pancreatic cancer xenograft mouse model. All animals were well tolerated without significant change of body weight during treatment (data not included).

IN VIVO ANTITUMOR EFFICACY IN VARIOUS PDX MODELS

- NTS071 dose-dependently inhibits tumor growth and induces tumor regression in the PDX models with different tumor types harboring Y220C mutation; The minimum effective doses of NTS071 are still significantly lower than those of PC14586.
- In the SCLC PDX model, NTS071 causes complete tumor regression at the dose levels of 50 mg/kg QD and 100 mg/kg QD; Tumor rebound was observed on the 12th Day (Day 32) and 43rd Day (Day 63) from the end of compound dosing for the 50 mg/kg QD and 100 mg/kg QD groups, respectively.

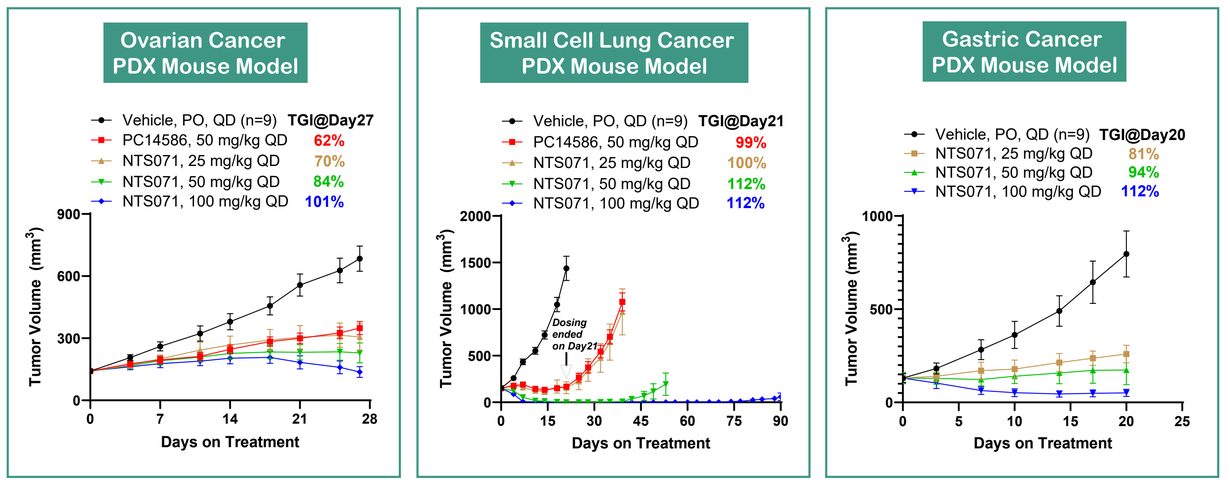

Figure 4. *In vivo* efficacy of NTS071 in ovarian cancer, small cell lung cancer and gastric cancer patient-derived xenograft models.

HUMAN DOSE PREDICTION

- NTS071 is predicted to be efficacious at the dose range of 150~1389 mg QD in human.

PK Parameter	Predicted Value	Features
CL (L/h)	4.2	• Extremely low clearance • Predicted by allometric scaling method corrected for maximum lifespan potential (MLP)
Vd_{ss} (L)	79.6	• Low to moderate volume of distribution
K_a (h^{-1})	0.92	• Fast absorption
$T_{1/2}$ (h)	13.1	• Sufficient half life to allow for QD dosing
F%	88%	• High bioavailability

Table 3. Human PK prediction of NTS071.

Assumption	Target Dose in Human PC14586	Target Dose in Human NTS071
Total AUC_{0-24} at steady state in human at efficacious dose ≥ the value in mouse (NUGC-3 model)	2403 mg	681 mg
Total AUC_{0-24} at steady state in human at efficacious dose ≥ the value in rat (NUGC-3 model)	868 mg	185 mg
Free AUC_{0-24} at steady state in human at efficacious dose ≥ the value in mouse (NUGC-3 model)	3124 mg	1389 mg
Free AUC_{0-24} at steady state in human at efficacious dose ≥ the value in rat (NUGC-3 model)	1546 mg	150 mg
Recommended Phase 2 Dose	**2000 mg**	**TBD**

Table 4. Human dose prediction of PC14586 and NTS071.

TOXICOLOGICAL STUDIES IN RATS AND DOGS

- NTS071 demonstrates sufficient safety margins in the 14-day DRF tox studies in both rats and dogs.

Category	Dose Level (mg/kg, QD)	AUC_{0-t} total (ng*hr/mL)	MOS total	AUC_{0-t} free (ng*hr/mL)	MOS free
Maximum Tolerated Dose (MTD) in rat	200	1,958,559	50	45,047	51
Maximum Tolerated Dose (MTD) in dog	225	804,809	21	61,165	69
Minimum Efficacious Dose (MED) in rat	6.25	38,784	-	892	-

Table 5. Margin of Safety (MOS) calculation for NTS071 based on the data of 14-day DRF tox studies in SD rats and beagle dogs.

CONCLUSIONS

- NTS071 is a novel small molecule p53 Y220C reactivator that stabilizes p53 Y220C protein, promotes conformational change to p53 WT, and restores binding with DNA as well as p53 transcriptional activity.
- Compared to PC14586, NTS071 exhibits superior *in vitro* potency, more desirable ADMET properties and better *in vivo* antitumor efficacy in multiple CDX and PDX models.
- In the preliminary tox studies in rats and dogs, NTS071 demonstrates excellent safety margins.
- NTS071 is predicted to require a remarkably lower minimum efficacious dose (MED) than PC14586 in human. It could be a superior therapeutic option for cancer patients harboring such a p53 Y220C mutation.

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References: 1) Hum. Mutat. 2016, 37, 865–876. 2) Trends Cell Biol. 2021, 31(4), 298–310; 3) PMV Pharma Webinar.

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