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The novel pan-RAF-MEK glue NST-628 is highly efficacious as monotherapy and as an anchor for vertical inhibition of the RAS-MAPK pathway in KRAS-mutant cancers

NESTED THERAPEUTICS

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Abstract

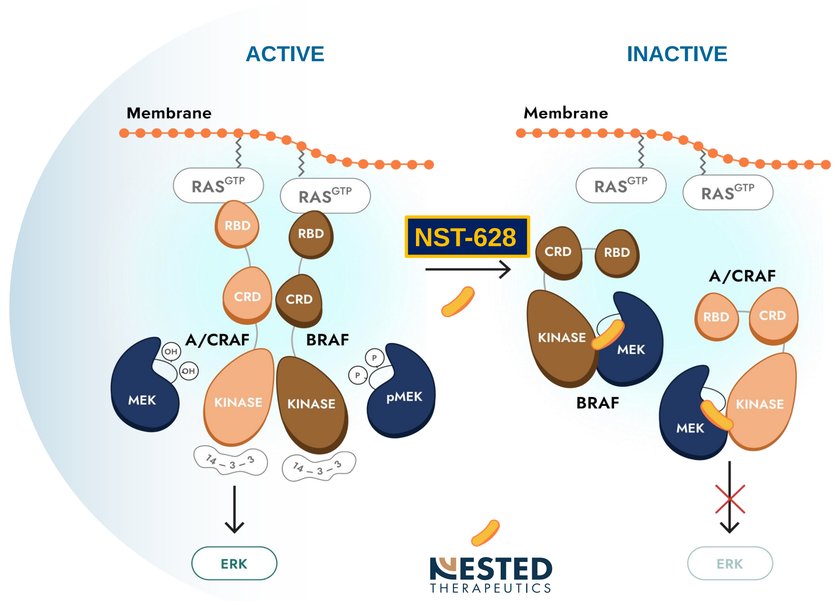
Background: Alterations in the RAS/RAF/MEK/ERK signaling cascade are common across multiple solid tumor types and aberrant signaling of the pathway is a driver for RAS- and RAF-driven cancers. Mutations in KRAS occur at the highest frequency, with G12D, G12V, and G12C mutations being the most common and apart from approved mutation selective inhibitors for KRAS G12C mutations, other mutations of KRAS are not directly addressable by currently approved inhibitors. NST-628 is a potent non-degrading pan-RAF-MEK molecular glue that prevents phosphorylation and activation of MEK by RAF, overcoming the limitations of traditional RAS-MAPK inhibitors and leading to deep durable inhibition of MEK kinase activity and downstream ERK signaling.

Methods: We designed *in vitro* potency assays (proliferation) to address the efficacy of NST-628 alone and in combination with KRAS G12D-inhibitors RMC-9805 (RAS "ON"), MRTX1133 (RAS "OFF"), and the pan-RAS inhibitor RMC-6236 (RAS "ON"). To address the synergistic interactions we applied Synergyfinder analysis of the combinations in a panel of KRAS-mutant pancreatic cell lines. *In vivo* efficacy of NST-628 monotherapy was assessed in a panel of patient derived xenografts (PDX) and cell line derived xenografts (CDX), collectively representing diverse solid tumor histologies. *In vivo* combination efficacy was assessed in a KRAS G12D mutant CDX model of pancreatic cancer.

Results: NST-628 demonstrates broad efficacy in cellular and patient-derived tumor models harboring KRAS G12A/C/D/R/V, G13D/x, Q61x, and exon 4 mutations both *in vitro* and *in vivo*. NST-628 is also active across multiple KRAS-mutant solid tumor histologies, including lung, colorectal, and pancreatic cancer. In a panel of KRAS G12D-mutant pancreatic cell lines, a combination synergy is seen with NST-628 and KRAS G12D-selective inhibitors RMC-9805 and MRTX1133 as well as the pan-RAS inhibitor RMC-6236. *In vivo,* the combination of the KRAS-G12D inhibitor RMC-9805 and NST-628 lead to deep tumor regressions in a KRAS G12D-mutant pancreatic model surpassing the monotherapy activity.

Conclusions: Collectively, this study warrants further investigation of NST-628 as a vertical combination partner with both RAS "ON" and RAS "OFF" inhibitors targeting KRAS G12D and other RAS mutations. NST-628 is positioned to make an impact clinically in areas of high unmet patient need in KRAS-mutant solid tumors. First-in-human trials of NST-628 in RAS- and RAF-mutant solid tumors are underway in 2024 (NCT06326411)

NST-628 is a non-degrading pan-RAF-MEK glue



NST-628 Prevents RAF paralog heterodimerization by promoting potent stabilization of CRAF-MEK, BRAF-MEK and ARAF-MEK complexes in inactive confirmations, blocking downstream signaling through ERK

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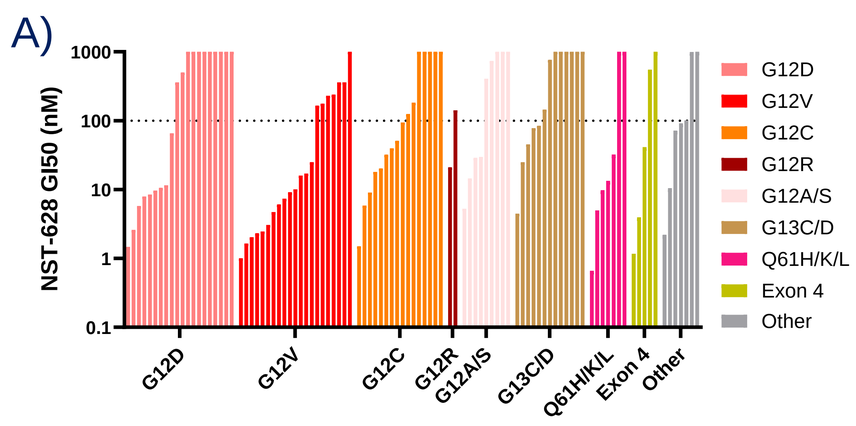
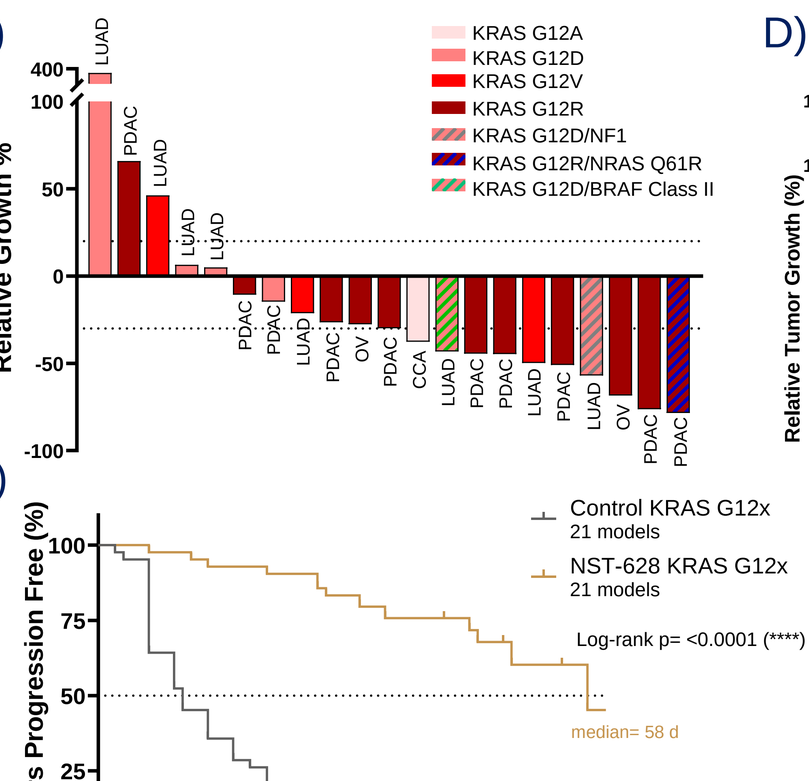
NST-628 is broadly effective in KRAS-mutant cancers

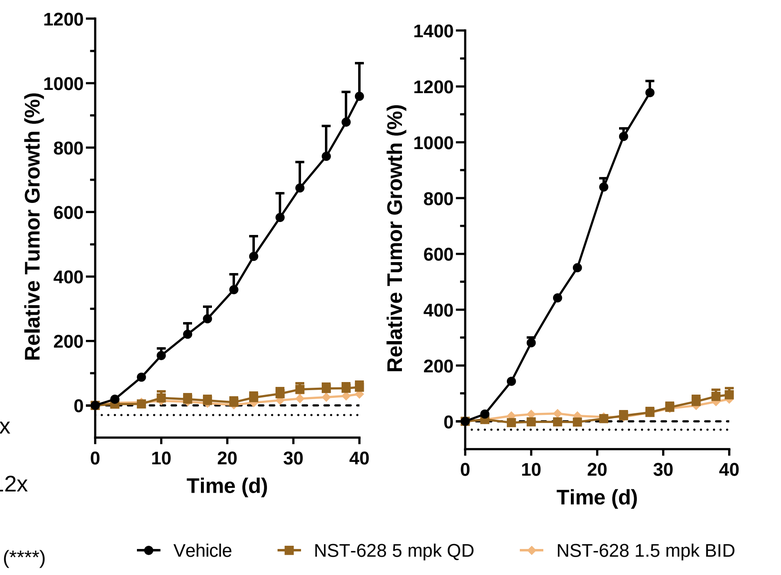


A) OMNI cell line screen in KRAS-mutant cell lines with NST-628 demonstrating efficacy (GI50<1 uM) across diverse KRAS-mutant cell line models. Response rates at clinically predicted achievable concentration (100 nM, dotted line) are G12D (45%), G12V (67%), G12C (56%), G12R (50%), G12A/S (44%), G13C/D (36%), Q61x (71%), Exon 4 (60%), other (71%)

B) *In vivo* assessment of NST-628 anti-tumor efficacy in patient derived xenograft (PDX) models with KRAS-mutations, treated at 3 mg/kg QD of NST-628 for a duration of up to 60 days. Data is representative of Day 13/14 or maximum tumor response normalized to day 0. 52% of models have partial responses (≤ -30% tumor growth) and 86% of models have stable disease (≤ 20% tumor growth)

Key: LUAD (lung adenocarcinoma), PDAC (pancreatic ductal adenocarcinoma), CCA (cholangiocarcinoma), OV (ovarian)

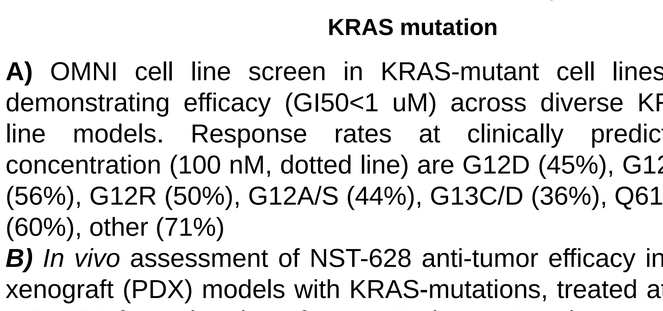




C) Kaplan-Meier survival plot of PDX models in **(B)** represented as a time to progression (tumor doubling). Comparison of survival curves measured by log rank test (Mantel-Cox)

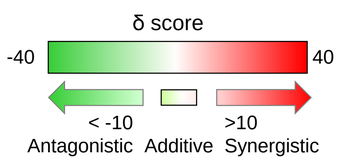
D) AsPC-1 and HPAC subcutaneous tumors implanted in Balb/c nude mice and treated with NST-628 at 5 mg/kg QD or NST-628 at 1.5 mg/kg BID

NST-628 synergistically combines with KRAS G12D inhibition in PDAC



B)

Cell line	RMC-9805	MRTX1133	RMC-6236
AsPC-1	6.465	6.789	7.257
HPAC	10.726	14.654	16.345
HPAF-II	4.085	5.785	5.89
Panc-1	-9.015	-9.038	-10.391
Panc 05.14	1.708	4.302	2.426
Panc 08.13	1.07	0.153	1.598
Panc10.05	2.514	2.414	5.771
SW1990	-5.654	-6.056	-5.801

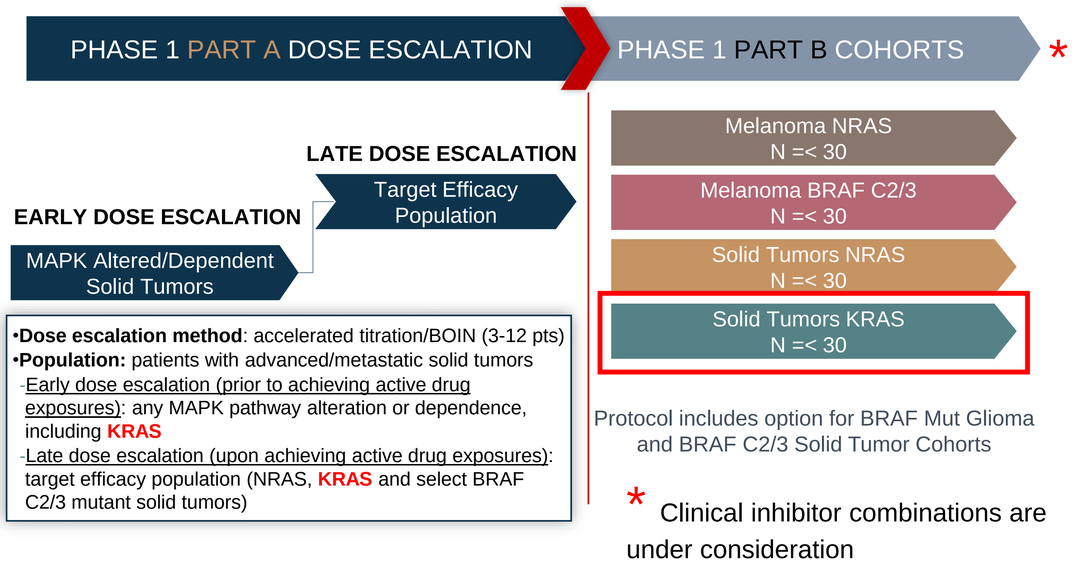


A) Bliss Synergy score (Synergyfinder) plot of the combination of a dose response of NST-628 combined with a dose response of RMC-9805 or MRTX1133 read out by CTG after 72 h of treatment in the AsPC-1 cell line

B) Summary of Bliss Synergy scores (δ) across KRAS G12D-mutant models treated with dose response combinations of NST-628 with RMC-9805, MRTX1133, or RMC-6236 treated as in A

C) Tumor volume of AsPC-1 tumors treated with 5 mg/kg NST-628, 30 mg/kg QD RMC-9805, or a combination of NST-628 and RMC-9805. Significance determined by Mann-Whitney test

NCT06326411 clinical trial design: refining for KRAS-mutant and other RAS-MAPK altered solid tumors



- **Dose escalation method**: accelerated titration/BOIN (3-12 pts)
- **Population:** patients with advanced/metastatic solid tumors
  - Early dose escalation (prior to achieving active drug exposures): any MAPK pathway alteration or dependence, including **KRAS**
  - Late dose escalation (upon achieving active drug exposures): target efficacy population (NRAS, **KRAS** and select BRAF C2/3 mutant solid tumors)

Protocol includes option for BRAF Mut Glioma and BRAF C2/3 Solid Tumor Cohorts

* Clinical inhibitor combinations are under consideration

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