NST-628 is a potent, best-in-class MAPK pathway molecular glue that inhibits RAS- and RAF-driven cancers

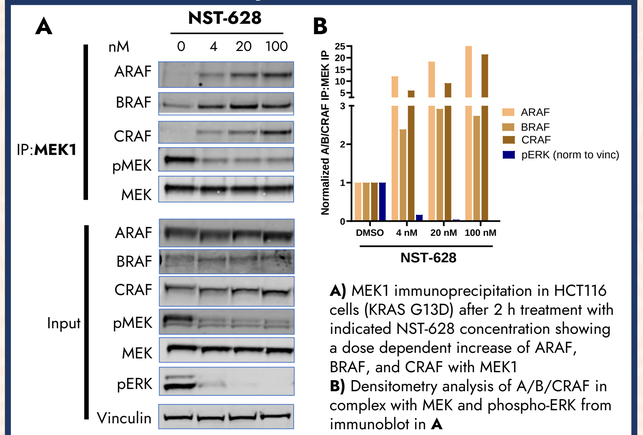
Meagan B. Ryan, Natasha Schenk, Marshall Zingg, Matthew Koehler, Brooke Swalm, Bradley Quade, Yongxin Han, Klaus P Hoeflich, Michael R Hale, Margit Hagel

Nested Therapeutics, 1030 Massachusetts Ave, Cambridge MA

Abstract

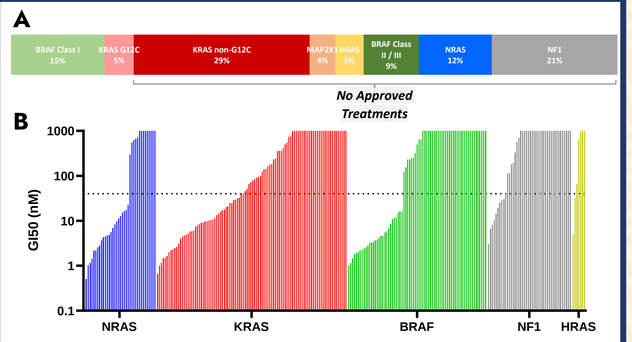
Alterations in the RAS/RAF/MEK/ERK signaling cascade are common across multiple solid tumor types and aberrant signaling of the pathway is a driver for RAS- and RAF-driven cancers. Apart from approved mutation selective inhibitors for BRAF Class I and KRAS G12C mutations, other mutations of RAS and RAF are not directly addressable by currently approved inhibitors and there is a need for inhibitors with superior efficacy, durability and tolerability for the MAPK pathway in RAS- and RAF-driven cancers. NST-628 is a potent pan-RAF-MEK molecular glue that prevents phosphorylation and activation of MEK by RAF, leading to deep durable inhibition of MEK kinase activity and downstream ERK signaling. In a MEK1 immunoprecipitation assay, NST-628 treatment glues ARAF, BRAF, and CRAF with unphosphorylated MEK1, stabilizing an inactive RAF-MEK complex and deeply inhibiting phospho-ERK signaling. In an unbiased cell line panel screen (550 models), NST-628 demonstrates efficacy across multiple tumor types with RAS-MAPK alterations, including melanoma, lung, and pancreatic models. NST-628 is broadly efficacious in models with BRAF Class II/III mutations, KRAS-mutations (G12C, G12D, G12V, G12R, Q61H), and NRAS-mutations (Q61x, G12x) as well as showing anti-proliferative effects in NF1-mutant/deficient models. Cell lines with NRAS Q61 mutations were particularly sensitive to NST-628 inhibition (GI50 average=150 nM). In vivo, NST-628 has a balanced metabolic profile and predicted half-life and exposure in humans is compatible with low dose daily dosing. NST-628 (3-5 mg/kg QD treatment) led to tumor regressions in HCT116 (KRAS G13D colorectal) and IPC-298 (NRAS Q61L melanoma) xenograft models, 53% and 38% respectively, correlating to inhibition of both phospho-MEK and phospho-ERK in tumors. In comparison to the MEK inhibitor trametinib, NST-628 led to deeper tumor responses and on target pathway inhibition in both models as well as significantly greater tolerability as measured by body weight. In a mini-mouse trial utilizing patient derive xenograft (PDX) tumors harboring NF1, KRAS G12D/R, BRAF Class II/III, and NRAS Q61x mutations, NST-628 (3 mg/kg QD) dosing demonstrates broad anti-tumor responses across melanoma, lung, pancreatic, glioma, and ovarian models. These data collectively, together with the predicted effective human half-life of NST-628 being consistent with daily dosing, support best-in-class potential for NST-628 and initiation of clinical trials in patients with solid tumors harboring RAS- and RAF-mutations is planned.

NST-628 engages ARAF, BRAF, and CRAF in complex with MEK



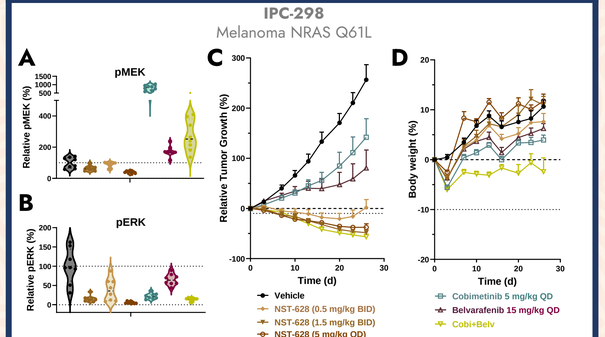
A) MEK1 immunoprecipitation in HCT116 cells (KRAS G13D) after 2 h treatment with indicated NST-628 concentration showing a dose dependent increase of ARAF, BRAF, and CRAF with MEK1
B) Densitometry analysis of A/B/CRAF in complex with MEK and phospho-ERK from immunoblot in **A**

NST-628 is highly active in RAS- and RAF-mutant cell lines



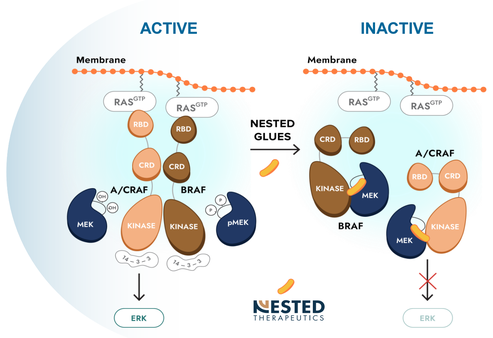
A) Distribution of RAS MAPK mutations with unmet patient need
B) OMNI cell line screen with NST-628 demonstrating efficacy (GI50<1 uM) across diverse RAS MAPK mutant cell line models. Response rates at clinically predicted achievable concentration (40 nM, dotted line) are NRAS (63%), KRAS (45%), BRAF (40%), NF1 (20%), HRAS (23%)

NST-628 demonstrates comparable efficacy and greater tolerability than RAF+MEK inhibitor combination



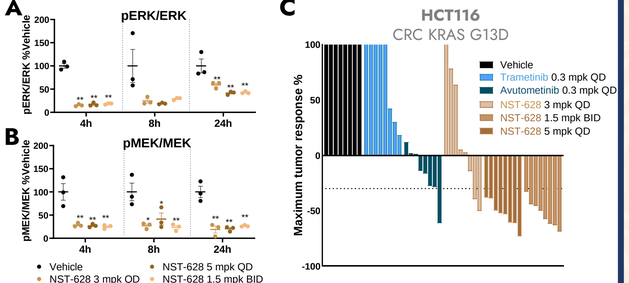
A) pMEK and **B)** pERK from IPC-298 tumors treated with the indicated inhibitors and collected 4 h post dose **C)** Volume of IPC-298 tumors treated with the indicated inhibitors, tumors normalized to starting volumes on D0 **D)** Mouse body weights of enrolled mice in efficacy study of **C)**

NST-628 is a pan-RAF-MEK glue



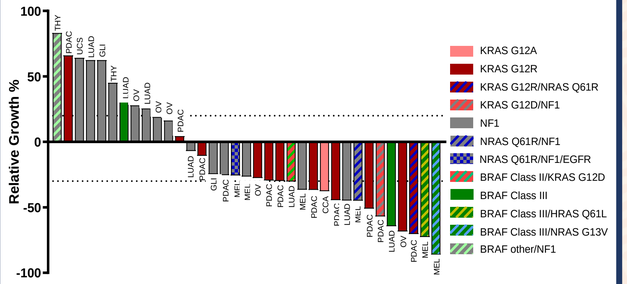
NST-628 Prevents RAF paralog heterodimerization by promoting potent stabilization of CRAF-MEK, BRAF-MEK and ARAF-MEK complexes in inactive confirmations, blocking downstream signaling through ERK

NST-628 potently inhibits the RAS MAPK pathway and inhibits tumor growth *in vivo*



A) ***In vivo*** assessment of phospho-ERK or **B)** phospho-MEK (lower panel) in HCT116 tumors after 4, 8, and 24 h of indicated compound treatment. Significance measured by one-way ANOVA vs Vehicle (* p<0.5, ** p<0.1)
C) Maximum tumor response (Day 10) of HCT116 tumors treated with the indicated inhibitors

NST-628 potently inhibits the growth of PDX models in a mouse mini trial



In vivo assessment of NST-628 anti-tumor efficacy in patient derived xenograft (PDX) models with RAS MAPK pathway 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. 46% of models have partial responses (≤ -30% tumor growth) and 71% of models have stable disease (≤ 20% tumor growth)

Conclusions and Acknowledgements

- NST-628 is a pan-RAF-MEK glue and engages ARAF, BRAF, and CRAF with MEK, shutting down the MAPK pathway
- NST-628 inhibits the phosphorylation of MEK and ERK ***in vivo,*** leading to potent anti-tumor activity
- NST-628 has broad efficacy against RAS- and RAF-mutant cancers, both ***in vitro*** and ***in vivo*** PDX models, addressing patient populations with no currently approved therapies
- NST-628 demonstrates comparable efficacy and greater tolerability than the cobimetinib (MEK) and belvarafenib (Type II RAF)

Thank you to our CRO partners for data generation, including Wuxi, Pharmaron, and Champions oncology

Please visit our other posters in today's session: #s A086, A089

For digital poster copies, visit our website www.nestedtx.com