A089

# NST-628 is a potent, fully brain-penetrant, RAS MAPK pathway molecular glue inhibitor with efficacy in CNS tumor models

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## Abstract

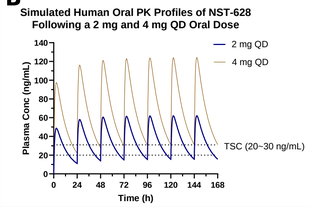
Alterations in the RAS/RAF/MEK/ERK signaling cascade are common across multiple solid tumor types and aberrant signaling of the pathway is a driver for RAS- and RAF-driven cancers. Apart from approved mutation selective inhibitors for BRAF Class I and KRAS G12C mutations, other mutations of RAS and RAF 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. Currently approved inhibitors of the RAS-MAPK pathway also have limited central nervous system (CNS) exposure and an area of significant unmet patient need exists for patients with primary and metastatic tumors harboring RAS-MAPK pathway alterations. NST-628 has CNS permeability, with a Kp,uu >1, that is far higher than trametinib or avutometinib (VS-6766) (Kp,uu of 0.11 and 0.18 respectively). The predicted effective human half-life of NST-628 (~9h) is amenable to daily dosing in the clinic. In vivo, NST-628 leads to a dose-dependent inhibition of the MAPK pathway in murine brain tissue as measured by phospho-ERK. In an intracranial luciferase-tagged SK-MEL-2 xenograft model (NRAS Q61R), NST-628 leads to tumor regressions with a daily dosing regimen (3 mg/kg QD). Tumor regressions as measured by bioluminescent imaging (BLI) were only seen with NST-628 (50%) in the SK-MEL-2 model, whereas trametinib and avutometinib showed minimal efficacy due to low exposure in the CNS. In the NF-1 mutant MeWo (NF1 Q1336*) melanoma intracranial xenograft model (luciferase tagged), NST-628 led to tumor regressions at two dosing regimens (1, 3 mg/kg QD) and tumor stasis at lower doses (0.3 mg/kg) as measured by BLI. In contrast, the brain penetrant type II RAF inhibitor tovorafenib (DAY101) showed no efficacy in the MeWo model and resulted in paradoxical pathway hyper-activation, as determined by enhanced PD response and tumor growth. Only NST-628 effectively inhibited MAPK signaling as measured by DUSP6 transcript in a time- and dose-dependent manner. Overall, survival of mice harboring MeWo intracranial tumors was also significantly increased versus vehicle control and tovorafenib treatment groups. These data collectively support best-in-class potential of NST-628 and initiation of clinical trials in patients with solid tumors harboring RAS- and RAF-mutations, including those presenting clinically with brain metastases and primary intracranial tumors.

## NST-628 has a best-in-class metabolic profile and is fully brain penetrant

**A**

| | NST-628 | Trametinib | Avutometinib |
|---|---|---|---|
| $IC_{50}$, nM pMEK, $HCT116^{KRASG13D}$ | 0.3 | 2.0 (partial inh) | 8.1 |
| $IC_{50}$, nM CTG $HCT116^{KRASG13D}$ | 20 | 8.3 | 278 |
| $IC_{50}$, nM CTG $IPC\text{-}298^{NRAS\text{-}Q61L}$ | 0.57 | 0.24 | 22.7 |
| In vivo tumor PD, *p*MEKi /*p*ERKi 4h post oral 0.5 mg/kg dose (vs tumor drug level) | >80% / >80% (140 nM) | Activation / >80% (152 nM) | >80% / >80% (392 nM) |
| Clinical $t_{1/2}$ | ~9 h (predicted) | 127 hr | 60 hr |
| Rat brain $K_p$ / $K_{p,uu}$ | 0.3 / >1 | 0.1 / 0.1 | 0.05 / 0.18 |
| Mouse brain $K_p$ (in vivo PK/PD) | 0.7 | 0.1 | 0.1 |

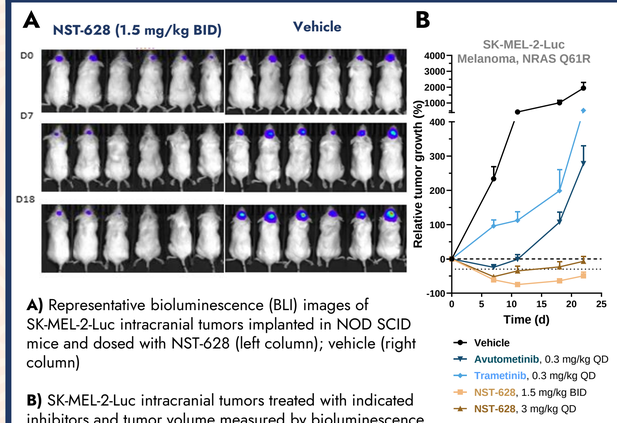
**B**




**A)** Properties of NST-628 in comparison to other MEK inhibitors, including on target pathway activity (pMEK, pERK), predicted half life, and CNS penetration (Kp, Kpuu)

**B)** Predicted human PK profiles and efficacious doses of NST-628 clinically at clinically relevant once daily dose and predicted tumor stasis concentration based on ***in vivo*** characterization of the compound

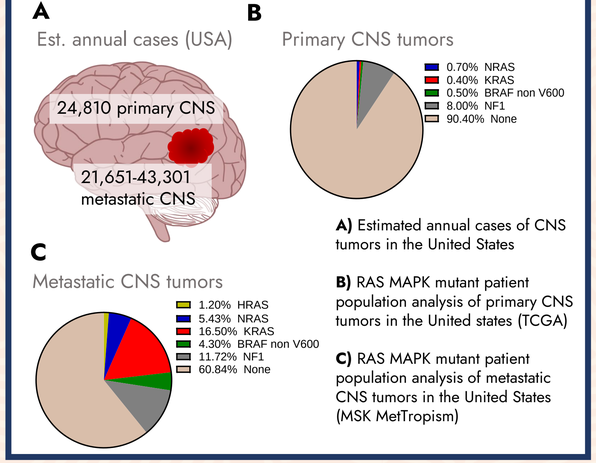
## NST-628 reduces the size of NRAS-mutant brain tumors in mice




**A)** Representative bioluminescence (BLI) images of SK-MEL-2-Luc intracranial tumors implanted in NOD SCID mice and dosed with NST-628 (left column); vehicle (right column)

**B)** SK-MEL-2-Luc intracranial tumors treated with indicated inhibitors and tumor volume measured by bioluminescence (BLI)

## NST-628 addresses an area of unmet patient need

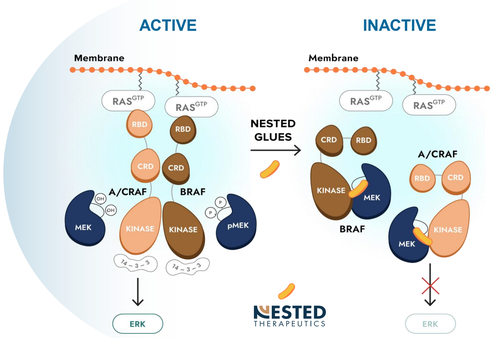



**A)** Estimated annual cases of CNS tumors in the United States

**B)** RAS MAPK mutant patient population analysis of primary CNS tumors in the United states (TCGA)

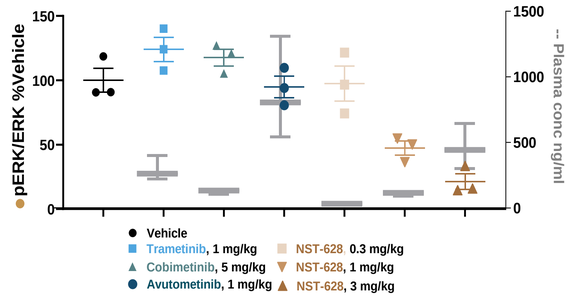
**C)** RAS MAPK mutant patient population analysis of metastatic CNS tumors in the United States (MSK MetTropism)

## 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

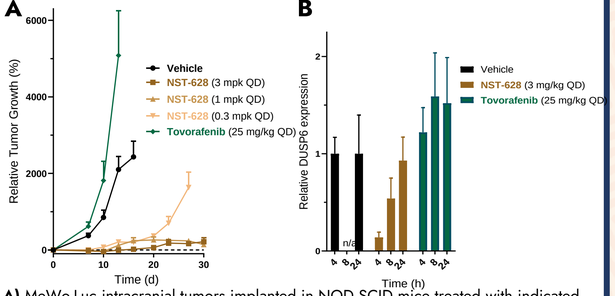
## NST-628 demonstrates superior CNS PK/PD




Plasma concentration of indicated inhibitors and on target pathway activity (phospho-ERK) in mouse brain tissue after 4 h of dosing, one dose. Only NST-628 inhibits phospho-ERK in mouse brain tissue (left y axis, colored points) comparable plasma concentration (right axis, gray bars)

## NST-628 inhibits the RAS MAPK pathway and growth of NF1-mutant brain tumors in mice

MeWo-Luc, NF1 Q1336* melanoma




**A)** MeWo-Luc intracranial tumors implanted in NOD SCID mice treated with indicated inhibitors and tumor volume measured by bioluminescence (BLI)

**B)** DUSP6 transcript expression measured by qPCR in MeWo tumors treated with the indicated inhibitors for 4, 8, or 24 h

## Conclusions/Acknowledgements

- NST-628 is an orally bioavailable potent inhibitor of RAF-MEK, and is predicted to have favorable human PK that is amenable for QD oral dosing at low efficacious doses in the clinic
- NST-628 is highly brain penetrant compared to trametinib or VS-6766 leading to greater inhibition of MAPK signaling in the brain
- NST-628 reduces the growth of NRAS-mutant intracranial tumor growth and has greater anti-tumor activity than both trametinib and VS-6766
- NST-628 inhibits MAPK signaling (DUSP6) and reduces the growth of NF1 mutant intracranial tumors
- NST-628 addresses an area of high unmet patient need in RAS MAPK mutant primary and metastatic CNS tumors

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

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