NST-628 is a novel molecular glue that inhibits signaling and pathway reactivation in oncogenic RAS-MAPK cancers
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Abstract
Alterations in the RAS-MAPK pathway are identified in nearly 20% of cancers and are associated with poor patient prognosis. Although the major targets and inhibitors in the RAS-MAPK pathway, clinical activity is limited by paradoxical bypass mechanisms, feedback signaling and pathway reactivation, and genetic resistance mechanisms. NST-628 aims to overcome the limitations of traditional RAS-MAPK pathway inhibitors by disabling all RAF-MEK complexes in a conformer that blocks MEK phosphorylation, tosequencerheseqences dynamicsignalprocessesthat promote RAR-mediated bypass signaling. In comparison to clinically approved RAF/MAPK pathway inhibitors, only NST-628 rapidly and durably decreases pathway reactivation, as measured by MEK phosphorylation, in MG-63/G10 tumor models. In vitro and in vivo, NST-628 distinguishes MEK from RAF and significantly decreases ERK1 phosphorylation. NST-628 promotes potent stabilization of CRAF-MEK, BRAF-MEK, and ARAF-MEK complexes with complete destruction of ERK-MEK. However, NST-628 treatment completely prevents RAF paragapatheterization in RAS-driven cells. Dose-dependent paragapathetic complex modification was also confirmed using recombinant-perfused recombinant CRAF-MEK, BRAF-MEK, and ARAF-MEK complexes, and both MEK and RAF paragapathy increases in the presence of NST-628 were quantified via surface plasmon resonance (SPR). In this assay, NST-628 significantly increased the affinity of MEK with both inactive monomeric and active dimeric BRAF complex, and was structurally characterized by circular dichroism (CD). CRAF paragapathetic complexes in the presence of NST-628 were analyzed via with mass spectrometry, and we observed increased stabilization of complex MEK-CRAF-MEK complex. NST-628 binds the interdialole alpha helix domain of the MEK isoforms, and directly prevents the MEK paragapathetic pocket features that NST-628 can engage, helping us rationalize the large increase in affinity observed for MEK and CRAF in the presence of NST-628. The increased stabilization of MEK-CRAF complex by NST-628 may also contribute to the decreased pathway reactivation in specific bioluminescence-driven assays. This study shows that the biophysical and cellular characterization coupled with novel structural insights, we have defined crucial mechanisms of action for NST-628 and provide insight into a broad class potential in RAS-MAPK activated cancers.

Fig. 1: NST-628 prevents inhibitor-induced RAS-MAPK pathway reactivation in vitro and in vivo.

Fig. 2: NST-628 enhances interactions between recombinant MEK and RAF in active or autoinhibited conformations.

Fig. 3: NST-628 stabilizes endogenous pan-RAF-MEK complexes and prevents RAF heterodimerization.

Designing a RAS-MAPK pathway inhibitor that acts on multiple nodes and blocks MEK phosphorylation

Conclusions and Acknowledgements

1. NST-628 durably inhibits RAS-MAPK pathway signaling and blocks MEK hyperphosphorylation
2. NST-628 engages endogenous and recombinant ARAF-MEK, BRAF-MEK, and CRAF-MEK complexes and blocks RAF heterodimerization
3. NST-628 engages MEK in active, dimeric and autoinhibited RAF complexes
4. NST-628 has best-class potential due to its unique mechanism of action, balanced metabolic profile, strong in vivo efficacy (see poster A088), and central nervous system permeance (see poster A089).

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