NST-628 is a Novel, Potent, Fully Brain-Penetrant MAPK Pathway Molecular Glue that Inhibits RAS- and RAF-Driven Cancers

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Disclosure Information

Klaus Hoeflich

I have the following relevant financial relationships to disclose:
   Co-Founder, employee: Nested Therapeutics
   Stockholder: Nested Therapeutics

My additional financial relationship disclosures are:
   Advisor and stockholder: Turbine AI
RAS/MAPK Pathway Is the Heartland of Cancer Research, but a High Degree of Unmet Need Persists

>80% of RAS mutations not addressed by current or research-stage precision treatments

**PATIENTS BY MUTATION CLASS**

<table>
<thead>
<tr>
<th>MUTATION CLASS</th>
<th>PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF Class I</td>
<td>15%</td>
</tr>
<tr>
<td>KRAS G12C</td>
<td>5%</td>
</tr>
<tr>
<td>KRAS non-G12C</td>
<td>29%</td>
</tr>
<tr>
<td>MAP2K1</td>
<td>4%</td>
</tr>
<tr>
<td>HRAS</td>
<td>5%</td>
</tr>
<tr>
<td>BRAF Class II/III</td>
<td>9%</td>
</tr>
<tr>
<td>NRAS</td>
<td>12%</td>
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<tr>
<td>NF1</td>
<td>21%</td>
</tr>
</tbody>
</table>

**RAS INHIBITORS HAVE LIMITATIONS**

Mutant-specific KRAS inhibitors have reduced ORR and PFS compared to other targeted therapies in the same tumor types.

Polyclonal resistance observed for KRAS inhibitors in the clinic. NST-628 MOI is effective for these mutant tumors.

Drs. Jessica Lin and Ryan Corcoran (MGH)
Current Therapies Are Vulnerable to Reactivation

- **PARADOXICAL REACTIVATION**
  - e.g., RAF type I/II inhibitors

- **FEEDBACK REACTIVATION**
  - e.g., MEK inhibitors

- **BYPASS REACTIVATION**
  - e.g., KRAS inhibitors

RAF Heterodimers

Tumor Growth, Resistance, and Escape
NST-628 Addresses Paradoxical Reactivation of MAPK Pathway by Preventing RAF Heterodimers

Prevents RAF paralog heterodimerization by stabilization and inactivation of CRAF-MEK, BRAF-MEK and ARAF-MEK complexes

Fully brain penetrant MEK/pan-RAF molecular glue shuts down MEK and ERK phosphorylation
NST-628 Modulates pan-RAF-MEK Complexes by Engaging Paralog-Specific Pocket Features
NST-628 is a Highly Potent and Selective pan-RAF-MEK Molecular Glue

**Properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>NST-628</th>
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</thead>
<tbody>
<tr>
<td>Phospho-MEK, HCT116&lt;sup&gt;KRASG13D&lt;/sup&gt; (IC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>0.3 nM</td>
</tr>
<tr>
<td>Cell TiterGlo, HCT116&lt;sup&gt;KRASG13D&lt;/sup&gt; (IC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>20 nM</td>
</tr>
<tr>
<td>Selectivity (proteomics, kinase panel)</td>
<td>Only MEK/RAF</td>
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<tr>
<td>hERG inhibition to 30 uM</td>
<td>Clean</td>
</tr>
<tr>
<td>Off target panel to 10 uM (n=44)</td>
<td>Clean</td>
</tr>
<tr>
<td>Cardiac ion channel to 30 uM</td>
<td>Clean</td>
</tr>
<tr>
<td>CYP DDIs (7 isoforms), TDI</td>
<td>&gt;19 uM, Clean</td>
</tr>
<tr>
<td>Oral bioavailability (all species)</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Predicted clinical CL (mK/min/Kg), t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>0.33 / 10-12h</td>
</tr>
<tr>
<td>Rat K&lt;sub&gt;p,uu&lt;/sub&gt;</td>
<td>1.3</td>
</tr>
<tr>
<td>Phototoxicity potential</td>
<td>Low</td>
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</tbody>
</table>

**NST-628**

SPR-based ternary complex assay quantifies increase in affinity between RAF and MEK by NST-628

GST-CRAF immobilized and MEK1 titrated in presence/absence of NST-628

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NST-628 Prevents Formation of RAF Heterodimers, Thereby Suppressing Pathway Reactivation

**NEST-628 pan-RAF-MEK endogenous ternary complexes**

<table>
<thead>
<tr>
<th>nM</th>
<th>ARAF</th>
<th>BRAF</th>
<th>CRAF</th>
<th>pMEK</th>
<th>MEK1</th>
<th>pERK</th>
<th>Vinculin</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>100</td>
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</tbody>
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**NST-628 does not induce RAF heterodimer formation**

**NST-628 Superior Efficacy in RAF Dimer-Dependent Cancer Cells**

* NST-628 potency also broadly observed across RAS pathway biomarkers in 550 cell line panel

HCT-116 (KRAS G13D), BRAF or MEK IP (endogenous), 100 nM, 2 h treatment
Similar ternary complexes observed for BRAF, CRAF and ARAF IPs
Significant Anti-Tumor Activity & Prolonged Survival in RAS or RAF mutant PDX models by NST-628

- 3 mg/kg QD NST-628 dose matches start of projected clinical efficacy
- Each tumor normalized to D0 starting size

• ORR: 16/26 models (61.5%) ≤ -30% tumor regression
• DCR: 22/26 models (84.6%) ≤ 20% tumor outgrowth

RAS/RAF-MUTANT ANTI-TUMOR EFFICACY

RAS/RAF-MUTANT PROGRESSION FREE SURVIVAL

Log-rank p= <0.0001 (****)
NST-628 ADME/PK Properties Optimized for Anti-Tumor Activity and Therapeutic Index in Patients

- 90% of pERK inhibition for 10-12 hours by NST-628 led to tumor regression in preclinical tumor models
- QD oral doses >2 mg predicted to be clinically efficacious with steady-state C_min achieving efficacious concentrations (20-30 ng/mL)
- NST-628 is amenable to flexible dosing schedules to optimize anti-tumor activity and therapeutic index
NST-628 has Broad Efficacy without Sacrificing Tolerability at Clinically Achievable Exposures

- NST-628 treatment results in higher response rate and is better tolerated than trametinib or Avutometinib
- Trametinib and Avutometinib doses selected based on clinically achievable drug exposures

* No body weight loss or skin keratinization observed in any NST-628 treated animals

**ANTI-TUMOR ACTIVITY**

**TOLERABILITY**

- Vehicle
- Trametinib (0.3 mg/kg QD)
- Trametinib (1 mg/kg QD)
- Avutometinib (0.3 mg/kg QD)
- Avutometinib (1 mg/kg QD)
- NST-628 (3 mg/kg QD)
- NST-628 (5 mg/kg QD)
- NST-628 (1.5 mg/kg BID)

HTC-116 (KRAS G13D) tumor xenograft model
NST-628 has Superior Pathway Inhibition and Anti-Tumor Efficacy in Intracranial Tumors

NST-628 Demonstrates Superior CNS PK/PD

Non-tumor bearing mice (fully intact blood-brain barrier)

Plasma concentration and phospho-ERK in mouse brain tissue measured 4 h after single dose

Only NST-628 inhibits phospho-ERK in mouse brain tissue at comparable plasma concentration

NST-628 Reduces the Size of NRAS-mutant Brain Tumors In Mice

SK-MEL-2-Luc, NRAS Q61R, Melanoma

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

NST-628 Inhibits the RAS MAPK Pathway and Growth of NF1-mutant Brain Tumors in Mice

MeWo-Luc, NF1 Q1336* Melanoma

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

Tovorafenib accelerates tumor growth due to paradoxical RAF reactivation in RAS-driven cancers

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NST-628 Differentiation (Broad Efficacy, Superior Drug Properties) Warrants Clinical Exploration

CLINICAL DEVELOPMENT FOCUS

- **RAS**: Robust pre-clinical data across tumor histologies with KRAS and NRAS codon mutations
- **BRAF**: Superior activity in select Class II and III mutant malignancies
- **High CNS exposure**: Offers efficacy for patients with primary CNS malignancies and CNS metastases
- **Optimal half-life (10-12 h)**: Offers superior therapeutic index and dosing optionality

DEVELOPMENT STRATEGY

- **Vertical Combinations**: 1st Line of Targeted Tx for K/NRAS
- **I/O & Other 1L SOC Combinations**: Indication and Biomarker Expansion
- **Advance Treatment Paradigms**: NRAS and BRAF Class II/III Solid Tumors
- **Establish Rapid POC**: Build Franchise Lifecycle

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GLP toxicology studies demonstrate improved exposure margins vs. MEKi’s in both non-clinical species.

Phase 1 clinical studies initiated (NCT06326411)

Fully Brain Penetrant

- Allows treatment of brain metastases;
- Only RAS/MAPK inhibitor with full intrinsic brain penetration

Superior Risk/ Benefit Profile vs. Other Pathway Combination Therapy

- Potential for monotherapy and to become backbone for various combination approaches

Broad Addressable Biomarker Populations that Have No Targeted Treatment Options as of Today

- BRAF class II and III, NRAS, KRAS, NF-1, MAP2K1, HRAS

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**MEK/PAN-RAF Glue**

- High potency and selectivity
- Potent stabilization of CRAF-MEK, BRAF-MEK and ARAF-MEK complexes
- Lack of paradoxical pathway activation through prevention of RAF paralog heterodimerization
- Balanced metabolic profile to maximize therapeutic index and provide dosing flexibility in clinic

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