Hit finding and assay enablement for VRK1, a paralog synthetic lethal kinase target in VRK2-deficient cancers

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TANGO therapeutics

Poster #484

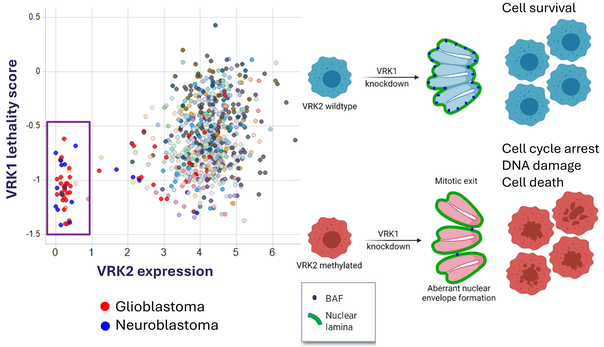
INTRODUCTION

Vaccinia-related kinases (VRKs) are a family of serine/threonine kinases that regulate diverse cellular processes, including transcription factor activity, chromatin remodeling, nuclear envelope formation, and cell-cycle progression. Among them, VRK1 has emerged as a paralog-selective synthetic lethal target in cancers with low VRK2 expression, encompassing nearly all neuroblastomas and over 60% of glioblastomas, with potential for therapeutic expansion into additional tumor types.

Here, we describe the biochemical, biophysical, and cellular assays that enabled the discovery and optimization of novel inhibitors of VRK1 activity found through variety of binding screens, activity-based screens, and rational design. High-resolution crystal structures of both VRK1 and VRK2 in complex with inhibitors revealed their binding poses, identified key interactions, and confirmed orthosteric binding. Medicinal chemistry optimization yielded chemical series capable of achieving >4000-fold selectivity over the VRK1 paralog VRK2 in a biochemical assay conducted with a physiologically relevant ATP concentration, and >70-fold selectivity for VRK2-deficient cells in cellular viability assays. Notably, strong correlations between biochemical potency, cellular target engagement, pharmacodynamic response, and functional viability supported both the specificity of the chemical matter and the robustness of the assay platform.

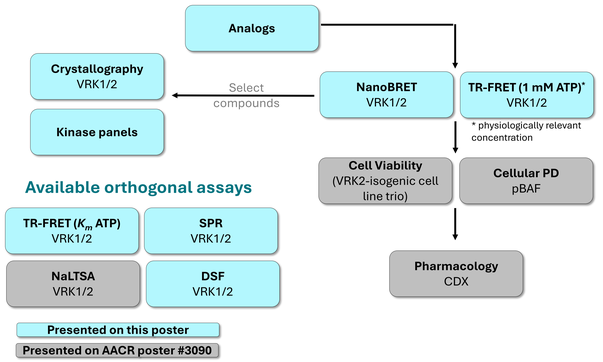
Together, these findings establish VRK1 as a tractable and structurally enabled target and demonstrate that high paralog selectivity in cells can be achieved through orthosteric inhibition. This work provides the strong foundation necessary for structure-guided optimization of VRK1 inhibitors with potential therapeutic applications in cancers with low VRK2 expression, such as glioblastoma and neuroblastoma.

VRK1 IS A SYNTHETIC LETHAL KINASE TARGET IN VRK2-LOW BRAIN CANCER

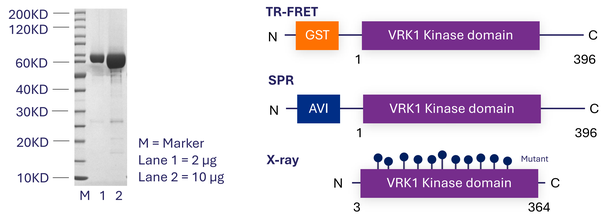


- ~66% malignant brain tumors have low VRK2 expression due to promoter methylation (TCGA). Most neuroblastomas are VRK2 low.
- VRK1 inhibition in VRK2-low cells causes aberrant nuclear envelope with blebbing, lobulation and micronucleation. VRK1 knockdown leads to cell cycle arrest, accumulation of DNA damage, and cell death

FULLY ENABLED SCREENING FUNNEL SUPPORTS VRK1 DRUG DISCOVERY

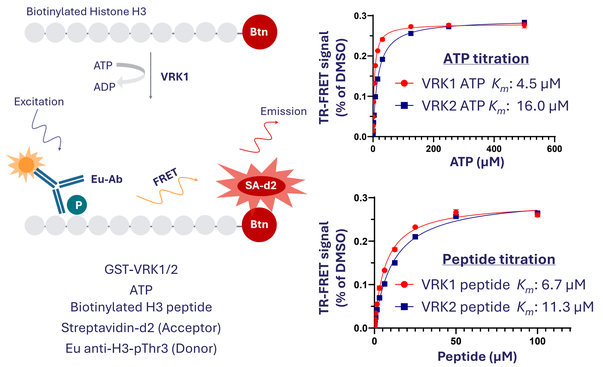


VRK1 AND VRK2 PROTEIN ROBUSTLY PRODUCED AND CHARACTERIZED IN BIOPHYSICAL AND BIOCHEMICAL ASSAYS

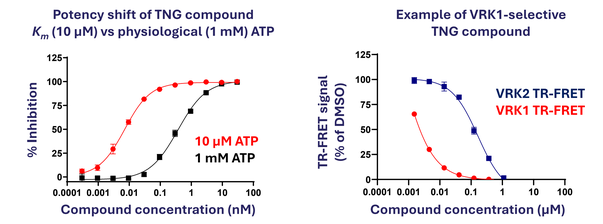


- Protein is >95% pure (SDS-Page), without post-translational modifications (LC-MS), and monomeric (Analytical SEC).

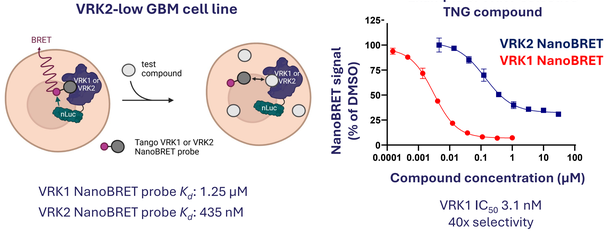
VRK1 AND VRK2 TR-FRET ASSAYS ESTABLISHED TO SUPPORT SAR DEVELOPMENT



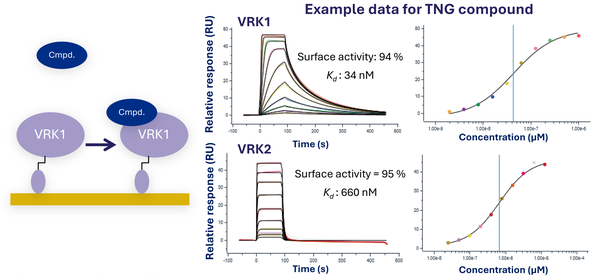
BIOCHEMICAL TR-FRET ASSAY DEVELOPED AT PHYSIOLOGICAL ATP CONCENTRATION TO REFLECT KINASE ACTIVITY IN CELLULAR CONTEXT



NANOBRET ASSAYS ESTABLISHED USING TANGO PROBES TO DETERMINE TARGET ENGAGEMENT AND CELLULAR SELECTIVITY

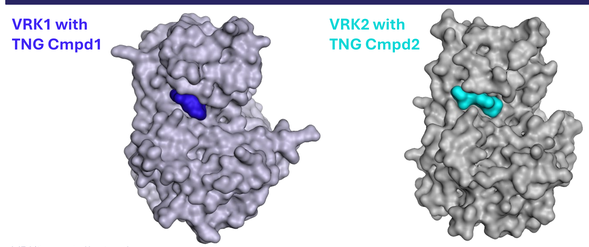


SPR ASSAY DEVELOPED TO EVALUATE COMPOUND BINDING KINETICS



- Full-length VRK1 or VRK2 immobilized on the chip surface
- High surface activity observed for both VRK1 and VRK2
- Enabled for selectivity assessment between VRK1 and VRK2
- Reproducible data on binding affinities and binding kinetics obtained

PROJECT ENABLED STRUCTURALLY WITH 170 CO-COMPLEX STRUCTURES OF VRK1 OR VRK2 WITH SMALL-MOLECULES OBTAINED



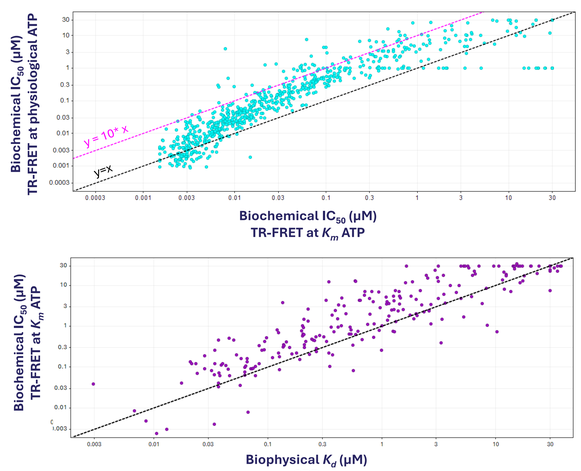
VRK1 crystallography:

- 158 unique VRK1 crystal structures were obtained via soaking and co-crystallization.
- Resolutions ranging from 1.8 to 3.3 Å, with median resolution of 2.4 Å.

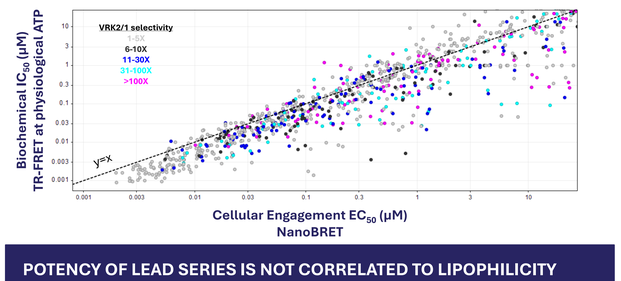
VRK2 crystallography:

- 12 unique VRK2 crystal structures were obtained through co-crystallization.
- Resolutions ranging from 1.7 Å to 2.7 Å, with a median resolution of 1.9 Å.

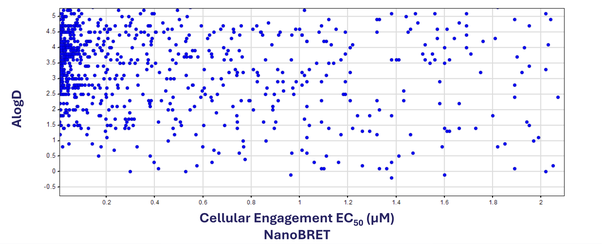
STRONG CORRELATION OBSERVED BETWEEN BIOCHEMICAL AND BIOPHYSICAL ASSAYS



BIOCHEMICAL ACTIVITY CORRELATES WITH CELLULAR TARGET ENGAGEMENT



POTENCY OF LEAD SERIES IS NOT CORRELATED TO LIPOPHILICITY



HIGH KINOME SELECTIVITY ACHIEVABLE WITHIN SERIES

Progressive Improvement in Kinome Selectivity within Lead Series

Eurofins ScanEdge at 1 µM compound (VRK1/2 are not represented in the panel)

NanoBRET VRK1 (µM)	0.02	NanoBRET VRK1 (µM)	0.03
TR-FRET at physiological ATP VRK1 (µM)	0.02	TR-FRET at physiological ATP VRK1 (µM)	0.008
TR-FRET at physiological ATP VRK2 (µM)	0.2	TR-FRET at physiological ATP VRK2 (µM)	4.4
Cell Viability VRK2 low (µM)	1.0	Cell Viability VRK2 low (µM)	2.2
Cell Viability VRK2 high (µM)	1.2	Cell Viability VRK2 high (µM)	>30

SUMMARY

- **VRK1 is a novel therapeutic target in brain cancers and neuroblastoma, particularly in tumors with low VRK2 expression and unmethylated MGMT (IHC).**
- **Strong drug discovery enablement, supported by excellent correlation across different assay formats and multiple protein–ligand crystal structures across diverse chemotypes.**
- **Excellent VRK1/2 biochemical selectivity translates into strong selectivity for VRK2-low cancer cells in viability assays.**
- **Large addressable patient population, including low-grade glioma, glioblastoma, and neuroblastoma.**
- **Biological validation is presented on AACR poster #3090**
- **VRK1 has been enabled for drug discovery and is available for partnering.**

References

1. Shields, J. A. et al. Cancer Res. 82, 4044–4057 (2022)

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