NUVISAN
The Science CRO

Methods for Characterization and Derisking of Small Molecule Hits and Leads
Learnings from RAS-SOS1 and Hippo Pathway Screens

Benjamin Bader, Volker Badock, Katrin Nowak-Reppel, Roman C. Hillig, Martin Lange, Jörg Weiske and Holger Steuber

NUVISAN ICB GmbH, Müllerstraße 178, 13353 Berlin, Germany

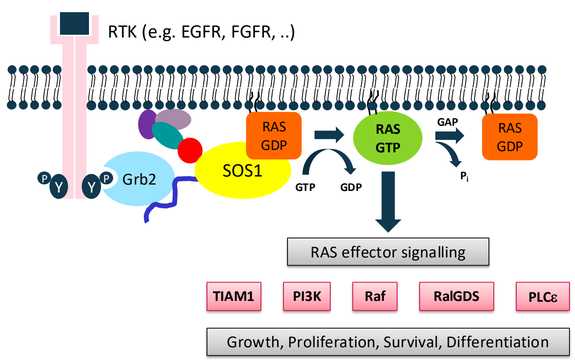
SCAN here to access NUVISAN posters →

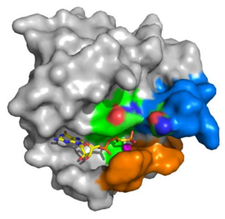
Successful drug discovery requires both, state-of-the-art technology platforms and in-depth experience of all disciplines involved along the value chain. Indicated by increasing attrition rates in drug discovery over the recent decades, various risks contribute to the success or failure of a drug discovery campaign. Potential liabilities could be linked to the validation of the target and disease hypothesis, low druggability, unsuitable assay systems, false hit selection, missing target engagement, toxicology and safety issues or lacking efficacy. We will discuss essentials based on our experience from both a biochemical and a cell-based HTS project.

Target significance

RAS-SOS1

Hillig et al 2018 PNAS
https://doi.org/10.1073/pnas.1812963116





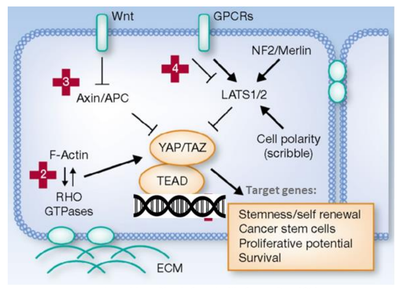
- 3 RAS isoforms (H-, N-, K-RAS) act as molecular switches, oscillating between GDP- and GTP-state
- 30% of human cancers feature RAS mutations (mainly positions 12 and 61) → render RAS constitutively active
- GxP bind with picomolar affinities, with millimolar concentrations in the cell → "undruggable target"

Figure 1. Target introduction

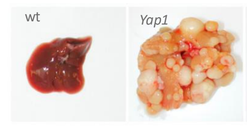
Hippo Pathway (YAP1/TAZ)

Graham et al 2024 Cell Chemical Biology
https://doi.org/10.1016/j.chembiol.2024.02.013

- Physiological functions: regulation of organ growth and size, cell proliferation and differentiation, embryogenesis, and tissue regeneration/wound healing
- Integration of upstream signaling, e.g. Wnt, GPCR and RHO
- YAP1/TAZ are overexpressed in human cancers, interact with TEAD transcription factors and activate target genes:
  - increased cell proliferation
  - resistance to apoptosis
  - induction of cell migration
- Therapeutic strategies that target dysregulated Hippo components might be promising approaches for the treatment of a wide spectrum of diseases



adapted from Piccolo S et al., Clin Cancer Res, 2013



Induction of liver cancer by *Yap1* overexpression in mouse

Liu-Chittenden Y et al., Genes Dev, 2012

Hit Identification

Parallel Hit Finding: HTS and Fragment Screening

HTS 3m cpds	SOS1-KRAS^{G12C} assay On-format
Retest 30.000 cpds	SOS1-KRAS^{G12C} assay On- / Off-format
IC50 3000 cpds	SOS1-KRAS^{G12C} assay On- / Off-format

KRAS^{G12C}-Raf interaction | KRAS^{wildtype}-SOS1 | KRAS^{G12C} intrinsic

KRAS^{G12C}-SOS1 interaction | Thermal shift assay KRAS^{G12C}, SOS1

Hitlist

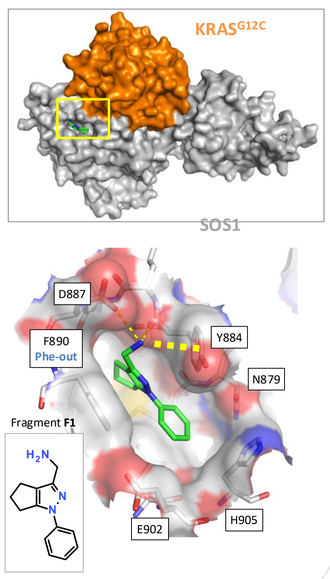
Fragment Screen (carried out at Evotec) of KRAS^{G12C}–SOS1 Complex by STD-NMR (Library of 3,000 Fragments)

310 Single Fragment Hits

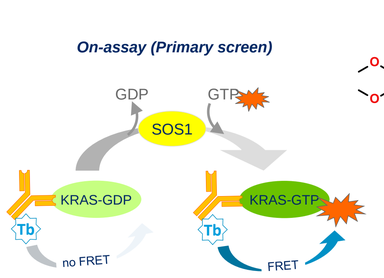
Hit Qualification: SOS1 and KRAS^{G12C} Binding Test (STD-NMR)

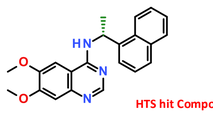
97 "Complex exclusive" Fragment Hits (42 clear hits, 55 ambiguous hits)

13 confirmed hits by soaking into KRAS-SOS1 complex crystals

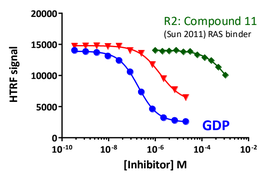


On-assay (Primary screen)

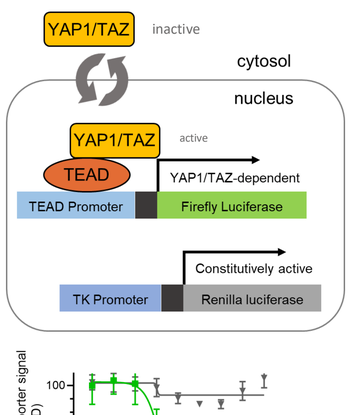




HTS hit Compound 1



Pathway Screen with Reporter Cell Line

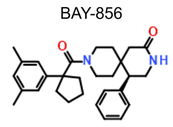


TEAD-luciferase pathway HTS (3.8 million compounds)

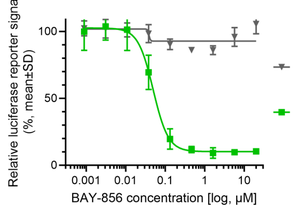
YAP1 cytoplasmic translocation (3994 hits)

YAP1/TAZ target gene expression (96 hits)

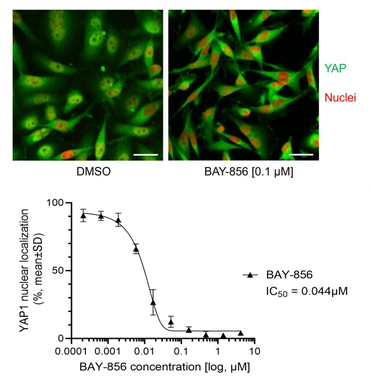
BAY-856



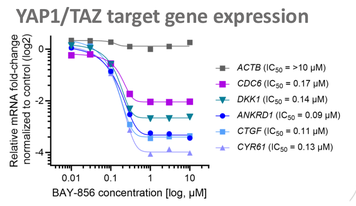
How does BAY-856 inhibit YAP1/TAZ ?



YAP1 cellular localization

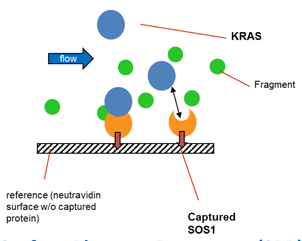


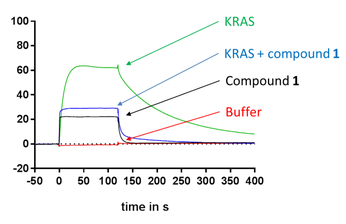
YAP1/TAZ target gene expression

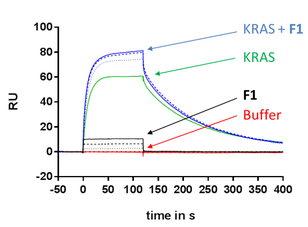


Derisking

Target Engagement & Mode-of-Action

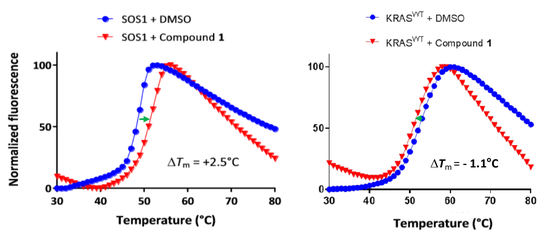




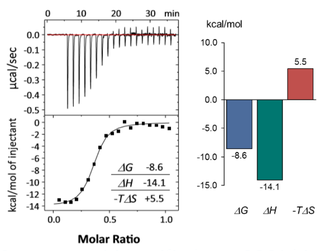


Surface-Plasmon-Resonance (SPR): Compound 1 is disrupting, but fragment 1 is stabilizing the KRAS-SOS1 interaction

Thermal Shift Assay (TSA)

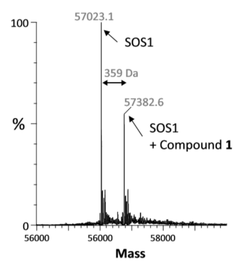


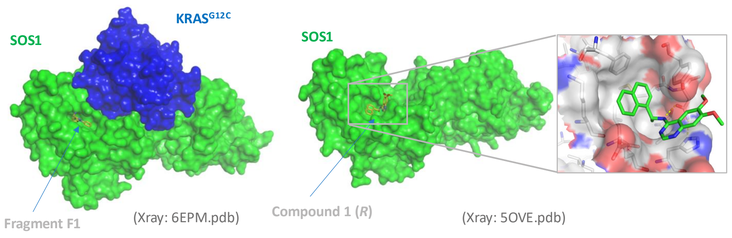
Isothermal Titration Calorimetry (ITC)



Thermal Shift Assay, Isothermal Titration calorimetry and native MS: Compound 1 identified as a SOS1 binder

Native Mass Spectrometry (MS)

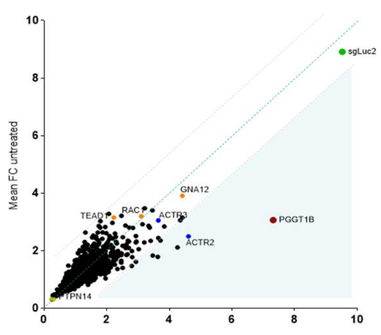




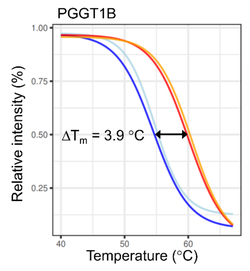
X-Ray: Fragment 1 and Compound 1 bind into same surface pocket of SOS1

Target Deconvolution: What is the Direct Target of BAY-856 ?

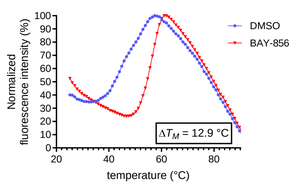
Pooled CRISPR/Cas9 screen in the TEAD-Luc reporter cell line Knock-down of ~ 6000 genes



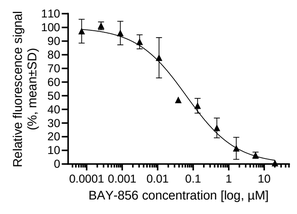
Cellular Thermal Shift Assay Mass Spectrometry (CETSA-MS)
(CETSA® experiments were carried out at Pelago Bioscience)



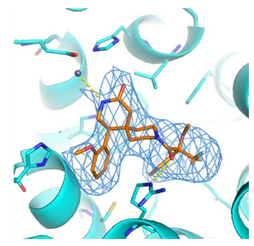
Thermal Shift Assay (TSA) purified PGGT1B

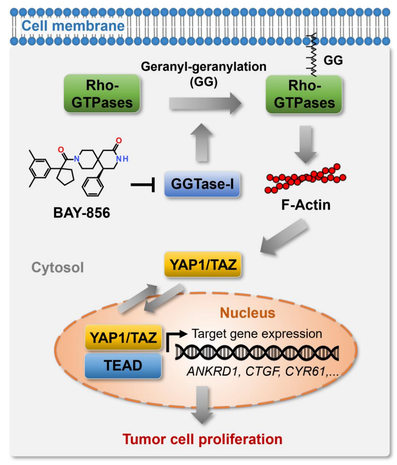


Biochemical GGTase-I assay



Target binding by X-ray crystallography





Lessons

Success factors for biochemical screens:

- Use more than one hit finding approach for low druggable targets
- Well established secondary and orthogonal assays for hit validation
- Biophysical confirmation of target engagement
- X-Ray support early on in the project

Success factors for cell-based phenotypic/pathway screens:

- Toxicity controls during primary / secondary HTS
- Well established secondary and orthogonal assays for hit validation
- Target deconvolution capabilities (CRISPR-KO, in silico, CETSA)
- Biochemical, biophysical and X-Ray confirmation of target interaction