small-scale *ROS1* Aberrations: functional impact and therapeutic potential

Moritz Glaser[1,3], Cornelia von Levetzow[1], Sebastian Michels[1], Lucia Nogova[1], Marianna Katzenmeier[1], Claudia Wömpner[1], Jaqueline Schmitz[1], Elisabeth Bitter[1], Inken Terjung[1], Elke Passmann[1], Diana Schaufler[1], Anna Eisert[1], Rieke Fischer[1], Richard Riedel[1], Sabine Hahne[1], Sabine Merkelbach-Bruse[2], Reinhard Büttner[2], Jürgen Wolf[1] and Matthias Scheffler[1]

[1]Department I of Internal Medicine, University Hospital of Cologne   [2]Institute of Pathology, University of Cologne   [3]presenting author declares no conflicts of interest

Background

ROS1-directed Tyrosine-Kinase-Inhibitors (TKIs) target efficiently **activating fusions in the *ROS1* proto-oncogene** in non-small cell lung cancer (NSCLC) patients. Besides solvent-front mutations (SFMs) in resistance to targeted therapy, *ROS1* **aberrations other than fusions remain biologically unexplored**. Driving on our thus far clinical investigations, we aimed at determining the **functional impact and the potential to act as a drug target**.

Methods

Tumor samples from NSCLC patients were screened with two next-generation sequencing (NGS) panels. Patients with activating *ROS1* fusions, SFMs and benign Single-Nucleotide Polymorphisms (SNPs) were excluded using the gnomAD database. The Provean and PolyPhen-2 (PP2) tools predicted the functional impact of the detected aberrations. ChimeraX and Pymol were used for drug binding analysis.

Results

Of 8072 patients analyzed by NGS between 2018 and 2022, 110 **(1.4%) patients harbored small-scale *ROS1* aberrations**. Our cohort consists of 113 aberrations leading to 95 **(84.1%) missense**, 12 (10.6%) truncating and 1 (0.1%) in-frame aberrations. In **10% of patients *ROS1* aberration was mutually exclusive** and most *ROS1* aberrations were transitions (55%).

PP2 predicts 75.5% of aberrations to be 'possibly' (6.4%), respectively **'probably damaging'** (68.1%, mean score 0.75, median score 0.999). **Provean predicts 50%** of aberrations to be **'deleterious'**. Polyphen-2 and Provean **coherently predict 46.8% of aberrations** to be **'deleterious'** respectively 'possibly/ probably damaging'.

Amid the tyrosine kinase domain almost all aberrations are predicted to be 'probably damaging' respectively 'deleterious' by both tools coherently and feature a higher-than-average score. Besides, no distinct molecular pattern occurs.

Figure 1 - Detected Subset of particularly deleterious Mutations

A **Comparison** of the PP2 Prediction with the Provean Prediction reveals a **distinct interval of consensus**, wherein **all mutations** are predicted to **have a deleterious effect on the protein function**.
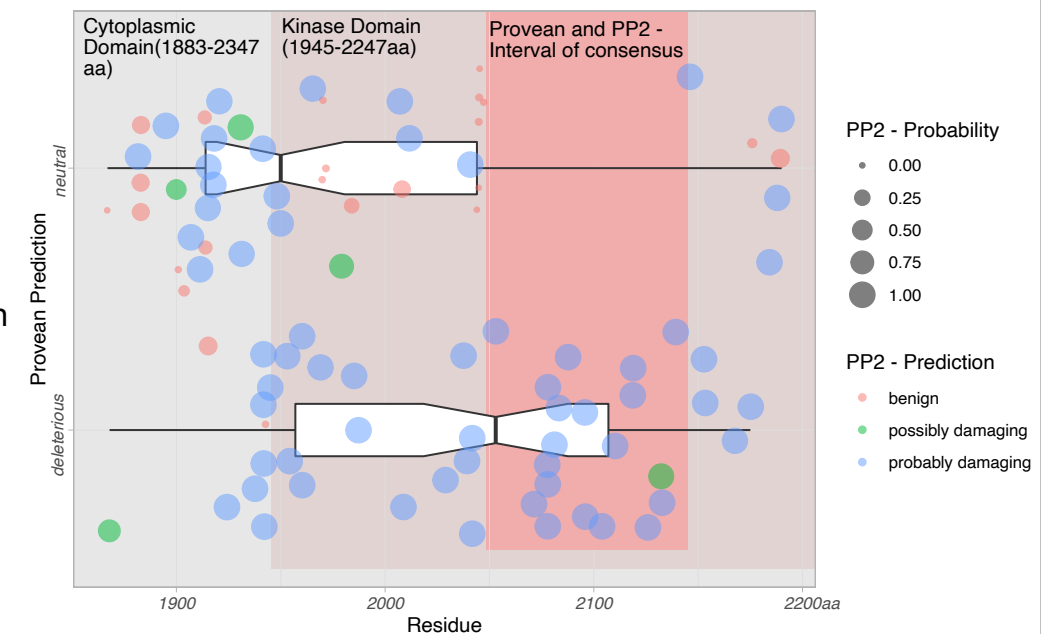

Conclusion

This evidence proves a **functional and deleterious impact of specific aberrations** and indicates a **high potential to be targetable**. We warrant further studies to elaborate these findings *in vitro* and *in vivo*.

Figure 2 - Mutations in the Drug-Binding Pocket

Comparative Analysis - binding of wild-type residue M2029 (left) and mutant residue M2029T (right) with Lorlatinib (top) or Crizotinib (bottom)
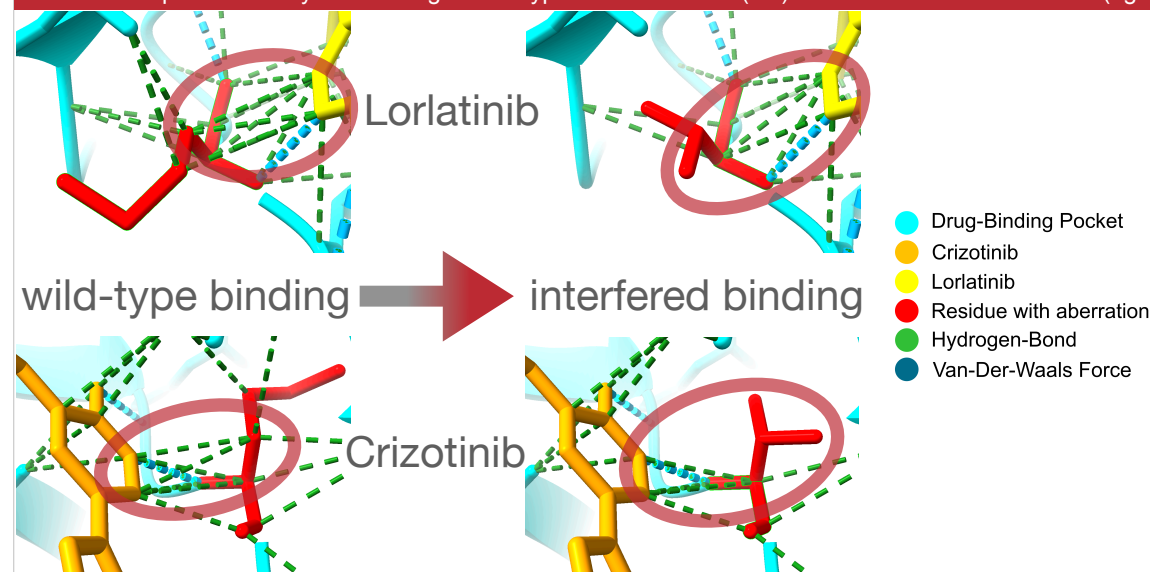
The **M2029T (and R2083T) missense mutations interfere with single hydrogen-bonds and Van-Der-Waals forces in** the *ROS1* **drug-binding pocket** between the respective residues and the TKIs crizotinib and lorlatinib. However, drug binding analysis revealed that the **overall binding of the TKIs is not affected**.

Contact Information

🐦 @MoritzGls

🐦 @trnsltnl

CIO
Center for Integrated Oncology
Aachen Bonn Cologne Duesseldorf