The return of RET GateKeeper mutations? An In-silico exploratory analysis of potential resistance mechanisms to novel RET macrocyclic inhibitor TPX-0046

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Abstract

Purpose: TPX-0046 is designed to overcome resistance to FDA approved RET inhibitors Selpercatinib and Pralsetinib. Early prediction of resistance mechanisms to investigational drugs may facilitate subsequent drug and trial designs. This study aims to predict potential mutations inducing resistance to TPX-0046.

Materials and methods: We conducted an in-silico analysis of TPX-0046 macrocyclic structure and predicted the binding mode on RET. We used as reference literary examples of resistance mechanisms to other macrocyclic inhibitors (Lorlatinib on ALK/ROS1) to construct RET secondary resistance mutations. We conducted docking simulations to evaluate impact of mutations on TPX-0046 binding.

Results: TPX-0046 binding mode on RET appears to not be influenced by Solventfront G810X mutation presence. Bulky Gatekeeper V804X mutations affect predicted TPX-0046 binding mode. Mutations in Beta 7 strand region L881F and xDFG S891L impair TPX-0046 docking.

Conclusions: Our findings suggest that development of second generation RET inhibitors focused mainly on Solventfront G810X mutations granting resistance to selective RET inhibitors Selpercatinib and Pralsetinib. If these findings are confirmed by identification of Gatekeeper V804X mutations in patients progressing to TPX-0046, explanation of acquired resistance and loss of benefit will be easier. These findings might accelerate development of third generation RET inhibitors, as well as clinical trial design in precision oncology settings.

Key Points

Question: To predict resistance mutations to second generation RET inhibitor TPX-0046 before observation in patients.

Findings: TPX-0046 binding mode on RET appears vulnerable to gatekeeper V804X mutations as well as L881F and S891L mutations.

Meaning: The development of second generation RET inhibitors focused on Solventfront G810X mutations granting resistance to Selpercatinib and Pralsetinib. The identification of Gatekeeper V804X mutations in patients progressing to TPX-0046 might explain acquired resistance and loss of benefit. If confirmed these findings might guide development of third generation RET inhibitors.

Background

In recent years the gene Rearranged during Transfection (RET), which encodes for a tyrosine-kinase selectively expressed in neuroendocrine healthy tissue, has raised a great deal of interest as a therapeutic target in cancer patients. Its activating alterations are detected with considerable frequency in medullary thyroid cancers (MTC, usually activating mutations, 70%), papillary thyroid cancer (PTC, gene fusions, 10-20%) and in 1-2% of Non-Small Cell Lung Cancer (NSCLC, gene fusions) [1], [2].

Unfortunately, the first therapeutical strategies employing multi-kinase inhibitors (MKIs) showed only little activity in RET-mutant MTC and RET-fusion positive NSCLC. For this reason, selective inhibitors were developed and demonstrated striking activity in tumors harboring RET alterations. In particular, two compounds are presently approved in the treatment of RET-altered cancers: Pralsetinib (BLU-667) and Selpercatinib (LOXO-292) [3][4][5].

In the phase 1/2 ARROW trial, Pralsetinib demonstrated an Overall Response Rate (ORR) of 61% and 70% in previously treated and treatment-naïve RET fusion-positive NSCLC, respectively [4]. In another cohort of the same trial, the ORR was 71% in patients with treatment-naïve RET-mutant MTC, 60% in patients who had previously received MKI Cabozantinib or Vandetanib, or both, and 89% in patients with RET fusion-positive thyroid cancer [5].

In a phase 1/2 study in RET-altered thyroid cancers, Selpercatinib showed an ORR of 69%, 73% and 79% in previously treated RET-mutant MTC, treatment-naïve RET-mutant MTC and previously treated RET-fused thyroid cancer [2]. Good activity was also seen in RET fusion-positive NSCLC patients (ORR 64%) and, notably, the intracranial ORR was 91% [6].

Despite the striking results of the two RET-specific TKIs and their design intrinsically capable of overcoming gatekeeper mutations (V804L/M), the acquisition of secondary resistance mutations has been described, especially solvent-front G810X mutations [7], [8]. In this perspective, a new generation macrocyclic RET/SRC-inhibitor (TPX-0046) was developed and is being tested in early-phase trials [9].

Unfortunately, the natural history of TKI treatments suggests even compounds belonging to the macrocycle class will eventually elicit evolution of secondary resistance mutations as a survival mechanism in cancer cells.

In recent years multiple macrocyclic TKI inhibitors have been discovered, among these Lorlatinib has recently achieved FDA approval for ALK rearranged NSCLC, clinical trials are ongoing for others such as Repotrectinib, Selitrectinib and TPX-0046.

Literary examples of secondary acquired resistance to Lorlatinib show recurrent compound kinase mutations such as Gatekeeper (GK, ALK L1196M) plus SolventFront (SF, ALK G1202R) or Solventfront+1 (SF+1, D1203N) [10], [11], alternatively SF or SF+1 plus xDFG mutation (G1269A) [12]. Additionally bulky hydrophobic mutations of a conserved beta7 leucine (B7L) can confer acquired resistance to Lorlatinib both on ALK (L1256F) and ROS1 (L2086F) [13], [14].

Based on these observations we conducted an in-silico exploratory analysis of TPX-0046 structure and employed structural modeling together with analogies with other notable examples of widely used macrocyclic inhibitors to predict which RET on-target alterations are more likely to confer resistance to TPX-0046

Materials And Methods

The Protein Data Bank (PDB) database was queried for available kinase-macrocyclic complexes to be used as references, Lorlatinib-ALK (5AA9), Lorlatinib-ROS1 (4UXL) and Repotrectinib-TRKa (7VKN) were thus obtained, all structures shared DFG-Din conformation.

Based on the afore mentioned observation the PDB database was queried for RET structures displaying the DFG-Din configuration, ligand deprived 2IVU was identified as the most suitable target for docking analysis requiring only minor adjustments in D892 orientation.

Both RET wildtype and RET secondary mutants (GK+SF, GK, SF+xDFG and B7L) were constructed with Pymol to be used for blind docking.

TPX-0046 formula was retrieved from Selleckchem.com, tridimensional conformers of this structure were generated with DataWarrior.dmg with Random, low energy bias algorithm [15]. Docking of conformers on purposely built RET structures was performed multiple times for every target with AutoDock Vina [16]. Docking result analysis was conducted with Pymol, docking on WT RET was considered successful if TPX-0046 conformer would be comparable to examples of other macrocycles. Docking on RET mutants was considered successful if at least one simulation result was superimposable to the predicted binding pose on WT RET.

Results

Docking of TPX-0046 on WT RET was successful (Figure 1). Analysis of docking pose showed the drug is predicted to engage in hydrogen bonding with amino group of A807 in the hinge region and contact via hydrophobic interactions GK, SF, xDFG and B7L residues. This binding pose was in line with what observed in other available macrocycle examples, namely Lorlatinib and Repotrectinib, and was used as a reference for subsequent docking on mutant targets.

Docking was successful on G810S (SF), S811N (SF+1) and G810S + S891A/T (SF + xDFG), as predicted binding pose was superimposable with the reference configuration on WT RET. Whereas docking on RET V804M/L (GK), V804M+G810S (SF + GK), G810S + S891L (SF + xDFG) and L881F (B7L) was unsuccessful on multiple iterations (Figure 1).

Discussion

In this in-silico exploratory analysis we employed structural modeling to predict RET on target mutations that could impair TPX-0046 binding.

Our computational model of TPX-0046 RET interaction is consistent with other available literary examples of macrocyclic TKIs bound to their target kinases. Docking of TPX-0046 tridimensional conformer was successful equally on WT RET and on SF G810S mutant, this is expected since this drug was purposely built to overcome this resistance mutation.

We subsequently designed various RET secondary mutant structures in order to reflect available examples of mutations conferring secondary acquired resistance to macrocyclic TKIs.

This analysis suggests the inability of TPX-0046 to bind RET when bulky hydrophobic GK or B7L mutation are present. Whereas effect of compound SF plus xDFG mutation appears to be limited, since only S891L was found to impair TPX-0046 docking on RET.

Based on these results we predict bulky GK mutations (V804L/M), and to a lesser extent L881F or G810S + S891L, could be a frequently observed on target acquired resistance mutation in patients experiencing PD after clinical benefit to TPX-0046.

This exploratory in-silico analysis has a number of limitations, ranging from the absence of reference conformers of TPX-0046, to the methods used for generating mutant protein targets, and finally to the docking algorithm used that is unable to perform flexible docking for macrocyclic structures. This last limitation however was secondary to our need to rely entirely on free, and readily available software.

Further research such as Ba/F3 cell models, or targeted NGS for patients experiencing PD to TPX-0046 might confirm our hypothesis.

If the scenario where GK mutations are a frequent secondary resistance mechanism to TPX-0046 is confirmed this might pose considerable challenges in the future for sequential TKI treatment strategies in patients with RET altered tumors. While Pralsetinib and Selpercatinib are able to overcome bulky GK mutations but are vulnerable to SF mutations, TPX-0046 might show the opposite activity spectrum being able to overcome SF mutations, but remaining vulnerable to GK mutations. Meanwhile GK and SF tolerant RET inhibitors have currently entered Phase-I clinical trials (TAS0953) [17].

Declarations

Author declarations

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not applicable.

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all data and materials are contained in the manuscript.

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All the authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Matteo Repetto and Edoardo Crimini. The first draft of the manuscript was written by Matteo Repetto and Edoardo Crimini and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

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Figures

Figure 1

A TPX-0046 structure, B: docking of TPX-0046 tridimensional conformer on WT RET, target obtained from modified ligand deprived 2IVU, C: bidimensional projection of predicted TPX-0046-RET interactions, Hydrogen bonds are shown with green dotted lines, Hydrophobic interactions are shown with red segments, D: Steric clash with bulky V804M (gatekeeper) highlighted as red sferes, E: Steric clash with bulky L881F (B7L) highlighted as red sferes, F: Steric clash with S891L (xDGF) highlighted as red sferes, G: Table showing failed and successful dockings of TPX-0046 conformers.