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Repotrectinib resistance in a Ba/F3 SLC34A2-ROS1 cell line

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Background:

- ROS1+ non-small cell lung cancer (NSCLC) is treated with tyrosine kinase inhibitors (TKI) such as crizotinib and lorlatinib.
- TKI Treatment eventually leads to resistance due to either on- or off-target mutations[1,2].
- Repotrectinib is a next-generation TKI that might overcome on-target resistance.
- No on-target resistance mutations were found in first line treatment with repotrectinib (in 14 patients)[3] .

Aim:

To generate a repotrectinib resistant Ba/F3 SLC34A2-ROS1 cell line by long term culture and study occurrence of on-target resistance mechanisms.

Results:

- Ba/F3 SLC34A2-ROS1 cells were sensitive to repotrectinib (IC50=0,16nM) confirmed by MTS and Western blotting for phosphorylation of ROS1 *(Figure 2)*.
- Acquired repotrectinib resistance was induced via long term treatment with repotrectinib and resulted in cell growth at a 100-fold increase of the IC50 *(Figure 3)*.
- Withdrawal of repotrectinib affected cell morphology while the cells remained resistant to repotrectinib *(Figure 4)*.
- Sanger sequencing of the R0 cells and three monoclonal cell lines created after limiting revealed no on-target resistance mechanisms *(Figure 5)*.

Discussion and Conclusions:

- Long term repotrectinib culture resulted in resistance to repotrectinib in Ba/F3 SLC34A2 ROS1 cells with an IC50 ~70-fold higher than parental cells.
- No on-target resistance mutations were identified in R0 and the three monoclonal derived cell lines (R1-R3). Additional clone analysis is ongoing.
- TKI resistance is likely cause by off-target mechanisms.

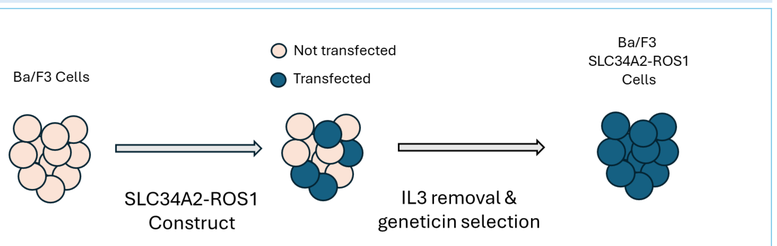

Figure 1: Generation of a Ba/F3 cell line overexpressing SLC34A2-ROS1 fusion protein using the pCDNA3.4 vector.

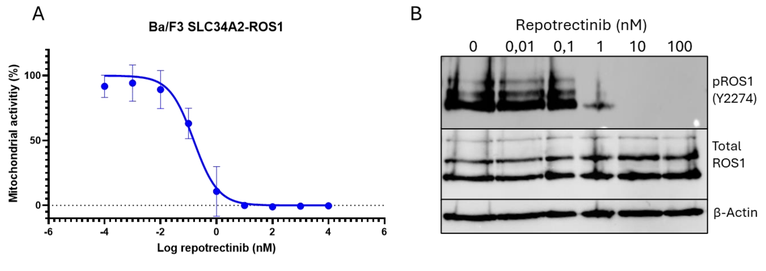

Figure 2: Analysis of sensitivity of Ba/F3 SLC34A2-ROS1 cells to reprotectinib. (A) Sensitivity to repotrectinib was measured using an MTS assay. IC50 value was calculated at 0,16nM (Celltiter proliferation assay, Promega, N=4, average ± SEM). (B) Western Blot analysis of phosphorylated ROS1 and total ROS1 in whole cell lysate from cells treated with repotrectinib for 3 hours. β-actin was used as a loading control. (Cell Signalling Technology, D4D6 and #3078 antibodies).

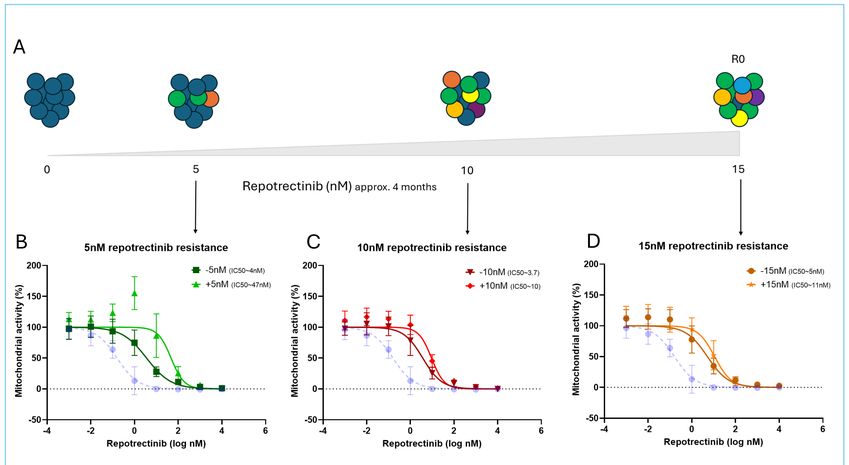

Figure 3: Inducing repotrectinib resistance in Ba/F3 SLC34A2-ROS1 cells via long term treatment. (A) Schematic overview of long-term treatment with increasing repotrectinib concentrations and time points of testing the IC50. The colors represent cells with hypothetical different resistance mutations in the cells, which can either be on- or off-target. (B-D) Sensitivity against repotrectinib with or without continuous pressure (Cell titer proliferation assay, Promega, N=4, average ± SEM). Dotted line represents parental cell line.

References:

1. Lin JJ, Shaw AT. Recent Advances in Targeting ROS1 in Lung Cancer. *J Thorac Oncol.* 2017 Nov;12(11):1611-1625. doi: 10.1016/j.jtho.2017.08.002.
2. Shaw AT, Ou SH, Bang YJ, Camidge DR, et Al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med.* 2014 Nov 20;371(21):1963-71. doi: 10.1056/NEJMoa1406766.
3. Drilon A, Camidge, DR, Lin JJ, et Al. Repotrectinib in ROS1 Fusion-Positive Non-Small-Cell Lung Cancer. *N Engl J Med.* 2024 Jan 11;390(2):118-131. doi: 10.1056/NEJMoa2302299

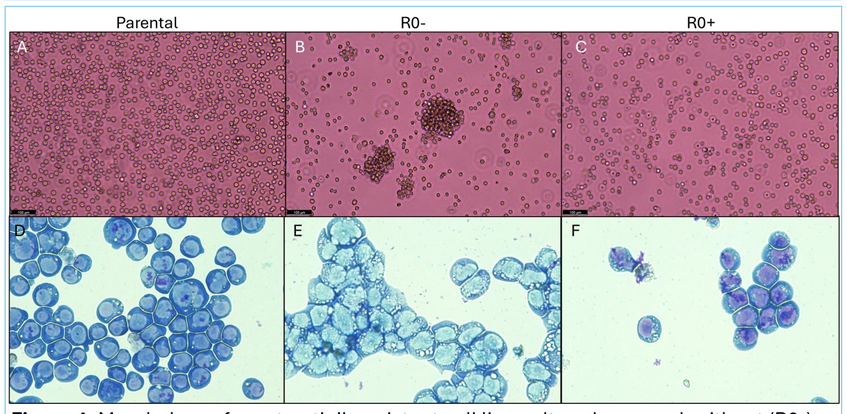

Figure 4: Morphology of repotrectinib resistant cell line cultured one week without (R0-) or with (R0+) 15nM repotrectinib compared to the parental cell line. (A-C) Image of cells at a 10x10 magnification. (D-F) MGG staining at a magnification of 40x10.

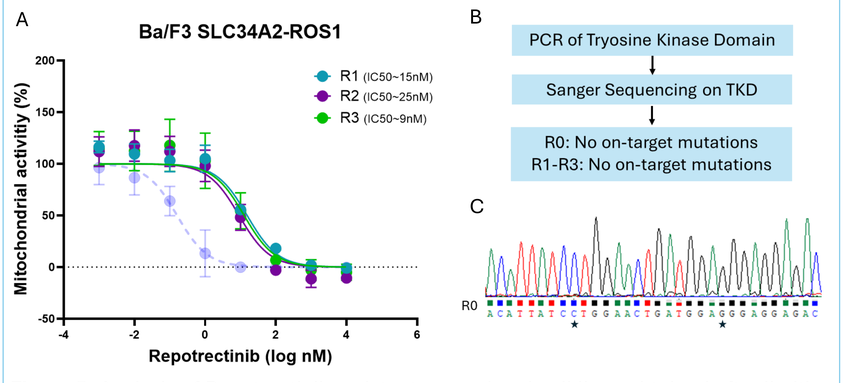

Figure 5: Analysis of Repotrectinib resistant monoclonal cell lines obtained after limiting dilution from resistant cell line R0. (A) Sensitivity against repotrectinib (Cell titer proliferation assay, Promega, N=4, average ± SEM), dotted line represents parental cell line.. (B) Sanger sequence results of the tyrosine kinase domain from all cell lines. (C) Chromatogram of R0 sequencing of TKD, star indicates hotspots for on-target mutations (L2026M C>A and G2032R G>C), analysis did not show heterogeneity.

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