

SLC34A2-ROS1[G2032R]/BaF3

CBP73192

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I. Introduction

Cell Line Name: SLC34A2-ROS1[G2032R]/BaF3

Host Cell: Ba/F3

Stability: 16 passages (in-house test, that not means the cell line will be instable beyond the passages we tested.)

Application: Anti-proliferation assay and PD assay

Freeze Medium: 90% FBS+10% DMSO

Complete Culture Medium: RPMI-1640+10%FBS

Mycoplasma Status: Negative

II. Background

Approximately 2% of lung tumors harbor ROS1 fusions (Bergethon et al. 2012). Like ALK fusions, ROS1 fusions are more commonly found in light smokers (<10 pack years) and/or never-smokers. ROS1 fusions are also associated with younger age and adenocarcinomas (Bergethon et al. 2012).

In preclinical models, ROS1 fusions are associated with sensitivity to tyrosine kinase inhibitors that have 'off-target' activity against ROS1, such as crizotinib (Bergethon et al. 2012; Davies et al. 2012). In addition, two patients—a previously treated metastatic NSCLC patient and a 65-year-old never smoker NSCLC patient—with tumors harboring ROS1 fusions have had partial responses to crizotinib (Bergethon et al. 2012; Davies et al. 2012). In an expansion cohort of a phase I study, 50 patients



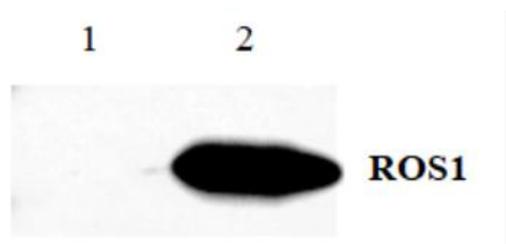
with ROS1-positive NSCLC demonstrated a 72% response rate and 19.2-month median progression-free survival interval when treated with crizotinib (Ou et al. 2013; Shaw et al. 2014). In a European case study, 32 ROS1-positive NSCLC cases treated with crizotinib were retrospectively reviewed, and an 80% response rate and a 9.1-month median progression-free survival interval was calculated in this cohort (Mazières et al. 2015).

Several different ROS1 rearrangements have been described in NSCLC. These include SLC34A2-ROS1, CD74-ROS1, EZR-ROS1, TPM3-ROS1, and SDC4-ROS1 (Figure 1; Davies et al. 2012; Rikova et al. 2007; Takeuchi et al. 2012). Clinically, the presence of a ROS1 rearrangement is detected by fluorescence in situ hybridization (FISH) with a ROS1 breakapart probe. FISH testing is not able to discern which particular ROS1 fusion is found in a clinical sample.

ROS1 rearrangements are non-overlapping with other oncogenic mutations found in NSCLC (e.g., EGFR mutations, KRAS mutations, ALK fusions, etc.; Bergethon et al. 2012).

III. Representative Data

1. WB of SLC34A2-ROS1[G2032R]/BaF3 expression



1. BaF3 host cell line
2. SLC34A2-ROS1 G2032R/BaF3

Figure 1. Protein Expression of ROS1 detected by antibody



2. Sanger Sequencing of SLC34A2-ROS1 Fusion and G2032R mutation

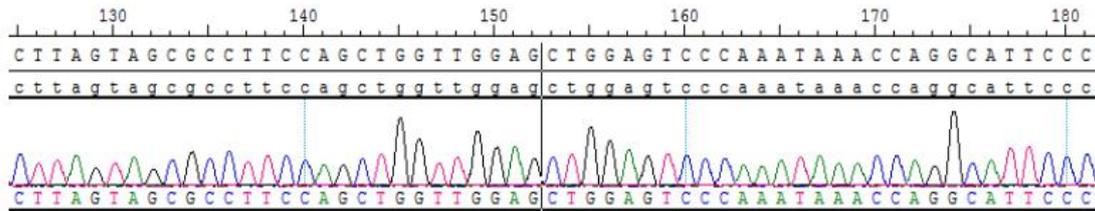


Figure 2. SLC34A2-ROS1 Fusion

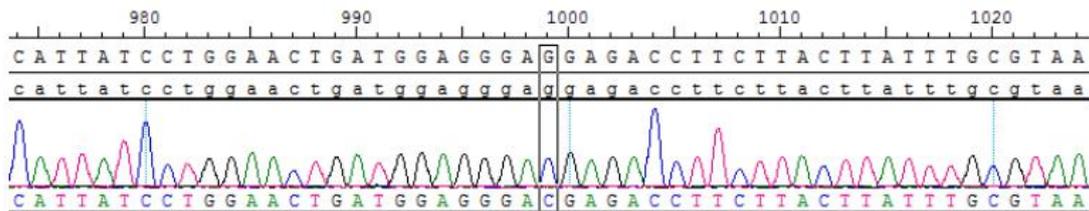
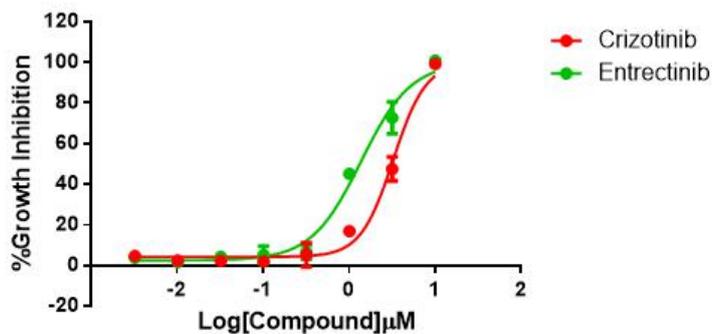


Figure 3. ROS1 p.G2032R

3. Anti-proliferation assay



Compound	GI50 (μM)
Crizotinib	3.258
Entrectinib	1.378

Figure 4. Anti-proliferation assay of two reference compounds on the SLC34A2-ROS1[G2032R]/BaF3 Stable Cell Line.



IV. Handling Procedure for Flask Cultures

The flask was seeded with cells grown and completely filled with medium at Cobioer.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination.

Using an inverted microscope (preferably equipped with phasecontrast optics), carefully check for any evidence of microbial contamination.

2. Incubate the flask in an upright position for several hours at 37°C.

After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 1000 rpm for 5 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 ml of this medium.

3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 3×10^5 viable cells/ml in the shipping medium.

4. Incubate the culture, horizontally at 37°C in a 5% CO₂ in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

V. Subculturing Procedure

Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 3×10^5 viable cells/mL. Do not allow the cell density to exceed 2×10^6 cells/mL.

Interval: Maintain cultures at a cell concentration between 3×10^5 and 2×10^6 viable cells/mL. Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density)



VI. Cryopreservation Procedure

1. Transfer cell suspension ($4-8 \times 10^6$ cells) to a centrifuge tube and spin at approximately 1000rpm for 5 minutes.
2. Discard supernatant and resuspend cells in cryopreservation medium (90% FBS+10%DMSO).
3. Transfer the cells into Freezing Tube, 1ml/vial.
4. Place Freezing Tube in a Frosty container that is kept at room temperature and has sufficient isopropanol.
5. Place the Frosty container into the -80°C freezer overnight.
6. On next day, transfer the vials into liquid nitrogen.

VII. Performing the Anti-proliferation Assay

1. Materials to Be Supplied by the User

- Compounds to be tested
- RPMI-1640 (Gibco #C11875500CP)
- FBS (Gibco #10099-141C)
- DMSO
- CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Cat.No.: G7573)
- 96 Well Assay Plate (White Plate, Clear Bottom with Lid Tissue Culture Treated Polystyrene 1/Pack, Corning #3610)
- T25 Flask
- 96 Well Storage Microplate(Corning#3357)
- Synergy H1 Hybrid Multi-Mode Reader(Biotek)

2. Protocol for the Anti-proliferation Assay



- 1) Take logarithmic growth cells, centrifuge and discard the culture supernatant, resuspend the centrifuged cells in fresh RPMI-1640+10%FBS medium.
- 2) Inoculate the resuspended cells into a 96-well cell culture plate with a white wall and transparent bottom, 100ul/well of cell suspension, inoculate two culture plates, and place in a 37°C cell incubator for overnight culture.
- 3) The next day, take one of the 96-well plates inoculated with cells, add 100ul/well cell titer glo detection reagent and leave it for 60 minutes, read the value, and define it as G0 data.
- 4) Take another parallel plate, and take 11.1 ul/well of compound from the previously diluted 10*compound concentration dilution plate to this plate , And set up another DMSO control well, continue to incubate in a 37°C cell incubator for 72 hours.
- 5) Take out the 96-well plate that has been treated with the compound for 72 hours from the incubator, add 100ul/well cell titer glo detection reagent and leave it for 60 minutes, read the value, and define it as G3 data.
- 6) Calculate the cell proliferation rate corresponding to each well according to the following formula $\%Proliferation = (\text{Test compound well G3-G0 average value}) / (\text{DMSO control well G3 average value-G0 average value}) * 100$.
- 7) According to the corresponding proliferation rate and concentration of each gradient concentration hole, use Prism Graphpad5.0 software to fit the gradient curve of cell proliferation, and calculate the GI50 of the compound (GI50 is defined as the corresponding compound concentration when the cell proliferation rate is 50% , The fitting formula



in the software is as follows: $Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1 + 10^{-(\text{LogIC50} - X) * \text{HillSlope}})}$.

VIII. References

1. Fusion of FIG to the receptor tyrosine kinase ROS in a glioblastoma with an interstitial del(6)(q21q21). (PMID: 12661006) Charest A ... Housman D Genes, chromosomes & cancer 2003
2. Characterization of ROS1 cDNA from a human glioblastoma cell line. (PMID: 2352949) Birchmeier C ... Wigler M Proceedings of the National Academy of Sciences of the United States of America 1990
3. Variations of specific non-candidate genes and risk of myocardial infarction: a replication study. (PMID: 19709766) Koch W ... Kastrati A International journal of cardiology 2011
4. Personalized smoking cessation: interactions between nicotine dose, dependence and quit-success genotype score. (PMID: 20379614) Rose JE ... Uhl GR Molecular medicine (Cambridge, Mass.) 2010 3 40
5. Genetic susceptibility to distinct bladder cancer subphenotypes. (PMID: 19692168) Guey LT ... EPICURO/Spanish Bladder Cancer Study investigators European urology 2010

