KIF5B(E15)-RET(E12)-Long/BaF3

CBP73195

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I. Introduction

Cell Line Name: KIF5B(E15)-RET(E12)-Long/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+2ug/ml puromycin

Mycoplasma Status: Negative

II. Background

Chromosomal rearrangements involving the gene that encodes the RET tyrosine kinase are known oncogenic drivers in 1% to 2% of patients with non–small cell lung cancer (NSCLC). These RET rearrangements occur with characteristic partners, most commonly KIF5B, but also CCDC6, NCOA, TRIM33, CUX1, KIAA1217, FRMD4A, and KIAA1468. They are typically identified in young patients with adenocarcinoma histology and minimal smoking history. Therapeutic targeting of RET-fusion–driven NSCLCs has taken the form of treatment with broad-spectrum tyrosine kinase inhibitors with anti-RET activity, such as cabozantinib (Cabometyx; Cometriq), vandetanib (Caprelsa), lenvatinib (Lenvima), RXDX-105, and sunitinib (Sutent). Cabozantinib and vandetanib have been the most heavily studied multi-kinase inhibitors

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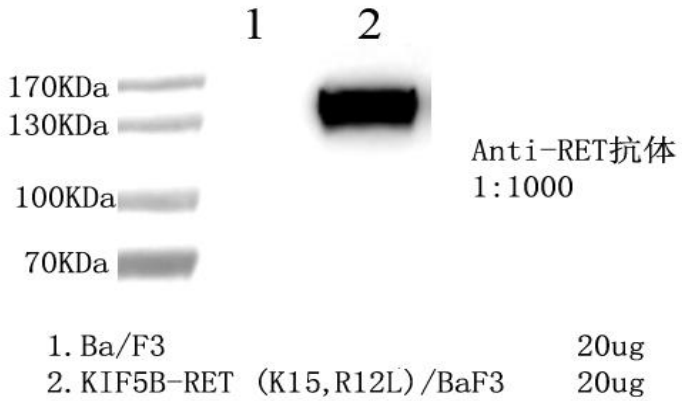
(MKIs), with response rates of 20% to 50% in largely pretreated patients with RET-rearranged NSCLC. Sunitinib has been used in fewer patients to date with initial results demonstrating a 22% response rate. RXDX-105 has exhibited uniquely impressive response rates (75%) in patients with non–KIF5B-RET-fusion NSCLC, compared with 0% response in patients with KIF5B-RET-fusion–positive NSCLC. BLU-667 has demonstrated an objective response rate of 50% in patients with RET-fusion positive NSCLC, and LOXO-292 reported a 74% ORR in patients with RET-fusion positive NSCLC. Notably, RXDX-105, BLU- 667, and LOXO-292 have all demonstrated some central nervous system activity in these early phase trials. Future directions of RET inhibition in patients with RET-rearranged NSCLC include additional clinical validation of the next generation RET-selective inhibitors RXDX-105, BLU-667, and LOXO-292 and comparing multikinase inhibitors with RET-selective inhibitors to determine the optimal sequencing of RET-targeted therapies.

III. Representative Data

1. WB of KIF5B(E15)-RET(E12)-Long/BaF3



2. Anti-proliferation assay

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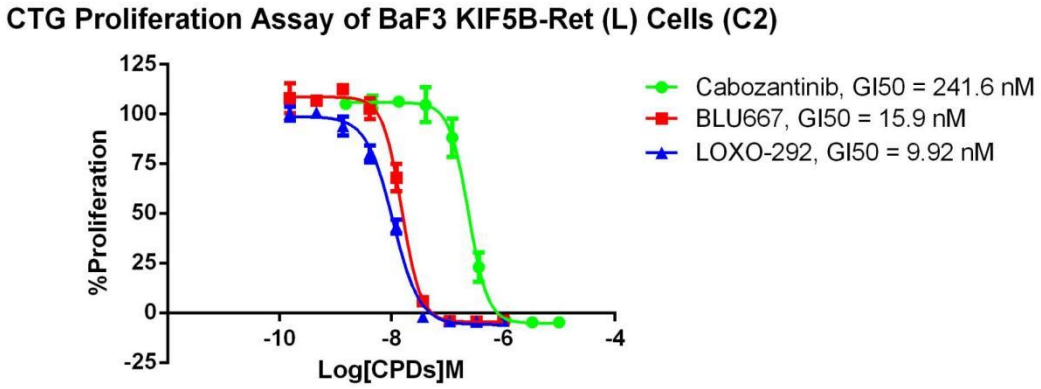

Figure 2. CTG Proliferation Assay of BaF3 KIF5B-Ret (L) Cells (C2).

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% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

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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($4-8*10^6$ 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

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- 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 = (Test compound

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well G3-G0 average value) / (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=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)).

VIII. References

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