Ba/F3 ETV6-NTRK3 G623E Cell Line

Cat. No: KC-1388 Version 19072001

Ⅰ. Cell Line Information

Catalog number	KC-1388
Cell line name:	Ba/F3 ETV6-NTRK3 G623E Cell Line
Gene ID/Accession #:	NM_001007156.2
Host cell line	Mouse Ba/F3 cell line
Cell type:	Pro-B cells
Description:	Stable Ba/F3 clone expressing exogenous ETV6-NTRK3 fusion protein bearing G623E mutation in NTRK3part.
Quantity:	One vial of frozen cells ($5X10^6$ per vial)
Stability:	Stable in culture over a minimum of 10 passages
Application:	Drug screening and biological assays
Freeze medium:	70% RMPI-1640 + 20% FBS + 10% DMSO
Propagation medium:	RPMI 1640 + 10% FBS
Selection marker:	Puromycin
Morphology:	Mostly single, round (some polymorph) cells in suspension
Subculture:	Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10^5 cells/ml
Incubation:	37 °C with 5% CO_2
Storage:	Frozen in liquid nitrogen with 70% medium, 20% FBS and 10% DMSO
Doubling time:	Approximately 20 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

Ⅱ. Background

NTRK3, also named Tropomyosin receptor kinase C (TrkC), is the high affinity catalytic receptor for the neurotrophin and play a major role in neuronal differentiation and survival, the overactivation or overexpression of NTRK3 fusion protein, due to chromosomal rearrangement, was originally found in congenital fibrosarcoma and subsequently found in secretory breast cancer and other type cancer, the identification of NTRK3 fusion genes as driver genes has led to the rapid development of anticancer therapeutics agents.

Ba/F3 cell, a murine interleukin-3 dependent pro-B cell line, is a popular system for exploring both kinases and their inhibitors, because some protein kinases can render the Ba/F3 cells to be depended on the activation of the kinases instead of IL-3 supplement, while their inhibitors can antagonize the kinase-dependent growth effects.

III. Cell Line Generation

Ba/F3 ETV6-NTRK3 G623E cell line was generated using retrovirus vector expressing human ETV6-NTRK3-G623E sequence.

IV. Characterization by RT-PCR sequencing

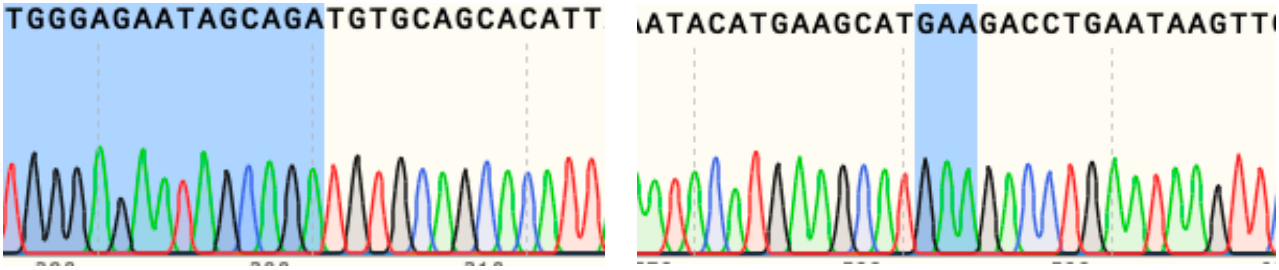
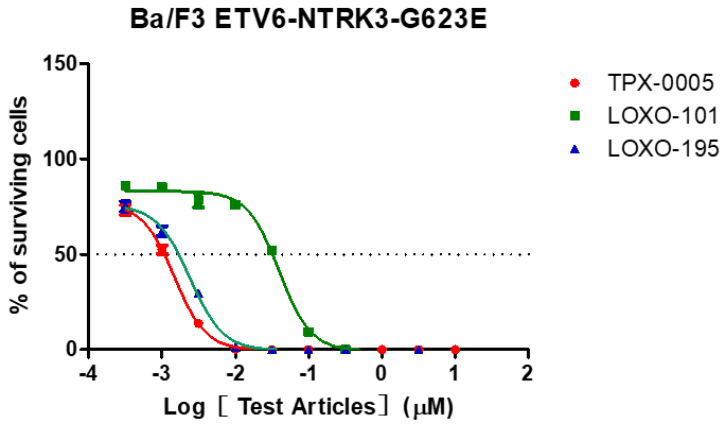


Figure: Characterization of ETV6-NTRK3-G623E overexpression in Ba/F3 stable clones.

V. Application

a. **Cell-based kinase inhibition screen**
b. **Cell viability assay**
c. **In vivo efficacy study**

Example: kinase inhibitors screening



IC50 of TRK inhibitors

Compound	IC50(nM)
TPX-0005	1.08
LOXO-101	32.16
LOXO-195	1.70

1. Harvest and seed the Ba/F3 cells in 96-well plate (3000 cells/90ul medium).
2. Next day, add 10ul 10X serially diluted compound solution each well and incubate the plates for another 72 hours.
3. Add 100ul Cell Titer-Glo each well, mixed and readout using Envision.
4. Plot the dose-responsive curve and fit the IC50 (the centration of 50% inhibition of DMSO vehicle treated clones) using GraphPad Prism software (Version 5).

VI. Cell Resuscitation

1. Prewarm culture medium (RPMI-1640 supplemented with 10% FBS) in a 37°C water bath.
2. Thaw the frozen vial in a 37°C water bath for 1-2 minutes.
3. Transfer the vial into biosafety cabinet, and wipe the surface with 70% ethanol.
4. Unscrew the top of the vial and transfer the cell suspension gently into a sterile centrifuge tube containing 9.0 mL complete culture medium.
5. Spin at ~ 125 x g for 5~7 minutes at room temperature, and discard the supernatant without disturbing the pellet.
6. Resuspend cell pellet with the appropriate volume of complete medium and transfer the cell suspension into a T25 culture flask.
7. Incubate the flask at 37°C, 5% CO_2 incubator.
8. Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10^5 cells/ml.

VII. Cell Freezing

1. Prepare the freezing medium (70% RPMI-1640 + 20% FBS + 10% DMSO) fresh immediately before use.
2. Keep the freezing medium on ice and label cryovials.
3. Transfer cells to a sterile, conical centrifuge tube, and count the cells.
4. Centrifuge the cells at 250 x g for 5 minutes at room temperature and carefully aspirate off the medium.
5. Resuspend the cells at a density of at least 3 x10^6 cells/ml in chilled freezing medium.
6. Aliquot 1 ml of the cell suspension into each cryovial.
7. Freeze cells in the CoolCell freezing container overnight in a −80°C freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

VIII. References

1. Vaishnavi, A, A T Le, and R C Doebele. 2015. “TRKing Down an Old Oncogene in a New Era of Targeted Therapy.” Cancer Discovery 5 (1): 25–34.
2. Jones, David T W, Barbara Hutter, Natalie Jäger, Andrey Korshunov, Marcel Kool, Hans-Jörg Warnatz, Thomas Zichner, et al. 2013. “Recurrent Somatic Alterations of FGFR1 and NTRK2 in Pilocytic Astrocytoma.” Nature Publishing Group 45 (8). Nature Publishing Group: 927–32.

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