# Genetically engineered Osimertinib-resistant cell lines and models

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OncoWuXi Newsletter

#### Outline



- Mechanisms of resistance to EGFR TKIs
- **Genetically engineered Osimertinib-resistant cancer cell lines and models**
- Genetically engineered Osimertinib-resistant Ba/F3 cell lines and models

### EGFR signaling pathway, resistance mechanisms and therapy strategy



- Gene amplification and mutation are the main mechanisms of acquired resistance to EGFR TKIs.
- Rewiring of cell signaling irrespective of EGFR is also a key mechanism to circumvent EGFR TKI therapy in the absence of EGFR mutations.
- Combinations of EGFR TKIs with different drugs (including other TKIs, monoclonal antibodies, chemotherapy and vaccines) might be strategies for overcoming the acquired resistance to third-generation inhibitors.

Nat Cancer. 2021 Apr;2(4):377-391.

#### **Mechanisms of resistance to Osimertinib**

Resistance mechanisms arising after second-line (a) and first-line (b) Osimertinib therapy



The most common tertiary EGFR mutation is C797S in exon 20 and it accounts for 15%-26% of cases of resistance to second-line Osimertinib treatment. Other uncommon mutations in exon 18 and exon 20 also lead to resistance to Osimertinib.

In EGFR off-target alterations, MET amplification is one of the most common mechanisms of acquired resistance to Osimertinib secondand first-line therapy.

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# Genetically engineered human cancer cell lines carrying EGFR C797S mutation 孕 餐 嗯 凍 德 IC50 of EGFR TKIs in parental vs. genetically engineered cell lines

|             | Coll line          | Madel Constice   | Reference compound IC50 (μM) |   |             |         |  |
|-------------|--------------------|--|------------------------------|---|-------------|---------|--|
| Cancer type | Cell line          | Model Genetics   | Erlotinib                    | Afatinib Osimertinib   / 0.078   / 4.951   / 0.018   / 3.2920 | Osimertinib | BI-4020 |  |
| lung concor | PC-9               | Carrying<br>(EGFR delE746-A750)  | /                            | /   | 0.078       | 0.032   |  |
| Lung cancer | PC-9 EGFR DTC      | Model Genetics Erlotinib Afatinib Osimertinib   Carrying<br>(EGFR delE746-A750) / / 0.078   Carrying<br>(EGFR delE746-A750) 4.483 / 4.951   Carrying<br>(EGFR L858R/T790M/C797S) 1.483 / 0.018   DTC EGFR exon 19<br>deletion/T790M/C797S 9.5850 / 3.2920   LTC EGFR<br>L858R/T790M/C797S 3.9480 2.1790 4.4580 | 0.047                        |   |             |         |  |
| Lung cancer | NCI-H1975          | Carrying<br>(EGFR L858R/T790M)   | /                            | /   | 0.018       | 0.250   |  |
|             | NCI-H1975 EGFR DTC | EGFR exon 19<br>deletion/T790M/C797S   | 9.5850                       | /   | 3.2920      | 0.180   |  |
|             | NCI-H1975 EGFR LTC | EGFR<br>L858R/T790M/C797S  | 3.9480                       | 2.1790  | 4.4580      | 0.372   |  |

#### Genetically engineered human cancer models carrying EGFR C797S mutation P NUX AppTec

In vivo efficacy of EGFR TKIs in parental vs. genetically engineered models

| Cancer type | Cell line  | Inoculation<br>method   | Drugs tested                           | Dosage<br>(mg/kg) | TGI (%)                 | MST (day) |
|-------------|--|---|--|-------------------|-------------------------|-----------|
|             | PC-9   | subcutaneous  | Osimertinib                            | 5                 | 108                     | /         |
|             | PC-9 EGFR DTC  | subcutaneous  | Osimertinib<br>BI-4020                 | 10<br>10          | 21.61<br>106            | /         |
| Lung cancer | PC-9-luc   | Inoculation<br>methodDrugs testedDosage<br>(mg/kg)TGI (%)subcutaneousOsimertinib5108subcutaneousOsimertinib1021.61subcutaneousOsimertinib1021.61intracranialVehicle-Osimertinib100BI-402010106intracranialVehicle-Osimertinib25/BI-40201010Bi-402010-Bi-402010-Bi-402010-Bi-402010-Bi-402010-Bi-402010-Bi-402010-Bi-402010-Bi-402010-Bi-402010-Bi-402010-Bi-402010-Birigatinib75-subcutaneousOsimertinib50SubcutaneousErlotinib50Birigatinib50-SubcutaneousOsimertinib2.5SubcutaneousOsimertinib-SubcutaneousOsimertinib-SubcutaneousOsimertinib-SubcutaneousOsimertinib-SubcutaneousOsimertinib-SubcutaneousOsimertinib-SubcutaneousOsimertinib-SubcutaneousOsimertinib-SubcutaneousOsimertinib-SubcutaneousOs | 17<br>43.5<br>Not reach<br>26.5<br>26  |                   |                         |           |
|             | PC-9-lucintracranialOsimertinib25BI-402010Brigatinib75PC-9Fight and the second se | /   | 13.5<br>19<br>18<br>23<br>13.5         |                   |                         |           |
|             | NCI-H1975  | subcutaneous  | Osimertinib                            | 1<br>5            | 51<br>108               | /         |
| Lung cancer | NCI-H1975<br>EGFR DTC  | subcutaneous  | Erlotinib<br>Brigatinib<br>Osimertinib | 50<br>50<br>2.5   | 12.46<br>22.44<br>30.13 | /         |
|             | PC-9<br>EGFR DTCintracranialung cancerNCI-H1975subcutaneousNCI-H1975<br>EGFR DTCsubcutaneousINCI-H1975<br>EGFR DTCsubcutaneousI  | Osimertinib   | 10                                     | 4.72              | /                       |           |

#### EGFR TKIs in PC-9 vs. genetically engineered PC-9 subcutaneous model





#### PC-9 EGFR-Del19/T790M/C797S



Days after the start of treatment

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#### **EGFR TKIs in PC-9-luc intracranial model**





Figure: PC-9-luc cells mixed with Matrigel were micro-injected into brain of mice. Treatments were started at day 4 after inoculation (A) Bioluminescent imaging of mice over 30 days (n=10). (B) Intracranial tumor growth curve as measured by average photon intensity in the experiments. (C) The average body weight curve of mice was used in the experiment. (D) Survival curve till day 51.

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0 5 10 15 20 25 30 35 40 45 50 55 60 Days after the start of treatment

40

20

0

#### EGFR TKIs in PC-9 EGFR-Del19/T790M/C797S-luc intracranial model





curve as measured by average photon intensity in the experiments. (C) The average body weight curve of mice was used in the experiment. (D) Survival curve till day 47.

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Days after the start of treatment

5 10 15 20 25 30 35 40 45 50 9

20

0

0

## EGFR TKIs in NCI-H1975 vs. genetically engineered NCI-H1975 subcutaneous model appreciation of the second s

NCI-H1975



Days after the start of treatment

NCI-H1975 EGFR-Del19/T790M/C797S

Brigatinib, 50 mg/kg, PO, QD, n=5

Osimertinib, 2.5 mg/kg, PO, QD, n=5

10

Days after the start of treatment

Osimertinib, 2.5 mg/kg, PO, QDx3w, n=5

Brigatinib, 50 mg/kg, PO, QD, n=5

15

15

20

20

Erlotinib, 50 mg/kg, PO, QD, n=5

Vehicle.PO.QD.n=5

5

Vehicle,PO, QD, n=5

5

3000

Ë 2000

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20

15

10

5

-5

-10 -15

-20-

0

Body weight change (%)

m <sup>3</sup>)

volum

Tum

#### NCI-H1975 EGFR-L858R/T790M/C797S



10

## **Genetically engineered Osimertinib-resistant Ba/F3 cell lines** IC50 of EGFR TKIs in genetically engineered Ba/F3 cell lines



|  | Reference compound IC50 (µM) |                   |                  |                    |             |             |         |  |
|--|------------------------------|-------------------|------------------|--------------------|-------------|-------------|---------|--|
| Cell line                                | Erlotinib                    | Gefitinib         | Afatinib         | Dacomitinib        | Brigatinib  | Osimertinib | BI-4020 |  |
| Ba/F3<br>EGFR-C797S                      | 0.011                        | /                 | 0.0043           | /                  | /           | 1.8040      | 0.0686  |  |
| Ba/F3<br>EGFR L858R/C797S                | $0.013 \pm 0.004$            | $0.011 \pm 0.004$ | 0.011±0.002      | 0.008±0.003        | 0.437±0.048 | >1          | 0.0073  |  |
| Ba/F3<br>EGFR L858R/T790M/C797S          | >10                          | 11.480±2.46<br>4  | 2.214±0.827      | 2.066±0.719        | 1.044±0.008 | 1.566±0.276 | 0.0012  |  |
| Ba/F3 EGFR<br>exon 19 deletion /C797S    | $0.004 \pm 0.000$<br>1       | 0.003±0.001       | 0.003±0.000<br>1 | <0.0030,<br>0.0019 | 0.169±0.090 | 1.147±0.116 | 0.0018  |  |
| Ba/F3 EGFR exon 19 deletion /T790M/C797S | >10                          | 4.17±0.30         | 1.39±0.014       | 1.45±0.17          | 0.20±0.13   | 1.75±0.015  | 0.0011  |  |

#### **Genetically engineered Osimertinib-resistant Ba/F3 models**

In vivo data summary of EGFR TKIs efficacy in genetically engineered Ba/F3 models



| Cell type       | Model ID   | Drugs tested                                       | Dosage (mg/kg)      | TGI (%)                | Model Genetics                           |
|-----------------|--|--|---------------------|------------------------|--|
|                 | Ba/F3<br>EGFR C  | Erlotinib<br>Afatinib<br>Osimertinib<br>Brigatinib | 50<br>15<br>5<br>75 | 111<br>92<br>-23<br>75 | EGFR<br>C797S                            |
|                 | Erlotinib50103Ba/F3Afatinib1535EGFR LCOsimertinib5-7Brigatinib7596 | EGFR<br>L858R/C797S                                |                     |                        |  |
| Murine<br>Pro-B | Ba/F3<br>EGFR LTC  | Erlotinib<br>Afatinib<br>Osimertinib<br>Brigatinib | 50<br>15<br>5<br>75 | 0<br>-4<br>4<br>42     | EGFR<br>L858R/T790M/C797S                |
|                 | Ba/F3<br>EGFR DC   | Erlotinib<br>Osimertinib                           | 50<br>5             | 115<br>18              | EGFR<br>exon 19 deletion<br>/C797S       |
|                 | Ba/F3<br>EGFR DTC  | Erlotinib<br>Afatinib<br>Osimertinib<br>Brigatinib | 50<br>15<br>5<br>75 | 8<br>20<br>-7<br>91    | EGFR<br>exon 19 deletion<br>/T790M/C797S |

## **Genetically engineered Osimertinib-resistant Ba/F3 models** *In vivo* data summary of EGFR TKIs efficacy in genetically engineered Ba/F3 models





| Cell type            | Model ID                    | Drugs tested                                       | Dosage (mg/kg)              | TGI (%)              | Model Genetics            |
|----------------------|-----------------------------|--|-----------------------------|----------------------|---------------------------|
| Murine<br>Pro-B<br>E | Ba/F3<br>EGFR LTL           | Osimertinib<br>Brigatinib                          | 5<br>75                     | 26<br>87             | EGFR<br>L858R/T790M/L792H |
|                      | Ba/F3<br>EGFR LTG           | Osimertinib<br>Brigatinib                          | 5<br>75                     | -3<br>69             | EGFR<br>L858R/T790M/G796R |
|                      | Ba/F3<br>EGFR exon20ins ASV | Erlotinib<br>Afatinib<br>Osimertinib<br>Poziotinib | 50<br>15<br>5/25<br>5/1/0.5 | 13<br>11<br>11<br>47 | EGFR<br>exon20ins ASV     |
|                      | Ba/F3<br>EGFR exon20ins SVD | Erlotinib<br>Afatinib<br>Osimertinib<br>Poziotinib | 50<br>15<br>5/25<br>5/1/0.5 | 4<br>-4<br>12<br>61  | EGFR<br>exon20ins SVD     |
|                      | Ba/F3<br>EGFR exon20ins NPH | Erlotinib<br>Afatinib<br>Osimertinib<br>Poziotinib | 50<br>15<br>5<br>0.5        | 13<br>5<br>12<br>40  | EGFR<br>exon20ins NPH     |

#### Showcase: EGFR TKIs in Ba/F3 EGFR LTL model



| Cell type | Model ID | Drugs tested | Dosage (mg/kg) | TGI (%) | Model Genetics    |
|-----------|----------|--------------|----------------|---------|-------------------|
| Murine    | Ba/F3    | Osimertinib  | 5              | 26      | EGFR              |
| Pro-B     | EGFR LTL | Brigatinib   | 75             | 87      | L858R/T790M/L792H |



#### Showcase: EGFR TKIs in Ba/F3 EGFR LTG model



| Cell type | Model ID | Drugs tested | Dosage (mg/kg) | TGI (%) | Model Genetics    |
|-----------|----------|--------------|----------------|---------|-------------------|
| Murine    | Ba/F3    | Osimertinib  | 5              | -3      | EGFR              |
| Pro-B     | EGFR LTG | Brigatinib   | 75             | 69      | L858R/T790M/G796R |





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