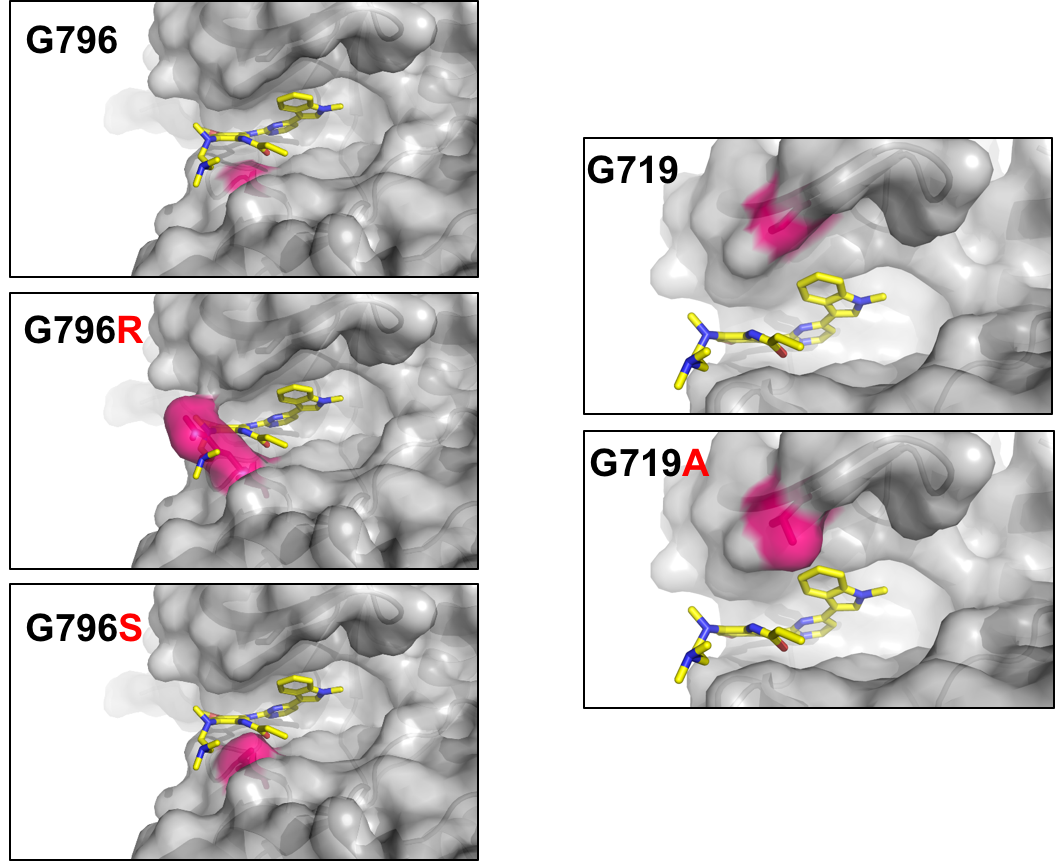
**Supplementary data for:**

**Investigating novel resistance mechanisms to third-generation EGFR tyrosine kinase inhibitor osimertinib in non-small cell lung cancer patients**

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**Supplemental Figure 1.** *In silico* protein structure modeling predicts that G796R/S and G719A substitutions could prevent osimertinib (PDB id: 4ZAU) binding by introducing spatial confliction (hot pink). EGFR are shown in surface.

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**Supplemental Figure 2.** Western blots showed that *EGFR* mutants were expressed at comparable levels in different cell lines.Total cell lysate containing 25 μg of protein were loaded for each sample. Blots were done for total EGFR using ERK2 as the loading control. Ba/F3 cells transfected with the vector *pBabe-puro* alone were used as the negative control. *EGFR* wild type was used as the positive control for the comparison of *EGFR* expression levels in different cell lines. Images were analyzed in LiCOR Image Studio Digits software (version5.2).

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**Supplemental Figure 3.** The growth curves of Ba/F3 cells harboring *EGFR* activating mutation, with or without T790M, *in cis* with indicated mutations. 104 cells/well were cultured in the absence of IL-3 during the entire course in 96-well plates. Experiments were repeated twice with mean ± standard deviation plotted at every 24-hour time point after setup. AU, arbitrary units of fluorescence.



**Supplemental Figure 4.** Ba/F3 cells expressing the *EGFR* L858R/L718Q mutant variant are resistant to gefitinib while those harboring L792 mutations remain sensitive. Ba/F3 cells harboring exon 19 deletion or L858R plus indicated mutations, either with or without T790M, were treated with gefitinib at the indicated doses, and cell viability was evaluated after 72 hours of treatment and plotted relative to untreated control cells.Experiments were repeated twice with mean ± standard deviation plotted at each concentration. The curves were fitted using a nonlinear regression model with a sigmoidal dose response in the Graphpad Prism 6.

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**Supplemental Table 1.** Information for cfDNA extraction, library preparation input and NGS coverage depth.

**Supplemental Table 2**. Primers used in site-directed mutagenesis

|  |  |  |
| --- | --- | --- |
| Point mutation | Primer | Sequence |
| T790M | Forward | gcaactcatcatgcagctcatgcccttcggc |
|  | Reverse | gccgaagggcatgagctgcatgatgagttgc |
| L718Q | Forward | aaagatcaaagtgcagggctccggtgcgttcgg |
|  | Reverse | ccgaacgcaccggagccctgcactttgatcttt |
| C797S | Forward | ctcatgcccttcggcagcctcctggactatgtc |
|  | Reverse | gacatagtccaggaggctgccgaagggcatgag |
| T790M+L792H | Forward | cgaagggcatgtgctgcatgatgagttgc |
|  | Reverse | gcaactcatcatgcagcacatgcccttcg |
| T790M+L792F | Forward | gcaactcatcatgcagttcatgcccttcggctgcc |
|  | Reverse | ggcagccgaagggcatgaactgcatgatgagttgc |
| T790M+L792Y | Forward | gcaactcatcatgcagtacatgcccttcggctgcc |
|  | Reverse | ggcagccgaagggcatgtactgcatgatgagttgc |
| L792H | Forward | ctcatcacgcagcacatgcccttcggc |
|  | Reverse | gccgaagggcatgtgctgcgtgatgag |
| L792F | Forward | gcaactcatcacgcagttcatgcccttcggctgcc |
|  | Reverse | ggcagccgaagggcatgaactgcgtgatgagttgc |
| L792Y | Forward | gcaactcatcacgcagtacatgcccttcggctgcc |
|  | Reverse | ggcagccgaagggcatgtactgcgtgatgagttgc |

**Supplemental Table 3.** Clinical, treatment information and the list of genetic alterations of the 12 patients with matched pre- and post- osimertinib samples.

**Supplemental Table 4.** Clinical and treatment information for 81 patients with post-osimertinib samples only.

**Supplemental Table 5.** Genetic alterations identified in the 81 patients with post-osimertinib samples only.

**Supplemental Table 6.** Experimental IC50 values for different drugs

|  |  |  |  |
| --- | --- | --- | --- |
| **Cells** | **IC50 value (n mole/L)** | | |
| Osimertinib | | Gefitinib |
| Ex19del | 0.78 | | 4.13 |
| Ex19del/T790M | 3.48 | | >1000 |
| Ex19del/T790M/C797S | >1000 | | >1000 |
| Ex19del/T790M/L718Q | >500 | | >1000 |
| Ex19del/T790M/L792H | 97.49 | | >1000 |
| Ex19del/T790M/L792F | 10.04 | | >1000 |
| Ex19del/T790M/L792Y | 33.04 | | >1000 |
| Ex19del/C797S | >1000 | | 3.95 |
| Ex19del/L718Q | NA | NA | |
| Ex19del/L792H | NA | NA | |
| Ex19del/L792F | 4.81 | | 3.89 |
| Ex19del/L792Y | 36.64 | | 2.85 |
| L858R | 3.55 | | 6.65 |
| L858R/T790M | 4.77 | | >1000 |
| L858R/T790M/C797S | >1000 | | >1000 |
| L858R/T790M/L718Q | >1000 | | >1000 |
| L858R/T790M/L792H | 54.75 | | >1000 |
| L858R/T790M/L792F | 38.65 | | >1000 |
| L858R/T790M/L792Y | 49.57 | | >1000 |
| L858R/C797S | >1000 | | 9.19 |
| L858R/L718Q | >1000 | | ~500 |
| L858R/L792H | 72.41 | | 31.25 |
| L858R/L792F | 36.29 | | 10.2 |
| L858R/L792Y | NA | NA | |

NA: No proliferation in the absence of IL-3.