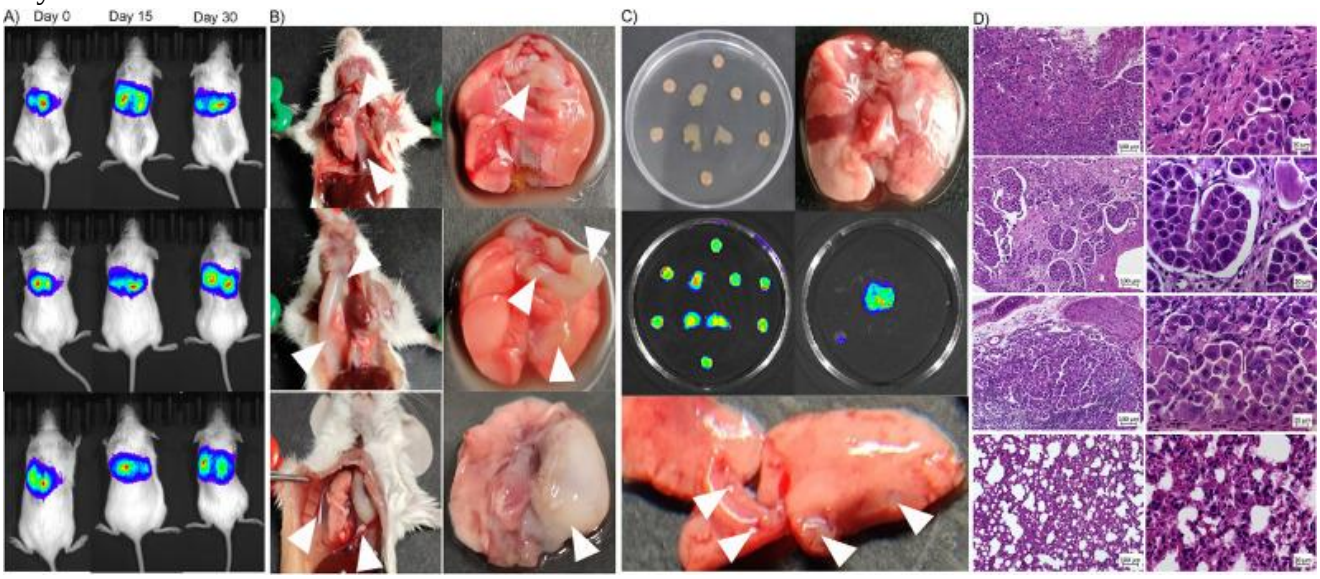
Osimertinib for lung cancer cells harboring low-frequency EGFR T790M mutation.

Joshi A, Butle A, Hait S, Mishra R, Trivedi V, Thorat R, Choughule A, Noronha V, Prabhash K, Dutt A

Osimertinib, a third-generation EGFR tyrosine kinase inhibitor (TKI), is approved for treatment for lung cancer patients who acquire EGFR T790M mutation following disease progression. The prevalence of the EGFR T790M among NSCLC patients ranges from one-half to three-fourths of re-biopsy samples. However, studies with real-life data, including the FLAURA trial, indicate the benefit of osimertinib is restricted to 25-31%. The EGFR T790M mutation occurs at varying allele frequency, wherein low allele frequency mutations are likely to be missed by conventional technologies in clinics with low sensitivity. Moreover, the clinical utility of osimertinib to the low- allele frequency of EGFR T790M in tissue biopsy remains sparsely explored.

To assess the prevalence of low-allele EGFR T790M mutation in primary tumors, we re-analyzed the NGS data of 48 samples collected longitudinally from 16 EGFR-mutated lung cancer patients representing treatment naïve primary tumors, paired re-biopsies upon disease progression with a negative EGFR T790M result, and their matched blood. Interestingly, 25% of EGFR T790M negative re-biopsy samples were found to harbor EGFR T790M mutation at a low-allele frequency of less than 5%, suggesting its significant occurrence. To explore the significance of low- allele EGFR T790M mutation in resistance to erlotinib, addressing this outstanding clinical question, we established erlotinib-resistant PC-9R cells from erlotinib-sensitive PC-9 cells harboring EGFR T790M at a low frequency, as confirmed by next-generation sequencing and allele-specific PCR. The low frequency of the chromosomal mutant allele was found to be stable across twenty sub-clones of PC-9R cells established by single-cell dilution. Next, we tagged the PC-9 and PC-9R cells with luciferase and implanted the same intercostally in mice to develop a clinically relevant orthotopic lung cancer mouse model. While the lung tumors formed by the PC-9 and PC-9R cells were sensitive and resistant to oral erlotinib, respectively, we found a potent response of osimertinib, but not erlotinib, on lung tumors harboring low-frequency EGFR T790M mutations—suggesting its clinical utility



Taken together, our findings establish a significant occurrence of EGFR T790M mutation at low allele frequency following disease progression, likely to be missed by conventional technologies in clinics. We also extend the benefit of osimertinib treatment to these patients with low EGFR T790M mutation allele frequency.