Engineering isogenic models harboring resistance mechanisms to the latest-generation EGFR inhibitor in non-small cell lung cancer

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Poster 7029

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Abstract

Background: EGFR-mutant lung cancer was among the first epithelial cancer subsets where directly targeting an oncogene yielded significant clinical benefit. While improved inhibitors, such as third-generation EGFR inhibitor Osimertinib, have significantly improved clinical outcomes of non-small cell lung cancer (NSCLC) patients, acquired resistance to targeted therapies remains a major barrier to durable responses. To address this challenge, we used a genetic engineering approach to develop three sets of isogenic NSCLC cell models harboring clinically resistant mutations to EGFR-targeted therapy.

Methods and results: To systematically investigate resistance mechanisms and associated vulnerabilities, we engineered isogenic NSCLC cell lines to model clinically relevant mechanisms of acquired resistance. Using CRISPR gene editing, three sets of resistant cell lines were generated from three Osimertinib-sensitive parental lines (HCC4006, NCI-H292, and HCC827). The engineered alterations included *BRAF V600E, KRAS G12D, PIK3CA E545K, EGFR C797S,* and additional fusion genes such as *TPM3–NTRK1*. Sequence verification and Osimertinib sensitivity assays were performed for all models. The engineered cell lines exhibited reduced Osimertinib sensitivity consistent with the introduced resistance mechanisms. Initial genomic validation confirmed the intended edits and additional genetic screening will be performed to further characterize the isogenic cell lines. Selected models were further evaluated in 3-D culture systems to assess phenotypic impact.

Conclusions: These validated novel models provide a robust platform for the research community and industry to dissect mechanisms of drug resistance, identify therapeutic vulnerabilities, and develop combination therapy strategies. This ATCC and Broad Institute collaborative effort will also support the establishment of the Resistance Map (ResMap) within DepMap to systematically characterize vulnerabilities in EGFR-driven NSCLC.

Graphical Abstract

Engineering and functional characterization of osimertinib resistance in NSCLC models

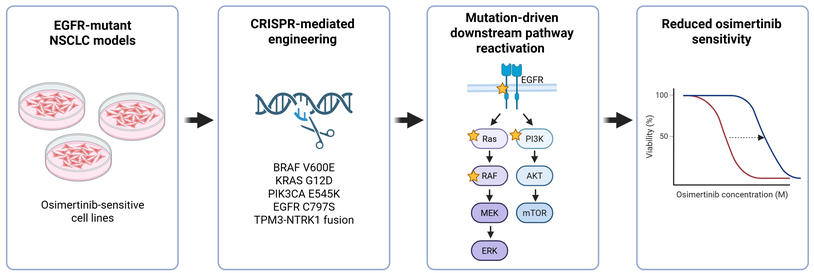

Figure 1: Modeling Osimertinib resistance in EGFR-mutant NSCLC. EGFR-mutant NSCLC cell lines (HCC4006, NCI-H292, HCC827) were engineered using CRISPR to introduce clinically relevant resistance alterations (BRAF V600E, KRAS G12D, PIK3CA E545K, EGFR C797S, and TPM3-NTRK1 fusion). These models exhibit reduced Osimertinib sensitivity and sustained downstream signaling (MAPK, PI3K-AKT) despite EGFR inhibition. The platform enables functional interrogation of resistance mechanisms and supports applications in combination therapy development, biomarker discovery, functional genomics, and 3-D phenotyping. Image created with Biorender.com.

Results

Osimertinib resistance across engineered alterations in HCC4006 models

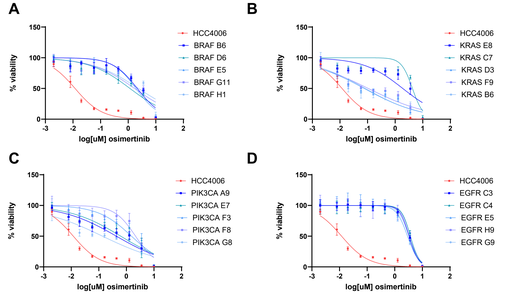

Figure 2: Engineered resistance mutations reduce Osimertinib sensitivity in HCC4006 isogenic clones. Dose-response curved to Osimertinib in HCC4006-derived clones harboring (A) BRAF V600E, (B) KRAS G12D, (C) PIK3CA E545K, and (D) EGFR C797S mutations. All engineered variants exhibit decreased drug sensitivity relative to parental controls. Data represent mean ± SD (n=4).

Osimertinib resistance across engineered alterations in NCI-H292 models

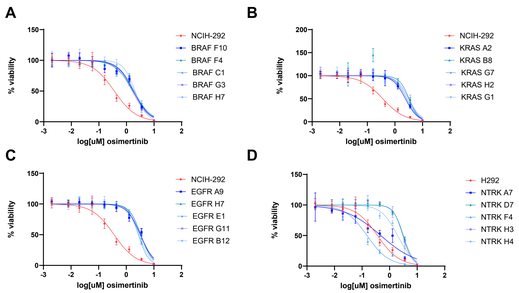

Figure 3: Broad spectrum of engineered alterations drives resistance in NCI-H292 isogenic clones. Dose-response curves of NCI-H292-derived clones carrying (A) BRAF V600E, (B) KRAS G12D, (C) EGFR C797S, and (D) TPM3-NTRK1 fusion. Resistance phenotypes are consistent across independent clones. Data represent mean ± SD (n=4).

Osimertinib resistance across engineered alterations in HCC827 models

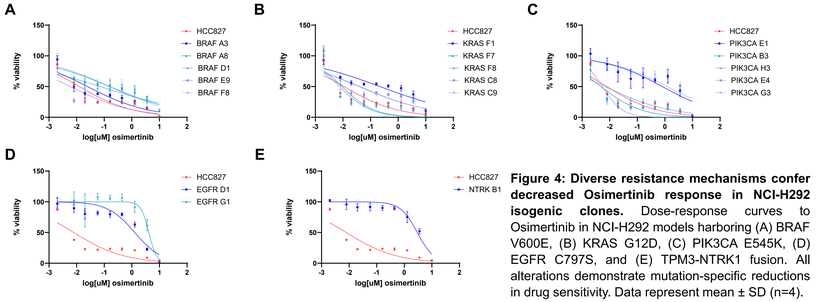

Figure 4: Diverse resistance mechanisms confer decreased Osimertinib response in NCI-H292 isogenic clones. Dose-response curves to Osimertinib in NCI-H292 models harboring (A) BRAF V600E, (B) KRAS G12D, (C) PIK3CA E545K, (D) EGFR C797S, and (E) TPM3-NTRK1 fusion. All alterations demonstrate mutation-specific reductions in drug sensitivity. Data represent mean ± SD (n=4).

Oncogenic pathway reactivation underlies resistance to EGFR inhibition

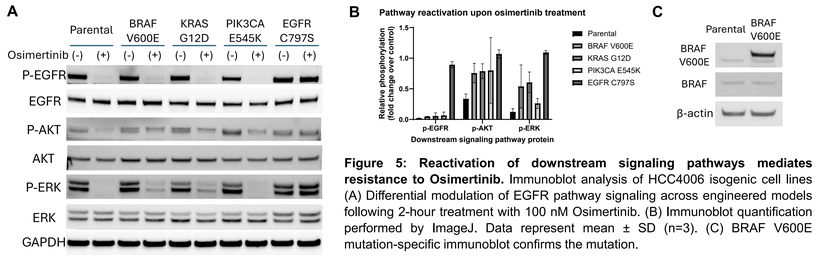

Figure 5: Reactivation of downstream signaling pathways mediates resistance to Osimertinib. Immunoblot analysis of HCC4006 isogenic cell lines (A) Differential modulation of EGFR pathway signaling across engineered models following 2-hour treatment with 100 nM Osimertinib. (B) Immunoblot quantification performed by ImageJ. Data represent mean ± SD (n=3). (C) BRAF V600E mutation-specific immunoblot confirms the mutation.

3-D spheroid models reveal phenotypic consequences of resistance mechanisms

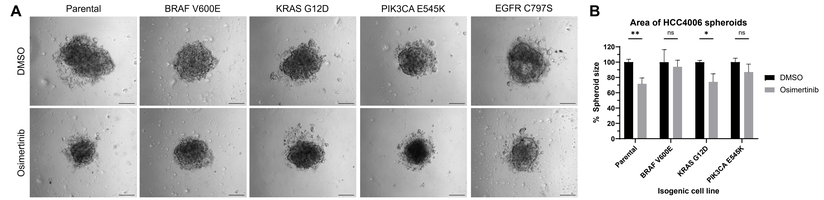

Figure 6: Resistance-associated phenotypes are recapitulated in 3-D spheroid models. HCC4006 isogenic cell lines were cultured as spheroids and treated with Osimertinib (35 nM, IC_{50}). (A) Phase contrast images were obtained by Zeiss Axiovert. (B) Dil-dyed cells were formed into spheroids and images were captured by Leica Mica confocal microscope. Spheroid area was calculated in ImageJ using maximum Z-projected images. Quantification of spheroid size demonstrates reduced drug response in resistant models. Data represent mean ± SD (n=3). ***, P<0.001; *, P<0.05; ns, P≥0.05.

Conclusion

- Isogenic NSCLC models enable precise interrogation of clinically relevant resistance mechanisms to Osimertinib by directly linking defined genetic alterations to drug response phenotypes.
- Engineered mutations consistently confer reduced drug sensitivity and promote reactivation of key downstream signaling pathways across multiple cellular backgrounds.
- Resistance phenotypes are reproducible in both 2-D and 3-D culture systems, supporting the robustness and translational relevance of these models.
- This platform provides a foundation for identifying genotype-specific vulnerabilities and informing rational combination therapy strategies.
- Integration into DepMap/ ResMap efforts will enable systematic mapping of resistance-associated dependencies and accelerate therapeutic discovery.

Table 1: Drug-resistant isogenic cell lines to be available from ATCC.

ATCC® Item ID	Cell Line	ATCC® Item ID	Cell Line	ATCC® Item ID	Cell Line
CRL-2871IG-1™	KRAS Mutant-HCC4006	CRL-1848IG-1™	KRAS Mutant-NCI-H292	CRL-2868IG-1™	KRAS Mutant-HCC827
CRL-2871IG-4™	EGFR Mutant-HCC4006	CRL-1848IG-4™	EGFR Mutant-NCI-H292	CRL-2868IG-4™	EGFR Mutant-HCC827
CRL-2871IG-5™	BRAF Mutant-HCC4006	CRL-1848IG-5™	BRAF Mutant-NCI-H292	CRL-2868IG-5™	BRAF Mutant-HCC827
CRL-2871IG-7™	PIK3CA Mutant-HCC4006	CRL-1848IG-6™	NTRK Fusion-NCI-H292	CRL-2868IG-6™	NTRK Fusion-HCC827
		CRL-1848IG-7™	PIK3CA Mutant-NCI-H292	CRL-2868IG-7™	PIK3CA Mutant-HCC827
		CRL-1848IG-8™	ROS Fusion-NCI-H292	CRL-2868IG-8™	ROS Fusion-NCI-H827

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