From Exploratory Research to Functional Diagnostics:

Scalability and Standardization of CRISPR/Cas9 Pipelines for Resistance Profiling

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We developed a standardized CRISPR knockout screening pipeline to identify mechanisms of resistance to brigatinib, an ALK inhibitor currently under clinical evaluation for pediatric Anaplastic Large Cell Lymphoma (ALCL). Using the GeCKO v2 CRISPR library (Addgene) and a second-generation lentiviral packaging system, we established a robust, high-throughput workflow in the ALCL cell line SupM2. Following two weeks of brigatinib selection, the screen successfully identified key modulators of drug response.

Among the top hits, we identified STK11, a well-established tumor suppressor, that was independently detected in other screenings conducted in ALK-driven Non-Small Cell Lung Cancer (NSCLC). This finding highlights the reproducibility of the platform across different ALK-dependent malignancies and supports its ability to identify clinically relevant resistance mechanisms.

A major strength of our approach is the implementation of a standardized methodology that overcomes the logistical challenges of genome-wide CRISPR screening, including large-scale cell expansion, accurate viral-to-cell ratio control, and maintenance of full library representation while ensuring a one-guide-per-cell design. Furthermore, the use of validated, ready-to-use commercial lentiviral particles containing pooled CRISPR libraries enables a modular, reproducible, and highly scalable workflow.

This platform has a dual translational potential. First, it enables rapid identification of genes and pathways regulating drug response, providing insights into mechanisms of therapeutic resistance. Second, its application to patient-derived samples could support functional risk stratification and prediction of treatment response, paving the way toward personalized therapeutic strategies.

Given its robustness, scalability, and compatibility with standardized workflows, this platform is well suited for implementation in centralized hospital facilities. We propose it as the technological foundation for a network of Point-of-Care functional genomics laboratories capable of generating real-time functional data to support clinical decision-making and accelerate the integration of CRISPR-based functional screening into precision oncology.