Title:
A comprehensive CRISPR-enabled functional genomics profiling platform in acute myeloid leukemia (AML): pilot study and validation of Fx Heme

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AML is the most common acute leukemia in adults over 50 years of age. Current therapeutic approaches are focused on high or low dose chemotherapy in the front line setting, with use of molecularly targeted agents in unfit patients or the relapsed/refractory setting. However, routine genomic profiling for common molecular lesions (e.g. FLT3 mutation) incompletely stratifies the target patient population, and more personalized and comprehensive approaches could be of significant clinical benefit. To this end, we have developed an in vitro functional genomics platform for the systematic characterization of gene dependency on drug targets using CRISPR/Cas9 technology.

Methods: Retrospective pre-therapy samples were obtained for a cohort of AML patients who subsequently went onto therapy with a combination of the tyrosine kinase inhibitor sorafenib plus chemotherapy (e.g. GCLAM). Unsorted primary tumor cells from these samples were transduced with lentivirus harboring Cas9 enzyme and an sgRNA library. This customized sgRNA library (Fx Heme) was designed to inhibit expression of genes encoding the targets of all FDA-approved oncology drugs, as well as internal positive and negative references. Transduced cells were harvested at multiple timepoints, and sgRNA distribution was assessed by amplicon sequencing of DNA barcodes. Changes in barcode abundance were quantitated and aggregated to calculate gene-level phenotype scores, enabling identification of gene dependencies. Dependency on targets of sorafenib was compared to clinical outcome (complete response (CR) without minimal residual disease (MRD), incomplete response, CR with MRD, or refractory) following treatment with sorafenib plus chemotherapy.

Results: Among the 22 AML patients analyzed, 14 reached CR and 8 were classified as incomplete responders (refractory or MRD). Gene dependency on sorafenib targets including FLT3, KIT, PDGFR, and all RAF isoforms was frequently observed (12/22 cases). Among cases indicating any sorafenib target gene dependency, 5/12 (42%) demonstrated dependence on 2 or more sorafenib target genes. Overall, Fx Heme achieved 78.6% sensitivity, 87.5% specificity, and 91.7% PPV for prediction of observed clinical outcome. In contrast, stratification based on FLT3 mutation (internal tandem duplication or tyrosine kinase domain mutation) demonstrated 64.3% sensitivity, 50% specificity, 69.2% PPV.

In summary, our results establish feasibility of CRISPR-based Fx Heme comprehensive functional genomic profiling for AML precision medicine, and validate this approach in a retrospective study cohort. Functional genomics may contribute to more effective personalization of AML treatment by uncovering drug target dependencies not readily identified by conventional genomic profiling, in an unbiased, comprehensive and patient-specific fashion.