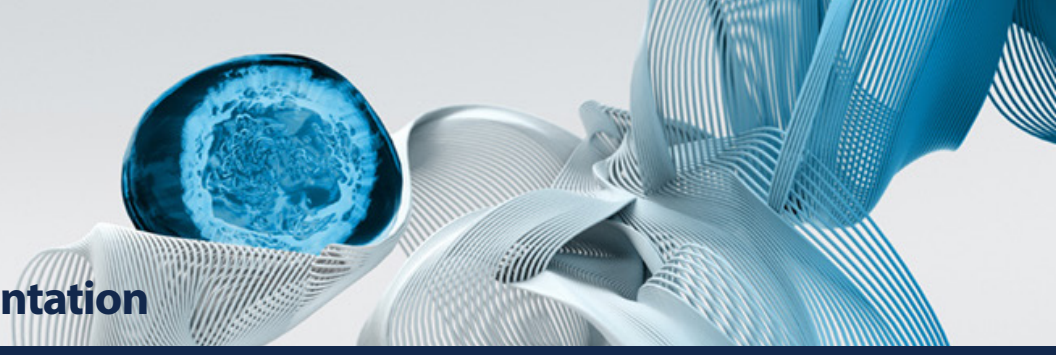
BioSkryb GENOMICS



AGBT 2021 Poster Presentation

Cells explored. Answers revealed.

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Distinct resistance modalities to targeted kinase inhibition exposed by single-cell Primary Template-directed Amplification in acute myeloid leukemia and triple-negative breast cancer.

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Cancer Omics Poster Session on Monday, March 1st from 3:45-5:45pm ET

Ascertainment of genomic lesions at the single-cell level is required to understand molecular mechanisms of resistance to targeted therapeutics in cancer. For this ascertainment to be comprehensive and encompass the functional genomic architecture of promoters, enhancers, and non-coding RNA in addition to protein coding regions, the single-cell genome amplification method must be robust in terms of coverage and uniformity. We have thus utilized Primary Template-directed Amplification (PTA) to amplify genomes from single drug-resistant cells due to the propensity for the method to prevent copying of daughter amplicons which consequently yields unprecedented coverage uniformity concomitant with reduced errors. We modeled acute myeloid leukemia (AML) drug resistance by generating MOLM-13 cells resistant to the FLT3 inhibitor quizartinib. Single-cell PTA verified the presence of the internal tandem duplication (ITD) mutation in both parental and quizartinib-resistant single cells and identified a secondary FLT3 mutation, N841K. N841K has been found in AML patient samples and its proximity to residues in the kinase activation loop suggest that the mutation is activating and likely counteracts quizartinib action. We utilized a quantitative PCR-based genotyping assay to detect wild-type N841 vs. mutant K841 in genomic DNA derived either the parental or the quizartinib-resistant cell lines. Mutant K841 was detectable at minute levels in parental DNA with wild-type K841 in vast excess but in resistant cell DNA mutant K841 was detectable at levels equal to that of wild-type N841. Our resistance model thus selected for a low-level, preexisting clone in parental MOLM-13 cells instead of a de novo DNA mutation leading to drug resistance—mimicking a minimal residual disease (MRD) scenario. Additionally, we have expanded upon these FLT3 insights and cataloged exonic single nucleotide variation in resistant vs. parental single MOLM-13 cells as well as stratified nucleotide variation exclusive of exons by genomic feature. Genomic insights from PTA were also extended to single triple-negative breast cancer (TNBC) cells in models of resistance to the MEK inhibitor trametinib. PTA in SUM-229PE cells revealed structural variation at the KRAS locus in the trametinib-resistant but not in parental single cells, while SNV analysis of SUM-159PT cells yielded marked intergenic variation between resistant and parental single cells as well as heterogeneity in this variation between single cells. Our single-cell studies with PTA whole genome amplification highlight distinct drug resistance mechanisms in preclinical models of cancer and illuminate the need to extend these studies to clinical samples.