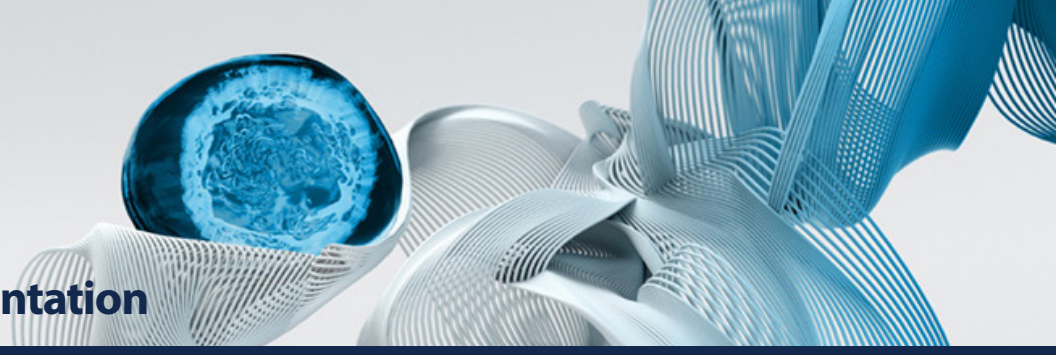
BioSkryb GENOMICS



AGBT 2021 Poster Presentation

Cells explored. Answers revealed.

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Unifying genomics and transcriptomics in single cells to illuminate cancer drug resistance mechanisms with ResolveOME™ amplification chemistry.

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Technology Development Poster Session on Tuesday, March 2nd from 3:30pm-5:30pm ET

The remarkable resilience of cancer cells to drug treatment can occur via genomic, transcriptomic, or epigenomic modulation. While one of these modes can be the primary driver, there is increasing evidence that the modes are not mutually exclusive and instead can synergize to change the cell state, leading to resistance. Thus, a nucleic acid mutation that partially contributes to drug resistance may be also accompanied by transcriptional adaptation and chromatin remodeling to more fully overcome the action of the drug. It will therefore become paramount to assay these multiple omic tiers in single cells, because bulk sequencing is not capable of revealing the inherent heterogeneity in each of these tiers, either in isolation or in concert. We developed a method, ResolveOME™, to ascertain the genome and transcriptome simultaneously from the same individual cell. The workflow unifies template-switching single-cell RNA sequencing (scRNA-seq) chemistry and whole-genome amplification (WGA) with Primary Template-directed Amplification (PTA), followed by affinity purification of first-strand complementary DNA (cDNA) and subsequent separation of the RNA/DNA fractions for sequencing library preparation. To demonstrate the efficacy of the technique in the context of cancer drug resistance, we generated a model of acute myeloid leukemia (AML) resistant to the FLT3 inhibitor quizartinib. The PTA arm of the ResolveOME™ protocol validated the presence of the internal tandem duplication (ITD) in FLT3 in both parental and quizartinib-resistant MOLM-13 AML cells, and, intriguingly, identified a secondary FLT3 mutation, N841K, that is exclusive to the quizartinib-resistant population. The RNA arm of the protocol identified a clade of differentially expressed transcripts between parental and resistant cells, which included the upregulation of GAS6, a ligand for the receptor tyrosine kinase AXL and a known bypass pathway for FLT3 inhibition. The degree of GAS6 expression upregulation varied between single cells. These data suggest a model that the N841K FLT3 mutation drives resistance to quizartinib in conjugation with transcriptional adaptation. Combined genomic and transcriptomic insights were also extended to single triple-negative breast cancer (TNBC) cells in a model of resistance to the MEK inhibitor trametinib. Not limited to drug resistance studies, the utility of ResolveOME™ extends to any application requiring defining DNA-level changes paired with simultaneous information about cell state revealed by transcriptomics in the same cell.