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Integrated cfDNA Methylation and Genomic Profiling Enables Tissue of Origin Prediction from a Single Liquid Biopsy

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Abstract

Background

Treatment selection for advanced cancers has traditionally relied on a tumor's tissue of origin (TOO), but 3-5% of cancers present as cancer of unknown primary (CUP) where standard workup fails to identify a definitive tumor source. TOO determination by pathology assessment can also be limited by tissue availability. We investigated whether methylation status and variant profiling from circulating cell-free DNA (cfDNA) could predict tumor TOO from liquid biopsy alone.

Methods

A retrospective analysis of 6,025 advanced cancer patient samples previously submitted to BillionToOne's clinical laboratory was conducted. Methylation profiling of >2,000 regions with single molecule precision and ultrasensitive comprehensive genomic profiling were performed using the Northstar Response and Northstar Select commercial assays, respectively. A supervised classifier was trained on liquid methylation features from 4,447 patients and tested on a validation cohort of 1,578 patients. A

sex-conditional Bayesian prior and orthogonal signal from tissue-specific somatic variants detected by Northstar Select were then applied. Class-specific rule-out thresholds were calibrated to a $\geq 97.5\%$ sensitivity exclusion threshold.

Results

In the validation cohort, cfDNA methylation successfully classified the correct TOO as the single most likely choice in 86% of cases and among the top three diagnostic predictions in 91% of cases. The model provided tissue-specific rule-out information, excluding 10 of 12 tissue types at a $\geq 97.5\%$ sensitivity threshold. Tissue-specific somatic variants from the same blood draw were used to resolve a subset of difficult cases.

Conclusion

cfDNA methylation and variant profiling enabled accurate, noninvasive TOO prediction through a supervised classifier, supporting liquid biopsy as a decision aid in CUP and other scenarios where TOO is ambiguous. Prospective studies expanding to additional tumor types are planned to confirm the practical utility of this novel approach.

