Longitudinal monitoring of pediatric acute myeloid leukemia using duplex sequencing of patient-specific panels reveals ultra-low frequency MRD that marks persistent carcinogenesis and complex clonal evolution

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Rationale:

Accurate and sensitive detection of measurable residual disease (MRD) is critical for clinical management and the development of new therapies for pediatric acute myeloid leukemia (AML). The presence of MRD is a well-established predictor of disease relapse and mortality. Ideally, MRD detection would be both broadly applicable across patients and highly specific, thus enabling clinicians to make timely treatment decisions to improve outcomes.

Background:

In pediatric AML, unlike adult AML, there are few cross-patient recurrent somatic driver mutations. This makes it impractical to use a fixed gene panel for MRD monitoring. One solution is to perform disease tracking with patient-specific mutations that are present in individual AMLs at time of diagnosis (TOD). However, following modern therapies, should residual disease remain, the telltale mutations will be present in only a very small subpopulation of cells–far below the ~1% limit-of-detection of conventional NGS methods. Duplex sequencing (DS) is an ultra-accurate NGS error-correction method which increases the accuracy of base calls by more than 10,000-fold. Here we demonstrate the use of tumor-informed DS to detect patient-specific MRD in pediatric AML samples with unprecedented sensitivity and specificity.

Methods:

Marrow aspirates were collected from 14 pediatric AML patients at TOD, during treatment, remission, and at relapse. Among the cohort, 9/14 patients ultimately relapsed. Personalized hybrid capture panels were designed to target hundreds of somatic mutations that were present in each patient's TOD sample, as identified by whole genome sequencing of tumor-normal pairs. DS was used for longitudinal monitoring of these mutations in blood or marrow at up to 5 time points following initial induction.

Results:

We detected MRD in one or more samples from all 9 patients who went on to relapse. However, we also observed high-confidence TOD variants in the last collected sample from 4/5 patients who did not relapse during at least 24 months of clinical follow-up. As a technical control, we tested a subset of patient panels on a blood sample from a healthy subject and found no disease-specific mutations. Interestingly, we observed a marked degree of heterogeneity of response-to-therapy in the cohort. In some cases, all mutations logarithmically decreased uniformly, whereas in others there was clear dispersion of subclones over time, with some declining 100-times more quickly than others.

Conclusions:

While DS in conjunction with personalized panels represents a powerful approach for MRD tracking, simply the existence of very low frequency TOD mutations in pediatric AML is not specific enough for predicting relapse without additional interpretation. Lingering mutations in non-relapsers might represent preneoplastic field defects, pre-existing somatic mosacism, or perhaps even true residual disease under immune suppression. Our study highlights an unappreciated degree of complexity that arises when using ultra-accurate mutation detection for cancer monitoring.

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