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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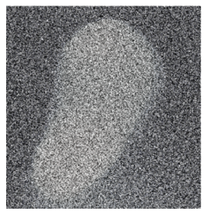
Introduction

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.

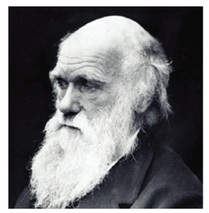
In pediatric AML, unlike adult AML, there are few cross-patient recurrent somatic driver mutations. This makes it impractical to use a fixed gene panel for MRD monitoring. One solution is to perform disease tracking with patient-specific mutations that are present in individual AMLs at time of diagnosis (TOD). However, following modern therapies, should residual disease remain while in morphologic remission, the telltale mutations may be present in only a very small fraction of cells, likely below the ~1% limit-of-detection of conventional NGS methods. Duplex sequencing (DS) is an ultra-accurate NGS error-correction method that compares both strands of each original DNA molecule to eliminate technical errors and achieve extreme accuracy and sensitivity, with an error rate $<10^{-7}$. Here we demonstrate the use of tumor-informed DS to detect patient-specific MRD in pediatric AML samples with unprecedented sensitivity and specificity.

Sequencing Errors Obscure Truth



Next-Generation Sequencing (NGS)

Single Strand Error-Corrected NGS

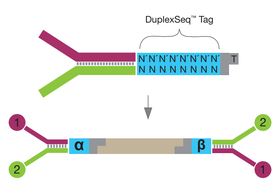


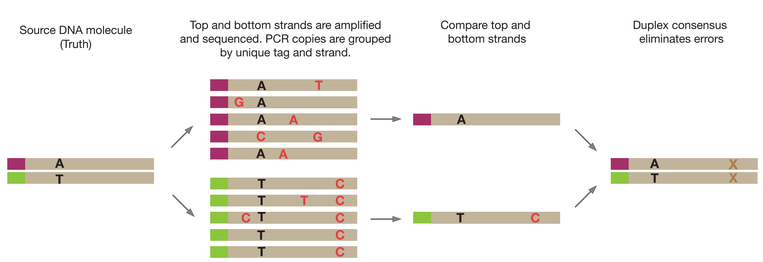
Duplex Sequencing

TwinStrand Duplex Sequencing™ Technology

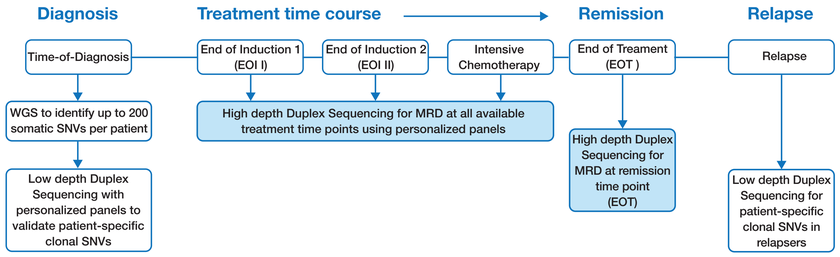
A DuplexSeq™ Adapter has:

1. Identical (or relatable) degenerate tags in each strand.
2. An asymmetry allowing independent strand identification.





Study Design



Marrow aspirates were collected from 13 pediatric AML patients at time of diagnosis (TOD), during treatment, remission, and at relapse. Among the cohort, 9/13 patients ultimately relapsed. Personalized hybrid capture panels were designed to target up to 200 somatic mutations that were present in each patient's TOD sample, as identified by whole genome sequencing of tumor-normal pairs. Duplex Sequencing was used for longitudinal monitoring of these mutations in blood or marrow at up to 5 time points following initial induction.

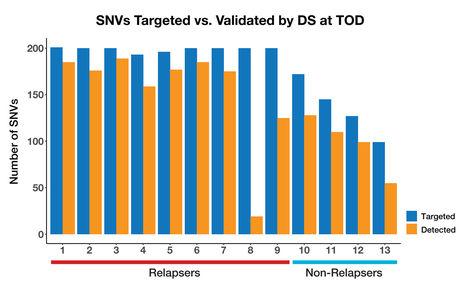
Panel Design

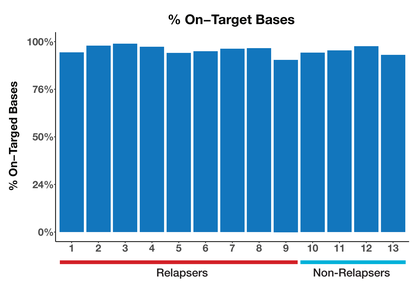
Identify high confidence somatic variant calls from WGS data of diagnosis
- Read depth >= 20 in tumor & variant allele frequency (VAF)VAF >= 5%

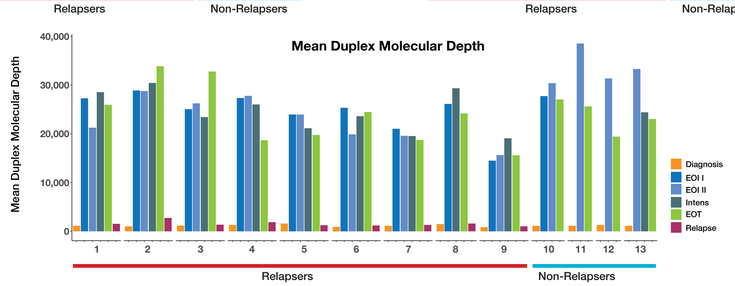
Select variants to be included in custom panel
- All coding mutations passing filter
- Noncoding variants are included to bring the total to 200 variants per patient where possible.
- Each panel also includes 10 multi-SNP loci that can be used to confirm sample identity and to screen for inter-patient contamination, if it exists.

Custom probe design
- Probes were tested for specificity with BLAST and for secondary structure with RNAfold to assess potential effectiveness in a hybrid selection experiment.

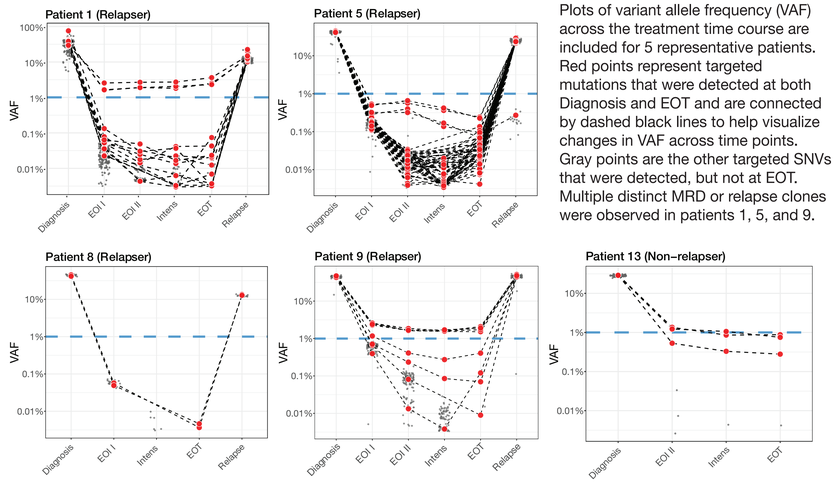
Duplex Sequencing with Patient-Specific Panels Achieved High Mean On-Target Depth Across Samples







Duplex Sequencing Enables Tracking of Rare MRD Clones



Plots of variant allele frequency (VAF) across the treatment time course are included for 5 representative patients. Red points represent targeted mutations that were detected at both Diagnosis and EOT and are connected by dashed black lines to help visualize changes in VAF across time points. Gray points are the other targeted SNVs that were detected, but not at EOT. Multiple distinct MRD or relapse clones were observed in patients 1, 5, and 9.

Results Summary

Patient Number	Group	SNV Targets	Coding SNV Targets	Targeted SNV Detected	Targeted SNV Detected at EOT	Average VAF at Diagnosis	Average VAF at EOT	Average VAF at Relapse
1	Relapser	200	11	185 (93%)	15	32.6%	0.541%	12.0%
2	Relapser	200	7	176 (88%)	7	47.4%	1.513%	47.5%
3	Relapser	200	18	189 (95%)	7	40.5%	11.303%	8.2%
4	Relapser	193	5	159 (82%)	2	32.8%	13.224%	4.1%
5	Relapser	197	6	177 (90%)	64	41.2%	0.042%	22.6%
6	Relapser	200	10	185 (93%)	52	35.7%	0.022%	34.5%
7	Relapser	200	11	175 (88%)	7	47.1%	0.208%	47.3%
8	Relapser	200	31	19 (10%)	2	44.6%	0.004%	12.5%
9	Relapser	200	15	125 (63%)	9	44.6%	0.978%	45.4%
10	Non-relapser	172	11	128 (74%)	8	37.7%	1.820%	
11	Non-relapser	145	5	110 (76%)	6	43.6%	2.914%	
12	Non-relapser	127	2	99 (78%)	46	33.4%	0.092%	
13	Non-relapser	99	5	55 (56%)	3	28.3%	0.478%	

Conclusions

- We detected MRD in one or more treatment/remission time points from all 9 patients who went on to relapse. However, we also observed high-confidence TOD variants in the last collected sample from all 4 patients who did not relapse during at least 24 months of clinical follow-up.
- In some cases, all mutations decreased at a similar rate by many orders of magnitude, whereas in others there was clear dispersion of subclones over time, with some declining 100 times more quickly than others.
- 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 clones, pre-existing somatic mosaicism, 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. Determining which somatic mutations are most biologically meaningful in pediatric AML remains an open question, but DS combined with personalized panels will be a valuable tool in future studies.

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