Long-Read Sequencing of Acute Leukemias Allows for Rapid Genome-Wide Copy Number and Methylation Analysis: A Pilot Study

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Introduction

- The diagnosis of acute leukemia requires integration of morphology, flow cytometry, cytogenetics, and molecular studies.
- Standard-of-care is time-consuming, costly, and occasionally inconclusive.
- DNA methylation profiling has emerged as a robust epigenomic marker of lineage and subtype.
- MARLIN (Methylation Analysis for Rapid Leukemia INference) is a machine-learning classifier that assigns acute leukemias into 38 methylation classes with diagnostic and prognostic significance, offering the potential to streamline classification and accelerate diagnosis (<24hrs TAT).

Methods & Objectives

- We multiplexed 10 cases of acute leukemia with established conventional diagnoses in a single 72-hour run using a PromethION flow cell (Oxford Nanopore Technologies, ONT).
- Basecalled data were processed using the wf-human-variation workflow in Epi2me to generate genome-wide copy number variation (CNV) plots and methylation profiling files, which were analyzed with MARLIN classifier.
- Concordance between conventional diagnostic testing, including in-house short read sequencing, cytogenetics/FISH and MARLIN classification was assessed by confusion matrix and Cohen's kappa.

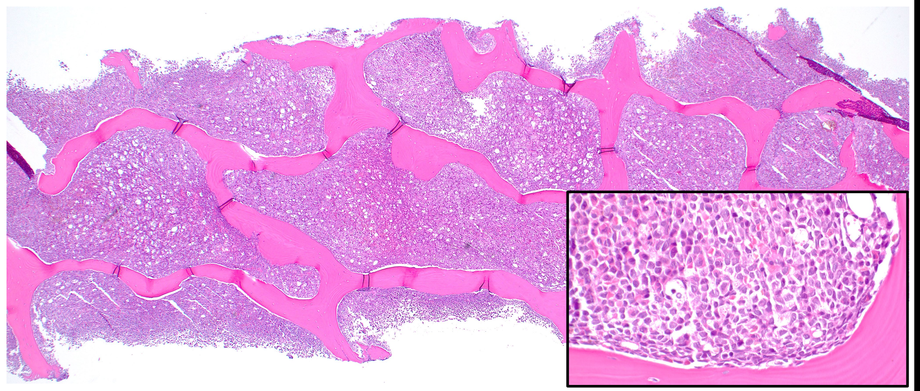

Figure 1. Microscopic image of bone marrow biopsy extensively involved by acute myeloid leukemia (AML). H&E, 40X and 600X (inset)

Results

- Low-pass whole genome CNV analysis demonstrated 100% concordance with conventional karyotype and/or FISH results (8/8).
- In two cases without karyotype, CNV analysis revealed no abnormalities.
- MARLIN achieved 90% concordance with phenotypic classification.
- All AML and AML-related cases (n=9) were classified as AML by MARLIN. One case of B-ALL was classified as T-ALL by MARLIN, representing the only discrepancy.

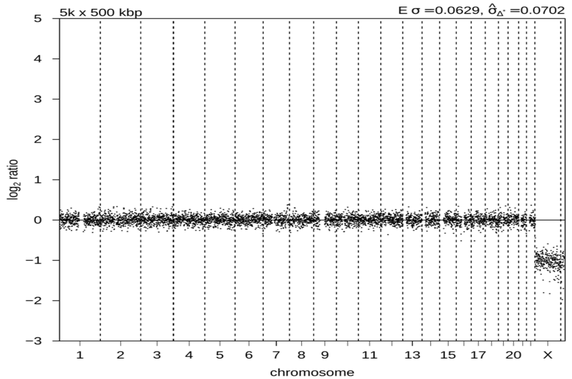

Figure 2. Normal Genome-Wide CNV Profile (Barcode01)
Copy number variation (CNV) plot demonstrating a normal diploid genome with no structural abnormalities. Log2 ratios remain close to zero across all chromosomes, confirming absence of significant copy number gains or losses.

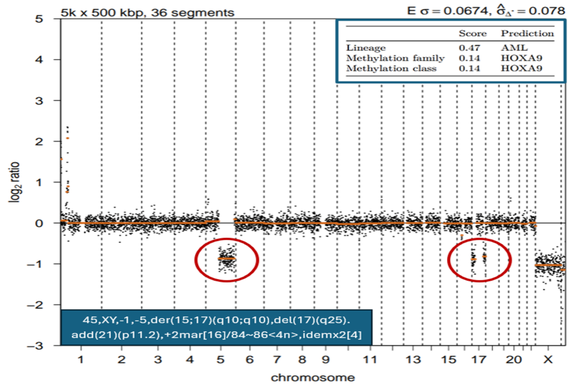

Figure 3. Integrated CNV and Epigenetic Classification of AML (Barcode02)
Genome-wide CNV plot with MARLIN classification and corresponding karyotype showing significant 5q and 17q losses (red circles).

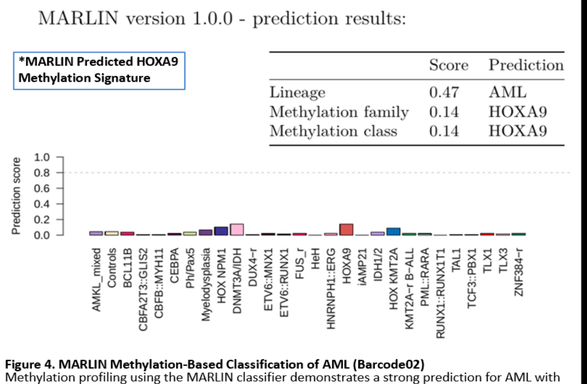

Figure 4. MARLIN Methylation-Based Classification of AML (Barcode02)
Methylation profiling using the MARLIN classifier demonstrates a strong prediction for AML with a HOXA9 methylation signature (Lineage score 0.47, Methylation family/class score 0.14). This molecular result aligns with the CNV abnormalities detected (5q and 17q losses) and supports an integrated genomic–epigenomic diagnostic approach.

Conclusions

- ONT + MARLIN methylation analysis demonstrates strong concordance with conventional methods and offers the potential to decrease turnaround time and the number of ancillary testing.
- Real time data analysis could provide results in less than 24 hours.
- By lowering costs, expediting therapeutic decisions, and improving diagnostic confidence, ONT + MARLIN represents a promising adjunct to conventional hematopathology practice, with direct benefits for pathologists, clinicians, and patients.

References & Acknowledgements

Steinicke, T.L., Benfatto, S., Capilla-Guerra, M.R. *et al.* Rapid epigenomic classification of acute leukemia. *Nat Genet* **57**, 2456–2467 (2025). https://doi.org/10.1038/s41588-025-02321-z

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