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Unravelling Prognostic Significance: Long-Read Nanopore Sequencing of TP53 and IGHV in Chronic Lymphocytic Leukaemia Patients in Tanzania

Collin Nzunda[1], Eliud Buchard[1], Jeffer Bhuko[1], Heavenlight Christopher[1], Emmanuel Saukiwa[3], Caro Mollel[1], Rehema Shungu[1], Doreen Kamori[2], Clara Chamba[1], Helene Dreau[4], Kate Ridout[4], Antony Cutts[4], Ahlam Nasser[1], Anna Schuh[1,4] , Dimitris Vavoulis[4], Adam Burns[4], William Mawalla[1]

[1]Department of Hematology and Blood Transfusion, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, [2]Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, [3]Department of Biochemistry and Molecular Biology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania. [4]Department of Oncology, University of Oxford, Oxford, United Kingdom, nzundacr@gmail.com

OBJECTIVES

- We aim to establish a feasible, cost-effective strategy using long-read nanopore sequencing to generate actionable prognostic information locally.

CONCLUSIONS

- By delineating mutations within the *TP53* gene and uncovering diverse *IgHV* mutation profiles, we have gained invaluable prognostic insights despite inherent resource constraints. These findings underscore the pivotal role of advanced sequencing technologies in informing clinical decision-making and enhancing patient outcomes in the management of CLL, even in resource-limited environments.

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INTRODUCTION

- Chronic lymphocytic leukaemia (CLL) exhibits considerable clinical heterogeneity, necessitating the identification of reliable prognostic markers for disease stratification.
- While integration of molecular diagnostics and targeted therapies has transformed outcomes in high-income settings, comparable gains remain elusive in many low- and middle-income countries (LMICs). Structural barriers including limited access to reliable molecular testing, delayed diagnosis, constrained formularies for novel agents, and fragmented care pathways translate into avoidable morbidity and mortality.
- Addressing this inequity requires scalable, cost-conscious diagnostic platforms, streamlined testing algorithms centred on prognostic markers such as *TP53* and *IGHV*, strengthened laboratory and clinical capacity to ensure that the prognostic insights afforded by modern CLL biology are translated into tangible improvements in patient care.

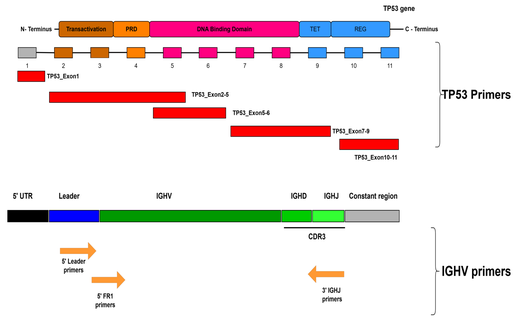
METHODS

- We prospectively enrolled 35 treatment-naïve CLL patients at Muhimbili National Hospital, Tanzania.
- Genomic DNA from CLL peripheral blood was amplified for *TP53* (exons 1–11) and *IGHV* (leader/FR1–JH), prepared as amplicon libraries, and sequenced on the Oxford Nanopore platform.
- Sequencing reads underwent basecalling, demultiplexing, QC, and GRCh38 alignment; *TP53* variants were called and annotated, while *IGHV* consensus sequences were analysed with *IMGT/V-QUEST* to assign germline gene/allele, and classify *IGHV* status. All reportable variants were orthogonally confirmed using a commercial multi-gene targeted panel.

RESULTS

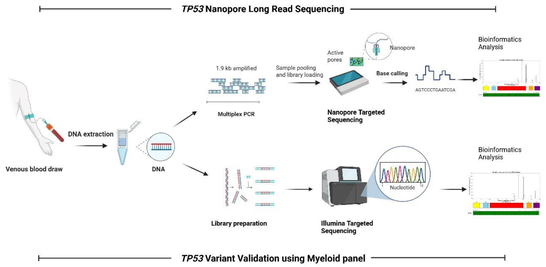
- Median age 65 years; 63 % male; 63 % Rai III–IV.
- Clinical follow-up data were available for 26 patients in the study cohort, including three individuals with *TP53* mutations.
- Among all patients, **10 of 26 (38.5%) experienced progressive disease**, and 5 (19.2%) died during the observation period.
- Of the **three patients with *TP53* mutations**, two died during follow-up. In the *TP53* unmutated group, 10 of 23 patients (43.5%) experienced progressive disease, and three died.

Figure 1. Primer design



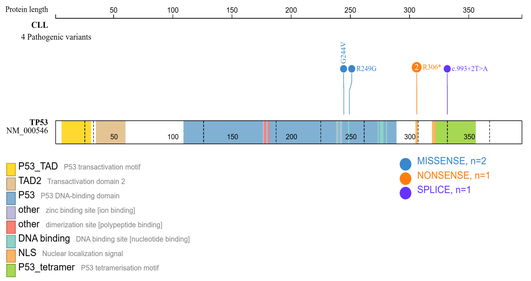
TP53 primers cover exons 1–11; IGHV primers (leader/FR1–JH) capture CDR3

Figure 2. Laboratory Workflow



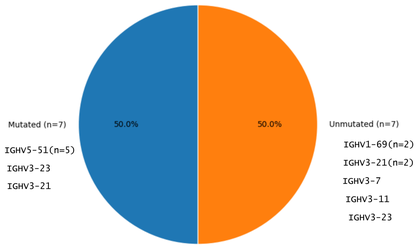
overview of the laboratory procedures using Nanopore long read sequencing and validation of the variants using Myeloid panel

Figure 3. Distribution of *TP53* Variants Across Functional Domains



Schematic representation of *TP53 (NM_000546)* mutations identified in the cohort, mapped across the major functional domains.

Figure 4. IGHV rearrangements



Mutated defined as IGHV V-region identity <98% per ERIC; productive rearrangements only

Table 1. *TP53* Pathogenic Variants platform comparison

Sample ID	TP53 Variant	Classification	Pathogenicity	VAF % (Nanopore)
CLL022	*p.Arg306Ter*	Nonsense	Pathogenic	13.5
CLL034	*c.993+2T>A*	Splice_Site	Pathogenic	94.9
	p.Gly244Val	Missense	Pathogenic	10.1
CLL035	*p.Arg306Ter*	Nonsense	Pathogenic	10.1
CLL024	*p.Arg249Gly*	Missense	Pathogenic	38.4

Notes: VAF: Variant Allele Frequency

VAF, variant allele frequency; HGVS nomenclature; ACMG/AMP classification; "Not detected" = below assay limit of detection.

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DISCLOSURES

The authors declare that they have no conflicts of interests