Genomic distinctions of HIV- and EBV-associated DLBCL in a diverse African cohort

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Introduction

Diffuse large B-cell lymphoma (DLBCL), the most common type of lymphoma globally, displays significant genomic heterogeneity. Despite its prevalence and poor prognosis, there is a notable lack of high-quality genetic studies on DLBCL among black individuals and in people living with Human Immunodeficiency Virus (PLHIV) in Africa. This study aims to elucidate the impact of HIV status and Epstein-Barr Virus (EBV) on the genetic landscape and molecular subtypes of DLBCL in ethnically diverse Sub-Saharan Africa patients.

Methods

We identified 66 consecutive DLBCL cases (Table 1) diagnosed at a tertiary hospital in Cape Town between 2010 and 2022 with FFPE or FF tissue available. Tumor specimens underwent histological review, whole exome sequencing (WES), and bulk whole transcriptome sequencing. Wherever possible, paired germline was obtained through microdissection or saliva collection in surviving patients (n = 32). Clinical records were obtained at Groote Schuur Hospital.

Table 1. African cohort overview

Characteristics		Total	HIV status: Negative	HIV status: Positive
Sample size number		66 (100%)	17 (26%)	49 (74%)
Age, years median (range)		49 (18–83)	57 (27–83)	43 (18–83)
Sex number	Female	31 (47%)	7 (41%)	24 (49%)
	Male	35 (53%)	10 (59%)	25 (51%)
Ethnicity number	Black African	48 (73%)	5 (29%)	43 (88%)
	Mixed Race	14 (21%)	8 (47%)	6 (12%)
	White	4 (6%)	4 (24%)	0 (0%)
Stage number	I – II	19 (29%)	8 (47%)	11 (22%)
	III – IV	44 (66%)	9 (53%)	35 (71%)
	Unknown	3 (5%)	0 (0%)	3 (6%)

Results

1 Figure 1. Common alterations in DLBCL-associated pathways

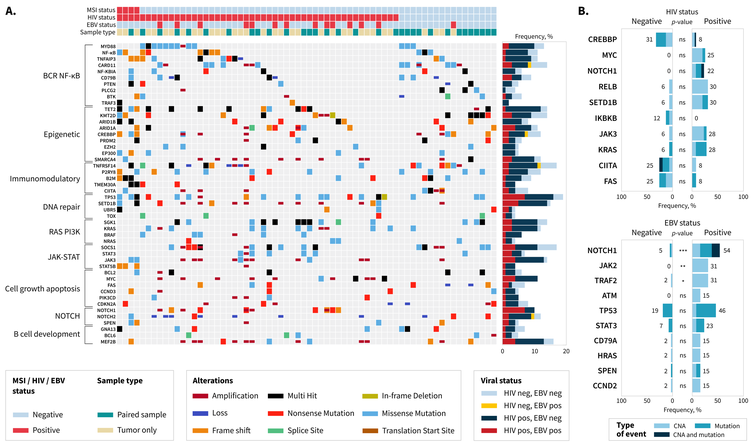

Figure 1. A. The spectrum of protein-coding alterations was examined by WES for paired (n = 32) and tumor only samples (n = 34) from 66 patients. While most alterations detected were mutations, some genes showed only copy number alterations (CNA). We identified genetic aberrations in well-described pathways affecting B-cell lymphoma pathogenesis, including DNA repair, NOTCH and JAK-STAT signaling, B-cell signaling and development, and apoptosis. The breakdown of mutation frequency by HIV and EBV status is given in the bar-plot on the right side. **B.** Top 10 genes with the highest difference in number of alterations between samples with or without viral infection for both HIV and EBV. *NOTCH2*, *JAK2* and *TRAF2* mutations showed significant enrichment in EBV-positive patients. * *p*-value ≤ 0.05, ** *p*-value ≤ 0.01, *** *p*-value ≤ 0.001 derived from the Mann–Whitney U test; ns — not significant.

2 Figure 2. The African cohort differs from published cohorts in its gene mutation landscape

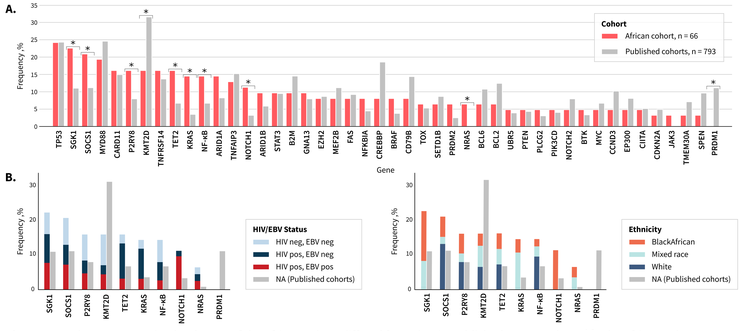

Figure 2. A. The gene mutation landscape of the African cohort differed from that in published DLBCL cohorts[1,2]. The African cohort presented with significantly higher mutation frequencies in *SGK1*, *SOCS1*, *P2RY8*, *TET2*, *KRAS*, *NF-κB*, *NOTCH1*, and *NRAS*, and lower mutation frequencies in *KMT2D* and *PRDM1*. **B.** Statistically significant difference observed for these 10 genes could not be attributed to EBV status, HIV status, or inherent ethnic and population differences between the African cohort and other established cohorts, except for *NOTCH1* associated with EBV. *p-value ≤ 0.05 derived from the Mann–Whitney U test.

3 Figure 3. Hodgkin-like morphology in EBV+ patients with DLBCL

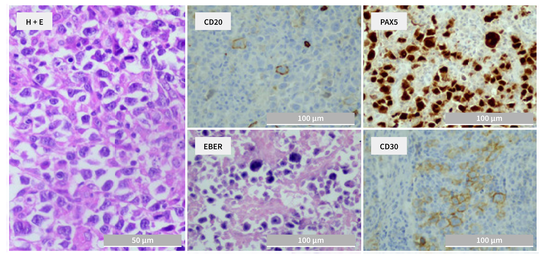

Figure 3. Several *NOTCH1* altered cases exhibited variable Hodgkin-like morphology, patchy downregulation or loss of CD20 and aberrant CD30 expression. These cases are confirmed as EBV+ (determined with Epstein–Barr encoding region (EBER) in situ hybridization). Despite the loss of CD20, these cells strongly expressed PAX5, corroborating their B-cell lineage. A hematopathology review of this unusual morphology concluded these cases to most likely be EBV+ DLBCL. Slides of a representative patient are shown.

4 Figure 4. *NOTCH1* mutations were associated with worse OS

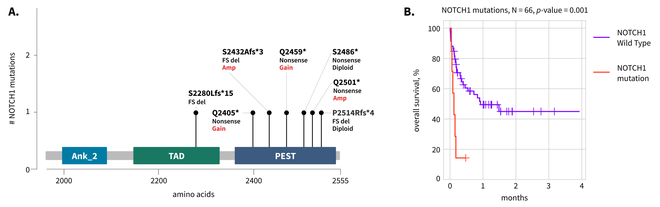

Figure 4. A. Alterations in *NOTCH1* were found in 7/66 (11%) samples. Most of these alterations were gain of function mutation and were located in the PEST domain. **B.** Cases with *NOTCH1* mutation had a strikingly poor survival .

5 Figure 5. A significant portion of cases could not be genomically subtyped using the current LymphGen classifier

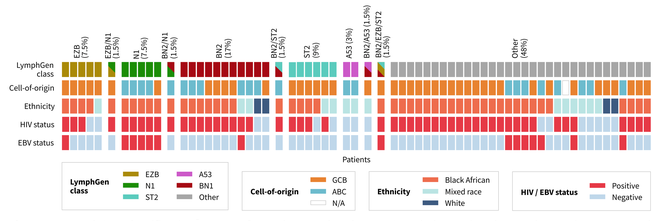

Figure 5. LymphGen classification[3] was performed to resolve the DLBCL subclass using detected mutations, CNA and fusions. EBV-positive cases showed clustering in the N1 cluster. HIV-positive cases were evenly distributed among clusters, implying an absence of connection between HIV infection and tumor subtype. A high proportion of HIV (45%) and EBV (43%) cases were classified as "Other". None of the samples were assigned to the MCD cluster.

6 Figure 6. Microsatellite instability was associated with HIV infection

MSI status		High	High	High	High
Viral status	HIV	Pos	Pos	Pos	Pos
	EBV	Neg	Neg	Neg	Neg
Protein	MLH1	Neg	Pos	Pos	N/A
	MSH2	Pos	Pos	Neg	N/A
	MSH6	Pos	Pos	Neg	N/A
	PMS2	Pos	Pos	Pos	N/A
Gene	*MSH2*				
	MSH6				
	MLH1				
	PMS2				
	MSH3				
	BRAF				

Figure 6. Microsatellite instability (MSI) was detected in 4/49 HIV-positive cases (p = 0.5). We identified mutations affecting key DNA-mismatch repair (MMR) genes in 3 of 4 MSI-high cases. IHC for MMR proteins was performed in 3/4 cases, confirming loss of the associated protein in 2/4 and in the remaining case the mutation *MSH2* (R524P) is described in association with normal expression MSH2 despite functional impairment.

Alterations: Frame shift; Missense Mutation; Multi Hit

Conclusions

1. The African cohort exhibits many of the same genetic aberrations observed in DLBCL, but with a notably higher mutation rate in *TET2* and genes within the PI3K/RAS pathway (*SGK1, KRAS, NRAS*).
2. EBV-positive cases were genomically distinct and characterized by frequent alterations in *NOTCH*, *TRAF2*, and *TP53*.
3. A subset of cases with *NOTCH1* gain-of-function (GOF) mutations exhibited morphological features overlapping with those of Hodgkin lymphoma. Cases with *NOTCH1* alterations showed poor overall survival.
4. LymphGen analysis revealed a substantial proportion of unclassified cases, indicating that genomically, these cases do not align well with existing classifications. Intriguingly, there were no cases of the MCD subtype.
5. Four cases (6% of patients), all HIV-positive, demonstrated MSI with protein-altering mutations in DNA mismatch repair (DNA-MMR) genes.
6. This study lacked the statistical power to assess mutational differences between HIV-positive and HIV-negative cases. Panel-based sequencing is currently underway to enhance the representation of HIV-negative cases in the same cohort.

References:

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2. Schmitz, R., et al. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med* 2018; 378: 1396-1407.
3. Wright, G.W., et. al. A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. *Cancer Cell* 2020 Apr 13; 37(4): 551-568. e14.

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