Transcriptomic Analysis of KSHV-Associated Multicentric Castleman's Disease from a Malawi Cohort

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Background

- Multicentric Castleman's Disease (MCD) is a rare polyclonal B cell lymphoproliferative disorder
- MCD is associated with Kaposi sarcoma-associated herpesvirus (KSHV) and prevalent in HIV+ individuals
  - Incidence of MCD has increased with access of antiretroviral therapy
  - The high rates of HIV/KSHV in Africa and underdiagnosis potentially points to underreporting of MCD in this population
- Preliminary analysis of MCD's biological characteristics can promote further research on improving clinical outcomes and treatment strategies

Methods

- Cohort from the prospective Kamazu Central Hospital Lymphoma Study
- Patients were diagnosed with MCD or non-MCD benign lymphadenopathy for the purpose of this research
- Clinical Whole Exome Sequencing performed on DNA extracted from FFPE tumor and blood
- Differential expression analysis of RNA-Seq and statistical analysis of clinical data
- Virdetect workflow to compare viral reads of oncogenic viruses within each sample
- BCR MiXCR identified B-cell receptor profiles, focusing on IG-H due to heightened variability.

Patient cohort

	Benign (N=15)	KS (N=1)	KS + MCD (N=1)	MCD (N=19)	Total (N=36)
Age					
Median [Min, Max]	44.0 [22.0, 53.0]	57.0 [57.0, 57.0]	31.0 [31.0, 31.0]	40.0 [27.0, 57.0]	41.5 [22.0, 57.0]
Sex					
Male	NA	1 (100%)	0 (0%)	13 (68.4%)	14 (38.9%)
Female	NA	0 (0%)	1 (100%)	6 (31.6%)	7 (19.4%)
Survival (months)					
Median [Min, Max]	NA	60.0 [60.0, 60.0]	0.723 [0.723, 0.723]	43.6 [1.15, 60.0]	43.6 [0.723, 60.0]
CD4 Count					
Median [Min, Max]	NA	149 [149, 149]	2.00 [2.00, 2.00]	417 [62.0, 1150]	360 [2.00, 1150]

Table 1. Characteristics of sequencing cohort. Benign controls from UNC Hospitals with limited clinical characteristics

Results

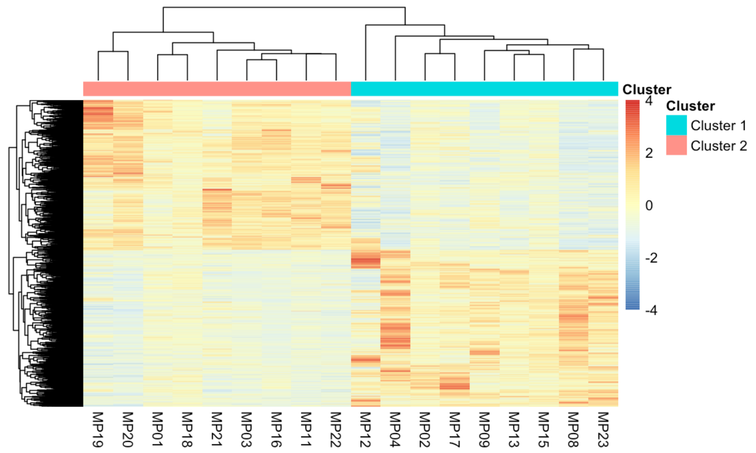

Fig 1. Heatmap visualization of the gene expression across the samples. Aligns with the previously identified clusters in the MCD cohort with similar genes being upregulated and downregulated in each group.

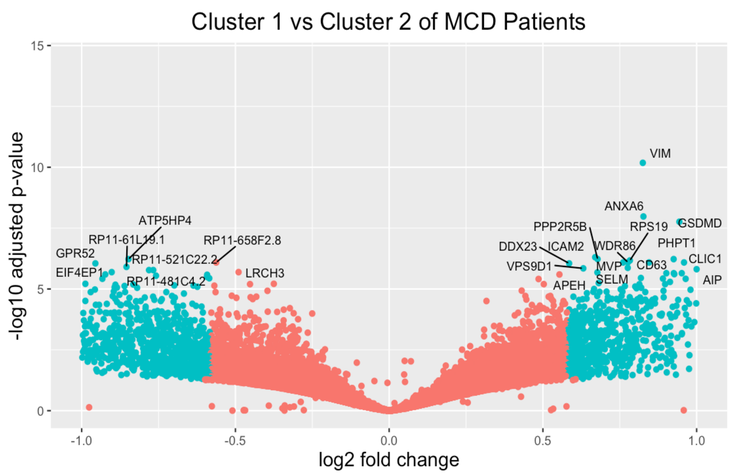

Fig 2. Top 50 significant genes labelled (padj < 0.05, fold change > 0.58) on volcano plot. Log2 fold change greater than zero are overexpressed in Cluster 2. Gene set enrichment analysis reveals that Cluster 2's significant genes are involved in complement system activation of the innate immune system.

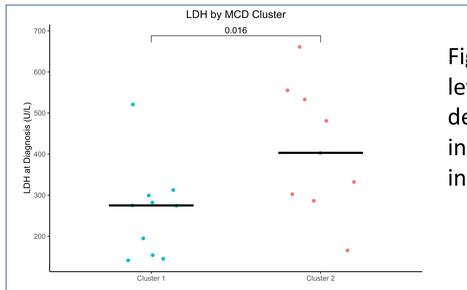

Fig 3. Wilcoxon rank test of LDH levels in MCD Patients. Lactase dehydrogenase (LDH) when high indicates cell and tissue damage in the body

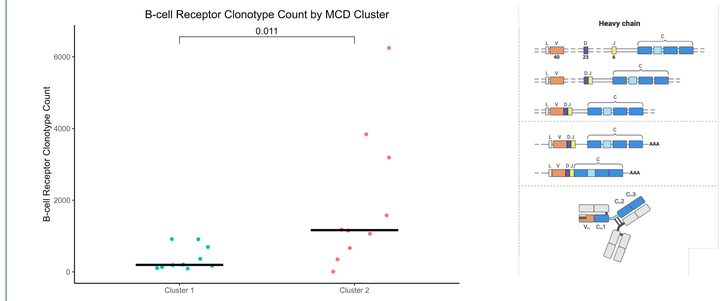

Fig 4. Wilcoxon rank test of B-cell receptor clonotypes with unique VDJ sequences present in MCD cluster 1 and 2. Cluster 2 patients had higher diversity in clonotypes and total number of clones.

Discussion

- MCD patients can be distinguished biologically by gene expression as well as increased clonality of the B cells present
- Inflammatory biomarkers, such as LDH and increased diversity in B-cell receptors
- Findings show that the possible differences between the identified clusters is in how the patient's immune system reacts to the

Future Directions

- Confirm whether the B cells observed in cluster 2 represent background activity of somatic hypermutation and increased activation, or KSHV infected cells
- Identify MCD latency patterns by quantifying virus-transcribed mRNAs of Epstein Barr virus and KSHV.

Acknowledgments

UNC PROJECT Lilongwe, Malawi

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