ELSEVIER

Blood 142 (2023) 2556–2557

The 65th ASH Annual Meeting Abstracts

# POSTER ABSTRACTS

## 203.LYMPHOCYTES AND ACQUIRED OR CONGENITAL IMMUNODEFICIENCY DISORDERS

### Somatic Mutational Landscape and Transcriptomic Profiles of Lymphoproliferative Disorders with Epstein-Barr Virus Infection in Activated PI3K Delta Syndrome

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**Introduction:** Germline gain-of-function variants in *PIK3CD* and *PIK3R1* cause activated PI3K delta syndrome (APDS) types 1 and 2, respectively. Lymphoproliferative disorder (LPD) and susceptibility to various infections, including Epstein-Barr virus (EBV), are well-known features of APDS. EBV-associated LPDs are frequently observed in patients with APDS, and oligoclonal LPD might transform malignant lymphoma (ML). However, somatic molecular alterations in LPD and ML are not fully elucidated. Here, we report clinical characteristics and somatic mutational landscape and transcriptomic profiles of LPDs with EBV infection in APDS.
**Methods:** LPD and ML in APDS diagnosed during 2011-2022 were recruited all over Japan. Clinical characteristics and outcomes were analyzed. EBV infection status was analyzed by EBER ISH and RNA sequencing (RNA-seq). Whole-exome sequencing (WES), RNA-seq, and single cell RNA-seq (scRNA-seq) were also performed.
**Results**: Five LPD and 5 ML (borderline ML: n = 2, diffuse large B-cell lymphoma (DLBCL): n = 2, classical Hodgkin lymphoma: n = 1) cases were enrolled. Seven cases were APDS1 and 3 cases were APDS2. The median follow-up period was 2.9 (0.5-8.4) years. LPD cases were treated with rituximab monotherapy (n = 1), prednisolone (n = 3), and hematopoietic cell transplantation (HCT) (n = 1). Rituximab-combined chemotherapy was performed in borderline ML and DLBCL (n = 4) or ABVD regimen in Hodgkin lymphoma cases (n = 1). Sirolimus was used in two ML cases. HCT was performed in 9 cases. Two-year overall survival and event-free survival (events: relapse and death) were 78.8% and 70.0% in total LPDs, respectively, and 60% and 40% in ML cases.
EBV was negative in 3 cases and positive in 2 cases of LPD cases, and positive in 4 cases, and negative in 1 case of ML cases. The median of 18 (10-48) and 42 (18-189) somatic variants were detected by tumor/germline pair WES in LPD (n = 5) and ML (n = 7, 2 samples were from the same patient at different time points), respectively. The recurrent variants were identified in *ZFHX3* (n = 4), *TRPM2* (n = 3), *BEND3*, *CDH23*, *UBR4*, *ARAF*, *TET2*, *FBXO10*, *MAP4K1*, *KMT2C*, *KMT2D* and *PIK3R1* (n = 2) in LPD and ML cases. In transcriptome analysis, genes associated with lymphocyte activation including B-cell receptor signaling were upregulated in LPD, cell-cell adhesion, cellular response to TNF, and IL-18 signaling pathway were upregulated in ML samples. We performed correlation analysis to elucidate a relationship between EBV infection status and host transcriptomic profiles using RNA-seq data. Interestingly, in addition to cell-cell adhesion and inflammatory machinery, the upregulated molecules of the p53 pathway and negative regulation of the immune system were correlated with transcripts associated with EBV latency, such as EBNA, LMP-1, and LMP-2. In single-cell transcriptome analysis, LPD sample consisted of two large clusters of T cells and B cells with 10 subsets, and EBV-positive DLBCL sample consisted of one independent large cluster with 9 subsets. In LPD sample, components indicative of the normal lymphocyte differentiation process, including the

germinal center, were preserved. The molecules involved in lymphocyte activation were upregulated in LPD subsets. In DLBCL sample, the genes associated with mitotic G2-M phases, chromatin organization and RNA metabolism were upregulated in the subsets in which EBV transcripts were more actively transcribed.

**Discussion and Conclusions**: Our retrospective analysis demonstrated that the outcome of LPD and ML in APDS is not satisfactory. scRNA-seq data revealed that cells in LPD still retain the signature of normal lymph node. However, once transformed with EBV, EBV infection may affect epigenetic machineries including chromatin organizer followed by active RNA transcription involved in cell-cell adhesion, inflammatory machineries, molecules of p53 pathway and negative regulation of immune system in the host genome. As the outcome of ML is inferior to LPD, preemptive HCT would be an option for LPD cases.

**Disclosures** No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-177663