

## CAN EBV CPG METHYLATION IN PLASMA DISTINGUISH EBV LYMPHOMA (+) SUBTYPES?

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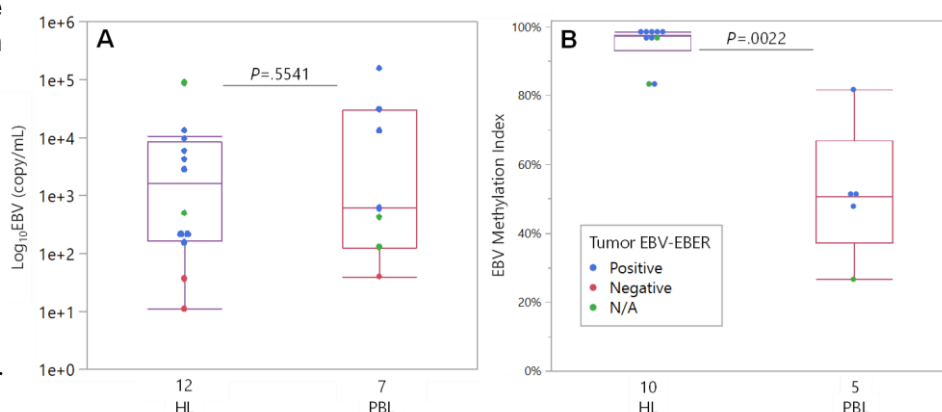
**Background:** We have presented data suggesting that CpG methylation of EBV DNA in plasma is consistently found in people with EBV(+) Hodgkin lymphoma and nasopharyngeal carcinoma [1]. Others have presented evidence that patterns of CpG methylation of EBV in plasma may vary between people with nasopharyngeal carcinoma and non-malignant EBV(+) conditions [2]. Among people living with HIV (PLWH), classical Hodgkin lymphoma (HL) and plasmablastic lymphoma (PBL) are almost uniformly associated with the virus. While we have shown that there is often high levels of plasma EBV in patients with EBV(+) tumors [3], we are now investigating the utility of detection of CpG methylated EBV DNA in plasma in PLWH with lymphoma.

**Methods:** With appropriate approvals from human investigations and ethics committees, we collected plasma from newly-diagnosed untreated patients with HIV and HL or PBL in Johannesburg, South Africa. Diagnostic biopsy specimens were reviewed in Baltimore by a Hematopathologist (RRX) to confirm the diagnosis and EBER in situ hybridization was also performed to confirm EBV association. Plasma EBV copies were assessed with standard qPCR targeting the BamW repeats. qPCR using the same primers was also performed after capture with methyl-

DNA binding protein 2 (MBD2) magnetic beads where captured fractions represent methylated DNA and flow-through represents unmethylated DNA.

**Results:** A total of 19 patients were recruited, 12 HL and 7 PBL, and 15 have EBV-EBER results. Eight of ten HL tumors were EBV(+) and 4 of 5 PBL tumors were EBV(+). Analysis of EBV DNA copy number in plasma showed overlapping levels of EBV DNA (median 3.2 Log<sub>10</sub> copy/mL in HL and 2.7 Log<sub>10</sub> copy/mL in PBL). Patients with at least 1.5 Log<sub>10</sub> copy/mL of EBV underwent methylation evaluation, which showed very different EBV CpG methylation in the two tumor types. The EBV methylation index was uniformly high in HL (median of 97%), whereas the methylation index of EBV was more variable in PBL (median of 51%).

**Conclusions:** High levels of plasma EBV is present in EBV(+) lymphoma in PLWH, but HL and PBL show very different methylation indices of the BamW repeat of EBV. Exploration of the entire methylome of the viral genome may reveal lymphoma subtype specific patterns.



**Fig. 1. Plasma EBV and methylated EBV in HIV(+) lymphoma. A.** EBV qPCR in patients with HIV (+) HL and PBL. Median EBV is 3.2 Log<sub>10</sub> in HL and 2.7 Log<sub>10</sub> in PBL. **B.** EBV methylation index in plasma in HL and PBL. Median EBV methylation index is 97% in HL (95% CI 90-99%) and 51% in PBL (95% CI 27-76%). Wilcoxon Rank Sums tests performed. Tumor EBV-EBER expression by in-situ hybridization is color coded in blue for positive, red for negative and N/A for not assessed.

Topic: Hodgkin lymphoma

Preferred presentation: Oral

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3. Kanakry JA, Hegde AM, Durand CM, Massie AB, Greer AE, Ambinder RF, Valsamakis A. 2016. *Blood* 127:2007-2017.