Establishment of a ddPCR method for detecting Epstein-Barr Virus in MS samples

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Background & Aims: Accumulating evidence shows that Epstein-Barr virus (EBV) infection, particularly in B cells, may be involved in the pathogenesis of multiple sclerosis (MS). ATA188, an investigational off-the-shelf, allogenic T-cell immunotherapy that targets EBV-infected cells, is in Phase 2 clinical evaluation for the treatment of progressive MS (EMBOLD Trial, NCT03283826) and has shown promising initial safety and efficacy (ECF 2020; ECTRIMs 2021). The quantification of EBV infected cells in clinical trial subjects will help support ATA188's proposed mechanism of action (MoA) of targeting and eliminating EBV infected B cells and plasma cells and help substantiate its encouraging clinical profile (ECTRMS 2021). Though numerous commercial and in-house quantitative PCR formats are used to detect EBV viral load, a standardized method using clinical peripheral blood mononuclear cells (PBMCs) and cerebral spinal fluid (CSF) cells to assess cell associated EBV has not yet been established. Here we describe the establishment of a highly sensitive and quantitative droplet digital PCR (ddPCR) method for the purpose of testing EBV viral load in clinical samples from the EMBOLD study.

Methods: To optimize detection of varying EBV strains, we designed primer/probes specific to conserved EBV associated target genes BWRF1 and BCLF1. We assessed and optimized assay performance using EBV naïve Jurkat and PHA blast T cells as well as EBV positive RAJI cells and EBV seropositive PBMCs.

Results: Both BWRF1 and BCLF1 show specificity to EBV DNA, and the ability to detect EBV in RAJI and in B cells from seropositive donors.

Conclusions: This highly sensitive ddPCR approach combined with optimized EBV associated target genes can be used to quantify the precise viral load in clinical samples collected from participants in the EMBOLD study before and following treatment with ATA188. Data generated using this technique may be used to support the ATA188 proposed MoA of targeting and eliminating EBV infected B cells and plasma cells thought to be involved in the pathogenesis of MS.