Introduction
Multiple sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system. Although the precise etiology of MS is unknown, data from epidemiological, genetic and twin studies suggest that MS develops in genetically susceptible individuals through the interaction with environmental factors. Among the environmental factors, the B-lymphocytotropic γ-herpesvirus Epstein-Barr virus (EBV) plays a unique role. While 90 – 95% of the general population are EBV positive, practically all patients with MS have evidence of prior EBV infection. Likewise, symptomatic primary EBV infection (infectious mononucleosis) is associated with an increased MS risk. Longitudinal studies have shown that initially EBV-seronegative persons became EBV seropositive before the development of MS (1). Furthermore, patients with MS have elevated antibody levels against EBV antigens as compared to healthy controls (2). Even though some knowledge on the targeted antigens is available, specific information on the relevant epitopes is scattered. Detailed information on these epitopes may help to reveal pathogenetic mechanisms and develop new diagnostic tests.

PepStar™ Peptide Microarrays are efficient tools for the differential analysis of serum-antibody levels on epitope level. Libraries of thousands of peptides spanning multiple annotated or potential antigens can be presented on a single peptide microarray. Sequence variants and post-translational modifications can easily be covered. Incubation of the microarray with a very small amount of patient serum (~1µl) leads to a comprehensive dataset of antibody reactivities. PepStar™ Peptide Microarrays have been successfully used in infectious & autoimmune diseases, allergy research, and cancer. Here we present an application in MS.

Results
A peptide library containing 1465 peptides spanning 8 EBV antigens (EBNA-1, EBNA-3, EBNA-4, EBNA-6, BLRF2, BZLF1, LMP1, VP26) was designed as scans of 15-meric peptides with an overlap of 11 and 4 amino acids offset. Peptides were synthesized using SPOT technology (3) and printed onto functionalized glass slides (4). For screening 1µl serum of MS-patients (n=29) and healthy donors (n=22) was diluted and incubated on the microarray. Detection was performed in a secondary incubation step with a fluorescently labeled secondary anti-human IgG antibody (Fig. 1). 39 of all peptides (2.7 %) showed significantly higher reactivities in MS patients compared to healthy controls (p<10⁻², Mann-Whitney U test). On the other hand, no single peptide showed significantly higher reactivities in the control cohort.

44 % of the peptides with higher reactivity in MS originate from EBNA1, 18 % from EBNA-3, 15 % from EBNA-4 and EBNA-6, respectively. Two peptides are from VP26 and one from LMP1. The majority of significantly different EBNA-1 peptides was located in the Gly-Ala-rich region of this protein from amino acids 87 – 352 (Fig. 1). The remaining 4 EBNA-1 peptides are found in the C-terminal region of the protein. As expected, several of the peptides with significantly altered signals between the groups overlapped (e.g. EBNA1_151-165, EBNA1_154-168, EBNA1_157-171). Due to the high multiplicity of the assay a correction for multiple comparisons is advisable. After Bonferroni correction three peptides met requirements for significance (p<10⁻⁵). Two of these peptides originate from the Gly-Ala-rich region of EBNA-1 (EBNA1_109-123, EBNA1_214-228) and one from the C-terminal region of EBNA-6 (EBNA6_841-855) (Fig. 2).

The latter peptides were used to retrospectively discriminate between healthy controls and MS-patients. The areas under the curve were 0.86 for EBNA1_109-123, 0.86 for EBNA1_214-228, 0.86 for EBNA6_841-855, and 0.9 for antibody reactivities against all 3 peptides combined.
Conclusions
Here, we present the use of JPT’s high density peptide microarrays for the serological screening of human serum samples in a cohort of healthy control individuals and patients with MS. In agreement with and extending previous reports (summarized in (5)) elevated antibody titers towards peptides from EBV antigens were detected in MS-patients as compared to healthy controls. The identified antigens include EBNA-1, EBNA-3, EBNA-4, and EBNA-6. Interestingly, most of the antibody responses against EBNA-1 are specific for the long Gly-Ala-rich stretch of the protein, which points to an important role of this region in the MS-specific B-cell immune response. The reasons for this phenomenon are currently unclear, but the elevated immune response to the Gly-Ala repeat of EBNA-1 may represent a clue to the pathogenesis of MS. Using three peptides with the best separation between the patient groups, an area under the curve of 0.9 could be achieved in a ROC analysis (Fig. 2B), indicating the potential of the screening technology for the fast and efficient identification of powerful serological biomarkers.

References
5. “Multiple sclerosis: The elevated antibody response to Epstein–Barr virus primarily targets, but is not confined to, the glycine–alanine repeat of Epstein–Barr nuclear antigen-1.” Ruprecht et al., J Neuroimmunol (2014)

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The Company
JPT Peptide Technologies is a DIN ISO 9001:2015 certified and GLP compliant integrated provider of innovative peptide solutions for: cellular and humoral immune monitoring, seromarker discovery & validation, vaccine target discovery, peptide lead identification & optimization, targeted proteomics, and enzyme profiling.

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