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LETTER TO THE EDITORS

Array CGH-based analysis of post-transplant plasmacytic hyperplasia reveals 'intact genomes' arguing against categorizing it as part of the post-transplant lymphoproliferative disease spectrum

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Dear Sirs,

Post-transplant lymphoproliferative disorders (PTLD) are defined as lymphoid proliferations arising after solid organ or hematopoietic stem cell transplantation [1]. The majority of them are associated with Epstein–Barr virus (EBV) infection facilitated by the continuous immunosuppression of the patients. PTLD are categorized by the World Health Organization (WHO) into four categories. They range from early lesions (ePTLD) to overt lymphomas. ePTLD are further subdivided into plasmacytic hyperplasia (PH) and infectious mononucleosis-like lesions [1]. ePTLD are known to be polyclonal [2]. In 2007, Vakiani *et al.* [3] proposed a third entity of ePTLD described as follicular hyperplasia, which showed cytogenetic abnormalities using

Table 1. Patients' characteristics.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at transplantation (years)	40.4	17.4	57.5	57.5	5.3
Age at diagnosis of ePTLD (years)	52.3	28.0	61.9	64.6	10.3
Type of organ transplant	Kidney	Heart	Heart	Kidney	Kidney
Time from transplantation to detection of ePTLD (months)	143.3	126.6	52.4	85.9	60.4
Site of ePTLD	Tonsil	Tonsil	Paracolic lymph node	Inguinal lymph node	Tonsil
Serological EBV-status at transplantation	Positive	Positive	No serological information available	Positive	Positive
Presence of EBV in the ePTLD specimen	Negative	Positive in 1–2% of cells	Positive in 1–2% of cells	Negative	Negative
Treatment of ePTLD	No specific treatment	No specific treatment	No specific treatment	No specific treatment	Reduction of immunosuppression
Development of other forms of PTLD	No	No	No	No	No
Recurrence of ePTLD	No	No	No	No	No
Follow-up	Squamous carcinoma of the tongue 2 years prior to the development of ePTLD. Several recurrences of squamous carcinoma afterwards	Alive with good transplant function 5 years after diagnosis of ePTLD	Death because of colon carcinoma 4 years after diagnosis of ePTLD	Death because of cardiac failure 2 years after diagnosis of ePTLD	Transplant loss 6 years after diagnosis of ePTLD
Duration of follow-up (years)	4.9	4.5	4.2	2.1	6.9

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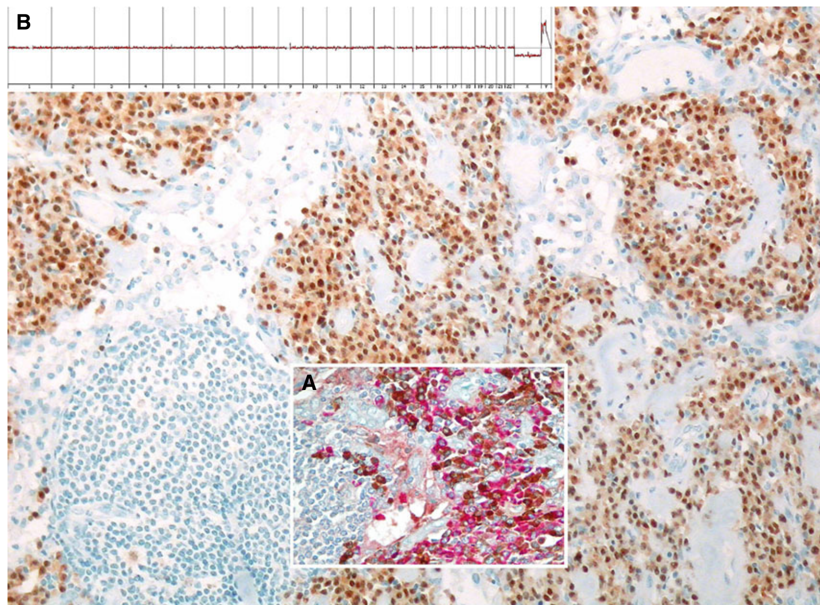

Figure 1 MUM1-staining highlighting abundance of plasma cells in the parafollicular areas of a lymph node. (A) Double immunostaining for kappa and lambda light chains showing an equal (polyclonal) distribution. (B) Array CGH genome overview plot showing no copy number imbalances in the chromosomes of isolated MUM1-positive plasma cell nuclei of PH-ePTLD. The shown male sample was hybridized to female reference genomic DNA, thus a single copy of chromosome X and a gain of chromosome Y are visible.

Giemsa banding and spectral karyotype analysis. Most ePTLD arise in the adenoids or lymph nodes; however, there are also rare case reports on ePTLD arising in the transplanted organs as well [4].

The absence of clonality does not exclude malignancy and cytogenetic abnormalities have been documented in ePTLD previously [3]. Therefore, we aimed to look for genomic aberrations in PH at higher resolution. We selected five PH-ePTLD cases with available formalin-fixed paraffin-embedded (FFPE) material from the archive of our institution (for patients' characteristics see Table 1). Applying our recently developed technique ([5], see also Supplementary material) we processed FFPE tissue sections to specifically extract intact and immunogenic cell nuclei. Using a monoclonal antibody reactive against the nuclear protein multiple myeloma oncogene 1 (MUM1), plasma cell nuclei of PH were labeled and then isolated by flow-sorting. Purity of the sorts was evaluated by subsequent morphologic examination and was estimated to be higher than 85% in all cases. Genomic DNA from the sorted plasma cell nuclei was extracted and used for array-comparative genomic hybridization (aCGH) on the Agilent SurePrint G3 CGH 180k arrays following the procedures described previously [6]. Microarray slides were scanned and then data was analyzed using AGILENT GENOMIC WORKBENCH v.7.0 software with the aberration detection algorithm ADM2 [7].

We did not find any genetic copy number aberrations detectable by aCGH in our cohort (Fig. 1). None of the patients developed another form of PTLD or a relapse of the ePTLD in the follow-up (mean: 4.5 years; range: 2.1–6.9 years). Only two of the five cases showed *in situ* single detectable EBV-RNA-positive cells. However, four patients had been EBV-seropositive at the time of transplantation. After the diagnosis of ePTLD, immunosuppression was reduced in one of the five patients, while in the others no adjustments of the immunosuppressive regimens were done (Table 1).

We could thus confirm on purified cellular populations that beyond being not clonal, PH-ePTLD do not yield genomic aberrations assessable by aCGH. Since the classification of PTLD in the late 1980s by Nalesnik *et al.* [8] there is a debate whether PH should be included into the spectrum of PTLD. Our findings might add momentum to the hypothesis that events other than those occurring within PH-ePTLD are necessary to develop a neoplastic lymphoproliferation leading to more aggressive forms of PTLD. Thus, PH categorization as PTLD might rather be reconsidered both because of the indolent clinical course and based on these genetic findings.

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Conflicts of interest

The authors have no conflict of interest to declare.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Isolation of plasma cell nuclei form the FFPE tissues

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