# Phenogenomic heterogeneity of post-transplant plasmablastic lymphomas

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## SUPPLEMENTARY MATERIAL

## SUPPLEMENTARY METHODS

### Immunohistochemistry/in situ hybridization

Immunohistochemistry was performed with the following panel of antibodies; CD45 (clone 2B11+PD7/26), PAX5 (clone 1EW), CD19 (clone CT 51E), CD20 (clone MJ1); CD10 (clone 56C6); BCL6 (clone LN22); BCL2 (clone D5); OCT2 (clone Oct 207), BOB1 (clone SP92), LMO2 (clone SP51), CD138 (clone BA38), CD43 (clone L60), CD56 (clone CD564), ALK (clone ALKO1), CD30 (clone BER-H2), P53 (clone Bp53-11), CD117 (clone CD117), LANA (clone HHV8-13B10), C-MYC, (clone Y69), all from Leica, Illinois, USA; CD79a (clone AP18); Cyclin D1 (clone SP4-R); CD138 (clone B-A38); Ki-67 (clone 30-9), MLH1 (clone M1), MSH6 (clone 44) all from Ventana, Arizona, USA; and MUM-1 (clone MUM1p) from DAKO, CA, USA; MSH2 (clone G219-1129) and PMS2 (clone EPR3947) both from Cell Marque, California, USA. In-situ hybridization was performed for EBER (clone EBV), kappa (clone ISH-5748A), and lambda (clone ISH-5770A) (Leica, Illinois, USA). Staining was performed with automated staining machines (Benchmark Ultra, Ventana, Arizona, USA and Bond III, Leica, Illinois, USA) and visualized with the ultraView Universal (Ventana, Arizona, USA) and Bond Polymer Refine (Leica, Illinois, USA) DAB detection kits according to the manufacturer's protocols. Expression of the following markers was quantified in deciles: P53, MYC, Ki-67, and PDL1 and positivity on neoplastic and non-neoplastic cells was recorded for the latter. The percentage of PD-1+ lymphoma infiltrating lymphocytes was graded semiquantitatively (rare <5%, mild 5-20%, moderate >20-50% and marked >50%). The cut-offs for MYC and P53 (aberrant nuclear) overexpression were ≥40% and ≥30% respectively.[1-5] Other markers were scored (–) if <10% of cells were positive, (-/+) if 10-20% were positive, (+/-) if >20-50% were positive, and (+) if >50% were positive. IHC for mismatch repair (MMR) proteins was performed only in cases that demonstrated microsatellite instability (MSI) based on genomic studies described below.

**Flow cytometry**

Flow cytometric analysis was performed on lymphoma cell suspensions using FACScan (Becton Dickinson, San Diego, CA) after staining with a comprehensive panel of antibodies including those directed against B- and T-lineage antigens (CD20, CD19, CD79a, CD3), plasma cell markers (CD38, CD138), and others (CD10, CD56, CD117, CD30) according to standard procedures. Data were analyzed with FCS Express software (De Novo Software, Los Angeles, CA).

**DNA extraction**

Tumor DNA was extracted from FFPE or fresh tissue depending on availability (Table 3) using the Qiamp mini kit or the Qiamp FFPE kit (Qiagen, Germantown, Maryland, USA). Non-tumor DNA was extracted from FFPE tissue samples from 9/11 patients.

**Genomic sequencing and variant calling**

Briefly, library preparation was performed using the Sure Select Hybrid Capture system according to the manufacturers specifications (Agilent Technologies, Santa Clara, USA), with custom designed probes. Pooled libraries were sequenced using Illumina HiSeq2500 and Illumina TruSeq v3 chemistry (Illumina, San Diego, USA). Variant calling required at least 5% variant allelic fraction. Germline variants in the normal samples and variants with an allele prevalence >0.01% in gnomAD or “benign” or “likely benign” designation in ClinVar were excluded. Non-synonymous variants that were not known driver mutations were classified as likely pathogenic if the predictive score in two of three in silico algorithms exceeded the following thresholds: REVEL rank score > 0.8; MetaSVM score > 0.75, and CADD score > 25. MSI analysis of genomic data was performed using the RepeatFinder tool (MANTIS software 18).[6]

**Microsatellite instability (MSI) analysis by PCR**

Microsatellite instability (MSI) testing was performed using a fluorescent PCR-based assay (MSI Analysis system, Version 1.2, Promega, Madison, WI). Briefly, this test assessed 5 mononucleotide repeats (BAT25, BAT26, NR-21, NR-24, and MONO-27) and 2 pentanucleotide repeats (PentaC and PentaD) on genomic DNA and matched normal, where available. The fluorescently labeled PCR products were analyzed by capillary gel electrophoresis. MSI was determined if the tumor alleles showed a size difference ≥3 bp. Tumors with 2 or more microsatellite unstable markers were classified as MSI-H.

**Statistical analyses**

Differences in proportions between groups were assessed with the Fishers Exact test and differences in the number of genetic lesions between groups were analyzed with the nonparametric Mann-Whitney U test using R software (version 3.6.1).

**SUPPLEMENTARY DATA**

**Therapy and clinical outcomes**

The immunosuppressive regimens of the patients included different combinations of tacrolimus (n=6), azathioprine (n=8), mycophenolate mofetil (n=2), cyclosporine (n=6), and prednisone (n=9). In all patients, reduction of immunosuppression, including reduction of calcineurin inhibitors by at least 50% and discontinuation of antimetabolic agents (i.e. azathioprine and MMF) was implemented at the time of PTLD diagnosis.

Eight patients received various combinations of immunochemotherapy, including rituximab etoposide, vincristine, doxorubicin cyclophosphamide and prednisone (R-EPOCH), n=4; bortezomib-EPOCH followed by autologous stem cell transplant (ASCT), n=1; rituximab, cyclophosphamide and prednisone (R-CP) followed by fludarabine, cyclophosphamide and mitoxantrone (FCM) at relapse, n=1, cyclophosphamide/prednisolone (CP) followed by gemcitabine/oxaliplatin (GemOx) at progression, n=1; bortezomib/dexamethasone, n=1. Two

patients received external beam radiotherapy, including one who also received chemotherapy, and one underwent surgical resection for removal of PTLD after completing chemotherapy. Two patients presented with multiorgan failure and sepsis and did not receive any lymphoma-directed therapy. The median survival after diagnosis was 1.4 years (range 0-15.9 years) and 2 of 11 patients were alive at the most recent follow-up (15.9 and 6.0 years post diagnosis), including both pediatric patients in the series. Four patients died of PTLD, 4 of unrelated causes, and one of unknown causes.

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Table S1. Summary of all genetic variants detected in PT-PBLs

See separate Excel file

Table S2. Laboratory data and radiographic findings at diagnosis of PT-PBLs

| Patient | EBV serostatus before tx | EBV viremia at PTLD dx | Hgb (g/dL) | Ca (mg/dL) | Cr (mg/dL) | LDH (U/L) | Alb (g/dL) | β2M (mcg/ml) | SPEP | SFLC | UPEP | Bone (lytic) lesions |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Pos | No | 7.9 | 8.2 | 8.2 | 264 | 3.2 | ND | IgG λ 1.2 g/dL | 2:80 | ND | No |
| 2 | Pos | Yes | 11.5 | 7.1 | 0.7 | 167 | 3.3 | 3.5 | IgM κ 0.1 g/dL | 2.1:4.4 | ND | No |
| 3 | Pos | ND | 14.0 | 9.7 | 1.7 | 191 | 4.2 | 3.9 | IgM κ 0.4 g/dL | ND | ND | No |
| 4 | Pos | Yes | 12.4 | 8.5 | 2.1 | 251 | 3.5 | 23.0 | IgA κ 1.0 g/dL | 55:4 | ND | No |
| 5 | Neg | ND | 8.7 | 8.8 | 6.4 | 242 | 2.7 | ND | ND | ND | ND | No |
| 6 | ND | No | 11.0 | 8.6 | 0.7 | 127 | 2.6 | ND | ND | ND | ND | No |
| 7 | Pos | No | 11.1 | 8.3 | 1.3 | 149 | 4.0 | ND | Neg | ND | ND | No |
| 8 | Neg | Yes | 8.9 | 8.8 | 3.0 | 455 | 1.8 | 23.1 | Neg | ND | ND | No |
| 9 | ND | Yes | 10.7 | 8.5 | 1.5 | 1554 | 2.9 | ND | Neg | ND | Neg | No |
| 10 | Neg | Yes | 9.4 | 9 | 0.5 | 210 | 3.7 | ND | ND | ND | ND | No |
| 11 | Pos | Yes | 7.1 | 8.4 | 3.3 | 445 | 2.9 | ND | ND | ND | ND | No |

Abbreviations: Hgb Hemoglobin; Ca Calicum; Cr Creatinine; LDH Lactate dehydrogenase; Alb Albumin; β2M Beta-2 macroglobulin; SPEP Serum protein electrophoresis; SFLC Serum free light chain ratio; UPEP Urine protein electrophoresis; ND Not determined; Pos Positive, Neg Negative

Table S3. Flow cytometric phenotyping of PBLs

| Case | CD138 | CD38 | CD10 | CD19 | CD20 | CD45 | CD56 | CD117 | s/c K/L | CD30 | cCD79a |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | - | - | - | + | - | | s- c L | | - |
| 3 | + | | - | - | - | -/+ | - | | s<c K | | + |
| 4 | + | | + | - | - | + | + | - | s- c K | | - |
| 6 | + | | - | + | - | + | - | - | s c K | | + |
| 10 | +/- | + | + | - | - | -/+ | - | - | c K | - | +/- |
| 11 | + | | + | -/+ | - | -/+ | -/+ | | s- | | |

Abbreviations: s surface; c cytoplasmic