

Safety and efficacy of brentuximab vedotin as a treatment for lymphoproliferative disorders in primary immunodeficiencies

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Table S1: Characteristics of the patients

Patient	Sex	Gene defect	Genetic alteration	Clinical manifestations	Age at onset of the manifestations (years)
1	F	<i>DOCK8</i>	Compound heterozygous deletion c.5490-12/5499-5520	Chronic severe dermatitis and ulcerative perineal skin lesions, recurrent RSV1, <i>candida albicans</i> and <i>staphylococcus</i> skin infections. Severe food allergy. Growth failure. Dysimmune colitis. Recurrent bacterial infections, respiratory infections with bronchiectasis	4
2	M	<i>ATM</i>	Homozygous point mutation c.103C>T	Ataxia - telangiectasia	4
3	F	<i>ITK</i>	Homozygous point mutation c.85C>T	None	5
4	F	<i>CD27</i>	Homozygous point mutation c.329G>A	Recurrent acute anterior uveitis. Knee arthritis, cervical polyadenopathy. Recurrent upper respiratory tract infections	3
5	M	Unknown	Unknown	Neonatal Omenn syndrome (erythroderma, diarrhea, and polyadenopathy) that regressed after etoposide treatment. Lung infections with bronchiectasia. Sinus aspergillosis. Macrophage-activation syndrome in a lung infection. Stomach ulcer, intestinal polyps. Two squamous cell carcinomas <i>in situ</i> . Stage IV EBV+ classical Hodgkin lymphoma at the age of 24 years. Remission after 1x R-CHOP and 8x ABVD	0
6	M	<i>ADA</i>	Unknown	Pneumocystosis, inefficacious GT (at the age of 4 years), on enzyme replacement therapy	0.5
7	M	<i>SH2D1A</i>	Deletion of exon 3	Two episodes of acquired bone marrow failure, succesfully treated with immunosuppressants (age: 22 years). Digestive stage IV EBV+ Burkitt lymphoma at the age of 11 years, complete remission after 2x COPADM	19

LPD: lymphoproliferative disorder. EBV: Epstein-Barr virus. GT: Gene therapy ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine. COPADM: cyclophosphamide, vincristine, prednisone, doxorubicin, high-dose methotrexate. R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.

Table S2 is available as separate excel table

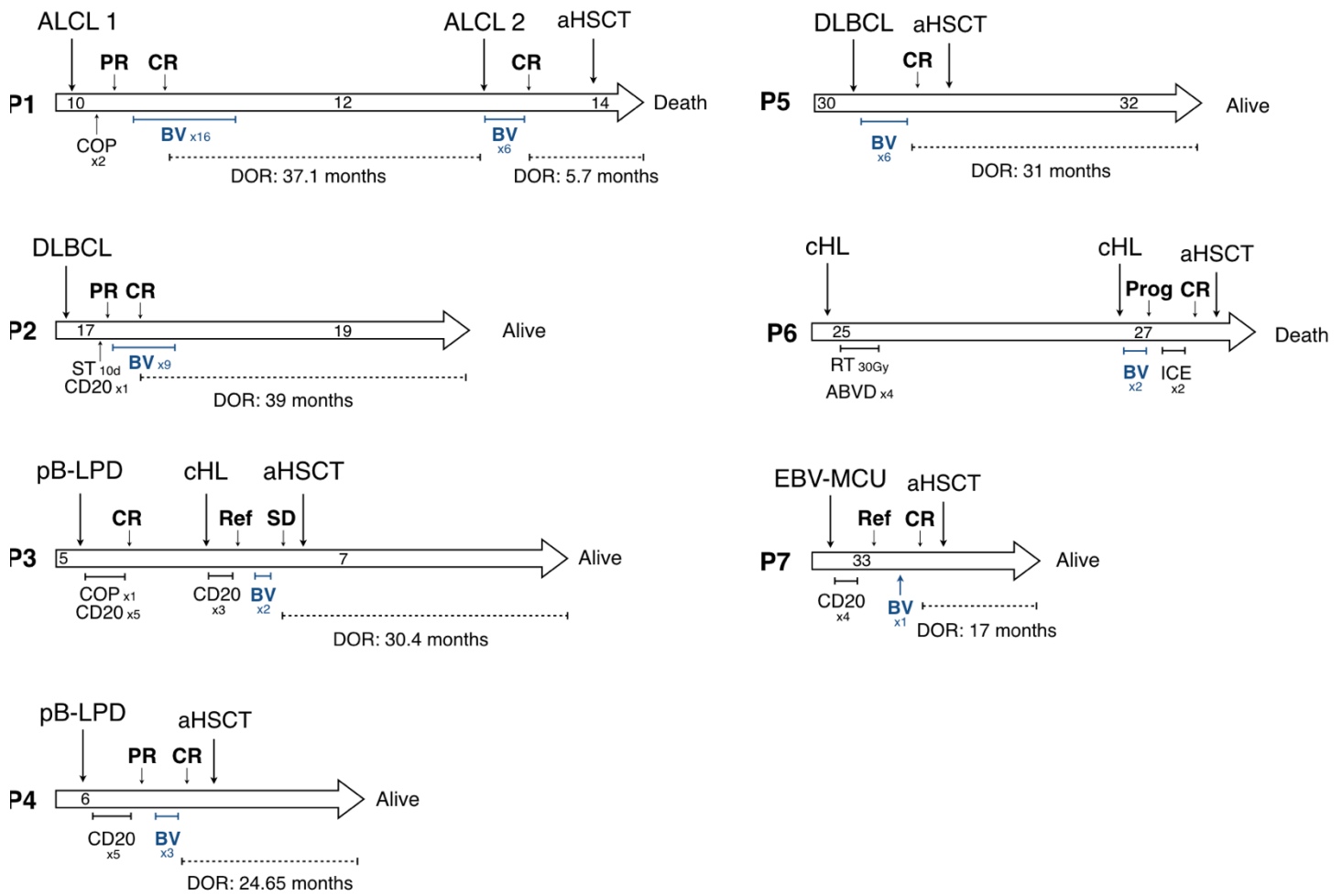


Figure S1: Individual summaries of treatments and outcomes

Each arrow represents a patient. The arrow length is proportional to the elapsed time. Ages are indicated inside the arrow. The objective duration of response (DOR) is shown for each patient. Despite different histopathologic pattern between the 2 lymphoproliferative disorders (LPD) of P3, both shared the same *IGKAPPA* monoclonality and thus the second LPD was considered as a relapse.

ALCL: anaplastic large cell lymphoma, DLBCL: diffuse large B-cell lymphoma, cHL: classical Hodgkin lymphoma, pB-LPD: polymorphic B-cell lymphoproliferation, EBV-MCU: Epstein-Barr virus-positive mucocutaneous ulcer, aHSC: allogeneic hematopoietic stem cell transplantation, CR: complete response, Prog: progression, Ref: refractory, SD: stable disease, BV: brentuximab vedotin, COP: cyclophosphamide, vincristine, prednisone, ST: steroid therapy, CD20: anti-CD20 monoclonal antibody, CD38: anti-CD38 monoclonal antibody, RT: radiotherapy, ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine, ICE: ifosfamide, carboplatin, etoposide.

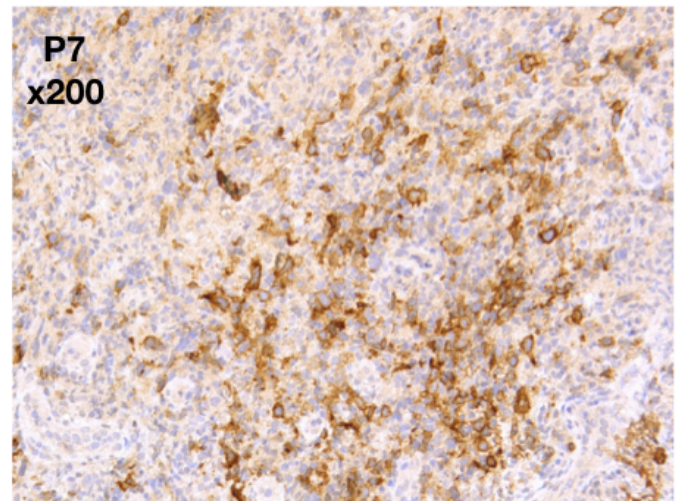
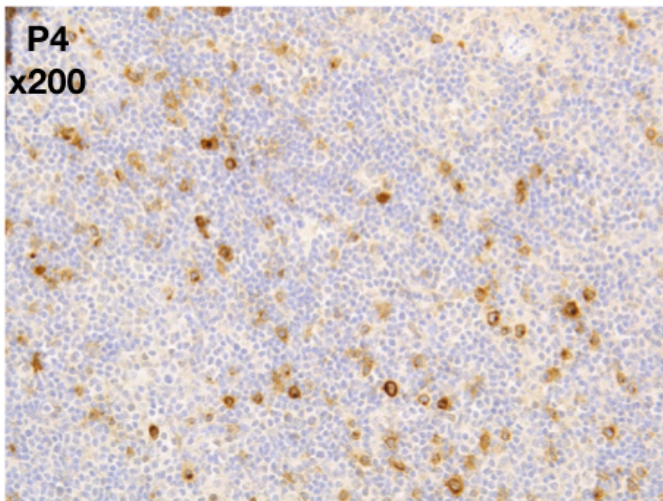
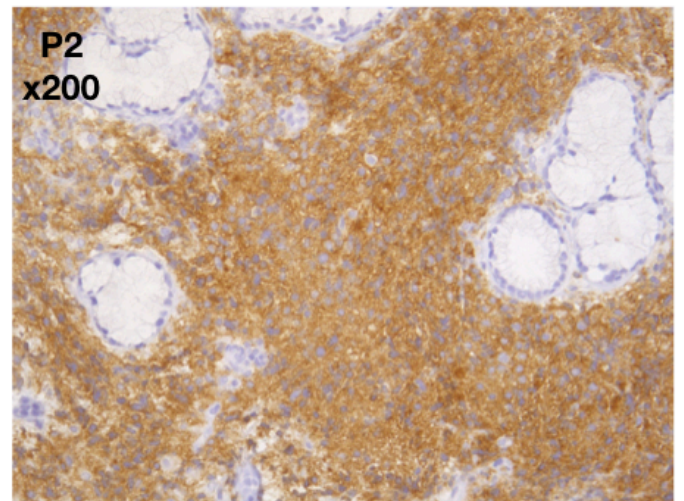
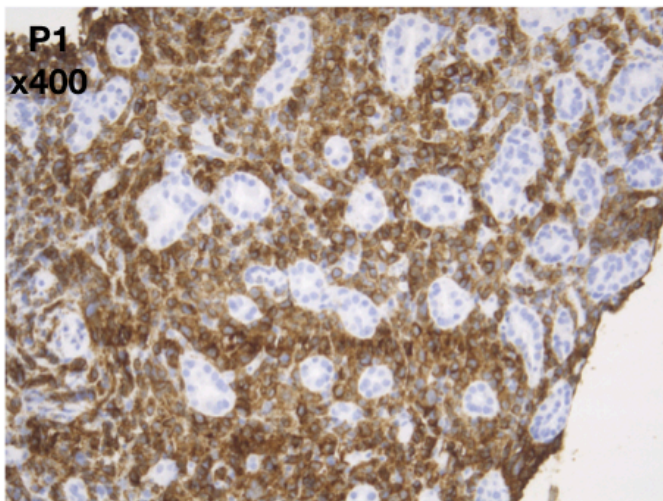


Figure S2: Representative CD30 immunostaining of lymphoproliferative disorders

Immunostaining showing CD30-positive tumoral cells at x200 (P2, P4 and P7) or x400 (P1) magnification.

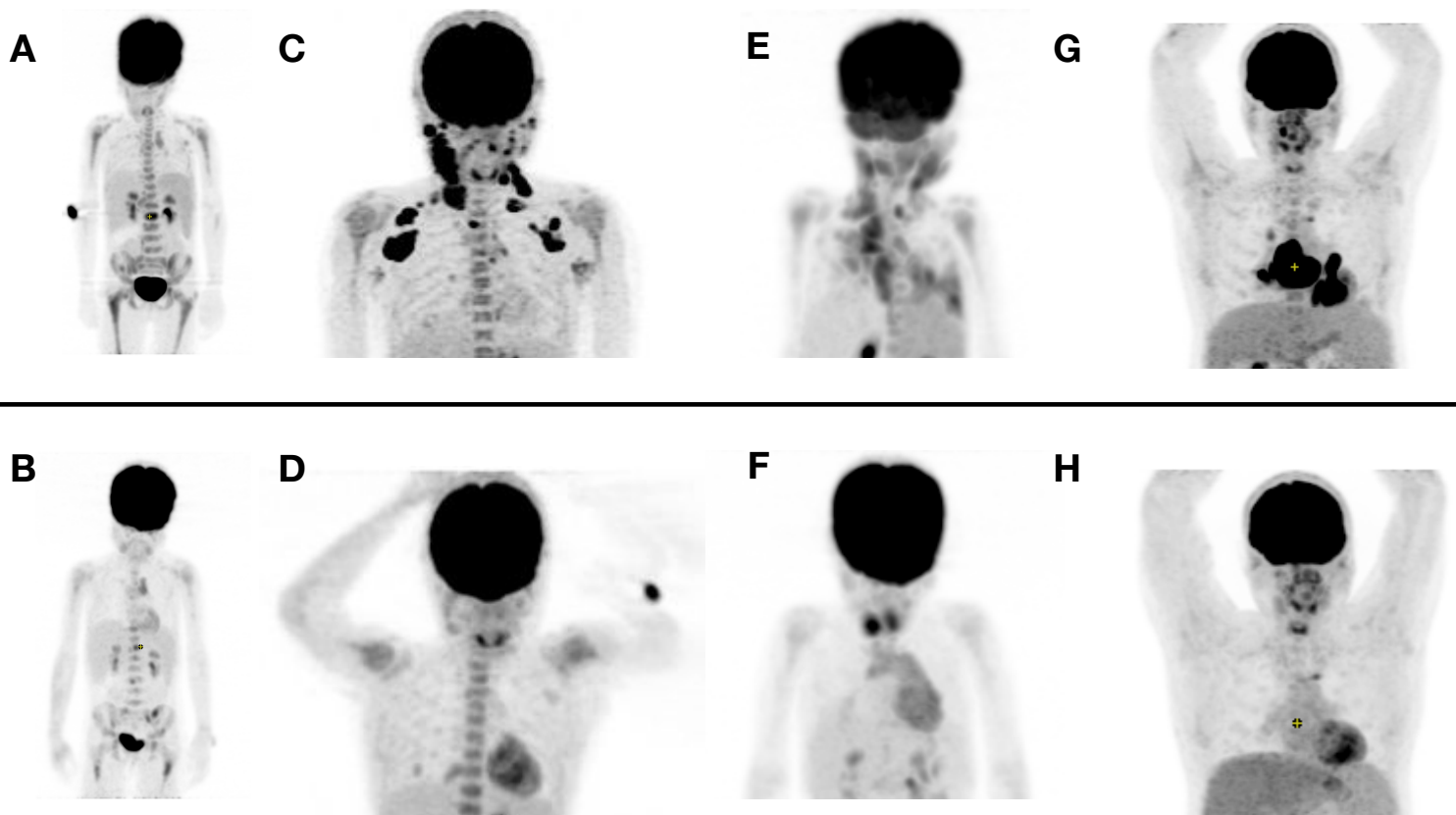


Figure S3: Representative examples of the treatment response on PET-CT

Comparison of PET-CT images before (upper part) and after treatment (lower part) with brentuximab vedotin in P3 (A and B), P1-LPD2 (C and D), P4 (E and F) and P5 (G and H).

Supplemental data: Individual courses

Patient 1

Patient 1 suffered from DOCK8 deficiency. Since early childhood, she had suffered from recurrent bronchitis, pneumonia, severe skin dermatitis, and perineal ulcerations related to chronic herpes simplex infection. At the age of 9 years and 10 months, she presented with persistent fever, cervical lymphadenopathies and an abdominal mass that had developed from the stomach. A pathologic assessment of cervical and celiomesenteric lymph nodes showed infiltration with large lymphoid CD3+, CD2+, CD5+, CD7-, CD4+, CD8-, CD30+, Anaplastic lymphoma kinase (ALK)-, CD56-, CD68-, Granzyme B-cells in both samples, and the LPD was classified as a T cell lymphoproliferative disorder, ALK- anaplastic large cell lymphoma (ALCL). *T-cell receptor gamma (TRG) V9-Jg1/2* monoclonality was present in both samples, with two different subclones. Two courses of COP (cyclophosphamide, vincristine and prednisone) resulted in a 50% reduction in tumor volume. Due to poor overall health status, the treatment was switched to BV to limit the chemotherapy's toxicity; the first evaluation evidenced a CR. Treatment with BV was pursued for a total of 16 injections, with no dose-limiting side effects. Allogenic hematopoietic stem cell transplantation (aHSCT) could not be performed due to the lack of a compatible donor.

Two years and 3 months after the last dose of BV, the patient presented with asthenia, anorexia, diarrhea, and swollen cervical lymph nodes. Imaging evidenced mediastinal and mesenteric lymphadenopathies. A pathologic assessment of a cervical lymph node showed the same morphologic and immunostaining pattern as in the first LPD (ALK- ALCL). The *TRG* repertory was oligoclonal but the previous clone was not found, which profile was compatible with either the presence of two distinct LPDs or clonal evolution/emergence of a distinct subclone rather than a relapse of the initial clone. Treatment with BV was initiated but was limited to 6 injections. A CR was observed in the first evaluation after 3 months of treatment. The patient underwent myeloablative conditioning and then aHSCT with her haploidentical father 2.2 months after the last BV injection. The graft was CD19- and TCR $\alpha\beta$ -depletion. The engraftment was successful but the patient died of an infection 3 months after aHSCT.

Patient 2

Patient 2 had been diagnosed with ataxia-telangiectasia (AT) in childhood. Progressive dysphagia appeared at the age of 16, followed by progressive anorexia and severe denutrition over a 9-month period. A palatine ulcer and submandibular cervical adenopathy were noted. Imaging did not reveal lymph node involvement other than the IIA cervical adenopathy. Pathologic assessment of the palatine ulcers and amygdale biopsies identified large monomorphic CD20+, CD10-, BCL6+, MUM1+, Bcl6-, Bcl2-, CD30+, Ki67++ (80% of tumor cells), latent membrane protein 1 (LMP1)-, EBER- B cell. *IGH* monoclonality was found. The LPD was classified as a monomorphic non-germinal center B-cell (GCB) DLBCL not otherwise specified (NOS). A short (10-day) course of steroids and a rituximab injection (375 mg/m²) allow to obtain a PR. To limit cytotoxic chemotherapy (due to AT), BV was initiated. The first evaluation at 3 months evidenced a CR. A total of 9 BV injections were given. The patient's general health status improved rapidly as he gained 30 kg over the following 8 months. Type 2 diabetes and liver steatosis appeared as the same time as grade II peripheral neuropathy. The latter resolved quickly after BV was withdrawn.

Patient 3

Patient 3 had an uneventful personal medical history until the age of 5 years and 2 months, when she was admitted to an intensive care unit for respiratory distress syndrome related to severe alveolar-interstitial pneumonia with hepatosplenomegaly and diffuse enlarged lymph nodes. A histopathologic assessment of a lymph node biopsy found B-cell lymphoproliferation CD19+, CD20+, MUM1+, CD138-, CD30+, EBER+ associated with Reed-Sternberg like cells expressing CD20 PAX5 CD30 LMP1 and EBER. *IGKAPPA* monoclonality was found. LPD was difficult to classify, named polymorphic B-cell LPD EBV+. There was a disseminated involvement, with lymphadenopathies on both sides of the diaphragm and bone marrow infiltration. The blood EBV load (PCR) was very high (8 log copies/ml). A course of COP and 4 rituximab doses resulted in a CR. The child was subsequently diagnosed with ITK deficiency.

Six months later, she developed asthenia, anorexia, fever, and cytopenia with a concomitant positive blood PCR test for EBV. Clinical and radiologic assessments identified a mediastinal mass, cervical lymphadenopathies, and hepatomegaly. A bone marrow biopsy revealed cHL with typical immunostaining (CD20-, CD30+, CD15-, PAX5 weakly+, EBER+). Despite a mild different histopathologic pattern (loss of CD20 expression on Reed-Sternberg like cells), the same *IGKAPPA* monoclonality than the previous LPD was identified and thus the cHL was considered as a relapse of the B-LPD. The patient received 3 additional doses of rituximab but no benefit was gained. Two doses of BV resulted in a PR. After myeloablative conditioning (including a last dose of BV), she subsequently received a T-repleted haploidentical transplant from her father, followed by post-transplant cyclophosphamide. This strategy resulted in prolonged remission with good immune reconstitution.

Patient 4

Patient 4 had suffered from recurrent respiratory infections since early childhood. At the age of 5, she presented with polyarthritides and uveitis. At the age of 6, she developed progressive fatigue, anorexia, persistent fever associated with dysphagia, snoring, sialorrhea, and canker sores. A clinical examination revealed widespread lymphadenopathies (cervical, inguinal and axillary) and splenomegaly. A pathologic assessment of a cervical adenopathy highlighted the presence of large and numerous scattered B cells CD20+, CD30+, Ki67+, EBER+ B cells with an abundant CD8+ Granzyme B+ T cell background and sheets of necrosis, mimicking infectious mononucleosis histology. No B cell monoclonality was found and a *TRG* monoclonality was present. The LPD was classified as a polymorphic B cell lymphoproliferative disorder. The blood EBV DNA load was 5.8 log copies/ml. The patient was diagnosed with CD27 deficiency.

After 5 rituximab injections (375 mg/m²), the clinical, morphologic, virologic and metabolic responses were incomplete. A second line of treatment with BV was initiated. After 3 injections, the patient was in stable disease according to RECIL criteria; however the clinical response was complete and the blood EBV load has dropped of 1.8 log cp/ml. This response was considered to be a valid indication for aHSCT. After myeloablative conditioning, the patient received a graft from a 9/10 HLA-identical unrelated donor 1.64 months after the last dose of BV. The engraftment failed but the patient then underwent a second aHSCT with another 9/10 HLA-identical unrelated donor, resulting in prolonged remission.

Patient 5

Patient 5 had suffered from a combined immunodeficiency of unknown molecular origin since early childhood. At the age of 24, he presented an episode of macrophage activating syndrome, revealing an EBV-positive stage IVBb scleronodular cHL with typical immunostaining (CD20- CD79a -, CD30+ CD15-). He was successfully treated with a course of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) and 8 courses of ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine).

At the age of 30, he was admitted for cough, hemoptysis, and fever, revealing lung lesion and marked hypermetabolic mediastinal adenopathy. A pathologic assessment of a mediastinal lymph node identified in a diffuse disrupted architecture, sheets of large B-cells CD20+, CD10-, Bcl6+, MUM1+, CD15-, CD30+, EBER+, LMP+ B-cells with *IGKAPPA* and *IGH* monoclonalities. The disease was classified as non-GCB DLBCL-NOS. The PCR blood test for EBV was positive (3.8 log copies/ml). Due to the cumulative doses of previously received cytotoxic drugs, toxicity-sparing treatment with BV was suggested. The patient received 6 injections, enabling a complete clinical, morphologic and metabolic response. The blood EBV load was no longer detectable. After myeloablative conditioning, the patient underwent aH SCT 1.1 months after the last BV. Engraftment was successful, and the LPD did not recur.

Patient 6

The patient was diagnosed with ADA deficiency in early childhood. Gene therapy was attempted at the age of 4 years but was unsuccessful. Long-term enzyme replacement therapy was initiated; it produced good detoxification but only partial correction of the immunodeficiency. At the age of 24 years and 8 months, the patient presented with an isolated cervical adenomegaly revealing an EBV-positive stage IIBb scleronodular cHL with typical immunostaining results (CD20-, CD79a+, CD15+, PAX5+, CD30+). A CR was obtained after 4 courses of ABVD and then mediastinal radiotherapy (30 Gy).

At the age of 27, P6 developed fever, anorexia and weight loss associated with a paryngeal ulcer and cervical adenomegaly. A PET-CT scan revealed a large number of cervical and abdominal hypermetabolic adenopathies, together with nodular hepatic and pulmonary lesions. A pathologic assessment of a pharyngeal biopsy revealed granulomatous inflammation, Reed-Sternberg cells and a typical immunostaining profile (CD15+, PAX5+,

CD20-, CD30+). The tumor cells were EBER+. The blood EBV load (in a PCR) was 3 log copies/ml. To avoid the toxicity of chemotherapy, first-line treatment with BV was initiated. After 2 BV injections, PET-CT revealed lesion progression. The patient received 2 courses of ICE (ifosfamide, carboplatin and etoposide), which resulted in a CR. He underwent aHSCT but died as a result of transplant-related complications.

Patient 7

At the age of 11, P7 was diagnosed with EBV-positive intra-abdominal Burkitt lymphoma. This also revealed an underlying X-linked lymphoproliferative syndrome type 1, which was confirmed by genetic testing (a hemizygous *SH2D1A* mutation). Hemicolectomy (due to bowel obstruction) was followed by 2 courses of COPADM (cyclophosphamide, vincristine, prednisone, doxorubicin, and high-dose methotrexate), which gave a CR.

At the age of 32, P7 presented with persistent fever, weight loss, diarrhea, and splenomegaly. A PET-CT scan showed a mesenteric panniculitis associated with hepatosplenic 18-fluorodeoxyglucose uptake. Colonoscopy revealed ulcerated colitis. A histopathologic analysis of mucosa biopsies identified polymorphic B-cell lymphoproliferation (CD20+, CD10-, MUM1+, Bcl6-, CD30+, Ki67+ (100% of tumor cells) with *IGH* monoclonality). The disease was classified as a polymorphic B-cell lymphoproliferative disorder and now classified as EBV+ mucocutaneous ulcer.. The blood EBV load (PCR) was 6.3 log copies/ml. After 4 doses of rituximab, disease progression prompted the initiation of BV treatment. A week after the first BV injection, the patient developed transient bowel obstruction and pancytopenia that resolved with conservative treatment and G-CSF injection. Colonoscopy and PET-CT evidenced a CR. The patient was bridged to aHSCT (3 months after the last BV) following a reduced-intensity conditioning regimen. The outcome was good, and the LPD did not recur.