Complete remission of immunochemotherapy-refractory monomorphic post-transplant lymphoproliferative disorder mediated by endogenous T-cell recovery

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DOI:
10.1080/10428194.2019.1571203

Complete remission of immunochemotherapy-refractory monomorphic post-transplant lymphoproliferative disorder mediated by endogenous T-cell recovery

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Keywords: Refractory, PTLD, Endogenous T-cell recovery

A 56 year old woman with a history of cadaveric renal transplant in 1986 for renal failure of unknown cause was found to have an 8.1x6.3cm right iliac fossa mass, adjacent to the transplanted kidney, following investigation into a Proteus urinary tract infection in February 2015. Biopsy demonstrated sheets of large atypical lymphoid cells staining for CD20, CD79a, MUM1, Bcl-2 but not CD10, Bcl-6, or Cyclin D1. EBV-encoded small RNAs (EBER) were strongly positive, confirming EBV positive monomorphic PTLD (diffuse large B cell lymphoma [DLBCL] histology). High levels of EBV DNAemia were evident by qPCR on peripheral blood (142 962 copies/ml). As a result of prior significant cytomegalovirus (CMV) reactivation, and recurrent episodes of bacterial infection of ulcerated skin lesions, significant reductions in immunosuppression (RIS) had already been necessary. At PTLD diagnosis, the patient had been on MMF 500mg twice daily and prednisolone 5mg once daily for the previous year with an estimated glomerular filtration rate of 33mls/min. Past medical history included hyperparathyroidism, hyperlipidaemia, osteoporosis, and obesity. The patient had an ECOG performance status of 3 and required walking aids to mobilise. The large right iliac fossa mass was palpable and invading through the anterior abdominal wall with visible tissue destruction and ulceration. Further large areas of ulceration were evident under both breasts, together with a grade two sacral pressure ulcer. The left chest wall mass was biopsied and was consistent with polymorphic PTLD.

Staging by PET-CT confirmed a highly FDG-avid 10.0x5.9cm mass extending cutaneously as well as two regions of intense activity in cutaneous and subcutaneous tissues of the left breast and right buttock (figure 1A). The international performance index (IPI) [NEJM 1993] was three on the basis of stage IV disease, LDH 506 and poor performance status. Further RIS was not deemed feasible given the relatively modest doses of immunosuppression and renal function. Baseline echocardiogram was normal and virology negative, and the patient received four cycles of rituximab 375mg/m^2 monotherapy with regular monitoring of EBV and CMV by peripheral blood qPCR. Clinical response to Rituximab was evidenced by improvement in performance status and minor healing of cutaneous ulceration, but no clinical change in her palpable mass. The patient subsequently received four

cycles of R-CHOP three weekly to June 2015. The EBV viral load reduced significantly during therapy but remained detectable throughout and following the final cycle of treatment (<500 copies/ml). Treatment was complicated, but not delayed, by CMV reactivation treated with oral valgancyclovir and anaemia requiring red cell transfusion. The areas of cutaneous ulceration improved but did not resolve. Six weeks post treatment, PET-CT imaging showed the abdominal mass to have enlarged (figure 1A) while peripheral blood EBV viral load had rapidly increased to $2.7x10^6$ copies/ml. Flow cytometric analysis of peripheral blood showed complete absence of CD19 positive B-cells, suggesting the viraemia was derived from tumor cell-free DNA, and further rituximab was considered futile. An application for NHS funding for third-party HLA-matched EBV-specific cytotoxic T-lymphocytes (CTLs) was not supported. Given the lack of other therapeutic options, after discussion with the renal transplant team, MMF was ceased and prednisolone was rapidly weaned and stopped, with no clinical suggestion of subsequent adrenal insufficiency. Notwithstanding the expectation of a dismal prognosis of RCHOP-refractory monomorphic PTLD, we observed a rapid reduction in the palpable mass and improvement in areas of cutaneous ulceration. A dramatic reduction in EBV viral load to 875 copies/ml was documented four months following cessation of immunosuppression, which became undetectable at six months. Repeat PET-CT imaging in January 2016 showed substantial reduction in tumor volume with minimal residual FDG-uptake present, consistent with a dramatic response to endogenous T-cell recovery following failure of immuno-chemotherapy (figure 1A). Within this timeframe the absolute lymphocyte count increased into the normal range, mirrored by a slow progressive rise in serum creatinine (figure 1B).

Evaluation of the patient's T-cell repertoire at six months following cessation of immune-chemotherapy demonstrated functional CD8+ and CD4+ T-cell responses against a range of EBV-derived peptides. Assays of CD8+ T cells using HLA A*11 tetramers carrying the EBNA3B-derived peptides IVT and AVF showed detectable frequencies of antigen-specific cells, within the ranges typically seen in healthy carriers [1]. Similar assays of CD4+ T-cells using a HLA DRB3*02 tetramer containing the PRS epitope (EBNA2) also detected a response of comparable magnitude to healthy

carriers [2]. Within the total CD8+ subset, the majority of T cells displayed a differentiated effector phenotype, based on CCR7 negativity with heterogeneous CD45RA positivity. Furthermore, there was increased expression of CD38 on both the total CD8+ subset and on the tetramer-positive EBV-specific CD8+ T-cells compared to a simultaneously analysed healthy carrier, suggesting an increase in T-cell activation. The IFNγ Elispot assay demonstrated both CD4 and CD8 T-cell responses to EBV epitope peptides representing a range of viral antigens expressed in both the virus lytic and latent phases, typical of healthy carriers [1]. Secretion of IFNγ in this assay showed that the T cells were functional in response to cognate peptide stimulation (figure 1C).

Analysis of the diagnostic biopsy showed that the EBER positive tumour cells expressed a range of viral proteins, including antigenic targets of the detected T cell responses (figure 1D). Extensive expression of EBNA1, EBNA2 and LMP1 was seen, typical of the EBV latency III growth-transforming infection that involves coordinated expression of six nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C and LP) and two latent membrane proteins (LMP1 and 2). However, LMP2-positivity was scarce in the tumour cells. Detection of BZLF1 in a small frequency of cells indicates that at least some tumour cells entered into lytic cycle, although lack of gp350 expression suggests that virus replication may not have progressed to completion [3].

One year following cessation of systemic chemotherapy, follow-up CT-PET was performed, demonstrating multiple avid mediastinal and abdominal lymph nodes together with widespread bone marrow uptake. The abdominal mass at presentation had the same minimal FDG uptake as previous, whilst serum LDH was normal and EBV PCR weakly positive at 1800 copies/ml. Functional T-cell assays were repeated and were unchanged. Bone marrow trephine showed granulomata and serum ACE was elevated at 73u/l (range 8-52), consistent with sarcoidosis or sarcoid-like reaction. The patient remained well and declined confirmatory bronchoscopy and endoscopic bronchial ultrasound. A further year on, the patient restarted haemodialysis due to slowly progressive renal impairment. The patient is now three years post failure of immuno-chemotherapy, remains well and

EBV-PCR remains negative. To our knowledge, this is the first report of a monomorphic DLBCL subtype PTLD, refractory to immuno-chemotherapy responding to endogenous T-cell recovery following cessation of immunosuppression.

EBV is a γherpes virus able to induce blastoid transformation and proliferation in B-cells, but in healthy individuals, infection is controlled by both T-cell mediated immune responses. EBV driven PTLD occurs in the setting of chronic immunosuppression and decreased T-cell immune surveillance and represents a spectrum of disease that ranges from an EBV positive polyclonal lymphoproliferation early post-transplant, often resolving on reduction of immunosuppression, to monomorphic PTLD[4], the majority of which are DLBCL [5, 6]. The cornerstone of treatment remains RIS and immuno-chemotherapy. RIS to the lowest tolerated level is used in all PTLD subtypes, and may be the only intervention required in polymorphic PTLD, although increases the risk of graft rejection [7, 8]. Monomorphic PTLD generally does not respond to RIS alone [9]. The anti-CD20 monoclonal antibody rituximab is able to induce complete remission in around 20% of monomorphic PTLD as a single agent [10, 11], however, chemotherapy is used generally used concurrently or, based on response to rituximab sequentially. The treatment schema for our patient was based on results from the PTLD-1 trial, which used single agent rituximab weekly induction for four weeks, followed by four cycles of CHOP or R-CHOP [11, 12]. Adoptive immunotherapy using autologous or banked HLA matched EBV-specific allogeneic CTLs derived from EBV seropositive blood donors has been established as potentially curative in patients failing immuno-chemotherapy for PTLD [13, 14]. For our patient, no funding was available for EBV CTLs.

In summary, our patient presented with EBV driven monomorphic PTLD with poor prognostic factors, including a high conventional IPI, hypoalbuminaemia and no response to rituximab monotherapy. The patient had immuno-chemotherapy refractory disease, and the only available clinical option was total cessation of immunosuppression. Endogenous T-cell recovery with demonstrable CD4 and CD8 EBV-specific responses resulted in rapid clearance of the EBV viral load

and sustained lymphoma response, and the patient remains well and in remission over three years later.

This work was supported by Bloodwise, UK (15021)

MJB, CPF and CB designed the research study, wrote the paper, and were involved in clinical decisions. HML wrote the paper. HML, ACD and BJM performed the correlative studies, contributed essential reagents or tools and analysed the data.

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Figure Legends

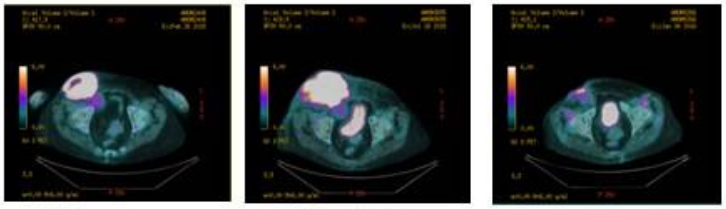
Figure 1A. CT-PET imaging at diagnosis, post R-CHOP immune-chemotherapy and post cessation of immunosuppression.

Figure 1B. Changes in EBV DNA and absolute lymphocyte count from diagnosis of PTLD throughout immuno-chemotherapy and cessation of immunosuppression.

Figure 1C. Analysis of the EBV-specific T cell response. (i) The gating strategy employed in all subsequent flow cytometry analyses showing single, live, CD3+ lymphocytes. (ii) Patient PBMCs stained with anti-CD8 and either no tetramer, or HLA A*11 tetramers carrying the EBNA3B-derived IVT or AVF epitope peptides. (iii) Patient PBMCs stained with anti-CD4 and either no tetramer or HLA DRB3*02 tetramer carrying PRS epitope peptide. (iv) CD38 expression on total CD3+ cells and HLA A*11 IVT tetramer-positive cells from the patient and a representative healthy carrier. (v) IFNγ Elispot of patient PBMCs stimulated overnight with a panel of CD8 and CD4 epitope peptides of relevant HLA restriction derived from the proteins shown. Results are shown as mean spot forming units (SFC) per $4x10^5$ PBMCs.

Figure 1D. EBER in situ hybridisation and EBV protein expression. Sections were taken from the formalin fixed paraffin embedded diagnostic biopsy specimen. EBER ISH staining is shown in blue (DCIP) with Fast Red counterstaining (pink). IHC staining for EBNA1, EBNA2, LMP1, LMP2, BZLF1 and gp350 is shown in brown, with haematoxylin counterstaining (blue). EBV+ and EBV- controls are from known EBV-positive and EBV-negative PTLD cases, respectively. Exceptions are LMP2, which shows LMP2+ L591 Hodgkin lymphoma cells, and gp350, which shows SUDHL-4 DLBCL cells infected with an MVA expressing gp350 (EBV+ control) or Ovalbumin (EBV- control).

Figure 1A



Diagnosis | Post R-CHOP | Post cessation of immuno-suppression

Figure 1B

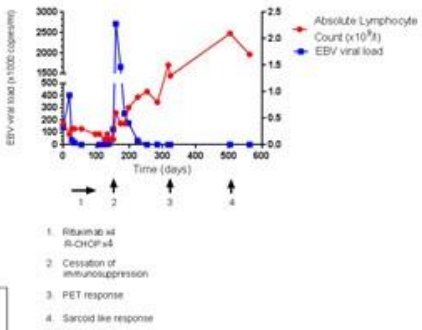


Figure 1C

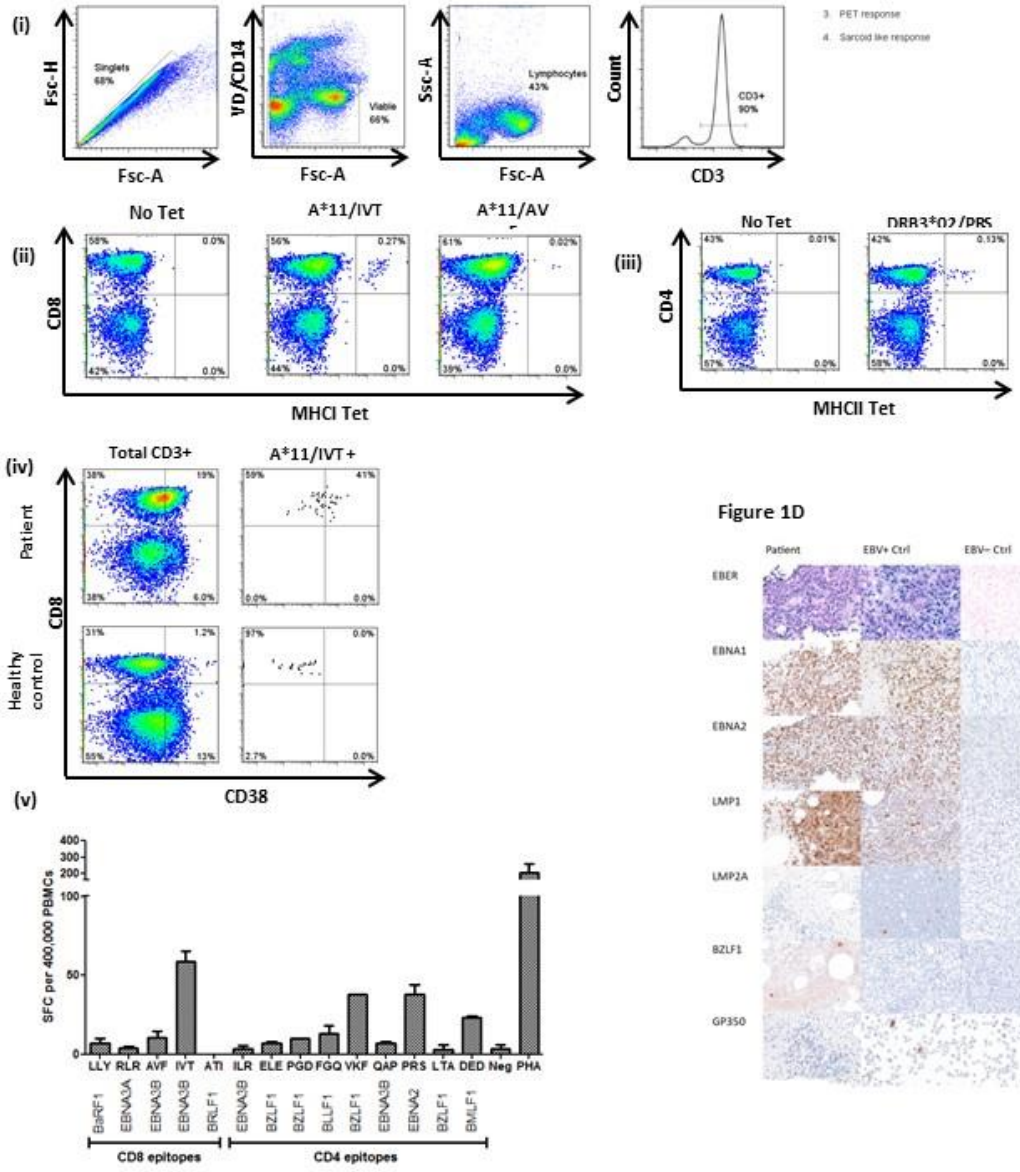


Figure 1D