

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

Bishton, Mark; Long, Heather; Dowell, Alexander; Meckiff, Benjamin; Byrne, Catherine; Fox, Christopher

DOI:

[10.1080/10428194.2019.1571203](https://doi.org/10.1080/10428194.2019.1571203)

License:

Other (please specify with Rights Statement)

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Bishton, M, Long, H, Dowell, A, Meckiff, B, Byrne, C & Fox, C 2019, 'Complete remission of immunochemotherapy-refractory monomorphic post-transplant lymphoproliferative disorder mediated by endogenous T-cell recovery', *Leukemia and Lymphoma*, vol. 60, no. 8, pp. 2075-2078.  
<https://doi.org/10.1080/10428194.2019.1571203>

[Link to publication on Research at Birmingham portal](#)

## **Publisher Rights Statement:**

This is an Accepted Manuscript of an article published by Taylor & Francis in *Leukemia & Lymphoma* on 05/02/2019, available online: <https://doi.org/10.1080/10428194.2019.1571203>

## **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

Download date: 26. Apr. 2024

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

<sup>1</sup>Mark J Bishton, <sup>2</sup>Heather M Long, <sup>2</sup>Alexander C Dowell, <sup>2</sup>Benjamin J Meckiff <sup>3</sup>Catherine Byrne,  
<sup>1</sup>Christopher P Fox

<sup>1</sup>Department of Haematology, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom, NG5 1PB

<sup>2</sup>Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom, B15 2TT.

<sup>3</sup>Department of Renal Medicine, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom, NG5 1PB

Corresponding author: Dr Mark Bishton email: [mark.bishton@nuh.nhs.uk](mailto:mark.bishton@nuh.nhs.uk) Tel: +44 115 965 3467

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<sup>2</sup> 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  $2.7 \times 10^6$  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 immunotherapy (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 immunotherapy 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 IFN $\gamma$  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 IFN $\gamma$  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  $\gamma$ herpes 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.

## References

1. Taylor GS, Long HM, Brooks JM, et al. The immunology of Epstein-Barr virus-induced disease. *Annual review of immunology*. 2015;33:787-821. doi: 10.1146/annurev-immunol-032414-112326. PubMed PMID: 25706097.
2. Long HM, Chagoury OL, Leese AM, et al. MHC II tetramers visualize human CD4+ T cell responses to Epstein-Barr virus infection and demonstrate atypical kinetics of the nuclear antigen EBNA1 response. *The Journal of experimental medicine*. 2013 May 6;210(5):933-49. doi: 10.1084/jem.20121437. PubMed PMID: 23569328; PubMed Central PMCID: PMC3646497.
3. Rea D, Fourcade C, Leblond V, et al. Patterns of Epstein-Barr virus latent and replicative gene expression in Epstein-Barr virus B cell lymphoproliferative disorders after organ transplantation. *Transplantation*. 1994 Aug 15;58(3):317-24. PubMed PMID: 8053055.
4. Dolcetti R. B lymphocytes and Epstein-Barr virus: the lesson of post-transplant lymphoproliferative disorders. *Autoimmunity reviews*. 2007 Dec;7(2):96-101. doi: 10.1016/j.autrev.2007.02.012. PubMed PMID: 18035317.
5. Kremers WK, Devarbhavi HC, Wiesner RH, et al. Post-transplant lymphoproliferative disorders following liver transplantation: incidence, risk factors and survival. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2006 May;6(5 Pt 1):1017-24. doi: 10.1111/j.1600-6143.2006.01294.x. PubMed PMID: 16611339.
6. Sebelin-Wulf K, Nguyen TD, Oertel S, et al. Quantitative analysis of EBV-specific CD4/CD8 T cell numbers, absolute CD4/CD8 T cell numbers and EBV load in solid organ transplant recipients with PLTD. *Transplant immunology*. 2007 Apr;17(3):203-10. doi: 10.1016/j.trim.2006.10.006. PubMed PMID: 17331848.
7. Tsai DE, Hardy CL, Tomaszewski JE, et al. Reduction in immunosuppression as initial therapy for posttransplant lymphoproliferative disorder: analysis of prognostic variables and long-term follow-up of 42 adult patients. *Transplantation*. 2001 Apr 27;71(8):1076-88. PubMed PMID: 11374406.
8. Reshef R, Vardhanabhuti S, Luskin MR, et al. Reduction of immunosuppression as initial therapy for posttransplantation lymphoproliferative disorder( bigstar). *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2011 Feb;11(2):336-47. doi: 10.1111/j.1600-6143.2010.03387.x. PubMed PMID: 21219573; PubMed Central PMCID: PMC3079420.
9. Swinnen LJ, LeBlanc M, Grogan TM, et al. Prospective study of sequential reduction in immunosuppression, interferon alpha-2B, and chemotherapy for posttransplantation lymphoproliferative disorder. *Transplantation*. 2008 Jul 27;86(2):215-22. doi: 10.1097/TP.0b013e3181761659. PubMed PMID: 18645482; PubMed Central PMCID: PMC4029101.
10. Blaes AH, Peterson BA, Bartlett N, et al. Rituximab therapy is effective for posttransplant lymphoproliferative disorders after solid organ transplantation: results of a phase II trial. *Cancer*. 2005 Oct 15;104(8):1661-7. doi: 10.1002/cncr.21391. PubMed PMID: 16149091.
11. Trappe R, Oertel S, Leblond V, et al. Sequential treatment with rituximab followed by CHOP chemotherapy in adult B-cell post-transplant lymphoproliferative disorder (PTLD): the prospective international multicentre phase 2 PTLTD-1 trial. *The Lancet Oncology*. 2012 Feb;13(2):196-206. doi: 10.1016/S1470-2045(11)70300-X. PubMed PMID: 22173060.
12. Trappe RU, Dierickx D, Zimmermann H, et al. Response to Rituximab Induction Is a Predictive Marker in B-Cell Post-Transplant Lymphoproliferative Disorder and Allows Successful Stratification Into Rituximab or R-CHOP Consolidation in an International, Prospective, Multicenter Phase II Trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2016 Dec 19;JCO2016693564. PubMed PMID: 27992268.



13. Doubrovina E, Oflaz-Sozmen B, Prockop SE, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic hematopoietic cell transplantation. *Blood*. 2012 Mar 15;119(11):2644-56. doi: 10.1182/blood-2011-08-371971. PubMed PMID: 22138512; PubMed Central PMCID: PMC3311278.
14. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood*. 2007 Aug 15;110(4):1123-31. doi: 10.1182/blood-2006-12-063008. PubMed PMID: 17468341.

### 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) IFN $\gamma$  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  $4 \times 10^5$  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

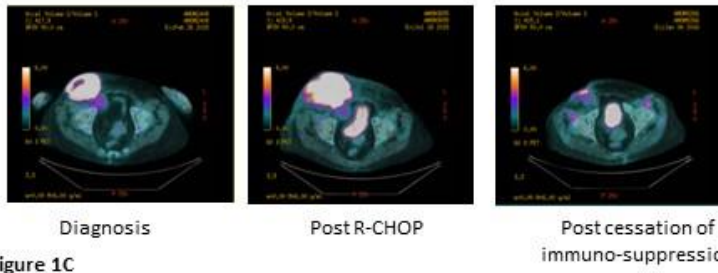
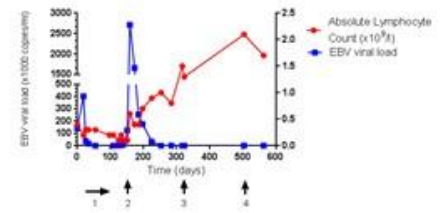


Figure 1B



1. Rituximab w/ R-CHOP x4
2. Cessation of immunosuppression
3. PET response
4. Sarcoid like response

Figure 1C

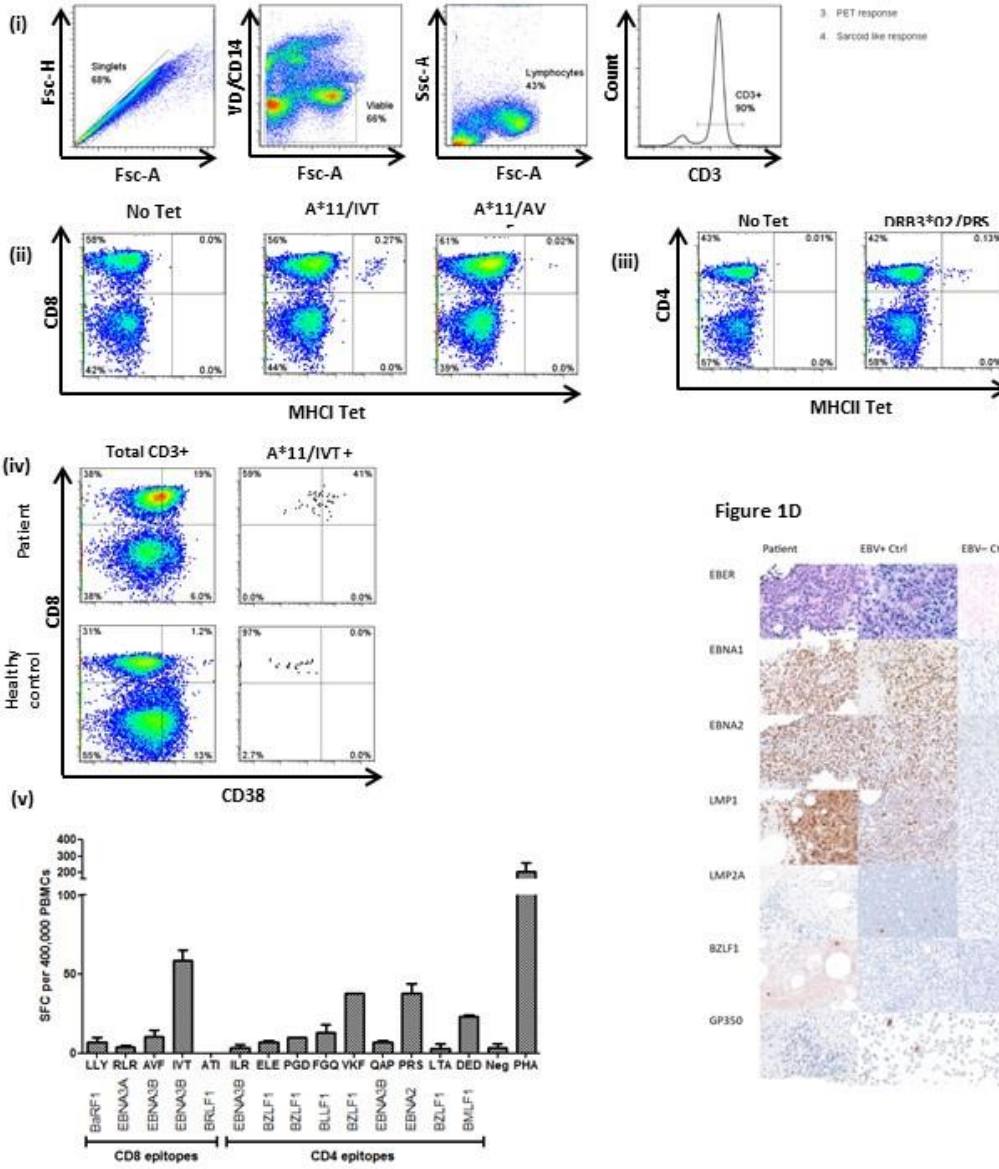


Figure 1D

