bjh short report

# Evolution to plasmablastic lymphoma evades CD19-directed chimeric antigen receptor T cells

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## Summary

A patient with relapsed and refractory chronic lymphocytic leukaemia with Richter transformation was treated with chimeric antigen receptor (CAR)-modified T cells targeted for CD19 but later relapsed with a clonally related plasmablastic lymphoma. The loss of most routine markers of pre-plasma cell or B lymphoid differentiation (including CD19) highlights the ability of such mature lymphomas to evade lineage-specific targeted immunotherapy by differentiating along pathways comparable to their normal cellular counterparts. Molecular genetic evaluation demonstrated multiple independent lines of CD19-negative disease that eventually evolved in this single patient. Such plasticity represents potential challenges for antigen-directed CAR-T cell therapy, while serving as a testament to the selective pressure exerted by these engineered T cells over time.

**Keywords:** leukaemia, chronic lymphocytic leukaemia, plasmablastic, lymphoma, chimeric antigen receptor T cells.

Treatment with CAR-T cells specific for CD19 has resulted in complete responses in B cell acute lymphoblastic (B-ALL) and chronic lymphocytic leukaemia (CLL) (Brentjens *et al*, 2013; Grupp *et al*, 2013; Maude *et al*, 2014; Lee *et al*, 2015). One recurrent observation among trials in B-ALL, however, is the emergence of CD19-negative blasts at relapse in a substantial minority (up to 10%) of patients (Grupp *et al*, 2013; Maude *et al*, 2014; Lee *et al*, 2015). Fewer published studies are currently available for comparison of response rates against mature B cell neoplasms and non-Hodgkin lymphoma and no examples of relapse attributable to CD19-negative escape variants have yet been reported (Brentjens *et al*, 2011; Porter *et al*, 2011; Kochenderfer *et al*, 2012, 2015). Here we report a patient with transformed CLL/small lymphocytic lymphoma (SLL) treated with CD19-directed CAR-T cell (CTL019) therapy who relapsed not only with CD19-negative disease, but overt plasmablastic lymphoma (PBL), which is inherently resistant to such treatment. These findings demonstrate the ability of a mature B cell malignancy to exhibit alternative pathways of lymphoid differentiation and thereby evade control by one or more antigen-specific targeted immunotherapies.

## Case report

The patient was a 62-year-old man initially diagnosed with CLL (Fig S1) three and a half years prior to receiving CTL019 therapy on clinical trial**.** Diagnostic features at the time of diagnosis, as well as relevant findings in multiple subsequent tissue biopsies, are outlined in Fig 1A. Progression occurred despite brief therapeutic responses achieved on multiple chemotherapeutic regimens and clinical trials over the next 3 years (see Table SI). Six months prior to CTL019 infusion, large B cell lymphoma/Richter transformation and extensive marrow involvement by CLL were demonstrated on lymph node and bone marrow biopsies, respectively. Importantly, flow cytometry at that time showed divergent immunophenotypic features exhibited by the CLL/SLL of the bone marrow (including partial loss of CD19 in addition to other B cell markers), suggesting that altered B cell differentiation was ongoing (Fig 1A). The patient was referred for the CTL019 (formerly CART-19) CLL trial at the University of Pennsylvania (NCT01029366) where he had successful apheresis and expansion of autologous T cells. Following infusion there was marked expansion of CTL019 cells and he experi-



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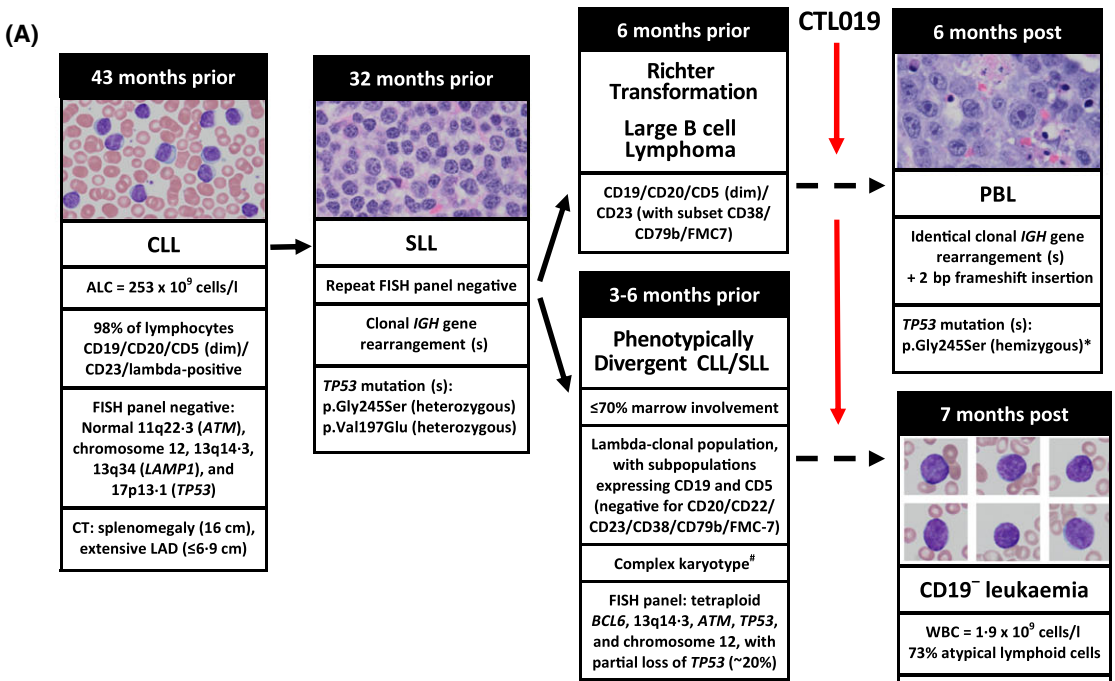


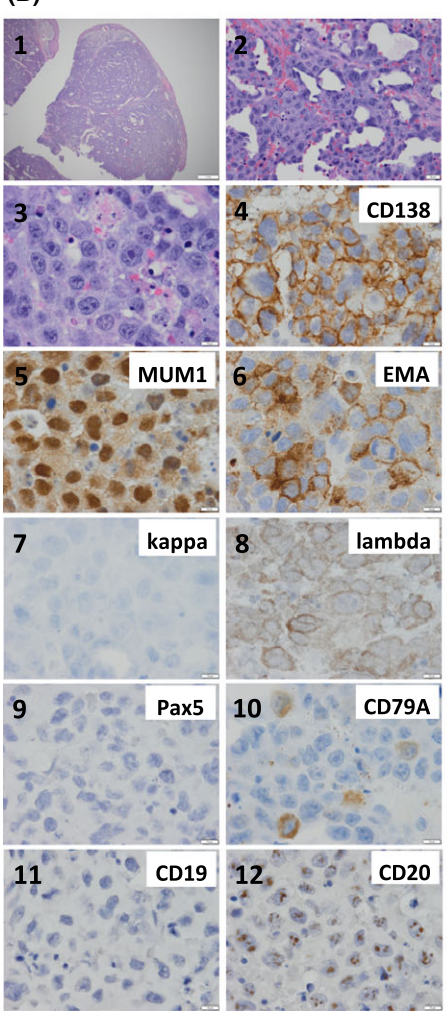


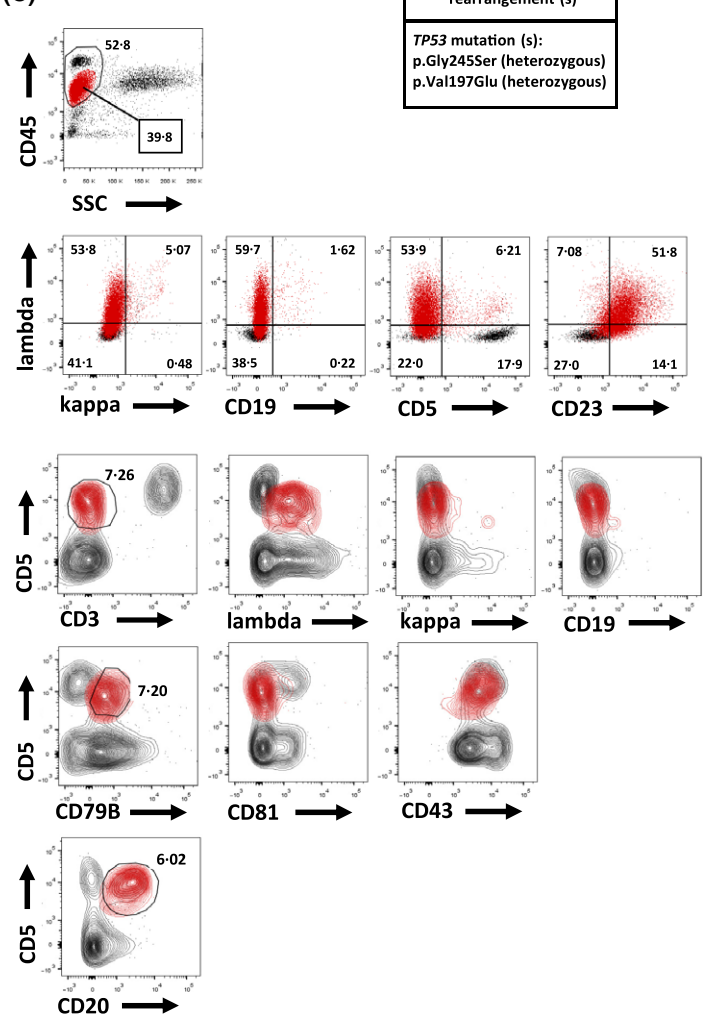

**Fig 1.** Course and clonal evolution of CLL/SLL through transformation, CTL019 selection, and evolution to PBL and CD19-negative leukaemia. (A) Schematic diagram demonstrating the pathological and genetic features of clonal evolution from CLL to PBL over time. Both the known clonal relationships (solid arrows) and pattern of presumptive subclonal evolution (dotted arrows) are shown, pre- and post-treatment with CTL019 therapy (red arrows). CLL, chronic lymphocytic leukaemia; PBL, plasmablastic lymphoma; ALC, absolute lymphocyte count; LAD, lymphadenopathy. [#]Karyotype reported as: 87–88,<4n>,XXYY,+X,+X,-1,add(3)(q27)x2,-6,-6,der(?;9)(?;q10)x2,-14,-14,-15,-15,-17,add(21)(q22)x2,+mar1, +mar2,+mar6[cp3]/83,sI,add(1)(q25),der(4)t(4;8)(q35;q13),-8,add(8)(p11.2),der(16)t(1;16)(q23;p13.3),+mar5[cp2]/nonclonal with clonal abnormalities[3]/46,XY[11]/nonclonal[1]. *Presumed hemizygous mutation. (B) Pathology of transformed PBL on gingival biopsy. (1–3) Haematoxylin and eosin stained sections; 1) 1·25× (scale bar = 1 mm); 2) 40× (scale bar = 20 μm); 3) 100× (scale bar = 10 μm). (4–12) Immunohistochemical and *in situ* hybridization (ISH) staining (as indicated for each); scale bar = 10 μm. Tumour cells were positive for CD138, MUM1 (IRF4), CD56 (subset), CD79a (subset), and EMA, but negative for CD19, surface CD20, and PAX5 (BSAP). There was some residual intracellular vesicular staining for CD20, but tumour cells were otherwise negative for CD5, CD23, BCL2, cyclin D1 (CCND1), CD30, ALK1, human herpesvirus-8 (HHV-8), and Epstein-Barr virus (EBV)-encoded RNA (EBER), while the Ki67 proliferation index was greater than 90% (data not shown). (C) Flow cytometric analysis of peripheral blood at 1 month post-relapse. Standard analysis (top panel, dot plots) revealed a predominant CD45-dim, lambda-restricted population with a plasmacytoid phenotype (gated in red) comprising 39·8% of total events (75·4% of lymphocytes) that expressed CD23, but was negative for CD19 and CD5. Note that a small population of CD5-positive cells expressing dim lambda light chain (~6% of lymphocytes) was also present. Concurrent MRD analysis (bottom panel, density plots) revealed a corresponding small CD19-negative CLL clone (6–7% of lymphocytes) with co-expression of dim lambda light chain, dim CD20, CD5, dim CD79b, dim-to-negative CD81, and positive CD43. Cells are gated (and highlighted in red) as follows: top row gated on CD5-positive, CD3-negative events; middle row gated on CD5 and CD79b-dim co-positive events; bottom row gated on CD5 and CD20-dim co-positive events.

enced cytokine release syndrome. Treatment response was estimated at 50% or more reduction in nodal areas by computerized tomography (CT) scan and 80% reduction in marrow involvement 1 month after infusion. Two months after infusion, CTL019 cells were readily detectable in peripheral blood (5% of all CD3-positive cells). At 6 months, however, they were no longer detectable. He remained lymphopenic and received monthly intravenous immunoglobulin replacement.

Approximately 6 months after the CTL019 infusion, he developed right jaw pain with a gingival mass noted on examination. Biopsy revealed PBL that lacked CD19 expression and all other markers of pre-plasmacytic B cell differentiation (Fig 1B). Prior to initiating further treatment, he received radiation therapy to the jaw, with partial clinical response. About 1 month after relapse, a predominant population of atypical cells with a plasmacytoid phenotype emerged in the peripheral blood, and a minor population of residual CD19-negative (but otherwise immunophenotypically normal) CLL was detected by minimal residual disease (MRD) flow cytometric analysis (Fig 1C). Shortly thereafter, the patient developed severe sepsis with neutropenia and pancytopenia. He died 7 months following his CTL019 cell infusion.

Molecular analysis (summarized in Fig 1A) confirmed the clonal relationship between the pre-treatment SLL and both the subsequent PBL and CD19-negative leukaemia. Each specimen exhibited identically sized immunoglobulin heavy chain (*IGH*) and kappa light chain (*IGK*) gene rearrangements. *IGH* gene sequencing revealed the same productive and non-productive gene rearrangements in each, the only exception being a two base-pair (bp) insertion in the complementarity-determining region 1 (CDR1) which destroyed the productive *IGH* reading frame in the PBL. *TP53* sequencing revealed two identical mutations (p.Gly245Ser and p.Val197Glu, both heterozygous) from the prior SLL lymph node and recent peripheral blood. Both mutations were predicted to be non-functional by the International Agency for Research on Cancer (IARC) *TP53* database (Petitjean *et al*, 2007). Only the p.Gly245Ser (presumed hemizygous) mutation was present in the PBL, however, consistent with its evolution from the subclone in which *TP53* deletion had been previously documented. Selected sequencing of additional mutational hotspots in the *NOTCH1*, *SF3B1* and *BIRC3* genes in the above specimens showed no evidence of mutation (data not shown).

Transformation from CLL/SLL to PBL is an exceedingly rare event, with few cases reported and only a portion of these proven to be clonally related (Martinez *et al*, 2013). The disease course and pattern of clonal evolution seen here suggests that CTL019 therapy selected for an uncommon pathway of malignant B cell differentiation or survival. The presence of a two bp insertion/frameshift mutation in the functionally rearranged *IGH* gene locus also suggests that, similar to normal plasma cells, this transformed PBL did not require surface immunoglobulin/B cell receptor (BCR) signalling, an otherwise critical pathway for proliferation and survival in CLL and certain types of diffuse large B cell lymphoma (DLBCL) (Davis *et al*, 2010). In contrast, the *IGH* reading frame and surface immunoglobulin expression was still intact in the CD19-negative circulating leukaemic cells, suggesting alternative survival signals were required.

The pattern of *TP53* mutation indicates at least two CD19-negative clones were selected during CTL019 therapy with their most probable recent common progenitor dating back prior to the Richter/large cell transformation (see Fig 1A). Although seemingly improbable, there is precedence for two clonally related but phenotypically divergent haematological malignancies to evolve under CAR T cell-mediated selection. One B-ALL patient is known to have developed a myelodysplastic syndrome with monosomy 8 and cytogenetic features that resembled the original B-ALL following CTL019 therapy (Maude *et al*, 2014). Such findings raise the possibil-

ity of an immature malignant progenitor (i.e. leukaemic stem cell) that is capable of alternative differentiation and expansion in an environment that becomes devoid of overt B cell leukaemia.

The mechanism by which CD19 expression is lost in patients treated with CD19-directed CAR T cells has not yet been demonstrated, and is reminiscent of immunological escape through altered immunoglobulin expression in patients with follicular lymphoma treated with anti-idiotype vaccination (Meeker *et al*, 1985). A single missense or nonsense mutation within CD19 (or an alternative component of the BCR complex) could result in loss of expression, as has been documented in human germline mutations responsible for antibody deficiency syndromes (van Zelm *et al*, 2006, 2010). Single gene mutation or selective down-regulation of CD19 in the face of selective pressure by CTL019 cells may explain the occurrence of the small CD19-negative CLL clone seen here, but it is unlikely that similar straightforward mechanisms of antigen loss occurred in the PBL given the other complex surface phenotypic differences that evolved. CAR T cells directed against multiple alternative B cell antigens, including CD22, CD23 and the receptor tyrosine kinase-like orphan receptor 1 (ROR1), have been developed to overcome limitations of single antigen targeting (Maus *et al*, 2014). Importantly, however, the presence of a lambda-restricted clonal population with only partial CD19 expression that was negative for CD20, CD22 and CD23 (among other markers) in our patient *prior* to CTL019 therapy suggests that even a combinatorial therapy approach would have limited anti-tumoural activity against a malignancy with this potential for plasmablastic or poorly differentiated transformation.

Overall, these findings suggest that, in addition to simply selecting for CD19-negative escape variants, CTL019 selective pressure may divert lymphoid differentiation into alternative pathways that are otherwise less competitive in a heterogeneic tumour environment. This case raises a note of caution regarding the screening of patient for targeted immunotherapy, while at the same time attesting to the efficacy and continued activity of these engineered T cells. Further characterization of the frequency, phenotype and behaviour of CTL019 escape variants will significantly inform our understanding of the promise and potential for this new and exciting clinical modality, and help to define rational approaches to overcome resistance.

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## Authorship contributions

AGE performed pathological evaluation and flow cytometric analysis, analysed genetic data and wrote the manuscript; PGR performed molecular analysis and analysed data; SFH, DLP and JLL provided patient care and collected clinical trial data; WRB, DLP, JWF and JLL provided overall guidance and edited the manuscript.

## Disclosure of conflicts of interest

DLP received research funding from Novartis. The remaining authors declare no competing financial interests.

## Supporting Information

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

**Fig S1.** Flow cytometry of circulating chronic lymphocytic leukaemia at time of diagnosis.

**Table SI.** Treatment history.

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