Plasmablastic Richter transformation as a resistance mechanism for chronic lymphocytic leukaemia treated with BCR signalling inhibitors

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We read with interest the report by Evans et al (2015), describing a patient with transformed chronic lymphocytic leukaemia (CLL) treated with CD19-directed chimeric antigen receptor-modified T-cells (CTL019), who developed disease escape with clonally-related plasmablastic lymphoma (PBL). This unusual observation illustrates how B-cell lymphomas may evolve to evade antigen-specific immunotherapy – a scenario previously seen only in B-cell acute lymphoblastic leukaemia, where patients may relapse with CD19-negative disease (Grupp et al, 2013). Herein, we report two cases of transformation from CLL to PBL in patients receiving ibrutinib, demonstrating that this PBL escape phenomenon is also a pathway for CLL resistance to Bruton tyrosine kinase (BTK) inhibition.

Case 1, a 63-year-old man, was diagnosed with kappa light chain-restricted CLL in 2005, which was ultimately refractory to seven lines of chemoimmunotherapy. Peripheral blood fluorescence in situ hybridization (FISH) was performed after third-line treatment, demonstrating 17p13 deletion in 94.5% of cells scored. Targeted next generation sequencing (NGS), using a panel of 26 genes frequently mutated in lymphoid malignancy, identified a pathogenic TP53 mutation (NM_000546.5:c.731G>A;p.Gly244Asp). No mutations in SF3B1, NOTCH1, BIRC3, BTK (including C481S) or PLCG2 were detected.

Prior to ibrutinib commencement in January 2013, the patient had generalized lymphadenopathy without clinical evidence of disease transformation. He achieved partial response after 12 weeks of therapy, but presented at 24 weeks with a new, symptomatic anal tumour. Biopsy of the mass revealed infiltration by PBL cells, which were positive for CD45, CD138, MUM-1 and CD79a (cytoplasmic) but negative for CD3, CD20, PAX-5, CD56 and cyclin D-1, with kappa light chain restriction (Figure 1). MYC was strongly positive by immunohistochemistry and tests for Epstein-Barr virus (EBER) and human herpesvirus-8 (HHV-8) were negative. Targeted NGS testing performed on DNA extracted from this anal tumour detected the same TP53 mutation previously identified, as well as acquisition of an activating NRAS mutation (NM_002524.4:c.35G>T; p.Gly12Val). PBL and baseline CLL samples were proven to be clonally related by IGH sequence analysis showing identical sequences.

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Following refractoriness to high-dose dexamethasone, the patient declined further active treatment and died two weeks after PBL was diagnosed.

Case 2, a 67-year-old man, was diagnosed with kappa light chain-restricted CLL in 2006 and referred to our institution in September 2009 after developing progressive disease refractory to two lines of chemoimmunotherapy. FISH on pre-treatment bone marrow identified 17p13 deletion in 37% of cells scored. NGS mutation analysis detected a pathogenic \textit{TP53} mutation (NM_000546.5:c.817C>A;p.Arg273Ser) and an activating \textit{NRAS} mutation (NM_002524.4:c.38G>T;p.Gly13Val).

Prior to ibrutinib commencement in May 2013, the patient had generalized lymphadenopathy without clinical evidence of disease transformation. Although partial response was achieved after nine weeks of therapy, enlarging retroperitoneal lymphadenopathy developed after 21 weeks, at pre-ibrutinib sites of disease. Retroperitoneal lymph node biopsies revealed sheets of PBL cells that were positive for CD45, CD138 and CD79a (focal) but negative for CD3, CD20, PAX-5, CD56 and cyclin D-1, with kappa light chain restriction. A background CLL infiltrate was also noted (Figure 2). MYC was positive in 70% of cells by immunohistochemistry, while EBER and HHV-8 were negative. NGS was unable to be performed on the tumour tissue. PBL and baseline CLL samples were proven to be clonally related by \textit{IGH} sequence analysis showing identical sequences.

The patient received three cycles of salvage chemotherapy but had disease progression and died 12 weeks after PBL was diagnosed.

As noted by Evans \textit{et al} (2015), transformation from CLL to PBL is an extremely rare event in the era of traditional cytotoxic therapies. Although Richter transformation to diffuse large B-cell lymphoma is a common form of CLL escape from BTK (Maddocks \textit{et al}, 2015) and BCL2 inhibition (Roberts \textit{et al}, 2015; Tam \textit{et al}, 2015), the histological and genomic characteristics of these Richter cases have not been well-studied. Known ibrutinib resistance mechanisms include BTK binding site mutations (C481S) and gain-of-function mutations in the downstream pathway enzyme phospholipase C gamma 2 (PLCG2), both of which occur predominantly in patients with CLL progression and are uncommon in those with Richter transformation (Maddocks \textit{et al}, 2015). Loss of \textit{TP53} and/or \textit{CDKN2A} (with \textit{MYC} activation) and trisomy 12 (with \textit{NOTCH1} mutations) are genetic abnormalities implicated in Richter transformation following cytotoxic therapies (Chigrinova \textit{et al}, 2013), but it is
unknown whether these mutations also drive breakthrough transformation in patients on novel therapies.

At our institution, four out of 60 patients have developed Richter transformation post-ibrutinib, two of which were PBL; another PBL case was reported in the series from Maddocks et al (2015). The mechanisms through which evolution to PBL occurs in ibrutinib-treated patients remain speculative. Most PBL cases carry MYC rearrangements or amplifications (Castillo et al, 2015), which may be the driver mutation in the transformation process and enable PBL cells to overcome BTK inhibition. BTK expression is also downregulated in non-malignant plasma cells (de Weers et al, 1993); thus, PBL may be intrinsically resistant to BTK inhibition if this characteristic is shared. Furthermore, myeloma cells exhibit relative ibrutinib resistance in vitro (Tai et al, 2012).

Activating NRAS mutations were detected in both of our patients – acquired at transformation in Case 1 and present at baseline in Case 2. While NRAS mutations are established drivers of carcinoma tumorigenesis through the RAS/MAPK pathway, their role in CLL (if any) is unknown. However, it is noteworthy that NF-κB pathway mutations (including KRAS mutations, though NRAS mutations were not examined) have been implicated in ibrutinib resistance and cell survival in an in vitro CLL model (Impgrogo et al, 2013).

Considering the case presented by Evans et al (2015) and those reported here, it is plausible that in the emerging era of potent, targeted antigen-specific immunotherapy and small molecule inhibitors, stem cell plasticity and conversion to plasmablasts that are CD19-negative (in the case of CTL019) and BTK-independent (in the case of ibrutinib) may be an increasingly seen mode of tumour escape. Further genomic characterization will be critical in elucidating the pathophysiology underpinning plasmablastic transformation in low-grade B-cell lymphoproliferative disorders and guiding novel treatment strategies for this rare and challenging disease.

Authorship Contributions
KLC collected data and wrote the manuscript; PB and KJ analysed and interpreted molecular NGS data; SL analysed and interpreted histological data; DC and HT provided patient care and intellectual content; JFS and CT provided critical input to intellectual content. All authors edited the manuscript and gave final approval for the manuscript to be published.
Conflict of Interest Disclosures
CT and JFS receive honoraria from Janssen; all other authors declare no competing financial interests.

References


**Figure Legends**

**Figure 1. Biopsy of the perianal mass from Case 1.** Haematoxylin and eosin stained sections (200X magnification) demonstrated sheets of large, hyperchromatic plasmablastic lymphoma cells with high nuclear-cytoplasmic ratio and a “starry sky” pattern. The tumour cells were positive for CD45, CD138 and MYC, but negative for CD20 and cyclin D1, with kappa light chain restriction. The Ki-67 proliferative index was close to 100%.

**Figure 2. Retroperitoneal lymph node biopsy from Case 2.** Haematoxylin and eosin stained sections (100X magnification) demonstrated extensive infiltration by plasmablastic lymphoma, with large

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cells that had prominent nucleoli and moderate amphophilic cytoplasm. The tumour cells were positive for CD45, CD138 and MYC, with kappa light chain restriction and a Ki-67 proliferative index greater than 95%. Note was also made of a background population of small lymphocytes that were negative for CD3 but positive for CD5 and PAX-5, consistent with the patient’s underlying chronic lymphocytic leukaemia.
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