However, its cost is prohibitive in many areas and significant dose increases compared to the *E. coli* formulation have been required to achieve therapeutic drug levels; thus, further studies into its pharmacokinetics are required.^{1,12} Finch *et al.* found that the formulation impacted triglyceride levels, with peg-asparaginase associated with higher triglycerides compared to L-asparaginase.¹¹

Interestingly, all four of our patients had T-cell malignancies. Studies involving much larger numbers of young adults with ALL/lymphoblastic lymphoma would be required to determine whether the lymphoid blast phenotype (B- or T-cell) has any causative role in the complications described. Pancreatitis, severe hyperlipidaemia and diabetes have all been reported as adverse events related to use of L-asparaginase, but are rarely problematic in children. Our experience suggests these toxicities may occur more frequently in the young adult population. Thus, we suggest consideration of baseline biochemical screening as well as serial monitoring of triglyceride, glucose and amylase/lipase in this population during L-asparaginase therapy, in addition to current routine blood tests. We would also advise remaining alert to the possibility of hyperlipidaemia first presenting as pseudohyponatraemia or other similar laboratory biochemical abnormalities that seem discordant with the clinical picture. Further review of large study populations may allow the identification of baseline markers to identify those young adults at increased risk of developing such toxicities, as well as clearly identifying the appropriate frequency of monitoring and thresholds for intervention.

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Diagnostically challenging immunophenotypic shift in mantle cell lymphoma following ibrutinib and venetoclax therapy

Sir,

A 57-year-old Caucasian male without a significant medical history presented with B symptoms, widespread lymphadenopathy and peripheral blood lymphocytosis. A diagnosis of mantle cell lymphoma (MCL) was confirmed on bone marrow histology and typical immunophenotype. He was treated with four cycles of R-HyperCVAD (rituximab, cyclophosphamide, vincristine, doxorubicin and dexamethasone) chemotherapy and obtained a complete metabolic remission on positron emission tomography (PET) scan. He then received maintenance rituximab 3 monthly (Fig. 1). One year later while on rituximab maintenance he had biopsy proven relapse of MCL in the right orbit and subcutaneously in the left posterior thigh. This was treated with local radiation to the thigh and orbit and he was commenced on ibrutinib. Six months later, he progressed with central nervous system (CNS) relapse of MCL. This was managed with intrathecal methotrexate and high dose cytarabine, followed by two further cycles of R-HyperCVAD chemotherapy and craniospinal radiation, achieving a complete remission of the CNS disease. He was subsequently recommenced on ibrutinib maintenance. Three months later he presented with multiple skin lesions over the legs and torso, with a biopsy of both the left knee mass and skin lesions demonstrating blastoid MCL. He received palliative radiotherapy to the lower legs and proceeded to venetoclax monotherapy with achievement of complete response on PET. After 5 months of venetoclax therapy a bone marrow aspirate demonstrated no definite evidence of involvement with MCL; however, there were two focal areas of a lymphoid-appearing infiltrate which did not appear congruent with mantle cell lymphoma on immunohistochemistry (CD20, CD79a, PAX5, cyclinD1 negative). Flow cytometry did not demonstrate an aberrant or clonal B-cell population.

One month after this biopsy he presented with widespread bony pain, B symptoms, pancytopenia and a rapidly rising white cell count. Peripheral blood film demonstrated a

Fig. 1 Timeline of events with relapse and response assessment above and treatment below.

predominantly mature lymphocytosis and rare blasts. A PET scan demonstrated splenic enlargement with diffuse moderate metabolic uptake and diffuse mild to moderate bone marrow activity. The bone marrow demonstrated extensive diffuse replacement by a haematological neoplasm which was negative for B-cell, myeloid and other T-lymphoid markers. Immunohistochemistry demonstrated diffuse positivity with CD4 and SOX11. P53 was positive in 10–15% of cells. BCL2 was weakly positive. Flow cytometry demonstrated an expanded abnormal population within the ‘typical blast region’ which was positive for CD45, CD4, partial CD117, negative for CD34, CD56, CD123 and for all other B-cell,

myeloid and lymphoid markers. Cytogenetics demonstrated a complex pseudodiploid karyotype with deletion of chromosome 9 and fluorescence *in situ* hybridisation (FISH) identified a cytogenetically masked $t(11;14)(q13;q32)$. Next generation sequencing (NGS) demonstrated that the malignant clone expressed a clonal immunoglobulin heavy chain (IGH) (IGHV3-21*01/IGHD1-26*01/IGHJ6*06) with unmutated immunoglobulin heavy chain variable region genes (IGHV). Additional findings included gains and losses of chromosomal material of chromosomes 1p, 1q, 6q, 9q, 10q, 12p, 12q, 13q, 14q and a high order amplification of the BCL2 gene locus on 18q (Fig. 2). To confirm clonal relatedness to the previously

Fig. 2 Next generation sequencing results. Green star, mutations present in all samples (including initial relapse). Red star, clonal evolution in blastic morphology samples.

Fig. 3 Images from time of last relapse of disease. (A) Peripheral blood (MGG $\times 1000$). (B) Bone marrow trephine (H&E). (C) CD4 on bone marrow; trephine flow cytometry panels, abnormal population in black. (D) Side scatter v CD45, (E) CD5 v CD19, (F) CD7 v CD4.

diagnosed MCL, next generation sequencing was retrospectively performed on all previous diagnostic samples from the patient. The same IGH clonal sequence (IGHV3-21*01/IGHD1-26*01/IGHJ6*06) was demonstrated in all previous samples. The chromosomal abnormalities seen in each of the tumour samples were also consistent with clonal relatedness, with some evidence of clonal evolution including acquisition of the amplification of the BCL2 gene locus (Fig. 2). No single nucleotide variants or indels were detected in BTK, BCL2 or TP53, nor was TP53 copy number loss detected either at diagnosis or blastic transformation.

The patient developed progressive cytopenias and septic shock secondary to a respiratory tract infection and died prior to further therapy being commenced at 31 months following initial diagnosis of MCL. Figure 3 shows images from last relapse of disease.

It has been well described that B-cell lymphomas may evolve to evade antigen-specific immunotherapy; for example, B-cell lymphomas and leukaemias may relapse with CD20-negative disease after treatment with anti-CD20 antibody therapy. Immunophenotypic aberrancies and morphological shifts have also been reported in chronic lymphocytic leukaemia (CLL) patients treated with BCR signalling inhibitors including plasmablastic Richter transformation as a resistance mechanism for CLL.¹ There are also reported cases of transdifferentiated histiocytic sarcomas and dendritic cell neoplasms with preserved IGH rearrangements arising in the setting of pre-existing B-cell lymphomas.^{2–4} Demonstration of a clonal relationship between malignancies using IGH studies, polymerase chain reaction or NGS studies does not elucidate the stage of haematopoietic maturation at which the detected mutation occurs. In cases of relapsed clonally related neoplasm, treatment refractory disease clone may arise through either intrinsic or acquired resistance with several studies demonstrating that lymphomagenesis can occur in early haematopoietic precursors and prior to VDJ rearrangement.⁵

Although MCL cells lost all B-cell markers and were cyclinD1-negative by IHC in our patient, FISH and NGS studies demonstrated changes suggestive of a clonally related process

with persistent t(11;14)(q13;q32) translocation, unmutated IGHV (IGHV3-21*01/IGHD1-26*01/IGHJ6*06), and numerous additional chromosomal abnormalities. Clonal evolution such as acquisition of a BCL2 gene amplification may reflect selective pressure from exposure to venetoclax therapy.^{6,7}

In summary, our case of MCL which had been treated with multiple therapies including venetoclax and ibrutinib during the 31 month clinical course, developed blastic morphology and markedly altered immunophenotype with loss of all B-cell markers and acquisition of CD4 antigen expression in the late stages of disease. The significant immunophenotypic shift in our case illustrates that under treatment pressure of novel biological agents targeting BCR signalling and/or BCL2 pathways, malignant cells may be prevented from typical lymphoid maturation resulting in the development of an atypical immunophenotype and morphology which is diagnostically challenging. This hampers early recognition and correct diagnosis of the disease, thereby delaying initiation of appropriate therapy.

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Lymphocyte nuclei of nodal marginal zone lymphoma mimicking granulocytic morphology with Pelger–Huët-like features

Sir,

Nodal marginal zone lymphoma (NMZL) with peripheral blood involvement is observed in only a small proportion of patients and usually shows small-to medium-sized centrocyte-like neoplastic lymphocytes in a peripheral blood smear.^{1–3} This disease may present with frequent plasmacytoid differentiation or occasional monocytoid appearance; however, the granulocytoid appearance of nuclei is rarely

Fig. 1 Morphology of abnormal lymphocytes on peripheral smear. (A–D) Metamyelocyte-like kidney bean-shaped nuclei; (E–H) band-shaped nuclei; (I–L) multi-lobed nuclei; and (M–P) pseudo Pelger–Huët-like pince-nez-shaped nuclei (Wright-Giemsa staining $\times 1000$).