approach for ABO incompatible transplants, whereby red cell depletion of stem cell products is considered for major ABO mismatch, and plasma depletion for minor ABO mismatch. The same principle is essentially employed for transfusion support in patients with pre-transplantation-ABO alloantibody. Recipient plasma antibody clearance with therapies such as plasmapheresis or rituximab is followed by transfusion of products negative for the stimulating antigen.¹⁵

This case highlights the role of donor red cell extended phenotyping for intended transplant recipients with known clinically significant alloantibodies, to guide donor selection.

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Monocytoid switch in an adult with B-cell precursor acute lymphoblastic leukaemia characterised by the PAX5 P80R mutation

To the Editor,

Lineage switch leukaemias are a rare entity, thought to be a result of genetic instability in the malignant clone or through unmasking of a dormant subclone following initiation of cytotoxic chemotherapy. In most reported cases, the switch has become evident after completion of induction therapy. Here we report a case of lineage switch after administration of steroids alone, in the context of a PAX5-P80R mutation.

A 60-year-old nurse was referred to hospital by her general practitioner who noted moderate to severe cytopenia, with a neutrophil count of $0.8 \times 10^9/L$, haemoglobin of 85 g/L, platelets of $9 \times 10^9/L$ and 71% circulating blasts. She presented clinically with mild abdominal pain and fatigue in the preceding week. She had no significant past medical history. Bone marrow examination confirmed acute leukaemia with 90% pro-lymphoblasts, having an immunophenotype of CD10, CD19, cytoplasmic CD79a, and partial expression of TDT and CD34. Positron emission tomography scan demonstrated increased FDG uptake in an enlarged spleen, pancreas and numerous lymph nodes above and below the diaphragm. After diagnostic work-up, dexamethasone 4 mg thrice daily was commenced as pre-phase therapy on day 3. Lumbar puncture was deferred due to profound thrombocytopenia refractory to transfusions. She was then transferred to a neighbouring institution which offered blinatumomab and abbreviated chemotherapy for frontline treatment, in the context of a clinical trial. The white cell count was $4.9 \times 10^9/L$ after 4 days of steroids, with the blast percentage falling to less than 1%.

The white cell count rose steadily from this point, however, and reached $108 \times 10^9/L$ on day 6 following commencement of steroids. Flow cytometry (Fig. 1) was repeated and revealed a single population of blasts with a monoblastic immunophenotype, positive for CD4, CD14, CD64, and CD11b, and negative for CD34, not noted at the initial diagnostic analysis. The erstwhile lymphoblast population had disappeared. This finding was confirmed on repeat bone marrow examination (Fig. 2).

Due to the loss of CD19 expression on the prevailing blast cell population, the patient was deemed ineligible for the clinical trial and returned to our institution. With a revised diagnosis of mixed phenotype acute leukaemia (MPAL), induction chemotherapy with 7+3 (idarubicin) was commenced. The induction phase was complicated by

Fig. 1 Immunophenotypical evolution. Flow cytometric analysis performed on bone marrow on day 1, and blood on days 4 and 8 demonstrate gradual disappearance of the population expressing cytoplasmic CD79a and CD19. By day 8, a previously unseen CD4, CD14, CD64 and CD11b positive population had emerged, negative for CD34 and CD19.

Fig. 2 Morphological changes of leukaemic blast population. (A) Peripheral blood day 0, circulating blasts, Wright-Giemsa stain. (B) Bone marrow aspirate day 0, heavy infiltrate of monomorphic lymphoblasts with scant cytoplasm and absence of normal haematopoiesis, May-Grunwald-Giemsa stain. (C) Peripheral blood day 38, pancytopenia with atypical monocytes dominating, no blasts, Wright-Giemsa stain. (D) Bone marrow aspirate day 38, moderate haemophagocytic activity with foamy macrophages, erythrophagocytosis and a suppressed trilineage haematopoiesis, May-Grunwald-Giemsa stain.

respiratory sepsis requiring intensive care unit admission and non-invasive ventilation, colitis, acute cholecystitis, and delayed count recovery. The patient remained cytopenic at day 37 of induction, and a bone marrow biopsy demonstrated no residual blast population, but moderate haemophagocytic activity. Cyclosporin and intravenous immunoglobulin were commenced for secondary haemophagocytic lymphohistiocytosis. However, the patient continued to deteriorate, developing severe hyperbilirubinaemia and worsening respiratory failure. She died 48 days after commencing chemotherapy.

Cytogenetics showed an abnormal karyotype with trisomy 6 and 7, though standard molecular tests were negative. Whole transcriptomic sequencing was performed on bone marrow CD19-positive mononuclear cells at initial diagnosis. Two recurrent acute lymphoblastic leukaemia (ALL) driver lesions were observed: *PAX5* variant p.P80R at a variant allele frequency (VAF) of 0.93, as well as *NRAS* p.G12S, also at a VAF of 0.93, both suggestive of copy neutral loss of heterozygosity. A t-distributed stochastic neighbour embedding (tSNE) visualisation of the gene expression profile, based on analysis of the top 2000 differentially expressed genes, localised this case within the *PAX5*-P80R patient cluster. Using multiplex ligation-dependent probe amplification (MLPA), a biallelic deletion of *CDKN2A* and *B* was identified, as well as duplication of *IKZF1* (associated with trisomy 7). Importantly, no copy number changes were observed in *PAX5*. The *CDKN2A/B* deletion together with *RAS* pathway mutations are not uncommonly associated with *PAX5*-P80R.¹ Marrow collected 6 days after prednisolone was also subjected to sequencing and MLPA. No additional genomic alterations were identified. The expression of *PAX5* is B-cell specific, thus consistent with morphological lymphoblast clearance. The *PAX5*-P80R variant remained detectable at a similar VAF, but the read count across exons was significantly reduced (from >1000 at diagnosis to 10–20). Of interest the *NRAS* p.G12S variant persisted with similar exon read counts between diagnosis and post-steroid samples.

Lineage switching in acute leukaemia is a rare entity, most frequently described in paediatric cases.² The process is postulated to be due to clonal selection through cytotoxic therapy, or genetic plasticity inherent in the original leukaemia, with the haematopoietic microenvironment also influencing the direction of cell differentiation and reprogramming.³ Among anti-neoplastic therapies, those exerting an immune pressure on the malignant clone though CD19 blockade, such as CAR-T cells and blinatumomab, can cause switching through clonal selection or genetic reprogramming,^{4,5} however, conventional cytotoxic therapies have also had this effect.

Certain conventional cytogenetic patterns, such as hyperdiploidy and the Philadelphia chromosome, are well established as having prognostic significance; among these, rearrangements involving *KMT2A* (formerly known as *MLL*) are known to have a predilection towards lineage plasticity³ and heterogeneity, and such rearrangements have been designated as a subtype of MPAL as well both myeloid and lymphoid leukaemia according to the World Health Organization classification.⁶

More recently, there has been growing understanding of the molecular patterns of other oncogenic subtypes in B-cell precursor acute lymphoblastic leukaemia (BCP-ALL)

precursor leukaemia,⁷ denoted B-other ALL. Of these, mutations involving *PAX5* were found in 53% of cases in a paediatric cohort, with *PAX5*-P80R found in 5% of cases.⁸ *PAX5* is a transcription factor crucial for cell commitment to the B cell line,⁹ and knock-out mutations can result in lineage promiscuity. Therefore, it is unsurprising that a *PAX5* mutation was discovered in our patient with early lineage switch of a previously established BCP-ALL. A recent large study of paediatric ALL identified a positive association between mutations in *PAX5*-P80R, *DUX4r*, and *ZNF384r*, and lineage switching, which occurred in 61 patients.¹⁰ As with our case, patients underwent monocytic switch within the first 35 days of therapy, though it is noteworthy that in spite of this, all patients continued to receive risk-based ALL treatment, whereas our approach had been to change to a myeloid leukaemia protocol. Contrary to previous reports of uniformly poor outcomes in paediatric lineage switch leukaemia,² these patients had the same overall survival and relapse rate as the 655 patients who did not undergo lineage switch. *PAX5*-P80R in a French adult cohort was found to have a favourable prognosis, though no switching was reported.¹ In a separate paediatric study, however, it was associated with a significantly reduced overall survival.¹¹ The poor outcome for our patient is perhaps related to her age and other complications, chiefly the development of haemophagocytic lymphohistiocytosis, and no inferences can be made regarding the role of her genetic profile in her prognosis.

This case supports existing evidence that BCP-ALL harbouring *PAX5*-P80R is associated with monocytoid switch, a phenomenon that has been hitherto unreported in adults. Such clinical characteristics may indicate a discrete subtype among the B-other ALL group.

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EBV-positive low-grade follicular lymphoma transforming to EBV-negative high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements

To the Editor,

Epstein–Barr virus (EBV) is associated with some types of lymphoma, notably of B-cell lineage, such as Burkitt lymphoma, Hodgkin lymphoma, EBV-positive diffuse large B-cell lymphoma (DLBCL) not otherwise specified (NOS), DLBCL associated with chronic inflammation, plasmablastic lymphoma, primary effusion lymphoma, and immunodeficiency-associated lymphoproliferative disorders.¹ However, the association of follicular lymphoma with EBV has not been described until recently.² Two large cohorts of follicular lymphoma conducted by Mackrides *et al.* found that less than 3% of follicular lymphomas were EBV-positive, and these cases were mostly high grade (grade 3A or 3B) and tended to transform to EBV-positive DLBCL or high-grade follicular lymphoma with worse prognosis. Whether this virus plays a role in lymphomagenesis or participates in a later stage of tumour progression is yet to be determined.^{2,3} We present a rare case of EBV-positive low-grade follicular lymphoma transforming to EBV-negative high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (so-called double-hit lymphoma) showing clonal relationship, which has not been previously reported. According to the chronological change in this case, we propose that *BCL2* rearrangement occurred early in the follicular lymphoma, and then EBV infected some lymphoma cells with clonal expansion, forming EBV-positive follicular lymphoma. Subsequently, *MYC* rearrangement in some EBV-negative cells induced transformation to EBV-negative high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements.

A 76-year-old man presented with poor appetite and general discomfort for 2 months. He had medical history of DLBCL, Ann Arbor Stage IVB, diagnosed in another hospital 7 years previously. He had received chemotherapy with nine cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) and two cycles of R-ESHAP (rituximab, etoposide, methylprednisolone, high-dose cytarabine, and cisplatin) with disease progression. The positron emission tomography-computed tomography (PET-CT) scan revealed multiple fluorodeoxyglucose (FDG) uptakes in the lymph nodes of bilateral neck, bilateral supraclavicular, mediastinum, bilateral pulmonary hila, upper abdominal, mesentery, abdominal, para-aortic, bilateral iliac and bilateral inguinal areas, compatible with lymphoma involvement. The right inguinal lymph node biopsy showed small to medium-sized lymphocytes in follicular pattern with mostly centrocytes and few scattered centroblasts (Fig. 1A,B). Focal geographic necrosis with nuclear debris and vague granuloma were noted, suggesting EBV infection. No obvious Hodgkin/Reed–Sternberg (HRS)-like cells or angioinvasion were seen. No microorganisms were found by periodic acid–Schiff (PAS) and acid-fast stains. Immunohistochemically, most of these lymphocytes expressed BCL2, BCL6, CD10, CD20 and CD79a (Fig. 1C,D). CD3, CD5, CD30, CD138, cyclin D1, HHV8 LANA1 (human herpes virus 8 latency-associated nuclear antigen 1) and MYC were negative (Fig. 1E). CD21 stain highlighted follicular dendritic cell meshwork, indicating follicular pattern. Ki-67 proliferation index was up to 20% (Fig. 1F). Interestingly, EBV-encoded small RNA (EBER) *in situ* hybridisation (ISH) was positive in more than 75% of the neoplastic lymphocytes in follicular pattern (Fig. 2A). EBV LMP1 was positive, while EBNA2 was negative, indicating latency type II EBV infection. Thus, EBV-positive follicular lymphoma, World Health Organization (WHO) grade 1–2, was diagnosed. Blood tests of EBV antibodies were not available. The bone marrow biopsy showed involvement of follicular lymphoma with characteristic paratrabecular pattern and EBER positivity in about 5% of the lymphocytes. The previous diagnosis of DLBCL in another hospital 7 years ago was revised to follicular lymphoma, WHO grade 1–2, with about 3% of EBER-positive lymphocytes after reviewing the sections. He received further chemotherapy with RICE (rituximab, ifosfamide, carboplatin, and etoposide) regimen.

Several months later, the patient complained of limb weakness. Magnetic resonance imaging (MRI) demonstrated an extradural spinal tumour from T2 to T7. The tumour biopsy exhibited totally different morphology with diffuse infiltrate of monotonous medium-sized atypical lymphocytes showing Burkitt lymphoma-like morphology, brisk mitoses and starry sky pattern (Fig. 1G,H). Immunohistochemically, the lymphoma cells expressed BCL2, CD10, IRF4/MUM1, MYC and PAX5 (Fig. 1I–K), but not BCL6, CD3, CD20, cyclin D1, CD34 or TdT (Fig. 2H). Ki-67 proliferation index was high, up to 80% (Fig. 1L). EBER ISH was totally negative in the lymphoma cells with positive external control in the same slide (Fig. 2G). Fluorescence *in situ* hybridisation (FISH) study confirmed concurrent *MYC* and *BCL2* rearrangements in the tumour cells without *BCL6* rearrangement, indicating double-hit lymphoma (Fig. 2I,J). In comparison, the previous follicular lymphoma carried *BCL2* rearrangement, but no *MYC* rearrangement by FISH study (Fig. 2C,D).