Pathology (■ xxxx) **xxx(xxx)**, xxx

CORRESPONDENCE

KMT2A amplification in B lymphoblastic leukaemia

To the Editor

KMT2A gene rearrangements are known to occur in both myeloid and lymphoid leukaemia.[1] However, *KMT2A* amplification is rarer and generally reported to occur in only 1% of cases of acute myeloid leukaemia (AML). *KMT2A* amplification is rarely reported in B-lymphoblastic leukaemia (B-ALL) with only a handful of cases[2–6] in the literature. To our knowledge, we present only the second case report of *KMT2A* amplification with corresponding *TP53* deletion in adult *de novo* CD10 negative B-ALL.

A 69-year-old male presented to the emergency department with symptoms of melena, coffee ground vomit, syncope and confusion, on a background of 2 weeks of abdominal pain. His only other significant medical history was consumption of 8−10 beers per day. He was sent for an urgent gastroscope which showed sigmoid diverticulitis with localised perforation. He denied any past significant medical history. His FBC results showed he was pancytopenic (Hb 52 g/L, WCC 3.9×10^9/L, Neut 0.5×10^9/L and platelet count 7×10^9/L) with 17% circulating blasts (Fig. 1). Flow cytometry was performed on the peripheral blood and showed that the blasts were positive for CD45(dim), CD19, CD34, CD38, CD15(variable) and HLA-DR. They were negative for CD3, CD4, CD5, CD7, CD10, CD13, CD20,CD33,CD56,CD64,CD117.

Plans were made for a bone marrow biopsy the following day, however the patient self-discharged from hospital, accepted referral to palliative care and died shortly after.

As a result of this and the prospect of no bone marrow sample, additional flow cytometry testing was performed on the peripheral blood. The additional flow cytometry markers showed the blasts to be positive for intracellular CD79a, CD22 and negative for MPO and intracellular CD3, confirming B cell lineage. At the same time rapid fluorescence *in situ* hybridisation (FISH)[7] for *BCR::ABL1* gene fusion and a *KMT2A* rearrangement was performed on directly harvested peripheral blood cells using the LSI BCR/ABL dual colour dual fusion (DCDF) probe and the LSI MLL (KMT2A) dual colour break-apart (DCBA) probe (Abbott Molecular, USA) to further classify the leukaemia/allow access to targeted therapy depending upon the results.

The FISH showed *KMT2A* amplification with >20 signals per cell (Fig. 2A−C). There was no evidence of *BCR::ABL1* gene fusion; however, there were three to four copies of *BCR* and three copies of *ABL1* suggesting aneuploidy for chromosomes 9 and 22 (Fig. 2D). Once the *KMT2A* amplification result was known, a diagnosis of AML was favoured, as the literature states this abnormality is seen in approximately 1% of cases of AML.[8] However, the additional positive markers (CD79a and CD22) by flow cytometry confirmed that the diagnosis was B-ALL.

Further review of the literature found only six papers reporting a total of seven cases of *KMT2A* amplification in B-ALL.[2–6,9] These consisted of two cases reported as occurring in childhood B-ALL,[6,9] with the remaining five cases being reported in adults. Of the two childhood cases, one contained only three copies of *KMT2A*[9] and is considered segmental duplication rather than true amplification. Of the five cases reported in adults, three were referred to as therapy related B-ALL[3,4] and one as lineage switching acute leukaemia,[2] with only one adult B-ALL case without a history of previous chemotherapy.[5] Therapy related ALL is not recognised by the World Health Organization classification of tumours of haematopoietic and lymphoid tissues;[1] however, this is how the respective authors have referred to these cases. The adult *de novo* case[5] was an 86-year-old female presenting with pancytopenia who was diagnosed with CD10 negative B-ALL with *KMT2A* amplification and *TP53* gene deletion.

Gene amplifications are rare in haematological malignancies, with *MYC* amplification the most commonly seen.[2] They lead to overexpression of the gene and are generally associated with a poor prognosis. In the literature *KMT2A* amplification has been reported in association with a *TP53* gene deletion in AML[2,10] but this has also been reported in ALL.[5] FISH for *TP53* deletion using the Vysis TP53/CEP17 FISH probe (Abbott Molecular, USA) confirmed that our patient also had a *TP53* gene deletion (Fig. 2E). It also suggested that a doubling clone exhibiting *TP53* deletion was present (Fig. 2F) which would be in keeping with a complex karyotype. Due to the patient discharging himself from hospital, no sample was available for conventional cytogenetics. Consequently, we could not determine if the amplification was due to extrachromosomal double minutes or intrachromosomal homogeneously staining regions by examining a G-banded karyotype. Additional image capture of over 50,000 images from the directly harvested peripheral blood cells was then performed and found three normal and three abnormal spontaneously dividing metaphases. These abnormal metaphases (Fig. 2A) favour that the amplification was localised to intrachromosomal regions and not due to extrachromosomal double minutes; but was not conclusive as double minutes can tether to chromosomes.

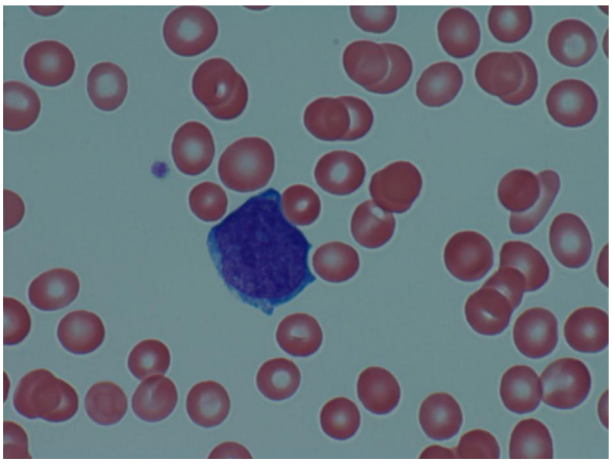

Fig. 1 Blasts consist of large cells with a high N:C ratio, variable nuclear morphology (some with folded nuclei), intermediate chromatin, multiple nucleoli, and a scant quantity of basophilic cytoplasm. No cytoplasmic granules or Auer rods are seen.

Print ISSN 0031-3025/Online ISSN 1465-3931

Please cite this article as: Wren C et al., *KMT2A* amplification in B lymphoblastic leukaemia, Pathology, https://doi.org/10.1016/j.pathol.2022.12.356

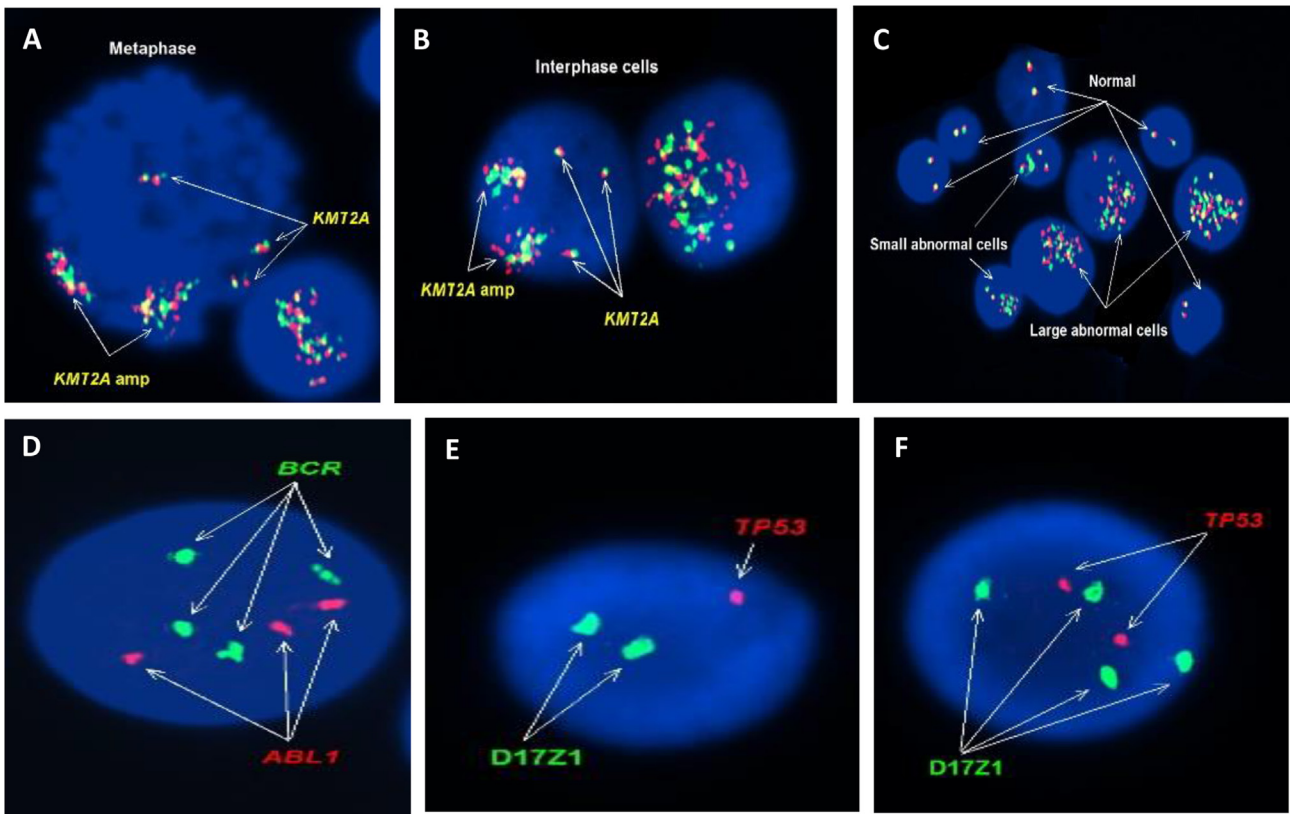

Fig. 2 (A) Metaphase and interphase showing *KMT2A* amplification with >20 signals per cell. (B) Three *KMT2A* signals (yellow fusion) and *KMT2A* amplification possibly localised to two intrachromosomal regions. (C) Proposed two *KMT2A* amplification clones (smaller cells with one localised region of *KMT2A* amplification beside larger cells with two localised regions of *KMT2A* amplification) as well as normal cells, raising the possibility of clonal evolution. (D) Three copies of *ABL1* (red) and four *BCR* signals (green). (E) Loss of one *TP53* signal (red) and two copies of the centromere 17 probe D17Z1 (green). (F) Doubling clone of the *TP53* deletion with two *TP53* signals (red) and four copies of *D17Z1* (green).

Review of these additional images also further supported the possibility of clonal evolution (Fig. 2C). The first proposed clone was comprised of small cells with 2 *KMT2A* signals and a smaller number of *KMT2A* amplification signals localised to a single region, and the proposed second clone consisted of larger cells with 3−4 *KMT2A* signals and two localised regions of *KMT2A* amplification (Fig. 2B). If the amplification signals have an intrachromsomal association with 11q, this would assume there are five+ chromosomal copies of this region present in the larger cells. The finding of clonal evolution would be in keeping with the presence of a doubling clone (Fig. 2F) detected using the Vysis TP53/CEP17 FISH probe (Abbott Molecular, USA). Multiplex PCR was negative for *TCF3/PBX1*, *ETV6/RUNX1*, *KMT2A/AFF1* and *BCR/ABL1* gene fusions. Unfortunately no further array or sequencing was performed due to the patient withdrawing from treatment and therefore the presence of other driver mutations[11] for B-ALL were not assessed. It is generally reported that acute leukaemias with *KMT2A* rearrangements have a lower additional mutation rate, however we accept the literature is limited in regard to *KMT2A* amplification in B-ALL.[12] This is a fascinating case of *KMT2A* amplification with associated *TP53* gene deletion and clonal evolution in adult *de novo* precursor B-ALL.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose. Funding was provided by the ICPMR-NSWHP ROPP trust fund for research salary and consumables.

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Please cite this article as: Wren C et al., *KMT2A* amplification in B lymphoblastic leukaemia, Pathology, https://doi.org/10.1016/j.pathol.2022.12.356

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DOI: https://doi.org/10.1016/j.pathol.2022.12.356

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