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Case report: Exome sequencing identifies T-ALL with myeloid features as a *IKZF1*-struck early precursor T-cell malignancy

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1. Introduction

Within the group of patients with T-cell acute lymphoblastic leukemia (T-ALL), which comprises about 15% of childhood ALL and 25% of adult ALL, a marked heterogeneity has recently become apparent. Thus, a sizeable fraction of leukemia patients exhibits a mixed phenotype (15%), with up to one third of this morphological inconclusive group presenting a T/myeloid type, when analysed by multiparameter flow cytometry (MFC) [1]. Notably, and concordant with early malignant transformation at the oligopotent progenitor level, such ambiguous cases of T-ALL, have a poor prognosis. More recently, the suggested diagnosis of Early T-cell Precursor (ETP) leukemia, likewise adverse, has entered the scene [2,3]. These observations on the underlying heterogeneity, and different states of differentiation, emphasize the need for more personalized molecular characterization of the single patient.

We hypothesized that exome sequencing (WES), combined with thorough analysis and evaluation of allele frequencies, could provide much needed information on lineage origin and clonal evolution in the individual patient with leukemia of ambiguous origin. Here, we present evidence to support this concept in an apparent T-ALL patient.

2. Case presentation

Following a one-month-long period of dyspnoea, a 21-year old man sought his general practitioner after noticing enlargement of a cervical lymph node and sore gums. A hematological screen revealed a leukocyte count of 80.3 $\times$ 10^9/L, a hemoglobin of 7.4 g/dL and a thrombocyte count of 45,000 $\times$ 10^9/L. Bone marrow (BM) sample, aspirated at the Department of Hematology, Aarhus University Hospital, was found to be dominated by small blasts with no granules and no myeloperoxidase enzyme activity. Flow cytometric analysis showed the blasts to be CD34+, CD2+, CD7+, CD13+, CD117+ and CD3- on the cell surface, but CD3+ and TdT+ intracellularly, hence leading to a diagnosis of T-ALL. The cytogenetic analyses revealed a normal karyotype, albeit tetraploidy was observed in a minor clone (4–5%). Consequently, the patient was treated with a combination of cyclophosphamide, anthracycline (daunorubicin and doxorubicin), vincristine, L-asparaginase, corticosteroids, etoposide, high dose methotrexate, high dose cytarabine and intrathecal methotrexate followed by mercaptopurine/methotrexate maintenance treatment for two years.

Four and a half year after initial diagnosis his peripheral blood counts started to decline over a period of several months. While a new BM aspirate was found to be compatible with relapse, only 15% malignant cells were found with continued expression of CD34, CD117 and CD7. Interestingly, revealed by cytogenetic analysis the, tetraploid clone now constituted 15–20% of the metaphases.

The patient was re-induced with nelarabine single agent [4] due to side-effects of the induction regimen. He received a full allogeneic BM transplantation from a matched unrelated donor after which he relapsed again, 857 days after transplant. Flow cytometry revealed a malignant clone continually positive for CD34, CD117, CD13 and CD7 and reduced cytoplasmatic CD3 (Fig. S1). A tentative diagnosis of therapy-related acute myeloid leukemia (AML) was established. At this stage he proved to be therapy resistant, and 7 years and four months after the initial diagnosis he succumbed to his disease.

2.1. Molecular characterization

Whole exome sequencing (AROS Applied Biotechnology, Aarhus, DK) was performed, post-mortem, on purified DNA from cryopreserved BM sampled at time of diagnosis (*day 0*), at relapse (*day 1693*), secondary relapse (*day 2687*) and cultured skin cells. The keratinocytes and fibroblasts from a skin biopsy, drawn 2 weeks before the patient succumbed to his disease, were cultured in order to yield enough material for control sequencing.

WES yielded an average of 94 million reads and general sequencing QC consistency between samples (Table S1). 31, 43 and 44 non-synonymous somatic mutations, with read depths exceeding 30, were detected in the diagnostic, relapse and second relapse samples,

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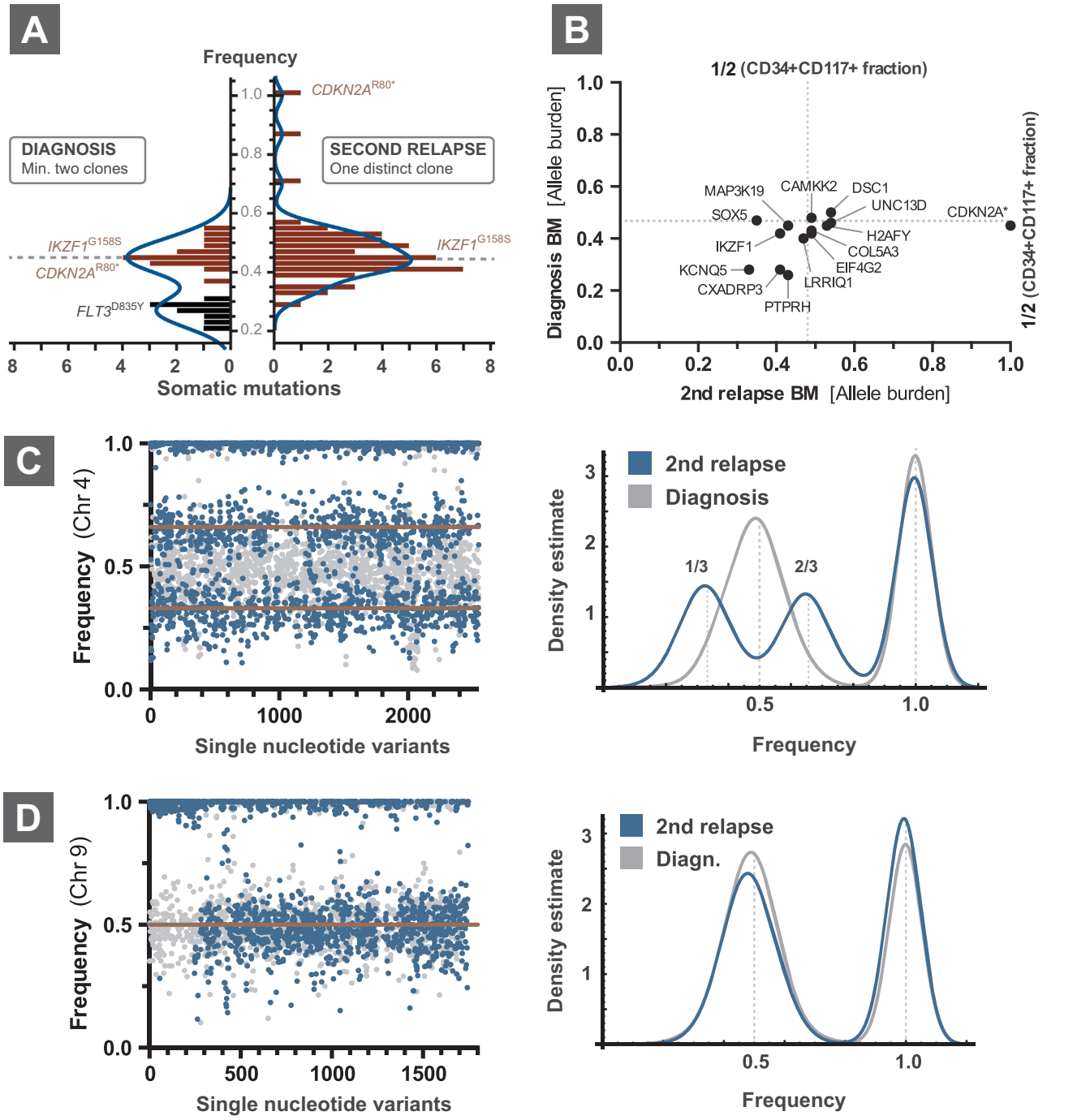

Fig. 1. Allele frequency comparison of 14 persistent mutations between diagnosis and 2nd relapse showed shift in CDKN2A^{R80*} (A), evidently arising from copy neutral loss of heterozygosity involving a 33 million basepair stretch of chromosome 9, and thus not detected by array-CGH. Some deviation from the hypothetical frequency is expected, when performing exome sequencing, but the allele frequency distributions are in agreement with leukemic burden (B), where dominant clone and some subclonality at diagnose can be postulated from low resolution kernel density estimations (left). Only one distinct high frequency peak is detected at second relapse (right). The clonal pattern is supported by the mutations held in each peak, i.e. the persistent mutations (*IKZF1*, *UNC3D* etc.) are generally found in the dominant clone, whereas FLT3^{D835Y} is located in the disappearing subclone. Frequency scatter plots (C, left) and kernel distribution estimation (C, right) demonstrated trisomy of chromosome 4 (C) at 2nd relapse and copy-number neutral loss of heterozygosity on chromosome 9 (D), when comparing to diagnosis.

respectively (Table S-II), with and average depth of coverage of 71. Kernel distribution estimation (KDE, read depth > 19) of all allele frequencies enabled exclusion of low frequency variants and potential background noise and facilitated enhanced clonal resolution as described below. Leukemic burden of first relapse was too low to confidently resolve allele distributions, although KDE was informative for clonal surveillance in comparison with diagnosis and second relapse (Fig. S2). Further description of the bioinformatics is included in the supplement.

In order to determine the clonal architecture and progression of the malignant cells during the eight-year course we correlated sequencing read frequencies to the percentage of malignant cells in the BM samples, based on CD34 and CD117 flow cytometry measurements at time diagnosis (> 90%), relapse (approx. 15%) and second relapse (> 90%). Comparing these figures with the allele burden derived from kernel distribution estimations two apparent high-frequency peaks (f ≥ 0.2)

could be resolved from the diagnostic sample, whereas only one distinct peak was present at secondary relapse (Fig. 1A). A single homozygous SNV (*CDKN2A*^{R80*}) was retrieved at this time point, affecting several CDKN2A isoforms, such as tumor suppressor P16.

By intersection of the variant sets we identified a subgroup of 14 somatic point mutations (10 coding), persistently present throughout the clinical course (Fig. 1B). Importantly, the highest frequency cluster contained genes with possible roles in malignant transformation; *IKZF1*^{G158S}, before-mentioned *CDKN2A*, and others of more indirect interest and unknown relevance (e.g. in *SOX5*, *PTPRH* and *UNC13D*. Somatic mutations are covered in Table S-II A–C). Finally, a special feature of this patient pertained to the *FLT3* myeloid signature gene. Thus, at time of diagnosis the highly recurrent gain-of-function *FLT3*^{D835Y} mutation was present in the apparent non-dominant clone (Fig. S5), and lost at second relapse along with low frequency *EZH2*^{Y675*} mutation. In contrast, a novel *FLT3* internal tandem repeat mutation was detected at the second relapse, as resolved by fragment analysis. The allele frequency analysis could also clearly distinguish trisomy 4 (Fig. 1C), as was confirmed by 24-color karyotyping and array-CGH (Fig. S3–4). Interestingly, the *CDKN2A* nonsense mutation was observed in combination with copy neutral loss of heterozygosity (CN-LOH) on chromosome 9 at late relapse, thus not detected by conventional cytogenetics, but clearly resolved by SNV allele frequency plot (Fig. 1D).

3. Discussion and conclusions

We believe that this case presentation adds two important aspects to the literature: One pertains to the lineage assignment of leukemia cases, here evidently involving early multipotent progenitors, the other to the contribution of WES in cases with unknown origin of the dominating clone. Thus, the striking difference in 2nd relapse kinetics suggested a therapy related AML. However, WES provided conclusive evidence for a unified monoclonal origin in all phases of the disease with *IKZF1*^{G158S}, a dominant-negative driver mutation in a hematopoietic transcription factor as major player, which has a pivotal role in early thymic progenitors with multipotent capabilities in hematopoietic differentiation [3,5]. We thus corroborate the leukemogenic role of this specific driver variant, which is currently described in a single case of B-ALL (COSM86966) [6,7], and extend it to play a role in the course of ETP-ALL. The role of *IKZF1* transcription factor, which have been shown to herald a poor prognosis in B-cell acute lymphoblastic leukemia (B-ALL) [6,8] with high risk of relapse [9], is underlined by the fact that is also known to be involved in primary leukemogenesis in childhood T-ALL [10,11]. Other aberrations of the gene have been described in ALL [6,12], along with loss-of-function in mixed phenotype (see also [13–15]). Its functional implication is evident by data from murine models revealing that dominant-negative ikzf1 mutation, with the murine equivalent shown here, drives aggressive T-cell leukemia [16]. In light of its pivotal driver role, and as a possible biomarker for multipotency [15,17], it is perhaps not surprising that *IKZF1* deletions are frequent molecular aberration in leukemia arising from an early T-progenitor. This is further supported by the phenotypic ambiguity of the presented case. We suggest that, as the cellular program behind leukemogenesis is combinatorially complex, thorough sequencing analysis of each individual ambiguous case should become a routine measure. Although the distinction between Pre-T and Early Pre-T may be difficult, it is known that *FLT3* mutations is also a frequent hallmark of ETP-ALL [18], along with overlap of both myeloid and lymphoid signatures [8,19] and frequent copy-number alterations [8]. Whereas it is yet unclear whether *CDK2NA* loss holds prognostic value [20,21], its appearance at the time of progressing to a bi-allelic deletion, does point to such a role [21].

The approach to leukemia lineage assignment employed here can be applied to other patients identified at diagnosis as potentially difficult to cytoreduce or prone to a similar course of disease as in the young male described here.

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We dedicate this manuscript to the memory of our patient, a very brave young man. We thank Dr. Anne Roug for helpful suggestions.

DeclarationsEthics approval and consent to participate

All sampling was performed in accordance with approvals from the Committee on Health Research Ethics (1-10.72-380-13). Informed consent on genomic analyses was obtained antemortem. The case study focused entirely on somatic aberrations.Consent for publication

The authors concur.Availability of data and material

Sequencing raw data is available upon specific and relevant request. This request may rejected by the PI on the basis of ethical concerns or if deemed irrelevant.

Competing interests

The Authors declare *no conflict of interest*.

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Authors' contributions

All authors contributed substantially from sampling to analysis, interpretation and elaborated manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.lrr.2017.11.002.

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