LETTER TO THE EDITOR

The PML-RARA fusion is not detectable in historical blood samples of acute promyelocytic leukaemia patients

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Dear Editor,

Acute promyelocytic leukaemia (APL) is characterized by the presence of the t(15;17) chromosomal translocation that generates the PML-RARA fusion gene [1]. PML-RARA, formed by the fusion of the promyelocytic leukaemia (PML) and retinoic acid receptor α (RARA) genes, is a constitutively active nuclear receptor that exerts transcriptional repression of RARα target genes leading to enhanced self-renewal capacity and myeloid differentiation block [2]. In approximately half of all cases, APL cells harbor additional mutations in genes such as FLT3, WT1, NRAS or KRAS [3, 4]. Mice expressing PML-RARA under the control of various promoters develop APL-like disease with variable penetrance and only after a significant period of latency (6–16 months) [5, 6]. These observations suggest a long latency between PML-RARA acquisition and APL onset, as reported for somatic mutations associated with acute myeloid leukaemia with a normal karyotype (AML-NK) [7]. Here, to investigate whether PML-RARA is detectable in the blood before APL onset, we study four APL cases with blood DNA available, from the same individual, 2–12 years before diagnosis.

Four individuals with paired APL and pre-leukaemic blood DNA samples were identified (Fig. 1a). APL DNA samples were shallow whole-genome sequenced on Illumina HiSeq using a 150 bp paired-end protocol preceded by DNA sonication to generate fragments of 450 nucleotides (nt) average length. FASTQ files were aligned to DNA sequences of the PML and RARA genes. Aligned reads were analyzed using RNAmut v1.0 [8] and this directly identified the precise location of 3/4 patient-specific breakpoints (by reads crossing the breakpoint), whilst the location of the fourth breakpoint was narrowed to a 20 nt window (Fig. 1b). Patient-specific primer pairs were then designed to amplify across each breakpoint using the polymerase chain reaction (PCR) (Fig. 1c). To determine the sensitivity for detecting each breakpoint by PCR, leukaemic DNA samples were suspended at 20 ng/μL and iteratively diluted tenfold to a dilution of 1:10,000 into equimolar (20 ng/μL) pooled normal blood DNA from 10 healthy subjects. All 4 primer pairs specifically amplified their cognate patient-specific breakpoints (Fig. 1d). Serial dilutions demonstrated sensitivity down to a 1:1000 dilution of cognate APL DNA (equivalent to 200 pg/μL of DNA). Despite this high sensitivity, none of the PML-RARA fusions was identified in their relevant pre-leukaemic samples (Fig. 1d, representative of triplicate PCRs).

In summary, our study of paired DNA samples suggests that the pre-clinical clonal history of APL may not be as long as that of AML-NK, where mutation-bearing clonal hematopoiesis (CH) clones can be detected years in advance of their progression to frank leukaemia [11]. However, with the exception of JAK2 V617F, CH is almost entirely driven by mutations in epigenetic (DNMT3A, TET2, ASXL1, IDH1, IDH2), splicing (SF3B1, SRSF2, U2AF1) or apoptosis-related (TP53, PPM1D) genes [9, 10]. By contrast, bona-fide leukemogenic mutations in genes such as NPM1, CEBPA and MLL have not been identified prior to the diagnosis of AML-NK, reflecting what we have observed for PML-RARA. Collectively these...
observations suggest that acquisition of such mutations leads to the development of AML after a relatively short time. Alternatively, mutation-bearing cells may not reach the circulation as a consequence of the effects of these mutations in blocking myeloid differentiation [6]. In either case, our findings suggest that, unlike AML-NK [7], APL does not commonly have a long pre-clinical phase that can be detected in the DNA of circulating blood cells.

Code availability The RNAmut package used here to identify breakpoints is available from https://github.com/muxingu/rnamut.

Author contribution G.S.V., P.C. and J.N. conceived and designed the study. G.S.V. supervised the study. W.G.D and G.S.V. design PCR assays. W.G.D. and J.C. performed experiments. M.G. and M.A.F. performed bioinformatic analyses. K.N. and J.X. identified patient samples. W.G.D. and G.S.V wrote the manuscript with input from all co-authors.

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Declarations

Ethics approval The study was approved by the Cyprus National Bioethics Committee (EEBK/EII/2014/11) and performed in accordance with the Declaration of Helsinki of 1975, as revised in 2008.

Consent to participate Informed consent was obtained from all participants.

Consent for publication The authors affirm that all patients who provided peripheral blood samples for the study gave consent for the publication of their anonymized data.

Conflict of interest G.S.V. is a consultant for STRM.BIO and a minor stockholder in KYMAB.

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