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Remission is good - relapse is bad

Paul S. Gaynon

Children's Hospital Los Angeles, University of Southern California, Los Angeles, CA, USA

E-mail: pgaynon@chla.usc.edu

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The prognostic significance of minimal residual disease (MRD), or perhaps 'measurable' residual disease,[1] is well-established acute and chronic leukemia.[2,3] The vast effort of European investigators in standardizing MRD assessment by polymerase chain reaction (PCR) and flow cytometry merits recognition and credit.[4,5] At present, we have several independent quantitative monitoring strategies, namely, PCR on DNA targets, reverse transcription (RT)-PCR on abnormal ribonucleic acid (RNA) transcribed from fusion genes or overexpression of normal messenger (m)RNA, and flow cytometry. Their relative implications remain under investigation.

MRD results, whatever the target, depend on specimen quality. Marrow aspirates represent a variable mixture of marrow and peripheral blood. Sensitivity depends on the number of cells or amount of nucleic acid interrogated. Leukemia may present with uniform marrow replacement and remit homogeneously across the marrow. Early relapse, however, may be patchy or perhaps anatomically localized with only later dissemination. Peripheral blood may be of use, despite a consistently lower and not always predictable presence of leukemic blasts in the peripheral blood relative to the bone marrow.[6]

The comparison of quantitative MRD strategies based on DNA and RNA is complex. The DNA target may persist from residual dying cells or in cells lacking leukemogenic potential, vis-à-vis the persistence of *DNMT3A* mutations in acute myeloid leukemia (AML),[7] representing clonal hematopoiesis and not always associated with relapse. While one or two copies of DNA targets are present per cell, the expression of both the target RNA and the housekeeping genes employed as denominators can vary from patient to patient, and from cell to cell for individual patients. Interventions may affect gene expression as well as cell number. The RNA target may also be present in cells lacking leukemogenic potential. RNA is more labile than DNA.

In this issue of Haematologica, Cazzaniga *et al.* compare MRD monitoring by RQ-PCR of DNA-based rearranged immunoglobulin/ T-cell receptor gene rearrangements (IG/TR), and of RNA-based *BCR/ABL1* fusion transcript in 90 young people with Philadelphia chromosome-positive acute lymphoblastic leukemia (PH+ ALL) who were allocated to imatinib on the European intergroup study of post-induction treatment of PH+ ALL (EsPhALL; EudraCT 2004-0014647-30; *clinicaltrials.gov Identifier: 00287105*). Of the 57 patients characterized, about 90% had the p190 transcript and 10% the p210 transcript.[8] Imatinib treatment was initiated after the first time point (tp1), at the completion of Induction IA at 5-7 weeks from diagnosis, and continued intermittently. Contemporary protocols for PH+ ALL begin tyrosine kinase inhibitors earlier and continue them without interruption.

None of the nine patients with undetectable MRD by PCR targeting IG /TR after one month of therapy (end induction IA) relapsed. MRD positive patients had a similar ~35% relapse rate, whether MRD was quantifiable (≥

$5x10^{-4}$) or positive below the quantifiable range (< $5x10^{-4}$).

Imatinib began with Induction 1B. MRD by IG /TR at the end of Induction IB (time point 2, tp2) was again prognostic. Fourteen of 64 patients first became negative at tp2 and had a 14% relapse rate. The relapse rate was about 40% for those who remained positive at any level.

MRD was monitored with each subsequent high-risk (HR) Block. Eleven of 37 and 7 of 21 patients first became negative after HR Block 1 and HR Block 2, respectively. Attaining negativity after tp2 carried no apparent benefit. One might attribute this revelation to the vagaries of small numbers. Alternatively, one might ask whether the persistence of excessive disease for too long a period of time provided an opportunity for mutation and the eventual emergence of resistant clones, despite the eventual eradication of the clones detectable from diagnosis.

Of interest, MRD response correlated well with conventional age and white blood cell count-based risk classification. In addition, while 7/10 patients with positive but unquantifiable MRD at tp1 prior to treatment with imatinib became negative at tp2 after initiating imatinib therapy, only 7/54 quantifiable MRD positive patients became negative at tp2, despite the imatinib regimen (P<0.01, chi-squared test). The response to the initial conventional cytotoxic chemotherapy and the response to subsequent therapy, including imatinib, appear to be linked.

BCR/ABL1 negativity at tp1 and tp2, like IG/TR negativity, carried a favorable prognosis. *BCR/ABL1* and IG/TR estimates of MRD were concordant for 69% of paired samples, although numerical values for *BCR/ABL1* were higher at tp1 and tp2, where sample numbers were sufficient to make a useful comparison.

Curiously, when MRD is assessed by flow cytometry, outcomes worsen stepwise with increasing values.[9] With PCR-based assays, results which are positive but below the quantifiable range still carry a high risk of relapse, both in PH+ ALL and in other patients with B-cell ALL (B-ALL). The Berlin-Frankfurt-Münster risk assignment algorithm is based on the persistence of MRD, more than the absolute MRD level.[10] Any positivity at tp1 or tp2, quantifiable or non-quantifiable, excludes patients from the standard-risk group. The persistence of MRD ≥ 10^{-3} at tp2 places patients in a higher risk group.

RQ-PCR for *BCR/ABL1* assesses fusion transcript. The marker is clonal, not sub-clonal, and perhaps even 'supra-clonal'. Expression may not be limited to fully leukemogenic clones or even to lymphocytes. The authors cite Hovorkova *et al.* who found discordance in about 20% of cases with *BCR/ABL1* positivity in T-lymphocytes, unlike chronic myelogenous leukemia (CML), but not in putative stem cells ($CD4^+$, $CD38^-$, $CD133^+$).[11] This was true both for patients with p190 transcripts associated with ALL and patients with p210 transcripts associated with CML. Similarly, in AML the persistence of *DNMT3A* mutations are common, representing clonal hematopoiesis and not always associated with relapse.[7]

Remission is good and relapse is bad. Therapy fails weeks or months before relapse is clinically apparent. Aggressive monitoring for submicroscopic relapse (molecular failure) has received little attention in pediatric B-ALL due to the generally low rates of relapse and prolonged years of risk.[10] In the past, two-thirds of pediatric relapses occurred in the first 3 years after diagnosis. Masurekar *et al.* have now established that on the contrary, two-thirds of relapses now occur after 3 years.[12] Early recognition of treatment failure has received more attention in adult ALL, where relapse is more common and the time to relapse is shorter.[13] However, certain subsets of pediatric ALL, such as PH+ ALL, severe hypodiploid ALL, and infant *KMT2a*-rearranged ALL still have substantial early failure rates. New therapeutic modalities, such as blinatumomab, inotuzumab, and chimeric antigen receptor (CAR)-T cells,[14] may place a new premium on prompt recognition of treatment failure. Our ability to detect MRD reliably will lead to new definitions of clinical treatment failure.

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