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Letter to the Editor:

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Post-treatment positivity of *BCR::ABL1* in acute lymphoblastic leukemia: should we keep track?

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To the editor

We read with a great interest the paper focused on different MRD approaches and its significance in Ph+ ALL patients, authored by Short et al. from the MD Anderson Cancer Center [1]. We would like to thank to the authors for this important data, and offer a short comment from our pediatric experience.

In our previous work we have defined the CML-like leukemias and described clinical features and differences between the "typical Ph+ ALL" and the "CML-like disease" in children [2-4]. We are happy to see confirmation of the CML-like phenomenon also in this adult study and we believe that particularly the clinical data on patients with long-term low *BCR::ABL1* positivity while IG/TR-negative are interesting and very important – particularly the (non-)response of such *BCR::ABL1* positivity to a TKI treatment. We have a very similar, albeit anecdotal experience with a patient retaining low-level *BCR::ABL1*-positivity (with negative IG/TR) after the end of maintenance therapy. We treated him with imatinib for one year, then switched to dasatinib for another 15 months and finally we withdraw the TKI completely, and the patient is now one year without any treatment. In accord with the data published by Short et al., after an initial modest decline, further prolonged TKI treatment (as well as its withdrawal) had no notable impact on the *BCR::ABL1* burden and the patient is still low-level positive (Figure 1A,C).

However, there is an important caveat that we would like to stress. Recently (during the last 18 months), we have experienced in our center three late relapses (4.2, 4.4 and 9.0 years from diagnosis) of *BCR::ABL1*-positive ALL patients (age at original diagnosis 3, 2 and 17 years, respectively), all of them previously assessed as having the CML-like disease but not monitored after completion of the treatment protocol (EsPhALL) (Figure 1B,C). While the patient-specific *BCR::ABL1* genomic breakpoint was maintained in all three cases at the relapse, they all relapsed with new

IG/TR rearrangements, all completely unrelated to the rearrangements from the original diagnosis (as confirmed by both PCR and NGS analyses and supplemented by a negative backtracking of the "relapse"-specific IG/TR rearrangements to diagnosis). Altogether, such data strongly suggest that a new fully leukemic clone has developed in these patients from the *BCR::ABL1*-positive cell reservoir (likely from its more "stem" or "multipotent" compartment). These recurrences imply that the residual *BCR::ABL1*-positive cells might still pose a risk of relapse – or rather a second presentation of the *BCR::ABL1*-positive leukemia – even several years after the original fully leukemic ALL clone (defined e.g. by the clonal IG/TR diagnostic rearrangements) is successfully treated and eradicated.

Our data from childhood leukemias suggest that while in the typical ALL (with concordant IG/TR and *BCR::ABL1* levels) the early MRD clearance is highly predictive of outcome, it is not the case in the CML-like patients where neither IG/TR nor *BCR::ABL1* levels showed a prognostic significance [4]. Unfortunately, the data presented by Short et al. do not enable outcome assessment separately for the typical ALL and CML-like cases (with definition based not on the MRD discordance of mere positivity vs. negativity, but rather considering a significant (> 1 log) differences between the two targets) and to disclose the association of early MRD levels/clearance and prognosis. It would be interesting to see how the early MRD dynamics is associated with outcome in adult patients and we are looking forward to further studies on this issue. Until the exact relationship between MRD and prognosis in patients with typical ALL and CML-like is revealed, and until all the differences in the clinical behavior of the two subtypes (and whether they are identical in adults and children) are defined, we believe that both targets (IG/TR and *BCR::ABL1*) should be monitored - especially in the early stages of treatment to distinguish between the two subtypes. Only then will it be possible to assess the prognostic significance of treatment response as well as its toxic complications in both groups separately, and only then will it be possible to confirm whether differences may lead to a different optimal treatment approach for each subtype (as suggested by our data from the pediatric cohorts). Considering all the recent data, we recommend in patients who do not reach the *BCR::ABL1*-negativity and a complete molecular remission (while IG/TR-negative) to continue with monitoring of the fusion in peripheral blood after the treatment, e.g. in 3 to 6 months intervals. Only then we can gradually collect more complete data on the dynamics of these clones, risk of its re-activation into a recurrence and possibly also on the efficacy of TKI treatment in cases where it is continuously administered. Moreover, the monitoring would allow a rapid response to any increase in *BCR::ABL1* levels. (IG/TR rearrangements can be also monitored; however, the BCR::ABL1 monitoring covers also the whole IG/TR-positive clone - in contrast to the opposite situation - and thus the IG/TR assessment can be in stable long-term IG/TR-negative/BCR::ABL1-positive patients reserved only for situations of BCR::ABL1 re-increase.) In case of recurrence, these patients usually still have a good chance of being rescued by stem cell transplantation (SCT) in a "second remission", at least in the pediatric setting.

In contrast to the approach used by Short *et al.* [1], we clearly prefer the DNA-based monitoring of *BCR::ABL1* dynamics, especially in the early phases of treatment [3,4]. It has usually a higher sensitivity and mainly a better quantifiability (one DNA target per positive cell, unlike in RNA approach where the number of targets - corresponding to the level of gene expression - can be in a wide range from zero to thousands, influenced by treatment, type of positive cell etc.). We believe that DNA-based monitoring does not represent a major technical obstacle in laboratories already using NGS diagnostics, and it can be expected that this approach will be more and more available. However, where the RNA approach is established and routinely used, it is certainly also an option, although its

prognostic value is lower [5] and the definition of typical ALL and the CML-like subtypes using *BCR::ABL1* transcript quantification is less clear.

IG/TR rearrangements should be assessed with similar (or higher) sensitivity as *BCR::ABL1* to distinguish persistence of the original fully leukemic cells. Whether the IG/TR levels are assessed by NGS or qPCR approach does not play a major role, in our opinion, as the sensitivity of both approaches is generally very similar and depends mostly on the amount of DNA used in reaction(s); to reach the 10^{-6} sensitivity, at least seven (to ten) micrograms of DNA must be tested, which is not always possible in a routine practice.

In conclusion, we would like to thank once again for the important data presented by colleagues from the MD Anderson Cancer Center, as with more and more centers providing detailed monitoring, the question of how to manage patients with continuing long-term *BCR::ABL1*-positivity (while IG/TR-negative) after the end of treatment protocol returns more and more often. Taking into account all the available data – although still rather scarce - we would recommend continuous monitoring of *BCR::ABL1* from peripheral blood in three to six months intervals in these patients. Since the data by Short *et al*. as well as our own experience suggest that prolonged TKI administration in these cases does not significantly affect the level of residual BCR::ABL1-positive cells, we believe that these patients (at least in pediatric hematology) can be spared the toxicity of TKI treatment as long as BCR::ABL1 levels are low and do not increase. In case of molecular or even hematological relapse (no matter whether with or without the original IG/TR markers), TKI administration should be resumed and subsequent SCT indicated where possible.

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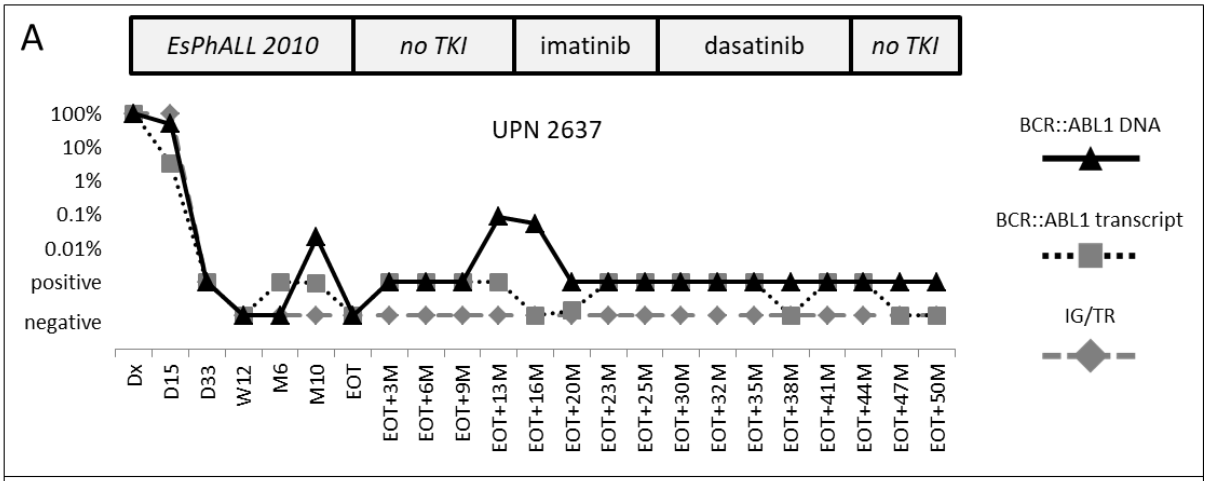
Figure 1:

MRD course of the patient with sustained low-level BCR::ABL1 positivity (A) and of the three relapsed CML-like patients (B); basic characteristics of the four patients (C).



C

UPN	Gender	Age at Dx [years]	WBC [/µl]	BCR::ABL1 type	Treatment protocol	TKI	TKI start [day]	TKI duration [years]	Relapse [years from Dx]
2637	M	7.5	18600	minor (p190)	EsPhALL2010	imatinib	15	2.1	-
1893	F	16.6	6670	minor (p190)	EsPhALL2010	imatinib	15	2.0	9.0
2882	F	2.4	356800	minor (p190)	EsPhALL2010	imatinib	15	2.1	4.4
2961	M	2.9	8800	minor (p190)	EsPhALL2017	imatinib	10	2.1	4.2

Dx – diagnosis; D – day, W – week; M – month; EOT – end of treatment; F – female; M – male; WBC – white blood cell count.