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Blood 142 (2023) 3180–3182

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

632.CHRONIC MYELOID LEUKEMIA: CLINICAL AND EPIDEMIOLOGICAL

Correlation between BCR::ABL1 Transcript LEVEL in Circulating Extracellular Vesicles and BOTH the Molecular Response and the Ongoing Therapy: A Study on Adult CML Patients

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Small extracellular vesicles (sEVs) have been shown to play a function in tumor growth and have been studied in Chronic Myeloid Leukemia (CML). Analysis of sEVs and their contents offers a non-invasive strategy that might identify messages transmitted by persisting leukemic cells. The sEVs recovered from CML patients may contain the BCR::ABL1 transcript, but its relationship to clinical and biological factors is still unclear. This study aims to connect the disease status and several clinical features with the BCR::ABL1 transcript levels in sEVs (BCR::ABL1-EV). sEVs were isolated by a commercial kit from 104 plasma samples of adult CML patients in molecular response: 18/104 (17%) in MMR and 86/104 (83%) in DMR assessed by RT-qPCR following the international scale. At sampling, 21/104 (20%) patients were treated with imatinib, 14/104 (13.5%) with nilotinib, 6/104 (5.5%) with dasatinib, 1/104 with bosutinib (1%), 27/104 (26%) with intermittent TKI therapy, and 24/104 (23%) were in treatment free remission (TFR). Taking advantage of digital PCR's (dPCR) sensitivity, it was used to detect the presence of BCR::ABL1 transcript in sEVs.

The undetectable samples by RT-qPCR on cells were 38/104 (36.5%), while by dPCR on sEVs were 13/104 (12.5%). This difference resulted statistically significant ($p<0.0001$) (Fig 1A). In terms of sensitivity, dPCR was able to detect more BCR::ABL1 transcript in the sEVs than in the cells ($p= 0.0052$), suggesting the release of sEVS shuttling BCR::ABL1 transcript by CML cells resident in the bone marrow (Fig 1B).

Considering the quantity of BCR::ABL1 transcript molecules, the BCR::ABL1-EV showed a decrease at the deepening of the MR classes and there was a significant difference between MR3.0 and MR4.5 and MR5.0 ($p= 0.0226$ and $p=0.0101$, respectively) (Fig 1C). By gathering overall samples according to MMR and DMR definition, the BCR::ABL1-EV resulted statistically different ($p= 0.0017$), presenting an increased significance when compared with the analysis on the different MR classes (Fig 1D).

A significant difference between BCR::ABL1-EV and the ongoing therapy was observed. For this analysis, nilotinib, dasatinib, and bosutinib were considered together as "2 nd generation TKI". The BCR::ABL1-EV were significantly higher in samples from patients in intermittent therapy compared to those from patients treated with imatinib ($p=0.0398$). No statistical difference resulted by other comparisons, even if a trend can be appreciated (Fig 1E). This evidence may be due to the therapy duration, since the mean therapy duration is 111 months and 45 months for imatinib and 2 nd generation TKI, respectively. Despite that, no linear regression between therapy duration and BCR::ABL1-EV. Another fascinating hypothesis is that the difference

could be related with mechanisms activated by the different drugs. Further analysis will clarify this point. Interestingly, when comparing the results obtained by dPCR on cells and sEVs, a significant difference was observed in samples of patients under intermittent treatment. A trend can be appreciated in the other conditions, with except for imatinib (Fig 1F).
In conclusion, dPCR associated with sEVs analysis confirmed its higher sensitivity to detect even low levels of target and it is able to identify the presence of not-circulating leukemic cells releasing BCR::ABL1 positive sEVs. Moreover, the analysis of the BCR::ABL1 transcript on sEVs revealed a transcript reduction at the deepening of the MR classes, discriminated between MMR and DMR, and correlates with the ongoing therapeutic strategy. Further analysis will clarify the mechanisms at the bases of this correlation. This is the first report about the application of this approach on a cohort of adult CML patients, but strongly suggests how a liquid biopsy approach is able to identify active leukemic cells not otherwise detectable. It opens to the identification of different sub-sets of patients presenting different vesicular BCR::ABL1 transcript levels. The different sub-sets could be related to the therapy response as well as the eligibility to TKI suspension, two of the main hot points in the present CML patients' management.

Disclosures Polverelli: *BMS:* Honoraria; *GSK:* Honoraria; *Abbvie:* Honoraria; *Novartis:* Honoraria. **Breccia:** *Incyte:* Honoraria; *Pfizer:* Honoraria; *BMS:* Honoraria; *AOP:* Honoraria; *AbbVie:* Honoraria; *Novartis:* Honoraria. **Abruzzese:** *Takeda:* Consultancy; *Pfizer:* Consultancy; *Novartis:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Incyte:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *BMS:* Consultancy, Membership on an entity's Board of Directors or advisory committees. **Malagola:** *Biotest, MSD:* Consultancy, Honoraria. **Russo:** *Medac, Abbvie, MSD, Jazz Pharma, Gilead, Novartis:* Membership on an entity's Board of Directors or advisory committees; *MSD, Novartis, Gilead, BMS, Medac:* Honoraria.

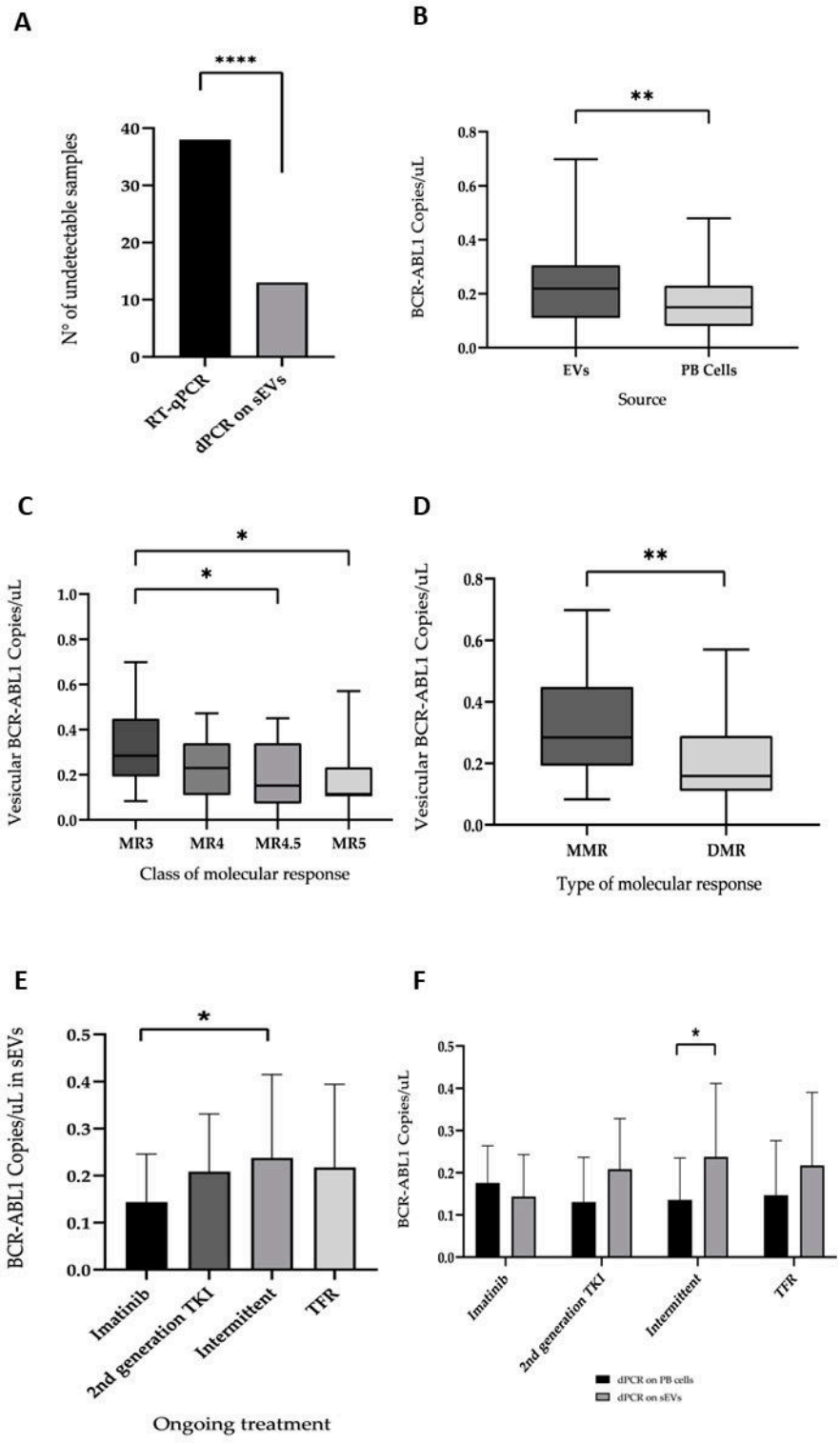

Figure 1

https://doi.org/10.1182/blood-2023-185733