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CORRESPONDENCE

Use of Peripheral Blood to Measure Residual Disease by Flow Cytometry in Adult Acute Myeloid Leukemia: Final Report of the Prospective GIMEMA AML1310 Trial

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To the Editor,

Measurable residual disease (MRD) assessment by multiparametric flow cytometry (MFC) is now a standard tool to refine risk stratification in acute myeloid leukemia (AML), guiding post-remission therapy and the choice between autologous (AuSCT) and allogeneic transplantation (ASCT). MRD-positive patients carry a more than twofold relapse risk compared to MRD-negative ones [1–5]. Although MRD testing is conventionally performed on bone marrow (BM), peripheral blood (PB) is increasingly being investigated as a less invasive, more accessible alternative. PB-based MRD assessment closely correlates with BM evaluation, thereby sparing patients the discomfort of repeated BM aspirations [3]. Prior reports supporting this concept were retrospective and based on limited, heterogeneously treated cohorts [6–8]. We now report a post hoc analysis of paired BM and PB MRD data from a large, uniformly treated cohort of patients enrolled in the prospective GIMEMA AML1310 trial [9]. The analysis aimed to assess the concordance between the two compartments, the analytical performance of PB testing, and the prognostic value of PB MRD after induction and consolidation cycles.

The risk-adapted, phase II AML1310 trial (EudraCT 2010-023809-36; NCT01452646), enrolled patients aged 18–60 with newly diagnosed non-APL AML. Treatment allocation to AuSCT or ASCT was guided by pre-treatment genetic risk and, for intermediate-risk patients, post-consolidation BM MRD [9]. Diagnosis followed WHO criteria, and genetic risk was classified per NCCN 2009 criteria. At diagnosis, leukemia-associated immunophenotypes (LAIPs) were characterized by 8-color MFC and used to monitor MRD in paired BM and PB samples simultaneously collected after induction (Ind) and consolidation (Cons). PB samples were collected in 5 mL of EDTA anticoagulant and processed within 24 h following a protocol identical to that used for BM aspirates [4–6, 10]. Patients had to provide informed consent and comply with inclusion or exclusion criteria, which were published elsewhere [9]. The study was approved by the ethics committees of the participating hospitals/academic institutions and was conducted in accordance with the Declaration of Helsinki. Residual leukemic cells (RLC) were quantified as a percentage of CD45-expressing events; limit of detection (LOD) and limit of quantification (LOQ) were established at 20 and 50 clustering events expressing a LAIP, respectively, as detailed

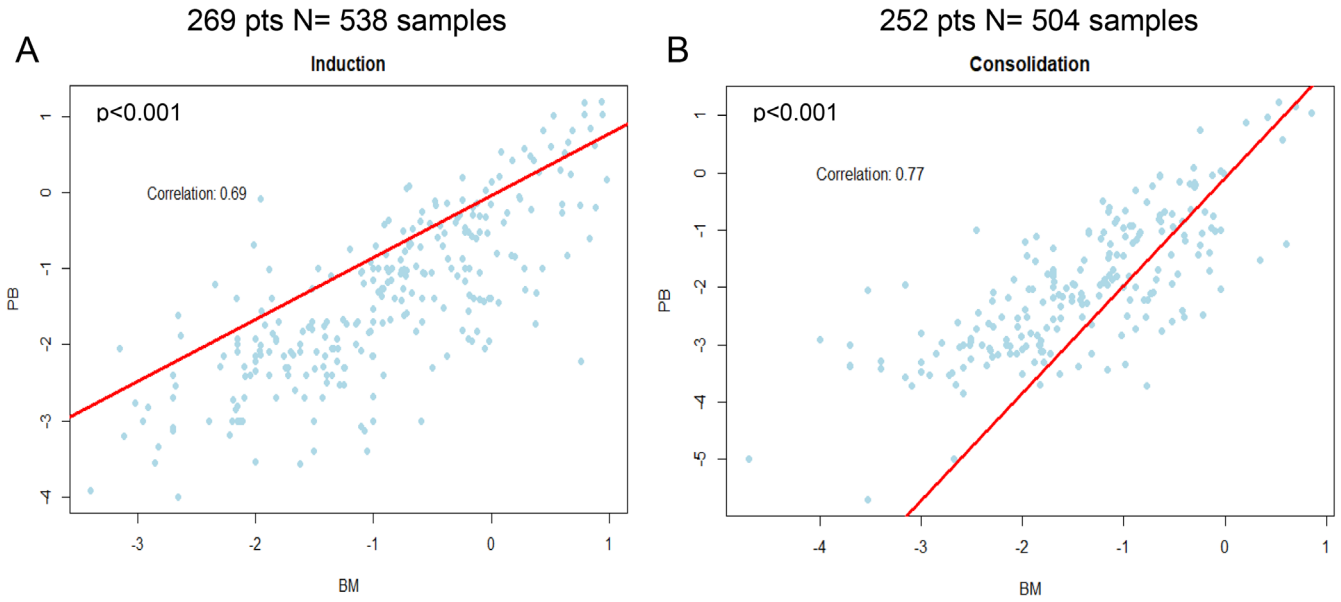

FIGURE 1 | Comparison of measurable residual disease (MRD) levels in peripheral blood (PB) and bone marrow (BM) samples showing a significant correlation between the cell sources. (A) Regression curve of the 269 paired PB and BM samples collected simultaneously after induction therapy. (B) Regression curve of the 252 paired PB and BM samples collected simultaneously after consolidation therapy. BM, bone marrow; PB, peripheral blood.

elsewhere [3, 10–12]. Patients were classified as MRD-negative if RLC were < LOD (LOD^{neg}), MRD-positive non-quantifiable if RLC were > LOD and < LOQ (LOD^{pos}-LOQ^{neg}) and MRD-positive quantifiable if RLC were > LOQ (LOQ^{pos}). Concordance between PB and BM RLC was assessed by Spearman correlation; positive and negative predictive values (PPV, NPV) of PB LOQ^{pos}/LOD^{neg} status for the corresponding BM status were calculated. Overall survival (OS) and disease-free survival (DFS) were estimated by Kaplan–Meier and compared by log-rank test; prognostic PB RLC thresholds were derived using maximally selected log-rank statistics applied independently at the post-Ind and post-Cons time points.

Paired post-Ind and post-Cons BM/PB samples were available for 269 and 252 patients in complete remission, respectively (median age 49 years, range 40–55). Demographic and clinic-biological characteristics are summarized in Table S1. Ninety-three (35%), 81 (30%), and 26 (9%) patients received ASCT, AuSCT or high dose cytarabine (HDAC) respectively, as post consolidation therapy, while 69 (26%) patients did not receive any post consolidation therapy due to early relapse, treatment-related toxicity, or patient preference. Median RLC number was consistently lower in PB than BM, both after induction (0.015% vs. 0.079%) and after consolidation (0.006% vs. 0.021%; $p < 0.001$ for both comparisons, Figure S1). Nevertheless, a significant correlation between PB and BM RLC values was observed at both time points (Spearman $r = 0.69$ post-Ind, $r = 0.77$ post-Cons; $p < 0.001$ for both, Figure 1), aligned with previous reports [7, 8, 13]. Notably, these correlations remain comparable across studies despite technical differences [13], implying that PB kinetics may closely mirror BM disease dynamics. In 55% of paired samples BM MRD exceeded PB MRD, in 40% the two were equivalent, and in only 5% PB MRD exceeded BM MRD, a distribution comparable to that previously described [6, 7]. Detailed

TABLE 1 | Relationship between PB and BM measurements by flow cytometry.

	PB *n*		
	LOD^{neg}	**LOD^{pos}-LOQ^{neg}**	**LOQ^{pos}**
(A) Post-induction (N = 269)			
BM *n*			
LOD^{neg}	50	2	4
LOD^{pos}-LOQ^{neg}	16	4	0
LOQ^{pos}	43	33	117
	NPV 46%		PPV 97%
	PB *n*		
	LOD^{neg}	**LOD^{pos}-LOQ^{neg}**	**LOQ^{pos}**
(B) Post-consolidation (N = 252)			
BM *n*			
LOD^{neg}	72	1	0
LOD^{pos}-LOQ^{neg}	22	4	3
LOQ^{pos}	32	31	87
	NPV 57%		PPV 97%

Note: Patients were defined LOD^{neg} when residual leukemic cells (RLC) < 20, LOD^{pos}-LOQ^{neg} in case of RLC were between 20 and < 50 events, and LOQ^{pos}: when RLC ≥ 50. Positive predictive value (PPV) is the proportion of cases that are MRD pos in PB and are also MRD pos in BM. Negative predictive value (NPV) is the proportion of cases that are MRD neg in PB that are also MRD neg in BM.
Abbreviations: BM, bone marrow; LOD, limit of detection; LOQ, Limit of quantification; PB, peripheral blood.

cell counts, LOD, and LOQ values are summarized in Table S2. Applying LOD/LOQ criteria, of 269 paired post-Ind samples 72% and 45% were LOQ^{pos} 7% and 14% LOD^{pos}-LOQ^{neg}, while 21% and 41% were LOD^{neg} in BM and PB, respectively. Of 252 paired post-Cons samples, 60% and 36% were LOQ^{pos}, 11% and 14% LOD^{pos}-LOQ^{neg} and 29% and 50% were LOD^{neg} in BM and PB, respectively (Table 1). PB LOQ^{pos} status accurately predicted BM LOQ^{pos} status with a PPV of 97% at both post-Ind and post-Cons time points (Table 1A,B). Accordingly, PB LOQ^{pos} patients after induction showed shorter OS and DFS compared to PB LOQ^{neg} patients, although the difference did not reach statistical significance (Figure S2). Notably, 65% of patients who relapsed between the first and second chemotherapy cycles were already PB LOQ^{pos} after induction, and 52% of post-Ind PB LOQ^{pos} patients remained so after consolidation.

Since LOD/LOQ stratification failed to define statistically prognostic groups in PB, we applied maximally selected log-rank statistics to identify significant prognostic thresholds; using DFS and OS as dependent variables and PB RLC values as the independent variable, this approach yielded optimal cutoffs of 0.014% post-Ind and 0.0008% post-Cons. Patients below these thresholds (PB MRD^{neg}) did not reach the median OS and DFS at last follow-up, whereas PB MRD^{pos} patients had median OS of 41 (post-Ind) and 63 (post-Cons) months, and median DFS of 22 and 24 months, respectively ($p = 0.0033$ and 0.0022 for OS; $p = 0.028$ and 0.016 for DFS, Figure 2A,B). The post-Cons threshold of 0.0008% is markedly below the LOD and could not always be supported by the required number of acquired events. However, patients below this cutoff had a median of 0 positive events (range 0–4) and very low corresponding BM MRD (median 0.0011%, with only 8/77 BM LOQ^{pos}), supporting its biological plausibility despite the analytic limitations. Equivalent survival plots stratified by trial-defined BM MRD threshold (0.035%) are provided in Figure S3. On multivariate analysis, independent predictors of shorter DFS included post-Ind MRD-positive status ($p = 0.038$), adverse cytogenetic risk ($p < 0.001$), and age ($p = 0.003$; Table S3A). Shorter OS was independently associated with post-induction MRD-positive status ($p = 0.009$), intermediate and adverse risk ($p = 0.004$ and $p < 0.001$), age ($p < 0.001$), and baseline WBC count ($p = 0.008$; Table S3B).

These findings, confirm that PB MRD by MFC is measurable, correlates with BM MRD, and carries prognostic value, extending earlier retrospective observations [6–8, 14]. This

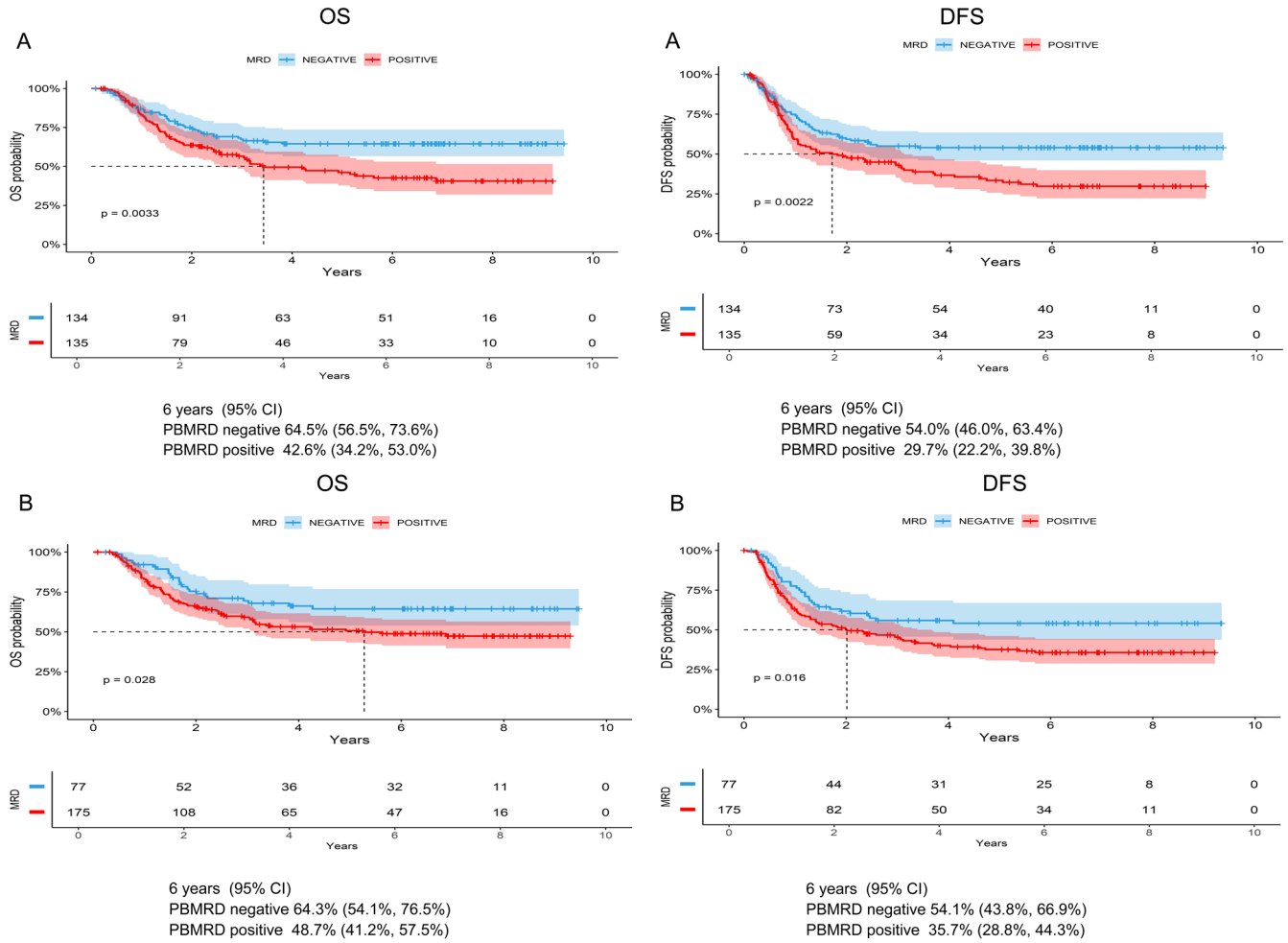

FIGURE 2 | Overall survival (OS) and disease-free survival (DFS) plotted according to the experimentally determined cut-off values by applying maximally selected log-rank statistics to residual leukemic cells at (A) post-induction and (B) post-consolidation timepoints. The experimental cut-off points identified were 0.014% and 0.0008% at post-induction and post-consolidation timepoints, respectively. Accordingly, patients below or above these thresholds were named PB $MRDInd^{neg}$ and PB $MRDCons^{neg}$ or PB $MRDInd^{pos}$ and PB $MRDCons^{pos}$, respectively.

parallel is reinforced by recent paired samples analyses, which reported strong Spearman correlation coefficients [8, 13]. Although sample quality and event acquisition remain critical determinants of analytical sensitivity, these observations suggest that PB kinetics reflect medullary dynamics and lower RLC burden in PB likely reflects a genuine, partial compartmentalization of residual disease within the marrow niche. Several non-mutually exclusive mechanisms may explain the cases with concordant or PB-predominant MRD, including BM hemodilution during aspiration and differential propensity of specific LAIPs, such as CD56-expressing or adhesion-molecule-associated clones, to circulate [15, 16]. A comparable pattern of PB-BM discordance has been described in acute lymphoblastic leukemia [17], suggesting shared biology governing leukemic cell trafficking across lineages, and some AML subtypes may simply have a greater intrinsic propensity to disseminate into the circulation.

Our data also indicate that PB MRD positivity after the first induction cycle, a time point not currently used for treatment decisions, may allow an earlier identification of chemoresistant disease, in line with previous reports linking early PB blast clearance to subsequent BM MRD status and survival [18, 19]. From a practical standpoint, a PB LOQ^{pos} result, obtained via simple venipuncture, can flag patients at high risk of BM MRD positivity (PPV 97%) without requiring an additional BM aspirate. Conversely, a PB LOD^{neg} result does not reliably exclude BM MRD positivity and warrants confirmatory BM sampling. This asymmetry suggests a rational use of PB testing as a rapid, minimally invasive first-line screening to triage patients who require BM confirmation and closer surveillance, rather than as a stand-alone substitute for BM MRD assessment at present. A similar paradigm occurs in molecular MRD monitoring for NPM1-mutated patients, where PB PCR testing serves as an effective screening tool but remains complementary to BM assessments [3].

Although the optimal prognostic PB threshold appears to lie below the conventional LOD—particularly post-consolidation—our analysis is limited by its post hoc design and a sub-optimal number of acquired events at this time point; consequently, formal validation of this very low cutoff warrants prospective confirmation with the higher-cellularity acquisitions (at least 1 000 000 events per sample) currently planned in the ongoing GIMEMA AML1819 trial.

In conclusion, our findings support PB as a clinically informative, minimally invasive complement to BM for MRD monitoring in AML, particularly for early identification of high-risk, PB LOQ^{pos} patients after induction. Post-induction PB MRD positivity provides an immediate surrogate indicator of chemoresistance and poor prognosis, potentially flagging high-risk patients. Conversely, due to its lower sensitivity, a negative PB result cannot definitively rule out residual disease, and BM assessment remains necessary to confirm PB MRD-negative status. Further studies are warranted to define the role of serial PB MRD monitoring in guiding treatment decisions and anticipating relapse. Multicenter validation with harmonized, high-event protocols is required before integrating these data-driven PB thresholds into routine decision-making algorithms.

Author Contributions

L.M., F.B., A.V., and A.P.: conceptualization. L.M., F.B., R.P., M.C., A.P., and A.V.: methodology. M.C. and A.P.: software. L.M., F.B., R.P., M.C., A.P., M.I.D.P., M.T., M.P.M., P.S., A.Cu., F.M., E.M., M.T.V., and A.Ca.: data curation. L.M., F.B., R.P., M.I.D.P., G.P., M.T., M.P.M., P.S., A.Cu., M.I.C., T.O., M.D.D., F.M., E.M., M.T.V., and A.Ca.: investigation. L.M., F.B., R.P., M.I.D.P., M.I.C., T.O., M.D.D., F.M., E.M., M.T.V.: validation. M.C. and A.P.: formal analysis. P.F. and A.V.: supervision. L.M., F.B., R.P., and A.V.: visualization. P.F. and A.V.: project administration. L.M., F.B., R.P., M.I.D.P., G.P., M.T., M.P.M., P.S., A.Cu., M.I.C., T.O., M.D.D., M.T.V., A.Ca., P.F., and A.V.: resources. L.M., F.B., and A.V.: writing – original draft; all authors writing – review and editing.

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The authors have nothing to report.

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki after approval by the ethics committees of the participating hospitals/academic institutions as part of the clinical research protocol EudraCT 2010-023809-36; NCT01452646.

Consent

Written informed consent for participation in the clinical protocol and for the publication of de-identified clinical data was obtained from all individual patients included in this study prior to their enrolment.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The clinical and biological data supporting the findings of this study were collected within the framework of the GIMEMA AML1310 clinical trial (NCT01452646). Due to institutional data sharing policies and patient privacy regulations, the data are not publicly available. De-identified individual-level data may be made available to eligible researchers upon reasonable request, subject to formal review and approval by the GIMEMA Foundation Data Ownership Committee. Inquiries regarding data access should be directed to the corresponding author or the GIMEMA Data Center.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Comparison between median residual leukemic measured in bone marrow (BM) and peripheral blood (PB) after induction (A) and consolidation (B) timepoints: median measurable residual disease (MRD) in PB was expressed at lower logarithms than in BM (Wilcoxon test for difference between medians, $p < 0.001$ for both comparisons). **Figure S2:** Overall survival (OS) and disease-free survival (DFS) after induction (A) and consolidation (B) by analyzing separately PB LOD^{neg}, LOD^{pos}-LOQ^{neg} and LOQ^{pos} status. OS, overall survival; DFS, disease free survival; PB, peripheral blood; LOD, limit of detection; LOQ, limit of quantification. **Figure S3:** Overall survival (OS) and disease free survival (DFS) plotted according to the AML1310 BM threshold (0.035%) at (A) post-induction and (B) post-consolidation timepoints. Accordingly, patients below or above these thresholds were named BM $MRDInd^{neg}$ and BM $MRDCons^{neg}$ or BM $MRDInd^{pos}$ and BM $MRDCons^{pos}$, respectively. No significative difference was observed in duration of OS and DFS between MRD $< 0.035\%$ and MRD $\geq 0.035\%$ patients. **Table S1:** General characteristics of the study population. **Table S2:** Total number of events acquired according to limit of detection (LOD) and limit of quantification (LOQ) coding and details on CD45 expressing cells denominators. **Table S3:** Multivariate Cox regression model for disease free survival (A) and overall survival (B).