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TO THE EDITOR:

Prospective validation of the prognostic relevance of CD34+CD38− AML stem cell frequency in the HOVON-SAKK132 trial

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Measurable residual disease (MRD) assessment is incorporated in the clinical decision-making of acute myeloid leukemia (AML) because of its prognostic value.[1] Therefore, accurate MRD assessment is essential to ensure the appropriate consolidation therapy. Established MRD assessment includes the detection of persistent mutant *NPM1* using real-time quantitative polymerase chain reaction and detection of leukemic myeloid progenitor cells using multiparameter flow cytometry. Refinement to current MRD assessments may include the detection of relapse-initiating leukemic stem cells (LSCs), defined as CD34+CD38−LSC+ marker cells.[2] The Dutch-Belgian Cooperative Trial Group for Hematology-Oncology (HOVON) or the Swiss Group for Clinical Cancer Research (SAKK) 102 (HO102) clinical trial and several other trials have retrospectively shown the prognostic value of CD34+CD38−LSC+ burden at diagnosis[3-8] and follow-up,[3,9,10] in particular after 2 cycles of induction chemotherapy[11] (C2). To prospectively validate the prognostic value of LSC burden, we measured the LSC burden during the HOVON-SAKK132 (HO132) randomized phase 3 trial. In this trial, the addition of lenalidomide to induction therapy was prospectively investigated in 927 patients aged between 18 and 65 years with newly diagnosed AML or high-risk MDS.[12] MRD results after C2 (not LSCs) were reported to inform the consolidation treatment for the patients allocated to the 2017

European LeukemiaNet intermediate–risk group. The HO102 LSC assay was optimized and used in the HO132 trial as a standardized one-tube LSC assay[13] to acquire more white blood cell (WBC) events (4×10^6).[6] The LSC tube contains the backbone markers for immature myeloid progenitors (CD45, CD34, and CD38) and leukemia-specific LSC markers in the CD34+CD38− compartment (CD45RA, CD123, CD33, and CD44 and a combination of inhibitory C-lectin like receptor, T cell immunoglobulin and mucin domain 3, CD56, CD7, CD22, and CD11b in 1 fluorochrome). For details of MRD and LSC detection, we refer to our previous publications[14-16] and supplemental Materials, which are available on the *Blood* website.

At the diagnosis, 764 of 883 (87%) eligible patients had a suitable sample, with sufficient cells for LSC detection (supplemental Figure 1). After conducting C2 in patients who achieved complete remission (CR, including CR with incomplete hematologic recovery), LSC burden could be assessed in 357 of 496 (72%) samples available for MRD measurement. The inability to assess LSC burden when the sample was available for MRD measurement was mostly because of the insufficient number of cells left after measuring MRD via multiparameter flow cytometry. No statistically significant difference in overall survival (OS) was

observed between patients with or without LSC results at diagnosis and after C2 (data not shown).

Analogous to HO102 at diagnosis, 3 prognostic risk groups could be defined based on CD34 status[3,17] and LSC burden. Patients were said to be with CD34−AML (CD34^{neg}) if they had <1% CD34$^+$ blasts of WBC, no leukemia-associated–immuno-phenotype with CD34 phenotype, and no LSCs. A cut-off of 0.03% CD34$^+$CD38$^-$LSC$^+$ of WBC was defined in previous studies[3,18] and used to distinguish LSC^{low} and LSC^{high}. The LSC status at diagnosis was associated with several patient characteristics (supplemental Table 1). OS and cumulative incidence of relapse (CIR) were significantly different between the 3 groups, with the patients with CD34^{neg} performing the best (Figure 1A,C). Prospectively assessed LSC remained an independent prognostic factor for OS and CIR after correction for WBC count at diagnosis, AML type, and ELN2017 risk group (Figure 1B). The last treatment before reaching CR/CR with incomplete hematologic recovery was also included for CIR (Figure 1D).

LSC status after C2 was categorized into LSC^{neg} and LSC^{pos} based on the formerly defined 0.00000% cut-off.[3] Patient characteristics based on their LSC status after C2 are summarized in supplemental Table 2. The HO132 data confirms the prognostic value of LSC in univariate and multivariate analysis of OS, (Figure 2A,B) but it did not reach statistical significance for CIR (Figure 2C,D). The combination of MRD and LSC showed that the MRD^{pos}LSC^{pos} group had a worse prognosis compared with the MRD^{neg}LSC^{neg} group in univariate (supplemental Figure 2A,C) and multivariate analyses for OS and CIR (supplemental Figure 2B,D). After the sensitivity analysis, the multivariate prognostic values (Figures 1C,D and 2C,D) were corrected for the first consolidation treatment (supplemental Tables 4-6).

In accordance with the ELN2022 recommendations for AML,[1] we determined the prognostic value of LSC after conducting C2 in the different ELN2017 risk groups. For patients in the intermediate-risk group in HO132, treatment decision for an autologous (MRD^{neg}) or allogeneic (MRD^{pos}) stem cell
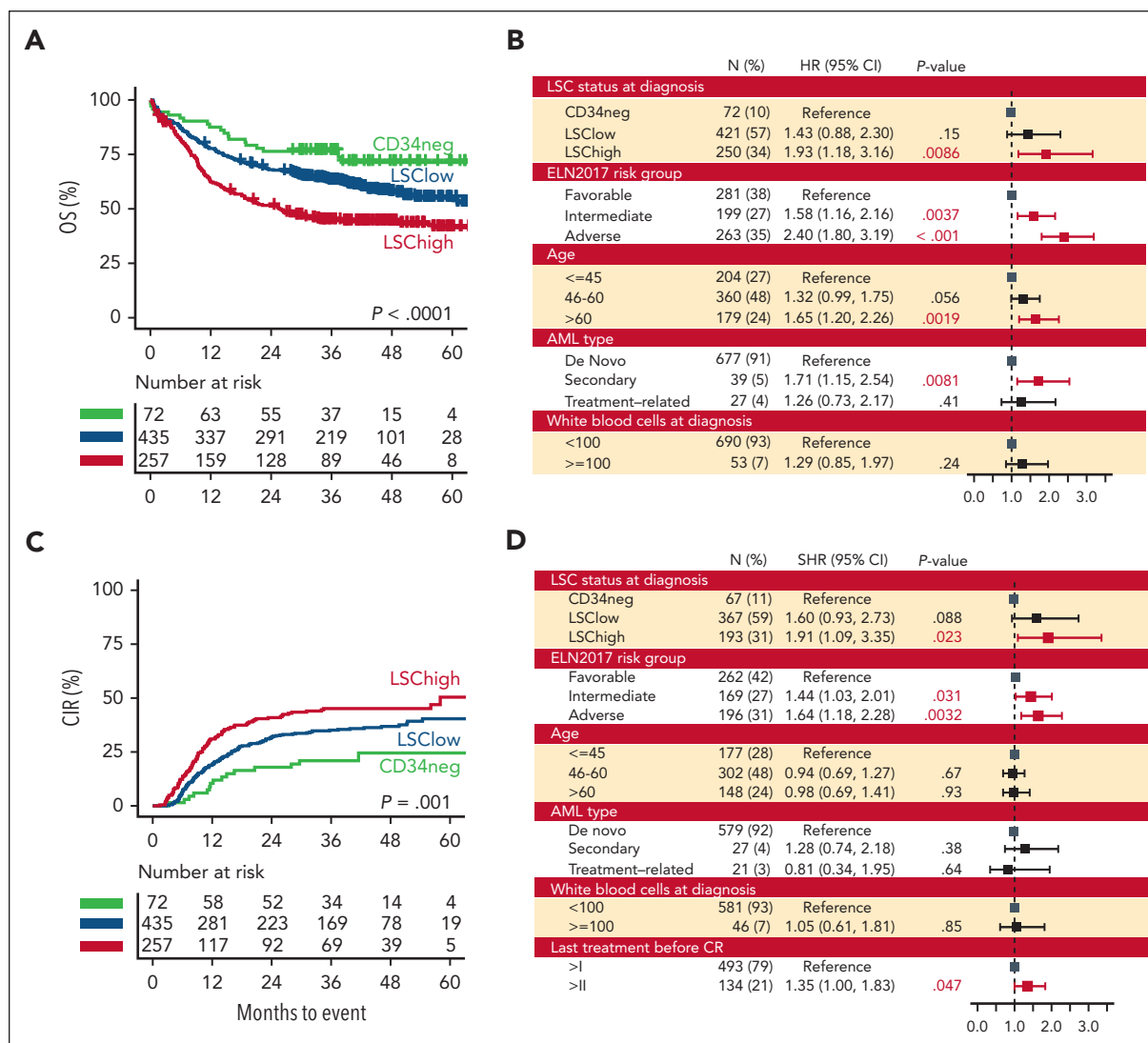
Figure 1. Prognostic value of LSC burden at diagnosis. (A) Kaplan-Meier curve for OS. (C) CIR. The overall group (A,C) is divided into CD34^{neg}, LSC^{low}, and LSC^{high}, with the cut-off of 0.03% CD34$^+$CD38$^-$LSC$^+$ population of WBCs. (B,D) Multivariate analysis adjusted for age, AML type, ELN2017 risk group, WBCs at diagnosis, and last treatment before reaching CR (only for CIR) of (B) OS (Cox regression) and (D) CIR (Fine and Gray regression).
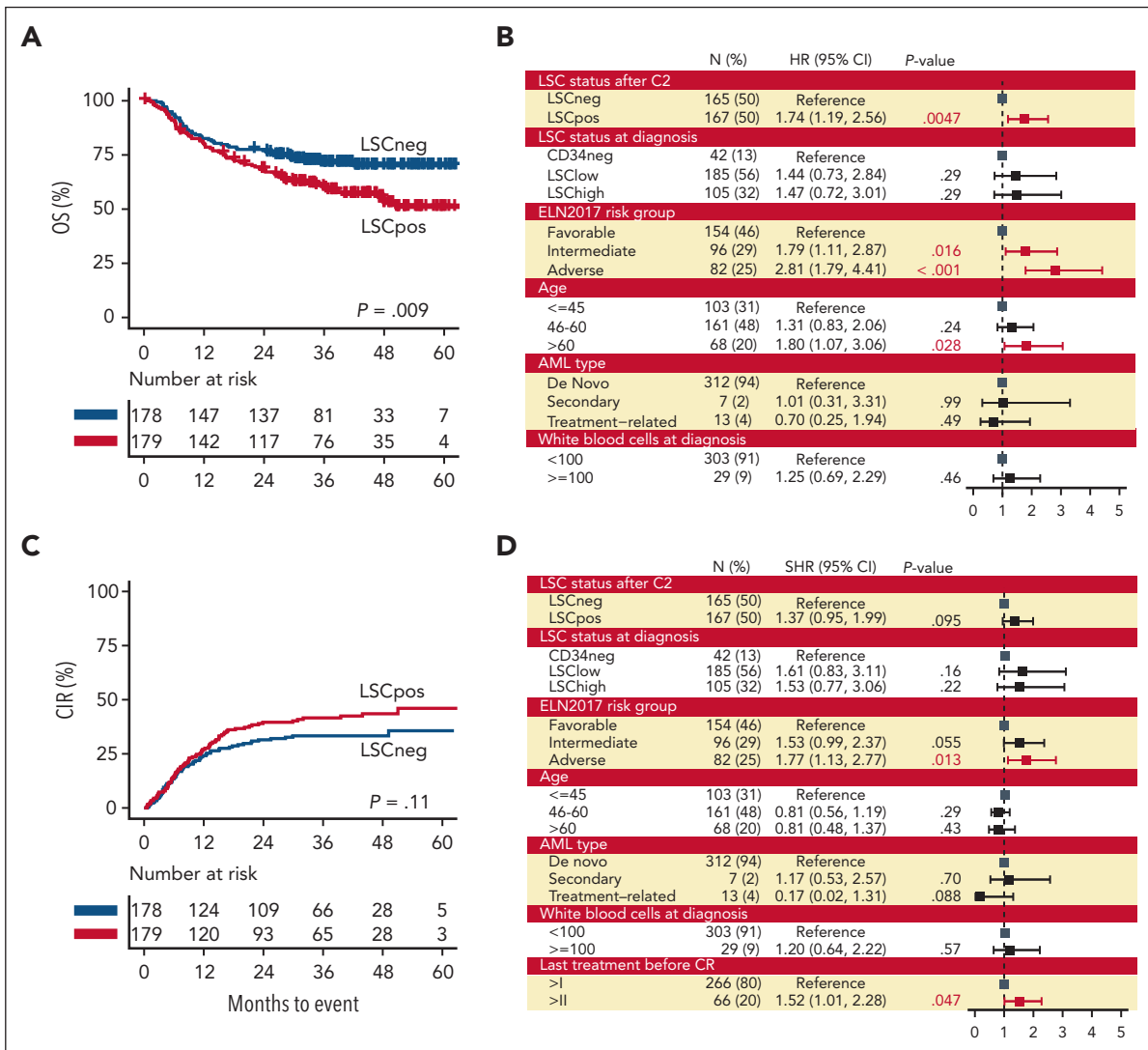
Figure 2. Prognostic value of LSC burden after 2 cycles of chemotherapy. Kaplan-Meier curves of (A) OS, (C) CIR after C2. The overall group in A and C is divided into patients with LSC^{pos} and LSC^{neg} with the cut-off of 0.00000% CD34$^+$CD38$^-$LSC$^+$ population. B and D show multivariate analysis adjusted for LSC status at diagnosis, age, AML type, ELN2017 risk group, WBCs at diagnosis, and last treatment before reaching CR (only CIR) of (B) OS (Cox regression) and (D) CIR (Fine and Gray subdistribution hazard regression).

transplantation (SCT) was guided by classical MRD assessment; this may explain why MRD itself was no longer a significant prognostic risk factor in this risk group.[12] Remarkably, in this MRD-guided–intermediate- risk group, LSC could still be used as a marker to distinguish a group of patients with relatively poor prognosis (supplemental Figure 3E,G). The adverse-risk group comprised a relatively high percentage of patients with LSC^{pos} (supplemental Table 2) who did significantly worse than the patients with LSC^{neg} (supplemental Figure 3I,K). For the favorable-risk group, LSC did not have a prognostic value (supplemental Figure 3A,C), suggesting improved risk classification. The favorable-risk group as per the ELN2017 risk stratification used in the HO132 trial included less specific adverse mutations such as *RUNX1* and *ASXL1*[19,20] compared with the ELN2010[21] risk stratification used for the HO102 protocol.

Overall, our data validate the prognostic value of LSC burden in AML at diagnosis and after C2. Interestingly, the recent

publication of Li et al[9] showed the prognostic value of LSC burden in AML after transplantation using a similar LSC assay. However, for the further clinical implementation of LSCs and to reach the level of standardization and harmonization that is currently established for the MRD assay, additional analyses need to be performed. For example, the use of 0.00000% cut-off for LSCs after C2 may be challenging because each positive LSC event should be specific. By acquiring a large number of WBCs in the HO132 trial (supplemental Figure 4), some (aspecific) LSC events may render the current cut-off of 0.00000% as too stringent and could potentially lead to false positives. Other optimal cut-off levels[22] and/or optimization steps may be required to assess statistically significant distinct groups. Standardization and harmonization of the LSC assay, such as applicability and reproducibility was already shown at the diagnosis.[14] However, to implement the LSC assay in clinical decision-making, further assay qualification experiments are

warranted. In addition, the applicability of the LSC assay may be improved by including a different denominator such as CD34$^+$ blasts or additional flow cytometric markers that can identify LSC populations in patients who are CD34$^-$.[23]

Our findings on the prognostic value of LSC can have implications on clinical decision-making, for example, in cases in which LSC levels have additive value to standard MRD assessments (eg, in patients who are MRD^{neg}; supplemental Figure 3B,D,F,H,J,L). In particular, for patients who are in the intermediate-risk MRD^{neg} group, LSCs can play a role in refining risk group assessment, indicating a need for extensive monitoring of patients with LSC^{pos} after autologous-SCT or even considering allogeneic-SCT for post-induction therapy. In the adverse-risk group, patients are currently allocated to allogeneic-SCT. Adaptive MRD guidance strategies have been suggested, for example, choosing different conditioning regimens; selection of donors; application of donor lymphocyte infusion or more frequent monitoring after transplantation;[24] in which LSC measurement could play an additional role.[25] To establish the broad implementation of LSC at different time points, extra analyses about the kinetics, subgroup analyses and the influence of therapies such as conditioning regimens and postallogeneic stem cell treatment is needed.

In conclusion, the data collected in the HO132 trial prospectively validated the prognostic value of LSCs at diagnosis and after C2 for intermediate and adverse-risk groups. Results of this study show that incorporating LSC load in clinical trials has additional value in identifying subgroups with worse prognoses.

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Authorship

Contribution: Sample collection was done by D.A.B., T.F., B.T.G., L.G., G.J., A.A.v.d.L., J.A.M., M.G.M., T.P., J.R.P., K.P., B.L., G.J.O., and J.J.W.M.J. in the HOVON-SAKK132 trial; experiments and/or analysis of flow cytometry LSC data was performed by L.L.N., D.H., F.J., J.C.-H., L.O.-M., M.F., M.H., A.K., W.S., A.S., and P.V.; Statistical analysis was performed by L.L.N. and checked by P.G.; the manuscript was written by L.L.N. and revised by J.C., G.J.O., D.H., J.M.T., C.B., J.M.T., B.L., A.A.v.d.L., J.J.W.M.J., D.C.d.L., D.A.B., T.F., B.T.G., L.G., G.J., J.A.M., M.G.M., T.P., J.R.P., K.P., and P.J.M.V.; and the results were reviewed and the manuscript was approved by all the authors.

Conflict-of-interest disclosure: B.T.G. serves as a consultant for Ber-GenBio, Pfizer Inc, and Novartis and holds stock options in Alden Cancer Therapy and KinN Therapeutics. L.G. holds a membership on an entity's board of directors or advisory committies for Miltenyi Biomedicine. K.P. has received honoraria for Pfizer, Novartis, Incyte, Bristol Myers Squibb, Astellas, and AbbVie and has received Celgene/Bristol Myers Squibb, Incyte, Pfizer, and Novartis. A.A.v.d.L. has received honoraria from Amgen, Novartis, Celgene/BMS, and Takeda and has received research funding from Alexion. M.GM. serves as a consultant for CDR-Life Inc; holds stock options in CDR-Life Inc; and has a patent licensed to the University of Zurich. B.L. serves as a consultant and has received honoraria from Clear Creek Bio; holds membership on an entity's board of directors or advisory committies for Celgene, Bristol Myers Squibb, Catamaran Bio Inc, Astellas, AbbVie, F.Hoffmann La Roche. and D.C.d.L; participates in the sponsored speaker's bureau of Servier, Roche and AbbVie; is part of the scientific advisory board of Takeda and Servier. J.J.W.M.J. serves as a consultant for Bristol Myers Squibb, Novartis, Pfizer Inc; has received research funding from Bristol-Myers Squibb, Novartis, Incyte Biosciences Benelux BV, Uppsala County Council, Glycomimetics, Avillion, and Ellipses Pharma; is a speaker bureau member for Incyte Biosciences Benelux BV, Roche, and Celgene; and holds membership on an entity's board of directors for Celgene. G.J.O. serves as a consultant for Novartis, Pfizer Inc, Celgene, Janssen, AGIOS, Amgen, Gilead, Astellas, Roche, Jazz Pharmaceuticals, and Merus; has received honoraria from Novartis, Celgene, AGIOS, Gilead, and Astellas; received research funding from Novartis; and holds membership on an entity's board of directors for Roche. J.C. serves as a consultant for Novartis; receives royalties from Navigate and BD Biosciences; and has received research funding from Takeda, DC-one, Genentech, Janssen, Novartis, and Merus. The remaining authors declare no competing financial interests.

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Footnotes

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