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CLINICAL TRIALS AND OBSERVATIONS

MRD-2 in the GHSG HD21 trial assessed by a validated circulating tumor DNA sequencing assay

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KEY POINTS

- **In the GHSG HD21 trial, MRD-2–negative patients are at lower risk for progression, relapse, or death compared with MRD-2–positive patients.**
- **Combining MRD-2 with PET-2 allows for the identification of patients at very low and very high risk of relapse, progression, or death.**

Beyond cure, major goals in patients with Hodgkin lymphoma (HL) are tailoring treatment to a patient's individual risk for relapse to reduce acute and late toxicities, identifying candidates for early incorporation of novel agents, and making treatment affordable on a global level. Minimal residual disease (MRD) assessment by circulating tumor DNA (ctDNA) sequencing emerged as a promising strategy to achieve these goals; however, previous studies differed in sampling time points, assay validation, and definitions for MRD negativity. Here, we applied LymphoVista, a validated ctDNA sequencing assay for genotyping and MRD monitoring in lymphoma, to samples obtained from the German Hodgkin Study Group (GHSG) HD21 trial after 2 cycles of treatment (MRD-2) using a case-cohort design. Patients with positive MRD-2 result were at higher risk for relapse, progression, or death compared with MRD-2–negative patients (4-year progression-free survival [PFS], 36.7% vs 82.2%; hazard ratio, 5.3; 95% confidence interval, 2.0-13.8; *P* = .0008). After inverse probability weighting accounting for the number of events in the full reference set, patients with positive and negative MRD-2 results had 4-year PFS rates of 72.2% vs 95.3%. Combining MRD-2 with positron emission tomography after 2 cycles of BrECADD/eBEACOPP (PET-2) can identify patients at very low and patients at very high risk of relapse, progression, or death. In summary, these results suggest that MRD-2 assessment by LymphoVista allows for early outcome prognostication in patients with HL and could be used as a tool to improve treatment guidance on its own or in conjunction with PET-2.

Introduction

Hodgkin lymphoma (HL) is a B-cell lymphoma often affecting young adults.[1,2] Current frontline treatment regimens are highly effective, leading to long-term remission in most cases.[3-7] Accordingly, limiting acute toxicities and potential late effects[8-12] and making treatment affordable on a global level have become major goals beyond cure.

Recently, 2 strategies have been developed to address these goals.

First, the incorporation of novel agents such as immune checkpoint blockade (ICB)[7] and the antibody-drug conjugate brentuximab vedotin[5] into frontline treatment has increased efficacy but comes with different toxicities and increased financial burden.

Second, response-adapted treatment guided by positron emission tomography (PET) has helped to decrease toxicity while maintaining efficacy.[4,13,14] However, this strategy is limited by a high rate of false-positive interim PET results.

Several studies provided proof of concept for minimal residual disease (MRD) assessment by circulating tumor DNA (ctDNA) sequencing in HL, suggesting that MRD might be able to overcome the limitations of PET.[15-19] MRD was assessed using individualized targeted gene panels[17,19] with improved error suppression leading to increased sensitivity through the use of duplex molecular identifiers or phased variants.[16,18]

However, sampling time points (ie, the amount of treatment already received before MRD is assessed), the definition of MRD negativity (ie, a certain degree of reduction in molecular tumor burden vs drop below the limit of detection [LoD]), and sensitivity and specificity of the assays used for MRD assessment varied across these studies.

Here, we used LymphoVista, a validated ctDNA sequencing assay for genotyping and MRD monitoring in lymphoma,[20] to assess the prognostic value of MRD-2 in patients with previously untreated advanced-stage HL in a subcohort of the German Hodgkin Study Group (GHSG) HD21 trial. The aim of this study is to assess the clinical utility of LymphoVista and determine whether MRD-2 prognosticates progression-free survival (PFS) in HD21.

Materials and methods

Study design

Patients and samples were obtained from the GHSG HD21 trial (ClinicalTrials.gov identifier: NCT02661503).[5] HD21 is an international, open-label, randomized phase 3 trial for patients with previously untreated advanced-stage HL. In the intention-to-treat population, 1482 patients were randomly assigned to receive either BrECADD (brentuximab vedotin, etoposide, cyclophosphamide, doxorubicin, dacarbazine, dexamethasone) or escalated (e)BEACOPP (etoposide, doxorubicin, cyclophosphamide, bleomycin, vincristine, procarbazine, prednisone). Initially, all patients received 6 cycles of BrECADD/eBEACOPP. Following an amendment after enrollment of ~10% of patients, treatment was guided by PET after 2 cycles of BrECADD/eBEACOPP (PET-2) due to the results from the HD18 trial.[13,14] Patients with Deauville score (DS) of 1 to 3 in PET-2 received a total number of 4 cycles of BrECADD/eBEACOPP, whereas patients with DS 4 to 5 in PET-2 received a total number of 6 cycles of BrECADD/eBEACOPP.

Peripheral blood samples were collected as part of an ancillary substudy accompanying the GHSG HD21 trial[5] in 386 consenting patients of 1058 patients treated at trial centers participating in the ancillary substudy before the initiation of treatment and after 2 cycles of BrECADD/eBEACOPP.

Of these 386 patients, 95 were selected with a case-cohort design[21] (random sample of 75 patients enriched by all [n = 20] patients with a documented PFS event at the time of sample definition). The inclusion of additional patients without a PFS event into the analysis would not have led to a substantial gain of additional power and/or insight and was thus omitted. After exclusion of patients with nonidentifiable plasma samples, patients without a plasma sample obtained after 2 cycles of BrECADD/eBEACOPP, and patients where plasma samples did not meet prespecified presequencing quality criteria (cell-free DNA [cfDNA] purity of at least 60%), 71 of 95 patients were eligible for assessment of MRD-2. Of note, in contrast to this retrospective design, these dropouts can be avoided in real-world application of the assay by ensuring that 2 full vials with 10 mL of peripheral blood each are available per sampling time point and patient, which is the recommendation for use of the test. Of these 71 patients (including 55 patients from the random sample), 62 (87.3%) fulfilled all postsequencing quality control (QC) criteria (number of trackable mutations ≥30 and mean mutated allele fraction of tracked variants in the baseline sample ≥0.25% of the validated LymphoVista assay) and were thus selected for the primary analysis of MRD-2. A sensitivity study including all 71 patients who underwent sequencing is presented separately (supplemental Figure 1, available on the *Blood* website).

Patient characteristics, response evaluation by PET, outcomes, and follow-up information were derived from the GHSG HD21 trial as previously reported.[5] Data cutoff for the final analysis was January 2024.

Preanalytical procedures

Peripheral blood samples were collected in 10 mL PAXgene blood circulating cfDNA tubes (Qiagen, Hilden, Germany) and processed within a maximum of 3 days after withdrawal. PAXgene tubes were centrifuged at 1900*g* for 15 minutes at room temperature to separate the plasma from the cell pellet fraction. The cell pellet fraction was aliquoted into cryotubes and stored at −80°C. Next, the plasma layer was pipetted into a clean 15 mL LoBind tube (Eppendorf, Hamburg, Germany) and centrifuged at 1900g for another 10 minutes at room temperature to achieve further plasma purification. Plasma was then aliquoted into cryotubes and stored at −80°C. DNA extraction was performed as previously described.[18,22] cfDNA was extracted from 2 to 4 mL plasma to obtain the required amount of cfDNA input for this study.

Sequencing, bioinformatic analysis, and assessment of MRD

MRD-2 was assessed using LymphoVista, a validated assay for ctDNA-based genotyping and MRD monitoring in patients with lymphoma. LymphoVista HL[20] is a version of LymphoVista specifically designed for patients with HL based on previous studies.[18,19] LymphoVista HL also includes validation targets used for analytical validation and QC. LymphoVista HL targets 305 regions across ~100 kbp (65.9% coding, 34.1% noncoding) of the human genome.

Robust sensitivity for MRD monitoring is achieved by using duplex molecular identifiers for improved error suppression,[23] leading to LoD of 6.54 × 10^{-6} (ie, LymphoVista HL can detect ~6 ctDNA molecules among 1 000 000 total cfDNA molecules) from 16.5 ng of cfDNA assessed with reference material (Oncospan, cfDNA HD833, batch ID 57404, Horizon Discovery, Cambridge, United Kingdom) representative of the average HL patient with regard to the number of trackable mutations for MRD monitoring.

Statistics

The primary aim of this study was to assess the prognostic relevance of MRD-2 regarding PFS. We aimed to demonstrate superior PFS of MRD-2–negative vs MRD-2–positive patients. Secondary aims were to describe the association of MRD-2 with demographic and clinical baseline characteristics and PET-2.

MRD-2 status was categorized as positive or negative based on the LoD (6.54 × 10^{-6}) of LymphoVista HL.[20] Any detectable MRD above the LoD was considered MRD positive.

PFS was defined as the time from enrollment to progression, relapse, or death from any cause, whichever occurred first. Patients without any of these events were censored at the date of the last information on disease status.

Analyses of PFS were done in the enriched sample according to Kaplan-Meier, including Cox regression.

The following hypotheses were to be tested on a significance level of $\alpha = 0.05$: H0, hazard ratio $(HR)_{MRD\text{-}2\text{–}negative/MRD\text{-}2\text{–}positive} = 1$; and H1, $HR_{MRD\text{-}2\text{–}negative/MRD\text{-}2\text{–}positive} \neq 1$.

Of note, the sample size for the case-cohort design described previously was determined pragmatically based on feasibility considerations rather than through formal power calculations.

For the primary analysis, time-dependent sensitivity and specificity at 24 months were calculated by Cox regression analysis.

To determine whether the association between MRD and PFS is independent of baseline risk factors, a sensitivity analysis was performed using Cox regression adjusted for the International Prognostic Score (0-2 vs 3-7).

All other analyses including subgroup and sensitivity analyses are of descriptive nature.

To enable generalizing PFS results of the event-enriched analysis population to the full cohort of HD21 patients participating in the ancillary substudy (n = 1058, reference cohort), inverse probability weighting[24] was used. Patients were weighted by the inverse probability of being selected for the analysis, assuming that blood sample collection was randomly done in the full reference cohort. By that, the number of events included in the analysis is weighted to approximately equal the number of events in the full reference cohort, as is with the controls.

Statistical analyses and visualizations were performed using SAS version 9.4.

All human subject research was approved by the ethics committees responsible for the respective trial centers and in accordance with the Declaration of Helsinki.

Results

Patient cohort for primary MRD analysis

Adhering to the predefined QC criteria of LymphoVista HL, 62 of 71 patients (87.3%) from the case-cohort design fulfilled postsequencing QC specifications and were thus selected for the primary MRD analysis (supplemental Figure 1). Of 11 patients not meeting postsequencing QC specifications, 8 patients had <30 trackable mutations, and 3 patients had a mean baseline mutated allele frequency across trackable mutations of <0.25%. Of these patients, 2 did not meet both criteria.

Patient characteristics

Median age was 32 years (range, 18-58 years), and 31 of 62 patients (50%) were female. Furthermore, 38 of 61 (62.3%) and 23 of 61 (37.7%) patients had negative and positive PET-2 results and thus received 4 and 6 cycles of BrECADD/eBEACOPP, respectively. PET-2 was unavailable in the remaining patient due to enrollment before amending the GHSG HD21 trial (see Materials and methods for details). During a median follow-up of 50 months, 17 of 62 patients (27.4%) experienced progression, relapse, or death. As expected, the number of these events was higher than in the GHSG HD21 trial due to the case-cohort design. Characteristics of the patient cohort evaluable for MRD-2 compared with the nonanalyzed patients of the reference cohort are depicted in Table 1.

Prognostic value of MRD-2

In the random sample (ie, the patients selected randomly for the case-cohort design), 10 of 50 patients (20.0%) had positive MRD-2, whereas the MRD-2–positive rate was 16 of 62 (25.8%) in the enriched cohort (including the random sample and all patients with a documented PFS event at the time of sample definition).

Patients with positive MRD-2 result were at higher risk for progression, relapse, or death compared with patients with negative MRD-2 result (4-year PFS, 36.7% vs 82.2%; HR, 5.3; 95% confidence interval [CI], 2.0-13.8; $P = .0008$; Figure 1), with time-dependent sensitivity of 56% and specificity of 85% at 2 years. The difference was preserved in a sensitivity analysis adjusted for International Prognostic Score at baseline (HR, 5.0; 95% CI, 1.9-13.3) and in a weighted analysis considering the proportion of progressions and relapses in the full reference set (weighted analysis, 4-year PFS: MRD-2 positive 72.2% vs MRD-2 negative 95.3%; Figure 1). To assess whether MRD-2 might be of prognostic value in patients where the strict predefined postsequencing QC specifications of LymphoVista HL (number of trackable mutations ≥30 and mean mutated allele fraction of tracked variants in the baseline sample ≥0.25% of the validated LymphoVista HL assay) were not met, we performed a sensitivity analysis (supplemental Figure 2) yielding similar results.

Table 1. Patient characteristics

	MRD-2 cohort	Reference cohort
	n = 62	n = 996
Age, y, median (range)	32 (18-58)	31 (18-60)
Sex, n (%)		
Female	31 (50.0)	453 (45.5)
Male	31 (50.0)	543 (54.5)
Median follow-up, mo (95% CI)	50 (39-50)	51 (50-52)
Clinical stage, n (%)		
II	9 (14.5)	155/995 (15.6)
III	29 (46.8)	419/995 (42.1)
IV	24 (38.7)	421/995 (42.3)
IPS, n (%)		
0-2	38 (61.3)	550 (55.2)
3-7	24 (38.7)	446 (44.8)
PET-2 result*, n (%)		
DS 1-3 (→4 cycles)	38 (61.3)	557/873 (63.8)
DS 4 (→6 cycles)	23 (37.1)	316/873 (36.2)
Failure event for PFS, n (%)		
No	45 (72.6)	929 (93.3)
Yes	17 (27.4)	67 (6.7)
Type of event, n (%)		
Progression or relapse	16 (25.8)	59 (5.9)
Death w/o prior relapse	1 (1.6)	8 (0.8)

IPS, International Prognostic Score.

*PET-2 and central review not done in 124 of 1058 patients before amendment 3.

Relationship between PET-2 and MRD-2

Next, we compared MRD-2 with PET-2. Among 23 patients with positive PET-2 results (DS 4-5), MRD-2 was positive in 7 of 23 cases (30.4%). Of 38 patients with negative PET-2 results (DS 1-3), MRD-2 was positive in 8 patients (21.1%).

The fact that treatment intensity (4 vs 6 cycles of BrECADD/eBEACOPP) was guided by PET-2 results within the HD21 trial might potentially introduce a bias regarding the prognostic value of MRD-2 when assuming at least moderate correlation with PET-2.

To address this, we separately assessed the prognostic value of MRD-2 within the uniformly treated subgroups of patients with negative (4 cycles of BrECADD/eBEACOPP) or positive (6 cycles of BrECADD/eBEACOPP) PET-2. MRD-2 retained its prognostic value in PET-2–negative patients (4-year PFS: MRD-2 positive 50.0% vs MRD-2 negative 93.3%; HR, 10.1; 95% CI, 1.8-56.6; Figure 2A). In patients with positive PET-2 result, there was a trend toward higher risk of relapse, progression, or death for patients with positive MRD-2 result (4-year PFS: MRD-2 positive 28.6% vs MRD-2 negative 61.9%; HR, 2.6; 95% CI, 0.8-8.8; Figure 2B).

Prognostic value of MRD-2 across treatment arms

BrECADD demonstrated superior PFS and lower treatment-related morbidity compared with eBEACOPP in the GHSG HD21 trial.[5] Thus, separately assessing the prognostic value of MRD-2 in each treatment arm is of relevance. Patients receiving eBEACOPP were at higher risk for relapse, progression, or death with positive MRD-2 as compared with negative MRD-2 (4-year PFS: MRD-2 positive 50.0% vs MRD-2 negative 75.2%; HR, 2.3; 95% CI, 0.6-8.7; Figure 3A). Of note, the confidence interval of the hazard ratio was wide and crossed 1, potentially due to small patient numbers. Strikingly, this effect was even more pronounced in patients receiving BrECADD (4-year PFS: MRD-2 positive 17.1% vs MRD-2 negative 88.0%; HR, 13.8; 95% CI, 3.2-59.4; Figure 3B).

Discussion

Here, we present, to our knowledge, the first study evaluating the prognostic value of MRD-2 as a preplanned analysis with

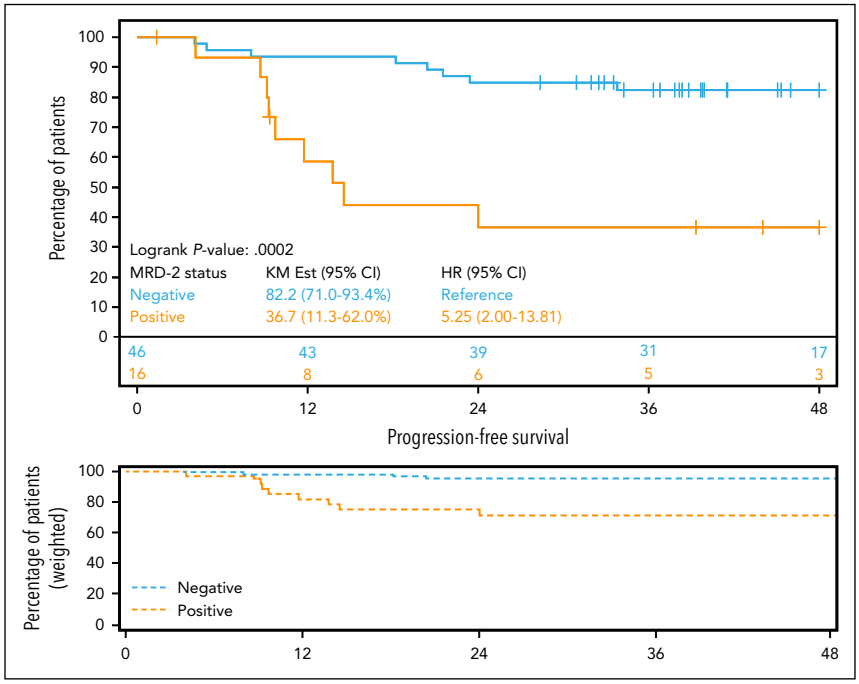

Figure 1. Prognostic relevance of MRD-2 regarding PFS. PFS of 62 patients by MRD-2 status. To illustrate PFS of subgroups defined by MRD-2 in the reference cohort, the lower inset illustrates the analysis results weighted by the inverse probability of being selected for the analysis. KM Est, Kaplan-Meier estimates.

prespecified QC criteria and using a validated genotyping and MRD-assay (LymphoVista HL) in patients with HL treated in a prospective clinical trial. The major findings were as follows:

1. Using LymphoVista HL, MRD-2 was evaluable in ~90% of all patients using strict predefined assay QC criteria.
2. Patients with positive MRD-2 were at higher risk for progression, relapse, or death compared with MRD-2–negative patients across both treatment arms.
3. Combining MRD-2 with PET-2 allows for the identification of patients at very low and patients at very high risk of relapse, progression, or death.

Before this study, several other studies have provided proof of concept for the prognostic value of MRD in patients with HL.[15-17,19] However, most of these studies were performed using samples obtained from patients treated in heterogeneous clinical settings in terms of treatment regimens and intensity. Moreover, MRD negativity was not uniformly defined.

For routine use and the development of clinical trials evaluating treatment guidance based on MRD, assays must be validated to ensure reliable results and alignment with regulatory requirements (eg, in vitro diagnostic regulation). Importantly, LymphoVista HL requires an input amount of total cfDNA (16.5 ng) that is achievable from 1 blood tube (10 mL peripheral blood) in most patients with HL.

Using this amount of input cfDNA, LymphoVista HL provides an LoD of 6.54×10^{-6}, meaning it can detect ~6 ctDNA molecules among 1 000 000 total cfDNA molecules.[20] Furthermore, this LoD was determined using reference material (Oncospan cfDNA HD833, batch ID 57404, Horizon Discovery, Cambridge, United Kingdom) representative of the average HL patient with regard to the number of trackable mutations for MRD monitoring, which is a key determinant of the LoD of a cfDNA MRD test.[25]

Importantly, there are several technical possibilities to achieve the required sensitivity for MRD detection in patients with HL that might differ from LymphoVista HL but could also be used for MRD detection in future clinical trials or routine care given they underwent technical and clinical validation.

Based on MRD-2, the rate of patients with residual disease after 2 cycles of BrECADD/eBEACOPP in the random sample was lower than that measured by PET (20% MRD-2 positive vs 33% PET-2 positive) in this study. Importantly, 16 of 23 patients (69.6%) with positive PET-2 were MRD-2 negative and might thus have received a higher treatment intensity than required. With PET scanners gaining more sensitivity for metabolic activity as they improve, the proportion of false-positive PET results might further increase in the future.[26] Accordingly, the use of MRD-2 offers the potential to identify patients, in whom the lymphoma has already been successfully eradicated, despite PET-2 still indicating residual disease. Consequently, these patients might achieve favorable outcomes without the need for 2 additional cycles of BrECADD/eBEACOPP. Of note, because patients with positive PET-2 received 2 additional cycles of BrECADD/eBEACOPP, the effect of PET-2 and MRD-2 alone might be underestimated.

Importantly, MRD-2 retained its prognostic value among patients with negative and positive PET-2, respectively. Combining PET-2 status with MRD-2 status might allow to detect patients at very low risk for relapse and patients at remarkably high risk of relapse. In patients with negative PET-2 and negative MRD-2, treatment intensity might be further reduced, for example, by switching to non-BEACOPP–based treatment regimens.

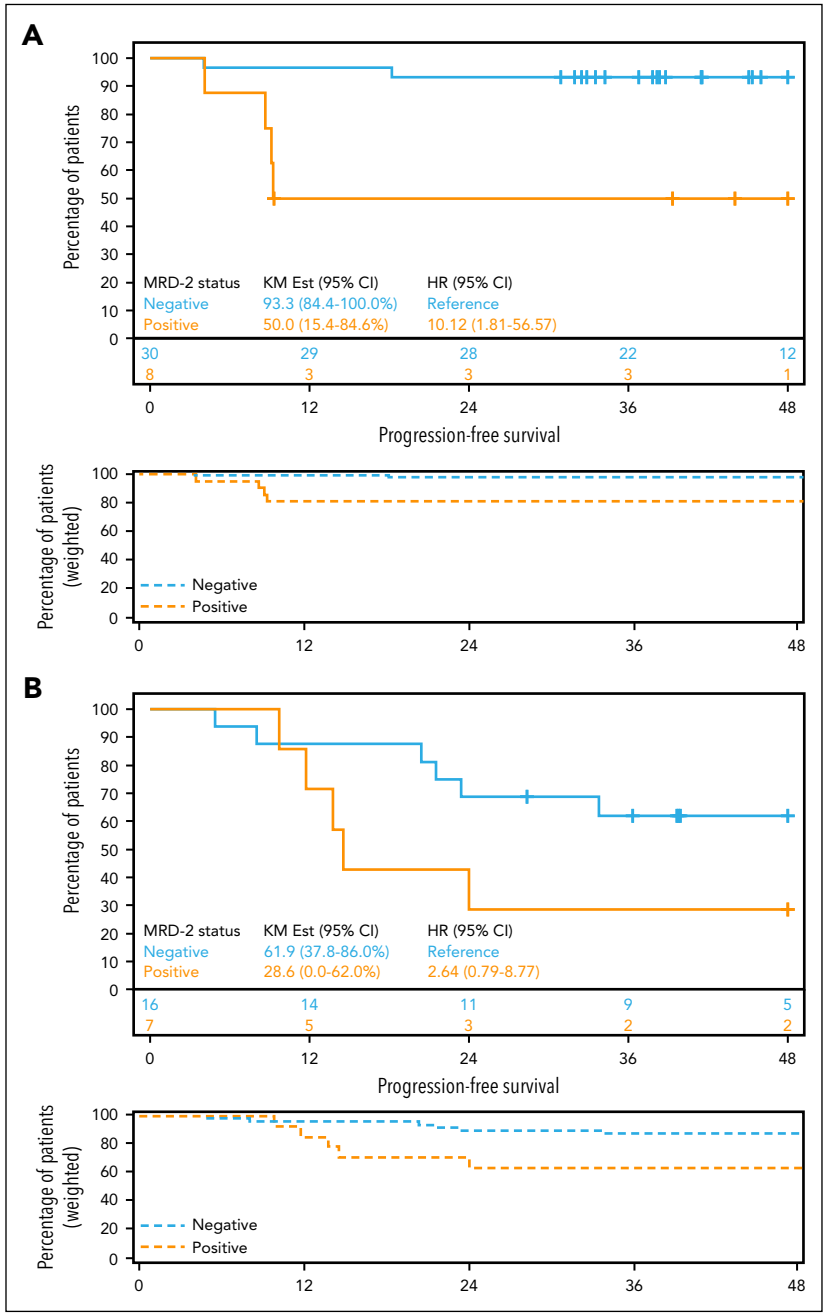

Figure 2. Prognostic relevance of MRD-2 regarding PFS among PET-2–negative and PET-2–positive patients. (A) PFS of 38 patients with negative PET-2 by MRD-2 status. To illustrate PFS of subgroups defined by MRD-2 in the reference subcohort, the lower inset illustrates the analysis results weighted by the inverse probability of being selected for the analysis. (B) PFS of 23 patients with positive PET-2 by MRD-2 status. To illustrate PFS of subgroups defined by MRD-2 in the reference subcohort, the lower inset illustrates the analysis results weighted by the inverse probability of being selected for the analysis. KM Est, Kaplan-Meier estimates.

In contrast, patients with positive PET-2 and positive MRD-2 in our study had a very high risk of progression, relapse, or death (4-year PFS: 28.6%) despite the use of BrECADD/eBEACOPP, the most effective regimen for patients with advanced-stage HL to date.[5] In patients with conflicting results, repeated MRD assessment or PET at the end of treatment might help to clarify these findings. Of note, the prognostic value of MRD-2 was numerically higher in patients treated with BrECADD compared with patients who received eBEACOPP. Because BrECADD has been found to be more effective compared with eBEACOPP in the GHSG HD21 trial,[5] these results suggest that patients with positive MRD-2 after BrECADD are not sufficiently responding despite the use of the most effective polychemotherapy for HL. These patients might thus be candidates for early incorporation of alternative treatment strategies such as ICB-based salvage therapy[27] or clinical trials evaluating novel approaches.

Whether assessment of MRD at certain landmarks is of prognostic value after combinations of ICB with chemotherapy for patients with previously untreated HL[7,28] or relapsed/refractory HL[27] remains to be evaluated. However, given the results from this study and recent early evidence,[29] it is likely that MRD might allow for treatment guidance after less intensive approaches, too.

This study used a case-cohort design drawing a subset from an existing database. The number of cases was determined

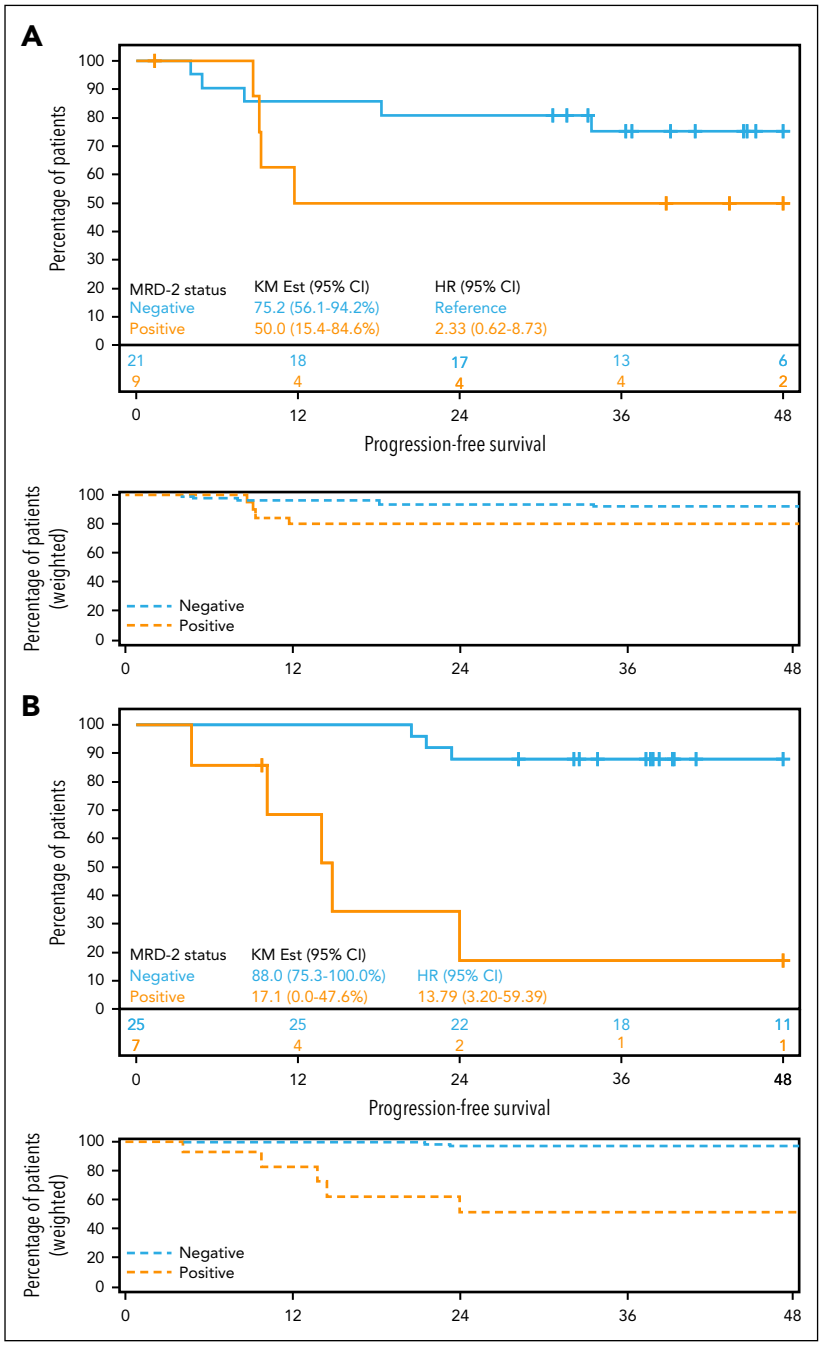

Figure 3. Prognostic relevance of MRD-2 regarding PFS across treatment arms. (A) PFS of 30 patients treated with eBEACOPP by MRD-2 status. To illustrate PFS of subgroups defined by MRD-2 in the reference treatment group, the lower inset illustrates the analysis results weighted by the inverse probability of being selected for the analysis. (B) PFS of 32 patients treated with BrECADD by MRD-2 status. To illustrate PFS of subgroups defined by MRD-2 in the reference treatment group, the lower inset illustrates the analysis results weighted by the inverse probability of being selected for the analysis. KM Est, Kaplan-Meier estimates.

pragmatically, as described in "Materials and methods," which can lead to limited statistical power in detecting small effects. In addition, there is a potential for bias introduced by the sampling method, especially if the selected sample is not fully representative of the underlying population. These potential limitations should be considered when interpreting the results. Finally, an additional assessment of MRD at the end of treatment might further increase its predictive value and should be evaluated in future clinical trials.

In summary, we present, to our knowledge, the first study assessing MRD-2 using the validated LymphoVista HL assay in a patient cohort that was treated with contemporary treatment regimens in a multicenter, international phase 3 trial for patients with previously untreated HL. Our results provide strong evidence for response assessment and treatment guidance by LymphoVista HL in future clinical trials and its potential introduction into routine care in selected patients with HL.

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Authorship

Contribution: J.-M.H., J. Mattlener, H.K., J.F., P.B., and S.B. contributed to the study design; J.F., V.S., M. Hänel, J.C.H., J.D., S. Martin, S. Mathas, J. Meissner, D.M.P., J.M.Z., M. Hallek, B.v.T., P.B., and S.B. contributed to study material, resource, and/or patient acquisition; J. Mattlener and J.S. contributed to sample preparation; A.O. and K.B. contributed to sequencing; J.-M.H., J. Mattlener, and S.B. contributed to bioinformatics; J.M.-H., J. Mattlener, H.K., J.S., G.S., J.K.S., and S.B. contributed to data analysis; J.-M.H., J. Mattlener, H.K., and S.B. wrote the original draft; and all authors contributed to the writing of the manuscript and approved its final version.

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Footnotes

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Primary sequencing data cannot be deposited publicly due to legal requirements and patient data protection. Upon entering a collaboration agreement and in consultation with the local ethics committee access to primary sequencing data might be granted. All secondary data derived from primary sequencing data are available within the article, supplementary information, or supplementary data files, or are available from the authors on reasonable request. Please contact the corresponding author, Sven Borchmann (sven.borchmann@uk-koeln.de), for any data-sharing requests.

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

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