HemaSphere

EHA EUROPEAN HEMATOLOGY ASSOCIATION

Letter
Open Access

Blood-based Monitoring of Relapsed/Refractory Hodgkin Lymphoma Patients Predict Responses to Anti-PD-1 Treatment

Esther E. E. Drees[1,2], Yvonne W. S. Jauw[2,3], Erik van Dijk[1,2], Sven Borchmann[4], Sandra A. W. M. Verkuijlen[1,2], Phylicia Stathi[1,2], Nils J. Groenewegen[1,2,5], Nathalie J. Hijmering[1,2], Daniella R. A. I. Berry[1,2], Eric J. Meershoek[6], Danielle Hoogmoed[6], Anne Kwakman[1,2], Tessa J. Molenaar[1,2], Dirk M. Pegtel[1,2], Bauke Ylstra[1,2], Daphne de Jong[1,2], Josée M. Zijlstra[2,3], Margaretha G. M. Roemer[1,2]

Correspondence: Josée Zijlstra (j.zijlstra@amsterdamumc.nl).

Many refractory classical Hodgkin lymphoma (cHL) patients are uniquely susceptible to immune checkpoint PD-1 blockade (anti-PD-1). Because of these encouraging clinical results, anti-PD-1 is rapidly becoming an integral component of cHL treatment regimens. Unclear is how patients that might benefit from anti-PD-1 can be selected and evaluated during response, highlighting a need to evaluate and compare currently available tissue- and blood-based biomarkers. Blood-based biomarkers may provide a minimally invasive, patient-friendly alternative for response assessment and can be easily obtained during treatment. One of the promising blood-based response biomarkers studied in cHL, focuses on the detection of cell free DNA (cfDNA) in the blood of cancer patients. A proportion of the cfDNA derived from tumor cells is known as circulating tumor DNA (ctDNA). Current cfDNA studies of cHL revolve around the detection of minimal residual disease (MRD) on the basis of mutational profiling of ctDNA.[1,2]

[1]Department of Pathology, Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit Amsterdam, The Netherlands
[2]Program Imaging and Biomarkers, Cancer Center Amsterdam, The Netherlands
[3]Department of Hematology, Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit Amsterdam, The Netherlands
[4]Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, University of Cologne, Germany
[5]ExBiome B. V., Amsterdam, The Netherlands
[6]Leica Biosystems/Kreatech Diagnostics B.V., Amsterdam, The Netherlands
Individual participant data that underlie the results reported in this Article, after de-identification, are available immediately following publication. Data are available for researchers who provide a methodologically sound proposal that fits within the scope of the study and is in accordance with the informed consent of the patients. Proposals should be directed to m.roemer@amsterdamumc.nl. Sequence CNA-data can be found under EGA number: EGAS00001005894. Supplemental digital content is available for this article.

HemaSphere (2022) 6:7(e749).
http://dx.doi.org/10.1097/HS9.0000000000000749.
Received: March 28, 2022 / Accepted: June 1, 2022

We and others have shown that detection of genome-wide chromosomal copy number alterations (CNAs) by next generation sequencing (NGS) from ctDNA is relatively straightforward and could serve as a cost and labor attractive alternative to mutational profiling for disease monitoring.[3,4] Extracellular vesicle associated microRNAs (EV-miRNAs) are described as potential monitoring strategy in cHL and reflect FDG-PET status.[5,6] Additional blood-based biomarkers described in cHL include the protein thymus and activation-regulated chemokine (TARC), of which expression correlates with progression free survival[7,8] and circulating soluble PD-L1, which has been detected in cHL patients.[9,10] In this letter, we address the potential applicability of these biomarkers for response monitoring of anti-PD-1 in cHL.

In an observational cohort study (BioLymph-study, NL60245.029.17), we longitudinally collected blood samples during anti-PD-1 in 4 R/R cHL patients. Additionally, matched tissue biopsies were collected at different timepoints and evaluated for 9p24.1/*PD-L1/PD-L2* genetic alterations by fluorescence in situ hybridization (FISH)[11,12] and expression of PD-L1 and antigen presentation molecules (HLA-I/II) by immunohistochemistry.[11,13] Blood samples were assessed for CNAs in ctDNA[4] in all patients and MRD-detection by mutational profiling[2] in 2 patients. Furthermore, expression of EV-miRNAs: miR-155-5p, miR-127-3p and let-7a-5p by qRT-PCR,[6]and serum TARC and soluble PD-L1 protein expression levels by ELISA were measured. Detailed protocols are described in the Suppl. Methods.

Promising initial responses to anti-PD-1 were observed in all 4 patients, of which 2 patients had a durable response to anti-PD-1 and 2 other patients responded initially but eventually progressed. Clinical and biomarker characteristics and results of biomarker analyses of all 4 patients are shown in Figures 1 and 2, respectively.

Patient 1 is a chemo- and brentuximab vedotin (BV)-treatment-refractory patient. In tissue, copy gain at 9p24.1/*PD-L1/PD-L2* was detected by FISH. The PD-L1 H-score decreased during anti-PD-1 at progression, with only very weak PD-L1 expressing Hodgkin-Reed Sternberg (HRS) cells in the biopsy (Figure 2B). HLA-I expression on HRS cells reverted from positive to negative at progression (Figure 2C). The patient showed no HLA-II expression on HRS cells at baseline and progression (Figure 2E,

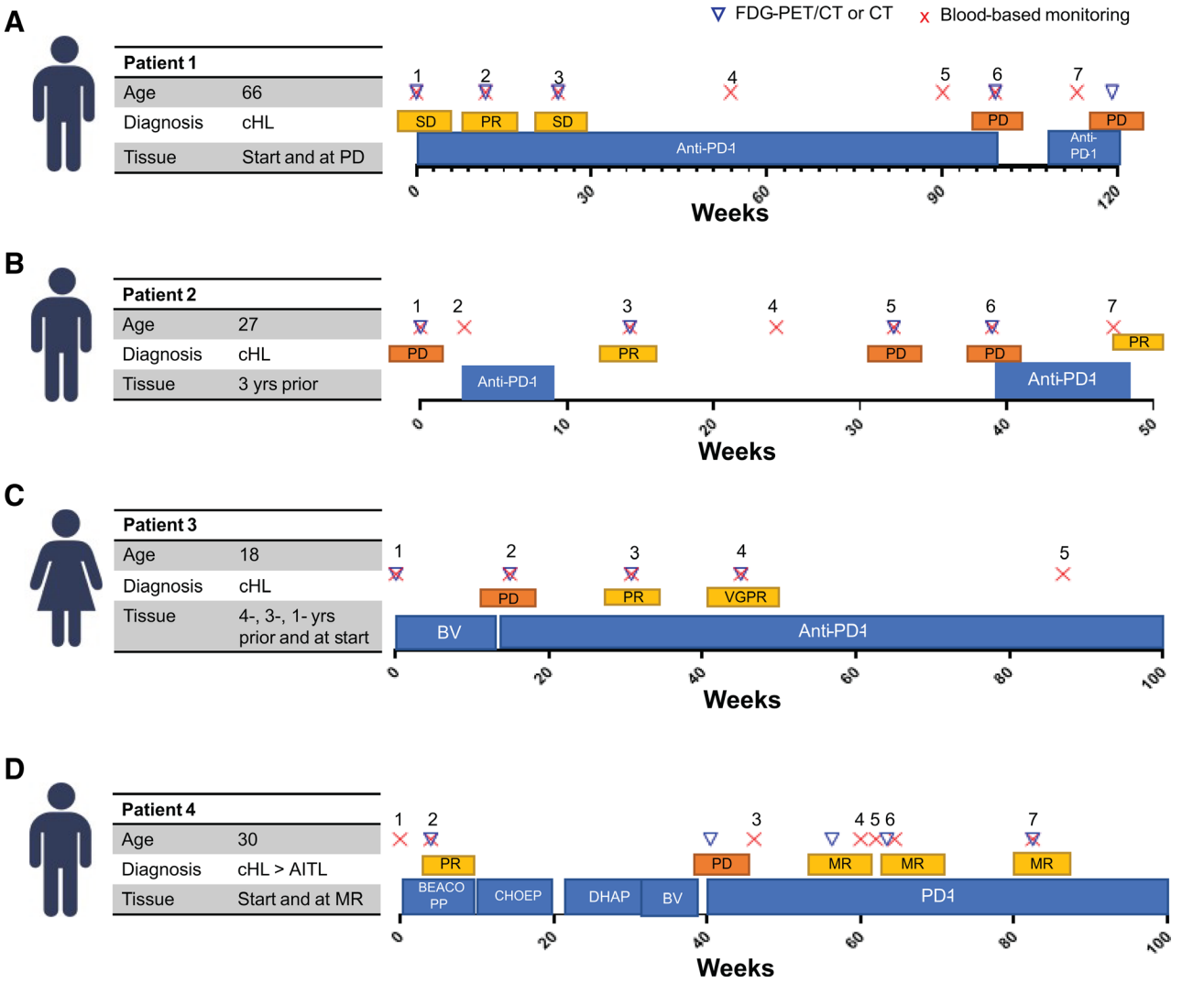
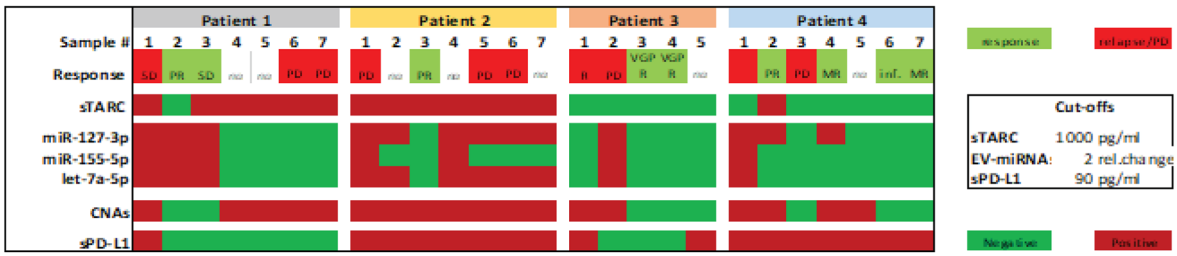


Figure 1. Clinical and biomarker characteristics of cHL patients that received anti-PD-1. Treatment timelines of patient 1–4 together with the timepoints of the blood-based monitoring. Imaging response (by FDG-PET/CT) are shown in the timeline. (A) Patient 1 is a chemorefractory cHL patient who, at baseline, showed no response (SD) after BV as third-line treatment. After 4 cycles of the anti-PD-1, the patient had a PR with a SD after 8 cycles on CT. Two years after the start of anti-PD-1, the patient progressed which was confirmed with FDG-PET/CT and tissue biopsy. After a 2-month without any treatment, followed by 2 additional cycles of anti-PD-1, the patient showed progression on FDG-PET/CT. (B) Patient 2 is an advanced chemo- and BV-treatment-refractory cHL patient who received a matched unrelated allogenic stem cell transplantation, complicated by GvHD. After progression under BV, the patient started anti-PD-1. After 2 cycles of anti-PD-1, the GvHD reactivated and the anti-PD-1 was put on hold. Once the GvHD was under control, the cHL had progressed and the patient received radiotherapy on the pulmonal and abdominal lesions, whereafter anti-PD-1 was restarted. The patient showed response to retreatment (PR, after 4 cycles) but the PET/CT scan was showed signs of pulmonary infectious disease. The patient died shortly thereafter of severe infectious complications. (C) Patient 3 is a chemorefractory cHL patient, who relapsed after allogeneic stem cell transplantation. The patient initially started on BV treatment but showed progression after 3 cycles and switched to anti-PD-1. The patient responded very well (VGPR) and at last consultation (follow-up time: 33 months), the patient has shown no clinical signs of cHL progression under maintenance. (D) Patient 4 is an advanced stage cHL patient. After 2 cycles of BEACOPPesc, the diagnosis was revised as AITL with secondary HRS-like large B-cells, and treatment was switched to CHOEP. The patient progressed under treatment and continued with salvage therapy (DHAP). After PR to DHAP, the patient continued with BV treatment, showed progression and received anti-PD-1. After 8 cycles of anti-PD-1, the patient had a MR on FDG-PET/CT and no signs of disease were seen in the tissue biopsy. AITL = angioimmunoblastic T-cell lymphoma; BEACOPPesc = escalated dose of bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone; BV = brentuximab vedotin; cHL = classical Hodgkin lymphoma; CHOEP = cyclophosphamide, doxorubicin, etoposide, vincristine, prednisone; DHAP = dexamethasone, high-dose cytarabine, cisplatin; FDG-PET/CT = fluorodeoxyglucose positron emission tomography/computed tomography; GvHD = graft-versus-host disease; HRS = Hodgkin-Reed Sternberg; MR = mixed response; PD = progressive disease; PR= partial response; SD = stable disease; VGPR= very good partial response.

left panel). For blood-based analyses, CNAs in ctDNA were detected at baseline but not at partial response (PR) and stable disease. At timepoint 4 and 5, before progression was observed by fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT), CNAs were detected (Figure 2A; Suppl. Figure S2). All EV-miRNAs were increased at baseline and decreased during treatment. sTARC was increased at baseline and decreased markedly at PR. Notably, sTARC levels gradually increased after every cycle of anti-PD-1 until progression was detected on FDG-PET/CT (n = 31 samples; Suppl. Figure S3).

Patient 2 is a chemo- and BV-treatment-refractory, postallogenic stem cell transplantation patient. In the preceding 3 years to starting anti-PD-1, tissue PD-L1 expression was high, along with copy gain of 9p24.1/*PD-L1/PD-L2*. As for the blood-based biomarkers, CNAs in ctDNA were detected throughout the complete disease period. A clear decrease relative to the baseline sample of ctDNA fraction was seen at PR (Suppl. Figures S1 and S4). EV-miRNAs decreased steeply after start of anti-PD-1 at PR, increased during time of graft-versus-host disease (GvHD) and progression, and decreased after restart

A



B

Patient ID	Timing of biopsy	PD-L1 H-score	HLA class I	HLA class II
1	At start of PD-1 treatment	130	Positive	Negative
1	During PD-1 treatment (PD)	30	Negative	Negative
2	3 years prior PD-1 treatment	260	Positive	Positive
3	4 years prior PD-1 treatment	150	Decreased	Decreased
3	2 years prior PD-1 treatment	50	Negative	Negative
3	1 year prior PD-1 treatment	280	Decreased	Decreased
3	At start of PD-1 treatment	200	Decreased	Decreased
4	At start of PD-1 treatment	30	Negative	Positive
4	During PD-1 treatment (MR)	-	-	-

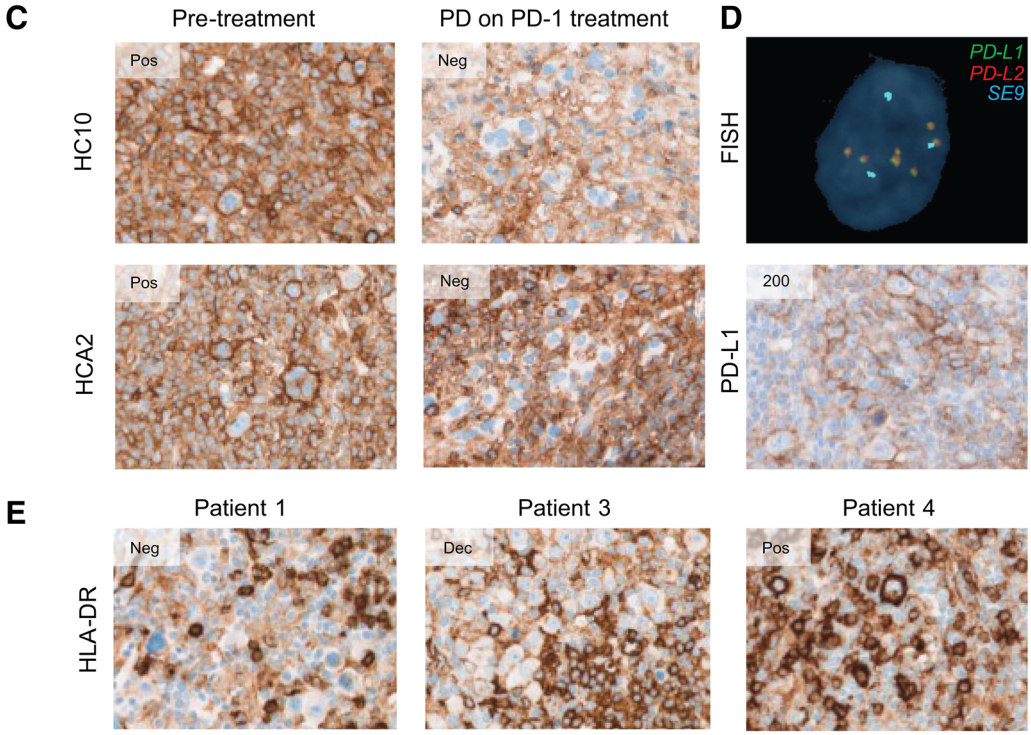


Figure 2. Blood-based and tissue biomarkers in cHL patients that received anti-PD-1. (A) Blood-based biomarkers are shown as positive or negative for disease-detection based on the cutoffs depicted on next to the figure. Response on the second line is the imaging response (by FDG-PET/CT). (B) Overview table with all the different samples added to the analysis, together with PD-L1 H-score and HLA class I and II. (C) Immunohistochemistry staining's pretreatment and at progression (PD) during treatment. (D) Fish and example of PD-L1 H-score. (E) HLA-DR staining of patients 1, 3, and 4. cHL = classical Hodgkin lymphoma; FDG-PET/CT = fluorodeoxyglucose positron emission tomography/computed tomography; inf. = PET-positive inflammation which is tissue biopsy confirmed; MR= mixed response; n.a.= not applicable; PD = progressive disease; PR= partial response; SD = stable disease; VGPR= very good partial response.

of anti-PD-1. The sTARC levels dropped sharply at PR and steadily increased until progression. Similar to sTARC, sPD-L1 increased throughout treatment until start of the second period of anti-PD-1 whereafter it decreased (Suppl. Figure S1).

Patient 3 is a chemorefractory, postallogeneic stem cell transplantation patient, progressive after 3 cycles of BV, switching to anti-PD-1. In tissue, significant fluctuation was observed in PD-L1 positive HRS cells. Largest changes were observed after allogeneic stem cell transplantation, before anti-PD-1: only low PD-L1 and no HLA expression were observed. At start of anti-PD-1, the majority of HRS cells were positive for PD-L1, and showed copy gain of 9p24.1/*PD-L1/PD-L2* (Figure 2D). HL-I and -II were expressed, although it was at lower levels than surrounding reactive cells (Figure 2E, middle panel). As for blood-based biomarkers, ctDNA fraction was overall low, but CNAs were seen before start BV and anti-PD-1 (Suppl. Figure S5). This patient was also analyzed for MRD in ctDNA by mutational profiling.[2] Compared with timepoint 2, patient 3 had an MRD reduction of only 0.02 log-levels, which means that almost the same amount of ctDNA was detected in both samples. EV-miRNAs initially increased at progression, and thereafter showed a stable decrease. sTARC initially decreased, and was already low before start of BV (Suppl. Figure S1).

Patient 4 is an advanced stage patient, progressive under salvage treatment and BV before starting anti-PD-1. In tissue before start of anti-PD-1, PD-L1 was expressed in a few HRS cells. In cells where PD-L1 expression was observed, the intensity of expression was very high (3+), consistent with 9p24.1/*PD-L1/PD-L2* amplification observed in a minority of the HRS cells. No HLA-I was observed, but HRS cells were positive for HLA-II (Figure 2E, right panel). As for blood-based biomarkers, CNAs were detected at start of BEACOPPesc and at PR, although a clear decrease in ctDNA fraction was seen (Suppl. Figures S1 and S6). After 8 cycles, the patient had a mixed response on FDG-PET/CT and no signs of disease were seen in the tissue biopsy, still a very low ctDNA fraction was seen, that disappeared at later timepoints (Figure 2A). Compared with the baseline timepoint, patient 4 had no detectable MRD, the best possible response with this assay. EV-miRNAs and sTARC were detectable at start of BEACOPPesc, and then decreased. sPD-L1 was very high in this patient initially, and then also decreased (Suppl. Figure S1). In line with this, 9p24.1/*PD-L1/PD-L2* amplification was observed in the tissue and in ctDNA.

In summary, we performed longitudinal response evaluation during anti-PD-1 in 4 patients using tissue- and blood-based biomarkers. Here, we show that expression of PD-L1 in tissue, as well as HLA-I and -II may modulate over time and during treatment, in line with previous findings.[14] PD-L1 expression is decreased on HRS cells in consecutive biopsies before and during anti-PD-1, suggesting that anti-PD-1 may cause a depletion of PD-L1 positive HRS cells. However, in a previous report of Sasse et al., PD-L1 expression seemed unaffected by anti-PD-1.[15] The observed expression of HLA-I and -II was quite heterogeneous. In 1 patient, HLA-I expression changed from positive to negative during anti-PD-1, suggesting that additional immune evasion strategies were obtained. Our results demonstrate that expression of various tissue-related biomarkers is not stable over time prior to anti-PD-1. Therefore, to potentially predict response to anti-PD-1, such biomarkers should be determined shortly before start of anti-PD-1.

As shown, blood-based technologies can be valuable for response monitoring, each with their own strengths and limitations. In general, the presence or absence of CNAs correspond with treatment response. In 2 patients, an increase in CNAs was observed before progression was detected by FDG/PET-CT. The main advantage of ctDNA using genome-wide CNA detection by NGS is that it can be easily included in routine high-throughput workflows. Also promising is mutational profiling of ctDNA, as shown for patients 3 and 4. Although sensitivity with this method may be higher than CNA evaluation,[1,2] mutation-based analyses (ie, CAPPseq) is more time-consuming and costly. EV-miRNAs have the benefit of being highly abundant in the circulation of cHL patients and for response monitoring of anti-PD-1, there is a good correlation with disease status in all patients. The only potential confounder for detection of EV-miRNAs is the presence of infection and GvHD, as seen here for patient 2, and reported previously.[6] We show that sTARC levels correlate to disease status in 3 of the 4 patients. Interestingly, 2 patients already had relatively low sTARC prior to start of anti-PD-1, potentially hampering sTARC as biomarker. In our set of patients, sPD-L1 did not appear to be a suitable biomarker, in contrast to other studies.[9,10] One of the potential explanations for this discrepancy is that we used serum to detect sPD-L1 instead of plasma, as done by Veldman et al.[9]

In conclusion, blood-based biomarkers are very promising for disease detection and monitoring of anti-PD-1 responses in cHL. For the most accurate response monitoring, our results suggest a multianalyte approach, using a combination of ctDNA, EV-miRNA and sTARC detection.

ACKNOWLEDGMENTS

We are thankful to the cHL MRD Consortium for their fruitful collaboration and support. We are thankful to Mai Tran, Leah Prins, Melissa Fidler and Jennifer Perez Boza for helping with the sample collection and Mandy Kerkhoff for help with scanning the immunohistochemistry slides. We are thankful to Helge Dörr and Elisabeth Kirst for excellent technical assistance.

AUTHOR CONTRIBUTIONS

EEED and MGMR did conception and design. EEED, JMZ, YWSJ, and NJH did provision of study and patient materials. EEED, EvD, SB, SAWMV, PS, NJG, DRAIB, EM, DH, AK, TJM, DMP, BY, DdJ, JMZ, and MGMR did collection and assembly of data. MGMR, BY, DMP, JMZ, EvD, and EEED did data analysis and interpretation. EEED, BY, DDJ, JMZ, and MGMR did article writing. All authors did final approval of article.

DISCLOSURES

DMP is cofounder and CSO of Exbiome BV. DMP served as an advisor for Takeda for which he received travel compensation. SB is founder, shareholder and CEO of Liqomics. All the other authors have no conflicts of interest to disclose.

SOURCES OF FUNDING

The blood-based biomarker work was funded by the Dutch Cancer Society (KWF-5510), Cancer Center Amsterdam Foundation (CCA-2013), the Technology Foundation STW (STW Perspective CANCER-ID) grants awarded to D.M. Pegtel. This publication is part of the Veni project of M.G.M. Roemer with project number 101737 which is (partly) financed by the Dutch Research Council (NWO).

STUDY APPROVAL

Samples were collected in the BioLymph-study. The study is registered in the Dutch CCMO-register (toetsingonline.nl, NL60245.029.17) and is being conducted in accordance to the Declaration of Helsinki (7th revision, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

REFERENCES

1. Spina V, Bruscaggin A, Cuccaro A, et al. Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma. *Blood*. 2018;131:2413–2425.
2. Sobesky S, Mammadova L, Cirillo M, et al. In-depth cell-free DNA sequencing reveals genomic landscape of Hodgkin's lymphoma and facilitates ultrasensitive residual disease detection. *Med (N Y)*. 2021;2:1171–1193.e11.
3. Buedts L, Wlodarska I, Finalet-Ferreiro J, et al. The landscape of copy number variations in classical Hodgkin lymphoma: a joint KU Leuven and LYSA study on cell-free DNA. *Blood Adv*. 2021;5:1991–2002.

4. Beagan JJ, Drees EEE, Stathi P, et al. PCR-free shallow whole genome sequencing for chromosomal copy number detection from plasma of cancer patients is an efficient alternative to the conventional PCR-based approach. *J Mol Diagn*. 2021;23:1553–1563.
5. van Eijndhoven MA, Zijlstra JM, Groenewegen NJ, et al. Plasma vesicle miRNAs for therapy response monitoring in Hodgkin lymphoma patients. *JCI Insight*. 2016;1:e89631.
6. Drees EEE, Roemer MGM, Groenewegen NJ, et al. Extracellular vesicle miRNA predict FDG-PET status in patients with classical Hodgkin Lymphoma. *J Extracell Vesicles*. 2021;10:e12121.
7. Guidetti A, Mazzocchi A, Miceli R, et al. Early reduction of serum TARC levels may predict for success of ABVD as frontline treatment in patients with Hodgkin Lymphoma. *Leuk Res*. 2017;62: 91–97.
8. Hsi ED, Li H, Nixon AB, et al. Serum levels of TARC, MDC, IL-10, and soluble CD163 in Hodgkin lymphoma: a SWOG S0816 correlative study. *Blood*. 2019;133:1762–1765.
9. Veldman J, Alsada ZND, van den Berg A, et al. Soluble PD-L1 is a promising disease biomarker but does not reflect tissue expression in classic Hodgkin lymphoma. *Br J Haematol*. 2021;193:506–514.
10. Guo X, Wang J, Jin J, et al. High serum level of soluble programmed death ligand 1 is associated with a poor prognosis in Hodgkin Lymphoma. *Transl Oncol*. 2018;11:779–785.
11. Roemer MGM, Redd RA, Cader FZ, et al. Major histocompatibility complex class II and programmed death ligand 1 expression predict outcome after programmed death 1 blockade in classic Hodgkin Lymphoma. *J Clin Oncol*. 2018;36:942–950.
12. Roemer MG, Advani RH, Ligon AH, et al. PD-L1 and PD-L2 genetic alterations define classical hodgkin lymphoma and predict outcome. *J Clin Oncol*. 2016;34:2690–2697.
13. Roemer MG, Advani RH, Redd RA, et al. Classical Hodgkin Lymphoma with reduced β2M/MHC Class I expression is associated with inferior outcome independent of 9p24.1 status. *Cancer Immunol Res*. 2016;4:910–916.
14. Hollander P, Amini RM, Ginman B, et al. Expression of PD-1 and PD-L1 increase in consecutive biopsies in patients with classical Hodgkin lymphoma. *PLoS One*. 2018;13:e0204870.
15. Sasse S, Reddemann K, Diepstra A, et al. Programmed cell death protein-1 (PD-1)-expression in the microenvironment of classical Hodgkin lymphoma at relapse during anti-PD-1-treatment. *Haematologica*. 2019;104(1):e21-e24.