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

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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 ctDNA studies of cHL revolve around the detection of minimal residual disease (MRD) on the basis of mutational profiling of ctDNA.1,2 We and others have shown that detection of genome-wide chromosomal copy number alterations (CNAs) 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 survival7,8 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-III) by immunohistochemistry.11,13 Blood samples were assessed for CNAs in ctDNA4 in all patients and MRD-detection by mutational profiling2 in 2 patients. Furthermore, expression of EV-miRNAs: miR-155-5p, miR-127-3p and let-7a-5p by qRT-PCR,5 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).
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.
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).

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.
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. 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. 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. 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.5,10 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.

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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.

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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).

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