Received: 13 August 2023 | Accepted: 8 October 2023

DOI: 10.1111/bjh.19162

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

ORIGINAL PAPER

Haematological Malignancy - Clinical

Baseline circulating tumour DNA and interim PET predict response in relapsed/refractory classical Hodgkin lymphoma

Eleonora Calabretta[1,2] | **Martina di Trani**[2] | **Francesco Corrado**[1,2] | **Martina Sollini**[1,3] | **Vanessa Cristaldi**[2] | **Fabrizio Marino**[1,2] | **Lodovico Terzi di Bergamo**[4] | **Alessio Bruscaggin**[4] | **Maria Cristina Pirosa**[4,5] | **Stefania Bramanti**[2] | **Arturo Chiti**[1,3] | **Davide Rossi**[4,5] | **Carmelo Carlo-Stella**[1,2]

[1]Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy

[2]Department of Oncology and Hematology, Humanitas Cancer Center, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

[3]Nuclear Medicine, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

[4]Laboratory of Experimental Hematology, Institute of Oncology Research, Università della Svizzera Italiana, Bellinzona, Switzerland

[5]Clinic of Hematology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland

Correspondence
Carmelo Carlo-Stella, Department of Oncology and Hematology, Humanitas Cancer Center, IRCCS Humanitas Research Hospital, Via Alessandro Manzoni, 56, 20089 Rozzano, Milan, Italy.
Email: carmelo.carlostella@hunimed.eu

Funding information
Associazione Italiana per la Ricerca sul Cancro, Grant/Award Number: 20575

Summary

Reliable biomarkers for early identification of treatment failure in relapsed/refractory (r/r) classical Hodgkin lymphoma (cHL) are lacking. Circulating tumour DNA (ctDNA) profiling has emerged as a powerful predictive and prognostic tool in several haemopoietic and non-haemopoietic malignancies and may guide rational treatment choices in r/r cHL. To assess the predictive and prognostic value of ctDNA, we performed a retrospective analysis on 55 r/r cHL patients treated with the bendamustine, gemcitabine and vinorelbine (BEGEV) regimen and additionally evaluated the potential utility of integrating ctDNA with interim [^{18}F]-FDG positron emission tomography (iPET). Baseline ctDNA genotyping in r/r cHL mirrored gene mutations and pathways involved in newly diagnosed cHL. We found that baseline ctDNA quantification and serial ctDNA monitoring have prognostic value in r/r cHL receiving salvage chemotherapy. Lastly, integrating ctDNA quantification with iPET evaluation may improve the early identification of patients at high risk of failing standard salvage therapy, who may benefit from an early switch to immunotherapeutic agents. Collectively, our results support the implementation of non-invasive methods to detect minimal residual disease in recurrent cHL and justify its prospective evaluation in appropriately designed clinical trials.

KEYWORDS
circulating tumour DNA, Hodgkin lymphoma, PET, prognostic factors

INTRODUCTION

Despite the introduction of highly active novel immunotherapies and targeted therapies for the treatment of relapsed/refractory (r/r) classical Hodgkin lymphoma (cHL), chemotherapy followed by consolidation with stem cell transplantation (SCT) is still the standard of care for young patients failing first-line therapy. The bendamustine, gemcitabine and vinorelbine (BEGEV) regimen is routinely offered as induction therapy prior to SCT.[1] Early identification of patients at high risk of failing this protocol may allow to select patients who may benefit from novel immunotherapies earlier in the treatment course, thereby mitigating the burden of chemotherapy-related adverse events and allowing to optimize patient management. Contrary to first-line setting, the role of interim 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) positron emission tomography/computed tomography (PET/CT) during salvage therapy is

Eleonora Calabretta and Martina di Trani contributed equally.

still not standardized in guiding clinical decisions, and reliable biomarkers for early identification of treatment failure are lacking. In this context, circulating tumour DNA (ctDNA) profiling is emerging as a powerful tool for outcome prediction and response monitoring in large B-cell lymphomas treated both with chemotherapy[2] and cellular therapy,[3] as well as front-line cHL treated with chemotherapy.[4] Pretreatment burden and dynamics during treatment course have the potential to impact treatment and adjust the therapeutic plan. However, lack of standardization of ctDNA monitoring and validated thresholds still prevent its real-life application. Furthermore, its prognostic value has yet to be validated when compared to more commonly used prognostic tools such as [^{18}F]-FDG PET/CT, and in recurrent lymphomas.

We therefore sought to characterize the mutational profile of a cohort of r/r cHL patients uniformly treated with BEGEV chemotherapy to explore possible biomarkers of chemoresistance. Then, we performed a retrospective analysis to assess the efficacy of quantification of baseline plasma ctDNA in prognosticating outcomes and monitored ctDNA dynamics during the treatment course to assess minimal residual disease. Lastly, we evaluated whether integrating ctDNA genotyping with interim [^{18}F]-FDG PET further improves outcome prognostication.

TABLE 1 Main patients' characteristics.

	All $n = 55$
Gender	
Male	30 (55%)
Female	25 (45%)
Median age (range)	34 (18–73)
BEGEV timing	
Second-line therapy/>second-line therapy	34 (62%)/21 (38%)
Stage at BEGEV	
I–II	27 (49%)
III–IV	28 (51%)
Extranodal disease prior BEGEV therapy	20 (36%)
B symptoms prior to BEGEV therapy	16 (29%)
Bulky disease prior to BEGEV therapy	7 (13%)
Response to first-line therapy	
Refractory	36 (65%)
Responsive	19 (35%)
Median number of BEGEV cycles (range)	4 (2–4)
Prior BV	18 (33%)
Prior RT	17 (31%)
Prior PD-1 inhibitors	9 (16%)

METHODS

Patients and study design

The study was retrospective in nature. Fifty-five patients affected by relapsed/refractory (r/r) cHL (age ≥16 years) who were treated with the BEGEV regimen as salvage therapy from February 2014 to June 2022, were included in the study (Table 1). Patients were required to have a plasma sample collected at baseline for ctDNA genotyping to enter the study. Patients ($n = 55$) were all treated at the Humanitas Research Hospital (Milano, Italy). The BEGEV regimen was administered on a 4-day schedule, repeated every 21 days as follows: 800 mg/m^2 of gemcitabine on Days 1 and 4, 20 mg/m^2 of vinorelbine on Day 1 and 90 mg/m^2 of bendamustine on Days 2 and 3. Intravenous prednisolone (100 mg) was administered on Days 1–4.[5] Prior allogeneic stem cell transplantation (allo-SCT) was considered an exclusion criterion. Treatment programme included consolidation with autologous stem cell transplantation (auto-SCT) for eligible patients treated in second line, and allo-SCT for patients treated beyond second line, if deemed feasible by clinicians. Patients who exhibited progressive disease (PD) at the end of BEGEV chemotherapy did not proceed to consolidation and were assigned to additional salvage therapies. Disease assessment was performed by [^{18}F]-FDG PET/CT scan prior to BEGEV, after cycle 2 (interim PET [iPET]) and after cycle 4 (end of BEGEV treatment [EOBT]) (Data S1). Patients were classified into 'responsive' or 'failure' based on disease status after a follow-up of 18 months, as a determinant of chemo-refractoriness (duration of remission <12 months from the end of therapy, comprising both BEGEV and SCT). The primary end-point of the study was event-free survival (EFS), where the event was defined as need for additional treatment within 18 months from start of BEGEV chemotherapy or disease-related death. Secondary end-points included overall survival (OS) and progression-free survival (PFS). This study was approved by the local institutional Review Board, and all patients provided written informed consent in accordance with the Declaration of Helsinki.

Sample collection, CAPP-seq library preparation, ultra-deep next-generation sequencing (NGS) and somatic analysis

The CAPP-seq (Cancer Personalized Profiling by deep Sequencing) strategy, an ultrasensitive capture-based targeted sequencing, was used for ctDNA genotyping[4,6–8] (Data S1). Peripheral blood (PB) samples were collected prior to chemotherapy initiation (baseline, $n = 55$), at the time of iPET ($n = 45$) and at EOBT (after last cycle of BEGEV, $n = 34$).

Statistical analysis

GraphPad Prism (version 9) and R version 6.3.1 were used for statistical analyses. Categorical variables were expressed as proportions with the respective 95% confidence intervals, and continuous variables were expressed as the medians with the respective range. Continuous variables were

compared by Mann–Whitney test and *t*-test. We used the Spearman correlation coefficient to test correlation between ctDNA and total metabolic tumour volume (TMTV). We used a Kruskal–Wallis test to establish correlation between ctDNA and clinical prognostic scores. Multiple *t*-test were used to investigate the prognostic impact of ctDNA baseline somatic mutations on EFS; correction for multiple testing was not applied, given the exploratory nature of this analysis and the relatively small sample size.

Event-free survival was measured from date of BEGEV treatment start to date of re-treatment (event) or disease-related death (event); patients failing the BEGEV programme beyond 18 months were censored. PFS was measured from date of BEGEV treatment start to date of progression (event), death from any cause (event), or last follow-up (censoring). OS was measured from date of BEGEV start to date of death (event) or last follow-up (censoring). Survival analysis was performed by Kaplan–Meier method. Differences in cumulative survival between two groups were tested with the log-rank test. Survival analyses on the basis of molecular response was performed by calculating survival from iPET evaluation to reduce unwanted guarantee-time bias (landmark approach).[9]

We used the Cox proportional hazards model to assess the prognostic role of clinical characteristics, ctDNA load and iPET evaluation on EFS. A survival tree was generated based on the most statistically significant independent variables derived from the analysis. Rpart package for R was used to perform the partitioning analysis (minsplit = 10). We used log-rank test to determine whether EFS, PFS and OS differed significantly between terminal node groups generated by the decision tree.

Statistical significance was defined as $p < 0.05$.

Data sharing system

Individual participant data will not be shared. For original correlative data, please contact the corresponding author (carmelo.carlostella@hunimed.eu).

RESULTS

Patients' characteristics and response to therapy

Median age of study participants was 34 years (range, 16–73) and 30 patients (55%) were male Caucasian. At baseline before BEGEV therapy 51% ($n = 28$) of patients presented advanced-stage disease, with extranodal involvement in 36% ($n = 20$) of cases. Of note, the study cohort included a large proportion of patients who were refractory to first-line therapy, ($n = 36$, 65%). Thirty-four patients (62%) received BEGEV as second-line therapy, whereas 21 patients (38%) were treated after second line (Table 1). Forty-eight patients (87%) received four cycles of BEGEV, three ($n = 5$%) received three cycles due to transplantation timing, and four (7%) were in progressive disease (PD) at iPET and discontinued therapy. At the end of BEGEV therapy, 75% of patients ($n = 41$) achieved a complete remission (CR) and 7% ($n = 4$) were in PR, with an overall response rate of 82%. Ten patients (18%) exhibited PD. Efficacy was in line with previous reports, which, of note, only included patients treated in second line.[5] Of the responding patients, 28 (62%) proceeded to auto-SCT (all in CR) and 14 (31%) were consolidated with allo-SCT (12 in CR, 2 in PR). Among responsive patients, three (7%) did not receive consolidation due to early progression ($n = 1$) or medical decision ($n = 2$, one of whom ultimately required further treatment). At 18 months, EFS was 68.3% (95% confidence interval [CI] 56.9–82.1, median follow-up 18 months), with 17 patients requiring additional therapy. Twenty-four-month PFS and OS for the entire population were 62.0% (95% CI 50.0–76.9, median follow-up 20 months) and 85.4% (95% CI 76.0–96.1, median follow-up 30 months) respectively (Figure S1).

Non-invasive genotyping of r/r cHL patients

By study design, a baseline plasma sample was available in all patients. The median extracted amount of cfDNA per millilitre of plasma was 7 ng/mL (Figure S2A). An average of 10 somatic coding variants, including single nucleotide variants (SNVs) and Indels, were identified (Figure S2B). The median variant allele frequency (VAF) of ctDNA variants was 2.6% (range 0.2%–43%) and 85% of somatic coding variants in the whole patient population had a VAF higher than 1% (Figure S2C,D). The pretreatment mutational profile of the entire cohort of patients is illustrated in Figure S3. Genes recurrently affected by non-synonymous somatic variants in at least 15% of patients included *STAT6* (44%), *B2M* (38%), *TNFAIP3* (36%), *GNA13* and *SOCS1* (31%), *ITPKB* (29%), *XPO1* (22%), *TP53* and *PTPRD* (18%) and *BTG1* (16%).

There were no differences in mutated pathways between our cohort of r/r cHL and previously published reports of newly diagnosed patients.[4,10] Specifically, JAK–STAT was mutated in 67% of patients followed by PI3K/AKT (58%), NF-kB (51%), immune surveillance (44%), epigenetics (40%) and NOTCH signalling (11%) (Figure S4).

Although it appears that the frequency of some mutations may be different in BEGEV-responsive versus BEGEV-non-responsive patients (Figure 1A,B; Figure S5), the only gene with a significant difference was *PTPRD*, which was enriched in patients failing therapy ($p < 0.05$) (Figure 1C). The *PTPRD* gene encodes for a phosphatase that functions as a negative regulator of signalling pathways and is a known tumour suppressor with prognostic significance in numerous solid cancers as well as marginal zone lymphoma.[11–14]

Ten patients (18%) harboured one or more *TP53* mutations, mainly distributed in the DNA-binding domain (Figure S6A). However, contrary to other settings, *TP53* mutation did not confer disadvantage in clinical outcome (18 months EFS 57.1% [95% CI 32.6–100.0] for *TP53*-mutated patients versus 71.0% [95% CI 58.8–85.6] for *TP53* wild-type patients, $p = 0.52$) (Figure S6B).

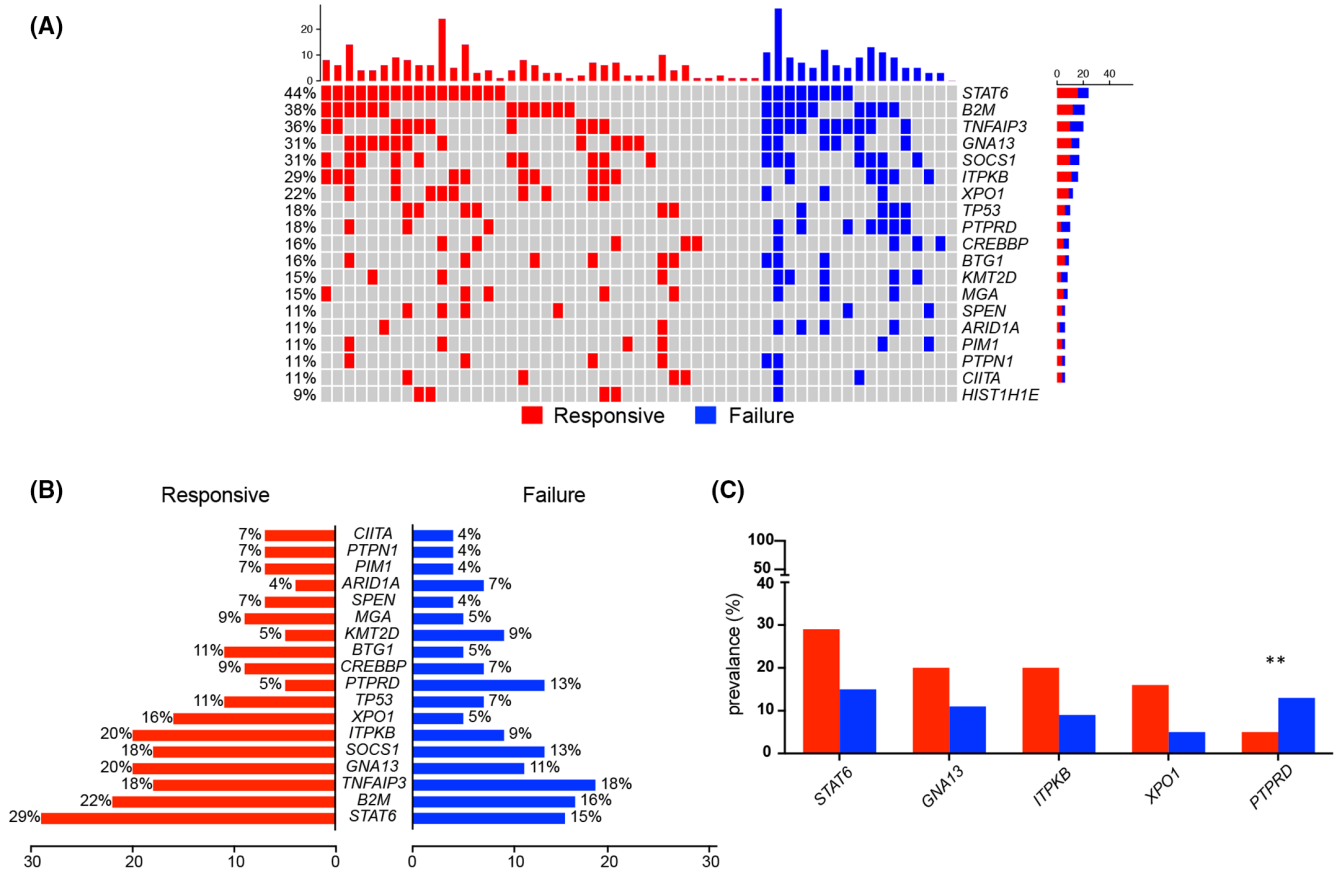


FIGURE 1 Pretreatment ctDNA genotyping ($n = 55$) in 'responsive' ($n = 38$) and 'failure' ($n = 17$) r/r cHL patients. (A) Oncoprint showing the distribution of pretreatment non-synonymous somatic variants, with a prevalence greater than 10%, according to EFS status (excluding silent SNVs and variants in non-coding regions). Each column represents a patient, and each row represents a gene. The information regarding the number of variants per each patient is shown in the histogram above the oncoprint. The frequencies of mutated genes are shown on the left and the relative absolute numbers are shown in the histogram on the right. (B) Bar graph showing genes with an overall prevalence of mutation greater than 10% and their distribution among 'responsive' and 'failure' patients. (C) Histograms showing the frequency of mutated genes with a prevalence >10% and in which for each gene the difference observed between responsive versus failure is greater or equal to 7%. The Fisher's exact test was used for comparisons (**$p < 0.01$).

ctDNA as early predictor of response or non-response in r/r cHL patients undergoing BEGEV therapy

The median concentration of pretreatment ctDNA, expressed as haploid genome equivalents per millilitre of plasma (hGE/mL), was 36 hGE/mL (range 4–4576). In line with previous findings,[7] pretreatment levels of ctDNA correlated with the TMTV (Spearman coefficient 0.51; $p < 0.0001$), as well as with the German Hodgkin Study Group (GSHG) prognostic score ($p = 0.025$), but not with stage or response to first-line therapy (Figure S7). Notably, the median baseline load of ctDNA was significantly higher in 'failure' (median 94, 95% CI 36–270) compared to 'responsive' patients (median 31, 95% CI 20–42) ($p = 0.0006$) (Figure 2A).

When longitudinally evaluating the ctDNA load, we observed two distinct patterns: (i) the majority of responsive patients had undetectable ctDNA levels at iPET ($n = 31$), which was maintained at EOBT ($n = 24$); on the contrary, (ii) patients requiring re-treatment after BEGEV chemotherapy showed a lower median value at iPET compared to baseline (median iPET = 7.6 hGE/mL, $n = 14$) but displayed a subsequent increase at EOBT (median EOBT = 38 hGE/mL, $n = 10$) (Figure 2B), indicating persistence of disease. In terms of mutational profile, patients failing BEGEV therapy showed a persistence of baseline mutations at EOBT in 60% of cases ($n = 6$), whereas 40% ($n = 4$) had no detectable variants; on the contrary, responsive patients cleared all baseline variants (Figure 2C). We did not find evidence of clonal evolution at EOBT, though this observation may be limited by the size of the targeted panel. Lastly, baseline ctDNA load significantly correlated with PFS ($p = 0.043$) and OS ($p = 0.0057$) (Figure 2D).

Interim PET analysis correlates with patient outcomes in r/r cHL

At iPET evaluation, 18 patients (33%) had persistent [^{18}F]-FDG uptake (13 PR, 1 SD and 4 PD) while 37 (67%) were in CR. Among iPET-positive patients, 13 (72%) ultimately required re-treatment: 10 (56%) due to persistent disease at EOBT and 3 (38%) who experienced treatment failure after transplant.

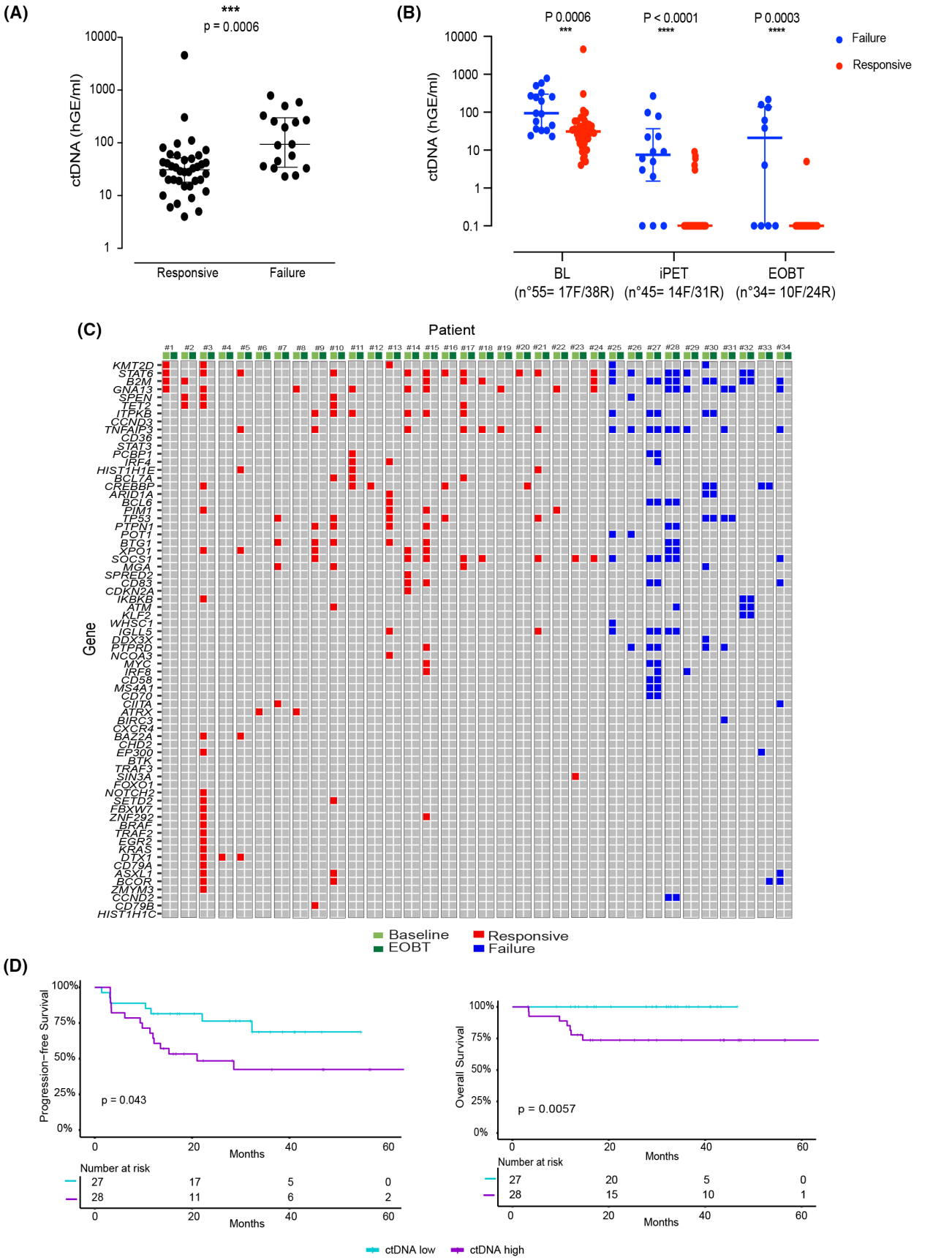


FIGURE 2 Pretreatment and on-therapy ctDNA load in 'responsive' and 'failure' patients. (A) Scatter dot plot showing pretreatment ctDNA load (hGE/mL) in 'responsive' ($n=38$) versus 'failure' ($n=17$) patients (median with 95% CI). Each dot represents a patient. The Mann–Whitney test was used for comparisons between groups. (B) Scatter dot plot showing the longitudinal (baseline, iPET, EOT) ctDNA load (hGE/mL) in 'responsive' versus 'failure' patients (median value and interquartile range). Each dot represents a patient. The Mann–Whitney test was used for comparisons. (C) Oncoprint showing the distribution of mutations in 34 r/r cHL patients for whom baseline and EOT plasma samples were available, according to response status. (D) Clinical outcomes stratified according to baseline ctDNA (greater or less than median). PFS 76.4% (95% CI 61.3–95.2) for low ctDNA and 48.5% (32.7–72.0) for high ctDNA. OS 100% for low ctDNA and 73.7% (58.7–92.5) for high ctDNA. Comparisons between groups were calculated with the log-rank test.

Among patients with a negative iPET ($n=37$), three (8%) were in PD at EOBT and only one patient (3%) relapsed 9 months after auto-SCT. At 18 months from start of BEGEV chemotherapy, EFS was 89.0% (95% CI 79.4–99.8) for iPET-negative patients versus 25.0% (95% CI 10.9–57.5) for iPET-positive patients ($p<0.0001$) (Figure 3A). Similarly, 24-month PFS and OS were 82.6% (95% CI 70.8–96.5) and 94.4% (95% CI 87.1–100), respectively, for iPET-negative patients versus 20.8% (95% CI 8.2–53.1) and 70.6% (95% CI 51.9–96.1), respectively, for iPET-positive patients (Figure 3B,C). The difference in outcomes persisted also when considering patients with DS 4 or 5 separately (Figure S8).

ctDNA quantification at the time of iPET correlates with clinical outcomes

At iPET evaluation, 45 r/r cHL patients had plasma samples available for ctDNA genotyping, which was detectable in 16 patients (36%) (Figure 4A). Of these patients, 11 (69%) also displayed persistence of significant FDG uptake, and 11 (69%) ultimately required additional therapy. Of the 29 patients with undetectable ctDNA, iPET was concordant (i.e. DS 1–3) in 80% of patients ($n=23$) and most ($n=26$, 90%) did not require additional therapy. Overall, the presence or absence of detectable ctDNA after two cycles of BEGEV chemotherapy correlated with iPET findings ($p=0.001$), disease assessment at EOBT ($p=0.0005$) as well as with EFS ($p=0.0001$) (Figure 4B). Interestingly, the evaluation of ctDNA was concordant with EFS in five of eight (63%) patients who were misclassified by iPET, suggesting that ctDNA assessment may complement standard imaging evaluation (Figure 4A, red arrows).

In addition, we assessed whether early ctDNA dynamics might predict response to therapy. By using a previously validated threshold (2-log drop of ctDNA levels after cycle 2 of chemotherapy),[2,4,15] we confirmed that early achievement of

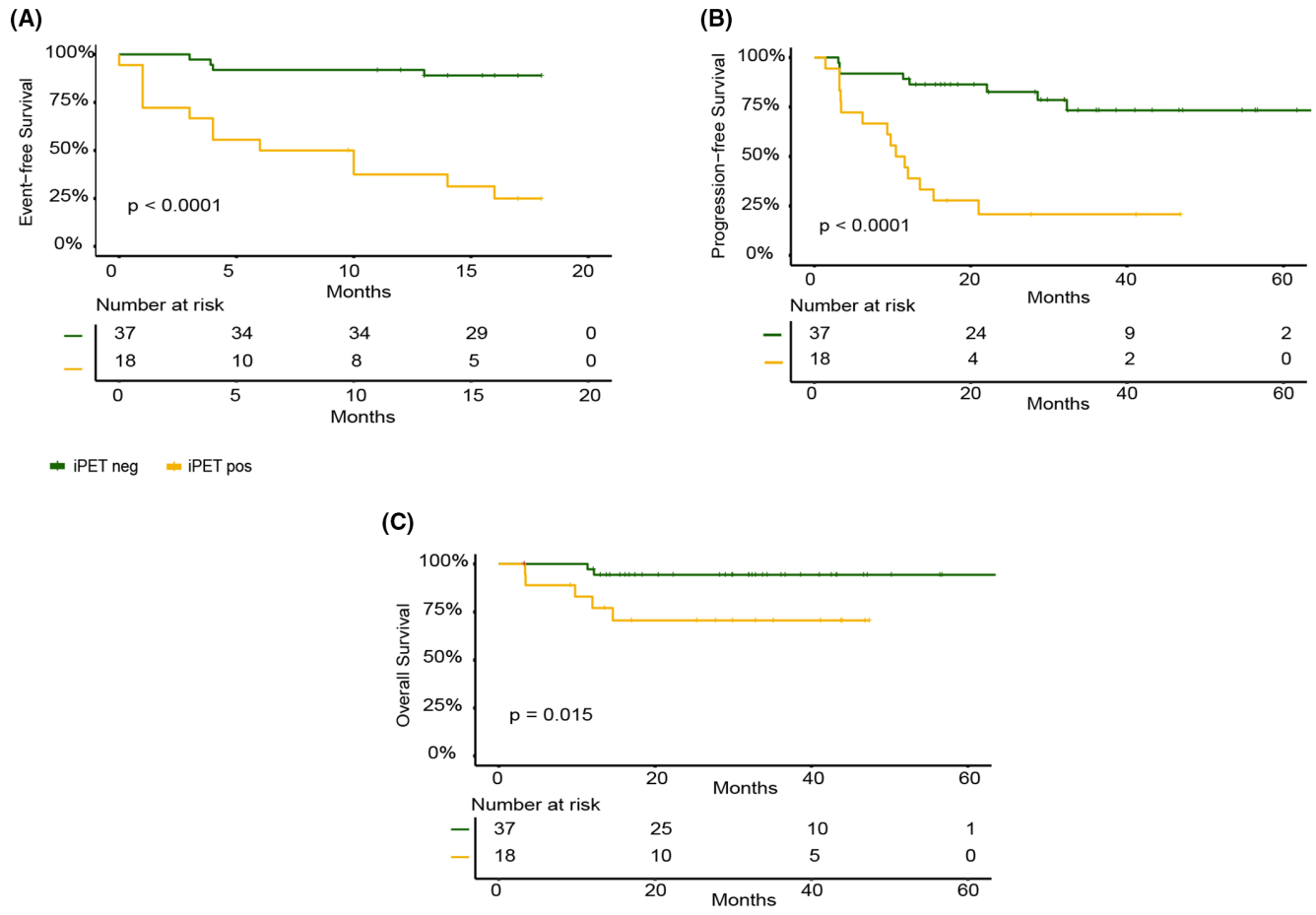


FIGURE 3 Clinical outcomes stratified according to iPET evaluation. Eighteen-month EFS (A), PFS (B) and OS (C). Comparisons between groups were calculated with the log-rank test.

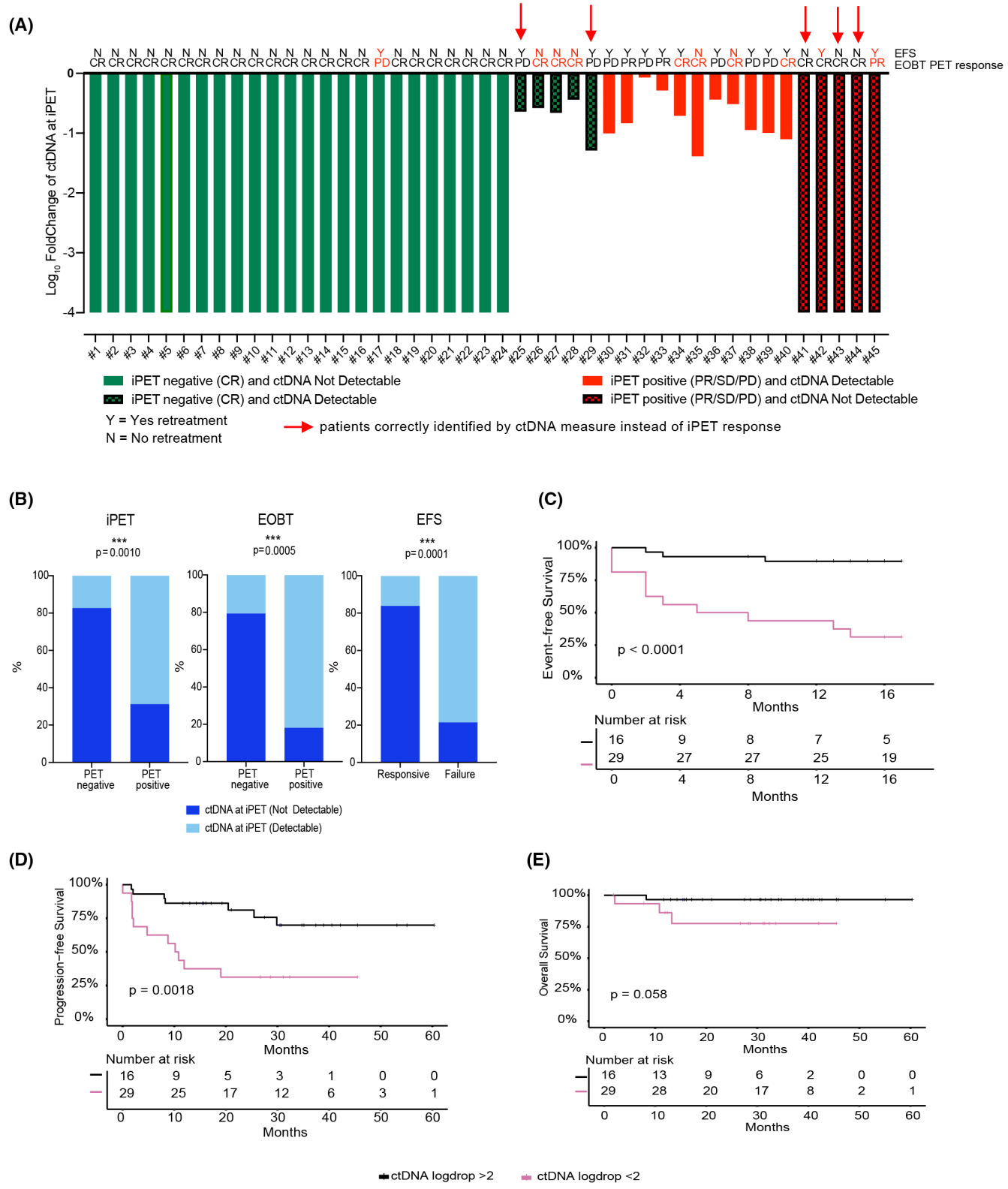


FIGURE 4 Analysis of interim ctDNA in 45 r/r cHL patients. (A) Waterfall plot showing the $\log_{10}$ fold change of ctDNA load measured at iPET compared to baseline. Each bar represents a patient. Above each bar are reported the EOT PET response (CR, PR, PD) and EFS status (in RED if clinical response not concordant with ctDNA measure). (B) Stacked bar graphs showing the proportion of detectable interim ctDNA at iPET, EOT PET and at study end-point (18-month EFS) according to response status. The *p* value calculated by Fisher's exact test is shown. (C) Clinical outcomes for all patients based on the level of reduction of ctDNA at interim evaluation. Eighteen-month EFS 89.5% (95% CI 61.3–100) for ctDNA logdrop >2 and 31.2% (15.1–64.6) for ctDNA logdrop <2. (D) Clinical outcomes for all patients based on the level of reduction of ctDNA at interim evaluation. PFS 69.9% (95% CI 52.8–92.5) for ctDNA logdrop >2 and 31.2% (15.1–64.6) for ctDNA logdrop <2. (E) Clinical outcomes for all patients based on the level of reduction of ctDNA at interim evaluation. OS 96.6% (95% CI 90.1–100) for ctDNA logdrop >2 and 77.5% (57.9–100) for ctDNA logdrop <2.

a molecular response predicted EFS and PFS, but not OS, although a trend was noted (Figure 4C–E).

Baseline ctDNA load combined with iPET evaluation can improve outcome prediction

Given the predictive significance of both baseline ctDNA load and iPET evaluation, we investigated whether integrating these findings could further improve outcome prediction. Based on the results of a Cox regression analysis, which included additional clinical variables (Table 2), we informed a recursive partitioning model to predict EFS (total events $n = 14/55$, 25%) (Figure 5A). The decision tree was generated based on the most statistically significant variables; however, the presence or absence of bulky disease did not contribute to the final nodes. Integrating baseline ctDNA and iPET resulted in an increased predictive value (accuracy rate 87.3%, sensitivity 70.6% and specificity 94.7%) when compared to baseline ctDNA or iPET alone (Figure 5B). In absolute terms, the combination of imaging analysis and molecular genotyping allows to correctly classify 48 of a total of 55 patients, compared to 46 with iPET evaluation alone and 44 patients with baseline ctDNA alone (Figure 5B). Despite the small sample size, combination of ctDNA and iPET can thus identify an additional 23% of patients for whom EFS is incorrectly predicted by iPET evaluation. Based on the results of the partitioning analysis, three categories of BEGEV-treated patients were defined. Patients with positive iPET and high baseline ctDNA (>31 hGE/mL) showed a significantly inferior treatment-free survival compared to the other two categories (iPET negative and iPET positive with low baseline ctDNA), with 91.4% (95% CI 45.2–98.7) of patients who required re-treatment within 18 months ($p < 0.0001$) (Figure 5C–E).

DISCUSSION

The outcome of patients with r/r cHL has markedly improved in recent years thanks to the use of highly active novel immunotherapeutic and targeted agents.[16–18] Nonetheless, chemotherapy followed by SCT is still the standard of care in patients failing first-line therapy, allowing to cure up to 70% of patients.[1] Integrating novel agents into chemotherapy-based induction regimens is likely to further increase the number of cured patients,[19] as is the use of maintenance therapy in the post-transplant period.[20–22] Here we assessed the potential utility of baseline plasma ctDNA quantification in improving the predictive value of iPET analysis in r/r cHL treated with the BEGEV chemotherapy protocol. Furthermore, we explored the mutational landscape of r/r cHL before treatment and longitudinally during treatment course.

TABLE 2 Univariate analysis of the association of clinical, imaging and ctDNA variables with EFS.

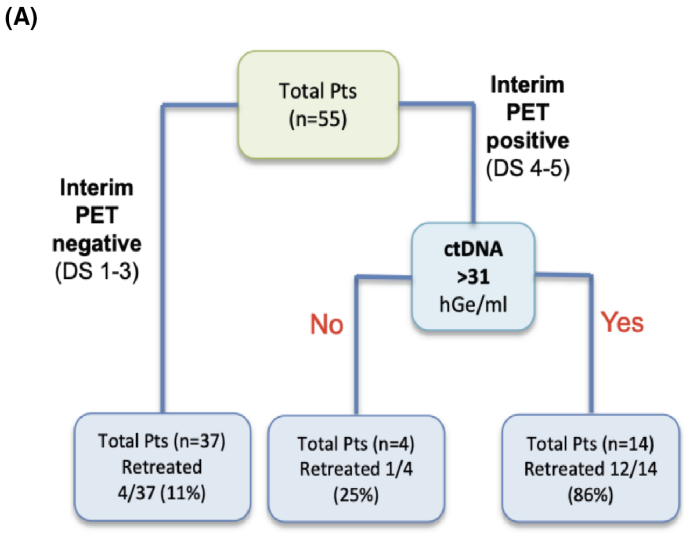
	Univariate analysis		
Variable	***p* value**	**HR**	**95% CI**
Interim PET positive	**<0.001**	10.292	[3.317–31.933]
Basal ctDNA ≥ median	**0.037**	3.056	[1.072–8.715]
Primary refractory	0.385	1.644	[0.535–5.050]
Bulky disease	**<0.001**	10.230	[3.194–32.761]
B symptoms	0.145	0.199	[0.023–1.745]
Advanced stage	0.864	0.920	[0.355–2.386]
BEGEV therapy beyond second line	0.866	1.073	[0.408–2.823]

Note: Bold values are statistically significant.

Using an ultraprecise NGS technology, we confirmed in a larger, uniform cohort that r/r cHL patients presented gene mutations that had been previously detected in a smaller similar series[4] and in the front-line setting.[4,23,24] Such mutations affect predominantly the NF-kB, JAK–STAT and PI3K–Akt pathways. Interestingly, our preliminary results confirm a previous report describing the increased frequency of *TP53* mutations in primary refractory cHL,[23] which, however, did not appear to translate into a clinical disadvantage. This finding, along with the absence of additional significant mutational patterns in patients who failed treatment, suggests that alternative mechanisms may confer chemoresistance, but requires confirmation in a larger cohort.

A growing number of reports have identified pretreatment levels of ctDNA as a predictive marker of response to therapy and outcome in lymphoid malignancies.[25] Our findings confirm such reports also in r/r cHL treated with salvage chemotherapy and consolidative SCT; elevated baseline ctDNA was significantly associated both with treatment failure within 18 months of start of chemotherapy, as well as with PFS and OS. By longitudinally monitoring ctDNA, we demonstrated that serial measurements can mimic disease evolution, with a persistence of mutated genes at the end of treatment in most patients who failed therapy. Furthermore, the absence of detectable ctDNA at interim evaluation was associated with response to therapy and favourable clinical outcomes. Thus, ctDNA may be effectively used as a tool to monitor minimal residual disease during treatment and guide clinical decisions in r/r cHL.

It is well known that the absence of CR assessed by [^{18}F]-FDG PET imaging prior to auto-SCT is a strong predictor of treatment failure.[26–28] However, the role of early [^{18}F]-FDG PET/CT assessment during induction therapy prior to SCT is still unclear. In our retrospective study, early evaluation of sensitivity to BEGEV therapy by iPET appears to have prognostic significance. However, [^{18}F]-FDG PET/CT evaluation requires a high level of expertise to integrate the numerous parameters (DS, SUVmax, disease extension) and its interpretation may be challenging. Baseline disease burden assessment by ctDNA is a candidate biomarker to complement iPET imaging data. In our study, incorporation of baseline ctDNA quantification allowed to identify a small group of patients with a positive iPET and a high baseline ctDNA characterized by a very high risk of therapy failure (91%). Most of these patients had a decreased tumour burden (metabolic PR) at iPET compared to baseline, often misguiding



(B)

Interim PET

Observed	Predicted: Responsive	Predicted: Failure
Responsive	33 (TN)	5 (FP)
Failure	4 (FN)	13 (TP)

Baseline ctDNA + interim PET

Observed	Predicted: Responsive	Predicted: Failure
Responsive	36 (TN)	2 (FP)
Failure	5 (FN)	12 (TP)

	Sensitivity	Specificity	Accuracy	Misclassified patients
Interim PET	76%	87%	84%	9
Baseline ctDNA	59%	89%	80%	11
Baseline ctDNA + interim PET	71%	95%	87.3%	7

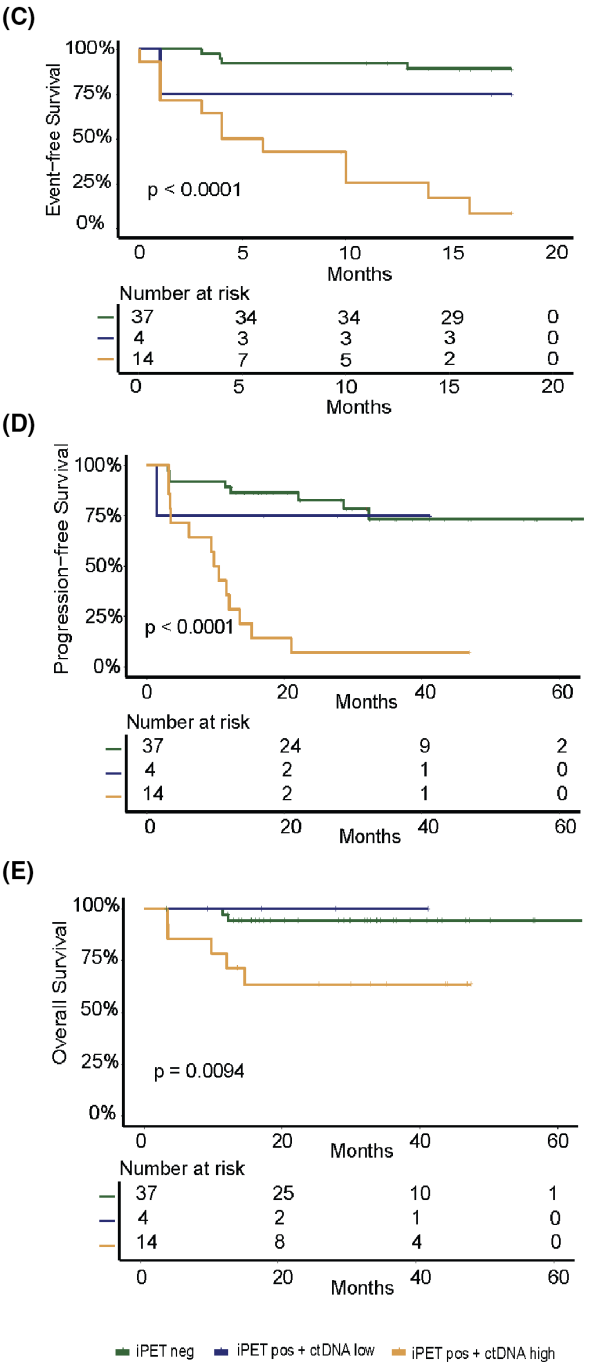


FIGURE 5 Recursive partitioning analysis. (A) Schematic representation of decision tree generated by recursive partitioning analysis integrating baseline ctDNA levels and iPET evaluation. (B) Confusion matrix obtained from recursive partitioning analysis for iPET alone (left) and iPET integrated with baseline ctDNA (right), together with sensitivity, specificity and accuracy measures. (C) Eighteen-month EFS according to categories defined by recursive partitioning analysis: 89.0% (95% CI 79.4–99.8) for iPET negative, 75.0% (42.6–100) for iPET positive and low baseline ctDNA and 8.6% (1.3–54.8) for iPET positive and high baseline ctDNA. (D) PFS according to categories defined by recursive partitioning analysis: 73.3% (95% CI 58.3–92.1) for iPET negative, 75.0% (42.6–100) for iPET positive and low baseline ctDNA and 7.1% (1.1–47.2) for iPET positive and high baseline ctDNA. (E) OS according to categories defined by recursive partitioning analysis: 94.4% (95% CI 87.1–100) for iPET negative, 100% for iPET positive and low baseline ctDNA and 63.5% (42.4–95.1) for iPET positive and high baseline ctDNA.

clinicians in completing treatment course and thus causing unnecessary toxicity. This high-risk group of patients may benefit from an earlier switch to brentuximab vedotin or PD-1 inhibitors prior to SCT to maximize chances of success and should be promptly identified.

We acknowledge that this study is limited by its retrospective nature and small sample size. Additionally, due to the rarity of the investigated patient population, it lacks a validation cohort. However, to our knowledge, it is the first report to describe the clinical utility of basal ctDNA

quantification, interim ctDNA reduction and integration of genomic data with iPET evaluation in predicting response to salvage chemotherapy in r/r cHL. Early switch to targeted and immune-based drugs should be evaluated in prospective trials to improve outcome in high-risk patients. Likewise, ctDNA is a radiation-free tool that can provide important prognostic information in r/r cHL. Current technological limitations, including potential absence of detectable variants, are likely to be overcome in the near future.[29]

AUTHOR CONTRIBUTIONS

Carmelo Carlo-Stella conceived the project. Eleonora Calabretta, Francesco Corrado, Martina Sollini, Fabrizio Marino collected clinical data. Martina di Trani and Vanessa Cristaldi performed ctDNA genotyping. Martina Sollini collected and reviewed imaging data. Eleonora Calabretta, Martina di Trani, Davide Rossi and Carmelo Carlo-Stella analysed and interpreted original data. Eleonora Calabretta, Martina di Trani and Francesco Corrado performed statistical analyses. Eleonora Calabretta and Martina di Trani, wrote the manuscript. Lodovico Terzi di Bergamo, Alessio Bruscaggin, Maria Cristina Pirosa, Stefania Bramanti, Arturo Chiti, Davide Rossi and Carmelo Carlo-Stella provided critical input and revised the manuscript.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Italian Association for Cancer Research (AIRC, grant #20575 to CC-S). Open access funding provided by BIBLIOSAN.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Individual participant data will not be shared. For original correlative data, please contact the corresponding author (carmelo.carlostella@hunimed.eu).

ETHICS STATEMENT

This study was approved by the local institutional Review Board, and all patients provided written informed consent in accordance with the Declaration of Helsinki.

ORCID

Eleonora Calabretta https://orcid.org/0000-0002-8105-1322
Carmelo Carlo-Stella https://orcid.org/0000-0003-3144-0124

REFERENCES

1. Santoro A, Mazza R, Pulsoni A, Re A, Bonfichi M, Zilioli VR, et al. Five-year results of the BEGEV salvage regimen in relapsed/refractory classical Hodgkin lymphoma. Blood Adv. 2020;4(1):136–40.
2. Kurtz DM, Scherer F, Jin MC, Soo J, Craig AFM, Esfahani MS, et al. Circulating tumor DNA measurements as early outcome predictors in diffuse large B-cell lymphoma. J Clin Oncol. 2018;36(28):2845–53.
3. Frank MJ, Hossain NM, Bukhari A, Dean E, Spiegel JY, Claire GK, et al. Monitoring of circulating tumor DNA improves early relapse detection after axicabtagene ciloleucel infusion in large B-cell lymphoma: results of a prospective multi-institutional trial. J Clin Oncol. 2021;39(27):3034–43.
4. Spina V, Bruscaggin A, Cuccaro A, Martini M, Di Trani M, Forestieri G, et al. Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma. Blood. 2018;131(22):2413–25.
5. Santoro A, Mazza R, Pulsoni A, Re A, Bonfichi M, Zilioli VR, et al. Bendamustine in combination with gemcitabine and vinorelbine is an effective regimen as induction chemotherapy before autologous stem-cell transplantation for relapsed or refractory Hodgkin lymphoma: final results of a multicenter phase II study. J Clin Oncol. 2016;34(27):3293–9.
6. Rossi D, Condoluci A, Spina V, Gaidano G. Methods for measuring ctDNA in lymphomas. Methods Mol Biol. 2019;1881:253–65.
7. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014;20(5):548–54.
8. Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AF, Esfahani MS, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. Sci Transl Med. 2016;8(364):364ra155.
9. Giobbie-Hurder A, Gelber RD, Regan MM. Challenges of guarantee-time bias. J Clin Oncol. 2013;31(23):2963–9.
10. Maura F, Zicchedda B, Xiang JZ, Bhinder B, Rosiene J, Abascal F, et al. Molecular evolution of classic Hodgkin lymphoma revealed through whole-genome sequencing of Hodgkin and reed sternberg cells. Blood Cancer Discov. 2023;4(3):208–27.
11. Spina V, Khiabanian H, Messina M, Monti S, Cascione L, Bruscaggin A, et al. The genetics of nodal marginal zone lymphoma. Blood. 2016;128(10):1362–73.
12. Ortiz B, White JR, Wu WH, Chan TA. Deletion of Ptprd and Cdkn2a cooperate to accelerate tumorigenesis. Oncotarget. 2014;5(16):6976–82.
13. Ortiz B, Fabius AW, Wu WH, Pedraza A, Brennan CW, Schultz N, et al. Loss of the tyrosine phosphatase PTPRD leads to aberrant STAT3 activation and promotes gliomagenesis. Proc Natl Acad Sci U S A. 2014;111(22):8149–54.
14. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008;455(7216):1069–75.
15. Bruscaggin A, di Bergamo LT, Spina V, Hodkinson B, Forestieri G, Bonfiglio F, et al. Circulating tumor DNA for comprehensive noninvasive monitoring of lymphoma treated with ibrutinib plus nivolumab. Blood Adv. 2021;5(22):4674–85.
16. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med. 2015;372(4):311–9.
17. Chen R, Zinzani PL, Fanale MA, Armand P, Johnson NA, Brice P, et al. Phase II study of the efficacy and safety of pembrolizumab for relapsed/refractory classic Hodgkin lymphoma. J Clin Oncol. 2017;35(19):2125–32.
18. Younes A, Bartlett NL, Leonard JP, Kennedy DA, Lynch CM, Sievers EL, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med. 2010;363(19):1812–21.
19. Moskowitz AJ, Shah G, Schöder H, Ganesan N, Drill E, Hancock H, et al. Phase II trial of pembrolizumab plus gemcitabine, vinorelbine, and liposomal doxorubicin as second-line therapy for relapsed or refractory classical Hodgkin lymphoma. J Clin Oncol. 2021;39(28):3109–17.
20. Moskowitz CH, Nademanee A, Masszi T, Agura E, Holowiecki J, Abidi MH, et al. Brentuximab vedotin as consolidation therapy after autologous stem-cell transplantation in patients with Hodgkin's lymphoma at risk of relapse or progression (AETHERA): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2015;385(9980):1853–62.
21. Armand P, Chen YB, Redd RA, Joyce RM, Bsat J, Jeter E, et al. PD-1 blockade with pembrolizumab for classical Hodgkin

lymphoma after autologous stem cell transplantation. Blood. 2019;134:22–9.
22. Herrera AF, Chen L, Nieto Y, Holmberg L, Johnston P, Mei M, et al. Brentuximab vedotin plus nivolumab after autologous haematopoietic stem-cell transplantation for adult patients with high-risk classic Hodgkin lymphoma: a multicentre, phase 2 trial. Lancet Haematol. 2023;10(1):e14–23.
23. Tiacci E, Ladewig E, Schiavoni G, Penson A, Fortini E, Pettirossi V, et al. Pervasive mutations of JAK-STAT pathway genes in classical Hodgkin lymphoma. Blood. 2018;131(22):2454–65.
24. Liang WS, Vergilio JA, Salhia B, Huang HJ, Oki Y, Garrido-Laguna I, et al. Comprehensive genomic profiling of Hodgkin lymphoma reveals recurrently mutated genes and increased mutation burden. Oncologist. 2019;24(2):219–28.
25. Roschewski M, Rossi D, Kurtz DM, Alizadeh AA, Wilson WH. Circulating tumor DNA in lymphoma: principles and future directions. Blood Cancer Discov. 2022;3(1):5–15.
26. Jabbour E, Hosing C, Ayers G, Nunez R, Anderlini P, Pro B, et al. Pretransplant positive positron emission tomography/gallium scans predict poor outcome in patients with recurrent/refractory Hodgkin lymphoma. Cancer. 2007;109(12):2481–9.
27. Mocikova H, Pytlik R, Markova J, Steinerova K, Kral Z, Belada D, et al. Pre-transplant positron emission tomography in patients with relapsed Hodgkin lymphoma. Leuk Lymphoma. 2011;52(9):1668–74.
28. Smeltzer JP, Cashen AF, Zhang Q, Homb A, Dehdashti F, Abboud CN, et al. Prognostic significance of FDG-PET in relapsed or refractory classical Hodgkin lymphoma treated with standard salvage chemotherapy and autologous stem cell transplantation. Biol Blood Marrow Transplant. 2011;17(11):1646–52.
29. Kurtz DM, Soo J, Co Ting Keh L, Alig S, Chabon JJ, Sworder BJ, et al. Enhanced detection of minimal residual disease by targeted sequencing of phased variants in circulating tumor DNA. Nat Biotechnol. 2021;39(12):1537–47.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Calabretta E, di Trani M, Corrado F, Sollini M, Cristaldi V, Marino F, et al. Baseline circulating tumour DNA and interim PET predict response in relapsed/refractory classical Hodgkin lymphoma. Br J Haematol. 2024;204(2):514–524. https://doi.org/10.1111/bjh.19162