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ORIGINAL PAPER

Chemotherapy after PD-1 inhibitors in relapsed/refractory Hodgkin lymphoma: Outcomes and clonal evolution dynamics

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Summary

Checkpoint inhibitors (CPIs) are routinely employed in relapsed/refractory classical Hodgkin lymphoma. Nonetheless, persistent long-term responses are uncommon, and one-third of patients are refractory. Several reports have suggested that treatment with CPIs may re-sensitize patients to chemotherapy, however there is no consensus on the optimal chemotherapy regimen and subsequent consolidation strategy. In this retrospective study we analysed the response to rechallenge with chemotherapy after CPI failure. Furthermore, we exploratively characterized the clonal evolution profile of a small sample of patients ($n = 5$) by employing the CALDER approach. Among the 28 patients included in the study, 17 (71%) were primary refractory and 26 (92%) were refractory to the last chemotherapy prior to CPIs. Following rechallenge with chemotherapy, response was recorded in 23 (82%) patients experiencing complete remission and 3 (11%) patients experiencing partial remission. The tumour evolution of the patients inferred by CALDER seemingly occurred prior to the first

cycle of therapy and was characterized either by linear or branching evolution patterns. Twenty-five patients proceeded to allogeneic stem cell transplantation. At a median follow-up of 21 months, median PFS and OS were not reached. In conclusion, patients who fail CPIs can be effectively rescued by salvage chemotherapy and bridged to allo-SCT/auto-SCT.

INTRODUCTION

Classical Hodgkin Lymphoma (cHL) is one of the most common malignancies in adolescents and young adults. Fortunately, most cases are cured by standard chemoradiotherapy. However, patients who relapse after standard regimens or are primary refractory have a poorer outcome. In this scenario, checkpoint inhibitors (CPIs) have shown impressive efficacy, producing significant remission rates with manageable toxicities, and have been approved for clinical use.[1,2] However, long-term disease control is uncommon, and most patients ultimately become resistant to CPIs and experience disease progression, with a median overall survival (OS) of around one year upon treatment discontinuation.[3,4] Such patients, together with those who do not respond to checkpoint inhibition, represent an unmet medical need. Though promising therapies directed against novel immunological targets are currently under evaluation in clinical trials,[5–7] no treatment alternatives have yet been validated.

In this setting, physicians have recently reported a considerable efficacy of conventional chemotherapy in a small series of solid tumours progressing after PD-1 inhibitors.[8,9] Accordingly, such results were confirmed in relapsed/refractory classical Hodgkin Lymphoma (r/r cHL) in two small French and Italian cohorts[10,11] and in a subsequent larger multicentric analysis.[12]

We here retrospectively analysed the outcomes of a multicentric cohort of chemo-refractory patients. These patients failed to respond to PD-1 inhibitors and were subsequently administered salvage chemotherapy and addressed to allogeneic stem cell transplantation (allo-SCT), in the attempt to identify the optimal therapeutic strategy. Additionally, we performed an exploratory analysis aimed at characterizing the clonal evolution profile of a small sample of patients, to investigate whether subclonal modifications and/or suppression could determine response to chemotherapy.

PATIENTS AND METHODS

Patients

A total of twenty-eight consecutive patients affected by cHL (age ≥ 18 years) who were refractory (n = 10, 36%) to PD-1 inhibitors or experienced disease progression after initial response (n = 18, 64%), from March 2017 to November 2020, were included in the study (Table 1). Patients were treated in three different hospitals: Humanitas Research Hospital (n = 17), Istituto Nazionale dei Tumori di Milano (n = 8), Istituto Europeo di Oncologia (n = 3). All the included centers shared similar institutional guidelines and, as such, addressed all patients failing PD-1 inhibitors to salvage chemotherapy. Sixteen patients (57%) received Nivolumab within the CA209-205 phase II clinical trial at a fixed dose of 3 mg/kg every 14 days, one patient (4%) received Nivolumab within an extended access program for patients relapsing after allo-SCT at a dose of 3 mg/kg once a month, eight patients (28%) received EMA-approved Nivolumab at a flat dose of 240 mg every 14 days, and three patients (11%) received EMA-approved Pembrolizumab at a flat dose of 200 mg every 21 days. Eligibility criteria included: (i) diagnosis of cHL; (ii) age ≥18 years; (iii) proven disease progression by PET-CT after therapy with

TABLE 1 Main patients' characteristics

	All n = 28
Gender	
Male	21 (75%)
Female	7 (25%)
Median age (range)	29 (19–71)
Stage prior to anti-PD1 therapy	
I–II	6 (21%)
III–IV	22 (79%)
Extranodal disease prior to anti-PD1 therapy	21 (75%)
B symptoms prior to anti-PD1 therapy	10 (36%)
Bulky disease prior to anti-PD1 therapy	6 (21%)
Response to anti PD-1 therapy	
Refractory	10 (36%)
Responsive	18 (64%)
Median number of anti PD-1 cycles (range)	13 (3–72)
Median duration of anti PD-1 therapy (mos)	6 (2–34)
Median number of prior therapies (range)	4 (2–11)
Prior BV	28 (100%)
Prior ASCT	18 (64%)
Prior RT	14 (50%)
Response to last chemotherapy prior to anti-PD1 therapy	
Refractory	26 (92%)
Responsive	2 (8%)

a PD-1 inhibitor and (iv) Eastern Cooperative Oncology Group performance status (0–1). Patients who exclusively received radiation therapy after disease progression were excluded from the study. Prior allogeneic stem cell transplantation was not considered an exclusion criterion. This study was approved by the institutional Ethical Committee of all participating centers, and all patients provided written informed consent in accordance with the Declaration of Helsinki.

Study design

The primary objective of this retrospective multi-centric study was to assess the effectiveness of salvage chemotherapy after failure of PD-1 inhibitors. The primary endpoint of the study was overall response rate. All patients received various chemotherapy regimens, some of which included agents that had been administered prior to CPIs. Disease response was evaluated by PET-CT according to the Lugano classification[13] at the end of the regimen; a PET/CT scored as 4 or 5 using the Deauville 5-point scale was considered positive; when clinically indicated, response assessment was anticipated. Visual analysis was independently performed by two experienced imagers blinded to patients' characteristics. If an objective response was reached, eligible patients were addressed to allogeneic stem cell transplantation (allo-SCT). Secondary objectives included overall survival (OS) and progression-free survival (PFS).

Circulating tumour DNA (ctDNA) genotyping

The CAPP-seq strategy (Cancer Personalized Profiling by deep Sequencing), an ultrasensitive capture-based targeted sequencing, was used to genotype ctDNA of 5 patients treated with BeGEV (Bendamustine, Gemcitabine, Vinorelbine).

Blood samples as source of plasma and peripheral blood mononuclear cells (PBMCs) were collected prior to chemotherapy initiation, at the time of interim PET and at the end of treatment (this time point is not present for 3 of the 5 patients). We analysed cfDNA (cell-free DNA) extracted from plasma and paired DNA from PBMCs, as source of germline DNA (gDNA) to filter out polymorphism and sequencing errors.

A targeted resequencing panel optimized to include the coding exons and splice sites of 133 genes (320 Kb) that are recurrently mutated in B-cell lymphomas was used. Sequencing was performed using the Nextseq 550 platform (Illumina) to obtain a depth of coverage >2000x in >80% of the target region in all samples. A robust and previously validated bioinformatics pipeline was used for somatic analysis.[14]

Additional methodological details can be found in the Supplementary Materials.

Reconstruction of longitudinal cancer evolution models via CALDER

We employed the Cancer Analysis of Longitudinal Data through Evolutionary Reconstruction (CALDER) approach,[15] a computational method for the inference of phylogenetic tumour trees from longitudinal bulk sequencing data, to reconstruct the evolution models of 5 treated R/R cHL patients. Notice that approaches with similar goals have been recently proposed to exploit single-cell sequencing data.[16]

In brief, CALDER takes as input the reference and alternative read count of a list of selected genomic variants, detected in samples collected from single patients at subsequent time points.

CALDER solves a Non-negative Matrix Factorization (NMF) problem and returns both the longitudinal phylogenetic tree describing the accumulation of genomic variants and the clonal prevalence variation at each time point, by maximizing the likelihood with respect to the input data. Every dataset includes the reference and alternative reads count for a panel of genomic variants, for each patient and each time point, as obtained via somatic analysis from ctDNA sequencing, as previously described. Furthermore, to reduce the number of features and the impact of noise, we here clustered all the variants occurring on the same gene, by summing their reference and alternative read counts. As a result, we here consider the counts of all variants hitting a specific gene in that sample. CALDER was run with default parameters and with the Gurobi 8.0.1 optimizer(http://www.gurobi.com). After the inference, we used the CALDER output as input for TimeScape[17] in order to obtain a user-friendly visualization of the clonal evolution model of every single tumour under analysis. The outcoming clonal evolution models are presented in Figure 3 of the main text.

Statistical analysis

Analysis was performed using SPSS version 21.0.0 and R version 3.6.1. Categorical variables were expressed as proportions with the respective 95% confidence intervals, and continuous variables were expressed as the medians with the respective range. Fisher's exact test was used for comparisons between categorical variables. The Kaplan–Meier method was used for OS and PFS analyses, which were calculated starting from the date of initiation of chemotherapy after CPI failure.

RESULTS

Patients' characteristics

The patients' characteristics are summarized in Table 1. From March 2017 to November 2020, 28 patients with relapsed/refractory cHL (r/r cHL) were included in the study.

Median age at diagnosis was 29 years (range, 19–71). Most patients had advanced stage (III/IV) cHL (70%) and had received a median of 4 (range, 2–11) therapies prior to CPI, including Brentuximab Vedotin (BV, 100%), autologous stem cell transplantation (ASCT, 64%) and radiation therapy (50%). One patient had relapsed after allo-SCT. Almost all patients (n = 26, 92%) were refractory to the last therapy prior to CPIs; 21 (75%) patients presented with extranodal involvement and 10 (36%) had B symptoms. Anti-PD1 therapy was administered for a median of 13 cycles (range, 3–72), for a median duration of 6 months (range, 2–34). The best overall response (BOR) during CPI treatment was CR in 3 patients (11%; 95% CI 0–22), PR in 15 patients (53%; 95% CI 35–71), SD in 7 patients (25%; 95% CI 9–41) and PD in 3 patients (11%; 95% CI 0–22); however, at end of treatment all patients had PD. Of the 3 patients who achieved CR, one had relapsed after allo-SCT, one interrupted treatment due to toxicity (grade 3 inflammatory colitis) and relapsed shortly thereafter, and one patient refused allo-SCT and ultimately progressed at 2 months.

Response to salvage chemotherapy

Disease progression after therapy with PD-1 inhibitors was assessed by PET-CT (all patients) and repeat histological evaluation (n = 9). All patients were then addressed to salvage chemotherapy. Median time to chemotherapy was 1.4 months (range 0.3–15.2). Choice of regimen was dependent on both prior administered regimens and center-specific guidelines/experience. The following chemotherapy regimens were administered: BEGEV (n = 11, 38%), BEACOPP (n = 4, 14%), L-PAM + ASCT (n = 4, 14%), FEAM +ASCT (n = 2, 7%), Gemcitabine (n = 2, 7%), IGEV (n = 1, 4%), HD-VP-16 (n = 1, 4%), BV (n = 1, 4%), Bendamustine (n = 1, 4%), DHAOx (n = 1, 4%). Chemotherapy was well tolerated; one patient developed encephalitis after one cycle of chemotherapy, which was treated with broad spectrum antibiotics and antiviral therapy, with complete resolution. Twenty-six patients (93%; 95% CI 84–100) were responsive to chemotherapy: 23

patients (82%; 95% CI 68–96) achieved CR and 3 patients (11%; 95% CI 0–22) achieved a partial response. Disease response achieved during treatment with anti-PD1 inhibitors did not correlate with response to chemotherapy (p = 0.357, Fisher's Exact test, see Figure S1). Interestingly, all patients who received BEGEV chemotherapy after anti-PD-1 failure achieved a CR. Furthermore, among such patients, two (18%) had been exposed to BEGEV just prior to PD-1 inhibition with evidence of progressive disease (PD) at the end of therapy (Figures 1 and 2). Duration of response to post-CPI chemotherapy was not assessable, as, according to internal policies, all eligible patients were addressed to allo-SCT, except two patients, one treated with autologous-SCT and another one, previously treated with allogeneic transplant, who was not transplanted. Therapies prior to and after CPIs with the responses obtained are summarized in Table 2.

ctDNA dynamics during treatment course and reconstruction of clonal phylogeny

We followed serial plasma samples during therapy with PD-1 inhibitors and subsequent salvage chemotherapy with BeGEV in a small cohort of 5 patients. Serial measurements of ctDNA were normalized to pretreatment levels and expressed as base 10 log hGE/ml.

After 2 cycles of salvage chemotherapy with BeGEV all patients displayed a drop in ctDNA, which is consistent with complete metabolic response assessed by interim PET-CT (figure not shown). These patients maintained the response, were addressed to allo-SCT and are currently disease free, supporting previous reports that ctDNA monitoring may be a useful tool in predicting response to chemotherapy also in the salvage setting.[18]

Additionally, we applied CALDER[15] to the Variant Allele Frequency profile of a panel of selected genomic variants, computed via variant calling (somatic analysis) from ctDNA sequencing data, for each longitudinal sample of the patients. Variants were aggregated at the gene level, i.e., the read counts of all variants detected
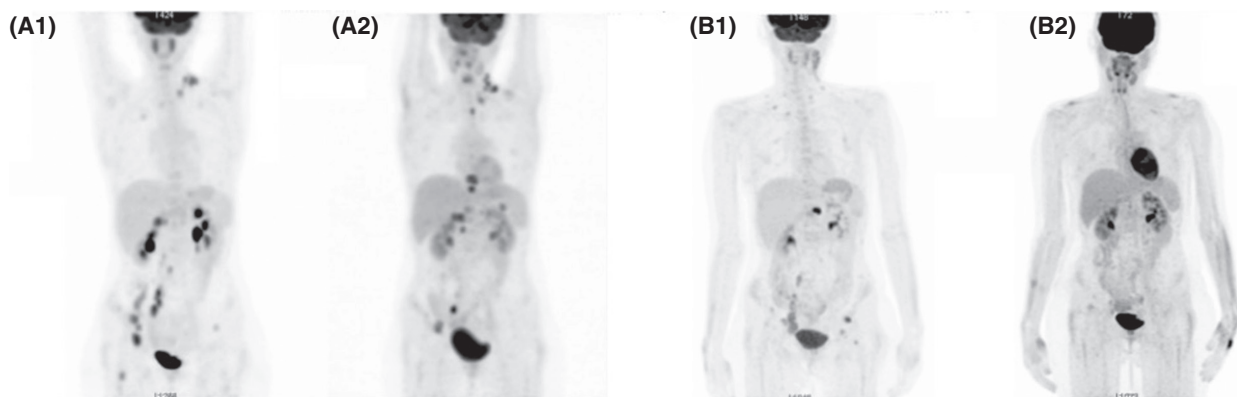

FIGURE 1 Response to BEGEV: (A) pre-Nivolumab: persistence of pathological uptake at the left latero-cervical level, at the hepatic hilum, and in proximity of right iliac vessels. New lesions at the left superior paratracheal level, at the splenic hilum, para-cavally. Two new bone lesions in two dorsal vertebral bodies; persistence of bone lesions in the left pelvis. **Deauville score (DS) 5**; (B) post-Nivolumab. Complete regression of previous areas of uptake. **DS3**
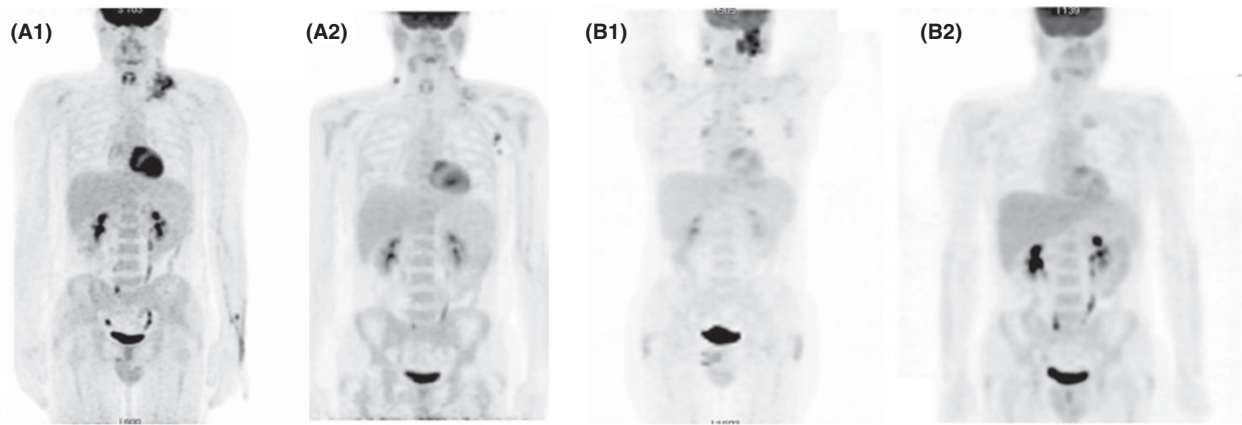
FIGURE 2 Response to BEGEV: (A) pre-Nivolumab: appearance of new small adenopathies in the right latero-cervical area and the left axillary area. **DS5**; (B) post-Nivolumab: complete regression of the lesions previously present in the bilateral laterocervical area, subclavicular, mediastinal and pulmonary hilar areas. Complete response to treatment. **DS3**

on a specific gene were added cumulatively, to reduce the impact of noisy measurements and limit the number of significant variables, as proposed for instance in[19] (See Methods section).

In Figure 3 one can see the longitudinal cancer evolution models returned by CALDER and visualized via TimeScape,[17] in which distinct clones are characterized by different sets of accumulating genomic alterations. Interestingly, the tumour evolution of all patients seems to occur prior to the first cycle of therapy and is characterized either by linear (0003) or branching evolution patterns (0005, 0008, 0024 and 0113), involving gene alterations in temporally ordered accumulation paths. In this respect, we note that the specific temporal orderings among genomic alterations should be considered with a certain caution. Even though the models returned in Figure 3 represent the optimal solutions returned by CALDER, equivalent and slightly different orderings might be possible (as for any deconvolution method), especially due the data-specific noise levels that are inherently present in the data.

Finally, for all patients, all (sub)clones seem to be depleted before the first ctDNA assessment (interim timepoint) and are not detected thereafter. This notable result shows that ctDNA monitoring can provide an effective tool to support clinicians for the definition (or discontinuation) of cancer therapies.

Patient outcomes: allogeneic stem cell transplantation

According to institutional policies, all patients who obtained an objective response were addressed to allo-SCT, if eligible (n = 25). Only one patient received consolidation with autologous stem cell transplantation. The patient who had relapsed after allo-SCT, continued solely with clinical observation and experienced PD after approximately 5 months. After a median follow up of 21 months from initiation of chemotherapy after CPIs failure, two-year OS was 80.0% months (95%

confidence interval [CI] 71.9–88.1), while two-year PFS was 70.7% months (95% CI 61.0–80.4) (Figure 4). Of the twenty-eight patients included in this study, twenty-one are alive and in CR, two are alive with PD, one died of PD, three died of PD after allo-SCT and one died of post-transplant toxicity (interstitial pneumonia) while in CR. Outcomes of the single patients are summarized in the Swimmer Plot (Figure S2).

DISCUSSION

We here demonstrate that administering chemotherapy in patients affected by r/r cHL who failed therapy with CPIs is an effective and feasible option and provides a potential bridge to allogeneic transplantation, thus redefining the prognosis in such patients. In our cohort of heavily pretreated chemo-refractory cHL patients, nearly all patients achieved an objective response; median OS and PFS were not reached after a follow up of 21 months. Our study confirms a previous retrospective report of 17 r/r cHL patients progressing on anti-PD-1 therapy, who were administered various chemotherapy regimens with an ORR of 59%.[10] Such results were also recently reported in a larger multicentric analysis including 81 patients, with an ORR of 62%[12]; in the latter study, however, post-CPI therapy included also allo-SCT conditioning and immune-based therapy. Lastly, an additional monocentric retrospective analysis has also addressed the same issue, with comparable results.[11]

Interaction and potential synergistic effects between immunotherapy and chemotherapy have been well established; chemotherapy induces cell death, thereby augmenting tumour antigen exposure to the immune system. Additionally, chemotherapy drugs can alter the tumour microenvironment by decreasing the number of regulatory T cells and myeloid-derived suppressor cells,[20,21] or by downregulating inhibitory checkpoint molecules such as PD-L1.[22] Such findings have been confirmed in the clinical setting, with combination of chemotherapy and immunotherapy producing enhanced response rates in non-small cell lung cancer,

TABLE 2 Summary of chemotherapy regimens administered before and after treatment with PD-1 inhibitors

Patient	Chemo-refractory[a]	Last chemo before anti-PD1	Disease response	PD-1 inhibitors (no. of cycles)	Chemo post anti-PD1	No. of cycles	Disease response
1	NO	BRENTUXIMAB	PD	10	BEACOPP	2	CR
2	YES	BENDAMUSTINE	PD	24	BEACOPP	2	CR
3	YES	BRENTUXIMAB+BENDA	PD	32	BEACOPP	2	PR
4	YES	FEAM + ASCT	PD	10	BEACOPP+RT	4	SD
5	YES	FEAM + ASCT	SD	41	BEGEV	4	CR
6	NO	BRENTUXIMAB	PD	53	IGEV	2	CR
7	YES	FEAM + ASCT	SD	55	BEGEV	4	CR
8	YES	BRENTUXIMAB	PD	37	BEGEV	4	CR
9	YES	RMACOBP	PD	15	BRENTUXIMAB	5	CR
10	YES	DHAP	PD	23	L-PAM + ASCT	/	CR
11	YES	BRENTUXIMAB	PD	74	BEGEV	1	CR
12	YES	BEGEV	PD	12	BEGEV	2	CR
13	YES	BRENTUXIMAB	PD	72	BEGEV	4	CR
14	YES	BEGEV	PD	12	BEGEV	1	CR
15	YES	GEM + VNR	PD	31	BEGEV	7	CR
16	YES	GVD	PR	8	BEGEV	4	CR
17	NA	BRENTUXIMAB	PD	3	GEM	4	PD
18	YES	BRENTUXIMAB	PD	17	HD-CTX + FEAM + ASCT	/	CR
19	YES	GDP	PD	12	FEAM + ASCT	/	CR
20	YES	FEAM + ASCT	PD	12	HD-VP-16	/	PR
21	YES	BRENTUXIMAB	PD	10	GEM	2	PR
22	YES	BRENTUXIMAB	PD	8	L-PAM+ ASCT	/	CR
23	YES	BRENTUXIMAB	PD	10	L-PAM+ ASCT	/	CR
24	YES	BRENTUXIMAB + BENDA	PD	7	L-PAM+ ASCT+RT	/	CR
25	YES	BRENTUXIMAB	PD	10	BENDA	3	CR
26	YES	BRENTUXIMAB	SD	29	BEGEV	2	CR
27	YES	BRENTUXIMAB	PD	13	BEGEV	3	CR
28	YES	CTX	PD	12	OxDHA + RT	1	CR

BENDA, bendamustine; ASCT, autologous stem cell transplantation; CTX, cyclophosphamide; RT, radiation therapy; HD, high-dose.

[a]Refractory to 1st line therapy.

advanced melanoma as well as cHL.[23–25] Furthermore, recent data have suggested that a sequential strategy of immunotherapy followed by chemotherapy may be equally effective, both in preclinical[26] and clinical settings.[8,27,28]

Our study confirms the significant efficacy of chemotherapy administered after immune-based therapy, especially considering that most patients were refractory to the last chemotherapy administered prior to CPIs and few of them were re-treated with drugs that were included in previously ineffective regimens. Due to the retrospective nature of this study, we cannot exclude a selection bias resulting in an over-estimation of the efficacy data. In particular, we cannot exclude that patients who were addressed to chemotherapy were selected among those with a pre-existing chemosensitivity. However, prior to CPIs, nearly all patients were overtly refractory to their last chemotherapy. Additionally, in our study, when compared to similar studies, most patients received either high-dose chemotherapy or a polychemotherapy regimen (n = 23, 82%), which has been suggested to increase the likelihood of response by Casadei et al. (Table 3).

Despite evidence from multiple reports in solid tumours and haematological malignancies that immunotherapy may restore chemosensitivity, there is lack of data on which chemotherapy may produce the best results. It is well established that Gemcitabine displays immunomodulatory effects[29–31]; indeed, among our small cohort of patients, those treated with Gemcitabine-containing regimens (n = 13, 45%) were more represented when compared to similar studies (Table 3) and achieved excellent responses. Specifically, the BeGEV regimen, which has been successfully studied as second-line salvage therapy in cHL,[32] produced CR in all patients. Furthermore, two patients who were refractory to BeGEV before CPIs overcame their chemo-resistance. Though this finding is limited by the small sample, our data suggest that this regimen may be considered as a primary choice for patients who fail PD-1 inhibitors.
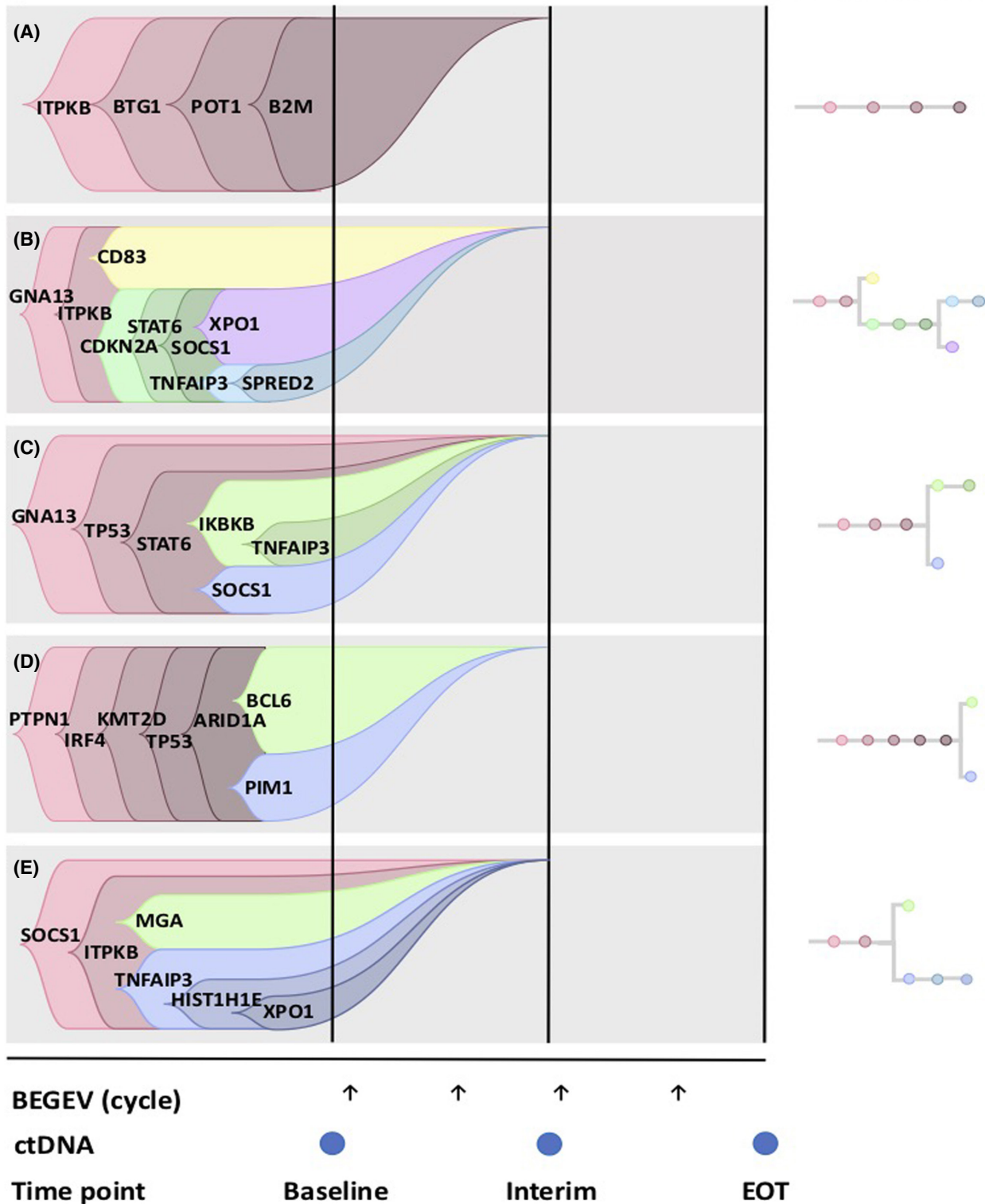
FIGURE 3 Longitudinal cancer evolution model returned by CALDER from the VAF profiles of 5 consecutive R/R cHL patients failing CPIs and subsequently addressed to BeGEV chemotherapy. Arrows represent BeGEV cycles, while dots indicate plasma collection for ctDNA profiling. Of note, patients C, D and E (0008, 0024 and 0113) do not have a plasma sample at end of treatment (EOT)

It is widely accepted that cancer is driven by the accumulation of somatic mutations. In this respect, data-science methods can exploit the temporal information of longitudinal samples to reconstruct the likely evolutionary history of the tumour, by leveraging accumulating genomic mutations as barcodes to identify putative (sub) clones, as proposed in[15] with bulk sequencing data and, more recently, in[16] with single-cell sequencing data.
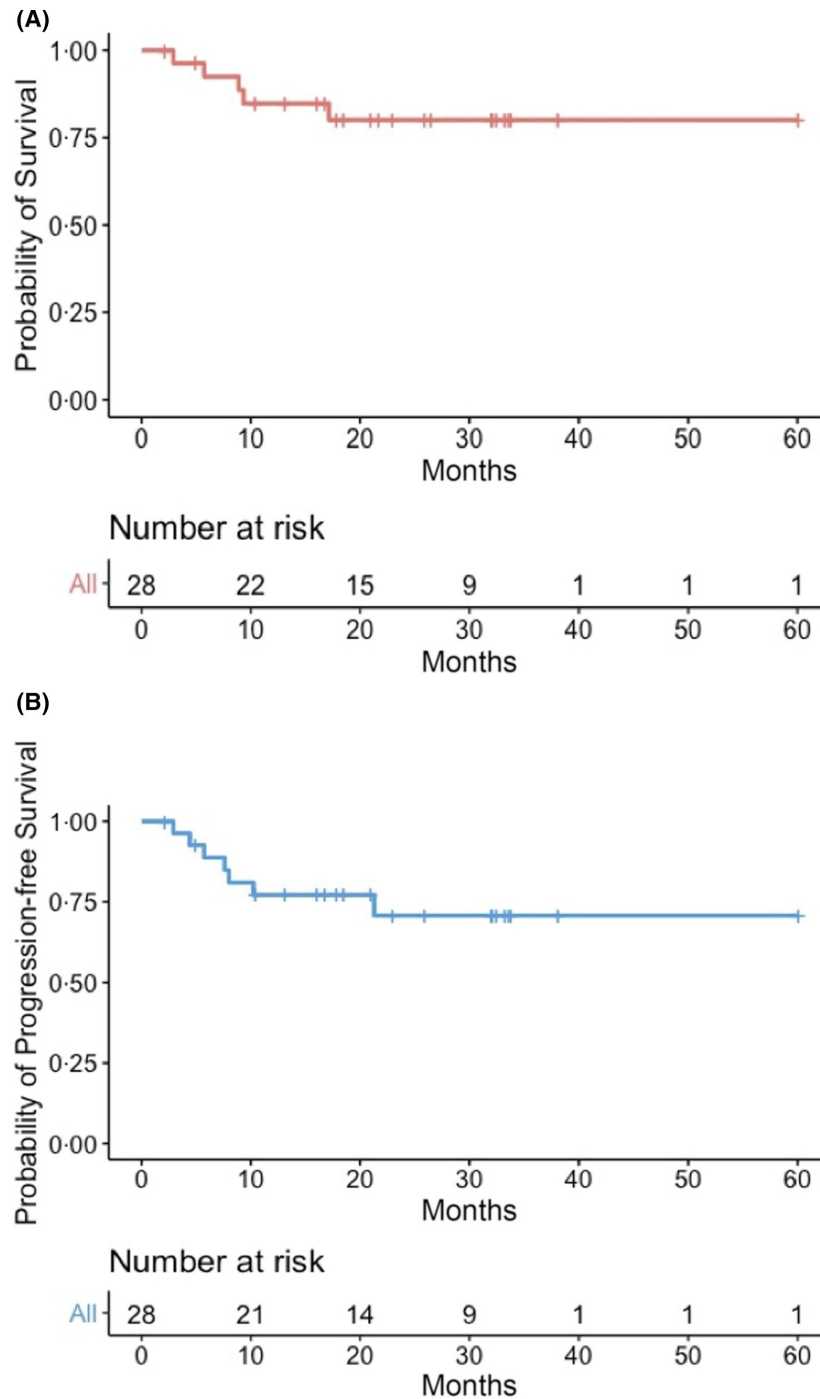
FIGURE 4 Kaplan-Meier curves. OS (A) and PFS (B) for the whole population [Colour figure can be viewed at wileyonlinelibrary.com]

Accordingly, the outcoming models allow to highlight the clonal prevalence variation in relation to the distinct therapeutic cycles in any given patient, possibly pinpointing sensitive and resistant subclones. To our knowledge, this is the first report of application of CALDER, a novel algorithm for the reconstruction of clonal phylogenesis, in r/r cHL patients uniformly treated with BeGEV chemotherapy. Surely, the sample is not sufficient to assess a possible predictive value of the inferred models, however the tumour evolution of all patients seems to occur prior to the first cycle of therapy and is characterized either by linear (0003) or branching evolution patterns (0005, 0008, 0024 and 0113), involving genomic alterations in temporally ordered accumulation paths, and is coherent with clinical course. Interestingly, TP53-mutated subclones were detected in two patients, supporting recent reports associating TP53 gene mutations with refractory cHL.[33] Further, we may speculate that Anti PD-1 priming followed by chemotherapy could overcome the adverse prognostic influence of TP53 mutations.

TABLE 3 Summary of studies assessing susceptibility to chemotherapy after checkpoint inhibition in r/r cHL

	Rossi et al.[10]	Carreau et al.[12]	Casadei et al.[11]	Calabretta et al. (this paper)
N of patients	19	81	25	28
Median age, years	44	39	32	29
Median N of prior therapies (N, range)	6 (2–14)	4 (1–11)	4 (1–10)	4 (2–11)
Prior auto-SCT (N, %)	10 (53%)	34 (42%)	11 (44%)	18 (64%)
Number of cycles of anti-PD1 therapy (median)	10	8	14	13
Disease status at end of anti-PD1 therapy (N, %)				
SD/PD	17 (89%)	55 (68%)	17 (68%)	28 (100%)
PR	2 (11%)	–	6 (16%)	–
CR	–	1 (1%)	2 (8%)	–
unknown	–	25 (31%)	–	–
Type of chemotherapy (N, %)				
Polychemotherapy	8 (42%)	30 (37%)	10 (40%)	23 (82%)
Single agent	11 (58%)	22 (27%)	15 (60%)	5 (18%)
Conditioning regimen[a]	–	11 (14%)	–	–
Non-chemotherapy	–	18 (22%)	–	–
Gemcitabine-containing regimens (N, %)	4 (21%)	17 (21%)	4 (16%)	13 (45%)
ORR (CR), %	59 (41%)	62 (42%)	60 (32%)	93 (82%)
Consolidation with allo-SCT (N, %)	3 (16%)	22 (27%)	4 (16%)	25 (89%)
Consolidation with auto-SCT (N, %)	–	16 (20%)	4 (16%)	1 (4%)
Median followup (mo)	12.1	18	32.4	21
Median PFS, mo	11	6.3	19.1	Not reached
Median OS, mo	Not reached	21	Not reached	Not reached

[a]Prior to allogeneic stem cell transplantation.

Patients who progress after PD-1 inhibitors have a poor prognosis, with a median OS upon treatment discontinuation of 13.5 months. Although prolongation of CPIs despite PD is associated with clinical benefit and a survival advantage,[3] patients will nonetheless require a new systemic therapy after a median of 8.8 months from initial progression. In this setting, administration of chemotherapy may rescue a substantial number of young patients who can be safely and effectively directed to allo-SCT.[34,35] Indeed, though our case series is limited, we report a 2-year OS of 80.0% from discontinuation of PD-1 inhibitors, which is likely affected by the high proportion of patients who underwent allo-SCT, as compared to similar studies. In our cohort, transplant-related mortality was low (n = 1), confirming that allo-SCT in this setting is feasible and can produce long-lasting disease control. Additionally, we can speculate that patients with chemo-refractory disease who are unable to undergo auto-SCT may benefit from exposure to CPIs in terms of re-sensitization to high-dose chemotherapy, thus potentially rediscussing the need for consolidation with allo-SCT.[36]

In conclusion, our study confirms previous reports describing the efficacy of chemotherapy when administered after checkpoint inhibition. In this setting, gemcitabine-containing polychemotherapy regimens may be considered a primary choice for salvage chemotherapy. Further, implementation of ctDNA profiling and bio-informatic models to current clinical practice, appears to be a promising, but still rather exploratory, approach to monitor disease and potentially predict clinical outcome.

AUTHOR CONTRIBUTIONS
C.C-S. conceived the project. E.C., A.G., F.R., C.M., M.Ma., S.B., A.d.R., M.Me., and E.D. collected clinical data. M.D.T., performed ctDNA genotyping. D.M. and A.G. performed CALDER analysis. E.C., M.D.T., M.A., D.R., and C.C-S., analysed and interpreted original data. E.C. performed statistical analysis. E.C., M.D.T., D.M. and A.G. wrote the manuscript. M.Me., S.B., E.D., D.R., P.C., A.S., and C.C-S. provided critical input and revised the manuscript.

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