Safety and efficacy of the combination of copanlisib and nivolumab in patients with Richter's transformation or transformed non-Hodgkin lymphoma: results from a phase I trial

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Abstract

Despite advances in targeted and cellular therapies, outcomes for patients with Richter's transformation (RT) and transformed non-Hodgkin lymphoma (tNHL) remain dismal. In this report of a phase I multicenter investigator-sponsored study we describe the safety and efficacy of the combination of copanlisib, a selective, small molecule inhibitor of phosphoinositide-3-kinase, and nivolumab, an antibody against programmed cell death protein 1. Twenty-seven adult patients with relapsed and/or refractory RT or tNHL were treated with escalating doses of copanlisib IV on days 1, 8, and 15 (dose level [DL] 1 - 45 mg, DL2 - 60 mg) combined with nivolumab 240 mg IV on days 1 and 15 of a 28-day cycle. Three dose-limiting toxicities occurred in two patients treated at DL2, hence 45 mg was determined to be the maximum tolerated dose and utilized in the expansion cohort. The most common treatment-related adverse events were diarrhea and anemia. All patients went off protocol, predominantly because of progressive disease and adverse events (67% and 26% of patients, respectively). The overall response rate (ORR) was 46%. Patients with transformed follicular lymphoma had an ORR of 67% (2 complete responses), with median progression-free survival of 4.4 months (95% confidence interval: 1.4-12.2). Patients with RT had an ORR of 31% (2 complete responses) with a median progression-free survival of 2.0 months (95% confidence interval: 0.7-4.9). Treatment resulted in downregulation of MYC and NFκB pathways in malignant B cells. Responding RT patients exhibited sustained activation of interferon-α and interferon-γ signaling pathways in $CD4^+$ and $CD8^+$ T cells. Overall, treatment with copanlisib and nivolumab demonstrated manageable toxicity and promising clinical efficacy in tNHL patients.

Introduction

Transformation from indolent non-Hodgkin lymphoma (NHL) to aggressive lymphoma (most commonly diffuse large B-cell lymphoma [DLBCL]) represents an entity that is challenging to treat. It can occur in any indolent NHL including follicular lymphoma (FL), lymphoplasmacytic lymphoma, marginal zone lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukemia (CLL). Transformation occurs at a rate of 0.5-3% per year with the median survival ranging from 16-60 months depending on the subtype.[1-4] There is variability in survival due to differences in the definition of transformation across studies as well as lack of a uniform approach to treatment.[5] Although there is no established standard of care, patients with transformed NHL (tNHL) are most often treated similarly to those with aggressive NHL histology.[5]

The prognosis of patients who develop Richter's transformation (RT) - the progression of CLL, most commonly to DLBCL - is particularly poor with a median survival of only 4-12 months.[6,7] RT occurs at a rate of ~0.5% per year, affecting 2-10% of CLL patients over the course of their disease, with similar incidences in both previously treated and treatment-naïve CLL patients.[8,9] A standard treatment for RT has not been defined, and most available data come from phase II studies, in which response rates range from 5-50%.[8] RT is frequently associated with aberrations in *TP53*, *CDKN2A/B*, *NOTCH1*, *SPEN*, *IRF2BP2* and *MGA* genes,

which portend worse overall prognosis and render RT cells resistant to chemoimmunotherapy.[10] Although allogeneic stem cell transplantation may be curative in RT, prospective data regarding efficacy remain limited.[11-13] In the era of novel agents, RT remains a significant concern, with evidence suggesting that RT rates have not decreased. Patients with RT exposed to both chemotherapy and novel agents have a limited survival of approximately 3 months.[14,15] Chimeric antigen receptor T-cell (CAR T) therapy has demonstrated efficacy in both transformed indolent NHL and RT, however, in retrospective studies, outcomes of patients with RT remain worse than those of patients with other aggressive lymphoma subtypes.[16, 17] Overall, despite advances in the treatment of aggressive NHL, novel therapeutic approaches for transformed lymphomas remain an unmet need.

Immune escape is one of the hallmarks of cancer, and restoring antitumor immunity has emerged as an effective therapeutic strategy in many cancers. Programmed cell death-1 (PD-1) and its ligands programmed death-ligand 1 (PD-L1) and PD-L2 constitute an important immune checkpoint axis, which modulates immune response under physiological conditions.[18] In B-cell NHL, neoplastic B cells as well as tumor-infiltrating non-malignant cells express high levels of PD-L1 as a mechanism to evade the immune system.[19-21] PD-1 therapy has shown promise in clinical trials for RT. In a phase II study of pembrolizumab in CLL, nine patients with RT were included, with four achieving a response and a median overall survival of 10.7 months.[22] A separate phase II trial of nivolumab combined with ibrutinib in CLL enrolled 24 patients with RT, achieving a response rate of 42% (10 patients).[23] The PD-1 inhibitor tislelizumab combined with the Bruton tyrosine kinase (BTK) inhibitor zanubrutinib in a phase II study showed a 58% response rate among 59 patients.[24] The depth and duration of these responses in B-cell malignancies is not yet known.

Copanlisib is an inhibitor of phosphoinositide 3 kinase (PI3K) α/δ isoforms which has shown preliminary efficacy in FL and DLBCL. The PI3Kα isoform is expressed in DLBCL and preclinical data suggest that dual inhibition of PI3K-α and -δ leads to tumor regression in non-germinal center B-cell DLBCL. In a phase II study in patients with relapsed/refractory FL, copanlisib achieved an overall response rate (ORR) of 59%.[25] Additionally, in a phase II study in DLBCL, the ORR in transformed FL (tFL) was 33.3%.[26] In a per-protocol analysis of another phase II trial in relapsed/refractory DLBCL, patients with non-germinal center B-cell DLBCL showed an ORR of 37.5%.[27] There is also a strong pre-clinical rationale for the use of PI3K inhibition in RT as several studies have demonstrated addiction to tonic PI3K signaling associated with transformation of CLL to RT.[10,28] Collectively, these results support the theoretical rationale for combining PI3K and PD-1 inhibition in tNHL. Herein, we report the results of a phase I investigator-sponsored, dose-escalation and expansion trial of copanlisib in combination with nivolumab in patients with RT or tNHL.

Methods

Study design and participants

We conducted a multicenter, open-label, phase I, investigator-sponsored trial (NCT03884998). Participating sites included the City of Hope National Medical Center, Dana Farber Cancer Institute and Oregon Health and Science University. All participating sites obtained institutional review board approval and all patients signed informed consent to participation in the study. Eligible patients were aged ≥18 years with histologically confirmed DLBCL arising out of CLL (RT) or indolent NHL (tNHL) with disease that relapsed or was refractory to at least one prior line of therapy. Patients with RT could have received prior therapy for CLL, RT, or both. Patients with tNHL had to have received at least one prior chemoimmunotherapy regimen and not be a candidate for, or had to have relapsed after, autologous stem cell transplantation. Other inclusion/exclusion criteria are summarized in the *Online Supplementary Methods*. The phase I dose escalation portion of the study followed a standard 3+3 design, followed by an initial dose expansion cohort of ten patients and a subsequent six-patient expansion in tFL after demonstration of promising initial efficacy. There were three planned dose levels (DL) of copanlisib, allowing for one dose escalation or one dose de-escalation as indicated. Copanlisib was to be administered intravenously (IV) on DL1 at 45 mg on days 1, 8 and 15, DL2 at 60 mg on days 1, 8 and 15 and DL "-1" at 45 mg on days 1 and 15 of a 28-day cycle, as outlined in Table 1. Nivolumab 240 mg was given IV on days 1 and 15 of each cycle. Patients could receive up to 24 cycles of therapy.

Study outcomes and statistical analyses

The primary study objective was to evaluate the maximum tolerated dose (MTD) of copanlisib in combination with nivolumab by evaluating adverse events (AE) and dose-limiting toxicities (DLT). DLT were monitored during the first cycle of treatment and were defined as grade ≥4 hematologic toxicities lasting longer than 7 days, grade ≥3 non-hematologic toxicities, grade 2 pneumonitis, or as described (*Online Supplementary Methods*) using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v5). Dose adjustments were allowed for copanlisib, but not nivolumab. Immune-related AE were managed as described in *Online Supplementary Table S1.* The secondary objective was to evaluate the preliminary

Table 1. Phase I dosing schema.

Dose level	Copanlisib	Nivolumab
-1	45 mg IV on days 1 and 15	240 mg IV on days 1 and 15
1	45 mg IV on days 1, 8, and 15	
2	60 mg IV on days 1, 8, and 15	

mg: milligrams; IV: intravenous.

efficacy of the combination, with endpoints including ORR, duration of response (DOR), and progression-free survival (PFS). Response was evaluated using the Lugano criteria.[29] Positron emission tomography (PET)-computed tomography (CT) (or CT, if PET-CT was not feasible) was performed on day 1 of cycle 3 and every three cycles thereafter while on treatment and every 6 months while in follow up. DOR and PFS were measured from the time of first response (DOR) or time of treatment start (PFS) to progression, subsequent anti-lymphoma therapy, or death, whichever was earlier. Patients were evaluable for efficacy if they completed at least one cycle of study treatment. During the dose finding phase, patients who did not complete the DLT period for reasons other than DLT were replaced and not included in the DLT assessment. Additional statistical methods, including sample size estimation as well as details related to the exploratory objectives including pharmacodynamic endpoints and correlative studies, are described in the *Online Supplementary Methods*. Baseline characteristics were summarized using descriptive statistics.

Results

Patients' and treatment characteristics

Twenty-seven patients were enrolled, 11 in the dose-finding portion (8 at DL1, and 3 at DL2) and 16 in the dose-expansion cohort. Fourteen had RT and 13 had tNHL (12 FL and 1 with transformed lymphoplasmacytic lymphoma). The patients' baseline characteristics are outlined in Table 2. The median age was 65 years (range, 32-77) with a 70% male predominance. Twenty-four patients (89%) had an Eastern Cooperative Oncology Group performance status ≤1. Patients had received a median of four prior lines of therapy (range, 1-10), including eight patients (30%; 2 RT, 6 tNHL) who had undergone prior autologous CD19-targeted CAR-T therapy. The median time from CAR-T therapy to the start of treatment with trial therapy was 7 months (range, 5-33 months). No patient had undergone prior autologous stem cell transplantation. Patients received a median of 3 cycles (range, 1-20), with a median of 2.5 cycles (range, 1-18) for those with RT and 4 (range, 1-20) for those with tNHL. Baseline genetic data were available for the antecedent CLL in eight of the 14 RT patients. All but one had a complex karyotype with del17p, two had *MYC* gene translocations, and three had del 11q. Altogether 18 patients discontinued therapy because of progressive disease (67%), seven because of AE (26%; 2 colitis, 1 prolonged lung infection, 1 esophagitis, 1 diarrhea, 1 neck pain), one patient refused to continue study treatment because of an infusion-related reaction to copanlisib, and one because of preexisting central nervous system disease discovered after enrollment.

Maximum tolerated dose

During the dose-finding part of the study, eight patients were treated at DL1 (45 mg copanlisib), five initially and three after DL2 had been explored. Among them six had RT and two had tFL. Two patients did not complete the DLT period because of rapidly progressive disease and were replaced. No DLT were observed in six evaluable patients at

Table 2. Patients' characteristics.

Characteristics	All patients N=27	RT N=14	tFL N=12
Age, years Median (range)	65 (32-77)	68 (41-77)	63 (32-76)
Prior lines of therapy, N Median (range)	4 (1-10)	3.5 (1-9)	5 (1-10)
Disease type, N (%) RT tFL Transformed WM	14 (52) 12 (44) 1 (4)	14 (100) - -	- 12 (100) -
Sex, N (%) Male Female	19 (70) 8 (30)	13 (93) 1 (7)	5 (42) 7 (58)
ECOG performance status, N (%) 0-1 2	24 (89) 3 (11)	12 (86) 2 (14)	11 (92) 1 (8)
Prior CAR T, N (%)	8 (30)	2 (14)	5 (42)
Prior lenalidomide, N (%)	5 (19)	1 (7)	4 (33)
Prior BTK inhibitor, N (%)	12 (44)	10 (71)	1 (8)

N: number; RT: Richter's transformation; tFL: transformed follicular lymphoma; WM: Waldenström macroglobulinemia; ECOG: Eastern Cooperative Oncology Group; CAR T: chimeric antigen receptor T cells; BTK: Bruton tyrosine kinase.

DL1. At DL2 (60 mg copanlisib) with three treated patients (2 RT and 1 tNHL), three DLT (grade 4 febrile neutropenia and grade 4 neutropenia in 1 patient, grade 4 thrombocytopenia in a second patient) were observed in two patients with RT. Therefore, the MTD and the recommended phase II dose of copanlisib in combination with nivolumab was determined to be 45 mg on days 1, 8, and 15.

Safety

Among all patients, the most common treatment-related AE (any grade) were diarrhea (44%), anemia (37%), fatigue (37%), neutropenia (37%), thrombocytopenia (37%), hyperglycemia (37%), and increased alkaline phosphatase (37%). The most common AE are presented in Table 3 and a complete list is provided in *Online Supplementary Table S2*. The most common severe AE (grade ≥3) were neutropenia (22%) and leukopenia (15%). Dose reductions of copanlisib from DL1 to DL-1 occurred in one patient because of elevated liver enzymes. Overall, there were ten cycles of treatment in which the dose was delayed in eight patients, with seven delays due to toxicity.

A total of 12 (44%) patients reported treatment-related diarrhea; in 75% of these cases the highest grade was initially reported in cycle 1 or 2. Two (7%) patients reported grade 3 diarrhea in cycles 2 and 4, respectively. Two patients had colitis, one with a grade 3 treatment-related colitis that occurred in cycle 3, and another with a grade 3 colitis that was considered unrelated to treatment and probably due to antibiotics. One patient had grade 3 pneumonitis in cycle 7 which was deemed treatment-related. There was one grade 3 treatment-related elevation of alanine aminotransferase in cycle 1 that lasted 7 days, and no report of grade ≥3 treatment-related elevation of aspartate aminotransferase. Hyperglycemia and hypertension were seen at a frequency of 37% and 33%, respectively. All cases were self-limited and there were no new cases of diabetes mellitus or chronic hypertension diagnosed during the course of the study.

Treatment-related infections occurred in four (15%) patients; of these infections, three were grade 3 (2 lung infections and 1 septic shock).

Efficacy

Efficacy analyses were conducted on 26 patients, excluding the one RT patient who was found to have preexisting central nervous system disease. For the entire cohort the ORR was 46% 4 complete responses [CR] and 8 partial responses [PR]. Patients with tFL had an ORR of 67% [CR] (2 CR, 6 PR, 1 stable disease [SD], 3 progressive disease [PD]); patients with RT had an ORR of 31% (2 PD, 2 PR, 2 SD, 7 PD). Both patients with RT who achieved CR developed immune-related AE (eosinophilic esophagitis and colitis) and both patients remain in remission from RT after a follow-up of 3.1 and 3.4 years, although one patient started a new therapy for relapsed CLL at 17 months and is technically counted as having PD. Among the eight patients who had received prior CAR-T therapy, the ORR was 63% (2 CR and 3 PR). The median follow-up among survivors is 17 months (range, 13-29) with follow-up until death, enrolment on long-term follow-up, consent withdrawal, or 1 year off-treatment. Twenty-four patients have progressed or started new therapy; two patients (1 RT and 1 tFL) were censored at 17.5 and 20.0 months, respectively, when they

Table 3. Attributable (possibly or higher) adverse events of all grades (>10% frequency overall) and grade 3-4 (in two or more patients) (N=27).

Adverse events	N (%)
All grades (>10%)	
Diarrhea	12 (44)
Anemia	10 (37)
Fatigue	10 (37)
Alkaline phosphatase increased	10 (37)
Neutrophil count decreased	10 (37)
Platelet count decreased	10 (37)
Hyperglycemia	10 (37)
Nausea	9 (33)
Hypertension	9 (33)
Aspartate aminotransferase increased	8 (30)
Alanine aminotransferase increased	7 (26)
Lymphocyte count decreased	7 (26)
White blood cell decreased	7 (26)
Maculopapular rash	6 (22)
Fever	5 (19)
Abdominal pain	5 (19)
Hyponatremia	4 (15)
Hypomagnesemia	3 (11)
Muscle cramp	3 (11)
Blood bilirubin increased	3 (11)
Chills	3 (11)
Grade 3-4 (≥2 patients)	
Neutrophil count decreased	6 (22)
Lymphocyte count decreased	4 (15)
Hypertension	3 (11)
Anemia	3 (11)
Platelet count decreased	3 (11)
Abdominal pain	3 (11)
White blood cells decreased	2 (7)
Lung infection	2 (7)
Diarrhea	2 (7)
Hyperglycemia	2 (7)

reached the end of study observation (1 year after treatment completion). Responses and DOR are outlined in Figure 1. The median DOR was 6.7 months (95% confidence interval [95% CI]: 2.0-15.5), the median PFS was 3.3 months (95% CI: 1.8-4.9) and the median OS was 23.8 months (95% CI: 5.4-not available [NA]) (Figure 2). Patients with RT had a median PFS of 2.0 months (95% CI: 0.7-4.9) and a median OS of 7.6 months (95% CI: 1.3-NA); the four responders had a median DOR of 15.2 months (95% CI: 3.0-NA). Patients with tFL had a median PFS of 4.4 months (95% CI: 1.4-12.2) and a median OS of 23.8 months (95% CI: 4.2-NA); the eight responders had a median DOR of 3.0 months (95% CI: 1.7-13.6) (Figure 2). The one patient with transformed lymphoplasmacytic lymphoma had progressive disease after two cycles of treatment.

Correlative studies

We performed an analysis of paired tumor and peripheral blood samples from patients with RT using flow cytometry and single-cell RNA sequencing. Peripheral blood samples were analyzed at baseline, after completion of one and five cycles of therapy and at the end of treatment, as described in the *Online Supplementary Methods*.

We analyzed samples from three patients with RT who achieved response on treatment and three non-responders. Flow cytometry analysis showed rapid downmodulation of PD1 expression in $CD4^+$ and $CD8^+$ T cells, regardless of response, consistent with the mechanism of action of nivolumab (Figure 3A). By contrast, we observed no changes in expression of CTLA4 (*data not shown*).

We next conducted single-cell RNA-sequencing analysis from two responders and three non-responders with RT who had sufficient material (Figure 3B). Following quality control, we clustered 56,189 cells from all samples based on their expression profiles and assigned cell types according to marker gene expression: B cells (*CD79A, CD19, IGHD*), $CD4^+$ T cells (*CD3D, CD3E, IL7R*), $CD8^+$ T cells (*CD8A, CD8B, CRTAM*), classical monocytes (***CD14, LYZ, CD36***), type-2 dendritic cells (DC2 [*LY75, CD1C*]), natural killer [NK] cells (*NCAM1, NKG7, NCR1, PRF1*), non-classical monocytes (*FCGR3A, TCF7L2, LYN*, *MTSS1*), and plasma DC (*IL3RA, CLEC4C*) (Figure 3C).[30-32] Clustering was not influenced by collection timepoints, except for malignant B cells, indicating minimal batch effects due to our multiplexing strategy. We identified distinct B-cell clusters with unique gene expression patterns, reflecting significant intra-tumoral heterogeneity between responders (CN09, CN10) and non-responders (CN13, CN14, CN15), and minimal intra-tumoral heterogeneity within the responder group. Compared with normal peripheral blood mononuclear cells, we observed altered copy number variations in our samples (*Online Supplementary Figure S1A*). We also identified distinct populations of circulating $CD5^+CD19^+$ B cells in four of five samples, likely representing circulating CLL cells (*Online Supplementary Figure S1B*). Gene expression analysis demonstrated that, interestingly, oxidative phosphorylation and *MYC* targets were enriched

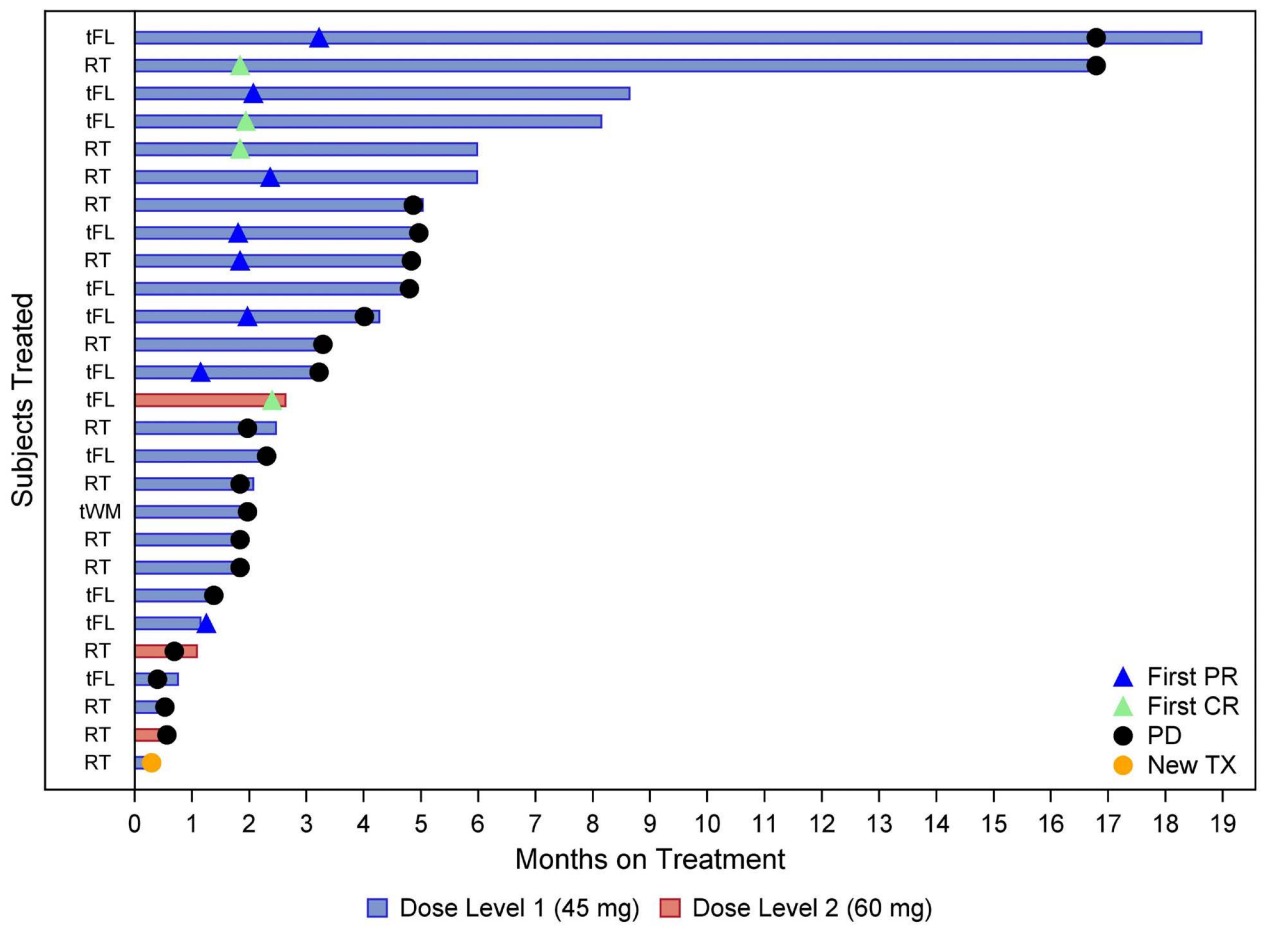

Figure 1. Swimmer plot showing response and duration of response by disease subtype. tFL: transformed follicular lymphoma; RT: Richter's transformation; tWM: transformed Waldenström macroglobulinemia; PR: partial response; CR: complete response; PD: progressive disease; TX: treatment.

at baseline in both CD4⁺ and CD8⁺ T cells obtained from responders (*Online Supplementary Figure S2*).

Subpopulation analysis demonstrated downregulation of MYC and NFκB signaling in circulating tumor cells as early as a month after therapy, which was still evident at later timepoints, regardless of response (Figure 4A). Analysis of T-cell subpopulations revealed upregulation of interferon (IFN)-α and IFN-γ signaling pathways in CD4⁺ and CD8⁺ T cells after one cycle of therapy in both responders and non-responders (Figure 4B, *Online Supplementary Figure S3*). However, T-cell activation was not sustained in non-responders, and was no longer evident at a later timepoint. Parry *et al.* previously reported that ZNF683 marks a CD8⁺ T-cell population associated with anti-tumor immunity following anti-PD-1 therapy for Richter syndrome.[33] We found that expression of ZNF683 was higher in responder

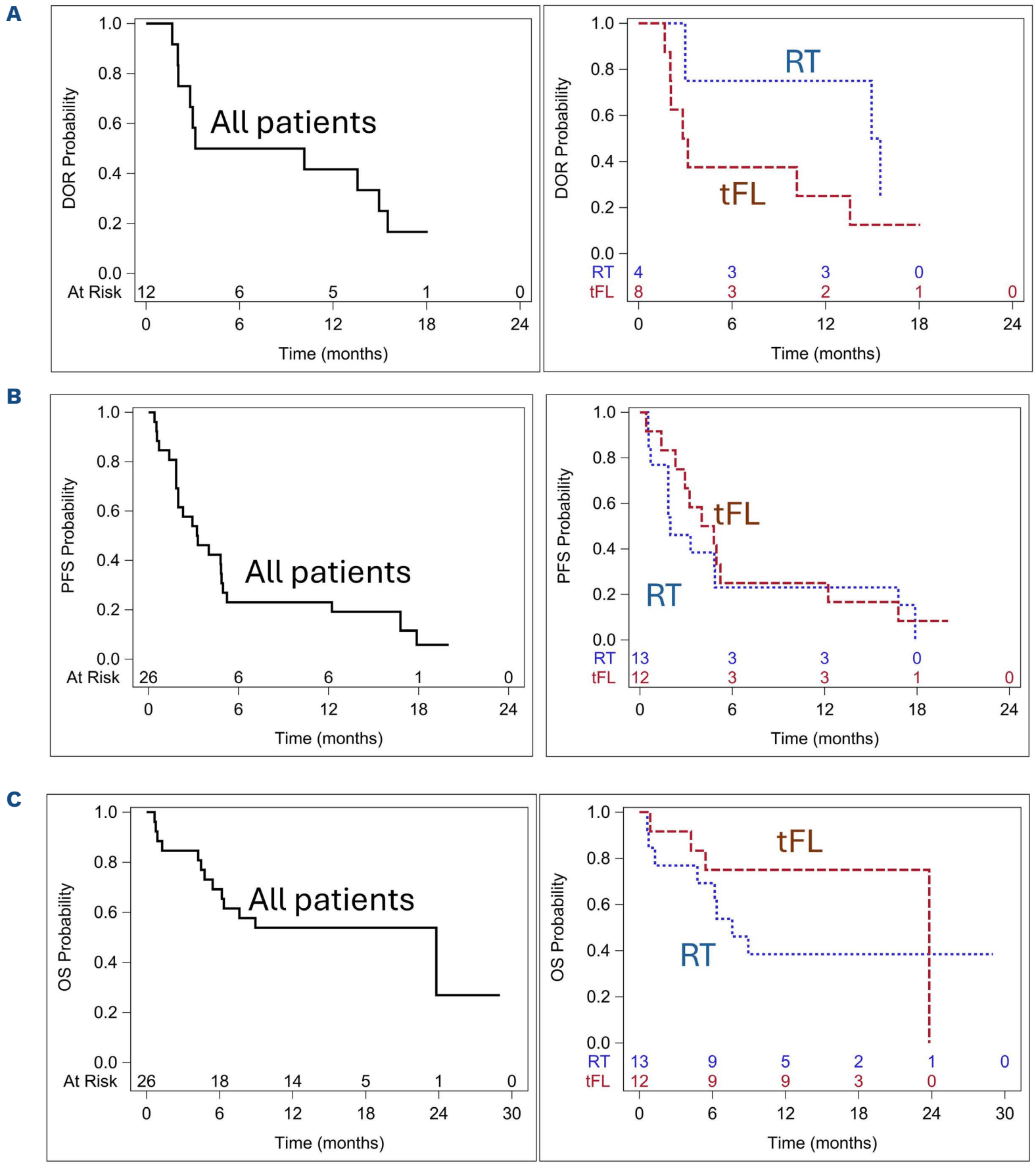

Figure 2. Outcomes of patients with Richter's transformation and transformed follicular lymphoma. (A-C) Duration of response (A), progression-free survival (B) and overall survival (C) in all patients (left panels) or in patients with Richter's transformation or transformed follicular lymphoma. DOR: duration of response; OS: overall survival; PFS: progression-free survival: RT: Richter's transformation; tFL: transformed follicular lymphoma.

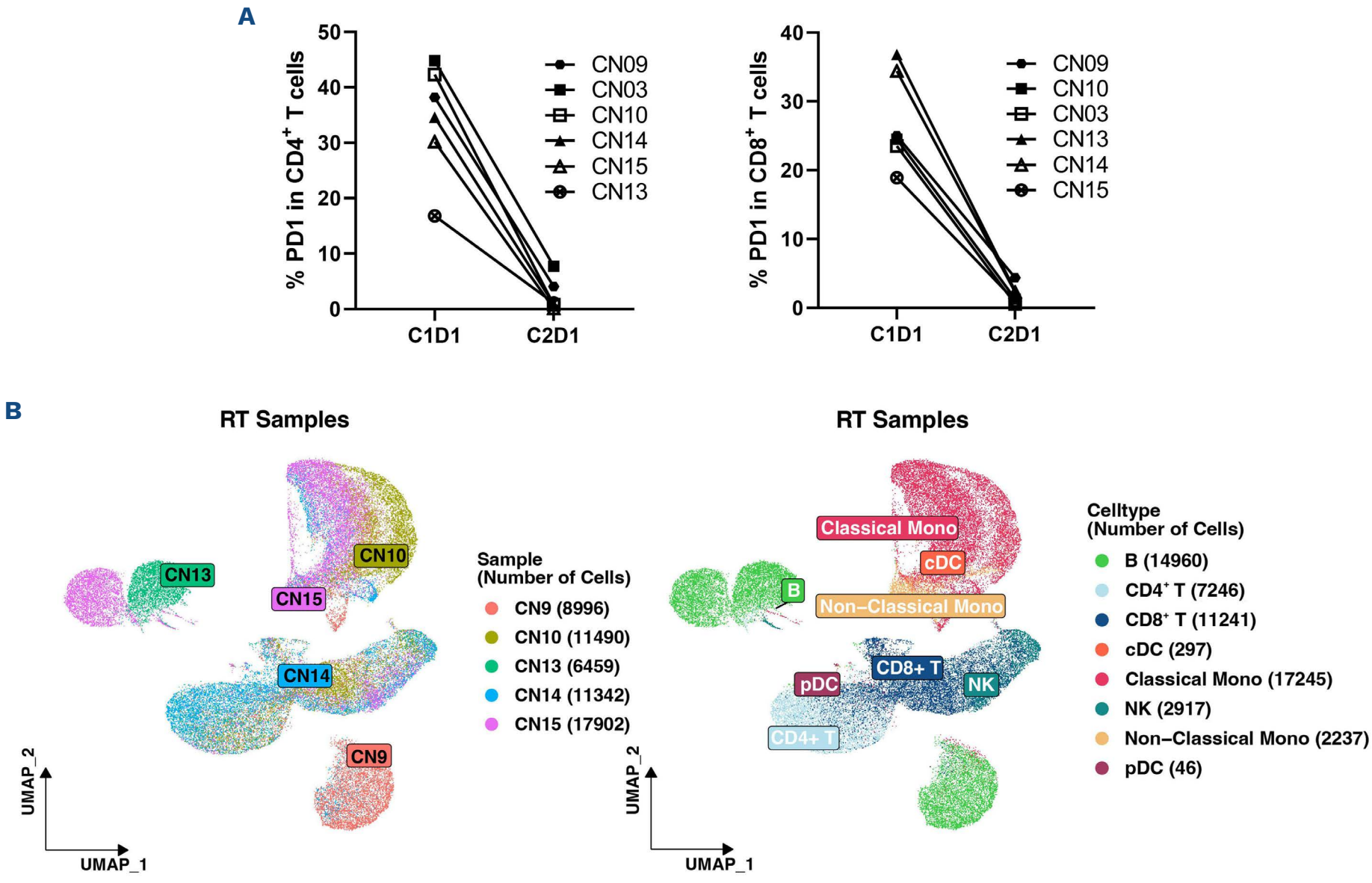

Figure 3. Correlative analysis of Richter's transformation samples. (A) Expression of PD-1 in peripheral blood T cells obtained from patients with Richter's transformation treated with copanlisib plus nivolumab assessed by flow cytometry. (B) Overview of cell types profiled in the single-cell RNA-sequencing cohort. The left uniform manifold approximation and projection (UMAP) plot displays a total of 56,189 cells profiled from five patients and 15 samples, categorized into eight distinct cell types (colors repre-

Continued on following page.

sent different cell types). The right UMAP plot shows the same cells, colored according to the originating samples. (C) Dot plot representing the expression of key marker genes across different cell types. The color scale indicates average expression levels, with red representing higher expression and blue indicating lower expression. The size of each dot corresponds to the percentage of cells expressing the gene within each population. PD-1: programmed cell death protein-1; C1D1: cycle 1, day 1; C2D1: cycle 2, day 2; RT: Richter's transformation; cDC: classical dendritic cells; mono: monocytes; NK: natural killer; pDC: plasma dendritic cells; Aver.Exp: average expression; Per.Exp: percentage expression.

CD8$^+$ T cells in our study, although the difference did not reach statistical significance likely due to small sample size (*Online Supplementary Figure S4*).

Flow cytometry analysis confirmed increased secretion of IFN-γ in T cells obtained from responding patients (Figure 4C). This was accompanied by an increased secretion of TNF-α in CD4$^+$ T cells, suggesting reprogramming towards a Th1 phenotype in response to treatment. Meanwhile, we observed no change in expression of interleukin (IL)-2, IL-4 and IL-17 in either CD4 or CD8 T-cell populations, while non-responders did not exhibit significant changes in cytokine expression by flow cytometry (*data not shown*). Together, these findings reveal distinct T-cell activation patterns within T-cell subpopulations between responders and non-responders following exposure to copanlisib and nivolumab.

Discussion

The combination of copanlisib and nivolumab showed promising efficacy in patients with transformed DLBCL arising from FL, whereas efficacy in RT patients was less encouraging. It is important to acknowledge that both PI3K- and PD-1-targeting therapies carry a risk of significant toxicities. The potential risk of autoimmune complications was a concern with the combination, and included colitis (reported risk of 1-2% for copanlisib and up to 10% for nivolumab), pneumonitis (1.4-9% for copanlisib and 0-8.5% for nivolumab), thyroiditis (0-18% for nivolumab) and hepatitis (1-2% for copanlisib and 1-6% for nivolumab).[34-39] Acknowledging these risks, the combination demonstrated manageable toxicity with the most common severe AE being neutropenia (22%) and leukopenia (15%). The DLT that occurred in patients with RT were also non-immune and included febrile neutropenia/neutropenia and thrombocytopenia, leading to 45 mg copanlisib as the MTD. Although 12 (44%) patients reported treatment-related diarrhea, only two cases were grade 3. Grade 3 colitis, pneumonitis, and elevation of alanine aminotransferase were uncommon (2, 1 and 1 episodes, respectively). In addition, in our study only 26% of patients discontinued treatment due to AE, similar to rates of discontinuation of single-agent copanlisib (25% in CHRONOS-1)[39] and nivolumab (28.3% from a pooled analysis of 4,677 patients treated with nivolumab).[40] Interestingly, the kinetics of response differed between tFL and RT with our regimen. While higher ORR and CR rates were achieved in tFL, the RT group had a longer DOR. Importantly, preliminary analyses of tumor cells and immune cell subsets from patients with RT treated on study suggest key differences in gene expression changes between responders and non-responders, which offer potential insights into mechanisms of resistance and may inform future strategies for optimizing immune therapies.

Despite a heavily pre-treated population of patients (up to 10 prior lines of therapy), the ORR among tFL patients was promising at 60%. This included 30% of patients previously treated with CAR-T therapy, a group for whom relapsed disease is typically associated with particularly poor prognosis.[16] Although the number of patients with tFL was small, the observed response rate was higher (60% *vs.* 33%) than previously reported for single-agent copanlisib in a phase II study with a tFL subgroup (N=6), in which both responses were partial.[26] Taken together, our data suggest that the addition of nivolumab provided clinical benefit. The immune microenvironment and, specifically, elevated expression of PD-1 on T cells, is thought to influence outcomes in FL, including the risk of transformation.[41-43] Thus, reversal of T-cell exhaustion and facilitation of tFL cell destruction in this context are likely the mechanisms driving the enhanced response observed.

The response rate among RT patients was less striking than in tFL, with only one-third of patients achieving a response. Where data were available, the underlying genetic alterations in the antecedent CLL were high risk including complex karyotypes and 17p deletion in all but one case suggesting that our cohort of patients was enriched for high-risk RT. Despite limited response rates, the DOR was significant, with a median of 15.2 months. This prolonged DOR is reminiscent of initial studies in which some melanoma patients achieved extended responses with anti-PD-1 therapy.[44] Interestingly, in our study, both RT patients who achieved CR discontinued treatment because of immune-related AE requiring corticosteroid therapy - esophagitis and colitis. Despite this, both patients remain in remission from RT 3.1-3.4 years after treatment. This observation suggests that immune "hyper"-activation may be a therapeutic mechanism in some cases of RT, a phenomenon observed with PD-1-directed therapy in other cancers.[45] Other studies have also shown success with PD-1-based combination therapies in RT. For example, in a phase II study combining the BTK inhibitor zanubrutinib with tislelizumab, the ORR was 58% (CR rate, 19%) among 48 patients evaluable for efficacy, with a median PFS of 10 months (95% CI: 3.8-16.3).[24] Similarly, a phase II study of ibrutinib and nivolumab demonstrated an ORR of 42% among 24 patients with RT.[23] In addition,

the combination of rituximab, gemcitabine and oxaliplatin with atezolizumab was evaluated in another phase II study that included patients with tFL and RT with ORR and CR rates, respectively, of 79% and 43% for those with tFL and of 22% and 11% for those with RT.[46] Finally, a phase II study of ibrutinib with durvalumab in patients with DLBCL and FL showed an ORR of 25% among the 61 patients treated.[47] Although these results are promising, there is still some room for improvement and future studies are needed to further improve response.

To further investigate the underlying molecular and immune mechanisms leading to therapy resistance in RT,

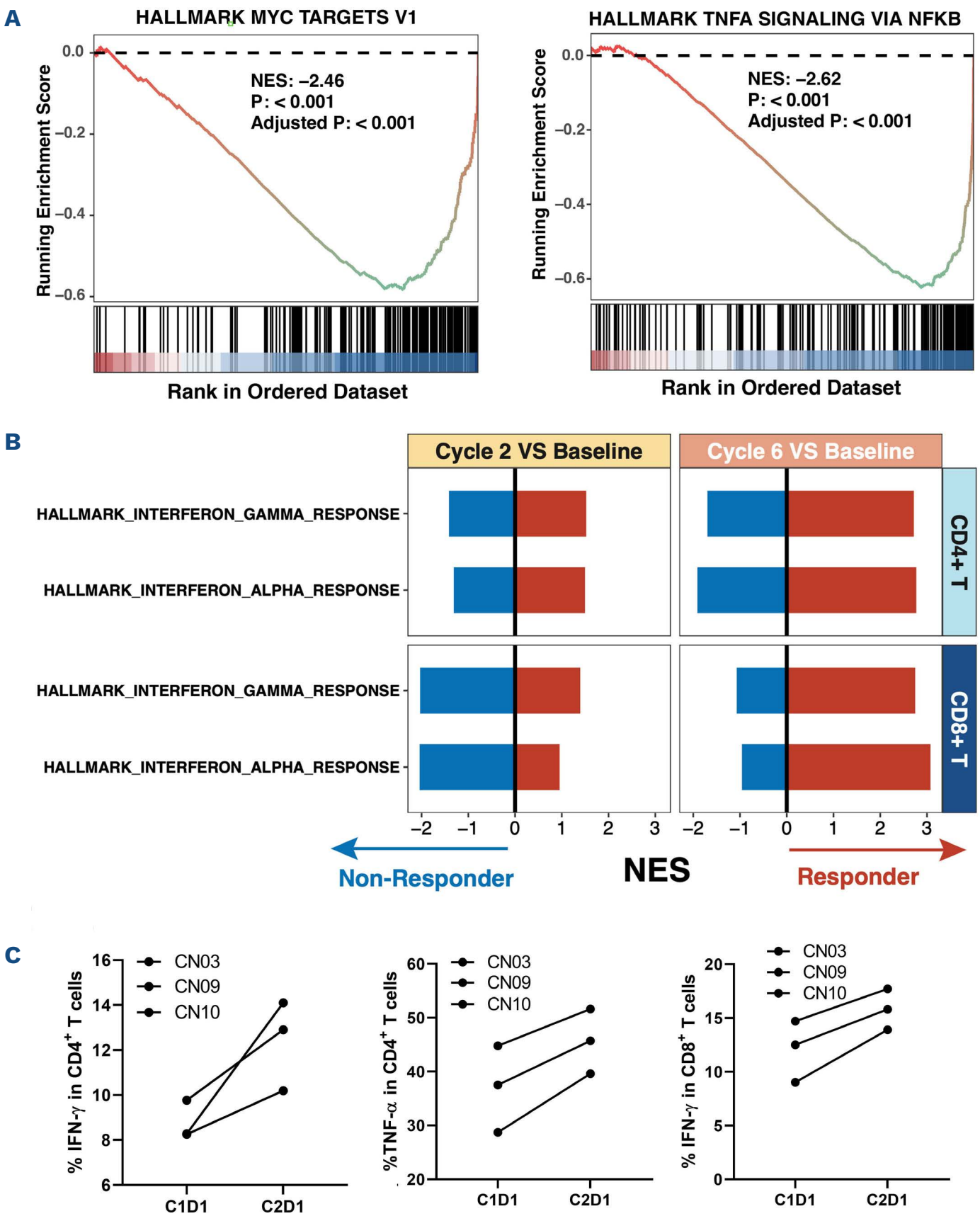

Figure 4. Pseudo-bulk gene set enrichment analysis of signaling pathways in malignant B cells and T-cell subpopulations. (A) Enrichment plots for MYC and NFκB signaling pathways in B cells at cycle 6, day 1 compared to baseline across all patients. (B) Normalized enrichment score (NES) of interferon response pathways in CD4⁺ and CD8⁺ T cells, comparing responders and non-responders across treatment cycles. The left panel (cycle 2 *vs*. baseline) and the right panel (cycle 6 *vs*. baseline) illustrate the activity of "HALLMARK_INTERFERON_GAMMA_RESPONSE" and "HALLMARK_INTERFERON_ALPHA_RESPONSE" pathways. Bars in blue indicate the NES score in non-responders, while bars in red show yhe NES score in responders. (C) Cytokine expression in peripheral blood T cells obtained from patients with Richter's transformation treated with copanlisib plus nivolumab assessed by flow cytometry. IFN: interferon; C1D1: cycle 1, day 1; C2D1; cycle 2, day 1; TNF: tumor necrosis factor.

we performed flow cytometry and single-cell RNA-sequencing analysis of paired samples from responders and non-responders. We observed a downregulation of PD-1 expression on T-cell subsets in all patients' samples over time, as expected with PD-1 treatment. We also found that MYC and NFκB signaling was decreased in circulating tumor cells from both responders and non-responders after 1 month of therapy. This finding aligns with preclinical observations of gene expression changes in lymphoma cell lines following exposure to copanlisib.[48,49] Furthermore, we observed increased IFN-α and IFN-γ signaling in both CD4 and CD8 T cells, irrespective of response, consistent with prior findings reported after copanlisib exposure.[48] However, while the increased IFN signaling was sustained in responders, it was short-lived in non-responders. We confirmed the elevation of IFN-γ signaling in responders through flow cytometry, which also revealed an increase in TNF-α expression in CD4 T cells, suggestive of a transition to a Th1 phenotype. By contrast, no significant changes in cytokine expression were observed in non-responders. IFN-γ expression has been shown to correlate with response to PD-1 treatment in melanoma and non-small cell lung cancer and may be a potential biomarker of response.[50-52] Further studies, however, are needed to fully characterize potential targets that could address these differences and guide future treatment strategies.

The limitations of this study include the limited DOR seen in tFL patients, however, there is still potential to use this combination as a bridging or induction regimen prior to more definitive consolidation. Evidence suggests that copanlisib may lead to downregulation of regulatory T cells and promote $CD8^+$ T-cell infiltration in the immune microenvironment.[53,54] This phenomenon, along with reversal of T-cell exhaustion by nivolumab, may prime patients for better efficacy with T-cell-based therapies such as CAR-T. Additional limitations include a small overall sample size; however, RT is a rare occurrence in CLL and few studies focus on this area, making our research relevant to the field. Additionally, multiple PI3K inhibitors, including copanlisib, have been withdrawn from the market due to concerns about toxicities. Nevertheless, our results suggest that there is potential for PI3K inhibitors to have utility in certain settings in which effective therapies are limited, such as RT.

In summary, we demonstrate that the combination of copanlisib and nivolumab produced no new safety signals and is efficacious in patients with previously treated RT and tFL. Copanlisib 45 mg was determined to be the MTD of the combination. The therapy had manageable toxicity. Response rates, especially among tFL patients, were promising despite the substantial burden of prior treatments. DOR of the two complete responders with RT suggest the potential for durable disease control in select patients. Additionally, our findings provide insights into potential mechanisms of response and resistance to therapy, which may inform future therapeutic strategies in these rare diseases.

Disclosures

GS is a consultant and member of a speakers' bureau for Beigene and Kite Pharma. AVD is a consultant for AbbVie, ADCT, AstraZeneca, BeiGene, Bristol-Myers Squibb, GenMab, Janssen, Lilly Oncology, MEI Pharma, Merck, Nurix, Regeneron and Roche and has ongoing research support from AbbVie, AstraZeneca, Beigene, GenMab, Lilly Oncology, Merck, Nurix and Regeneron. MSD is a consultant for AbbVie, Ascentage Pharma, Adaptive Biotechnologies, AstraZeneca, BeiGene, Bristol-Myers Squibb, Eli Lilly, Galapagos, Genentech, Genmab, Janssen, Merck, MEI Pharma, Nuvalent, Schrödinger, SecuraBio, Takeda and TG Therapeutics and has ongoing research support from Novartis, Ascentage Pharma and MEI Pharma. ASK is a consultant for AbbVie, AstraZeneca, Bristol-Myers Squibb, Eli Lilly and Galapagos, receives speaking honoraria from AstraZeneca, Eli Lilly and Janssen and research support from Beigene. AFH is a consultant for Bristol-Myers Squibb, Genentech, Merck, Seagen, AstraZeneca, ADC Therapeutics, Takeda, Genmab, Pfizer, AbbVie and Allogene Therapeutics and has ongoing research funding from Bristol-Myers Squibb, Genentech, Merck, Seagen, AstraZeneca and Pfizer. TS is a consultant for AstraZeneca, AbbVie, BeiGene, Bristol-Myers Squibb, Celgene and Gilead/Kite, a member of a speakers' bureau for AstraZeneca and receives research funding from Bristol-Myers Squibb. SEFS is a consultant for Genentech, Janssen, Beigene, ADC Therapeutics and Incyte, is an expert witness for AbbVie, and receives research funding from Beigene, Bristol-Myers Squibb, Incyte, Janssen, ADC Therapeutics, Shrodinger, Merck, Profound Bio, Gilead and Acutar. JZ is a consultant for Secura Bio, Citius, Myeloid and Kyowa Kirin, a member of a speakers' bureau for Kyowa Kirin and receives research support from Pfizer, Secura Bio, AstraZeneca, Myeloid, CRSPR and Daiichi Sankyo.

Contributions

AVD and ASK designed the study. GS, MSD, SEFS and AVD were the study investigators. GS, MSD and AVD enrolled the patients. GS, AM, LP, TS, JZ, AFH, OD and LC collected and assembled the data. OD, CL, LW, LC, CC and ZX analyzed the data and prepared figures. All authors interpreted the data. GS, CC, LC and AVD wrote the paper. All authors critically reviewed and approved the manuscript.

Data-sharing statement

Data from the clinical trial (NCT03884998) and correlative analysis are available upon request to the corresponding author at adanilov@coh.org.

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